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Pets as Sentinels, Forecasters and Promoters of Human Health

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To the pets that illuminate our lives…
to Maria Pia, the cat

Preface

More than a company or protection, more than attractive possessions or a set of playful personalities, pets have the potential to promote human health. As sentinels of exposure to environmental contaminants or forecasters of future health conditions, pets can help their human companions attain higher living standards.

This book presents recent advances on the uses of pets as sentinels, forecasters, and promoters of human health. The chapters compiled under this volume, written by leading specialists from a wide array of different and complementary areas (from biology, chemistry, toxicology, and psychology to veterinary and medical sciences), will provide a comprehensive understanding of the capabilities of pets regarding human health.

The first part of the book (Part I) focuses on the possible use of pets as sentinels and surrogates regarding exposure to the main classes of environmental contaminants. By sharing the same environment with their human companions, pets are exposed to the same type of contaminants, and by possessing a higher sensitivity and shorter periods for the establishment of symptoms and the onset of disease, they may be used as sentinels. The shared environment can be regarded in a more confined way, focusing, for example, on the household that is shared between human and animal or going in the opposite direction into a broader context, as, for example, an entire ecosystem. Both perspectives are addressed in this volume. The chapter by Sonne and collaborators offers an ecosystem-based perspective on the usefulness of sledge dogs as sentinel species of ecosystem health across the Arctic, a particular and vulnerable region that is especially sensitive to environmental stressors, as evidenced by the multilayered effects of climate change. The chapter by Poma et al. provides a global overview of the use of pets as sentinels of indoor contamination, explaining the rationale behind the use of pets as sentinels and describing the vast array of contaminants that pets are exposed indoors, paving the way for the subsequent chapters that deal with specific chemicals. Aslan et al. describes the different classes of pesticides to which pets and humans are exposed under a “One Health” perspective, highlighting the common food and water sources and the potential impacts on animal and human health. Luzardo and colleagues provide a detailed account of human and pet exposure to polycyclic aromatic hydrocarbons (PAHs).

These authors review the available information and make an important contribution to the enlargement of this highly limited amount of data by providing new, previously unpublished results of PAHs levels in the blood of pet dogs. The chapter by Pastorinho et al. describes the levels, the exposure pathways, and the impacts of neurotoxic metals in both cats and dogs and discusses the usefulness of this knowledge in the use of pets as sentinels and early warning systems of human neurodegenerative processes. The exposure of cats and dogs to brominated flame retardants (BFRs) is analyzed by Mizukawa and Nomiyama. These authors describe the tissue-specific accumulation and biotransformation of polybrominated diphenyl ethers (PBDEs) and their hydroxylated and methoxylated derivatives (OH-PBDEs and MeO-PBDEs) in pet dogs and cats and unveil the possible mechanisms that might be responsible for the differences observed between these animals. Weiss and Jones provide further insights on the deleterious health consequences of PBDEs exposure in cats. The authors summarize the available studies describing blood levels of PBDEs and other organic contaminants in cats and discuss potential effects on the thyroid hormone system.

The second part of the book (Part II) deals with the capacity of pets to be used as forecasters and promoters of human health, particularly regarding noncommunicable diseases, including obesity and metabolic disorders, cancer, and immunological and neurological diseases. The chapter by Tvarijonaviciute and co-workers explores the similarities, links, and differences between human and canine obesity in order to better understand this disease that is now considered a global epidemic. The authors describe the causes and effects of obesity and further provide a detailed description of obesity grade measurements as well as obesity biomarkers in both cats and dogs. Vilhena et al. in their chapter on canine and feline spontaneous mammary tumors use a comparative oncology approach to describe the histophatological, molecular, and genetic features of canine and feline mammary tumors and propose that these spontaneous tumors can act as models for human breast cancer. The chapter by Taborda-Barata provides a clinical perspective on the immunomodulatory effects of pets. The author reviews the effects of pets on the human immune system, addressing the role of pet guardianship in the risk of developing allergic diseases and other immunomodulators, such as the reduction of psychological stress and depression, as well as the increase in the levels of regular physical activity and exercise. The potential use of this immunomodulatory effect of pets is further addressed in the chapter by Gilbert and collaborators. The microbiome approach put forward by these authors provides novel insights on how pets may affect human health and well-being and examines whether or not pets may be able to function as a new microbiome-based therapy and thus as promoters of human health. The chapter by Vaz-Patto provides a clinical-oriented perspective on benefits and hazards of pets' interactions with the elderly. The impacts of pet guardianship are reviewed in the light of the more frequent neurological changes presented after 65 years of age, and the positive and negative aspects associated with pets and the elderly population are outlined. The effects of companion animals on human health and the use of pets in health promotion in a healthcare setting are critically reviewed by Silva and Lima. The authors describe the evidence suggesting companion animals have a positive impact

on human health as well as the available opposite findings. By critically evaluating the literature, the authors identify gaps still needing to be addressed and propose the implementation of a comprehensive research approach, which integrates confounding, mediating, and moderating variables.

The third part of the book (Part III) is dedicated to the psychosocial and psychophysiological aspects of human-animal interactions. The complex communication between cats and humans is summarized and described in the chapter by Schötz. The author explains how cats communicate and describes in detail the vocal communication of domestic cats through phonetic methods. The application of these methods provides a deeper understanding of the phonetic characteristics of cat vocalizations, which, according to the author, may improve cat-human communication and ultimately may contribute to improve health and well-being of cats and, by extension, their tutors. The final chapter on this section provides an alternative view on the human-pet interaction by essentially focusing on the pet perspective. The chapter by Glenck explores the canine perspective of animal-assisted interventions (AAI), describing the factors that modulate dog's welfare and addressing the physiological and behavioral welfare indicators available to identify stress in dogs used in AAI. The author reviews the limitations of the available studies and highlights the future directions in this emerging field.

We hope that the diverse, complementary, and sometimes discordant perspectives brought by the different authors on the usefulness of pets as sentinels, forecasters, and promoters of human health may contribute to increase the debate on this topic, improving our understanding on the human pet relationships and thus reinforcing the One Health Concept.

We would like to finish by thanking the Springer editorial team for all the support and all the authors who contributed to this work. Without their commitments and patience, this book would not have been possible.

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Part I
Pets as Sentinels of Human Exposure to Environmental Contaminants

Chapter 1
Pets as Sentinels of Indoor Contamination

Giulia Poma, Govindan Malarvannan, and Adrian Covaci

Abstract Historically, domestic and wild animals have been used as sentinels for human exposure to environmental contaminants, providing an early warning system for public health intervention. Since domestic animals, particularly cats and dogs, share their (indoor) environment with humans, they can respond to or be affected by toxic assaults like their owners. Given that, the potential for pets to act as biosentinels of human exposure to environmental contaminants has been explored in many scientific papers. In this chapter, an overview of literature studies of how pets have served as sentinels for human health effects resulting from exposure to several classes of environmental contaminants (such as metals, persistent organic pollutants, flame retardants, and polycyclic aromatic hydrocarbons) is reported and discussed. The possible links among the studies and/or the potential gaps in knowledge and research were also investigated. The presented studies indicated that cats and dogs are exposed to complex mixtures of industrial chemicals. The research outcomes demonstrated how pets well may be serving as sentinels for human health, as they breathe in, ingest, or absorb the same chemicals that are in our (indoor) environments.

Keywords Pets · Sentinels · Human exposure · Indoor contaminants · Organic pollutants · Metals

1.1 Introduction

In the beginning of the twentieth century, miners in Great Britain and the USA were encouraged to carry small animals, like canaries or mice, in the coal mines to detect dangerous concentrations of carbon monoxide in their working environment (Rabinowitz et al. 2009; Reif 2011; Sekhar and Rao-Chakra 2014). Besides being

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easily transportable, these animals have a high basal metabolic rate, making them exhibit symptoms of poisoning before gas concentrations became critical for the workers (Sekhar and Rao-Chakra 2014). In Minamata Bay, Japan, during the 1950s, neurobehavioral symptoms were first observed in cats that consumed methylmercury-contaminated fish, and their disturbed behavior has been called by the locals the "dancing cat disease" (Rabinowitz et al. 2005). In 1960, Rachel Carson's renowned book *Silent Spring* implied that bird mortality related to the use of pesticides was a warning that pesticides, including DDT and other organochlorine compounds, were causing widespread toxicity in the environment that could also be a threat to human health (Carson 1962; Rabinowitz et al. 2009). More recently, the presence of reproductive abnormalities in fish, birds, amphibians, reptiles, and invertebrates has been attributed to the presence of endocrine-disrupting chemicals (EDCs) in the environment (Van Der Schalie et al. 1999; deFur 2004).

These are few examples of how domestic and wild animals have been historically used as sentinels for human exposure to toxic substances, being sensitive indicators of environmental hazards and providing an early warning system for public health intervention (Bischoff et al. 2010; Reif 2011). In particular, companion animals, e.g., cats and dogs, are considered valuable sentinels for human exposures because they are physically and physiologically similar to humans and they share their living environment with humans (Barthold et al. 2009). They are also potentially exposed to a high number of human-made chemicals, e.g., by inhaling indoor air with contaminated dust, strolling in industrial urban neighborhoods, eating factory-made pet food added with a number of chemicals, and being in contact with a wide variety of house and garden products (Backer et al. 2001; Ruiz-Suárez et al. 2016). In addition, because animals have typically shorter and physiologically compressed life spans if compared with people, latency periods for the development of some diseases are shorter in animals (Backer et al. 2001).

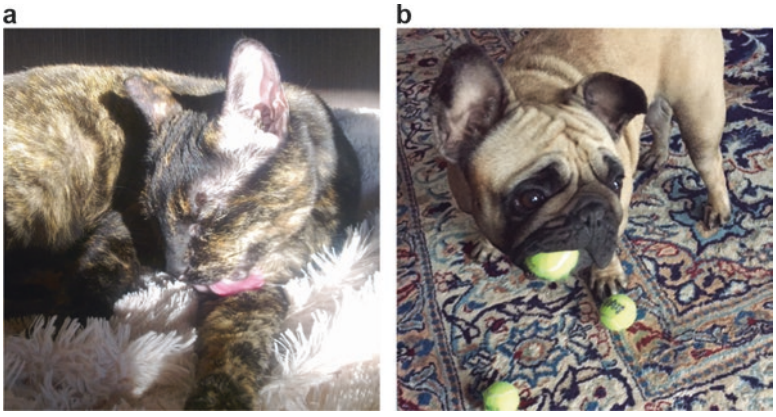

Fig. 1.1 A cat shows typical grooming behavior. (Photo of Brisbi taken by Giulia Poma) (**a**) and a dog shows chewing and mouthing behavior. (Photo of Pippa taken by Celine Gys) (**b**)

Like humans, the exposure of pets to chemical contaminants would likely occur through indoor dust ingestion, dermal contact or inhalation, diet, and contact with household materials (Ali et al. 2013; Ruiz-Suárez et al. 2016). Moreover, since certain behavioral patterns of pets (e.g., living close to the ground, chewing on domestic objects, licking and self-grooming, ingesting dust) (Fig. 1.1a, b) are similar to the behavior of human toddlers, the presence of toxic chemicals in cats and dogs can also be an early warning sign for the health of children (Betts 2007; Environmental Working Group (EWG) 2008). For example, the exposure of cats to indoor dust can be comparable to toddlers, assuming similar bioavailability for chemicals accumulated in dust, so the exposure in cats is expected to reflect the toddlers' exposure (Norrgran Engdahl et al. 2017).

Since the iconic "canary in the cage" began to be used in the coal mines, the potential for all kinds of animals (pets in particular) to act as sentinels for human exposure to toxic substances has been explored in many scientific papers (Reif 2011; Ruiz-Suárez et al. 2016). In this chapter, an overview of literature studies of how household cats and dogs have served as sentinels for human health effects resulting from exposure to several classes of environmental contaminants (e.g., metals, persistent organic pollutants (POPs) and polycyclic aromatic hydrocarbons (PAHs), flame retardants (FRs)) is reported and discussed. As such, this chapter aims at finding possible links among the studies and/or identifying potential gaps in knowledge and research.

1.2 Exposure of Pets to Indoor Contaminants

The investigation of chemicals present in an indoor environment has allowed the identification of the main sources of pollutants, and their bioavailability has been assessed by measuring chemical residues in tissues, organs, or fluids of animals living in appropriate habitats (López-Alonso et al. 2007). In this perspective, the use of pets as biosentinels of contamination makes it possible to determine the degree of environmental contamination and the extent of human chemical exposure, since they share the same environment as their owners and are exposed, at least in part, to the same pollutants (López-Alonso et al. 2007; Tomza-Marciniak et al. 2012). For example, as cats meticulously groom themselves (Fig. 1.1a), they lick off accumulated dust that can be contaminated with FRs, such as polybrominated diphenyl ethers (PBDEs); dogs eating scraps from the floor may swallow dirt and dust tracked in from the outdoors and become contaminated with heavy metals and pesticides (Environmental Working Group (EWG) 2008; Veterinary Learning Systems 2008); dogs chewing and mouthing behaviors (Fig. 1.1b) can lead to oral exposures to a variety of chemicals (Wooten and Smith 2013). In addition, pets often ingest food packaging materials that contaminate their food resulting in cumulative exposures with unknown health risks (Environmental Working Group (EWG) 2008). Therefore, the knowledge of the contamination status in these animal populations is an important first step, not only to estimate the magnitude of indoor pollution but also to predict human health risks from exposure to environmental contaminants (Storelli et al. 2009).

1.2.1 Pets as Biosentinels of Contamination with Metals

In recent years, there has been an increasing ecological and global public health concern associated with environmental contamination by toxic metals, able to induce toxicity at low level of exposure (Tchounwou et al. 2012). Human exposure to toxic metals has risen dramatically because of an exponential increase of their use in several industrial, agricultural, domestic, and technological applications. Reported sources of toxic metals in the environment include geogenic, industrial, agricultural, pharmaceutical, and domestic effluents (López-Alonso et al. 2007). While certain metals (e.g., copper (Cu), chromium (Cr), iron (Fe), magnesium (Mg), zinc (Zn)) are essential nutrients requested for various biochemical and physiological functions, other metals, such as arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg), have no established biological functions and are considered as non-essential metals. Because of their high degree of toxicity, these non-essential elements are known to induce multiple organ damage even at lower levels of exposure and rank among the priority metals that are of great public health significance (Tchounwou et al. 2012).

In 2006, a study aimed at determining blood Pb concentration in dogs from two urban areas and in surrounding rural areas of India and at analyzing Pb concentrations in dogs in relation to environmental (urban/rural) and animal variables (age, sex, breed, and housing) was published (Balagangatharathilagar et al. 2006). Blood samples were collected from 305 dogs of either sex from urban and unpolluted rural localities. The results clearly demonstrated that the urban dogs had significantly higher mean Pb concentration (0.25 μg/mL) than rural dogs (0.10 μg/mL) and that in stray dogs, either from urban or rural locality, the mean blood Pb level (0.27 μg/mL) was higher than that of pets (0.20 μg/mL). The locality (urban/rural) was considered as the major variable affecting blood Pb concentration in dogs. In particular, the blood Pb concentration in dogs was significantly influenced by breed and housing (pet/stray) in the case of dogs from urban areas and only by housing in dogs from rural areas. The higher Pb residues found in the blood of urban dogs were considered an indication of their exposure to excess lead from the environment, probably due to pollution of urban localities with the emission from industrial units and automobile exhausts (Balagangatharathilagar et al. 2006). In addition, the elevated environmental Pb content and possible contamination of food for dogs might have contributed for the higher blood Pb residue in the studied urban canine population.

To evaluate the utility of dogs as biosentinels of human exposure to metals, the concentrations of four toxic metals (viz., As, Cd, Hg, and Pb) in canine liver and kidney were investigated in Lugo (Spain) (López-Alonso et al. 2007). The authors have then compared between dogs from rural (most of the day outside, but coming indoors overnight) and urban (most of the time inside the house) habitats. The influence of diet, sex, and age on the accumulation of toxic metals was also considered. Mean As residues in the dogs were similar in the liver (12.6 ng/g wet weight, ww) and kidney (15.9 ng/g ww) and were not significantly affected by any of the variation factors

considered. On the contrary, the mean Cd concentrations were significantly higher in the dog kidney (175.5 ng/g ww) than in the liver (58.0 ng/g ww), varying significantly with both age and sex, but not being influenced by diet and habitat. Mean Hg residues were significantly higher in the kidney (53.4 ng/g ww) than in the liver (32.7 ng/g ww), significantly influenced only by the habitat: Hg concentrations in kidney of dogs from urban areas were higher than in dogs from rural areas. Mean Pb concentrations were significantly higher in the liver (57.7 ng/g ww) than in the kidney (23.1 ng/g ww), affected only by the diet: dogs fed commercial diets showed higher Pb residues than dogs fed homemade or mixed commercial and homemade feeds, respectively. The mean concentrations of toxic metal observed in these dogs were in general below concentrations considered high for dogs (liver 0.5–1.0 (As), 1.0–7.0 (Cd), and 3.6–5.0 (Pb) μg/g ww; kidney 0.5–1.0 (As), 4.0–17.0 (Cd), and 5.0–10.0 (Pb) μg/g ww) (Puls 1994). This is most likely because Lugo is a relatively unpolluted area.

In 2010, a case study involving a US farm in which Pb-containing paint was found as the major source of contamination for the animals was presented (Bischoff et al. 2010). Among the farm animals, measurable Pb concentrations were documented in the blood of a dog (0.15 μg/mL) and a cat (0.08 μg/mL), and two different sources of Pb exposure in the farm were identified: Pb paint on the barn for the cat and Pb paint on the house for the dog. The cat probably ingested Pb from the floor and barn dust that accumulated on his fur through grooming behavior, while the paint removal from the house (in undergoing renovation) was the most likely source of Pb contamination for the dog, via inhalation and ingestion of Pb paint dust (Bischoff et al. 2010).

The concentrations of Pb and Cd were investigated in serum of 48 healthy pet dogs from an urban area of northwestern Poland (Tomza-Marciniak et al. 2012). The mean concentrations of the analyzed metals were 0.49 and 0.31 μg/mL, respectively. Of all the factors analyzed, the body size had the largest effect on the concentrations of metals, while neither age nor sex had a significant effect on the metal concentrations in serum. In addition, it was hypothesized that small dogs could be more vulnerable, since they are lower to the ground and inhale larger amounts of dust, soil particles, and deposited particulates, which carry toxic metals (Tomza-Marciniak et al. 2012).

Literature data concerning Hg concentrations in household pets are still scarce. In order to fill this gap, the concentrations of total mercury (HgTotal) were assessed in 26 dog blood and hair, and the use of household pets as sentinels for human environmental exposure to Hg was investigated in Portugal (Sousa et al. 2013). The obtained results, independent of gender, age, and diet type, showed relatively low concentrations of HgTotal in the surveyed dogs, with values ranging from 0.16 to 12.4 ng/g in blood and from 24.2 to 826 ng/g in hair. Since inorganic Hg has a higher excretion rate in the organism than methylmercury, it is poorly accumulated, and therefore the measured HgTotal concentrations tend to reflect the methylmercury concentrations (Tchounwou et al. 2012). In this study, a highly significant positive correlation was found between blood and hair Hg concentrations, validating the latter as a surrogate, non-invasive matrix for the evaluation of Hg exposure (Sousa et al. 2013).

Table 1.1 Summary of reported data on metal concentrations in pet cats and dogs

Country	Year	Metal	Species	Sample type (units)	Concentrations	Reference
India	2006	Pb	Dogs	Blood (μg/mL)	Urban dogs = 0.25 ± 0.01	Balagangatharathilagar et al. (2006)
					Rural dogs = 0.10 ± 0.01	
					Stray dogs = 0.27 ± 0.01	
					Pets = 0.20 ± 0.01	
Spain	2007	As, Cd, Pb, Hg	Dogs	Liver and kidney (ng/g)	As: liver = 12.6; kidney = 15.9	López-Alonso et al. (2007)
					Cd: liver = 58.0; kidney = 175.5	
					Pb: liver = 57.7; kidney = 23.1	
					Hg: liver = 32.7; kidney = 53.4	
USA	2010	Pb	Cats/ dogs	Blood (μg/mL)	Cat = 0.08	Bischoff et al. (2010)
					Dog = 0.15	
Poland	2012	Pd, Cd	Dogs	Serum (μg/mL)	Pb = 0.49 ± 0.07	Tomza-Marciniak et al. (2012)
					Cd = 0.31 ± 0.05	
Portugal	2013	Hg	Dogs	Blood and hair (ng/g)	Blood = 0.16–12.4	Sousa et al. (2013)
					Hair = 24.2–826	

The presented studies (Table 1.1) fully support the idea that pet animals, living in both urban and rural areas, may be indicators of environmental metal pollution, providing information about the potential exposure of humans to these toxic substances.

1.2.2 Pets as Biosentinels of Contamination with Persistent Organic Pollutants and Polycyclic Aromatic Hydrocarbons

POPs are organic compounds that, to a varying degree, resist photolytic, biological, and chemical degradation, characterized by low water solubility and high lipid solubility, leading to their bioaccumulation in fatty tissues (Ritter et al. 1995). POPs include different classes of contaminants, among which organochlorines, such as polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs)

are persistent in the environment and of bioaccumulative nature (Kunisue et al. 2005). Although their production and use have been restricted or banned in most industrialized countries, considerable amounts of these compounds are still circulating in the ecosphere (Storelli et al. 2009). PAHs are ubiquitous environmental pollutants containing two or more fused benzene rings that are produced during the incomplete combustion of organic matter and during human or industrial activities (Guo et al. 2012b). Because of their efficient metabolization, PAHs are not POPs, but due to their high prevalence in the environment and their lipophilicity, PAHs are usually considered as pseudo-POPs (Lammel et al. 2013).

Exposure to these pollutants has been linked to a wide range of potent biological effects including immunosuppression, carcinogenicity, reproductive toxicity, and endocrine disruption in wildlife and humans (Ritter et al. 1995; Storelli et al. 2009). However, information on residue concentrations of POPs (and PAHs) in companion animals is still fragmented and limited (Storelli et al. 2009).

For example, the concentrations of OCPs and PCBs were determined in genital organs of pet dogs and cats and pet foods from Japan (Kunisue et al. 2005). Concentrations of POPs in dogs were relatively lower than those in cats, while residue concentrations in their diets were almost similar, implying that accumulation and elimination mechanisms of these contaminants in genital organs are different between the two species. This result suggests that pet dogs are at lower health risk by POPs, while pet cats may be at higher risk. Even if it has been reported that pet dogs may be valuable sentinels for environmental contamination by various chemicals, including POPs, this study showed that pet dogs have different accumulation patterns of OCPs and PCBs from pet cats and humans, suggesting that cats may serve as better sentinels of human exposure to environmental POP contamination rather than pet dogs (Kunisue et al. 2005).

With this background, the status of PCB and DDT contamination and the accumulation profile of individual PCB congeners were investigated in 84 pet cats and 91 dogs from Southern Italy (Storelli et al. 2009). In cats, the major component among DDTs was its metabolite p,p′-DDE, with an average value of 94.8 ng/g lipid weight (lw), while in dogs DDT and its metabolites were not detected in any animal, except in two specimens. Also PCB concentrations were higher in cats (199.0 ng/g lw) than in dogs (41.6 ng/g lw). These findings can reflect differences of size class, dietary exposure, and/or xenobiotic metabolizing systems between the species (Storelli et al. 2009). It has also been suggested that dogs might have greater metabolic capacity and elimination of POPs than cats (Kunisue et al. 2005).

The contamination levels of OCPs and PCBs were investigated in 2013 in a total of 36 pet serum samples (cats, $n = 20$, and dogs, $n = 16$) and 22 hair samples (cats, $n = 12$, and dogs, $n = 10$) collected from three large cities in Pakistan (Ali et al. 2013). Mean values of OCPs were higher in cat serum and hair (475 ng/g lw, 12.7 ng/g hair, respectively) than in dog serum and hair (32 ng/g lw, 10.3 ng/g hair). These results confirmed that pets may be valuable sentinels for indoor contamination by many, but not all, chemicals and with significant differences in the target species. This might be due to differences in their diet, accumulation and elimination mechanisms, and interspecies differences.

The hypothesis of an existing relationship between environmental exposure to PCBs and OCPs via indoor environments and adverse health effects was then investigated, targeting domestic cats suffering from diabetes mellitus (DM) (Dirtu et al. 2013). Mean concentrations of OCPs and PCBs of 0.65 and 1.75 ng/mL, respectively, were measured in plasma samples collected from diabetic cats. While lower concentrations of OCPs were found in cats than in UK human serum samples (Thomas et al. 2006), the PCB profiles in cat plasma were found to mirror the general profile. Although diet was not analyzed in this study, it is often acknowledged as the main source for exposure to "legacy" POPs in humans, and it seems probable that the same source is responsible for the observed POP profile in the cat samples.

The concentrations and accumulation patterns of PCBs and their metabolites (OH-PCBs) were determined in the blood of pet cats ($n = 11$) and dogs ($n = 17$) collected from a veterinary hospital in Japan (Mizukawa et al. 2016). To estimate the exposure routes to these chemicals, the extent of dietary exposure of these pets to PCBs and their metabolites from representative samples of dry and wet pet food products was also determined. Median PCB concentrations in cat blood samples (48 pg/g ww) were higher than those in dog blood samples (< 7.4 pg/g ww), attributed by the authors to cat's higher exposure levels. Interestingly, median PCB concentrations in dry pet food (120 pg/g ww for dogs and 350 pg/g ww for cats) were significantly higher than those of wet pet food (13 pg/g ww for dogs and 72 pg/g ww for cats), implying that PCBs in raw materials are concentrated during the manufacture of dry pet food products. OH-PCBs were detected in the blood samples of both species (median of 120 pg/g ww for dogs and 93 pg/g ww for cats), while only a few OH-PCB congeners, at extremely low concentrations, were found in the pet food, suggesting the biotransformation of PCBs to OH-PCBs in dogs and cats.

To explore metabolic capacity differences between dogs and cats and the hypothesis that domestic dogs might not be good sentinels for human exposure to POPs (Kunisue et al. 2005; Storelli et al. 2009; Ali et al. 2013), the role of the dog as monitor of human exposure to PCBs, OCPs, and PAHs was investigated (Ruiz-Suárez et al. 2015, 2016). In the first study, the authors determined the concentrations of 16 PAHs, 18 PCBs, and 19 OCPs in samples of typically consumed feeds for dogs and cats and calculated the daily dietary intake of these pollutants in both species (Ruiz-Suárez et al. 2015). The levels of the same pollutants were then measured in the plasma of 42 dogs and 35 cats, fed on the analyzed commercial feeds. The levels of pollutants were found higher in dog food (median $\sum$PAHs of 22 ng/g ww, $\sum$OCPs of 15 ng/g ww, $\sum$PCBs of 8.5 ng/g ww) than in cat food (median $\sum$PAHs of 7.6 ng/g ww, $\sum$OCPs of 6.2 ng/g ww, $\sum$PCBs of 2.4 ng/g ww), and the results showed that the median values of intake were about twice higher in dogs than in cats for the three groups of pollutants (ΣPAHs 275 vs. 142, ΣOCPs 233 vs. 83, ΣPCBs 102 vs. 44 (ng/kg bw/day), respectively). As expected, considering the median intake, the plasma levels of PAHs were higher in dogs than in cats. However, despite the higher intake in dogs, the plasma levels of OCPs and PCBs were found to be 2 to 23 times higher in cats than in dogs. This reveals a lower capacity of bioaccumulation of some pollutants in dogs, likely related with their higher metabolizing capabilities. Considering that exposure to POPs in vertebrates is likely

through food ingestion, these results suggest that dogs seem to be able to efficiently metabolize and eliminate some POPs and that domestic cats may represent a better model to assess human exposure to these chemicals (Ruiz-Suárez et al. 2015). In the second study, the authors determined plasma concentrations of 56 POPs (27 PAHs, 11 OCPs, and 18 PCBs) in the plasma of 87 dogs and 100 people from the Canary Islands (Spain) (Ruiz-Suárez et al. 2016). The mean values of PAHs were 782 ng/g lw for dogs and 1624 ng/g lw for humans, and it was hypothesized that the lower concentrations of PAHs detected in the plasma of dogs could be due to a higher rate of biotransformation and elimination thereof or to the presence of different routes and extent of exposure. Also for OCPs, the values of most of the target contaminants were much lower in dogs (75.6 ng/g lw) than in humans (mean 724 ng/g lw) and with different pollution profiles between the two species. With respect to PCBs values, the medians were almost 20 times lower in dogs than in humans (24.3 vs. 364.7 ng/g lw). It was then concluded by the authors that, in the light of the obtained results, it does not appear that pet dogs can be used as suitable indicators or sentinels for human exposure to POPs and PAHs (Table 1.2).

1.2.3 Pets as Biosentinels of Contamination with Brominated Flame Retardants

Brominated flame retardants (BFRs) are industrial chemicals produced to delay the spreading of fire and added to construction materials, indoor decorations, furniture, textiles, electronics, and electrical appliances (Alaee et al. 2003). Due to their extensive applications in these products, many of the BFRs are now ubiquitous environmental contaminants (Norrgran Engdahl et al. 2017). PBDEs, one of the major classes of BFRs, are of concern since they may target the endocrine system and also undergo debromination, leading to the formation of lower brominated PBDE congeners that possess higher bioavailability potencies (Hakk and Letcher 2003). Since they are not chemically bound to the product material, PBDEs can leach out of the products over time and accumulate in dust, which serves as a sink for these kinds of compounds in indoor environments. Due to their grooming behavior, pets are particularly exposed to chemicals accumulated in indoor dust, making them good markers for indoor exposure to BFRs (Norrgran et al. 2015; Norrgran Engdahl et al. 2017). To date, several international agreements on the regulation and use of some PBDEs have been introduced, opening the way for the introduction of other flame retardants (novel flame retardants – NBFRs), used as replacements to comply with fire safety regulations in commercial products (Dodson et al. 2012). However, most of these replacement compounds are also persistent and bioaccumulative, and their toxicological effects are still not well understood (Venier and Hites 2011).

In an early study, the US Environmental Working Group (EWG) investigated the extent of exposures of pets to several classes of contaminants (including PBDEs) in indoor environment and found that American pets are polluted with even higher

Table 1.2 Summary of reported data on the concentrations of persistent organic pollutants and polycyclic aromatic hydrocarbons in pet cats and dogs

Country	Year	Compound	Species	Sample type (units)	Concentrations	Reference
Japan	2005	POPs	Cats/dogs	Genitals/pet food (ng/g lw)	PCBs, M[a] dog = 8.8 ± 5	Kunisue et al. (2005)
					OCPs[b], M dog = 10 ± 6.3	
					PCBs, F[a] dog = 20 ± 19	
					OCPs, F dog = 30 ± 29.8	
					PCBs, M cat = 78 ± 32	
					OCPs, M cat = 189 ± 180.3	
					PCBs, F cat = 77 ± 55	
					OCPs, F cat = 166 ± 172.2	
					PCBs, dog food = 5.4 ± 4	
					OCPs, dog food = 25.5 ± 32.1	
					PCBs, cat food = 5.9 ± 7.4	
					OCPs, cat food = 7.1 ± 5.9	
Italy	2009	POPs	Cats/dogs	Adipose tissue (ng/g lw)	PCBs, dogs = 41.6	Storelli et al. (2009)
					PCBs, cats = 199	
					DDT, dogs = 76.9	
					DDT, cats = 99.8	
Pakistan	2013	POPs	Cats/dogs	Serum, hair (ng/g lw, ng/g hair)	PCBs, dog serum = 18 ± 3.9	Ali et al. (2013)
					PCBs, cat serum = 47 ± 32	
					OCPs[c], dog serum = 32 ± 23	
					OCPs, cat serum = 475 ± 635	
					PCBs, dog hair = 0.1 ± 0.15	
					PCBs, cat hair = 0.5 ± 0.4	
					OCPs, dog hair = 10.3 ± 10.2	
					OCPs, cat hair = 12.7 ± 6.8	

(continued)

Table 1.2 (continued)

Country	Year	Compound	Species	Sample type (units)	Concentrations	Reference
UK	2013	POPs	Cats	Plasma	PCBs = 1.75	Dirtu et al. (2013)
				(ng/mL)	OCPs = 0.66	
Japan	2015	PCBs	Cats/ dogs	Blood/pet food (pg/g ww)	Dog, blood <7.4[d]	Mizukawa et al. (2016)
					Dog, dry food = 120	
					Dog, wet food = 13	
					Cat, blood = 48	
					Cat, dry food = 350	
					Cat, wet food = 72	
Spain	2014	POPs, PAHs	Cats/ dogs	Pet food (ng/g ww)	PAHs = 21.86[d] dogs; 7.58[d] cats	Ruiz-Suárez et al. (2015)
					OCPs = 14.84[d] dogs; 6.24[d] cats	
					PCBs = 8.49[d] dogs; 2.37[d] cats	
				Plasma (ng/g lw)	PAHs = 423[d] dogs; 253[d] cats	
					OCPs = 25.1[d] dogs; 47.9[d] cats	
					PCBs = 50.8[d] dogs; 89.2[d] cats	
Spain	2015	POPs, PAHs	Dogs	Plasma (ng/g lw)	PCBs = 24.3 ± 26	Ruiz-Suárez et al. (2016)
					OCPs[e] = 75.6 ± 52.7	
					PAHs = 782 ± 323.8	

[a]*M* male, *F* female
[b]Sum of DDT, HCH, HCB, CHL
[c]Sum of HCB, DDT, HCH
[d]Median
[e]Sum of DDT, HCB, HCH, cyclodienes

concentrations of many of the same synthetic industrial chemicals that researchers have recently found in people (Environmental Working Group (EWG) 2008; Veterinary Learning Systems 2008). Composite blood serum samples were collected from 20 dogs and 37 cats and analyzed for PBDE contamination. Concentrations of PBDEs were 113 ng/g lw in dogs (on average 2.7 higher than in humans) and up to 986 ng/g lw in cats (23.4 times higher than in humans). For both species, the potential sources of exposure were suggested to include foam furniture and bedding manufactured before 2005, contaminated air and house dust, and food contaminated with PBDEs (Environmental Working Group (EWG) 2008), while, especially for cats, a significant portion of PBDEs may come from dietary sources (e.g., seafood) and dust ingestion (Dye et al. 2007; Environmental Working Group (EWG) 2008).

Concentrations of PBDEs and NBFRs were determined in cat and dog serum and hair samples from Pakistan (Ali et al. 2013). Mean concentrations of PBDEs in cat and dog serum samples were 72 ng/g lw, significantly higher than those found in human serum from the same region, and 1.9 ng/g lw, respectively. In the same study, the concentrations of PBDEs and NBFRs (including bis-2,4,6-tribromophenoxyethane (BTBPE), decabromodiphenyl ethane (DBDPE), and tetrabromophthalate (TBPH)), were also detected in hair collected from the same cats (mean 5.15 and 7.2 ng/g, respectively) and dogs (mean 0.65 and 4.7 ng/g).

To explore the main exposure routes, ingestion of PBDEs from cats via house dust and via cat food was investigated in Sweden (Norrgran Engdahl et al. 2017). House dust from 17 homes and pet cat serum were collected, while cat food was purchased to match the diet reported. Paired samples of cat serum, house dust, and cat food were analyzed for PBDEs and the NBFR, DBDPE. The mean concentrations of PBDEs analyzed in cat serum, house dust, and cat food were measured at 63 pmol/g lw, 1435 pmol/g dry weight (dw), and 2.0 pmol/g lw, respectively. DBDPE was found in high concentrations in all dust (mean 386 pmol/g dw) and food samples (mean 1.4 pmol/g lw), but was below detection limit in serum samples, suggesting low or no bioavailability for DBDPE in cats. A correlation between cat serum concentrations and household dust has been established for the first time, supporting the hypothesis that dust is a significant exposure route for cats (Norrgran Engdahl et al. 2017).

The serum concentrations of PBDEs were also measured in serum of 22 pet cats and cat owners from Gran Canaria (Spain) (Henríquez-Hernández et al. 2017). In this study, the mean PBDE concentrations were found as 5.48 and 1.62 ng/g lw in cats and humans, respectively, and the correlation of concentrations and the pattern of contamination (congener distribution and proportions) between both species were reported to be significant.

The growing use of PBDEs in consumer products over the past 30 years has paralleled the rising incidence of feline hyperthyroidism (FH), at present the most common endocrinopathy in cats (Dye et al. 2007; Nguyen et al. 2014). The risk of developing FH was associated with indoor living and consumption of canned cat food (Dye et al. 2007). Dye et al. (2007) hypothesized that increases in FH were, in part, related to increased PBDE exposure, with key routes of exposure being diet and ingestion of house dust. In their study, serum samples were collected from 23 cats and analyzed for PBDEs. Mean serum concentrations were up to 12.7 ng/mL, 20- to 100-fold greater than median concentrations in US adults, supporting the hypothesis that cats are highly exposed to PBDEs. It was concluded that, by extension, due to prolonged PBDE exposure, cats may be at increased risk for developing FH.

To evaluate the concentrations of BFRs in cats from Sweden and to determine whether body burdens of these compounds differ depending on thyroid status, 138 serum samples from Swedish cats, pooled into 21 pools in accordance with cat thyroid status and age, were analyzed (Kupryianchyk et al. 2009). The highest median level of contamination was found for the BDE-209, found at 52 ng/g lw and 88 ng/g lw in hyperthyroid and non-hyperthyroid cats, respectively. Yet, no association between PBDE concentrations and thyroid status of cats was revealed.

However, the PBDE concentrations in serum from Swedish cats were about 50 times higher than in the general Swedish human population.

As a follow-up to the study by Dye et al. (2007), the PBDE concentrations in serum samples from 26 California household cats were measured (Guo et al. 2012a). They found extremely high PBDE concentrations (mean 4505 ng/g lw, approximately 50 times higher than concentrations in California residents), linked, by the authors, to their presence in house dust. However, no evidence that linked concentrations of PBDEs with FH was found. This may be due to the small sample size, competing or confounding risk factors, or complicated causal mechanisms (Guo et al. 2012a).

The role of PBDEs in the occurrence of FH was investigated also by measuring the PBDE concentrations in serum from 62 client-owned (21 euthyroid, 41 hyperthyroid) and 10 feral cats, together with samples of commercial cat food and home dust (Mensching et al. 2012). Median serum PBDE concentrations in euthyroid cats (2850 ng/g lw) were not significantly different from those of hyperthyroid cats (2517 ng/g lw), while the median serum PBDE concentrations in feral cats (883 ng/g lw) were significantly lower than in either of the groups of client-owned cats. Of the two major PBDE sources investigated in this study, relatively small amounts of PBDEs were found in canned cat food (range 0.42–3.1 ng/g ww), while dust (150–95,000 ng/g lw) was identified as a risk factor for PBDE exposure in the hyperthyroid cat's home. However, like Dye et al. (2007), this study did not support a difference in contaminant load between euthyroid and hyperthyroid cats.

In addition to hyperthyroidism, it has been suggested that direct relationships may also exist between environmental exposure to BFRs and type 2 diabetes mellitus (T2DM) (Dirtu et al. 2013). Interestingly, cats may serve as a sentinel also in this scenario, since, apart from hyperthyroidism, cats also suffer from a type of diabetes mellitus (DM) akin to human T2DM. The PBDE contamination in plasma of domestic cats suffering from DM, particularly DM induced by acromegaly and T2DM, was thus investigated in 2013 in the UK (Dirtu et al. 2013). The mean total PBDE concentrations in cat plasma were 0.98 ng/mL in acromegaly-induced diabetes and 1.66 ng/mL in T2DM. In agreement with the study by Dye et al. (2007), the PBDE concentrations measured in cat serum were higher than those usually reported for human samples collected in the same geographical area (Dirtu et al. 2013). These data suggest a great potential for accumulation of BFRs in cats, providing further evidence to the cat's potential role as sentinel for the assessment of low-level human exposure to chemicals via indoor environments.

As listed above, the measured PBDE concentrations in cat serum were, on average, higher than those found in humans, suggesting different mechanisms of absorption and elimination of these compounds (Dye et al. 2007; Environmental Working Group (EWG) 2008; Kupryianchyk et al. 2009; Guo et al. 2012a; Ali et al. 2013). Because the previous studies have suggested that cats tend to accumulate BFRs to a higher extent than dogs and that dogs can metabolically better degrade these pollutants (Storelli et al. 2009), it was investigated if pet dogs might be better biosentinels than cats (Venier and Hites 2011). The authors hypothesized that dogs may resemble better humans in their response to BFRs and therefore are better indicators of human exposure to these contaminants. In their study, the

authors collected blood samples from 18 pet dogs and dog food samples, analyzing them for PBDEs and DBDPE. The average concentrations of PBDEs in dog serum and food were 1.8 ng/g ww and 1.1 ng/g ww, respectively. Lower concentrations were found for DBDPE in the dog food samples (average of 0.030 ng/g ww, probably related to processing or packaging rather than to the raw material used to produce the dog food), but this compound was not detected in any of the dog serum samples. The concentrations of PBDEs in these dogs were not correlated with any of the animal-specific variables (i.e., age, weight, or daily time spent outdoors), except for the dog's weight, suggesting that dogs might accumulate PBDEs mainly through their diet, since daily feeding portions are based on weight, rather than age (Venier and Hites 2011). The lower concentrations of PBDEs measured in dogs compared to cats (from the above-listed studies) can eventually suggest that cats biomagnify these compounds more than dogs, that dogs metabolize these compounds more rapidly than cats, or that dogs are exposed to much lower concentrations of these compounds (Venier and Hites 2011) (Table 1.3).

Finally, the potential role of pet cats as sentinels for human exposure to organophosphorus flame retardants (PFRs) was evaluated for the first time in cat serum from Gran Canaria (Canary Islands, Spain) (Henríquez-Hernández et al. 2017). PFRs are currently considered as more environmentally friendly and safer than the BFRs and are being increasingly employed in consumer products. Although PFRs have been present in industrial formulations since 40 years ago, information about their environmental fate or their effects in humans and biota is however still scarce (Henríquez-Hernández et al. 2017). In this study, a total of 11 compounds were measured in the serum of 22 pet cats and 20 humans. Mean of total PFRs in cats was 1049 ng/g lw, not significantly different from the mean of total PFRs measured in humans (712 ng/g lw). Although some PFR compounds were found at higher concentrations in serum of cats than in humans, in general terms both patterns of exposure were virtually overlapped, suggesting that cats may play a potential role as sentinels of the human exposure also to PFRs.

1.3 Conclusions

The present review of scientific literature indicates that cats and dogs are exposed to complex mixtures of industrial chemicals, often at concentrations higher than those found in people. Several links between chemical exposures and health risks for pets were identified, improving understanding of the risk related to low-level chronic exposure to indoor pollutants which may lead to a cumulative body burden and adverse health outcome. The presented studies showed how pets' unique behaviors may place them at risk for exposures and health risks from environmental pollutants in the home, food, and consumer products (for both people and pets). These studies demonstrate that the use of pets as sentinels for indoor contamination can eventually improve the integration of human and ecological research on persistent environmental contaminants.

Table 1.3 Summary of reported data on flame retardant concentrations in pet cats and dogs

Country	Year	Compound	Species	Sample type (units)	Concentrations	Reference
USA	2007	PBDEs	Cats	Serum (ng/mL)	12.7 ± 3.9	Dye et al. (2007)
USA	2008	PBDEs	Cats/ dogs	Serum (ng/g lw)	Cats = 986 Dogs = 113	Environmental Working Group (EWG) (2008)
Sweden	2009	PBDEs	Cats	Serum (ng/g lw)	Hyperthyroid = 52[a] Non-hyperthyroid = 88[a]	Kupryianchyk et al. (2009)
USA	2011	PBDEs, DBDPE	Dogs	Serum/ dog food (ng/g ww)	PBDEs, serum = 1.8 ± 0.4 PBDEs, dog food = 1.1 ± 0.2 DBDPE, serum < LOQ DBDPE, dog food = 0.03 ± 0.006	Venier and Hites (2011)
USA	2012	PBDEs	Cats	Serum (ng/g lw)	4505 ± 1006	Guo et al. (2012a)
USA	2012	PBDEs	Cats	Serum/ dust (ng/g lw) cat food (ng/g ww)	Euthyroid = 2850[a] Hyperthyroid = 2517[a] Feral cats = 883[a] House dust 150–95,000 Cat food = 0.42–3.1	Mensching et al. (2012)
Pakistan	2013	PBDEs, NBFRs	Cats/ Dogs	Serum and hair (ng/g lw, ng/g hair)	PBDEs, cat serum = 72 ± 285 PBDEs, dog serum = 1.9 ± 1.1 PBDEs, cat hair = 5.15 ± 7.3 PBDEs, dog hair = 0.65 ± 0.3 NBFRs, cat hair = 7.2 ± 4.6 NBFRs, dog hair = 4.7 ± 2.4	Ali et al. (2013)
UK	2013	PBDEs, NBFRs	Cats	Plasma (ng/mL)	Acromegaly-induced diabetes = 0.98 Type 2 diabetes mellitus = 1.66	Dirtu et al. (2013)
Sweden	2017	PBDEs, DBDPE	Cats	Serum/ dust/cat food (pmol/g lw)	PBDEs, serum = 63 PBDEs, house dust = 1435 pmol/g dw PBDEs, cat food = 2 DBDPE, serum < LOQ DBDPE, house dust = 386 pmol/g dw DBDPE, cat food = 1.4	Norrgran Engdahl et al. (2017)
Spain	2017	PBDEs	Cats	Serum (ng/g lw)	5.48 ± 4.35 1049.8 ± 558.9	Henríquez-Hernández et al. (2017)

[a]median

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Chapter 2
Sled Dogs as Sentinel Species for Monitoring Arctic Ecosystem Health

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Abstract Here we review sled dogs as a sentinel monitoring species of ecosystem health across the Arctic focusing on environmental changes including pollution, climate change, and infectious diseases. Studies on environmental contaminants have been carried out mostly in Alaska and Greenland. While the majority of reports focus on mercury exposure and health effects, a major classical case-controlled study of exposure and effects from persistent organic pollutants (POPs) has been

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carried out on Greenland sled dog bitches and their pups. Altogether, the studies show that mercury and POPs affect multiple health endpoints across physiological systems, including reproductive, endocrine, and immune systems, that ultimately affect systems such as the liver and kidney. Therefore, sled dogs have proved to be a good model for assessing the health effects from contaminant exposure of top predators and Northerners in the Arctic. Furthermore, they are widely distributed across the Arctic and show similar correlations to important health indicators reported in Northerners and polar bears. With respect to climate change and disease dynamics of zoonosis, most studies have taken place in Canada. However, at present sled dogs are not utilized in monitoring studies of zoonotic diseases. Such an inclusion will increase the understanding of environmental changes, pollution, and diseases dynamics in Northerners and wildlife. We therefore recommend that ecosystem health assessments in the Arctic including that of Northerners start to include analyses of sled dogs combined with modeling tools. Doing so in a circumpolar perspective will further increase our understanding and monitoring possibilities of ecosystem health and Northerners exposure to contaminants, diseases, and climate change in the Arctic.

Keywords Arctic · Arctic fox · Climate change · Contaminants · Diseases · Ecosystem · Endocrine · Energetics · Genetics · Histopathology · Hormones · Immune · Inuits · Mercury · OHCs · PCB · One Health · Organohalogen · PBPK · Persistent organic pollutants · Polar bears · POPs · Sentinels · Sled dogs · Vitamins · Zoonosis

2.1 Introduction: The Sled Dog

The domesticated sled dog (*Canis familiaris*) is widely used by indigenous people in Greenland, Alaska, Canada, and Russia to pull dog sledges (Fig. 2.1). It hence represents a unique mode of transport allowing, e.g., hunters and tourist guides to explore, hunt, and transport their items and food in over considerable distances during the winter where the sea ice and snow coverage serve as the "road" for this ancient and original form of transport. The use of sled dogs as an animal engine of transport as compared to modern forms of transport in the Arctic, like the snowmobile or outboard driven engines, is of course slower, but much more reliable and does not require expensive investments and expensive gasoline which may limit the range and cause functional problems. In addition, the dogs can warn the hunters against polar bears (*Ursus maritimus*) in the night. They are even used during the bear hunt where a few dogs will be cut loose from the dragging team and will catch up with and distract the bear. Sled dogs are excellent in helping the driver to avoid unstable ice conditions, and the sledge is extremely suitable for crossing difficult landscape routes due to its high flexibility. Finally, the dogs are more silent and reliant compared to, for example, a noisy snowmobile that may break down, which make dogs very suitable for hunting in remote areas.

Fig. 2.1 The dog sledge represents a unique transport medium allowing the hunters to explore, hunt, and transport their items and food over considerable distances. Northwest Greenland, spring 1984. (Photo: R. Dietz)

In Greenland, the number of sled dogs has decreased by ca. 50% over the last three decades (Sonne et al. 2018; Statistics Greenland 2017, Fig. 2.2). The reason for this is a substantial loss of sea ice, which has reduced the ability of locals to go hunting and fishing using sledges and ice as their transport platform. Such changes influence lifestyle and the way of living as well as dietary habits of Northerners (Cavalieri and Parkinson 2012).

2.2 The Sled Dog as Circumpolar Biomonitoring Species

The sled dog is equally distributed in the circumpolar areas of Europe, North America, and Asia, and despite some physiological differences, it may therefore be an ideal biomonitoring species for Northerners and predator health in the Arctic (Burger and Gochfeld 2001; Sonne 2010; Sonne et al. 2017a). Sled dogs live in proximity to Northerners, and since they are fed a local food web diet, sled dogs are ideal to monitor contaminants and diseases in a One Health perspective (Sonne et al. 2017a). Relatively few studies have used sled dogs as a sentinel species for One Health monitoring in the Arctic (Sonne et al. 2017a). Given that sled dogs have a known history and are easy to access, they are suitable as additional biomonitoring species to polar bears and Arctic fox (*Vulpes lagopus*).

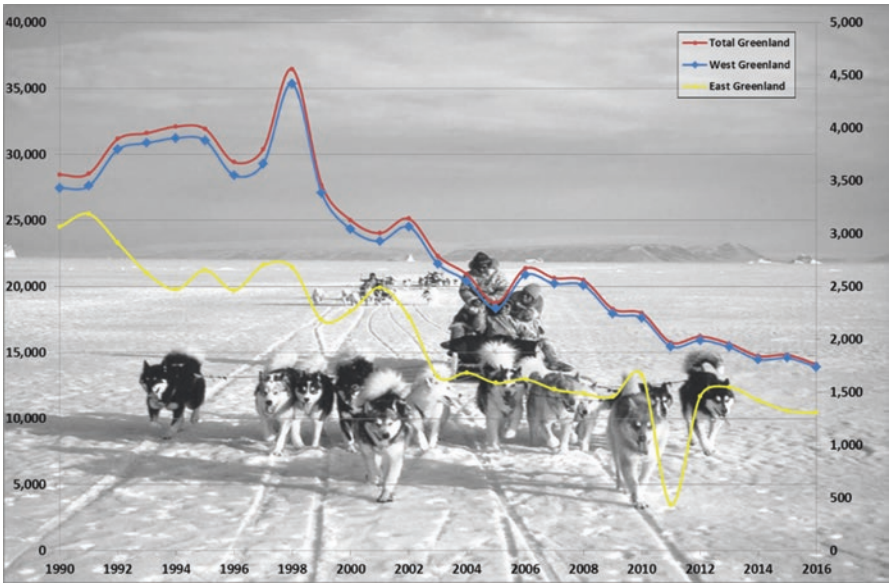

Fig. 2.2 Trends in the Greenland sled dogs population size from 1990 to 2016. The total (red) and West Greenland (blue) numbers refer to the Y-axis, while the East Greenland (yellow) numbers refer to the Z-axis. (Source: Statistics Greenland (2017))

2.3 Pollution in the Arctic

Pollution has been extensively monitored in the Arctic due the high exposure of Inuit populations appearing from high trophic consumption of marine mammals (AMAP assessment 2018; AMAP 2015). Since the 1940s, large amounts of lipophilic organohalogen compounds (OHCs) have been released into the environment and transported to the Arctic (AMAP 2014, Rigét 2019; de Wit et al. 2010; Butt et al. 2010; Hoferkamp et al. 2010; Hung et al. 2010). This transport is mainly caused by the so-called grasshopper effect being an evaporation-precipitation mechanism due to the relatively low vapor pressure of these pollutants. The OHCs include polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), brominated flame retardants (BFRs), and proteinophilic per- and polyfluoroalkyl substances (PFASs), such as perfluorinated carboxylic acids (PFCAs) and perfluorinated sulfonates (PFSAs). These chemicals typically originate from industrial and household use of insulating fluids and coolants in electric and electronic equipment and machinery, agricultural pest control, textiles, construction material, and firefighting foams (de Wit et al. 2010). Many of these compounds are persistent in the environment and organisms, undergo long-range transport by atmospheric and seawater currents, and ultimately end up in the cold Arctic environment (Rigét et al. 2016; Letcher et al. 2010, 2018). Arctic fish and wildlife rely on energy-rich fatty and waxy tissues as their main energy source (Butt et al. 2010; Dietz et al. 2013; Houde et al. 2011). Such fatty tissues typically accumulate various natural fat-soluble com-

pounds, such as specific vitamins (A and D) and endogenous steroid hormones depending on the species-specific fatty acid and lipid class composition (Sonne 2010; Sonne et al. 2014a, b; Kirkegaard et al. 2010a, b, Bechshøft et al. 2011). However, these tissues also easily absorb the lipophilic contaminants, which are consequently prone to biomagnify through the food web to top predators such as polar bears, Arctic fox, seals, whales, and seabirds and ultimately to indigenous Northerners and their pets that traditionally consume the aforementioned wildlife (Letcher et al. 2010, 2018; Rigét et al. 2016).

In addition to these substances, elevated emissions of mercury (Hg) since the 1850s (early industrialization) have led to a global spread and high concentrations of this heavy metal (Dietz et al. 2009). The Hg sources include burning of fossil fuels, metal production (ferrous and non-ferrous), large-scale gold production, artisanal and small-scale gold production, cement production, chlor-alkali industry, waste incineration, as well as release from dental amalgam upon body cremation (Outridge et al. 2011). Mercury is easily methylated by marine primary producers, biomagnifies, and thus poses health concerns for Arctic top predators and wildlife (AMAP 2015; Dietz et al. 2013; Outridge et al. 2011). In fact, predator mammals in East Greenland and Svalbard, such as sled dogs, polar bears, Arctic foxes, and killer whales (*Orcinus orca*), are among the most contaminated animals on our globe (Dietz et al. 2015; Letcher et al. 2010; Pedersen et al. 2015; Pedro et al. 2017). Several of the OHCs and Hg have been globally regulated through international treaties and conventions including the Stockholm and the Minamata Conventions, which initially resulted in environmental declines. However, over the last decade, the concentrations of the highly toxic PCBs and PFASs have remained essentially unchanged in polar bears inhabiting contaminant hot spots, such as East Greenland and Hudson Bay, due to climate-related changes in the food chains, generational transfer, and continued emissions (Dietz et al. 2013; Houde et al. 2011; Boisvert et al. 2019; Letcher et al. 2018). For Hg, body burdens even appear to be continuously increasing in most top predators in the central Arctic reaching up to 20-fold increases above baseline levels prior to the industrialization (Dietz et al. 2009, 2011; Rigét et al. 2011).

The abovementioned OHCs and Hg pose a health threat to Arctic top predators and northern people (Northerners) because most of the compounds, or their biotransformation metabolites, have structural similarities to hormones and may act via non-endocrine pathways as well. This causes these xenobiotic environmental compounds to have negative effects on immune and neuro-endocrine functioning, growth and development, reproduction, and general fitness (Grandjean and Landrigan 2006; Letcher et al. 2010; Sonne 2010). Since these compounds target different organ-tissues, exposure manifests in several health effects (Sonne 2010). Furthermore, seasonal cycles of energy requirement for fasting, breeding, lactation, and migration lead to increased metabolism of adipose tissue causing release and pulsed exposure to bioavailable contaminants circulating in the blood stream (Polischuk et al. 2002; Tartu et al. 2017). In polar bears, for example, up to 70% of the total OHC body burden is transported from mother to offspring during lactation, resulting in cub adipose tissue concentrations that are approximately three times

higher than in their mother (Dietz et al. 2004, 2007; Muir et al. 2006; Polischuk et al. 2002; Bytingsvik et al. 2012). There is evidence that such high exposure poses a great risk to neonatal individuals during their critical period of development. As for other mammals, a female polar bears' first litter is particularly vulnerable to OHC effects on normal development and growth as a higher percentage of the total body burden of contaminants in the mother is excreted to blood and milk (Letcher et al. 2010; Sonne 2010; Sonne et al. 2012). In a meta-study, it has recently been modeled that chlorinated and brominated OHCs, singularly or collectively, were better predictors for declines in population densities in 14 polar bear subpopulations than were human population density, harvest rate, and sea ice extension (Nuijten et al. 2016). From a population conservation point of view, contaminants that reduce pregnancy, fecundity, and survival are among the most important to monitor and assess in different Arctic subpopulations of polar bears and other predators.

2.4 Diseases

Polar bears have received considerable focus since loss of sea ice and access to seals as main prey has projected the species to go extinct around year 2100 (for instance: Amstrup et al. 2010; Castro de la Guardia et al. 2013; Whiteman 2018; Hamilton et al. 2014; Molnár et al. 2011). The current threats against polar bears are however echoed for other marine and terrestrial animals in the Arctic. But while the degree of bioaccumulation of environmental contaminants discriminates between animal groups, i.e., terrestrial vs. marine and high vs. low trophic levels, the threat of diseases is a universal one. Some disease agents readily cross trophic levels and main habitats; an example of such diseases is the so-called zoonoses, i.e., diseases that can infect Northerners via animal vectors (such as rabies). Zoonoses make up approximately 60% of all human infectious diseases. Zoonoses are therefore not rare, but more the rule than the exception of human infectious diseases (Jones et al. 2008).

Diseases can be indicators of ecosystem health. For example, climate change is, in addition to posing an increased level of energetic stress (such as in the classic example of the polar bear and loss of sea ice and hunting grounds), also projected to increase the risk of both disease spread and virulence (Burek et al. 2008; Harvell et al. 2002; Shope 1992). This risk may be further accentuated by the concurrent exposure to environmental contaminants known to pose immunotoxic effects (Desforges et al. 2016). It applies to both climate change and environmental contaminants that their effects/presence is particularly accentuated in the Arctic (Bard 1999; ASIA 2004). There is as such a risk that disease-related mortality of Arctic mammals may increase – as well as a risk of zoonotic infections in Northerners – in the aftermath of anthropogenic environmental pollution and climate change (Fig. 2.3) (Jenssen et al. 2015; Sonne 2010). Regarding human zoonotic infections, this is a highlighted concern in many Arctic indigenous communities where it is still common practice to ingest wildlife foods raw and where wildlife game continues to

Fig. 2.3 Often Northerners eat raw seafood, which increase the risk of disease transfer (zoonoses). Northwest Greenland, spring 1984. (Photo: R. Dietz)

be an important part of their diet (Chan et al. 1995). However, with infectious diseases, such as zoonoses, crossing species barriers and contrasting habitats, it points to the potential use of indicator species that they present a link between the environment, wildlife, and Northerners for monitoring the current challenges of overall ecosystem health: an indicator species such as the sled dog.

Since the domestication of the dog 18,000–33,000 years ago (Thalmann et al. 2013; Wang et al. 2016), humans and dogs have not only shared food items, space, and security but to a high degree also pathogens. Today, more than 60 different pathogens, i.e., zoonoses, are known to be shared between dogs and people, and some of these have developed a synanthropic life cycle, i.e., a life cycle specialized for the human-dog relation (Craig et al. 2003). Large studies of zoonotic diseases in Northerners and wildlife have taken place in Canada and Alaska (Jenkins et al. 2013; Sonne et al. 2017a); however only a few studies have focused on zoonoses harbored by sled dogs (Salb et al. 2008), and sled dogs have not been included in a large-scale study of Arctic zoonoses and Northerners health risk before. Moreover, almost no studies have investigated the zoonotic infection pressure on Inuit of Greenland – Greenland still holds one of the largest populations of working sled dogs.

The status, character, and extent of Arctic zoonoses are still generally poorly elucidated; known diseases are moreover likely highly under-reported and other diseases are probably yet to be acknowledged/recognized (Parkinson et al. 2014; Gilbert et al. 2010). Some of the known diseases that are causes for significant human health concern are parasitic diseases such as trichinosis, echinococcosis, anisakiasis, toxoplasmosis, and toxocariasis, viral diseases like rabies, and bacterial infections such as brucellosis and clostridiosis (Magnaval et al. 2016; Jenkins et al. 2013; Rausch 1972 and others). The sled dog acts as a significant or potential host for most of these (Salb et al. 2008, Rausch 2003). The importance of dogs in the

epidemiology of Arctic human zoonotic infections is exemplified by the parasitic disease echinococcosis. This was once an endemic disease in the Arctic affecting primarily those who hunted ungulates or were herders (Rausch 2003). Dogs act as host for all species of *Echinococcus* present in the Arctic and they are the definitive host for the dominant species causing human infections: *E. granulosus* (Gilbert et al. 2010, Rausch 2003). However, since the introduction of motorized snowmobiles and resultant decline in dog numbers, human cases of echinococcosis also declined drastically (Rausch 2003). The parasite and disease nevertheless still persist in the Arctic today, in particular where dogs are to be found, and in Canada, the highest human incidence of infection is found among Arctic indigenous communities (Gilbert et al. 2010; Himsworth et al. 2010).

As for infectious diseases of Arctic sled dogs per se, infectious epizootics of canine distemper virus (CDV, *Morbillivirus*) and parvoviral diarrhea probably top the list today – as they likely have since before the arrival of the Western societies. As such, canine distemper outbreaks have been recorded at least since 1860 in Greenland with concurrent reports of grave consequences for the owners and settlements that lost most of their dogs during such an event (Vernersen and Jensen 2018). These two viral diseases are not zoonotic; they are however carried by many other Arctic predators, in particular CDV which can be isolated from seals, polar bears, and foxes (Beineke et al. 2015). It is therefore unsurprising that there are strong indications of epizootics within dog populations being initiated by transmission from and further spread by wildlife (if not via main sled routes of travel) (Vernersen and Jensen 2018, Blixenkrone-Møller et al. 1989). This is an example of how the sled dogs may act as a mirror of Arctic ecosystem health via the health status of other top predators. Rabies is another example of a zoonosis with a broad host spectrum potentially including all Arctic mammals with the ability of causing mass die-offs among Inuit sled dog populations through transmission from wildlife (Tabel et al. 1974). Rabies is however no longer considered as great a concern as earlier with the advent of vaccines, general awareness, and control programs (Tabel et al. 1974). However, the occurrence of rabies is climate change sensitive, and problems may reoccur/rise, e.g., with the currently observed changes in rodent and fox populations (Parkinson and Butler 2005).

Summed up: Monitoring Arctic ecosystem health, including wildlife and human health, is an ongoing challenge of accelerating importance. The sled dogs represent an interesting link between Northerners, prey species, predators, and the Arctic environment. Sled dogs would be of value to achieve a high quantity and quality of samples from across the Arctic that could infer on the consequences and proper management of current environmental challenges that among others affect disease dynamics related to canine, wildlife, human, and overall ecosystem health.

2.5 Contaminants in Sled Dogs

Sporadic studies on sled dog health have mostly focused on Hg exposure (Harley et al. 2016; Lieske et al. 2011; Dunlap et al. 2007, 2011; Hansen and Danscher 1995; Hansen et al. 1989), wood smoke exposure (Montrose et al. 2015), hypospadias (penile malformation and PCB exposure) (Sonne et al. 2008), and infectious diseases which include zoonoses (Jenkins et al. 2013). At least six studies have been published on effects related to mercury exposure in sled dogs. These have been conducted in Alaska (Dunlap et al. 2007, 2011; Harley et al. 2016; Lieske et al. 2011) and Greenland (Hansen and Danscher 1995; Hansen et al. 1989). In Alaska, a cohort of sled dogs following a diet based on fish was investigated (Lieske et al. 2011). The study analyzed concentrations of Hg in blood and hair and found that sled dogs had a very high Hg exposure with a bioaccumulation similar to that seen in polar bears. Furthermore and based on concentrations in hair and hair-blood concentration ratios; the Hg toxicokinetics in sled dogs was found to be more similar to that of humans than that of laboratory rats, suggesting sled dogs as a potential biomonitoring species for exposure and effects of Hg in the Arctic environment. Another study by Dunlap et al. (2007) reported elevated Hg concentrations in hair of Arctic sled dogs as compared to other subpopulations of family dogs from temperate regions reflecting the high dietary intake of local Arctic communities. The study by Dunlap et al. (2011) is interesting as it showed that Yukon sled dogs suffer from Hg exposure since the dogs had antioxidant mechanisms that were induced due to high Hg concentrations, including the scavenging and neutralization of free radicals by vitamins. Such effects are known to lead to, e.g., vitamin deficiencies and immunotoxic effects affecting the overall health of the dogs. It is, however,

Fig. 2.4 Studies have revealed elevated Hg concentrations in hair of Arctic sled dogs as compared to other subpopulations of family dogs, which may cause them to suffer from Hg exposure health effects. Northwest Greenland, summer 2015. (Photo: R. Dietz)

important to study the confounding variables further before solid conclusions on toxicogenomics in sled dogs (and Arctic fox) can be drawn (Harley et al. 2016). Another study by Dunlap et al. (2012) showed that dietary habits of Yukon sled dogs are reflecting the ratio of healthy omega-3 and unhealthy omega-6 fatty acids as well as mercury exposure. This poses sled dogs as an ideal local biomonitoring species for human dietary exposure to fatty acids and mercury (Fig. 2.4).

In polar bears, high Hg concentrations have been associated with biological endpoints (Sonne 2010). It is likely that such impacts are also relevant for the health of Northerners as a similar tissue distribution and partitioning of Hg can be anticipated (AMAP 2015; Basu et al. 2009; Dietz et al. 2013; Sonne 2010). Sled dogs were also used as a sentinel model organism for Inuit exposure to investigate effects of local pollution from wood smoke (particles) on DNA hypomethylation and the risk of immunologically related gene expression (Montrose et al. 2015). The authors compared a wide range of dog kennels in order to reflect different exposure scenarios. Using dust trackers, the authors reported significant differences in smoke-related particle exposure among kennels, but there were no effects on DNA methylation and the functional expression of immunologically related canine genes. It is hard to conclude on this; however, the number of epigenetic studies is increasing for humans, and there are newer techniques that in the future may provide additional insights to such cause and effect relationships.

With respect to organic environmental contaminants; as part of a field survey in Tasiilaq on the southwest coast of Greenland, blood was sampled from four sled dogs in September 2014. The blood was analyzed for various PFASs (all PFSAs and PFCAs), and the concentrations of PFOS were by far the greatest of the analyzed compounds. This was in accordance with analyses of East Greenland polar bears and local Inuit (Dietz et al. 2008; Long et al. 2012; AMAP 2015) as well as in Hudson Bay polar bears (Boisvert et al. 2019; Letcher et al. 2018). From an ecological One Health point of view, it is therefore reasonable that sled dogs are good sentinel species for monitoring Arctic ecosystem health including that of Northerners (Sonne et al. 2017a).

2.6 Developmental Effects

A study of a male sled dog in Scoresby Sound in East Greenland in the year 2000 revealed that it was suffering from hypospadias which is a mal-closure of the ventral part of penis and urethra being part of the testicular dysgenesis syndrome (TDS). The TDS is characterized by hypospadias, testicular malign neoplasm (cancer), and cryptorchidism as described for humans by, e.g., Skakkebæk et al. (2001). The male dog from Scoresby Sound is the only reported case of hypospadias in Arctic mammals including polar bears and seals indicating that this is not a regular occurring event.

Previous studies have shown TDS being associated with exposure to environmental endocrine-disrupting chemicals, including PCBs, DDTs and genetics, which

disrupt the testosterone production by Leydig cells as well as the sperm production by Sertoli cells (Edwards et al. 2006; Skakkebæk et al. 2001). Such effects are of course vital if occurring in high frequencies in, for example, male sled dogs, in polar bears, or in Inuit populations. In humans, however, surgical reconstruction is possible in the less severe cases. The authors did not have a chance to examine the dam for other causes such as organ pathology or tumors that could explain the case study of hypospadias in its offspring. In Greenland Inuit, the prevalence of hypospadias is four times lower than in, e.g., the USA (approximately 0.08%) despite Inuit peoples carrying high body burdens of endocrine-disrupting chemicals (AMAP 2015; Giwercman et al. 2006; Sonne et al. 2013). The reason for this is unknown; however, one explanation could be that the genotype of the Greenland Inuit is less sensitive to develop hypospadias (Giwercman et al. 2006).

Local Inuit people and researchers of High-Arctic Canada have previously reported that female sled dogs in Iqaluit (Nunavut) that were fed seal blubber vs. traditional dog pellets had a higher incidence of females in their litters (Sonne 2010). As part of an interview investigation of polar bear hunters in Scoresby Sound in East Greenland, similar observations of a higher female-male ratio were reported (Dietz et al. 2001; Sonne 2010). Reports of skewed offspring sex-ratio indicate an endocrine-disrupting feminization of the prenatal environment and fetuses, which may increase the female-male ratio of sled dogs as previously suggested for humans (Taylor et al. 2007; Tiido et al. 2006).

2.7 Controlled Studies

2.7.1 Immune Effects

In the literature, one large cohort study of sled dogs has been published with the aim to complement field studies of polar bears with relevant controlled studies in order to unravel how contaminant exposure affects health endpoints. For that purpose, the Greenland sled dog was selected as the model species of OHC exposure and health effects as its nutrition physiology is similar to that of polar bears (Sonne 2010). The study was performed in Aasiaat (Egedesminde) in West Greenland during year 2004–2006 in order to include the specific Arctic environmental physical parameters (Sonne 2010).

The parental P generation of dogs was eight sister pairs obtained from Inuit hunters (Fig. 2.5). The exposed group of dogs were fed minke whale (*Balaenoptera acutorostrata*) blubber with high OHC levels to mimic polar bear exposure according to Sonne (2010), while the control group was fed pig (*Sus scrofa*) fat with low OHC levels. Overall, effects from exposure to the complex mixture of OHCs can be divided into immune and endocrine effects, organ pathology, and effects on vitamin concentrations and distribution. It has been shown that the cocktail of environmental contaminants in the Arctic affected cellular, humoral, and complement parts of

Fig. 2.5 One of the female sled dogs included in the controlled Aasiaat study over the period 2004–2006. (Photo: C. Sonne)

the immune system of the dogs in the controlled study (Sonne 2010) which has been supported by a recent review of marine mammals across taxa (Desforges et al. 2016). Such reduction in, e.g., lymphocyte proliferations and antibody production (IgG) may affect the ability to respond to intruding infectious pathogens as previously suggested for polar bears (Letcher et al. 2010) and Inuit and other northern peoples (AMAP 2015).

2.7.2 Endocrinology

Several steroids (including sex steroid) and thyroid hormones were analyzed from the experimental study on Greenland sled dogs. These were analyzed in both the mothers (P generation) and the pup offspring (F1 generation). For the P generation, an increase was observed for all major steroid hormones in the group of females exposed to minke whale blubber, indicating slight overcompensation of the negative feedback system (Sonne et al. 2014b). In the F1 generation of pups, there seemed to be reduced concentrations of testosterone and testes size although that was based on a very low sample size (Sonne 2010). With respect to reproductive steroid hormones, Svalbard polar bears are exposed to similar OH-PCB levels as the sled dogs, which caused concentration-dependent reduction of plasma concentrations of pregnenolone and androstenedione in female polar bears (Gustavson et al. 2015). The

authors suggested that CYP17 might be a potential target enzyme for these effects of OH-PCBs. Similarly for sled dogs the upregulated CYP activities may affect the concentrations of testosterone in the F1 generation (Sonne 2010). Analyses of thyroid hormones showed that concentrations of free T3, total T3, and T4 were lowest in the exposed female sled dogs after 10 months of age and that total T3 was lowest in the exposed group of F1 pups (Kirkegaard et al. 2011). Such relationships have also been found in Svalbard and East Greenland polar bears (Sonne 2010; Jenssen et al. 2015) as well as Inuits (AMAP 2015; Dallaire et al. 2008). Thus, in conclusion the sex steroid and thyroid hormone system appears to be affected by environmental contaminants in Arctic wildlife and in local Inuit.

2.7.3 Organ Pathology and Vitamins

Organ pathology has been investigated in Greenland polar bears for nearly two decades focusing on the liver, kidney, and thyroid gland (Sonne 2010; Sonne et al. 2011). Since the studies were of correlative nature, organ morphology was enrolled as an important parameter of the sled dog study. The results showed that lesions in the exposed group were similar to those of East Greenland polar bears and that up to 14% of the liver and ca. 60% of the kidney lesions could be ascribed to exposure to the environmental contaminants of the minke whale blubber (Sonne 2010). The liver and kidneys are very important organs for the overall metabolism and physiology, including metabolism and excretion of contaminants, and a reduced function is likely to have negative effects on mammals. Based on this it can be hypothesized that similar effects may exist in Inuit people eating at the same trophic level as polar bears and sled dogs. With respect to vitamins in the liver and kidney of the sled dogs, vitamins A, E, and D seemed to be affected by the exposure to environmental contaminants (Kirkegaard et al. 2010b; Sonne 2010; Sonne et al. 2014b). Similar findings have been reported for polar bears and Northerners (Sonne 2010; AMAP 2015) which are clear indices that there is a risk of suffering from hypovitaminosis with potential effects on immune system, reproduction, and development (Letcher et al. 2010; Sonne 2010).

2.8 Sled Dog Modeling

2.8.1 PBPK Modeling

Sonne et al. (2015, 2016) used a PBPK (physiologically based pharmacokinetic) model, also adapted for polar bears by Dietz et al. (2015), to calculate potential effects from contaminants on the sled dogs. This allowed for estimating risk quotients (RQs) based on critical daily dose (CDD) and critical body residues (CBR)

obtained from controlled studies. In PBPK models, the body is subdivided into anatomical compartments representing individual organs or tissue groups. The transport of chemicals in the body is described by mass balance differential equations that incorporate blood flows, partitioning into compartments and tissue volumes. Numerous specific PBPK models have been used for modeling fate and disposition of a certain drug or for cancer risk assessment of industrial chemicals. However, only a few studies have reported on specific PBPK models for Arctic organisms (Cropp et al. 2014; Hickie et al. 2013). The PBPK model applied to the sled dogs for estimating fate, distribution, and CBR of contaminants has been presented by Gustavson et al. (2008) and Sonne et al. (2009, 2015).

The modeling showed that RQs in exposed, but not control, dogs exceeded immune effect thresholds, corroborating results of in vivo immunotoxicity in the sled dog cohort studies (Sonne et al. 2016). The modeling also showed that the exposed dogs were in risk of having effects on reproduction, which to some extent is supported by data in Kirkegaard et al. (2010a) and Sonne et al. (2016). In addition, PBPK modeling of East Greenland polar bears exposed to similar cocktail of environmental contaminants suggests that these are in risk of effects on immune and reproductive systems or even carcinogenic effects as supported by empirical field data (Dietz et al. 2015; Letcher et al. 2010; Jenssen et al. 2015). For Inuit people, there are no publications showing similar effects on reproduction, while there are studies that support effects on the immune system (AMAP 2015; Dallaire et al. 2004, 2006).

2.8.2 Energetics and Health Effects Modeling

As mentioned above, the most important contaminant effects in terms of individual and population-level health are those that affect demographic-related parameters such as reproduction, growth, morbidity, and mortality. Changes in these parameters are often difficult to measure directly in wildlife populations; thus biomarkers are used to study various health endpoints. The problem is that molecular and cellular biomarkers of contaminant effects are often difficult to link to broader and observable health implications in individuals and provide only a descriptive snapshot of a given endpoint measured at that particular time (Jager et al. 2010). Biology-based and process-driven approaches are therefore necessary to describe the physiological mechanisms that underpin the effects of stressors in animals. Dynamic energy budget (DEB) models may be useful in this context as these provide a framework to understand the flow of energy through living organisms as it relates to important physiological processes such as growth, development, reproduction, and maintenance (Kooijman and Bedaux, 1996). DEB models are particularly useful in ecotoxicology since they can explain life-history traits over the entire life cycle of organisms as a function of their environment and ecology, as well as anthropogenic stressors.

There have been no mechanistic or individual-based modeling studies performed to date using sled dogs. However, a recent study using DEB theory to model environmental contaminant exposure in mink (*Mustela vison*) provides an example of the type of modeling necessary to understand the implication of contaminants for organism health (Desforges et al. 2017). The model of physiological energy demands over the entire lifetime of the animal accurately predicted growth, development, and reproductive output when compared to captive mink studies. The model also included the toxicokinetics and dynamics of PCBs in mink, accurately replicating dose-response relationships for effects on growth and reproduction resulting from changes in energy allocation during ontogeny. DEB models have also been used to describe and predict fat accumulation in polar bears and how it can relate to starvation, reproduction, and population effects with increased nutritional stress due to climate change (Molnár et al. 2011, 2010). When linked to individual-based models (IBMs), energy budget models can be used to extrapolate contaminant effects to the population levels (Martin et al. 2013). This approach can be easily applied to sled dogs as their food intake, growth, and reproductive output can be closely monitored thereby providing the necessary data for DEB modeling. Lastly, since the model is based on physiology and the mechanisms underpinning contaminant effects, the results can be more confidently extrapolated to other top predator species such as polar bears, Arctic foxes, and Northerners exposed to the same contaminants.

2.8.3 Modeling of Disease and Contaminant Source

Based on the above sections, sled dogs can be employed in answering the need to biomonitor selected Arctic communities for effects of contaminants and diseases on the hormone and immune systems and general health and development. Doing so, sources and pathways of contaminant and disease exposure, mainly through the diet, should be identified. Since the exposure to contaminants and zoonotic diseases mainly occurs through the diet, understanding the dietary physiology and ecology of the sled dogs is an important aim, best done in a quantitative manner in order to allow for extrapolation to those wildlife species for which it acts as surrogate.

The analysis for stable carbon and nitrogen isotopes, typically in muscle and keratinous tissues such as hair, has become the backbone of quantitative chemical investigations of the foraging region and trophic position of mammalian top predators (Boecklen et al. 2011; Jardine et al. 2006). It has been successfully used to show how spatiotemporal plasticity in dietary habits influences the sources and intensity of contaminant exposure in polar bears (Cardona-Marek et al. 2009, McKinney et al. 2009, 2010, 2011, Routti et al. 2012) and Arctic foxes (Fuglei et al. 2007). At the present day, stable isotope mixing models (Parnell et al. 2013) are a powerful modeling tool that can help elucidate how the diet of an individual sled dog is composed of different feed items or species. Hence, it will allow identifying dietary items and habits that lie at the source of increased exposure and risk to specific contaminants or diseases of interest. Furthermore, this chemical methodology of

measuring stable isotopes in bulk tissues, i.e., a homogenized tissue, has recently been refined to the precise measurement of individual compounds, such as the measurement of stable carbon isotopes of individual fatty acids and stable carbon and nitrogen isotopes of individual amino acids (Iverson et al. 2004; McMahon et al. 2013). This approach has in fact already showed to be promising and further increase the accuracy and power of reconstructive investigations of temporal variation in polar bear dietary habits and its impact on contaminant exposure (McKinney et al. 2013).

Despite the above promising conceptual quantitative framework and its suggested applicability for using sled dogs as sentinel species for monitoring Arctic ecosystem health, there have been no efforts to date to use either bulk or compound-specific stable isotope measurements for quantitative diet reconstructions in sled dogs. Nonetheless, these approaches have large potential to provide the quantitative ecological input required for effect-oriented modeling, such as the above-mentioned PBPK and DEB modeling.

2.8.4 Genetic Modeling

Studies of genetic makers or even full nuclear genomes have revolutionized the field of evolutionary biology by shedding light over phylogeny and population structure. However, gene and genomic information of an individual does not only constitute the specific individual's ancestry, but it also first codes for an individual's abilities and physiological operation. In the genome, the exome coding regions are throughout life expressed as RNA transcription, to maintain and regulate specific cell and overall body functions. The transcription of messenger RNA and microRNA, respectively, facilitates protein expression in a cell and various regulation of expression, and these expressions can vary as response to external influences and stress (Wasaki et al. 2003; Feder and Walser 2005; Todgham and Hofmann 2009; Jozefczuk et al. 2010; Chapman et al. 2011; Pujolar et al. 2012; Lemay et al. 2013). The relatively new field of transcriptomics made accessible by sequencing technology targeting RNA molecules is increasingly expanding (Wang et al. 2009; Martin and Wang 2011). Although no environmental change or stress-related transcriptomic investigation of sled dogs has been made to date, it is a potent methodology for future work.

2.9 Other Biomonitoring Species: The Arctic Fox

In a similar study to the one on sled dogs in West Greenland, domesticated Arctic foxes, also fed minke whale blubber, were used to study biological effects from environmental contaminants in the Arctic as well (Hallanger et al. 2012; Pedersen

et al. 2015; Rogstad et al. 2017; Sonne 2010; Sonne et al. 2017b). Similar for the sled dogs, the foxes were exposed to the complex mixture of organic contaminants, and effects on organ pathology, endocrine system, and vitamin concentrations and distribution were investigated (Hallanger et al. 2012; Rogstad et al. 2017; Sonne et al. 2017b). In the exposed group of Arctic foxes, higher hepatic CYP activity may have led to increased testosterone metabolism reducing the blood concentrations and combined with increased oxidative stress also lowered vitamin E (Helgason et al. 2013; Sonne et al. 2017b). Similar liver and kidney lesions as those found in the sled dogs were found and ascribed to the chemical cocktails of environmental contaminants in the minke whale blubber (Sonne 2010). In addition, lesions were found in the thyroid glands, which may be related to changes in thyroid hormone concentrations that again can be a support for the study of thyroid lesions in East Greenland polar bears (Sonne 2010; Sonne et al. 2011).

2.10 Inuit People and One Health

Due to their reliance on the marine food resources, Inuit people are heavily exposed to environmental chemicals and mercury (AMAP 2015; Bonefeld-Jørgensen 2010). This is of extreme concern and it is therefore important that exposure to toxic anthropogenic pollutants is closely monitored continuously in order to discover if there are significant changes. This is undertaken via AMAP showing that, e.g., East Greenland hunters are exposed to high PCB concentrations due to ingestion of seals, polar bears, killer whales, and narwhals (AMAP 2011; Bonefeld-Jørgensen 2010; Dietz et al. 2013; Sonne et al. 2013). As a supplement to the AMAP monitoring program, a PBPK model has been built as a first step to estimate potential health effects in Greenlanders (Sonne et al. 2014c). For the Greenlanders, significant correlations were found between chemically analyzed contaminant blood concentrations and calculated daily intake of OHCs; and the PBPK model predicted blood concentrations of a factor 2–3 within the actual measured values. Furthermore, the model itself estimated that the most important excretion route for higher chlorinated PCBs in Greenlanders was in fact via alveolar excretion and not as previously suggested via feces or urine. This combination of PBPK modeling, monitoring of Inuit people, and studies of sled dogs would be a very strong combination of tools to further understand and monitor human exposure to contaminants in the Arctic.

2.11 Conclusions

Based on the accumulated scientific data, sled dogs have proved to be a good model for assessing the health of top predators and Northerners in the Arctic. Sled dogs are widely distributed and when compared to polar bears and Inuit people they are similarly exposed and show similar correlations to important health indicators. We therefore recommend that ecosystem health assessments in the Arctic, including that of Northerners, should also be monitored using sled dogs combined with modeling tools such as PBPK and DEB. Performing this in a circumpolar perspective will further increase our understanding and monitoring possibilities of ecosystem health and human exposure to contaminants, diseases, and climate change in the Arctic.

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Chapter 3
Pets as Sentinels of Human Exposure to Pesticides and Co-exposure Concerns with Other Contaminants/Toxicants

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Abstract Companion animal exposures to pesticides were investigated to describe instances in which shared exposures may be useful to understand impacts on the health of humans. Several distinct types and classes of pesticides that have wide-ranging sources of exposure across animals, people, and environments are discussed in a "One Health" context. The ubiquity of pesticides in surface waters and drinking water may be an important source of persistent pesticide exposures to animals and people, and we can appreciate that low doses of chemical mixtures are the reality. While exposures may accumulate in any animal or human host over different lengths of time (i.e., days, months, years), not all mechanistic studies utilize relevant concentrations nor, can the levels be used to predict shared health risks. Pet animals already serve as a high sentinel value for humans in cancer research and other shared diseases. This chapter highlights common food and water sources from household and agricultural settings for selected pesticides and the potential impacts on animal and human health. The application of novel methodologies such as high-throughput chemical screening and predictive modeling, may be a future research opportunity to advance our understanding of pets as sentinels of exposure to chemical mixtures. Baseline data is needed if we intend to design interventions that will prevent or mitigate the negative, adverse effects of pesticide exposures throughout the lifespan and across generations of people and companion animals.

Keywords Pesticides · Pets · Water · Sentinels

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3.1 Introduction

Companion animals and humans share a common habitat that includes water and food sources in both rural and urban household types. We hypothesize that "pet animals" play an important role as sentinel species, classically defined as organisms that are used to detect risks to humans, primarily because these animals can provide a signal of caution or potential harm and injury to health. Human exposures to pesticides may occur across a wide range of chemical classes and ecosystems. Companion animals may also act as vectors for human exposure through handling of pet food, excrements, medications, and pesticides. Due to species differences in sensitivity and chemical exposure doses, companion animals may show a clinical response to a hazardous chemical after a shorter period of time than people, differential dose responses, and species-dependent chemical sensitivities. Identifying exposure to pesticides in companion animals may be a highly useful tool in cases where exposure results in subclinical or subtle effects on health that manifest or trigger risks for diseases due to shorter lifespans.

Establishing causation and direct and/or indirect relationships between chemical pesticide exposures and clinical outcomes remains challenging for both humans and pets. New diagnostic tools may be required in order to identify novel biomarkers of exposure for companion animals and for people. Further research is likely required in order to develop Such tools. This chapter addresses completed research that was focused on how companion animals have been used to identify relevant pesticide exposures and their effects on various health parameters. Currently, there is a paucity of relevant research directly linking pesticide exposures in animals and humans to compromised health that is, in part, due to a dearth of understanding on the significant use of animals as sentinels for human health. Integration of pesticide surveillance data for animals might improve current models for predicting potential risks to human health. The Canary Database has previously been used to identify 465 studies on animals, which were then assigned categories of linkage (Scotch et al. 2009). These studies suggest that use of animals as sentinels for human health may be a meaningful approach to continue for human health risk surveillance.

3.2 Pesticide Exposures of Potential Concern to Animal and Human Health

The assortment of pesticides aimed to kill, deter, or incapacitate pests fall into chemical classes that have both specific and non-specific applications in diverse environmental settings that include, but are not limited to, buildings, crop and livestock agriculture, food storage, and industrialized areas. Typically, all applications of pesticides are aimed to remove certain groups of unwanted organisms at a dose or exposure that would be considered safe for humans that may handle or be

otherwise exposed to the agent. However, excess pesticide exposure occurs for many reasons and can be a risk for adverse effects across multiple species.

Toxicological studies used to determine safety doses for pesticides are limited when it comes to long-term and low-dose exposures and therefore may often overlook subtle organ-specific effects (Rozman et al. 2010; Liao et al. 2004). Companion animals, farm animals, and wildlife are commonly exposed to pesticides under a multitude of scenarios; pesticide exposure may be intentional, as seen with flea and tick treatments of pets, or unintentional, as seen with exposure to air, water, plants, and soils that contain pesticide residues in the environment. Animal foods and feedstuffs can also be contaminated by pesticide residues when used to grow certain crops and during postharvest storage (Bhaskara Reddy et al. 2016). Human exposures to pesticides through the natural or built environment that contains pet animals have been identified and sometimes quantified in biological samples, yet continued assessments with relevant doses to clearly identify health-related effects over time are warranted. In addition to direct or acute toxicity seen with pesticide exposures, wider socio-ecological implications exist from pesticide use that may include population decline and shifts in biodiversity as a result of a loss of invertebrate prey/food supplies and contaminated water systems (Chagnon et al. 2015).

This chapter focuses on scientific reports that have evaluated pesticide exposures in animals and people across diverse chemical classes that can arise from surface, ground, and drinking water globally among other intentional use sources. The chemical properties of the compounds in the water sources that could cause lethal, toxic, and/or trace levels of contamination to animals are deliberately emphasized for selected pesticides. The context for water exposures to pesticides is crucial for pets and animals because they are often considered to experience higher doses of exposure than what is accessible to humans in treated drinking water systems. Humans and pets exposed to pesticides from aquatic environments or through drinking water and even at trace concentrations have shown to sometimes exceed the maximum regulated concentrations that pose threats to health (Benner et al. 2013; Moschet et al. 2014). Table 3.1 shows examples of pesticide exposures whereby pets and animals could be considered sentinels and the potential sources of exposure are provided as an essential variable when considering dose, mechanisms of action, and related health effects.

3.2.1 Insecticides, Acaricides, and Molluscicides

Insecticides used to kill insects or acaricides used to kill arachnids (including mites and ticks) are in high use in companion animals and in farm animals. These agents are common culprits in animal poisoning cases where they are used in excess of the maximum safe dose or when exposed to animals other than the intended species.

Table 3.1 Summary of pesticide sources, chemical classifications, and mechanisms of action

Pesticide name	Sources	Chemical classification	Animals exposed	Mechanism of action clinical signs	References
Brodifacoum	Ground water, biota, soil, foods	Rodenticide	Mammals	Ecchymosis, hematuria	Yu et al. (2013), Berny et al. (2010), World Health Organization (1995)
Bromadiolone	Ground water, surface water, soil, meat/food	Rodenticide	Mammals	Disruption of vitamin K pathway	Vindenes et al. (2008), Yu et al. (2013)
Chlorpyrifos	Flea control dip, ground and surface water	Organophosphate insecticide	Dogs	Musculoskeletal changes, immune system deregulation	Eskenazi et al. (1999), Rauh et al. (2006), Thrasher et al. (1993), Boone et al. (2001)
DDT	Agricultural land, sea, and river water	Organochlorine	Chickens	Seizures, tremors, low birth weight	Jayaraj et al. (2016), Arora et al. (2013), Nayak et al. (1995), Rajendran and Subramanian (1997), Pandit et al. (2002), Aulakh et al. (2006), Goel and Aggarwal (2007)
Diazinon	Home lawn, ground and surface water	Organophosphate	Dogs	Increased liver glucose release	Morgan et al. (2008), Teimouri et al. (2006), Alahyary et al. (2008), Thayer et al. (2012), Hasanuzzaman et al. (2017), Hossain et al. (2015), Bhattacharjee et al. (2012), Akan et al. (2014), Masia et al. (2015), Rahmanikhah et al. (2011)
Diuron	Agricultural runoff, ground water	Phenylurea herbicide	Fish, amphibians	Cytotoxicity, oxidative stress, enzyme induction	Moncada (2004), de Lima et al. (2011), Huovinen et al. (2015), Sunouchi et al. (2011), Rudzok et al. (2009), Marlatt and Martyniuk (2017)
Endosulfan	Dairy farms, marine/fresh, surface, and ground water	Organochlorine	Sheep, goats	Convulsions, hypoxia	Goel and Aggarwal (2007), Soto et al. (1994), Dalvie et al. (2003), Tsiplakou et al. (2010)

Pesticide name	Sources	Chemical classification	Animals exposed	Mechanism of action clinical signs	References
Fipronil	Topical flea and tick control, residential water, surface and wastewater	Phenylpyrazole	Cats, dogs	Decreased ATP production, seizures	Sengupta et al. (2014), Stone et al. (2014), Heidler and Halden (2009)
HCH	Agricultural land, river and sea water	Organochlorine	Chickens	Potential role in breast cancer	Jayaraj et al. (2016), Arora et al. (2013), Nayak et al. (1995), Rajendran and Subramanian (1997), Pandit et al. (2002), Zou and Matsumura (2003), Bronden et al. (2007), Guy et al. (2015), Aulakh et al. (2006)
Imidacloprid	Animal hairs, household use, aquatic media, i.e., ground and surface water	Neonicotinoid insecticide	Mammals, birds, fish, amphibians, reptiles	Immunosuppression, respiratory failure, prolonged sedation	Bonmatin et al. (2015), Federoff et al. (2008), Canadian Council of Ministers of the Environment (2007), Forster et al. (2014), Craig et al. (2005), Gibbons et al. (2015), Mohamed et al. (2009), Bal et al. (2012), Cardone (2015)
Linuron	Agricultural runoff, various waters	Phenylurea herbicide	Fish, amphibians	Enzyme induction	de Lima et al. (2011), Huovinen et al. (2015), Sunouchi et al. (2011)
Methaldehyde	Household	Molluscicide	Domestic animals	Ataxia, convulsions	Ruiz-Suarez et al. (2014)
Methiocarb	Household and agricultural water	Carbamate insecticide	Rabbits, rats	Musculoskeletal changes	Blazkova et al. (2009), Rodrigues et al. (2011), EPA (1994)
Paraquat	Agriculture, waters systems	Herbicide	Dogs	Lung failure	Caloni et al. (2016), Clark et al. (1966)
Permethrin	Natural waters	Pyrethroid	Cats	Loss of consciousness, metabolic acidosis	Dymond and Swift (2008), Gotoh et al. (1998)

3.2.1.1 Neonicotinoids

Imidacloprid, an insecticide that belongs to the neonicotinoid family, is one of many chemicals used on mammals, birds, fish, amphibians, and reptiles. Although invertebrates suffer greater toxic effects with the use of these pesticides, toxicity effects may also be seen in vertebrates. Imidacloprid is widely used in agriculture for the control of insects and has water solubility between 0.5 and 0.6 g/L. It is therefore highly persistent in aquatic matrices, with elevated potential to leach into ground water and run off into surface water (Bonmatin et al. 2015; Federoff et al. 2008). Imidacloprid breaks down in water slowly in the absence of light and persists in the environment thus with longer-term availability to animals (Canadian Council of Ministers of the Environment 2007). Imidacloprid has been approved by the EPA for use on dogs to kill fleas and was detected in the urine of healthy dogs (Forster et al. 2014). There is documented human exposure that is related to transferable residues from the topical treatment of dogs (Craig et al. 2005). Neonicotinoids were shown to suppress the immune systems of vertebrates, making them more susceptible to infectious diseases, which in some cases could then be spread to exposed humans (Gibbons et al. 2015). The effects of imidacloprid on humans were shown in a demographic cohort study in Sri Lanka that consisted of imidacloprid self-poisoning cases, where patients presented with symptoms of nausea, vomiting, headache, diarrhea, rhabdomyolysis, and respiratory failure (Mohamed et al. 2009). The effects of chronic exposure are often elusive for imidacloprid as it may result in decreased reproductive capacity in multiple species (Bal et al. 2012; Cardone 2015).

3.2.1.2 Organochlorine Pesticides

Organochlorine pesticides are synthetic pesticides widely used in agriculture for which pets should be considered as sentinels for exposure as they pose comparable concerns to animal and human health. Both dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH) belong to the organochlorine family. These chemicals are regulated under the Stockholm Convention and their use is (mostly) banned because of their established toxicity profiles in animals (Jayaraj et al. 2016) and humans (Arora et al. 2013). Given the prevalent use of DDT and HCH in agricultural systems, production animals such as chickens (which may also act as pets) merit concern for higher levels of exposure in humans (O'Shaughnessy 2008). DDT and HCH can enter the water through the discharge of sewage, industrial wastewater, and runoff from non-point sources (Ruiz-Suárez 2015). These pesticides in water exhibit bioaccumulation in aquatic organisms and are eventually transferred into the food chain. In India, the measured concentrations of DDT and HCH in several rivers and sea waters are higher than the safe upper limit of 1 μg/L described by the World Health Organization (WHO) (Nayak et al. 1995; Rajendran and Subramanian 1997; Pandit et al. 2002). A study was performed to assess pesticide contamination of DDT and HCH in chicken food or water sources, which can lead

to contamination of human food (via poultry meat or egg consumption). This study concluded that eggs and chicken feed contained greater concentrations of DDT and HCH when compared to chicken muscle (or meat); and therefore, chicken eggs appeared to be an exposure source of organochlorine pesticides by humans (Aulakh et al. 2006). Clinical signs related to ingestion of DDT in humans include nervous system excitation, tremors, seizures, sweating, headaches, nausea, vomiting, and dizziness (Goel and Aggarwal 2007). Minor changes in liver enzyme levels in the blood have been documented with chronic low-dose exposures to the pesticides (US Department of Health and Human Services 2002). Environmental exposure to DDT and its metabolites has also been linked to low birth weight in newborn babies and to obesity (Xu et al. 2017).

HCH is lipophilic and accumulates in human adipose and breast tissues (Zou and Matsumura 2003). This compound has been shown to mimic estrogen, thus resulting in endocrine system disruption. Pets may be sentinels to better understand associations between exposure to HCH and an increased cancer risk. Specifically, HCH acts as a precursor for the conversion of breast cancer cells into advanced stages of malignancy (Zou and Matsumura 2003). Previous studies have shown that 13 months of exposure to the pesticide resulted in increased in vitro transformation tendencies and promoted cancer cell invasiveness, as shown in MFC-7 cells. Companion animal cancer has an increasing prevalence and incidence for both cats and dogs (Bronden et al. 2007; Storelli et al. 2009). In addition to assessment of novel chemotherapeutic regimens for treatment of animals that are similar to people, there are ongoing efforts to assess environmental exposures and cancer risk (Guy et al. 2015).

Persistent organic pollutants (POPs), including organochlorine pesticides, are detectable in blood plasma. The levels of these chemicals were compared in 100 humans and 87 companion dogs that shared their living environments. Humans were found to have higher plasma levels of organochlorine pesticides than dogs, possibly suggesting that dogs were able to metabolize and eliminate these contaminants more efficiently than humans, or that dogs had lower overall exposures despite the shared environment (Ruiz-Suárez 2015, Ruiz-Suárez et al. 2016; Kunisue et al. 2005). It remains inconclusive as to whether dogs would be promising sentinels to POPs; further research across diverse environments will be required to strengthen conclusions that have diagnostic or prognostic utility for humans.

Endosulfan, another member of the organochlorine family, may cause severe clinical signs in people including epileptic seizures, hyperactivity, irritability, tremors, convulsions, and paralysis (Goel and Aggarwal 2007). In addition, this compound was found to have estrogenic properties comparable to DDT (Soto et al. 1994). The organochlorine endosulfan has been banned in over 60 countries, yet it is still in use across the globe (especially in the southern hemisphere). Measurable levels of endosulfan have been reported in marine and fresh water, surface water, and ground water (Dalvie et al. 2003; Ruiz-Suárez 2015). Endosulfan was studied in ten farms in Greece where samples of milk were obtained from dairy sheep and goats (Tsiplakou et al. 2010). These animals' feed was analyzed was found to have been contaminated with several pesticides. Endosulfan was determined to be the

main residue in both concentrates and in hay. There was no detectable amount of pesticide residue in the goat's milk, indicating that in this case, farm animal exposure to pesticides may have had less applicability for human exposure (Tsiplakou et al. 2010).

3.2.1.3 Organophosphate Insecticides

Organophosphates are another highly common class of insecticides. These act by inhibiting acetylcholine esterase, a neuromuscular enzyme, resulting in impaired neuromuscular function. Organophosphate toxicity in both animals and humans may result from exposure via ingestion, skin contact, or inhalation (Eskenazi et al. 1999).

Chlorpyrifos is an insecticide that belongs to the organophosphate family and was used in a now-withdrawn commercial, nonprescription flea control dip for pets. Chlorpyrifos exposure is suspected to have negative effects on musculoskeletal, neural, and cognitive development in children (Rauh et al. 2006). Chlorpyrifos has also been shown to suppress and enhance certain parameters of the immune system of exposed humans. Exposure to humans resulted in an increase in CD26 expression in white blood cells (WBCs) and lymphocyte subsets, decrease in percentage of CD5 phenotype, decreased mitogenesis in response to phytohemagglutinin and concanavalin, and an increased frequency of autoantibodies, which were directed towards smooth muscle, parietal cell, brush border, thyroid gland, myelin, and anti-nuclear antibodies (Thrasher et al. 1993). Therefore, this pesticide has overall shown to result in immunosuppression and may also trigger risks for autoimmunity (Thrasher et al. 1993). Chlorpyrifos was studied in 12 different dog breeds after 4 consecutive treatments in order to quantify transferable residues from treated dogs to exposed children in the household. Pesticide amounts were obtained from the dogs' hair and the butyrylcholinesterase and acetylcholinesterase activities were measured in the dogs' plasma (Thrasher et al. 1993; Boone et al. 2001). Chlorpyrifos residues decreased 50–75% or fivefold during the 3-week intervals in between treatments, but plasma cholinesterase activity did not return to control levels during this time. This study provides essential insight into using butyrylcholinesterase, rather than acetylcholinesterase, as a reliable biomarker for measuring the persistence of chlorpyrifos and possibly other organophosphates in treated pets. It was also determined that exposure risk is highest shortly after the flea control dip treatments (Boone et al. 2001).

Diazinon, another member of the organophosphate family, has been shown to exhibit toxicity in dogs. Canines come into contact with treated lawns, leading to exposures being brought inside to people in the household. One study used samples collected from six families that were obtained from soil, carpet, indoor air, dog paw swipes, and dog hair (Morgan et al. 2008). The children and parents provided urine samples to determine levels of toxicant exposure, and the monitoring occurred on 1, 2, 4, and 8 days post-application of diazinon. Dog paw swipes contained the highest amount of pesticide residue one day after application. This study served to verify

dogs as a pathway for transferring lawn chemical residues to owners and other household occupants (Morgan et al. 2008). In diazinon exposure studies with humans, liver cells were identified as a target site for toxic actions of diazinon. Diazinon increases glucose release from the liver into blood through activation of glycogenolysis and gluconeogenesis and as a detoxification non-cholinergic mechanism to overwhelm diazinon-induced toxic stress (Teimouri et al. 2006; Alahyary et al. 2008). Multiple mechanisms of action by organophosphates suggest that these chemicals may predispose or trigger alterations in metabolic processes that may lead to development of obesity and type II diabetes (Thayer et al. 2012). Taken together, there are emerging concerns for the endocrine-disrupting effects on pet owners with animals that are routinely exposed to pesticide-treated lawns (Teimouri et al. 2006). Organophosphate pesticides such as chlorpyrifos and diazinon also bioaccumulate in aquatic environments and have ground and surface water levels ranging from 0.003 ng to 0.8 mg chlorpyrifos/L in aqueous matrices (Hasanuzzaman et al. 2017; Hossain et al. 2015; Bhattacharjee et al. 2012) and 40 ng diazinon/kg to 4.3 mg diazinon/kg in solid matrices (Akan et al. 2014; Masia et al. 2015; Rahmanikhah et al. 2011).

3.2.1.4 Phenylpyrazoles

Fipronil is the active compound in a topical flea and tick control medication and belongs to the phenylpyrazole family. The metabolites or intermediate small molecule derivatives of fipronil were measured via cat and dog hair samples in order to determine the fate and distribution of the medication and its effects on pet owners (Bigelow Dyk et al. 2012). For this study, dog hair samples were obtained from human gloves during petting, human socks to pick up stray hairs, and indoor cloths placed on frequent locations of direct touching from pets in order to pick up surface residues. Urine specimens were also collected to quantify levels of the excreted chemical and its metabolites. Due to the lipophilic nature of fipronil, urine samples did not exhibit significant excretion, and hair clippings did display persistence of the medication/active chemical compound. Dog hair was also found to have much higher levels of fipronil than cat hair; this may have been affected by species differences in sebum levels on the skin. Results from this study supported that human exposure risk was relatively low for fipronil (Bigelow Dyk et al. 2012). Another study illustrated cytotoxic effects of fipronil on human HepG2 cells (liver origin) by induction of cytochrome p450 isoforms in hepatocytes (Das et al. 2006). Fipronil also mediated activation of caspase-3, resulting in compromised ATP production and apoptosis (Das et al. 2006). Since 1995, there have been greater than 1000 pesticide-related poisoning deaths documented, two of which were fipronil-related (Mohamed et al. 2004). Four self-poisoned patients were retrospectively reviewed, of which two cases had developed generalized tonic-clonic seizures and were treated with diazepam without complications (Mohamed et al. 2004). Fipronil is a phenylpyrazole insecticide that was reported in varied water systems including residential water, surface water, and wastewater (Sengupta et al. 2014; Stone et al. 2014). The

steady increase in fipronil concentrations in water from 2002 to 2011 was reported by the US Geological Survey to exceed the EPA aquatic limit set at 11 ng/L in about 70% of the urban water streams (Stone et al. 2014; Heidler and Halden 2009).

3.2.1.5 Pyrethroids

Pyrethrins and pyrethroids are highly toxic chemicals for both humans and other mammalian species (Caloni et al. 2016). The use of animals as sentinels for human exposure could be very significant, as these agents demonstrate toxicity to neuronal cells and promote cellular death by causing hyperexcitation of sodium channels (Soderlund 2012). While used as anti-parasitic agents in veterinary medicine, pyrethrin and pyrethroid insecticide exposure can occur via the environment due to their use in controlling pests in domestic and agricultural settings (Ray and Burr 2014). Exposure routes include house dust and residues on agricultural products, as well as their primary routes of exposure via skin and inhalation. A retrospective study assessed 20 cases of permethrin toxicity (also belonging to the pyrethroid family) in cats (Dymond and Swift 2008), which are highly susceptible to drug toxicity due to having low enzymatic activity needed for biotransformation capabilities. Dermal application was the most common route of toxicity noted. Cats that experienced toxicity had presented with seizures and tremors as seen in one emergency clinic (Dymond and Swift 2008). Different treatment protocols were utilized, including medical intervention and supportive treatment, such as the use of an Elizabethan collar to avert them from grooming and reintroducing the pesticide via oral ingestion to the body (Dymond and Swift 2008). Permethrin also has serious health consequences to exposed humans. Doses administered for attempted suicide with permethrin were observed in a 59-year-old male, resulting in vomiting and diarrhea after ingestion and with sustained loss of consciousness and metabolic acidosis (Gotoh et al. 1998). While no symptoms of tremors, hyperexcitation, ataxia, convulsions, or paralysis were reported that contrast signs typically seen in animals, it was suspected that vomiting and diarrhea decreased absorption of permethrin, resulting in dampened neurotoxic effects (Gotoh et al. 1998).

3.2.1.6 Carbamate Pesticides

Methiocarb, an N-methylcarbamate pesticide, is commonly used in households and agriculture as a molluscicide, insecticide, acaricide, and bird repellent around the world (Blazkova et al. 2009). Methiocarb is a widely-used carbamate insecticide often associated with animal poisonings (Rodrigues et al. 2011). It works through inhibition of acetylcholinesterase with an LD50 value of 10–25 μg/kg in dogs. Oral exposure to methiocarb results in acute poisoning that can cause death in both humans and animals (Environmental Protection Agency 1994). Methiocarb has also been shown to induce developmental toxicity effects in rabbit and rat fetuses (Rodrigues et al. 2011).

Methaldehyde is a molluscicide used against snails, slugs, and other gastropods. Acute intoxication exists due to domestic use in both humans and animals (Ruiz-Suarez et al. 2014). The most common human exposure pathway of this chemical is ingestion by children under three years of age. Exposure to methaldehyde may cause neurological symptoms such as ataxia, tremors, and convulsions along with gastrointestinal signs such as vomiting and diarrhea in both human and animals (Ruiz-Suarez et al. 2014).

3.2.2 Herbicides, Algicides, and Rodenticides

The contamination of ground and surface water with herbicides has become a global issue because of reported adverse effects on human health. Herbicides are found at trace levels in various water matrices. The prolonged exposures trigger chronic health effects (Ma and Chen 2005; Jones and Huang 2003; Adachi et al. 2001).

Paraquat, an herbicide widely used for controlling weeds, is known for its toxic profile in both humans and animals which has been especially seen in pet dogs (Caloni et al. 2016). Paraquat affects the central nervous system and also causes formation of peculiar lesions in lungs, the main target organ of this chemical (Clark et al. 1966). Exposure to this herbicide can result in lung failure in both dogs and humans. Elimination of paraquat from the body is challenging because of its effects on kidneys that may result in renal failure (Clark et al. 1966). Paraquat is also highly water-soluble, so it is easily transported by leaching or runoff and can contaminate various natural water sources.

Diuron, a broad-spectrum phenylurea herbicide, enters the ground water primarily through agricultural runoff. Due to its intrinsic physical properties of low (mobility of a substance in soil) KOC (418–560), its high chemical stability with soil photolysis half-life of 173 days and hydrolysis half-life of >1000 days, and it being bio-recalcitrant, it is mobile and persistent in ground water (Moncada 2004). A fish and amphibian study was performed in order to understand the mechanism of action of diuron, which has been present in agricultural runoff contaminated by phenylurea herbicides. Another phenylurea herbicide that is well documented in science literature, linuron (de Lima et al. 2011) was also present in the runoff from this study. Linuron and diuron were shown to elicit cytotoxic effects on human cell lines in vitro (Huovinen et al. 2015; Sunouchi et al. 2011). It has also been shown that diuron leads to CYP1A1 mRNA induction (Rudzok et al. 2009). Studies have also shown pregnane X receptor (PXR) activation, steroid biosynthesis, and cholesterol metabolism to be molecular metabolic targets of diuron (Marlatt and Martyniuk 2017).

Bromadiolone is a second-generation anticoagulant rodenticide that is shown to be both more effective and more toxic than its predecessors. This compound has been widely used against rodents and is readily available commercially. Bromadiolone is also known as super-warfarin due to its anticoagulant activity, as it works by interfering with vitamin K-dependent factors II, VII, IX, and X metabolism in the liver (Vindenes et al. 2008). This rodenticide is a major concern for

toxicity to humans, as well as other mammalians, because of its mechanism of action through the vitamin K pathway. Pet animals may be unintentionally exposed to this compound in areas where it has been intentionally applied, whereas for humans, accidental exposure to children and/or intentional exposures (in adults as seen in cases of suicide attempts) have been reported (Yu et al. 2013).

Brodifacoum is another anticoagulant rodenticide, which is another associated with frequent human and animal poisonings alongside bromadiolone (Berny et al. 2010). Brodifacoum has a similar mechanism of action and clinical symptoms to the effects of bromadiolone, and only slight differences in outcomes such as increased rates of ecchymosis and hematuria (Yu et al. 2013). Exposure to brodifacoum may cause continued internal bleeding for several months in humans. The lethal dose for an adult human was found to be approximately 15 mg (World Health Organization 1995).

3.3 Conclusions and Future Perspectives

The purpose of this chapter was to review and synthesize available research in the scientific literature regarding pets and companion animal exposures to pesticides and to describe instances where shared exposures may impact the health of humans. We selected distinct types and classes of pesticides that have wide-ranging sources of exposure across animals, people, and environments and therefore put forth that continued investigations should use a "One Health" context (Fig. 3.1). The ubiquity of pesticides in surface waters (i.e., rivers, lakes, oceans), ground water, and drinking water is well established (Pfister et al. 1969; Pinto et al. 2010) and has been reported to negatively influence water quality (Dale and Polasky 2007; Mineau and Whiteside 2006). Environmental and treated waters may be important sources of persistent pesticide exposures to animals and people and likely elicit different mechanisms from what is shown in controlled, single-exposure laboratory animal studies. Furthermore, we know that chemical mixtures are the reality (Bridges 2000; Carlos et al. 2001; Horrigan et al. 2002). Water pesticide levels may accumulate in any animal or human host over different lengths of time (i.e., days, months, years), and not all mechanistic studies utilize relevant concentrations found from the respective water sources in which exposure occurs (Bridges 2000; Carlos et al. 2001; Horrigan et al. 2002). Although it is clear that companion animals already serve as a high sentinel value for humans, water contamination in household and agricultural settings could be better understood for selected pesticides. Some current research was available on this topic, yet additional research using novel methodologies and predictive modeling capacity is needed to identify how animals may serve as sentinels to the real-life complexity of pesticide exposures that occur as chemical mixtures. It has become increasingly important to consider chemical mixtures when the majority of toxicological research studies have revealed mechanisms

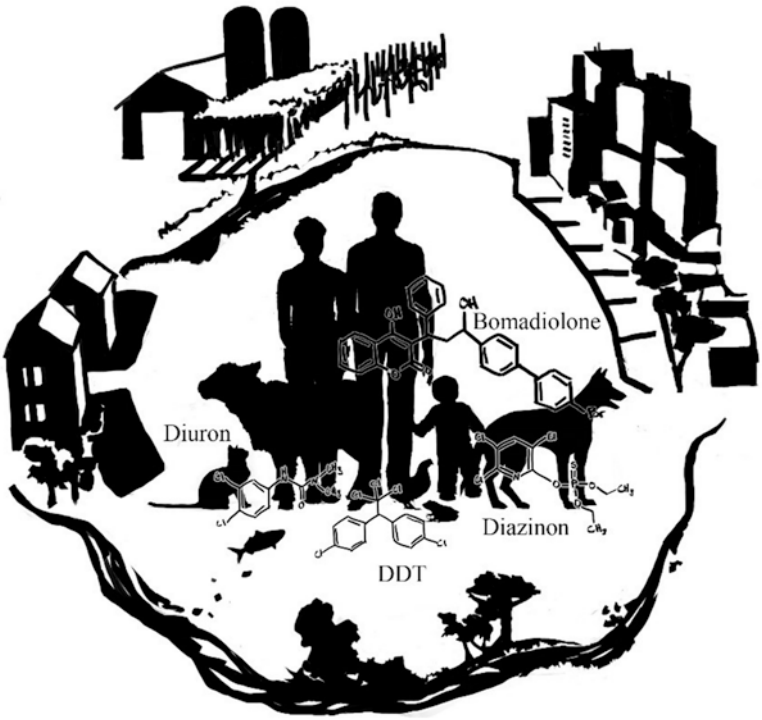

Fig. 3.1 One Health and pesticide exposures: Creating a systems view for evaluating pets and other animals as sentinels of human exposure to pesticides. The natural and built environment includes complex water systems that are shared sources of exposure to animals and people for many chemical classes of pesticides. (Original artwork and diagram by Hannah Haberecht)

of action for a single agent with defined dose levels. The notion that pets may serve as a stronger indicator of what people are exposed to than laboratory animals is not a new idea, but the challenge is that the myriad of chemicals that may exist in the host are often at levels that are expensive to detect and quantitatively measure. Although there are research limitations of using pets as sentinels to human exposures for pesticides, our synthesis of the literature suggests that there is a dire need for us to establish a stronger understanding of the relationships between animal exposures and owner/human exposures across the varied environments of cohabitation. The impacts of real-life and relevant exposures are needed if we intend to design interventions that will prevent or mitigate the negative, adverse effects that these pesticide agents can have throughout the lifespan and across generations of people and pets.

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Chapter 4
Role of Pet Dogs and Cats as Sentinels of Human Exposure to Polycyclic Aromatic Hydrocarbons

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Abstract Polycyclic aromatic hydrocarbons (PAHs) are a large group of chemical contaminants, predominantly produced via fossil fuel combustion. They spread easily worldwide, so they are considered as semipersistent pollutants. Many of them are considered as carcinogenic or mutagenic compounds, for example, interacting directly with DNA. Benzo(a)pyrene (BaP) is the most important and well-known PAH. Living beings are exposed everyday through air, water, plastic stuff and smoke and almost by food intake, because they are highly lipophilic. In human risk assessments, monitoring these compounds, or their products, in environment, biological or food samples has attracted enormous interest. Pets commonly share habitat and routine life with humans. In this chapter, the possibility that pets were good sentinels of human exposure to PAHs is studied in detail. Concentrations of parental PAHs and some metabolites between human and pets have been compared. In the case of dogs, their concentrations and profiles of PAHs are very different to those of humans when compared. Dogs had lesser concentration of parental compounds and higher concentration of their metabolites than humans. Similarly, cats present different concentrations and detection frequencies than humans. Therefore, the scarce data available indicate that dogs and cats seem to have different sources of exposition to PAHs than humans. Although more studies are needed, pets do not seem to be good sentinels for human exposure to PAHs.

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4.1 Introduction

Polycyclic aromatic hydrocarbons or polynuclear aromatic hydrocarbons (PAHs) are a large class of organic compounds (more than a hundred are known) made from carbon and hydrogen, formed by more than two benzene rings fused and organised on linear, angular or cluster structure. According to their molecular weight, they can be classified as low-molecular-weight PAHs (LMW-PAHs, up to three fused rings) or as high-molecular-weight PAHs (HMW-PAHs, minimum of four rings).

Generally, they are colourless, white or yellowish solid at room temperature; have low vapour pressure, high melting and boiling points and low water solubility; and are hence highly lipophilic (WHO 1998). The most harmful and best-known PAH is benzo(a)pyrene (BaP), but there are many other PAHs of concern (Fig. 4.1) because of their toxicity, human exposure, occurrence in the environment and scope of available information. According to the list of priority pollutants of the United States Environmental Protection Agency (USEPA), there are 16 priority PAHs, because of their occurrence and the fact that they are continuously emitted to the environment (ATSDR 1995). More important are those PAHs that have been identified as mutagenic/teratogenic/carcinogenic by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (Fig. 4.1). Based on data from oral bioassays conducted in mice with coal tar mixtures, the JECFA calculated margin of exposure values of 25,000 and 10,000 between the BMDL10 value of 100 μg of benzo[a]pyrene/kg bw/day and mean and 95th-percentile intake levels of 4 and 10 ng/kg bw/d, respectively.

According to their origin, PAHs can be classified as pyrolytic (high temperature), petrogenic (high pressure) or biological (synthesised by microorganisms). Besides, they can be disguised between anthropogenic (combustion of fossil fuels, principally) and natural (forest fires, volcanos, fossil fuel formation, vegetal matter decomposition) sources, although the latter have a minimal contribution to the total environment burden.

Granting that they have no utility per se, PAHs are used as intermediaries in different industries, namely, in the manufacture of pharmaceutical products, polyvinyl chloride (PVC) and plasticisers (naphthalene), pigments (acenaphthene, pyrene), dyes (anthracene, fluoranthene) and pesticides (phenanthrene) (WHO 1998). Nevertheless, production, processing and use of fossil fuels principally coal – and, to a lesser extent, oil and natural gas – for industries, heating or transportation in cities, are the main source of emission of these contaminants to the environment (Cabuk et al. 2014; Villar-Vidal et al. 2014). Concerning traffic, petrol-fuelled vehicles can emit greater amounts of fluoranthene and pyrene, whilst diesel-fuelled

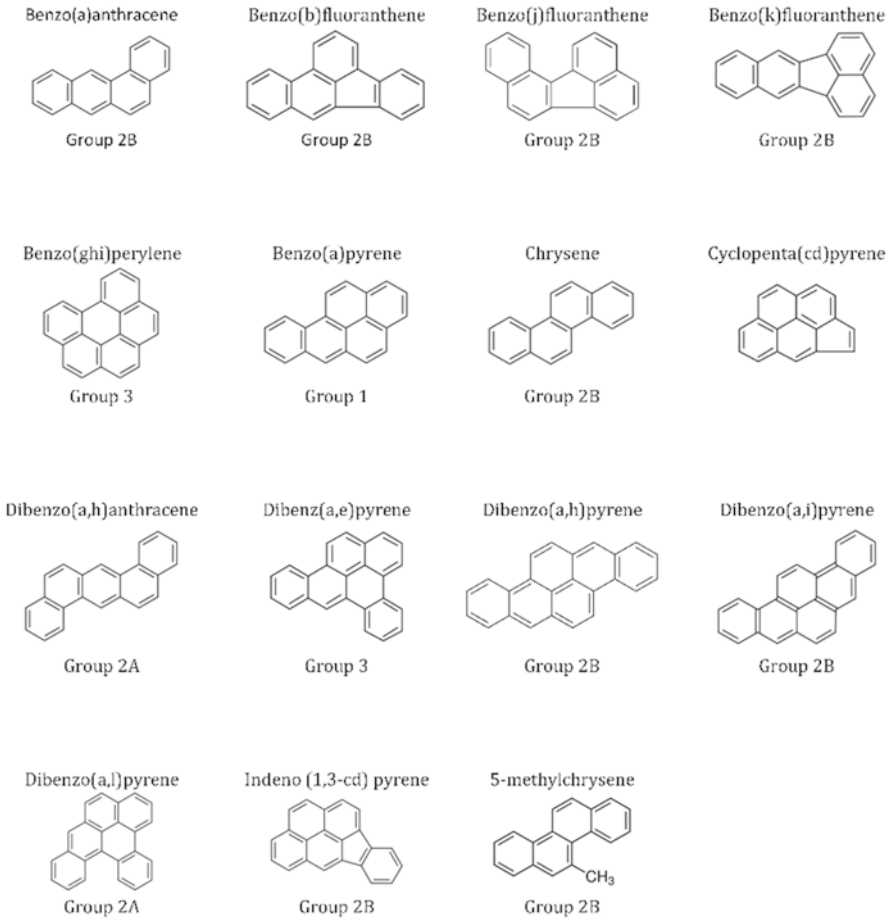

Fig. 4.1 Polycyclic aromatic hydrocarbons for which there is clear evidence of mutagenicity/genotoxicity in somatic cells in experimental animals in vivo and, with the exception of benzo(ghi)perylene, which have also shown clear carcinogenic effects in various types of bioassays in experimental animals

vehicles emit naphthalene and acenaphthene. In the case of smoking, cooking or burning (of stubble, garbage, tyres or other types of waste), a great variety of different compounds are emitted, including the ones already mentioned.

After being formed, these hydrocarbons are dispersed in the environment according to their molecular weight and climate conditions (Kozak et al. 2003). Thus, the HMW-PAHs can be adsorbed into the organic matter of the soil, water or air, whilst the LMW-PAHs will become a part of the gas phase in the atmosphere (Li et al. 2015). Both can be transported over long distances in several weeks until they are precipitated and/or degraded by solar light or microorganisms in the soil or sediments (Walgraeve et al. 2010). Along the way, they can react with different airborne

compounds, namely, sulphur oxides, nitrogen oxides or ozone, resulting in no less toxic combinations (Li et al. 2015; Walgraeve et al. 2010), like nitro-/oxy-PAHs and radicals formation.

4.2 Sources of Exposure and Health Effects

Humans and other living beings can be exposed to PAHs through inhalation or dermal/mucosa contact or mainly through water and food intake (Boada et al. 2016; Henriquez-Hernandez et al. 2017b; Hernandez et al. 2015, 2017; Luzardo et al. 2013a; Rodríguez-Hernández et al. 2015b, 2016, 2017). Inhalation is an important source in smokers and people who live near or in big cities or industrialised zones, where ten times higher concentrations of PAHs than in rural areas can be found (de la Gala Morales et al. 2015; Srogi 2007). Several authors have described higher concentration of PAHs in winter than in summer because of increased use of domestic heating (de la Gala Morales et al. 2015; Li et al. 2015; Villar-Vidal et al. 2014).

It has also been described that dermal exposure may be relevant, mainly when prolonged or continued contact with products made of petroleum derivatives occurs. Recently, the European Union, through the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) regulation, established new restrictions about PAHs in several day-to-day stuffs made of plastics or rubber, which are in direct, prolonged or short-term repetitive contact with human skin or mucosa. These items should not contain more than 10 mg/kg of the sum of benzo(a)pyrene (BaP), benzo(e)pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene and dibenzo(a,h)anthracene, or no more than 1 mg/kg of BaP alone (ECHA 2017).

Although parental PAHs are generally inert, once in the organism, PAHs can be metabolised to be eliminated, generally via urine (LMW-PAHs) or faeces (HMW-PAHs). By the way, the process may result in active PAH metabolites (m-PAHs) capable of forming adducts with the DNA (Boada et al. 2016; Ramesh et al. 2004; Rodríguez-Hernández et al. 2015a, Hernandez et al. 2017). The biotransformation process is carried out through a series of enzymes like cytochrome P-450, which catalyses mainly oxidation, reduction and hydrolysis reactions. In vertebrates, the liver is the major contributor in the biotransformation process. However, in other organs there are cytochromes, which are able to perform this function according to the entryway (i.e. lungs, intestine or skin) (Ramesh et al. 2004). In addition, conjugation enzymes such as sulphotransferases, epoxide hydrolase, glutathione transferase and UDP-glycosyltransferase can metabolise PAHs, producing a variety of phenols, catechols, quinones and radical cations. Once they are formed, these compounds may produce adverse effects by means of various mechanisms, such as DNA damage diol-epoxides (that give place to formation of adducts), interaction with membranes and oxidative stress (Li et al. 2015; Sikkema et al. 1994; Zhang et al. 2016). Given that, some PAHs are described as carcinogenic (c-PAHs), namely, human carcinogen BaP (Group 1), whilst others are considered as probably (Group 2A) or

possibly carcinogenic (Group 2B) by the International Agency for Research on Cancer (IARC 2005). All these compounds have been described as contributing causes of breast, bladder, lung, skin or gastrointestinal cancers (Alicandro et al. 2016; Boada et al. 2015, 2016; Flesher and Lehner 2016; Korsh et al. 2015).

Often mixtures of hydrocarbons and/or their derivatives (such as nitro-PAH) are more harmful, due in part to synergistic properties. In general terms, the lower the molecular weight, the lower the carcinogenicity potential, but they are more prone to cause acute health effects, such as cardiovascular diseases (thrombosis, haematopoietic effects), dyspnoea, asthma (Al-Daghri et al. 2014), diarrhoea, vomiting, nausea and eye, dermal or bronchial irritation or inflammation (Ramesh et al. 2004). Moreover, it is well known that some PAHs are endocrine disruptors in animals and humans. Neurological, congenital and development problems in the offspring and mothers (Jedrychowski et al. 2013; Neal et al. 2008; Oliveira et al. 2017) or immunosuppressant effects (Bolden et al. 2017; Ramesh et al. 2004) have been reported.

4.3 Biomonitoring of Polycyclic Aromatic Hydrocarbons

Given the toxicity and environmental prevalence of these compounds, the monitoring of PAHs is a relevant issue, and there is plenty of interest in control and assessment of these substances in food, environmental compartments, living beings and of course humans. Environmental monitoring of these substances is achieved by sampling and analysing samples such as air, water, food or soil (Bucchia et al. 2015; de la Gala Morales et al. 2015; García-Álvarez et al. 2014b; Hernandez et al. 2015; Kakuschke et al. 2010). Specifically, biomonitoring – the monitoring of these compounds in living beings – is usually considered the best approach as it provides a real picture of the exposure of living beings, meaning that it provides an assessment of the whole uptake through all exposure routes (Srogi 2007).

The biomonitoring of human populations may be done either by direct measurement in samples taken from study populations or extrapolating the data from the environmental exposure of other organisms (bioindicators or sentinels). This biomonitoring can be done by directly determining the individual PAHs and/or their metabolites, as well as by determining biomarkers of the effect they produce. In the case of PAHs, it is common to determine the presence of adducts of PAHs with DNA, or the detection of tetrahydroxy-PAHs that can also be measured as an indicator of tissue damage.

For reasons of practicality and ease of collection of samples, it is often considered that urinary metabolites of PAHs are better bioindicators of exposure, being considered the gold standard to determine recent exposure to a single PAH, in particular when multiple routes of exposure have to be taken into account (Jacob and Seidel 2002) or in occupational meaning (Unwin et al. 2006). The main m-PAHs that should be included in biomonitoring studies are 1-hydroxynaphthalene (1-napthol), 2-hydroxynaphthalene (2-naphthol), 1,2-dihydroxynaphthalene, 2-hydroxyfluorene (2-FLUO), 3-hydroxyfluorene (3-FLUO), 9-hydroxyfluorene

(9-FLUO), 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, 3-hydroxyphenanthrene, 4-hydroxyphenanthrene, 9-hydroxyphenanthrene, 1-hydroxypyrene (1-PYR) and 3-hydroxybenzo(a)pyrene (3-OHBaP) (Wang et al. 2014a, b). 1-PYR has been linked to dietary exposures, whilst both 1-PYR and 2-naphthol are well correlated with smoking in a non-occupational population (Nethery et al. 2012; Srogi 2007). Urinary 3-OHBaP may be a suitable biomarker to assess BaP genotoxic exposure in humans (Marie-Desvergne et al. 2010; Oliveira et al. 2017). One decisive factor to take into account when determining urinary metabolites is sampling time, due to the high rate of biotransformation of these compounds (Cathey et al. 2018; Grova et al. 2017a, b). Taken together, those results suggest that it is better to use a combination of metabolites, since each metabolite gives an information about a single or few parental PAHs (Castano-Vinyals et al. 2004; Grova et al. 2017b; Hilton et al. 2017; Singh et al. 2008).

Other excretion routes, such as nails, hair, sweat or feathers, amongst others, have been also investigated regarding their content in m-PAHs, as a means of determining long-term exposure to these substances. In fact, some authors have pointed out that these matrices are more appropriate for the determination of HMW-PAHs (Grova et al. 2017b; Marie-Desvergne et al. 2010).

On the other hand, not only for assessing exposure but also the toxicological effect of PAHs, some other authors prefer to determine the amount of PAHs-DNA adducts in peripheral white blood cells, or their binding to plasmatic proteins, especially in occupational studies (Oliveira et al. 2017; Pleil et al. 2010). Other authors correlate the level of oxidative stress induced by PAHs as an indirect indicator of the carcinogenicity of these compounds (Singh et al. 2008). However, these studies of biomarkers have the disadvantage in that the analytical techniques are complex, have low sensitivity and do not allow deriving the global exposure to these compounds.

Finally, some authors consider that the direct measurement of PAHs in blood is the best way to estimate the total body burden and also the most realistic way to estimate exposure (Boada et al. 2015; Pleil et al. 2010). It has the disadvantage in that sampling is invasive, especially taking into account that WHO recommends that biomonitoring studies include mainly children, because it has been estimated that children aged 6–11 are the sector of the population most exposed to these compounds (Singh et al. 2013). In addition, and as we said before, it is possible to evaluate human exposure to PAHs indirectly, using bioindicator species. In these cases also blood is often the easiest sample to take, so comparison with human levels is simpler (Boada et al. 2015; Bucchia et al. 2015; Camacho et al. 2012b, 2014; Camacho et al. 2013b; García-Álvarez et al. 2014a, b; Luzardo et al. 2014).

In this sense, studies of the effects of environmental exposures on vegetables or animals can corroborate or support epidemiological studies in humans or in the environment. In these cases, the levels determined in these easy-to-sample species may reflect the exposure of a group of environmentally related species, rather than the individual exposure. Thus, the use of microbial bioindicators in order to evaluate contamination of some PAHs in agricultural soils (Niepceron et al. 2013) and in the gas and aqueous phases (Cho et al. 2014) has been reported. In the same way, moss,

lichens and plants have been used as passive phytomonitors instead of the active samplers and several studies have found promising results. In wildlife, some authors have proposed different species as possible sentinels of exposure. Some invertebrates have been studied. Amphipod (*Talitrus saltator*) appears to be a good bioindicator of this class of organic compounds in supralittoral zone (Ugolini et al. 2012). The possibility that molluscs are good bioindicators of the contamination of PAHs from the waters or sediments in mudflats of Malaysia (Tavakoly Sany et al. 2014) and mangrove oysters (*Crassostrea rhizophorae*) (Ramdine et al. 2012) has also been reported. Studies on oil spills such as those occurring on the northern Cantabrian sea and in Guanabara Bay, Brazil, respectively, concluded that barnacles are good indicators for oil spill evolution (Soares-Gomes et al. 2010; Vinas et al. 2009). Other species in the highest levels of the food chain have also been described as efficient indicators of recent pollution. Fuentes-Rios et al. (2005) determined that the cat shark is a good bioindicator for exposure to PAHs on the Chilean Pacific coast, showing good correlation with the concentration of pyrene in water and urinary 1-PYR. On Atlantic eastern coast and Mediterranean sea, several authors (Bucchia et al. 2015; Camacho et al. 2012a, 2013a, 2014, García-Álvarez et al. 2014a) investigated serum levels of PAHs in different populations of sea turtles (*Caretta caretta*) and bottlenose dolphins (*Tursiops truncata*) indicating that both species could be good indicators of local and recent pollution in the marine environment.

Since the iconic 'canary in the cage' began to be used to detect the presence of toxic gases in the coal mines, pets and other animals in the human immediate environment have been used as sentinels of human exposure to many other chemical classes. In this case, they were used as an early warning system, since the canary is more sensitive to carbon monoxide poisoning than humans and other domestic animals like cats, dogs, pigeons or rabbits. Livestock, including bees, cattle, horses, sheep and goats, can be good bioindicators for outdoor air, whilst pet cats and dogs can share the indoor air, water, food or even household dust. However, daily routine and diet, especially in people who are occupationally exposed, smokers or on some kind of diets, are confounding factors. The different metabolism and elimination capacity amongst the species should be also taken into account as confounding factors.

4.4 Pet Dogs as Sentinels forHuman Exposure to PAHs

Pet dogs are particularly interesting as sentinels for human exposure to PAH, given that they share the habitat with humans and they respond to toxic assaults similarly than their owners (Backer et al. 2001). As far as we know, there is only one research article that has assessed exposure to PAHs in dogs and humans to date (Ruiz-Suárez et al. 2016). In this study, the authors included blood samples from 87 pet dogs (46 males and 41 females, 0.5–13 years old) visiting the veterinary hospital of the Faculty of Veterinary Medicine of the University of Las Palmas de Gran Canaria (Canary Islands, Spain) for routine care. Only clinically normal animals (negative stool sample, negative result on a heartworm test and no overt disease) were included

in the study, after owners' consent. In parallel, human blood samples from 60 males and 40 females (19–34 years old) were collected from a blood bank during the same period that dogs' samples were drawn. For logistical reasons the researchers could not get blood from the owners of the same dogs included in the study. Even so it has been estimated that there are about six million domestic dogs in Spain and that more than 40% of Spanish homes have at least one dog, so the authors assumed that a high percentage of these blood donors share habitat with some dog.

In this research work the authors determined 21 PAHs, including the 13 c-PAHs and also 6 common m-PAHs (Table 4.1), by means of solid phase extraction and gas chromatography coupled to tandem triple-quadrupole mass spectrometry. In this research the authors detected the totality of the PAHs and m-PAHs in any of the samples, both in humans and dogs, with the only exception of benzo(a)pyrene, which was not detected in none of the dog plasma samples.

The compounds most frequently detected in both species were phenanthrene, fluorene and fluoranthene and 2-naphthol, which were present in nearly 100% of the samples. The frequencies of detection of the rest of the compounds of this chemical group were highly variable and different between the two species (Table 4.1). The mean values of $\sum$PAH21 were much lower in dogs than in humans (782.2 vs. 1623.3 ng/g lw, respectively). Regarding the c-PAHs, the authors considered only seven compounds (PAH7, benzo(a)anthracene, chrysene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, dibenzo(a,h)anthracene and indeno(1,2,3-c,d)pyrene), and the mean values were also much lower in dogs than in humans (6.8 vs. 21.9 ng/g lw, respectively). On the opposite, according to the authors' results, it seems that dogs may have a higher capacity of biotransformation of these compounds, because in parallel to the lower levels of untransformed PAHs, dogs also had higher levels of PAHs metabolites than humans, in whom the relationship was inverse ($\sum$m-PAH = 198.1 and 131.6 ng/g lw in dogs and humans, respectively; $p < 0.0001$).

The importance of the employment of sentinel species for the assessment of human exposure to chemicals has been widely demonstrated for many chemical classes, since the sentinel species may reflect the actual human exposure of a given population, much more accurately than the comparison to other remote populations. However, it does not seem to be the case of pet dogs as sentinels of human exposure to PAHs, because the authors of the only study available in this regard found that there were many significant differences between these two species (Fig. 4.2), both in the levels of many parental compounds and in their metabolites. These results suggest that exposure of both species to this contaminant group could be different, but also may be indicating that dogs have a higher capacity to metabolise these compounds than humans. Obviously, to confirm this point, additional research is needed, but these results allowed the authors to hypothesise that the lower levels of PAHs detected in the plasma of dogs could be due to a higher rate of biotransformation and elimination thereof. Furthermore, as shown in Fig. 4.2c, neither the profiles of PAHs contamination were similar between dogs and humans, with a clear predominance of the four-ring compounds in humans and three-ring compounds in dogs. In fact, it is noteworthy that some compounds such as pyrene, which was

Table 4.1 Concentrations of individual PAHs and PAHs metabolites concentrations (ng/g lw) in dog (n = 87) and human (n = 100) serum samples from the Canary Islands, Spain

	Dog serum		Human serum		
	Mean ± SD	Freq. (%)	Mean ± SD	Freq. (%)	P^*
Benzo(a)anthracene	4.2 ± 5.1	12.6	6.0 ± 17.9	12.0	
Benzo(a)phenanthrene (chrysene)	5.4 ± 7.3	10.3	4.4 ± 17.1	13.0	
Benzo(a)pyrene	n.d.	0.0	4.4 ± 8.5	8.0	
Benzo(b)fluoranthene	4.6 ± 5.4	6.9	4.7 ± 9.2	10.0	
Benzo(k)fluoranthene	4.3 ± 6.9	11.9	17.9 ± 34.1	36.0	0.0015**
Dibenzo(a,h)anthracene	4.9 ± 6.1	2.3	4.43 ± 7.8	6.0	
Indeno(1,2,3-cd)pyrene	5.2 ± 7.4	2.3	5.2 ± 4.4	3.0	
Benzo(j)fluoranthene	4.5 ± 5.6	7.9	11.7 ± 3.7	14.0	0.0056**
Benzo(j,k)fluorene (fluoranthene)	6.6 ± 4.3	97.7	77.5 ± 26.4	99.0	<0.0001***
Dibenzo(a,e)pyrene	5.2 ± 5.6	1.2	4.3 ± 5.3	5.0	
Dibenzo(a,h)pyrene	6.3 ± 7.4	2.3	4.5 ± 5.8	6.0	
Dibenzo(a,l)pyrene	4.4 ± 6.1	1.2	6.2 ± 5.9	5.0	
5-Methylchrysene	6.3 ± 6.1	12.6	7.6 ± 17.9	14.0	
Acenaphthene	7.6 ± 25.5	13.8	8.7 ± 19. 2	16.0	
Acenaphtylene	51.2 ± 34.4	75.8	12.5 ± 6.3	6.0	<0.0001***
Anthracene	4.7 ± 26.2	4.6	6.8 ± 34.1	10.0	
Benzo(ghi)perylene	n.d.	0.0	4.5 ± 5.2	3.0	
Fluorene	76.9 ± 42.5	98.8	42.5 ± 17.2	98.0	<0.0001***
Phenanthrene	382.5 ± 0.21	100.0	313.3 ± 137.5	100.0	<0.0001***
Pyrene	7.6 ± 17.2	17.2	43.7 ± 25.9	94.0	<0.0001***
Naphtalene	34.1 ± 51.6	28.7	34.1 ± 59.6	41.0	
1-Naphthol	76.9 ± 69.8	79.3	5.2 ± 17.2	8.0	<0.0001***
2-Naphthol	95.1 ± 52.6	96.6	67.2 ± 23.5	98.0	<0.0001***
2-OH-Fluorene	5.8 ± 8.5	11.5	7.9 ± 12.3	21.0	0.1623
1-OH-Phenanthrene	17.4 ± 35.6	23.5	4.2 ± 6.6	10.0	0.0459*
7-OH-Benzo(c)fluorene	51.4 ± 8.5	36.8	4.5 ± 8.5	12.0	0.0089**
1-OH-Pyrene	4.5 ± 17.2	14.9	17.0 ± 43.5	6.0	0.4185
$\sum$PAH7[a]	6.8 ± 17.2	53.2	21.9 ± 43.5	78.0	0.01**
$\sum$PAH21[b]	782.2 ± 323.8	100.0	1623.5 ± 799.2	100.0	<0.0001***
$\sum$m-PAH[c]	198.1 ± 110.5	100.0	131.6 ± 148.5	100.0	<0.0001***

P^*: Mann-Whitney U test
[a] $\sum$PAH7, sum of carcinogenic PAHs
[b] $\sum$PAH21, sum of 21 priority PAHs
[c] $\sum$m-PAH, sum of 6 PAH metabolites

detected in almost 100% of the human samples, were barely detectable in 17% of samples from dogs, and yet, others such as acenaphthylene or 1-naphthol were much more frequently detected in dog plasma than in human plasma. Thus, in the light of the above, the authors concluded that the pet dogs do not seem to be good sentinels for human exposure to PAHs.

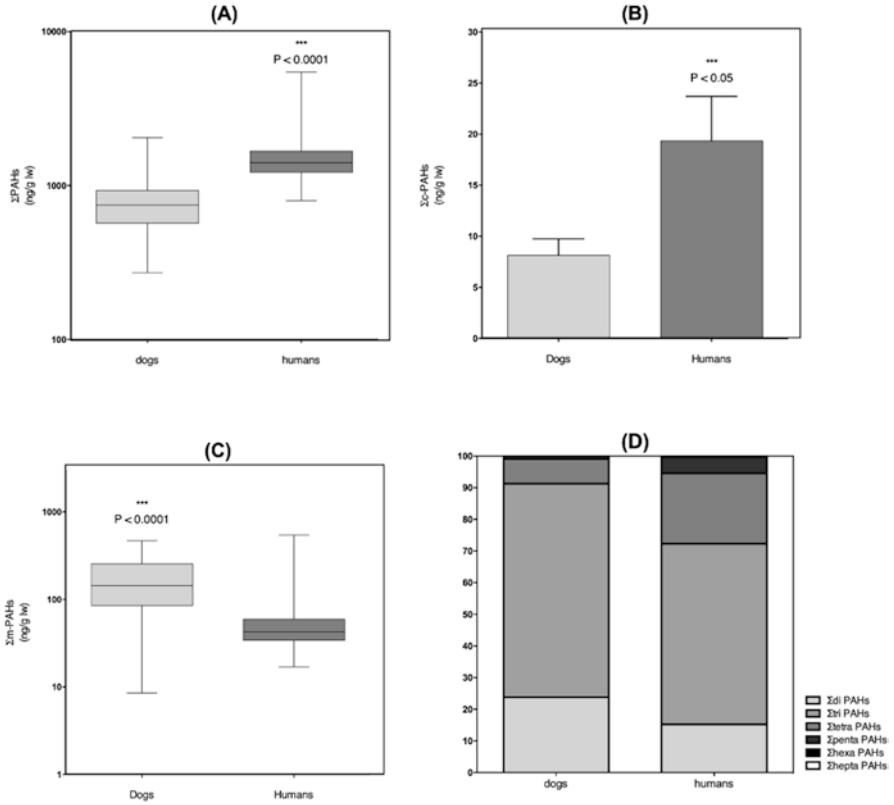

Fig. 4.2 Levels of PAHs in plasma samples. (**a**) (main body). Box plots of ∑PAH21 in dogs and humans. (**a**) (inset). Bar graph of ∑PAH7 (carcinogenic PAHs, median and interquartile range) in dogs and humans. (**b**) Box plots of ∑PAH metabolites in dog and humans. (**c**) Profile of distribution of PAHs in dogs and humans. The line inside the boxes represents the median, the bottom and top of the boxes are the first and third quartiles of the distribution, and the lines extending vertically from the boxes indicate the variability outside the upper and lower quartiles. (**d**) distribution profile of PAH in dogs and humans

4.5 Pet Cats as Sentinels for Human Exposure to PAHs

As far as we know, there is no published study that explored the role of domestic cats as sentinels of human exposure to PAHs. However, the cat that lives inside the house is usually considered a good bioindicator, even better than the dog, to assess the exposure of man to the contaminants present in the domestic environment. This is mainly due to their grooming habits, which cause cats to ingest high amounts of household dust, with all the load of contaminants associated with it. Thus, in different publications, it has been indicated that this pet is ideal for evaluating human exposure to different kinds of contaminants. (Bost et al. 2016; Chow et al. 2015; Dirtu et al. 2013; Henriquez-Hernandez et al. 2017a). In addition, other studies have

shown that dietary exposure to different contaminants (including PAHs) is different between dogs and cats (Ruiz-Suarez et al. 2015), so although, as we said earlier, dogs do not seem to be good sentinels of human exposure to PAHs, cats present differential facts that could make them suitable for this purpose, so this possibility is worth investigating.

With the purpose of completing the information in this chapter, we decided to shed light on the question whether or not cats would be good sentinels of human exposure to PAHs. For this, we collected venous blood from a total of 25 cats that were recently admitted for routine health check-ups and vaccination in the clinical hospital of the Faculty of Veterinary Medicine of the University of Las Palmas de Gran Canaria. In parallel, blood was collected from 25 volunteers from the same faculty, from amongst the staff and the students of the same, all of them owners of cats (although not from the same cats participating in the study). The serum was obtained, and the PAHs were extracted by solid phase extraction following the procedure described elsewhere (Camacho et al. 2012a). In this work, we included only the 16 priority PAHs for the USEPA, whose analysis was performed by gas chromatography coupled to tandem triple-quadrupole mass spectrometry (Luzardo et al. 2013b). All human volunteers and cat owners provided their written informed consent to participate in this study.

We found only 8 out of the 16 compounds analysed both in humans and cats. In addition, acenaphthylene was also detected in cats, but not in humans. The summary of the results of this study is shown in Table 4.2. As it can be seen, the most frequently detected compounds were acenaphthene, phenanthrene and fluorene, with frequencies of 90% or more in both species. For the rest of the substances, the detection percentage between both species was highly variable. We want to highlight the differences found between cats and humans for chrysene and fluoranthene (percentages of detection of 18.2 vs. 90% and 31.3 vs. 100%, respectively). The median of the $\sum$16PAHs was similar in both species (1.93 vs. 2.08 ng/mL or 232 vs. 257 ng/g lw, respectively). However, although the total concentrations do not show significant differences between both species, when we focus on carcinogenic compounds for EFSA, the outlook changes radically, since these compounds were practically undetectable in the group of cats, whilst they were present in the group of cat owners (Fig. 4.3). Obviously, this is only a preliminary study, and the conclusions that derive from it should be taken with caution because of the low sample size. However, based on the results obtained, it could not be considered that the cat is the ideal sentinel to assess human exposure to PAHs, although it does seem to be better than dogs in this sense.

4.6 Conclusions

Based on the scarce existing bibliography and limitations of the study, it can be concluded that pet dogs and cats are not good sentinels of human exposure to PAHs. The analyses of parental compounds and metabolites in serum and their concentra-

Table 4.2 Concentrations of polycyclic aromatic hydrocarbons (ng/mL) in the whole series of cats ($n = 22$) and humans ($n = 20$)

	Pet cats			Humans			
Congener	% detection	Median	p25th–p75th	% detection	Median	p25th–p75th	P^a
Acenaphthylene	63.6	0.02	0.00–0.04	0	–	–	0.003
Acenaphthene	100.0	0.56	0.42–0.99	100.0	0.52	0.29–0.79	ns
Anthracene	31.0	0.00	0.00–0.07	80.0	0.13	0.05–0.20	0.008
Benzo(a)anthracene	0	–	–	0	–	–	na
Benzo(a)pyrene	0	–	–	0	–	–	na
Benzo(b) fluoranthene	0	–	–	0	–	–	na
Benzo(ghi)perylene	0	–	–	0	–	–	na
Benzo(k) fluoranthene	0	–	–	0	–	–	na
Chrysene	18.2	0.00	0.00–0.00	90.0	0.03	0.01–0.04	0.003
Dibenzo(ah) anthracene	0	–	–	0	–	–	na
Fluoranthene	31.8	0.00	0.00–0.03	100.0	0.05	0.03–0.07	0.001
Fluorene	100.0	0.16	0.09–0.41	90.0	0.12	0.03–0.21	ns
Indeno(123,cd) pyrene	0	–	–	0	–	–	na
Naphthalene	18.2	0.00	0.00–0.00	30.0	0.00	0.00–0.07	ns
Phenanthrene	100.0	1.16	0.57–2.30	100.0	1.22	0.68–1.44	ns
Pyrene	13.2	0.00	0.00–0.00	60.0	0.01	0.00–0.04	0.047

Abbreviations: *p25th–p75th* percentiles 25 and 75 of the distribution, *ns* non-significant, *na* not applicable

[a]Mann-Whitney U test

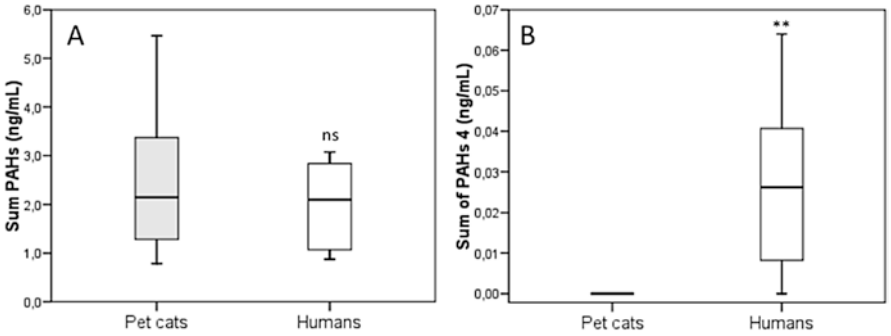

Fig. 4.3 Box plot showing the serum levels of sum of all PAHs (panel A) and sum of PAHs 4 (panel B), amongst cats ($n = 22$) and humans ($n = 20$). Sum PAHs included all the 16 congeners analysed. Sum of PAHs 4 included only benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene. *P* values were calculated with Mann-Whitney U test. The lines connect the medians, the boxes cover the 25th to 75th percentiles, and the minimal and maximal values are shown by the ends of the bars. Abbreviations: ns, non-significant. **, $p = 0.003$

tions and contamination profiles are not comparable between species. These results could indicate that different sources of exposure, such as smoking, occupational setting or food intake, in humans exist. In the analysis of PAH metabolites, higher levels in dogs suggest that they metabolise them more effectively than humans. Despite sharing a home and in some cases diet with humans, pets differ greatly from humans to consider them good sentinels for PAHs exposure.

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Chapter 5
Pets as Sentinels of Human Exposure to Neurotoxic Metals

M. Ramiro Pastorinho and Ana Catarina A. Sousa

Abstract The idea that animals may be used as sentinels of environmental hazards pending over humans and the associated public health implications is not a new one. Nowadays pets are being used as bioindicators for the effects of environmental contaminants in human populations. This is of paramount importance due to the large increase in the worldwide distribution of synthetic chemicals, particularly in the built environment. Companion animals share the habitat with humans being simultaneously exposed to and suffering the same disease spectrum as their masters. Moreover, their shorter latency periods (due to briefer lifespans) enable them to act as early warning systems, allowing timely public health interventions. The rise on ethical constraints on the use of animals and, consequently, on the sampling they can be subjected to has led to the preferential use of noninvasive matrices, and in this case we are looking into hair. This chapter focuses in three non-essential metals: mercury, lead, and cadmium, due to their ubiquitous presence in the built environment and their ability of affecting the mammal nervous system. There is a fairly short amount of studies reporting the concentrations of these metals in pets' hair, particularly for cats. These studies are characterized, and the metal concentrations corresponding to different parameters (e.g., age, sex, diet, rearing) are described in order to provide the reader with a general vision on the use of this noninvasive matrix on the studies conducted since the last two decades of the twentieth century.

Keywords Surrogacy · Early warning · Latency · Cadmium · Lead · Mercury

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5.1 Introduction

The idea that animals may be used as sentinels of environmental hazards pending over humans, and the associated public health implications is not new. The archetypal concept of the *canary in the coal mine* re-claims a new life in the twenty-first century. Miners used canaries in the early decades of the twentieth century to detect high levels of carbon monoxide and other toxicants in mine shafts (Pollock 2016). Nowadays, pets are being used as assessment and prediction tools (bioindicators) for the effects of environmental contaminants in human populations. In an age where increases in synthetic chemicals production and diversification are outpacing "classical" agents of global change (e.g., atmospheric CO_2 concentrations, nutrient pollution, habitat destruction, biodiversity loss) (Bernhardt et al. 2017), the need for sensitive indicators of the presence, and consequently, of the potential (and actual) effects of these chemicals is paramount. The rise on the awareness of the sentient capabilities of other species outside man compelled regulator bodies to impose ethical constraints on the use of animals and consequently on the sampling they can be subjected to. In this context, noninvasive matrices have become preferential targets for the evaluation of tissue contamination on animals (Sousa et al. 2013).

Companion animals share the habitat and are consequently exposed to similar agents as their human counterparts. A particular case is that of children that can be subjected to the same exposure sources (e.g., house dust). The spectrum of disease suffered by pets is similar to that of humans, enabling them as indicators of environmental hazards. Moreover, since they possess shorter latency periods (as they have shorter average lifespans), they can provide an early warning system, enabling timely public health interventions (Wallis et al. 2018). Also, the use of pet sentinels as models for epidemiologic studies of human diseases and environmental exposures has been long proven to present advantages over classical laboratory animal models (Bukowski and Wartenberg 1997). Moreover, the depth of the interface between humans, animals, and the environment is being made more apparent due to the global change our planet is undergoing.

However, these impressive capabilities were not always duly regarded. The notorious episode of the outbreak of neurologic manifestations in the population of Minamata Bay in the 1950s caused by the consumption of fish contaminated by methylmercury was preceded by neurobehavioral disorders in the cat population of the same area. The ataxic, "dancing," cats were a grossly disregarded warning sign, from which resulted a large loss of life (Reif 2011; Tsuchiya 1992).

The currently recognized connection between metals, neurodegeneration, and pets as early warning systems and predictors of human health risk will be the focus of this chapter. The use of a noninvasive matrix – hair – will be highlighted and concentrations found in studies conducted since the last two decades of the twentieth century reported.

Neurodegenerative diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease) represent a major threat to human health, with nearly 50 million people across the world suffering from dementia, with this number set to reach

150 million by 2050 (WHO 2019). The increasing proportion of elderly citizens is partially to blame. Conveniently, this reality finds a parallel in pets since their lifespan is also increasing (Wallis et al. 2018). The One Health concept (van Helden et al. 2013) by proposing coordinated efforts between human epidemiology, veterinary epidemiology, and environmental toxicology presents an optimal framework for approaching the current epidemic of non-communicable diseases (of which neurodegenerative disorders are an integral part) and their association with environmental contaminants.

5.1.1 The Importance of Using Bioindicators

The term "bioindicators" has been receiving wider acceptance in recent years, despite its definition being somewhat variable. Here, we define bioindicators as species or communities that are used to identify the influence of an environmental chemical, environmental changes, or pressure, by demonstrating a departure from a normal status. The most common origins of these disrupting elements are anthropogenic activities and the destruction of the biotic system (Martin and Coughtrey 1982). In ecosystems at large, multitudes of bioindicators are used to determine air, soil, and water quality and how they reflect in animal and plant health. This abundance of bioindicators does not have correspondence into heavily humanized areas (e.g., industrial, rural, urban). Far fewer bioindicators used to monitor these ecosystems can be found in the literature. Despite encompassing taxa across different levels of organization, such as lichens (Cicek et al. 2007), plants (Minganti and Drava 2018), soil invertebrates (Santorufo et al. 2012), and bats (Russo and Ancillotto 2015), it becomes evident that they are only remotely related to humans themselves (both in terms of phylogeny and daily habits) so as to become nearly unrepresentative. To circumvent this situation, companion animals can provide a very important contribution to monitor the most common human habitats.

5.1.2 Companion Animals as Bioindicators/Sentinels of Metal Exposure

Humans and animals share the same ecosystem and, in the case of companion animals, the same home environment, and in a large number of times, they share the same food items or entire diets. Pets may therefore serve as sentinels and/or early warning systems for human health hazards, since, as a norm, they are more sensitive to the offending agents, come in closer contact with the hazard (cats groom frequently, dogs crawl and eat food out of the floor), or have shorter latency periods for symptoms and/or disease. Examples include lymphoma in domestic dogs exposed to phenoxy herbicides (Hayes et al. 1991), lung cancer from passive smoking

exposure in dogs (Reif et al. 1992), and the mirroring of the human obesity epidemic by pet cats and dogs (German 2006). But probably the better-known example of the forecasting ability of pets regarding the human health was the Minamata incident. A factory (owned by the Chisso Corporation) located in Minamata Bay (Japan) started production of acetaldehyde in 1932. The chemical reaction used to produce the acetaldehyde used mercury sulfate as a catalyst, generating methylmercury (a powerful neurotoxic) as a side product that was discarded into the bay until 1968, contaminating the ecosystem, including the fish consumed by humans. The first patient, reported on April 21, 1956, was a five-year-old girl presenting walking and speaking difficulties and convulsions. Many more would follow with a death rate of 37% by the end of the same year. However, from around 1950 onward, far before the appearance of similar effects in humans, cats had been seen suffering convulsions, "go mad," and die, leading the locals to call the mysterious disease the "cat dancing disease." This exhibition of neurotoxic effects, brought upon by the consumption of very same contaminated fish captured from the bay, occurred years before the first human reported case. If properly contextualized and identified, this could have saved the life of 900 individuals and prevented the effects of poisoning in 2300 others who were left with lifelong sequels (Tsuchiya 1992; Aronson 2005).

Despite their potential, household pets, mostly cats and dogs, have been used as biomonitors in a limited number of studies, particularly in the context of the built environment (e.g., Hayashi et al. 1981; Doi et al. 1986; Berny et al. 1995; Sakai et al. 1995; Dunlap et al. 2007; Atanaskova et al. 2011; Rodriguez Castro et al. 2013; Sousa et al. 2013; Bischoff et al. 2010; Lanocha et al. 2012; López-Alonso et al. 2007; Park et al. 2005a, b; Tomza-Marciniak et al. 2012; Zaccaroni et al. 2014). Besides superimposition of exposure pathways, the use of pets to assess human health impacts has the added advantage of possessing fewer ethical issues associated with obtaining samples, particularly when compared to the case of young children and infants (Needham and Sexton 2000).

As early as the 1990s, Berny et al. (1995) demonstrated that dogs and cats represented reliable surrogates to assess lead exposure in humans. These authors reported that juvenile dogs recorded lead poisoning clinical symptoms ahead of young children and infants. This suggests the potential use of domestic dogs as surrogates for lead exposure in children. This study also described a strong correlation between blood lead concentrations (BLC) in indoor pets and younger children and that the presence of one pet with a high BLC in a house increased the likelihood of finding one person in the same house with a BLC > 10 μg/dl was significantly increased. Surprisingly enough, the study suggested that despite living in an area of heavy lead soil contamination, due to the vicinity of a closed lead smelter, the subjects investigated (pets, or their owners) did not show associations with high blood lead concentrations. This led the authors to focus in indoor sources and concluding that, given the same lead sources (e.g., dust and paint), domestic pets would register higher blood lead concentrations than children.

Subsequent studies came to confirm these seminal discoveries, with domestic animals being considered as good indicators of human metal exposure since they live in the same environment as their owners, being exposed, at least in part, to the same sources (Figs. 5.1 and 5.2). Yet, despite such similarities between humans and

their pets, some factors may differ. This preoccupation regarding confounding factors led several researchers to investigate specific pet traits on the bioaccumulation of metals. The effect of habitat, food, and sex (rural vs urban areas; commercial, homemade, mixed feeds; male vs female) in metal bioaccumulation (including arse-

Fig. 5.1 Indoor cats share the same environment as their human counterparts and thus are exposed to the same indoor contaminants. In the picture, Maria Pia, the cat, sleeps in the bed of her guardians. (Picture by A.C. Sousa)

Fig. 5.2 Barney, the dog, in the living room. Dogs by sharing the same environment as their human counterparts are exposed to the same indoor contaminants. (Picture by R. Teles)

nic, cadmium, mercury, and lead) was investigated by López-Alonso et al. (2007) in the liver and kidneys (main organs for metal accumulation) of pet dogs. The study showed that habitat had no significant effect on the levels of three of the studied metal(loid)s (Pb, As, and Cd) and that overall levels were low. However, marked differences were found when comparing kidney tissue Hg concentration, with urban dogs showing on average three times the concentration of rural dogs, this being attributed to the higher Hg urban environment concentrations due to atmospheric deposition. Commercial diets caused significantly higher liver lead levels (but not the other metals) as opposed to dogs fed homemade or mixed feeds. Finally, females had statistically significant higher kidney levels than males. Such results reinforce the need to take into account potential confounding factors when using pets as biosentinels.

5.2 Metals, the Nervous System, and Neurodegeneration

The nervous system and adjacent structures constitute a highly complex communication network enabling organisms to maintain homeostasis. It consists of sensory components detecting stimulus, pathways that conduct and process the collected information, and effector components that produce a reaction. In its essence, the mechanism is similar in all species. However, morphology and complexity have changed according to levels of organization of each species (Finsterer et al. 2014).

Neurodegeneration represents the malfunction or overall failure of one or all of the components in the nervous system. Presently, neurodegenerative diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease) represent a major threat to human health, with as many as 50 million people across the world suffering from dementia, an umbrella term for a series of neurodegenerative conditions that cause memory loss, with the figure set to triple by 2050 (WHO 2019). This increase is, in part, connected with the increasing longevity of humans, which will lead, by 2050, to a proportion of people above 60 years of 22% of the entire world population (WHO 2013). Simultaneously, demographic studies demonstrate that life expectancy of pet dogs and cats is also increasing, with a census conducted in the US indicating that an increase of 15% in the number of cats over 10 years and 6% for dogs over 6 years occurred in the last two decades (AVMA 2012).

Neurodegenerative processes have been observed in human, canine, and feline brains, including the progressive accumulation of β-amyloid (Aβ) as well as Tau aggregates, two signature hallmarks of neurodegeneration, and dementia progression, namely, Alzheimer's disease (AD) (Head et al. 2005; Ambrosini et al. 2019).

A strong correlation has been shown by epidemiological and clinical studies between aberrant metal exposure and a number of neurological diseases, including AD, amyotrophic lateral sclerosis, autism spectrum disorders, Guillain–Barré disease, Gulf War syndrome, Huntington's disease, multiple sclerosis, Parkinson's disease (PD), and Wilson's disease (Chen et al. 2016).

5.2.1 Metals with Neurodegenerative Potential

Metals are naturally present in the environment being released from natural (volcanic activity, erosion of ore-bearing rocks) and anthropogenic sources (burning fossil fuels; mining and processing of metal ores; mechanical, chemical, and automotive industries; transport; and agriculture). Since the industrial revolution that occurred in the XII century, increasing amounts of metals started to be introduced into the natural cycles, registering a sharp increase after World War II (Nriagu 1988). In the present, the anthropogenic mobilization of metals (as compared to natural mobilization) has increased the magnitude of natural metal cycles, sometimes merely by a fraction, but in other cases by factors of over 100. This means that for these metals the forcing of their cycles is not natural but driven by man (UNEP 2013).

From a living organism's perspective, metals are divided into two groups: essential (being part of structural proteins, enzymes, hormones) and non-essential (with no biological function) (Ferrari 2012). All metals entering an organism, despite being essential or non-essential, can exert toxicity after passing specific thresholds, causing disorders at molecular, cellular, tissue, and organ levels, which can lead to illness and death. As can be easily anticipated, this is particularly true for non-essential metals which can have near-zero thresholds. There is a long list of metals with neurodegenerative potential that include essential and non-essential metals and metalloids (e.g., Cu, Fe, Mn, Zn, Al, As, Cd, Pb, Hg, Tl).

This chapter is focusing on three non-essential metals: mercury (Hg), lead (Pb), and cadmium (Cd), due to the frequency of their study and the long-established fact that they possess the ability of affecting mammals (Keil et al. 2011). The major exposure routes for warm-blooded vertebrates to these metals are via food and inhalation. However, the latter is relevant solely at areas with high levels of air pollution. A third, minor, route is dermal contact, being significant only in very specific circumstances (Tchounwou et al. 2012). Once in contact with the gastrointestinal lining, the absorption of the three metals is residual if they are presented in their inorganic forms (below 3% to a maximum of 20%). However, the most common form of organic mercury (methylmercury – MeHg) can be assimilated on upward of 90%. These variations depend not only on the speciation state of the metal, which influences its bioavailability, but also on individual characteristics and physiological parameters of the exposed organism, such as fasting status, presence of competing elements, sex, and age (Keil et al. 2011).

Of these metals, only Hg is object to biomagnification (the presence of increased amounts of a contaminant in the organisms belonging to the highest levels of a trophic chain), being a good example the high concentrations attained by predatory fish, the main source of exposure of piscivorous mammals (Wolfe and Norman 1998; Clarkson and Magos 2006). The other two metals, despite not being object of biomagnification, are (together with mercury) bioaccumulated in the tissues of vertebrates, being the brain the most vulnerable to Hg and Pb, since they can penetrate the brain–blood barrier (as well as the placental barrier, causing teratogenic effects in the developing fetus) (Clarkson and Magos 2006; Caserta et al. 2013).

The effects of Hg in the brain include visual, cognitive, and neurobehavioral deficits, linked to structural degeneration of the organ, whereas Pb causes its weight reduction; lack of coordination; impaired motor skills, visual discrimination, and learning; convulsions; abnormal social behavior; and increased tendency for aggression (Tchounwou et al. 2012). Despite being mostly recognized as a carcinogenic, both in humans and animals, cadmium can also cause olfactory dysfunction, slowing of vasomotor functioning, learning disabilities, and behavioral disturbances due to its effects upon the nervous system (Minami et al. 2001).

The existence of solid evidence of the negative effects of mercury, lead, and cadmium on the human brain (leading to neurologic dysfunction), in parallel with the growing amounts of metals in circulation in the environment creating added opportunities for exposure, compel us to multiply the amount of studies reporting the levels of these metals in humans' brain tissue establishing correlations with the prevalence and incidence of neurodegenerative diseases. The obstacle here is quite evident: the difficulty in obtaining brain tissue samples, particularly in the numbers necessary to generate robust epidemiological studies. Based on the information provided so far, one could argue that if pets share the same habitat (being subjected to the same type of metal exposure), suffer the same type of damage to their nervous system, and can act as early warning systems, then pets' brains should be harvested in order to achieve that objective. Putting aside the doubtful willingness of owners to relinquish part of their pet's central nervous system, ethical constrains apply to that endeavor. As such, these obstacles need to be circumvented by using easily obtainable (i.e., noninvasive) but representative surrogate pet samples.

5.3 Hair as a Noninvasive Indicator

Blood, urine, liver, and kidney samples have normally been used for assessing levels of metals in the human body. In a much smaller measure, hair has also been used for this purpose (Matsubara and Machida 1985; Nowak and Chmielnicka 2000; Mikulewicz et al. 2013; Pozebon et al. 2017). Usually cited advantages of using hair as an indicator of metal contamination are the ease of sampling and storage (Wołowiec et al. 2013) and the fact that hair, being a concentrator tissue, can contain higher concentrations of metals when compared with blood and urine (Mikulewicz et al. 2013; Wołowiec et al. 2013).

Similarly to humans, levels of metal accumulation in animals can be determined by analyzing samples that include blood, vital organs (e.g., kidneys or liver), bones, and hair. Animal hair can potentially be a better biomonitoring tool for metal assessment (Hayashi et al. 1981; Doi et al. 1986; Sakai et al. 1995; Dwivedi et al. 2001; Dunlap et al. 2007; Vázquez et al. 2013) due to the more complex patterns of exposure to contaminated items. No permanent damage is caused to the animal during and after sampling, and it can be used as a surrogate method for determining the bioavailability of metals, can reflect long-term accumulation and concentration, and can serve as an indicator of exposure (Merian 1991; Rashed and Soltan 2005). As a consequence of growing usage in environmental, ecological, hygienic, and clinical

Table 5.1 Sources of error in hair analysis

Step	Sources of error
Sampling and storage	No unambiguous identification of the individual Insufficient sample amount and order of hair tuft Inadequate labelling, causing mix-ups with other samples Danger of contamination and degradation
Decontamination	Choice of wrong solvent or solvent sequence No analysis of the wash solution
Extraction	Inappropriate choice of extraction or digestion method Incorrect time and temperature of extraction Decomposition of the compounds High levels of impurities
Analysis	Insufficient specificity, sensitivity, and accuracy Loss of substance in clean-up False-positive or false-negative results

Adapted from Schramm (2008)

studies since the 1980s, dog hair has become one of the most reliable bioindicators of metal concentrations while, on a reverse trend, cat's hair being sparsely used.

5.3.1 Limitations of Hair Analysis

The use of hair to report contamination exposure and risk still has some detractors, not being universally accepted. Early criticism emerged from the validity of reference ranges depending on the analytical methods used, as well as sampling, sensitivity, accuracy, and precision (Rodushkin and Axelsson 2000; Druyan et al. 1998). Additionally, various sources of error could occur during the various steps of hair analysis (Schramm 1997). However, many of these criticisms have been voided by the evolution of techniques and equipment, the establishment of standard operating procedures, and the production of certified reference materials (Yoshinaga et al. 1997). Still, there is room for error during collection, processing, and analysis of the samples. Table 5.1 summarizes potential sources of error.

5.4 Levels of Metals with Neurodegenerative Potential in Pets' Hair

There are a fairly limited number of studies using cat and dog hair as a matrix of metals bioaccumulation and logically an even lower reporting the levels of metals with neurodegenerative potential. Among these there are great variation on the type of information provided, the locations of the studies, the number of animals involved (cats, min 15- max 44; dogs, min 8- max 204), the presence/absence of discrimination in terms of age or sex being present, and the number of metals analyzed (rarely all three metals – Cd, Pb, and Hg – are reported on the same study).

Other aspect making this description very hard is the unevenness in the way data is presented. Frequently, values in tables are solely presented as an average without standard deviation or standard error, or results are presented plainly on graphs from which values have to be extrapolated. This last fact excluded several studies from this chapter, as it was virtually impossible to read the data with a minimum of desirable accuracy.

Without the intention of being exhaustive (due to the circumstances described above, and because this chapter is not intended as a systematic review), in the following subchapters, we will present studies that determined the concentrations of Cd, Pb, and Hg in this matrix, by pet. The original intention was to solely describe the levels of the metals in hair of healthy dogs (used as reference or specifically selected to act as control) so as to provide the reader with a set of background levels for metals with neurodegenerative potential. However, the sum of all cited constraints together with this intention dramatically reduced the amount of information. This led to the inclusion, when available, of levels in the hair of cats and dogs with specific illnesses in order to complement the information on healthy animals. Hair mercury level is often not correlated with blood mercury concentration or symptoms of mercury toxicity, and reports of hair contamination by exogenous mercury are not uncommon (Nuttall 2006).

The establishment of baseline levels on the hair of healthy animals is a difficult endeavor, due to the lack of information. Additionally, the baseline itself will vary with food (commercial, wet or dry, homemade), breed, sex, age, rearing (outdoors, indoors, a mix of both), and environmental variables (e.g., temperature, which will influence metabolic rates). Normal reported levels (NRL) determined by the Committee on Minerals and Toxic Substances in Diets and Water for Animals from the US Board on Agriculture and Natural Resources will be provided when available. As additional information, ballpark non-peer-reviewed estimates can be mentioned and can indicate population averages at 0.041 ppm for cadmium, 1.3 ppm for lead, and 0.27 ppm for mercury. These values are reproduced here strictly for reference.

5.4.1 Cats

The number of studies using cat's fur in order to describe the contents in terms of neurodegenerative metals is very restricted. After going through the available literature, five studies can be reported and are summarized in Table 5.2.

These are studies that in some cases additionally reported levels of other metals and/or other species. However, since those are not the object of this chapter, they will not be described here.

Badea et al. (2016) aimed to determine the levels of Cd and Pb in the coat of 15 cats (six clinically healthy and nine suffering from renal failure) in Romania. Rzymski et al. (2015) investigated the contents of Cd and Pb in hair of 18 free-ranging and 36 household (14 outgoing and 22 not outgoing) cats from Poland. Concentration values also had to be extrapolated from graphs in this study.

Table 5.2 List of studies reporting levels of metals with neurodegenerative potential in cats' hair

Ref.	1st author	Year	Profile	Location	Metal	*N*
1	Badea	2016	Pet cats	Romania	Cd, Pb	15
2	Doi	1986	Pet cats	Japan, Philippines, Norway	Hg	nd
3	Rzymski	2015	Free and pet cats	Poland	Cd, Pb	44
4	Sakai	1995	Pet cats	Japan	Hg	41
5	Skibniewski	2013	Pet cats	Poland	Pb	20

nd denotes "Not detected"

Skibniewski et al. (2013) determined the lead content in 10 domestic and 10 urban feral (stray) cats from the Warsaw region. All animals were mature and disease-free cats. Doi et al. (1986) collected fur from domestic cats in Tokyo, Norway, and the Philippines, determining the concentrations of mercury. The results were presented solely as graphs, so we had to visually extract data here presented directly from those reproduced in the paper. The number of animals tested was not indicated. Hair mercury concentrations were also measured by Sakai et al. (1995) in 41 cats from the Kanagawa, Saitama, and Tokyo prefectures (Japan).

The concentrations obtained in each paper are presented by metal in the following sections. The concentration values are reported as averages (due to the inconsistent reporting) in parts per million (ppm), due to the wide variation of units used by the different authors.

5.4.1.1 Cadmium

Two papers reported cadmium values in cats' coats: Badea et al. (2016) and Rzymski et al. (2015). Clinically healthy cats (in particular males which present levels over eight times higher than females) in the study performed by Badea et al. (2016) registered higher cadmium levels when compared with those suffering from renal failure, which were comparatively the same regardless of age. The authors argue that this is because cats suffering renal failure have a reabsorption inability, which will impoverish the hair matrix in terms of metals. The results obtained by Rzymski et al. (2015) show a clear influence from the inhabited environment, with free-ranging animals registering lower cadmium levels as compared to animals totally or partially living indoors. This data illustrates the already mentioned difficulty in establishing a baseline for the values of metals in hair. Even more "traditional" matrices like blood or urine values for small animals are poorly defined, with an indication that blood levels ≥100 μg/dL reflect acute exposure, whereas the presence of cadmium in urine indicates chronic exposure (Osweiler et al. 2011) (Fig. 5.3).

5.4.1.2 Lead

Lead toxicity is not well defined in cats with toxicity set at 1000 ppm in diet or 3 mg/kg (Osweiler et al. 2011). Three papers reported lead values in cats' coats: Badea et al. (2016), Rzymski et al. (2015), and Skibniewski et al. (2013). The results

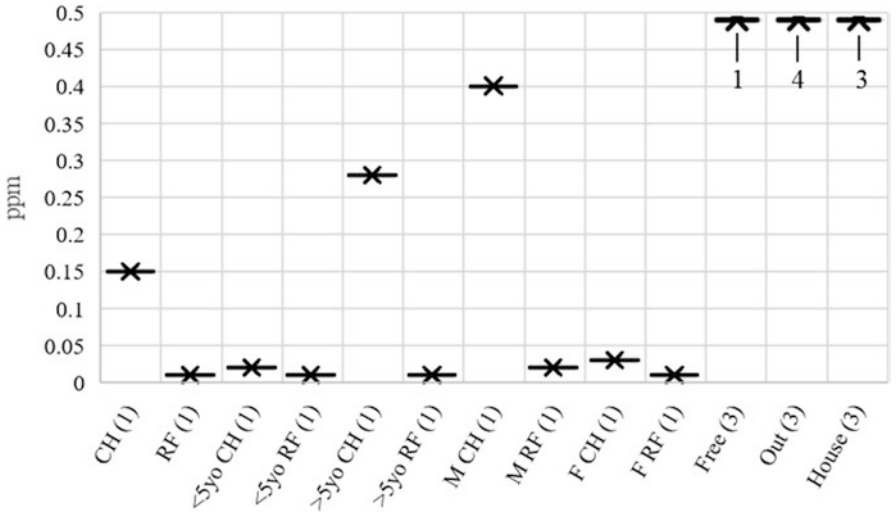

Fig. 5.3 Average cadmium concentrations in cats' hair as reported by (1) Badea et al. (2016) and (3) Rzymski et al. (2015). CH clinically healthy, RF renal failure, <5yo below 5 years of age, >5yo above 5 years of age, M male, F female, Free free-ranging, Out household outgoing, House household not outgoing. (Values from reference 3 were visually extracted from graphs reproduced in the paper)

obtained for cadmium by Badea et al. (2016), in which males exhibited much higher levels than females, are not replicated for lead, despite clinically healthy males registering slightly higher levels of lead among all sampled animals (Badea et al. 2016). In the study by Rzymski et al. (2015), house cats, once again, registered the highest levels of metal (lead in this case), but, and contrarily to what was noticed for cadmium, the lowest levels were registered in outgoing pet cats. In the study by Skibniewski et al. (2013), feral females registered the highest concentrations of lead for the Warsaw area, with household cats registering, on average, a concentration three times lower (Fig. 5.4).

5.4.1.3 Mercury

Cats are recognized as highly susceptible to mercury. Blood concentrations of >6.0 ppm and urine >1.5 ppm illustrate acute to subacute exposure. Hair concentrations >45 ppm are proof of chronic exposure (Osweiler et al. 2011).

Two papers reported mercury values in cats' coats: Doi et al. (1986) and Sakai et al. (1995). Japanese cats inhabiting Tokyo in the 1980s have, according to Doi et al. (1986), the highest mercury fur contents, followed by Norwegian and Filipino cats. Sakai et al. (1995) resampled Tokyo cats nearly a decade later finding concentrations a full order of magnitude lower and no differences between males and females (Fig. 5.5).

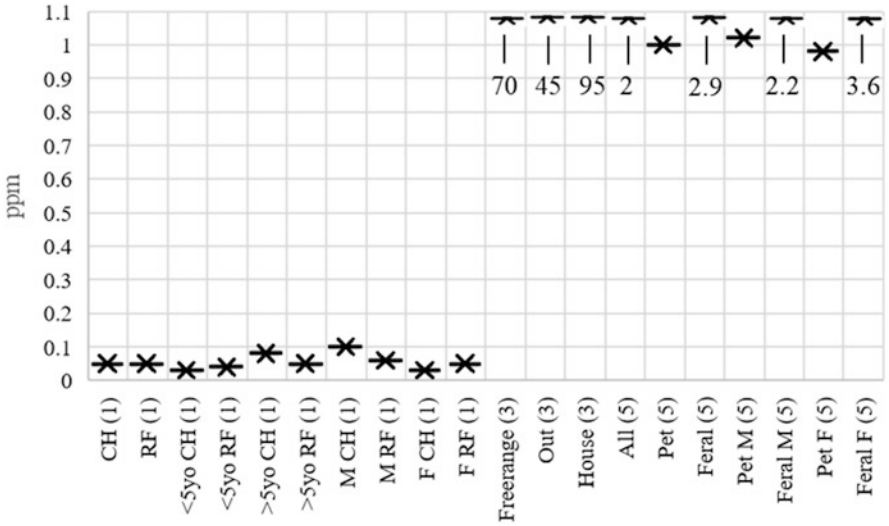

Fig. 5.4 Average lead concentrations in cats' hair as reported by (1) Badea et al. (2016), (3) Rzymski et al. (2015), and (5) Skibniewski et al. (2013). CH clinically healthy, R renal failure, <5yo below 5 years of age, >5yo above 5 years of age, M male, F female, Free-ranging stray cats, Out household out-going, House household not outgoing, All household + stray, Pet household, Feral stray. (Values from reference 3 were visually extracted from graphs reproduced in the paper)

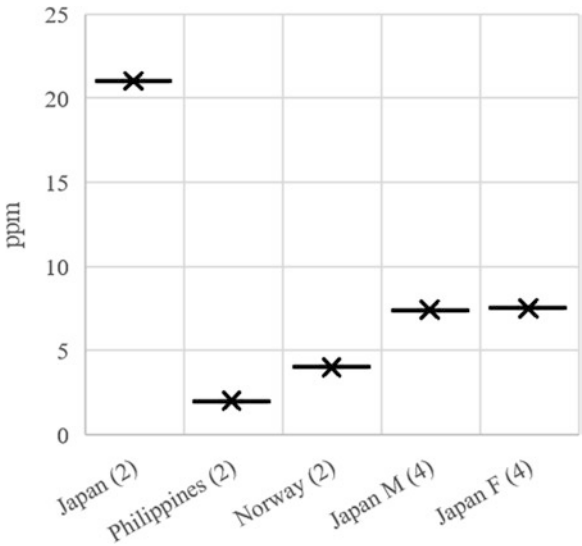

Fig. 5.5 Average mercury concentrations in cats' hair as reported by (2) Doi et al. (1986) (cats from Japan, the Philippines, and Norway) and (4) Sakai et al. (1995) (cats from Japan). M male, F female. (Values from reference 2 were visually extracted from graphs reproduced in the paper)

5.4.2 Dogs

The number of studies analyzing dog's fur for neurodegenerative metals is larger than that for cats, but still very limited. The same constraints applied, and after going through the available literature, 12 studies are here reported and summarized in Table 5.3.

Atanaskova et al. (2011) analyzed the content of Cd and Pb in 35 companion dogs' hair from three cities in the Republic of Macedonia. Badea et al. (2018) conducted his study solely in female dogs from Romania. All animals were older than 5 years old, and 15 suffered from mammary neoplasms, whereas 15 were used as a control group. Both groups included animals living indoor and outdoor. The results were presented in the form of graphs, so the results here presented were visually extracted. Kozak et al. (2002) evaluated the content of cadmium and lead mostly in indoor companion dogs' hair from Bratislava (32 individuals) and Kosice (66 individuals). Dogs varied their age between 1–11 and 1–13 years old (respectively), with 4–6 years old constituting the largest category. Zaccaroni et al. (2014) aimed at assessing the levels of Cd, Pb, and Hg in dog hair from three different areas of Campania (Italy) with different profiles of contamination. Thirty healthy dogs from each area (where they had been living since pups) had their hair sampled during normal health control examinations, the ages ranging from 2 to 15 years, with the dogs on the category 5–7 years being the most numerous. In order to quantify the contents of Cd, Pb, and Hg in dog hair from domestic districts and to assess effects of sex and living area, Park et al. (2005a) collected 204 samples from apparently healthy dogs with no history of occupational exposure from different localities of

Table 5.3 List of studies reporting levels of metals with neurodegenerative potential in dogs' hair

Ref.	1st author	Year	Profile	Location	Metal	*N*
1	Atanaskova	2011	Urban dogs	Rep. Macedonia	Cd, Pb	35
2	Badea	2018	Pet dogs (female)	Romania	Cd, Pb	30
3	Doi	1986	Stray dogs	Japan, Norway, Philippines	Hg	nd
4	Dunlap	2007	Sled dogs	USA (Alaska)	Hg	97
5	Hansen	1995	Sled dogs	Greenland	Hg	10
6	Kozak	2002	Pet dogs	Slovakia	Cd, Pb	98
7	Kral	2015	Pet dogs	Czech Republic	Hg	131
8	Lieske	2011	Sled dogs	USA (Alaska)	Hg	8
9	Nikolovski	2011	Pet dog	Rep. Macedonia	Cd, Pb	95
10	Park	2005	Pet dog	Korea	Cd, Pb, Hg	204
11	Sakai	1995	Pet dogs	Japan	Hg	75
12	Sousa	2013	Pet dogs	Portugal	Hg	27
13	Zaccaroni	2014	Pet dogs	Italy	Cd, Pb, Hg	90

Korea. Nikolovski and Atanaskova (2011) aimed to compare cadmium and lead exposure in different areas of the Republic of Macedonia, using dog's hair while taking into consideration the influence of age. For this purpose, 38 samples of dog hair were collected in low population cities (<20,000) and 57 in higher populated cities (<60,000). The age of the dogs varied between 1 and 10 years, with the largest group being that including dogs between 1 and 2 years old.

Generally, mercury levels in dogs' hair are very rarely reported. That is why the results reported by Doi et al. (1986), despite being determined in the hair of stray dogs (collected in Asahikawa city, Japan), were included in this report. These results were available only as graphs, so we had to visually extract data here presented. Dunlap et al. (2007) reported the contents of mercury in hair from 97 sled dogs fed commercial food and traditional village diets. Thirty-six individuals were fed commercial food (16 from New York and 20 from Salcha, Alaska), whereas 12 from Russian Mission, 12 from Galena, 12 from Rampart, 12 from Fort Yukon, and 12 from Salcha (all in Alaska) were fed a traditional diet. Mercury in sled dogs was also the object of study for Hansen and Danscher (1995). They reported results of hair analysis from 10 individuals (with ages between 6 weeks and >10 years) from the Thule District in Greenland. The work of Kral et al. (2015) was focused on the assessment of mercury contamination of dogs through the analysis of hair. A total of 131 animals were analyzed with 42 being fed granulated feed containing fish and 89 fed fish-free granulated food. Once again, results were presented as graphs so that data here presented had to be visually extracted. The major objective of the study conducted by Lieske et al. (2011) was to characterize changes in total Hg concentrations in hair of sled dogs over time due to long-term piscivory. For that purpose, four dogs were fed a fish diet and four a fish-free diet for twelve weeks. In order to evaluate the effects of environmental contamination, Sakai et al. (1995) analyzed the mercury concentrations in hair of 34 clinically healthy dogs (16 males and 18 females) living in the Kanagawa, Saitama, and Tokyo prefectures. More recently, Sousa et al. (2013) quantified the levels of mercury in the hair of 26 pet dogs from the northern area of Portugal, and the authors concluded that the mercury concentrations were independent of gender, age, and diet types.

The concentrations determined and reported in each paper are presented by metal in the following sections. Concentrations are reported as averages in parts per million (ppm), due to the wide variation of units used and the inconsistent statistical reporting.

5.4.2.1 Cadmium

Six papers reported cadmium values in dogs' hair: Atanaskova et al. (2011), Badea et al. (2016), Kozak et al. (2002), Nikolovski and Atanaskova (2011), Park et al. (2005a), and Zaccaroni et al. (2014).

Values reported for cadmium were generally low for all the studies. As with cats, dogs with renal problems registered lower levels than control animals, but only those living outdoors (Badea et al. 2016). A group of studies centered in central

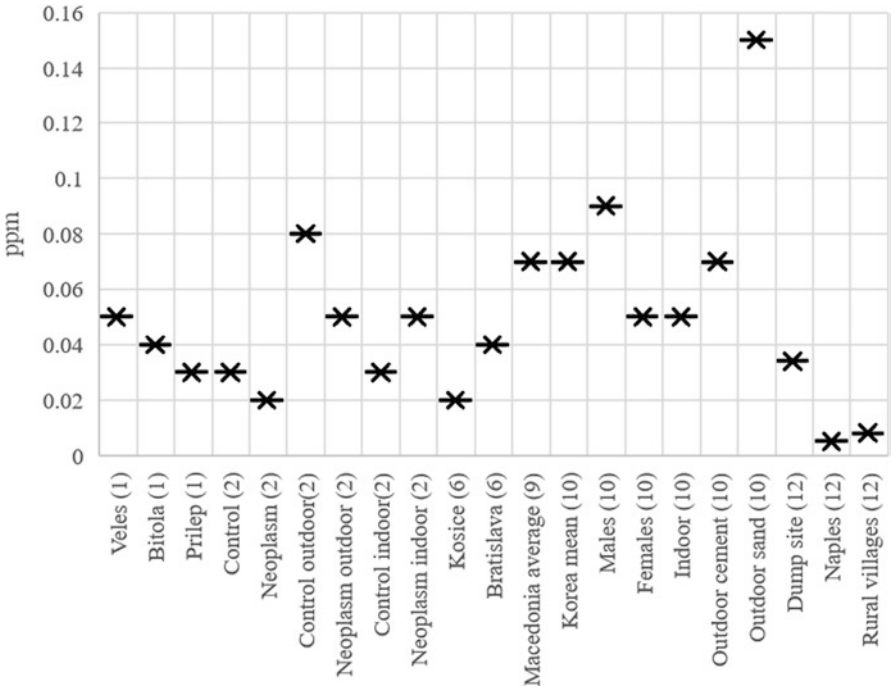

Fig. 5.6 Average cadmium concentrations in dogs' hair as reported by (1) Atanaskova et al. (2011), (2) Badea et al. (2016), (6) Kozak et al. (2002), (9) Nikolovski and Atanaskova (2011), (10) Park et al. (2005a), and (12) Zaccaroni et al. (2014). Veles, Bitola, Prilep: Rep. of Macedonia cities; Neoplasm: female dogs suffering from mammary neoplasm; Outdoor: females raised outdoor; Indoor: females raised indoor; Kosice, Bratislava: Slovakian cities; Outdoor cement: dogs raised outdoor on a cement floor; Sand: dogs raised outdoor on a sand floor; Dump site ("the death triangle"); Naples and rural villages: locations in the Campania Region of Italy. (Values from reference 2 were visually extracted from graphs reproduced in the paper)

Europe (Macedonia, Romania, Slovakia) present consistent results (Atanaskova et al. 2011; Badea et al. 2018; Kozak et al. 2002). The other study performed on Macedonia dogs (Nikolovski and Atanaskova 2011) presents higher concentrations, which are similar to those registered in Korea (Park et al. 2005a) where the highest levels were registered for the hair of dogs living outdoor in a sandy substrate. Italy, with the exception of animals living near a dumping site, registered the lowest concentrations (Zaccaroni et al. 2014).

Dogs tolerate 10 ppm in diet; chronic toxicity occurs at 50 mg/kg (Neiger and Osweiler 1992). Normal reported levels (NRL) are comprised between 0.1 and 0.9 μg/g (Klasing et al. 2005) (Fig. 5.6).

5.4.2.2 Lead

Six papers reported lead values in dogs' hair: Atanaskova et al. (2011), Badea et al. (2016), Kozak et al. (2002), Nikolovski and Atanaskova (2011), Park et al. (2005a), and Zaccaroni et al. (2014) (Fig. 5.7).

Overall levels of lead were lower when compared to those reported for cats. The group of studies centered in central Europe (Macedonia, Romania, Slovakia) again present consistent results, but this time the second study performed on Macedonia and that in Korea in agreement. However, the value for indoor control dogs in Romania patented higher than average levels (Atanaskova et al. 2011; Badea et al. 2018; Kozak et al. 2002; Nikolovski and Atanaskova 2011; Park et al. 2005a). In the last study, a little nuance is discernible when compared to cadmium results: The animals registering the highest levels are those living indoors. Italy (again with the exception of animals living near a dumping site) once more registered the lowest concentrations (Zaccaroni et al. 2014).

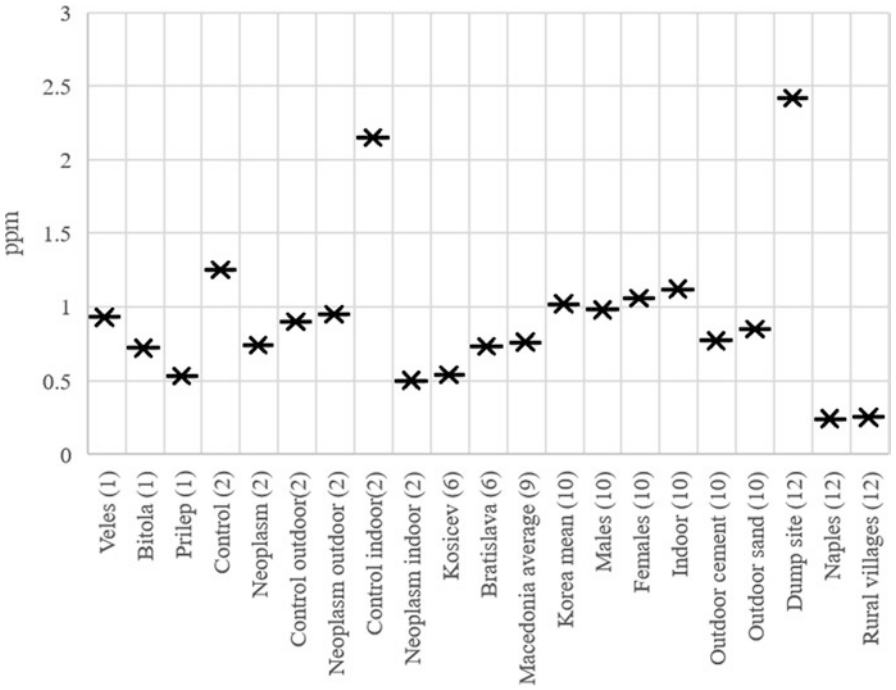

Fig. 5.7 Average lead concentrations in dogs' hair as reported by (1) Atanaskova et al. (2011), (2) Badea et al. (2016), (6) Kozak et al. (2002), (9) Nikolovski and Atanaskova (2011), (10) Park et al. (2005a), and (12) Zaccaroni et al. (2014). Veles, Bitola, Prilep: Rep. of Macedonia cities; Neoplasm: female dogs suffering from mammary neoplasm; Outdoor: females raised outdoor; Indoor: females raised indoor; Kosice, Bratislava: Slovakian cities; Outdoor cement: dogs raised outdoor on a cement floor; Sand: dogs raised outdoor on a sand floor; Dump site ("the death triangle"); Naples and rural villages: locations in the Campania Region of Italy. (Values from reference 2 were visually extracted from graphs reproduced in the paper)

For lead, the acute toxic dose for dogs is approximately 190–1000 mg/kg (dependent on lead form), whereas the chronic cumulative toxic dose is 1.8–2.6 mg/kg/day (Osweiler et al. 2011). NRLs are comprised between 0 and −88 μg/g (Klasing et al. 2005).

5.4.2.3 Mercury

Nine papers reported mercury values in dogs' hair: Doi et al. (1986), Dunlap et al. (2007), Hansen and Danscher (1995), Kral et al. (2015), Lieske et al. (2011), Park et al. (2005a), Sakai et al. (1995), Sousa et al. (2013), and Zaccaroni et al. (2014).

There is a wide variation in both inter- and intra-studies regarding mercury in dogs' hair. Observations on sled dogs returned very high fur concentrations (Dunlap et al. 2007), particularly when compared to dogs used as control (by being fed with commercial fodder), and if they were between 1 and 5 years old (Hansen and Danscher 1995). However, sled dogs undergoing a fish diet did not accumulate as much mercury as would be expected (Lieske et al. 2011) being on average, even lower than the study performed in Korean dogs (Park et al. 2005a). In this study and similarly to lead, the animals registering the highest levels are those living indoors.

In Portugal, levels of mercury in dog's hair found by Sousa et al. (2013) varied widely (0.024–0.826 ppm). However, the average levels were overall low, and no significant differences were obtained between average levels for females and males, being the only study to report such results (all the other had clear differences between males and females). Similarly, no differences between the types of diet (commercial or homemade food) could be found. Also, no differences between dogs reared outdoors and indoors (or a mixture of both) were found. The authors suggested that such results could be due to the small sample size. Contrary to the previous metals, the study performed by Zaccaroni et al. (2014) demonstrated that dogs sampled in Naples are the most contaminated among all studies (closely accompanied by those living near the dumping site), whereas the third location used in the study was on level with the one performed in Korea (Fig. 5.8).

5.5 Considerations on the Use of Pets as Sentinels for Neurotoxic Metals

All kinds of animals have been put under consideration for becoming sentinels of human exposure to toxic substances (Reif 2011). The majority of these studies have considered synthetic chemicals, the common conclusion being that, according to each specific contaminant, some species are more useful than others, based on their comparative metabolic capabilities toward man (e.g., D'Havé et al. (2005), Ruiz-Suárez et al. (2016), González-Gómez et al. (2018)).

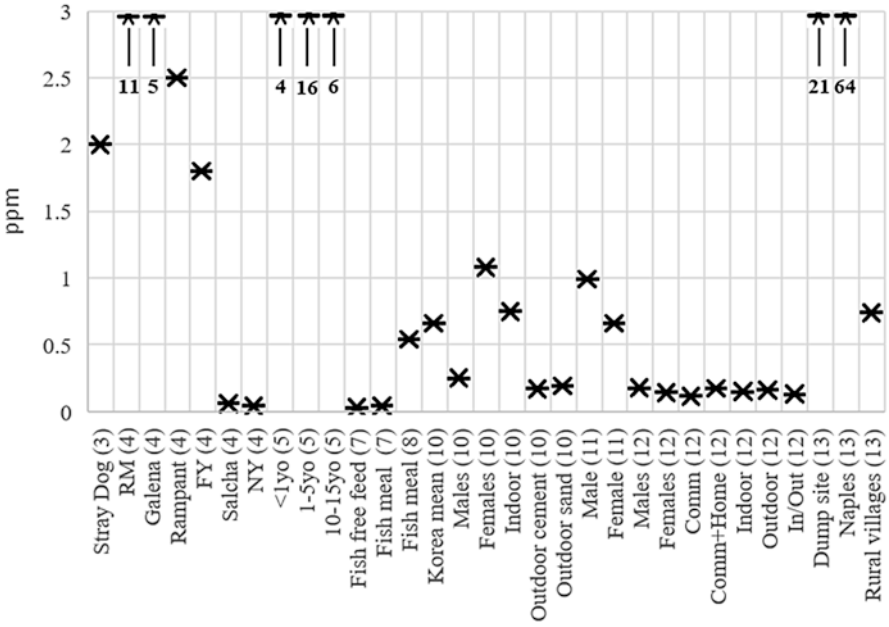

Fig. 5.8 Average mercury concentrations in dogs' hair as reported by (3) Doi et al. (1986), (4) Dunlap et al. (2007), (5) Hansen and Danscher (1995), (7) Kral et al. (2015), (8) Lieske et al. (2011), (10) Park et al. (2005a), (11) Sakai et al. (1995), (12) Sousa et al. (2013), and (13) Zaccaroni et al. (2014). RM (Russian Mission), Galena, Rampant, FY (Fort Yukon), Salcha, NY (New York): Sites of sampling; <1yo: below 1 year of age; 1–5yo: between 1 and 5 years old; 10–15yo: between 10 and 15 years old; Indoor: dogs raised indoors; Outdoor cement: dogs raised outdoor on a cement floor; Sand: dogs raised outdoor on a sand floor; Dump site ("the death triangle"); Naples, and rural villages: locations in the Campania Region of Italy. (Values from reference 2, 3, and 7 were visually extracted from graphs reproduced in the paper)

Metals are probably the oldest known toxins, and the evolution of living entities occurred in the omnipresence of metals. Maybe because of this evidence, they have not been object to the same level of scientific interest. As far as we can determine, only Patrashkov et al. (2003) simultaneously analyzed a non-disclosed number of human, cat, and dog samples for metals with neurodegenerative potential (Cd and Pb) in hair in farms. Results were quite similar for Pb in the three species (humans, 1.93 ± 0.28; cat, 2.42 ± 0.51; and dog, 1.08 ± 0.41), but not for cadmium in dogs, which showed average concentrations lower than the other two (0.06 ± 0.06 mg/kg, versus 0.48 ± 0.22 mg/kg for cat and 0.41 ± 0.07 mg/kg for humans). Despite this, it is evident (at least for this study) that humans exposed to the same exposure environment as pets will end up with very approximate concentrations of metals with neurodegenerative potential in their hair. But this was a single study. Variations between species metal content in hair exist mostly due to differences in metal metabolism. Atop of this, intraspecific differences also occur and can derive from a set of factors such as age, sex, rearing, and type of food consumed, physiological

condition, and habitat. In general, the overlapping of all factors contributes to variation in the concentrations within and between species. Further investigations will be necessary to establish robust baselines describing the distribution of these metals among species living in the same environment. There is an imperative need to verify if the strong, positive relationships between metal blood level concentrations in animals and their owners (especially pre-school children) are sustained for hair levels. If they are, the costly and stressful processes of population testing can be immensely simplified. The surrogate testing of cats and dogs instead of their owners can immediately indicate the need for further testing. If none of the animals tested in the household has hair levels that are above identified thresholds for each metal, it will be highly unlikely that the human inhabitants will, as pets integrate them in hair at higher levels than their owners. In either case, the observation by veterinarians of the emergence of neurological symptoms will always pay off, since pets, due to shorter latency periods (as they have shorter average lifespans), can act as early warning systems for human neurodegenerative processes. Fortunately, this trait is more important for chronic rather than acute toxic exposures, which is one of the etiological bases of the dementia disease spectrum.

5.6 Conclusions

The number of studies conducted in pet cats and dogs for the identification and quantification of metals with neurodegenerative potential is very scarce, particularly for cats. The information presented by these studies report (sometimes wide) discrepancies between metals and the influence of sex, age, diet and rearing of the animal. The geographic location of sampling (within and between countries) also clearly influences the concentrations of these metals in pets' hair (but always with significant correlations between this matrix and environmental metal concentrations). Since the number of individuals sampled for each study is generally low (mostly in the tens digit), the results should be carefully interpreted. However, the strong correlation shown in epidemiological and clinical studies between aberrant metal exposure and a number of neurological diseases and, simultaneously, the knowledge that canine and feline brains are subjected to the same neurodegenerative processes as those observed in humans, in a quicker time frame (due to their shorter latency periods), proves that pets should growingly be used as sentinels of exposure to neurodegenerative metals in humans. The archetypal concept of the *canary in the coal mine* re-claims a new life in the twenty-first century.

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Chapter 6
Biotransformation of Brominated Compounds by Pet Dogs and Cats

Hazuki Mizukawa and Kei Nomiyama

Abstract There are growing concerns about health risks in pet animals due to the exposure to brominated compounds. This chapter describes the available information on tissue-specific accumulation and biotransformation of PBDEs and their hydroxylated and methoxylated derivatives (OH-PBDEs and MeO-PBDEs) in pet dogs and cats. Cats tend to exhibit higher tissue and blood concentrations of PBDEs. Furthermore, brominated compounds are also found at relatively high concentrations in cat brains, suggesting that they can cross through the blood–brain barrier. Thus, cats might be at a high risk from PBDEs and their derivatives. In dogs, BDE47 is the dominant congener in the bile, which suggests a species-specific excretory capacity of the liver. Regarding PBDEs metabolites, the major congeners of OH-/MeO-PBDEs identified in both pet food products and blood were natural products (6OH-/MeO-BDE47 and 2′OH-/MeO-BDE68) from marine organisms. The profiles and tissue distribution of PBDEs and metabolites are described for both species, and possible explanations for the differences observed between these pets are put forward.

Keywords Dogs · Cats · Biotransformation · Brominated compounds

6.1 Introduction

Polybrominated diphenyl ethers (PBDEs) are ubiquitous environmental contaminants used as synthetic flame retardants. Because of their persistence and bioaccumulation potential, these contaminants are widely distributed in the environment and accumulate in both aquatic and terrestrial food webs (Alaee et al. 2003; Law et al. 2006; Letcher et al. 2010).

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PBDEs are metabolized to hydroxylated PBDEs (OH-PBDEs) by cytochrome P450 monooxygenases (CYPs) in the liver. OH-PBDEs can also be formed by the demethylation of the methoxylated PBDEs (MeO-PBDEs) which occur naturally in marine organisms (Wan et al. 2010). The detection of OH-PBDEs in the plasma of wild animals (Verreault et al. 2005; Houde et al. 2006; Nomiyama et al. 2011a; Weijs et al. 2014) and human blood (Qiu et al. 2007, 2009; Haraguchi et al. 2016) suggests that the biotransformation of PBDEs occurs in the livers of various animals (Hamers et al. 2008; Qiu et al. 2009; Stapleton et al. 2009). Compared with marine mammals, terrestrial carnivore species can have a higher metabolic capacity for organohalogen compounds such as PBDEs and polychlorinated biphenyls (PCBs) (Kunisue and Tanabe 2009; Mizukawa et al. 2013). In fact, the levels of hydroxylated PCBs (OH-PCBs) were found to be higher than parent PCBs in the blood of carnivorous species (Kunisue and Tanabe 2009; Mizukawa et al. 2013). For example, PBDE concentrations in red foxes from Belgium were lower than those of voles and mice, which are the main prey species of the red fox (Voorspoels et al. 2006). Furthermore, it was also reported that drug-metabolizing enzymes are induced depending on the hepatic levels of contaminants, which metabolizes PCBs and PBDEs in raccoon dogs (Kunisue et al. 2008). These studies on carnivorous species suggest that the toxicological risk of hydroxylated metabolites in the blood may vary among carnivorous species, and some may be at a higher risk from these metabolites.

Domestic pets such as dogs (*Canis lupus familiaris*) and cats (*Felis catus*) share living environments with humans. Therefore, they are exposed to various contaminants, including PBDEs and brominated phenols (BPhs), in their immediate surroundings, which raise concerns about possible health risks (Venier and Hites 2011; Norrgran et al. 2012, 2015). Recent studies have reported elevated PBDE levels in the sera of cats (Dye et al. 2007; Kupryianchyk et al. 2009; Guo et al. 2012; Chow et al. 2015; Henríquez-Hernández et al. 2017). Moreover, evidence suggests that the main routes of PBDE exposure in domestic cats are dietary intake and the ingestion of contaminated house dust (Guo et al. 2012; Chow et al. 2015; Mizukawa et al. 2016). Notably, compared with euthyroid cats, hyperthyroid cats have higher serum concentrations of some PBDE congeners (BDE99, BDE153, and BDE183), which suggests that feline hyperthyroidism (FH) might be associated with increased exposure to PBDEs (Chow et al. 2015; Norrgran et al. 2015). The number of cats diagnosed with FH has increased significantly during the last three decades (Peterson 2012), and studies have suggested that the pathogenesis of FH involves exposure to goitrogens, including PBDEs and phenolic metabolites such as hydroxylated PBDEs (OH-PBDEs) (Mizukawa et al. 2013, 2016).

Structurally, OH-PBDEs resemble the thyroid hormone (TH) thyroxin and can bind to TH transport proteins (e.g., transthyretin, TTR; thyroxine-binding globulin, TBG), disrupting homeostasis (Hamers et al. 2008; Li et al. 2010; Ucán-Marin et al. 2009, 2010) (Fig. 6.1). OH-PBDEs reportedly interrupt oxidative phosphorylation (van Boxtel et al. 2008) and elicit neurotoxicity (Hendriks et al. 2010; Ibhazehiebo et al. 2011). These studies suggest that the brain and liver are useful organs for understanding the toxicokinetics of OH-PBDEs.

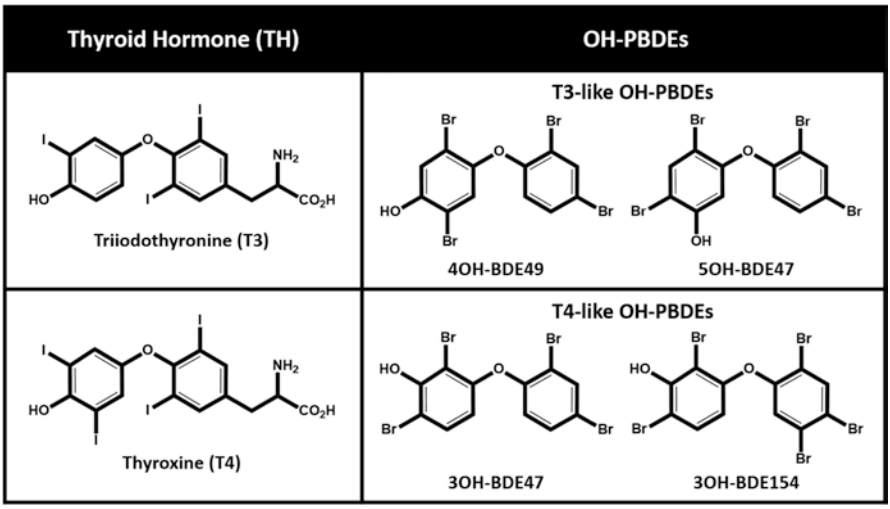

Fig. 6.1 Structures of thyroid hormone (TH) and OH-PBDEs. OH-PBDEs have some structural resemblance to the TH which hydroxyl group is adjacent with halogen atoms

Considering that the complex action of PBDEs and OH-PBDEs may be responsible for the increased incidence of FH, further intensive studies are required to assess the toxicokinetics of not only these parent compounds but also their derivatives in domestic animals. Based in our previous studies, this chapter describes the species-specific congener patterns of PBDEs and their derivatives (OH-PBDEs, MeO-PBDEs, and bromophenols) in the blood of cats and dogs and evaluates the differences in the accumulation pattern and metabolic capacities of PBDEs in cats and dogs (Mizukawa et al. 2013, 2016, 2017). Also, we further describe the tissue-specific congener patterns of PBDEs and their derivatives (OH-PBDEs and MeO-PBDEs) by summarizing the levels the livers, blood, bile, and brains of Japanese domestic dogs and cats reported in our studies (Nomiyama et al. 2017).

6.2 Accumulation Features of PBDEs in the Blood of Terrestrial Mammals

In 2013, when we determined the residue levels and patterns of PBDEs in the blood of various terrestrial mammals (cats, raccoon dogs, dogs, masked palm civets, foxes, raccoons, badgers, and mongooses) in Japan, the levels of PBDE in the blood of cats were higher than those of other carnivorous species (Mizukawa et al. 2013). Concerning pet cats and dogs, no significant differences were found in the PBDE levels in the blood of these Japanese pets (Mizukawa et al. 2016). However, differences between PBDEs levels in these species from Pakistan were reported. Ali et al. (2013) described that the levels of PBDEs were significantly higher ($p < 0.05$) in cat

serum compared to dog serum in Pakistan. Conversely, the residual levels of PBDEs in Japanese pet/stray cat blood were 1–3 orders of magnitude lower than those reported for the serum of pet cats in the USA (Dye et al. 2007; Guo et al. 2012; Mizukawa et al. 2013, 2016). In addition, the concentration of PBDEs in the blood of dogs from Japan was 1/8 of that of American pet dogs (Venier and Hites 2011; Mizukawa et al. 2013, 2016), which we suggested to be a consequence of the much higher amount of PBDE usage in the USA (Hites 2004). At that time, our results suggested that pet dogs and cats from Japan were exposed to low levels of PBDEs from furniture and household electrical appliances, and we also suggested that there was a lower PBDE contamination of indoor environments in Japan than in the USA.

The PBDE congener patterns that we found in terrestrial mammals indicated a high proportion of BDE209 (Mizukawa et al. 2013). Previously, it was argued that BDE209 should have a negligible bioavailability due to its large molecular size, low water solubility, and low vapor pressure. However, bioaccumulation associated with larger molecular size can be explained by factors other than molecular size, such as uptake and elimination (Arnot et al. 2010). For terrestrial mammals, there are specific uptake sources of BDE209 (e.g., municipal waste from waste material from building renovation or recycling), suggesting continuous dietary exposure of BDE209 or slow elimination rates. Compared with marine food webs, terrestrial mammals may directly uptake soil, dust, and municipal waste from the ambient environment, which contains higher proportion of technical deca-BDE products where BDE209 is a major congener (La et al. 2006). BDE209 was also found to be the dominant isomer in Japanese human blood (Takasuga et al. 2004; Inoue et al. 2006). Furthermore, the PBDE profiles of the blood reflect recent exposure, and thus given that technical deca-BDE was at that time in use in Japan, it was retained in terrestrial mammalian blood (Takasuga et al. 2004; Mizukawa et al. 2013, 2016). In the USA and Sweden, BDE47, BDE99, and BDE153, in addition to BDE209, were the predominant congeners in pet dog and cat serum (Dye et al. 2007; Venier and Hites 2011; Guo et al. 2012; Norrgran et al. 2015). Interestingly, the presence of BDE209 in the serum of pet cats and dogs is also associated with pet dry food, in which BDE209 was the dominant congener (Dye et al. 2007; Venier and Hites 2011, Mizukawa et al. 2016). Thus, the high proportion of BDE209 in the blood of pet dogs and cats may be caused by the consumption of these dry food products (Mizukawa et al. 2016). On the other hand, house dust may also be a source of the high BDE209 levels found in these pet animals, because previous studies have reported that BDE209 is a dominant congener in house dust in both Japan and the USA (Stapleton et al. 2005; Suzuki et al. 2009; Mizukawa et al. 2013).

6.3 Accumulation Features of OH-PBDEs in the Blood of Terrestrial Mammals

We have previously demonstrated that total OH-PBDEs exhibited higher median concentrations in mongooses, cats, and raccoons than other terrestrial mammals (Mizukawa et al. 2013). However, the concentrations of OH-PBDEs in the blood of

terrestrial mammals (raccoons, foxes, masked palm civets, raccoon dogs, badgers, and dogs) were lower than the ones found in marine mammals (Gebbink et al. 2008; Nomiyama et al. 2011a, Mizukawa et al. 2013). Yet, the concentrations of OH-PCBs in terrestrial mammals were 1–3 orders of magnitude higher than that of cetacean species. OH-PBDEs are metabolites of PBDEs and are also natural products found in marine organisms, such as red algae and sponges (Gribble 2000; Hakk and Letcher 2003). Remarkably, the concentration of total OH-PBDEs in cats was at levels comparable to that of marine mammals. We have hypothesized that such results could indicate high exposure of these species to natural OH-PBDEs through their feeding preferences in addition to the specific metabolic capacity (cats lack glucuronate conjugation ability). It should be mentioned that cats prefer to eat fish (Houpt and Smith 1981), which is a main ingredient of cat food in Japan.

In our study, the dominant congeners of OH-PBDEs were 6OH-BDE47 and 2′OH-BDE68, which accounted for up to 80% of quantified total OH-PBDEs in the blood of all terrestrial mammals (Mizukawa et al. 2013). 6OH-BDE47 and 2′OH-BDE68 are natural products in the marine environment (Gribble 2000; Hakk and Letcher 2003; Nomiyama et al. 2011a), but in our work we demonstrated that they were also accumulated in terrestrial mammals. The elevated levels of 6OH-BDE47 and 2′OH-BDE68 observed in the blood of cats suggested that cats are more likely to be exposed to these chemicals originating from the marine environment through food such as fish (Mizukawa et al. 2013, 2016). In fact, a high accumulation of 6OH-BDE47 and 6MeO-BDE47 were reported in Japanese amberjack and scalloped hammerhead shark collected from the Japanese coast (Nomiyama et al. 2011b). In addition to the uptake of natural marine products, the origin of the high percentage of OH-PBDEs (e.g., 6OH-BDE47 and 2′OH-BDE68) in the blood of cats could be a result of the higher rate of production via biotransformation of PBDEs or MeO-PBDEs and/or a slower rate of OH-PBDE elimination. 2′OH-BDE28 and 5OH-BDE47 were detected only in the blood of cats, while 4OH-BDE49 was detected in cats and foxes, and 3OH-BDE47 was detected in raccoon dogs and cats (Mizukawa et al. 2013). The debromination/hydroxylation of BDE47 originates several metabolites including 2′OH-BDE28, 6OH-BDE47, 5OH-BDE47, 4OH-BDE49, and 3OH-BDE47 (Qiu et al. 2007). We thus suggested that the hydroxylated metabolites detected in cats, foxes, and raccoon dogs could be metabolites of BDE47. The structure of 3OH-BDE154, 3OH-BDE47, and 4OH-BDE90 is similar to thyroid hormones where the binding of the OH group is adjacent to brominated atoms, and they have higher TTR-binding potencies, and they markedly inhibited the binding of T3 to TRα, acting as TH-like agents (Hamers et al. 2008; Kitamura et al. 2008). Besides, OH-PBDEs significantly activates TRβ reporter gene expression, and the naturally occurring 6OH-BDE47 is one of the several congeners that are strong activators of gene expression (Li et al. 2010).

Trace levels of 6OH-BDE47 and 2′OH-BDE68 have been detected in the blood of dogs, which indicates that dogs either metabolize OH-PBDE congeners more rapidly than cats or are exposed to much lower levels of these natural compounds (Ruiz-Suárez et al. 2015; Mizukawa et al. 2016). Thus, among carnivorous species, cats might be at high risk from 6OH-BDE47 and 2′OH-BDE68 exposure, and the metabolic capacities of CYPs and binding affinities to proteins such as TTR likely

differ in dogs and cats (Mizukawa et al. 2013, 2016). Although marine mammals may have developed a tolerance for naturally occurring OH-PBDEs in marine environments during the course of evolution, it is unlikely that terrestrial mammals have any tolerance for these compounds. Therefore, the toxic effects of these compounds may pose a risk to terrestrial mammals, particularly cats, which accumulate high levels of OH-PBDEs compared to other Carnivora species.

6.4 Exposure Routes to PBDEs

PBDEs levels in the sera of pet cats are generally higher than those detected in the sera of dogs or humans (Chow et al. 2015; Dye et al. 2007; Guo et al. 2012; Mizukawa et al. 2016). It is well established that for cats the main routes of exposure to PBDEs are diet and ingested contaminated house dust due to their grooming behavior (Chow et al. 2015; Dirtu et al. 2013; Guo et al. 2012; Mensching et al. 2012). Several authors already reported that BDE209 which is the dominant congener in pet blood was also present in house dust and animal feed (Stapleton et al. 2005; Mizukawa et al. 2016; Li et al. 2018). In our previous study, the major congeners of OH-/MeO-PBDEs identified in both blood and pet food were 6OH-/MeO-BDE47 and 2′OH-/MeO-BDE68 (Mizukawa et al. 2016). Some abundant congeners were previously found to be natural products in marine organisms (Teuten et al. 2005). Interestingly, MeO-PBDEs and the OH-PBDEs contents in fishmeal, which is an important ingredient of pet food, were influenced by the fishmeal-producing areas. High MeO-PBDEs levels were identified in the Southeast Asian fishmeal, which might be due to the suitable environmental conditions for the proliferation of bromoperoxidase-contained algae (Li et al. 2018).

6.5 In Vitro Biotransformation of OH-PBDEs from MeO-PBDEs

It has been previously suggested from in vivo studies that MeO-PBDEs and OH-PBDEs might be interconverted (Wan et al. 2010), which suggests that the production of OH-PBDEs from naturally occurring MeO-PBDEs may be an important contributor to OH-PBDEs occurrence in wildlife (Wiseman et al. 2011). We have demonstrated that for cat blood OH-PBDEs concentrations were higher than MeO-PBDE congeners, while for cat food MeO-BDEs were dominant. Thus, as previously mentioned, a high proportion of the OH-PBDEs detected in cat blood may be a consequence of the biotransformation of MeO-PBDEs to OH-PBDEs, alongside with the direct ingestion of cat food (Mizukawa et al. 2016).

We have also demonstrated that 6MeO-BDE47 and 2′MeO-BDE68 are demethylated to 6OH-BDE47 and 2′OH-BDE68 in both dog and cat liver microsomes, but

we could not detect any hydroxylated metabolite of BDE47. In cat microsomes, the estimated demethylation rates of 6MeO-BDE47 and 2′MeO-BDE68 were between 6.7–18% and 0–5.0%, respectively. With such results, we concluded that domestic cats were exposed to large amounts of MeO-PBDEs through cat food containing fish materials and that the OH-PBDEs in cat blood are derived from the CYP-dependent demethylation of naturally occurring MeO-PBDE congeners, and not from the hydroxylation of PBDEs (Mizukawa et al. 2016). As for dog microsomes, 2′MeO-BDE68 was mostly demethylated to 2′OH-BDE68 (95%), and the production rate of 6OH-BDE47 (44%) was also higher than the rate observed in cats. Based on such findings, at that time, we proposed that dogs have a higher MeO-PBDE demethylation capacity than cats. However, because 2′OH-BDE68 and 6OH-BDE47 were undetectable in the blood of dogs (Mizukawa et al. 2016), we hypothesized that the low levels of MeO-BDEs in dog food might be an important factor, alongside with the dog's efficient conjugation metabolism for these OH-PBDEs due to their high phase II enzymatic activity (Kakehi et al. 2015). On the opposite, cats have low conjugation ability for hydroxylated metabolites, and thus they might be slowly eliminated from the body; nevertheless, cats have a lower capacity for interconversion of MeO-PBDEs. Consequently, we proposed that the demethylation of MeO-PBDEs should be considered an important source of OH-PBDEs rather than the metabolism of anthropogenic PBDEs in cats (Mizukawa et al. 2016).

6.6 Accumulation Features and Biotransformation of BPhs in Dogs and Cats

Besides PBDEs and metabolites, we have also investigated the concentrations of bromophenols (BPhs) in Japanese domestic pets. BPhs concentrations in cats' blood were higher than in dogs' blood, although the differences were statistically insignificant (Mizukawa et al. 2017). The congener 2,4,6-tribromophenol (TBPh) represented over >90% of BPhs in both species. In what concerns pet food (wet and dry type), the most abundant congener in all the samples was 2,4,6-TBPh that accounted for >99% of total BPhs. Because this profile was similar to the blood samples of the pets, we suggested that diet was an important exposure route for BPhs in pets (Mizukawa et al. 2017). Furthermore, our results from in vitro exposure to PBDEs mixtures (BDE47, BDE99, and BDE209) showed that 2,4,5-TBPh was detected in dog liver microsomes but not in cats, which suggests species-specific metabolic capacities for PBDEs. Additionally, the formation of 2,4,5-TBPh occurred by hydroxylation at the 1′ carbon atom of the ether bond of BDE99 which is similar to what happens in humans, as previously reported by Erratico et al. (2012). Because hydroxylated PBDEs were not detected in the in vitro PBDEs metabolism assay, it was suggested that diphenyl ether bond cleavage of PBDEs can also be an important metabolic pathway for BPhs formation in cats and dogs (Mizukawa et al. 2017).

6.7 Tissue Distribution of PBDE in Dogs and Cats

We have recently reported the concentrations of PBDEs (47, 99, 100, 153, 154, 183, 196, 197, 206, 207, and 209) in the blood, livers, bile, and brain of Japanese pet dogs and cats (Nomiyama et al. 2017). Generally, the levels of PBDEs in the blood, livers, and bile of cats were one order of magnitude higher than those of dogs ($p < 0.05$). In addition, PBDE levels in the cat brains were also higher than those in dogs; nevertheless, they were not significantly different.

Concerning the PBDE congener profiles, BDE209 was found in the highest proportions in blood and the livers of dogs and cats from Japan. However, BDE47 was detected at low concentrations. Furthermore, BDE207 (debrominated metabolite) was predominant in cat livers.

In the bile of dogs, BDE47 was the dominant congener, which implies a species-specific excretion capacity for this lower-brominated BDE from the liver.

In dogs' brain, the dominant PBDE congener was BDE209, followed by BDE153, BDE47, and BDE28. Conversely, in the cats' brain, BDE209 accounted for approximately 50%, and the second most dominant congener was BDE207. To explain this, it is necessary to understand how these compounds cross the blood–brain barrier. Gabathuler (2010) suggested that compounds of smaller molecular size are easier to be transferred into the brain than larger-sized compounds due to the function of the blood–brain barrier (BBB). However, BDE209 (MW: 959.22) was detected from brains of both cats and dogs. These results suggest that physicochemical properties such as molecular size and log Kow of BDE209 are not so important for the passage of the compound from the blood into the brain. Previous studies showed that BDE209 disrupts the TH system in the cerebellar Purkinje cells of newborn rats via partial dissociation of the TH receptor from the TH response element acting through the TH receptor DNA-binding domain (Ibhazehiebo et al. 2011). Based on this, we suggested that BDE209 may disrupt normal brain development of cats via TH-dependent gene regulation. Yet, in order to fully understand the toxic mechanisms of these compounds, the evaluation of BDE209 in the brain of pet animals is necessary.

6.8 Tissue Distribution of OH-PBDEs and MeO-PBDEs in Dogs and Cats

Similarly, to PBDEs, the levels of OH-PBDEs in cat tissues were one order of magnitude higher than those of dogs ($p < 0.05$) (Nomiyama et al. 2017). However, in contrast to PBDE concentrations, the concentrations of OH-PBDEs were significantly higher in the bile than in the liver and blood ($p < 0.05$). Among OH-PBDE congeners, 6OH-BDE47 and 2′OH-BDE68 were predominant in the blood and livers of dogs and cats, and the concentrations in cats were 1–2 orders of magnitude higher. In all cat tissues, 6OH-BDE47 accounted for up to 80% of the total

OH-PBDEs, whereas trace levels of 3′OH-BDE28, 3OH-BDE47, and 5OH-BDE47 were detected in the bile. In contrast, compared with 6OH-BDE47 concentrations, the concentrations of 2′OH-BDE28, 3′OH-BDE28, 5OH-BDE47, and 4′OH-BDE49 were 1–2 orders of magnitude lower in the bile of dogs, and bile-to-blood concentration ratios were relatively higher. Based on these results, we suggested that these phenolic compounds are rapidly eliminated through the bile.

In our previous work, among the 15 MeO-PBDE congeners targeted, only 6MeO-BDE47 and 2′MeO-BDE68 were detected in the tissue samples from cats and dogs. In cats, MeO-PBDEs were found in the liver, blood, and bile (Nomiyama et al. 2017). In dogs, MeO-PBDEs were detected in the liver and blood. We reported that cats are exposed to 6MeO-BDE47 and 2′MeO-BDE68 through the intake of cat food containing fish (Mizukawa et al. 2016). Similarly to 6OH-BDE47 and 2′OH-BDE68, these MeO-PBDE congeners are natural products in marine organisms (Nomiyama et al. 2011a, b). However, the congener profile of MeO-PBDEs differed from that of OH-PBDEs in cats: 2′MeO-BDE68 concentrations were higher than those of 6MeO-BDE47 in the blood, liver, bile, and brain. Thus, it was hypothesized that this difference was probably due to variations in the demethylation rates between 2′MeO-BDE68 and 6MeO-BDE47.

6.9 Xenobiotic Metabolic Capacities by CYPs and UGT in Dogs and Cats

Xenobiotic compounds such as drugs and environmental pollutants are activated by phase I enzymes, conjugated by phase II enzymes, and eliminated in urine or bile through phase III transporters. Phase I enzymes include primarily the CYP superfamily, whereas phase II conjugating enzymes include many enzyme superfamilies such as UGT, sulfotransferase (SULT), and glutathione S-transferase (GST) (Xu et al. 2005). For phase II conjugating enzymes, the UGT superfamily plays the most important role in xenobiotic metabolism, since 55% of the 200 most frequently prescribed drugs are conjugated by UGT and eliminated in urine or bile (Guillemette et al. 2014). However, the interspecies differences in this UGT metabolism are significant.

UGT1A6 and 2Bs plays an important role in glucuronidation of xenobiotics, especially phenolic compounds as previously described by Maruo et al. (2005) and Kondo et al. (2017). Due to their hypercarnivorous diet, cats are less exposed to natural xenobiotics such as phytotoxins, and thus they experience fewer gene duplications of xenobiotic-metabolizing UGT genes and have UGT1A6 pseudogenes or low activity of UGT2Bs (Court and Greenblatt 2000; Shrestha et al. 2011; Kakehi et al. 2015; Kondo et al. 2017).

This weak ability to eliminate phenolic compounds can result in adverse effects in cats. In fact, their low xenobiotic glucuronidation capacity causes high accumulation to these compounds such as OH-PBDEs. As aforementioned, the concentra-

tions of 6OH-BDE47 and 2′OH-BDE68 in the blood of cats were comparable to those of marine mammals (Nomiyama et al. 2011a; Mizukawa et al. 2016). These findings indicate that halogenated phenolic compounds may be preferentially retained in cats, likely because they do not undergo robust UGT conjugation. As a consequence, this slow glucuronidation of phenolic compounds leads to the slow clearance and high sensitivity of cats to the adverse effects of these chemicals (Davis and Westfall 1972; Savides et al. 1984).

Another explanation may be the high exposure of this species to natural OH-PBDEs owing to their feeding preferences. As mention above, higher levels of OH-PBDEs were detected in the blood of cats which are derived from the CYP-dependent demethylation of naturally occurring MeO-PBDE congeners. On the other hand, hydroxylation of BDE47 was reported by the CYP2B6 in the human liver microsome (Erratico et al. 2013, 2015). These results may be a consequence of the CYPs species-specific mechanisms. Although information on feline CYP activity is limited, a previous report showed that the metabolic activities of the CYP2C subfamily in the cats were less inhibited by Tolbutamide than those of other species (human, horse, and dog), compared with the CYP1A, 2A, 2D, 2E, and 3A subfamily (Chauret et al. 1997). Further studies of the activities of the CYP subfamily involved in the metabolism of PBDEs in the cats' microsome are essential.

Because domestic cats routinely ingest natural MeO-PBDEs from cat food containing fish, they retain the demethylated metabolites, OH-PBDEs, in the blood for a prolonged time. The possible toxic effects of OH-PBDEs such as 6OH-BDE47 on thyroid homeostasis are necessary in order to establish the relation between exposure levels and the incidence of diseases such as FH.

6.10 Conclusion

This chapter revised the available information on the tissue distribution of brominated compounds in cats and dogs. Generally, among PBDEs, BDE207 was predominant in tissue samples, particularly in the liver of cats, which indicates that BDE207 forms via the debromination of BDE209 or accumulates through food intake. In dogs, BDE47 was the dominant congener in the bile, which implies that this lower-brominated PBDE is excreted rapidly through the bile. Higher concentrations of other phenolic compounds such as 2′OH-BDE68, 6OHBDE47, and 2,4,6-tri-BPh were also found in the bile, and bile-to-blood concentration ratios were relatively higher in dogs. These results suggest that these phenolic compounds are rapidly eliminated through the bile. Pet cats routinely ingest natural MeO-PBDEs in cat food products containing fish and retain their demethylated metabolites, OH-PBDEs, in the blood, liver, bile, and brain for a prolonged time. Because of the absence of UGT1A6, the metabolization phenolic compounds are difficult. Further studies are required to clarify the metabolic capacities and the toxic effects of these compounds particularly those related to neurotoxicity and thyroid hormone disease.

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Chapter 7
Using Cats as Sentinels for Human Indoor Exposure to Organic Contaminants and Potential Effects on the Thyroid Hormone System

Jana Weiss and Bernt Jones

Abstract Cats share the indoor environment with humans and has been shown to develop similar endocrine depending health effects, such as thyroid hormone diseases. The etiology behind feline hyperthyroidism, a relatively modern disease in cats, is not yet fully understood. It is likely induced by a combination of aspects, where the exposure to organic contaminants associated to common household dust might be a significant contributor. Dust is an efficient sink for functional chemicals that are added to, e.g., indoor products, textiles, and building materials, and are emitted by vaporization or abberation. Flame retardants, plasticizers, and surfactants are examples of chemicals commonly found in dust, which have endocrine-disrupting potency. Other relevant exposure pathways for organic contaminants are via food and drinking water, where typically the persistent organic pollutants can be found. Here we summarize the studies describing blood levels on organic contaminants in cats and discuss the potential effects on the thyroid hormone system. Investigating cats has been demonstrated to be a useful model for human indoor exposure, especially to small children and toddlers, sharing a similar behavior pattern (cat's grooming and children hand-to-mouth activity) and elevated dust intake.

Keywords Feline hyperthyroidism · Endocrine disruption · Organic contaminants

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7.1 Introduction

Before the end of the 1970s, feline hyperthyroidism (FHT) was a rare disease generally caused by hormone producing carcinomas of the thyroid gland. However, in 1979 Peterson and coworkers at the Animal Medical Center in New York published a report on a new type of FHT, with a still unknown etiology (Peterson et al. 1979). The first question raised among veterinarians was if this was really a new disease or if they had overseen it up until then. A screen of 7000 feline necropsies performed by Dr. Peterson showed that thyroid abnormalities were rare until the late 1970s (Anthes 2017). After that first report from the American East Coast, this new disease was described from all continents and in an increasing prevalence (De Wet et al. 2009; Kass et al. 1999; Scarlett et al. 1988; Wakeling et al. 2009).

Typically, elderly cats are more prone to develop FHT. An English study performed in 2009–2011 showed that the overall prevalence of FHT was 2.4%, and in cats >10 years of age, it was 8.7% (Stephens et al. 2014). Today, senior cats are routinely screened for hyperthyroidism, resulting in less severe symptoms when the disease is diagnosed (Broussard 1995). While clinical diagnosis and treatment of FHT has progressed, researchers are still puzzled by the origin of the disease.

Today's domestic cats (*Felis catus*) spend a significant time indoors, especially in urban areas. Due to their grooming behavior, i.e., cleaning their fur by licking, they have been shown to be highly exposed to chemicals accumulated in common household dust. Dust is a rather new matrix for exposure assessments of the indoor environment. It has become evident that dust is a significant source of exposure to organic contaminants (OCs), such as the polybrominated diphenyl ethers (PBDEs), which is for cats a much more important pathway than exposure via diet, which traditionally has been considered the main exposure source for OCs (Lorber 2008). In addition, cats, in relation to dogs, accumulate OCs to a greater extent in their bodies, i.e., the bioconcentration factors seem to be higher in cats (Kunisue et al. 2005). For these reasons, cats may accordingly be a suitable biomarker for exposure of indoor pollutants.

7.1.1 Thyroid Hormone System and Metabolic Capacity of Cats

As for humans and all higher vertebrates, the primary task of thyroid hormones (THs) is to regulate body metabolism, body growth, body weight, cholesterol metabolism, gluconeogenesis, maturation, reproduction, and brain development (Mullur et al. 2014; Zoeller et al. 2002). The THs, i.e., thyroxine (T4, 3,3′,5,5′-tetraiodo-L-thyronine) and triiodothyronine (T3, 3,3′,5-triiodo-L-thyronine) are produced by the thyroid gland, which is situated in the neck in front of the trachea below the larynx. The inactive T4 is bound to plasma thyroxine-binding proteins necessary to transport THs from the production site in the thyroid

gland(s) to target cells, where T4 is converted by deiodinases to T3. T3 is the active form binding to various TH cytosolic and nuclear receptors (Richardson 2009). Only a small fraction of the THs are circulating free in the blood plasma, whereas most of the THs are bound to one of the thyroxine-binding plasma proteins: albumin, transthyretin (TTR), and thyroxine-binding globulin (TBG) (Richardson 2009). TBG has not been demonstrated in cat plasma, and therefore TTR is believed to have a more pronounced role in the transport of THs in cats compared to other mammals (Larsson et al. 1985). TTR is unique in that it is the main carrier of THs to the brain and the cerebrospinal fluid (Richardson 2009).

THs are normally cleared from plasma through conjugation with glucuronic acid or sulfate. Cats have reduced activity of some phase II conjugation enzymes, i.e., glucuronosyltransferase and sulfotransferases, responsible for catalyzing conjugation with glucuronic acid and sulfate (Shrestha et al. 2011). Therefore, the levels of THs in cats are strongly dependent on the hormone regulation by the thyroid-stimulating hormone (TSH) released from the pituitary gland located below the brain. The pituitary is controlled by the thyrotropin-releasing hormone (TRH) secreted from hypothalamus in the brain. The thyroid hormones control the secretion of TSH and TRH by negative feedback mechanisms. The axis of the hypothalamus-pituitary-thyroid glands' main purpose is to maintain normal or euthyroid hormone balance in the body (Mullur et al. 2014).

Glucuronidation accounts for detoxification of a great variety of phenolic substances (not only TH), e.g., drugs such as acetaminophen and acetylsalicylic acid. As conjugation with glucuronic acid and sulfate is limited in cats, clearances of phenolic substances are slower. Cats exhibit therefore acute life-threatening effects of, e.g., acetaminophen, at much lower doses than is toxic to dogs and humans (Court 2013). It is suggested that the Felidae family has been less exposed to plant toxicants (Felidae family is considered hypercarnivores, i.e., meat makes up >70% of the diet) and consequently not subjected to an evolution of the metabolic system similar to, e.g., omnivores and herbivores (Shrestha et al. 2011). This low capacity to conjugate has consequences on the metabolism and excretion of several OCs, compared to the capacity of, e.g., humans.

7.1.2 Feline Hyperthyroidism and Etiology

The incidence of hyperthyroidism in cats worldwide is around 1.5–12% (Köhler et al. 2016; McLean et al. 2016; Peterson 2012). Hyperthyroidism is associated with elevated plasma concentrations of THs and decreased levels of TSH (Peterson and Ward 2007). In contrast to humans and dogs, hypothyroidism is very rarely occurring in cats, and hyperthyroidism is recognized as the most prevalent endocrine disorder in senior pet cats (Rijnberk et al. 2003).

Hyperthyroidism is simple to diagnose in cats, as the morphologic changes of the thyroid gland are clear. More than 95% of cats with FHT have functional thyroid adenomatous hyperplasia, i.e., benign thyroid enlargement, and the majority (70%)

of the hyperthyroid cats has an enlargement of both thyroid lobes (Köhler et al. 2016; McLean et al. 2016; Peterson and Ward 2007; Peterson 2012). The typical clinical symptoms are weight loss, polyphagia, polydipsia, hyperactivity, aggression, diarrhea, vomiting, and tachycardia (Peterson and Ward 2007). Hyperthyroidism in cats is treated in similar ways as in humans, by the anti-thyroid substance thiamazole to reduce serum TH or by surgical removal of the thyroid gland. Alternatively, administration of radioactive iodine to reduce the number of hyperactive cells in the gland by radiotherapy is also possible.

The etiology of FHT is debated and the causes remain uncertain. It is likely to be a multifactorial disease combining nutritional and environmental influences. In humans, women have a higher incidence of thyroid hormone-related disorders than men, which is not normally seen in cats (Vanderpump 2011; Zoeller et al. 2002). Only one study reported higher incidence in female cats (Olczak et al. 2005), whereas several studies reported no difference between gender (Edinboro et al. 2004; Stephens et al. 2014). Interestingly, as for humans, genetic factors might play a role for the etiology. Purebred cats, especially related to the Siamese and Himalayan breeds (two genetically similar breeds), have a decreased risk at developing FHT (Kass et al. 1999; Martin et al. 2000; Olczak et al. 2005; Peterson and Ward 2007; Stephens et al. 2014).

Studying the pathologic changes of the thyroid in hyperthyroid cats, several possible links or combinations thereof have been suggested, including immunologic factors (altered immunoglobulins), nutritional factors (e.g., unbalanced iodine intake, goitrogens in food or water), an infectious agent, or environmental factors (e.g., toxins or thyroid hormone-disrupting compounds) (Peterson 2012). Epidemiological studies have been suggesting several other risk factors for developing FHT, e.g., indoor lifestyle (e.g., use of litterbox), a diet based on canned food, eating fish, and environmental factors such as exposure to herbicides or regular use of flea powders/sprays (Peterson and Ward 2007; Wakeling et al. 2009).

7.2 Organic Contaminant Exposure to Cats

7.2.1 Exposure Pathways

The major exposure pathways for OCs (excl. medicines) to cats are via food and ingested dust. Generally, cats are not in need to drink large volumes of water, as they get most of their fluid via food. Even with the added water to dry food and the extra water you should have available for cats, we do not consider water a significant exposure pathway for anthropogenic OCs.

In manufacturing cat food, a batch of abattoir offal, e.g., lung, heart, liver, kidney, and throat, foremost from chicken which is neutral in taste but also from pig and cattle, is prepared before the flavor is added. The flavor must constitute a minimum of 4% by weight; hence 92–96% of the cat food from one producer has the

same ingredients across the various tastes (Norrgran et al. 2017). Canned or wet food has been identified as a risk factor by several studies (Edinboro et al. 2004; Martin et al. 2000; Olczak et al. 2005; Wakeling et al. 2009). Interestingly, despite being based on the same batch of ingredients, the contaminant profile is different between dry and wet food. The highly brominated PBDEs (flame retardants) and DDT (pesticide) were mainly found in the dry food, whereas the phenolic compounds, such as pentachlorophenol (PCP, pesticide), 2,4,6-tribromophenol (2,4,6-TBP, pesticide, and byproduct), and 6-OH-BDE47 (a metabolite and naturally occurring compound in the marine environment), were detected at a higher frequency and at higher concentration in the wet food (Norrgran et al. 2017). The phenolic compounds have a structural resemblance to THs and high binding potency to the transport protein TTR (Weiss et al. 2015). Other possible association between FHT and the cat food can be highly variable iodine content (Edinboro et al. 2004), or the presence of goitrogenic vegetable substances like isoflavones and phthalates coming from soy and corn in cat food (Kass et al. 1999; White et al. 2004). Several of these vegetable substances are conjugated by glucuronidation, the process known to be slow in cats (Hill and Shaw 2014).

In 2009 came the first suggestion that indoor household dust could be an exposure source for cats to ingest OCs, which could affect their health (Dye et al. 2007). They reported the observation that FHT started in parallel with the introduction of flame retardants in our household products, e.g., the PBDEs. The question was raised and several studies started to analyze for anthropogenic OCs in cat serum to search for a cause-effect relationship (Ali et al. 2013; Bost et al. 2016; Chow et al. 2015; Guo et al. 2016; Henríquez-Hernández et al. 2017; Mizukawa et al. 2013, 2016; Norrgran et al. 2015, 2017; Serpe et al. 2018; Walter et al. 2017; Yavuz et al. 2018). Not until recently could the exposure pathway be statistically confirmed by using paired dust/cat blood serum samples from the same household (Norrgran et al. 2017).

Cats have a high dust exposure from their grooming behavior, similar to small children and toddlers with their frequent hand-to-mouth activity. The estimated daily intake of dust for children is 40 mg dust/day (EPA 2017) and in worst-case scenarios approximately 200 mg dust/day (Jones-Otazo et al. 2005). To the best of our knowledge, there are no estimates of daily intake of dust for cats, but it is reasonable to believe that the intake is within the same range as the worst-case scenario for small children.

Other possible exposure sources for anthropogenic OCs can be pet toys and cat litter sand. This was investigated in a pilot project in Sweden 2017, where seven cat toys were screened for the content of halogenated (bromine and chlorine) OCs. The results revealed no commonly found OCs (PCBs, PBDEs, etc.) but in three pet toys, i.e., a vinyl mouse, a plush mouse, and a vinyl rubber ball, indicated the presence of chlorinated paraffin (Fijol 2018). Chlorinated paraffin are industrial chemicals primarily used in metalworking but also used as flame retardants and softeners in plastics and has, among others, been reported in plastic products of children (Miller and DiGangi 2017). Only in one cat litter sand sample, the only one of eight samples containing paper pellets, could DDT/DDE be quantified (18 ng/g sand) and in one

bentonite sample could low levels of 2,4,6-TBP be detected (0.5 ng/g sand). No other OCs were detected in cat litter sand (unpublished data).

7.2.2 Organic Contaminants in Cat Serum

In total, the authors could find 19 studies which have analyzed OCs in cat serum/plasma or blood. The data reported are summarized in Table 7.1, and a selection of OCs concentration in serum is illustrated in Fig. 7.1. Eight of the studies have separated the analysis of samples from healthy and unhealthy cats, i.e., cats with diagnosed feline hyperthyroidism and diabetes mellitus. These are the only two endocrine disorders investigated together with serum OC levels. Both diseases are relevant to humans (Dirtu et al. 2013). Healthy, or control, cats in Table 7.1 are cats visiting clinics to be examined for other reasons than endocrine disorders, e.g., bone fractures, vaccinations, sterilizations, etc. In Table 7.1, we have chosen to report all levels on wet weight (mL serum) basis and on molar instead of on molecular weight basis for comparison. Calculations from reported lipid weight based concentrations were made assuming a cat blood serum density of 1.042 g/mL and a relative lipid content of 0.6% (Norrgran et al. 2017).

Even though it looks like the serum levels of OCs in the unhealthy cats are higher, it is not possible to draw any conclusions from the material of serum analyzed from healthy or unhealthy cats as the internal sample variance is too big, i.e., sample year, location, known/unknown health status, and sample size. For example, 12 studies have analyzed and reported PBDE serum levels, and together they cover the USA, Europe, and Asia, and the sample size is 431 cats (Ali et al. 2013; Chow et al. 2015; Dirtu et al. 2013; Dye et al. 2007; Guo et al. 2012, 2016; Henríquez-Hernández et al. 2017; Mensching et al. 2012; Mizukawa et al. 2013; Norrgran et al. 2015, 2017; Walter et al. 2017). OPFRs, on the other hand, have only been reported in one study with 22 cats, living in Spain (Henríquez-Hernández et al. 2017). The median level of the sum of OPFRs is one of the OC groups dominating the serum chemical profile, but is the level representative? Dust, the suspected main source for exposure to the flame-retardants, has been analyzed all over the world, and, typically, the levels of the OPFRs are much higher than for the brominated flame retardants (Cristale et al. 2016). This is in accordance with the chemical profile of the cat serum (Fig. 7.1). It was recently suggested that OPFR could increase the binding affinity of T4 to TTR, an observation never reported before (Hill et al. 2018).

The chlorinated compounds, i.e., the persistent organic pollutants (POPs) regulated under the Stockholm Convention (e.g., polychlorinated biphenyls [PCB], DDT/DDE, hexachlorobenzene [HCB], PCP, etc.), have been reported in cats ($n = 438$) from Japan, Pakistan, Turkey, Italy, Spain, Sweden, the UK, and the USA (Ali et al. 2013; Dirtu et al. 2013; Guo et al. 2012, 2016; Henríquez-Hernández et al. 2017; Kunisue and Tanabe 2009; Mizukawa et al. 2013, 2016; Norrgran et al. 2015, 2017; Serpe et al. 2018; Walter et al. 2017; Yavuz et al. 2018). Of special interest, considering the thyroid hormone system are the phenolic compounds, i.e.,

Table 7.1 Median of all median and maximum levels (pmol/g lipid weight [lw] and pmol/mL serum for PFAS) of reported organic pollutants in cat serum from animals diagnosed as healthy (or with unknown health status) or non-healthy (hyperthyroid or diabetic)

			Healthy cats				Hyperthyroid or diabetic cats			
			Concentration		T4-REP		Concentration		T4-REP	
			pmol/g lw		nmol/L		pmol/g lw		nmol/L	
Group	Compound	T4-REP[a]	Median	Max	Median	Max	Median	Max	Median	Max
BFRs	BDE-47	0	11	1000	0.0	0.0	49	1200	0.0	0.0
	BDE-99	0	25	4000	0.0	0.0	84	2700	0.0	0.0
	BDE-183	0	6.2	41	0.0	0.0	20	22	0.0	0.0
	BDE-209	0	65	290	0.0	0.0	220	230	0.0	0.0
	BB-209	nt	55	86			69	69		
Phenols	6-OH-BDE47	0.78	110	210	0.53	1.0	11	170	0.05	0.82
	2'-OH-BDE68	3.75	2.3	13	0.05	0.31	3.9	6.0	0.09	0.14
	6-MeO-BDE47	0.29	7.4	15	0.01	0.03				
	2'-MeO-BDE68	nt	24	48						
	2,4,5-TBP	nt	3.6	3.6						
	2,4,6-TBP	4.73	100	130	3.0	3.8	88		2.6	
	2,3,4,6-TeBP	nt	0.74				0.74			
OPFRs	DPEHP	nt	1500							
	TEHP	nt	0.0							
	TBP	nt	270							
	TEP	nt	3.3							
	TiBP	0[b]	180		0.0	0.0				
	TPhP	nt	76							
	TBOEP	0[b]	110		0.0	0.0				
	TCEP	0[b]	25		0.0	0.0				
	TCIPP	nt	350							

(continued)

Table 7.1 (continued)

			Healthy cats				Hyperthyroid or diabetic cats			
			Concentration		T4-REP		Concentration		T4-REP	
			pmol/g lw		nmol/L		pmol/g lw		nmol/L	
Group	Compound	T4-REP[a]	Median	Max	Median	Max	Median	Max	Median	Max
	TCP	nt	28							
OCPs	PCP	1.74	590	1300	6.4	14	1100	1300	12	14
	HCB	nt	8.4	12			8.3	10		
	4,4′-DDT	0	22	310	0.0	0.0	81	420	0.0	0.0
	4,4′-DDE	0	300	1500.0	0.0	0.0	760	1200	0.0	0.0
PCBs	CB-99	nt	2.0							
	CB-101	0	63		0.0					
	CB-105	0	0.0	5.6	0.0	0.0	10	13	0.0	0.0
	CB-118	0	11	55	0.0	0.0	64	70	0.0	0.0
	CB-138	0	42	160	0.0	0.0	81	130	0.0	0.0
	CB-153	0	97	270	0.0	0.0	180	2900	0.0	0.0
	CB-180	0	39	250	0.0	0.0	51	1700	0.0	0.0
OH-PCBs	4’-OH-CB18	8.4[c]	9.1	12	0.48	0.64				
	4’-OH-CB19/26/31	8.4[c]	26		1.4					
	4-OH-CB70	3.3[c]	3.1		0.06					
	4-OH-CB107	3.40	6.1	49	0.13	1.0	14	19	0.30	0.41
	3’-OH-CB118	4.10	4.9		0.12					
	4-OH-CB101/120	4.0[c]	32	58	0.80	1.5				
	4-OH-CB-146	3.7[c]	1.1	3.1	0.02	0.07	7.4	10	0.17	0.23
	3-OH-CB153	3.3[c]	0.0	0.88	0.0	0.02	1.7	1.9	0.03	0.04
	4-OH-CB162	3.7[c]	3.5	11	0.08	0.26	4.2	4.8	0.10	0.11
	4’-OH-CB172	4.20	0.65		0.02					
	4-OH-CB187	4.00	1.7	3.6	0.04	0.09				

	4-OH-CB202	3.4[c]	0.45		0.01					
	4'-OH-CB199	3.4[c]	0.71		0.01					
PFASs	PFHpA	0.039	260	4200	0.06	1.0				
	PFOA	0.064	2600	23,000	1.0	9.0				
	PFNA	0.022	1700	6200	0.24	0.86				
	PFDA	0.007	500	1600	0.02	0.07				
	PFUnDA	0.003	620	1900	0.01	0.03				
	PFDoDA	0.001	30	250	0.0	0.0				
	PFBS	0.003	0.0	1300	0.0	0.02				
	PFHxS	0.085	1600	97,000	0.84	51				
	PFHpS	0.17[b]	0.0	59	0.0	0.06				
	PFOS	0.065	7500	45,000	3.1	18				
	FOSA	0.01	0.0	33	0.0	0.0				
	66PFPiA	nt	0.0	3.9						
	68PFPiA	nt	0.0	9.2						
	88PFPiA	nt	0.0	0.0						
	62PAP	0[b]	0.0	45	0.0	0.0				
	82PAP	0	0.0	22	0.0	0.0				
	62diPAP	0	1.1	26	0.0	0.0				
	82diPAP	0	1.4	10	0.0	0.0				
Summary T4 equivalents (nmol/L)					18	104			16	18

[a]Weiss et al. (2015) if not stated otherwise
[b]Zhang et al. (2015)
[c]The congener has not been tested, but the value is estimated using the (average if several congeners are available) value from similar congener with the same number of chlorine plus hydroxylated on the same position
References used: Ali et al. (2013), Bost et al. (2016), Chow et al. (2015), Dirtu et al. (2013), Dye et al. (2007), Guo et al. (2012, 2016), Henríquez-Hernández et al. (2017), Kunisue and Tanabe (2009), Mensching et al. (2012), Mizukawa et al. (2013, 2016, 2017), Norrgran et al. (2015, 2017), Serpe et al. (2018), Walter et al. (2017), Weiss et al. (Submitted), Yavuz et al. (2018)

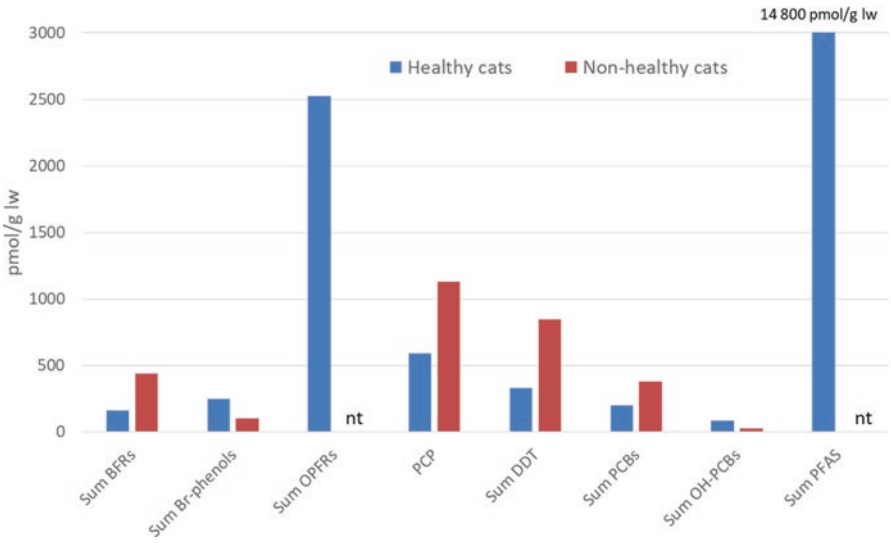

Fig. 7.1 Median levels of all reported studies on healthy (and control cats with unknown health status) and unhealthy cats (hyperthyroid or diabetic) (pmol/g lw). The different compound groups are added from Table 7.1 and are hence crude estimates of the median cat OC profile in serum. (nt, not tested)

PCP and the hydroxylated metabolites of PCBs (OH-PCBs) with a high relative binding potency to TTR (1.7 and 3.3–8.4, respectively, Table 7.1).

Several studies have specifically focused on the OH-PCBs, i.e., in cats from the UK (Dirtu et al. 2013) and Japan (Kunisue and Tanabe 2009; Mizukawa et al. 2013, 2016). Penta- to hepta-chlorinated OH-PCB congeners have been reported as predominant in human blood (Hovander et al. 2002), whereas in cats tri- and tetra-chlorinated were elevated, although many of them were not structurally identified (Kunisue and Tanabe 2009; Mizukawa et al. 2016). Many of these metabolites have not been tested for their binding potency to TTR, so here we have made a T4 relative potency (T4 REP) estimate based on related OH-PCBs reported in the review of TTR binding compounds (Weiss et al. 2015). The T4 REP estimate selected the related OH-PCBs based on chlorination degree and substitution pattern (Table 7.1). These phenols, together with PCP and the brominated phenols, are together the main contributor to the total T4 equivalents (eq) calculated in cat serum (Table 7.1). No studies have shown any elevated levels of hydroxylated PBDE (OH-PBDE) metabolites, except the naturally formed 6-OH-BDE47 and 2-OH-BDE68 from the marine environment, which theoretically could also be formed but are no major metabolites (Dirtu et al. 2013; Mizukawa et al. 2013; Norrgran et al. 2017).

The OCs of highest concentration found in cat serum is the group of per- and polyfluoroalkyl substances (PFASs). PFASs exhibit a high surface activity due to their lipophobic and hydrophobic characteristics. This gives them useful properties,

e.g., thermal stability and acid resistance, and has led to a broad application in a wide range of industrial and commercial products, e.g., as polymers, surfactants, lubricants, in textile coatings, non-sticking coatings, stain repellents, food packaging, and firefighting foams (Buck et al. 2011). The human exposure pathways are mainly via food and drinking water, but PFASs can also diffuse directly from applied household products and associate to dust particles (Vestergren and Cousins 2009). For cats this is considered a relevant exposure pathway as a correlation between serum levels, and dust levels from the same household was found (Weiss et al. Submitted).

The PFASs physicochemical properties are different than for the other OCs measured in cat serum, as they are both lipophobic and hydrophobic and instead associate to serum proteins (Jones et al. 2003). The other OCs measured in cat serum are either hydrophilic or lipophilic. The very elevated PFAS levels in serum, compared to, e.g., OPFRs, which have a much higher concentration in dust than PFAS, are probably a product of factors such as persistence, accumulation (proteinophilic), metabolism, and bioavailability.

The majority of compounds reported to be binding competitively to TTR are aromatic, hydroxylated, and halogenated, features in common with thyroxine itself. By coincidence, the PFASs were tested in the TTR-binding assay, and a clear structural-activity relationship could be reported (Weiss et al. 2009). All three chemical functionalities that varied (i.e., degree of fluorination, carbon chain length, and functional end group) in the test set affected the TTR binding potency of PFASs. Highest binding affinity was found for perfluorohexane sulfonic acid (PFHxS), followed by perfluorooctane sulfonic acid (PFOS), and perfluorooctanoic acid (PFOA).

Concentrations of PFASs have been reported from three studies, in healthy cats visited in their households while paired dust samples were collected (Weiss et al. Submitted), in cat serum collected in veterinary clinics in North Carolina, USA ($n = 72$) (Bost et al. 2016), and in California, USA ($n = 20$) (Wang et al. 2010). The profile is dominated by PFOS and PFOA in all three studies, with a much higher variation in the cats from the USA than from Sweden (Fig. 7.2). The Swedish study also included analysis of perfluorooctane sulfonamide (FOSA) and the emerging fluorinated organophosphorus compounds, such as perfluoroalkyl phosphonic (PFPA) and phosphinic (PFPiA) acids, and mono- and di-polyfluoroalkyl phosphates (PAPs). The levels of the emerging PFASs are very low in cat serum (observe that the scale is fmol/mL) compared to the more traditional PFASs. The dust levels in these paired samples, on the other hand, showed the opposite profile than the cat serum, with mono- and di-PAP levels five times higher than, e.g., PFOS (Weiss et al. Submitted). The PAPs do not have any binding affinity to TTR, but an in vitro study has showed that PAPs can affect the steroidogenesis, which could lead to lower androgen and higher estrogen levels, demonstrating that also these compounds are potential EDCs.

Although the TTR-binding potency is relatively low for the PFAS, the competitive binding to the natural ligand T4 can be significant, which is shown in the max levels in the cats from the USA. The contribution to the total T4 equivalents in the cat serum from PFHxS is the solely highest among all the compounds discussed here. No hyperthyroid or otherwise unhealthy cats have been analyzed for their PFAS serum levels.

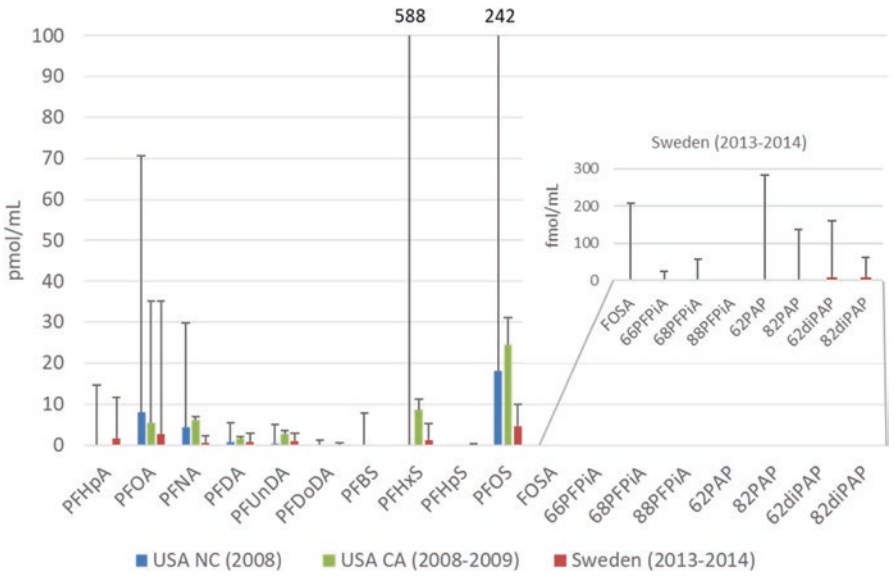

Fig. 7.2 Median levels of per- and polyfluoroalkyl substances in cat serum (pmol/mL and fmol/mL) from USA NC (Bost et al. 2016) and CA (Wang et al. 2010) and Sweden (Weiss et al. Submitted). Error bars show maximum levels

7.3 Discussion

Thyroid hormones are crucial for the development of the central nervous system, and even short durations of deficiency may lead to irreversible brain damage (Bernal 2015). The severity also depends on the specific timing of deficiency (Bernal 2015). Low free T4 levels in healthy pregnant women are related to a moderate delay in child neurodevelopment (Julvez et al. 2013). Even after birth, THs are still essential, since some neurodevelopmental processes are not completed until adolescence, and THs play a role in the behavior and cognitive functions of the young brain (Anderson 2001; Rice and Barone 2000; Schug et al. 2015). Therefore, the possible effects of OCs on thyroid function during fetal and childhood life are a matter of public concern.

Translating the analyzed OC levels to T4 equivalents (Table 7.1) shows that, hypothetically, the exposure could competitively bind to TTR with 16–18 nM T4 eq and in worst case with more than 100 nM T4 eq. This could be compared to an average span of T4 (total T4) in cat serum of 10–40 nM (Weiss et al. Submitted) and for human adults 60–150 nM (www.thyroidmanager.org/). A study on children's TH levels during development showed a wide span (50–250 nM) directly after birth and a clear age dependency (Lem et al. 2012). After the age of 2 years, the span of free and total T4, T3, TSH, and T4 binding globulin (TBG) was narrower. Puberty significantly increased the THs activity by an upregulation of T3, which probably con-

tributes to the acceleration of growth. The wide span of THs during the first period of life makes it difficult to discuss the potential effect of the OC exposure defined here. A child with 50 nM TH levels would obviously be affected by OC levels corresponding to 100 nM T4 equivalents.

The phenolic compounds and PFASs are the major competitive binders to TTR and could hypothetically disrupt the normal TH balance. A systematic review of the literature on TH and PFAS serum levels measured in pregnant women and children (<19 years) reported a consistent positive association between maternal or teenage male exposure to some PFASs and TH levels (Ballesteros et al. 2017). In addition, we can assume that we have not a full overview of the broad span of chemicals we are exposed to in our indoor environment, many of which are EDCs. Many more chemicals analyzed in dust, with TH-disrupting potency, have not yet been searched for in cat serum, e.g., phthalates, BPA, musk compounds, and parabens. Some of these chemicals are typically found in urine. Parabens were recently determined in urine from pet cats and dogs (Karthikraj et al. 2018). Considering the growing evidence of the endocrine effects of these compounds and the increasing number of monitoring data in dust and serum (both for cats and humans), it is reasonable to attribute chemical exposure as contributing to the observed increased incidence of endocrine disorders in cats and humans, such as FHT.

7.4 Conclusion

Cats are good sentinels for human exposure to indoor contaminants. A significant correlation between cat serum levels of OCs and their presence in paired dust samples has established dust as a relevant exposure pathway to cats, and likewise should be that for small children and toddlers. The observation reported in epidemiologic studies that cats spending the majority of time indoors have a higher incidence of FHT could possibly be associated to the vast number of TH-disrupting chemicals analyzed in dust. To determine the etiological importance of OC exposure for the development of FHT, more detailed biochemical mechanisms are needed to fully elucidate the mode of action behind this rather new disease, but also longitudinal studies should be useful to determine the etiology of FHT. To protect our animals, and our children, it is of outermost importance to identify and regulate the soup of EDCs in our indoor environment.

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Part II
Pets as Forecasters and Promotors of Human Health

Chapter 8
Obesity in Humans and Dogs: Similarities, Links, and Differences

Asta Tvarijonaviciute, Alberto Muñoz-Prieto, and Silvia Martinez-Subiela

Abstract Obesity is defined as an excess of adipose tissue and is considered as one of the most pervasive, chronic diseases leading to morbidity and decreased lifetime expectancy in both humans and dogs. For these reasons and because the prevalence of obesity continues to increase, the societal burden of obesity is increasing worldwide. Dog ownership has been related with improved lifestyle and lower obesity rates. However, the possible relationship between owner and dog obesity has been reported, suggesting common causes of obesity in humans and dogs. In this line, the specialists of both fields, human and veterinary medicine, agree not only about the main common causes but also about dangerous consequences of the obesity. Nevertheless, some discrepancies between human and dog obesity have also been described. For this reason, in this chapter, we aimed to look more deeply at the similarities, links, and differences between human and canine obesity. Better understanding of this disease would not only help to design obesity treatment approaches, but also, and more importantly, to prevent it in humans and dogs taking advantage of marvelous human-dog relationship.

Keywords Adipokines · Canine · Inflammation · Insulin resistance · Obesity consequences · Obesity grade · Overweight · Owner-dog relationship

8.1 Introduction

Obesity is defined as excess adipose tissue and is considered as one of the most pervasive, chronic diseases leading to morbidity and decreased lifetime expectancy in both humans and dogs. For these reasons and because the prevalence of obesity

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continues to increase, with prevalence exceeding half of general population of both humans and dogs in some countries (WHO 2018; Muñoz-Prieto et al. 2018), the societal burden of obesity is increasing worldwide.

In order to prevent health, economic, and social consequences of obesity in humans, close follow-up of obesity prevalence is performed among different countries and different health organizations, such as Organisation for Economic Co-operation and Development (OECD) or World Health Organization (WHO). Furthermore, a growing number of countries have adopted policies to prevent obesity from spreading further. These include awareness raising, health care, regulatory and fiscal measures, taxation of unhealthy food, and sugar-sweetened beverages (http://www.euro.who.int/en/health-topics/noncommunicable-diseases/obesity). In the same manner, in recent years, the increasing societal burden is being reflected in the increasing scientific literature dealing both human and canine obesity. The specialists of both fields, human and veterinary medicine, agree about the dangerous consequences of the obesity and the importance to study it further in order to better understand its pathophysiology. This knowledge would not only help to design obesity treatment approaches, but also, and more importantly, to prevent it. In addition, possibly common actions to prevent at the same time human and canine obesity, especially in dogs and owners, could be undertaken based on the studies about links and common causes of obesity in humans and dogs (Kushner et al. 2006; Bartges et al. 2017).

The main common causes and consequences have been reported for both humans and dogs. Dogs have even been suggested as an animal model for obesity investigations (Osto and Lutz 2015; Stachowiak et al. 2016). However, some discrepancies have also been described. For this reason, in this chapter, we aimed to look more deeply at the similarities, links, and differences between human and canine obesity.

8.1.1 Metabolic Syndrome

Both humans and dogs can suffer from metabolic syndrome (MetS). MetS is a metabolic disorder resulting from obesity and is diagnosed on the basis of a combination of the presence of obesity, impaired glucose metabolism, dyslipidemia, and hypertension (Eckel et al. 2005; Tvarijonaviciute et al. 2012a) (Table 8.1). While in humans the main risk factors associated with the presence of MetS are clearly defined (cardiovascular disease, type 2 diabetes, liver disease), in dogs, the doubts about usefulness of its determination still exist (Verkest 2014). However, in dogs, the presence of MetS was suggested to be related to lipid metabolism alterations, liver and lung function impairment, presence of a prothrombotic state, and a decrease in immune function (Tvarijonaviciute et al. 2019), although further studies are needed to corroborate these results.

Table 8.1 Definitions of the human and canine metabolic syndrome (MetS)

Human MetS criteria (Eckel et al. 2005)	Canine MetS criteria (Tvarijonaviciute et al. 2012a)
Generally by different organizations, reported criteria for MetS diagnosis in humans include: Overweight/obesity (more importantly central) ↑ triglycerides (or specific treatment for this lipid abnormality) ↓ high density lipoprotein (HDL) cholesterol ↑ glucose or previously diagnosed type 2 diabetes ↑ blood pressure Some criteria also suggest to determine: Microalbuminuria ↑ high-sensitivity C-reactive protein (hsCRP)	The guidelines of the International Diabetes Federation (2006) were modified in order to produce an accessible system for dogs: Overweight/obesity And two of the following: ↑ triglycerides (or specific treatment for this lipid abnormality) ↑ cholesterol ↑ glucose or previously diagnosed diabetes ↑ blood pressure

8.2 Causes

Obesity is a multi-causal disease (Ravussin and Ryan 2018). However, food-related and physical activity-related factors were labeled as "the big two" because of the importance that these exhibit in the development of obesity (Keith et al. 2006). Furthermore, genetics, environmental, and reproduction-related factors, among others, were also shown to contribute to obesity epidemic in both humans and dogs. It is important to point out that in the particular case of the dogs, different authors highlight that neglectful or misconceived strategies of the owners are the primary causes of obesity in dogs (Burkholder and Bauer 1998; Bland et al. 2009) since most of the obesity risks in dogs are related to the chosen way of life, habits, and values of the people (Muñoz-Prieto et al. 2018).

8.2.1 Genetic Susceptibility

Insights from human and animal model studies suggest that genetics, including monogenic disorders and, more recently, polygenic clues arising from genome-wide association studies (GWAS), presents a central role of the brain in the regulation of feeding behavior (Yeo and Heisler 2012). It was shown that, specifically, monogenic alterations in the melanocortin, leptin, 5-hydroxytryptamine (5-HT; serotonin), and brain-derived neurotrophic factor (BDNF) pathways could result in disturbed energy balance and body weight gain in humans (Yeo and Heisler 2012). Furthermore, mutations in proopiomelanocortin (POMC) gene have been associated with severe hyperphagia and obesity in humans, with a subsequent increase in

risk of obesity-related diseases such as type 2 diabetes (Farooqi et al. 2006). In a similar manner, in dogs, POMC gene has been associated with food motivation and obesity in Labrador Retriever and Flat-Coated Retriever dogs (Raffan et al. 2016; Mankowska et al. 2017). However, further studies are needed in order to confirm these observations, since these alterations were not detected in other obesity-prone dog breeds (Beagles, Cocker Spaniels) and have been not related to 5-point body condition score (BCS) (Mankowska et al. 2017).

Although being true that some individuals are more genetically susceptible to develop obesity, it is not correct to blame for the epidemic on genetics alone (Ravussin and Ryan 2018). Most of the increase in obesity has occurred in the past 40 years, and the genes have not changed such much to be responsible for the high rates of obesity (Hall 2018; Ravussin and Ryan 2018). Thus, other environmental-behavioral changes were suggested to be the main causal factors for the current obesity epidemic (Hall 2018).

8.2.2 Food-Related Obesity Risk Factors

Obesity occurs when energy intake exceeds energy expenditure over an extended period (Ravussin and Ryan 2018). However, not only the quantity but also the quality, availability, palatability, and food costs together with the eating behavior (snacking, eating out of home) were described to be related to the obesity development in humans (Ravussin and Ryan 2018). In a similar manner in dogs, the number of meals per day, the type of diet, and price were linked to increased risk to develop obesity (Kienzle et al. 1998; Robertson 2003; Bland et al. 2009; Mao et al. 2013). Some human-related dog feeding conduct could also predispose for canine obesity, e.g., the presence of the owner while dog is eating (Kienzle et al. 1998). In addition, the similarities between children and dog behavior related to food have been described (Pretlow and Corbee 2016). It was argued that if the owner is constantly eating, the dog may develop the habit of begging (Carballo et al. 2015), which could result in uncontrolled caloric intake leading to the development of obesity. Furthermore, there is the belief among some owners that the affection toward the dog is best reflected by giving treats or sharing their food while eating. This fact, together with the fact that the calories of the treats usually are not included in the general count, can result in increased caloric intake and subsequent development of obesity (Kienzle et al. 1998; Bland et al. 2009).

8.2.3 Physical Activity-Related Obesity Risk Factors

Humans as well as dogs with low physical activity are prone to suffer obesity. It was stated that the changes driven by behavioral factors triggered by the obesogenic environment resulted in reduction in physical activity in humans (Keith et al. 2006; Ravussin and Ryan 2018). Even a relatively small change in energy balance was

responsible for weight gain, especially, in genetically susceptible individuals (Hill et al. 2012). For this reason, reduced physical activity was stated to be the main reason of human obesity epidemic (Church and Martin 2018).

Currently, dogs are increasingly considered more as "fellows" than "pets." The "overhumanizing" of the pet dogs was suggested to result in ignorance of the some basic needs of the dog such as exercise and occupation (Kienzle et al. 1998) that, together with excessive calorie intake, results in obesity development.

Nevertheless, a recent questionnaire-based study involving ten different European countries suggested that obesity rates among dog owners are lower than the general population (Muñoz-Prieto et al. 2018). These observations could be related to the increased physical activity, since dogs were shown to present both social and physical activity support (Jennings 1997; Kushner et al. 2006; Cutt et al. 2007; Coleman et al. 2008). Furthermore, owning a dog has been suggested to be a way to combat a sedentary lifestyle by enhanced motivation for activity (Wohlfarth et al. 2013). However, the scientific basis is weak, and further studies are required in order to confirm these observations (Christian et al. 2013). And more importantly, owning a dog does not protect owner from being obese (Muñoz-Prieto et al. 2018).

8.2.4 Reproduction-Related Obesity Risk Factors

In humans and animal models, the influence of reproduction-related factors with the risk of developing obesity in offspring has been suggested. The development of obesity was related to extremes of energy imbalance in utero (both high and low birth weight) of mother or even two generations back, when oocytes were formed in the grandmother, suggesting that the environmental changes initiated one-two generations ago could be responsible for the obesity epidemics today (Keith et al. 2006; Davis et al. 2018). In addition to this, the late pregnancy age, as women delay reproduction beyond 30 years, increases risk of offspring obesity possibly due to the accelerated loss of the brown adipose uncoupling protein 1 levels in the offspring, which may act to increase white adipose tissue deposition in later life (Symonds et al. 2004; Keith et al. 2006; Davis et al. 2018). Furthermore, taken into consideration that estimated hereditability of adiposity reaches 65%, together with the increasing obesity rates and assortative mating in terms of adiposity, and the fact that positive relation between BMI and number of offspring exists, an increased frequency of the genotypes susceptible to obesity in the following generations would be expected (Davis et al. 2018).

In dogs, possibly because of the lack of knowledge, neutering is the most important reproduction-related obesity risk factor (Lund et al. 2006; Sandøe et al. 2014). The main reasons related to body weight gain after neutering are increased food consumption, decreased metabolic rate, and reduced activity (Houpt et al. 1979; Sloth 1992; Robertson 2003). For this reason, warning the owner that neutered dogs are more likely to develop overweight/obesity than entire ones is of high importance. However, it is also important to highlight that neutered dogs can be maintained at their ideal body weight through careful feeding and adequate exercising.

8.2.5 Environmental Obesity Risk Factors

In scientific literature, there are evidences that accessibility to and quality and availability of green spaces together with their safety have a direct effect on physical activity, a key mediator of obesity (Swinburn et al. 1999; Lee and Maheswaran 2011). On the other hand, the increase in atmospheric carbon dioxide (which promotes wakefulness and increased energy intake via increased secretion of the orexin as a result of decreased pH in the organism) and the use of thermoregulation systems to reach the thermoneutrality (resulting in declined energy waists) were suggested to further contribute to obesity spreading among people (Keith et al. 2006). Although similar studies in dogs are lacking, it could be postulated that these factors could also be risks for canine obesity development, since dogs share the same environment as humans.

Furthermore, in the last years, the term "obesogenic environment" has emerged referring to "the sum of influences that the surroundings, opportunities, or conditions of life have on promoting obesity in individuals or populations" (Swinburn et al. 1999). This definition includes societal (aging population, urbanization), economical (work opportunities, economic disparity and insecurity, national gross domestic product (GDP)), physical (high mechanization, sedentary lifestyle), and nutritional (consumption of processed high-energy food) drivers shifting the focus of this disease from the individual to the systemic level (Dempsey et al. 2018).

Table 8.2 includes some additional possible contributors of the obesity epidemics. Although these factors are frapped in uncertainty and contradictions, at least in part they should be considered as possible causal factors of increased obesity rates in the general population over the last decades (Keith et al. 2006; Davis et al. 2018).

8.3 Obesity Grade Measurements

One of the cornerstones of obesity diagnostic and monitoring is an accurate determination of body adipose tissue content (Baldwin et al. 2010; Freeman et al. 2011). However, measurement of adipose tissue is not simple because it is not perfectly compartmentalized being tightly interconnected with the lean tissue. For the determination of obesity, the main conceptual division of importance is between body fat (BF) (Armstrong 1996) and lean body mass (LBM) (Burkholder 2001). The correct measurement of body condition is basic for a correct diagnosis and even more important for the weight loss monitoring to ensure that the BF, and not the LBM or bone mineral content, is lost (Diez et al. 2002).

A number of methods were developed to assess body condition in both humans and dogs. From a practical point of view, they can be divided into (1) *simple*, which are easy to perform and non-invasive and do not need specialized personnel or equipment; thus, they are economic; and (2) *complex*, which usually require specialized personnel and/or equipment. The most frequently used methods in clinic and investigation are disclosed in Table 8.3.

Table 8.2 Additional risk factors suggested contributing to the obesity development

Obesity causes	Described in humans	Described in dogs
Age	Obesity rates were positively related with age. Taken into account that in the last decades the proportion of older people have increased, the obesity epidemics at least in part could be attributed to this fact (Keith et al. 2006; Muñoz-Prieto et al. 2018)	The age of dogs and the age of the owner were associated with an increased probability of dogs being overweight (Muñoz-Prieto et al. 2018)
Behavioral Smoking Sleep debt	Since smoking has anorexigenic effects and increases metabolism, in the last decades, observed decreasing smoking could possibly contribute to the obesity epidemic (Davis et al. 2018). Nevertheless, negative health effect of smoking is profound, and its cessation should be further recommended. Decreased sleeping hours were related to increased hunger and appetite leading to increased risk of adiposity, and inversely correlated to BMI (less sleeping hours – higher BMI) (Reutrakul and Van Cauter 2018).	The dogs owned by the smokers were shown to be prone to obesity development. This could be related to the unhealthy habits of the owner (Muñoz-Prieto et al. 2018)
Gut microbiome	The possible mechanisms resulting in obesity development in persons were suggested to include an increase in energy harvest, modulation of free fatty acids (e.g., butyrate), lipopolysaccharides, gamma-aminobutyric acid (GABA), an impact on Toll-like receptors, the endocannabinoid system, and "metabolic endotoxinemia" as well as "metabolic infection" (Harsch and Konturek 2018)	Different composition of gut microbiota was described in obese dogs when compared with normal weight ones (Handl et al. 2013). Furthermore, weight loss was associated with modifications in microbiota composition (Kieler et al. 2017)
Infections	Although contradictory results exist, adenovirus 36 was suggested to accelerate the differentiation and proliferation of pre-adipocytes (Davis et al. 2018)	

8.3.1 Complex Body Condition Measurement Methods

The complex methods for body condition determination are highly accurate, precise, and objective; for these reasons, they are considered as reference methods in both humans and dogs (Burkholder and Toll 2000). However, they are mainly used for investigation purposes being limited in clinical practice due to their difficult performance and cost or time required. The mostly used methods include dual energy X-ray absorptiometry, deuterium oxide dilution, and bioelectrical impedance.

8.3.1.1 Dual-Energy X-Ray Absorptiometry (DEXA)

This method determines simultaneously the BF, LBM, and bone content (Pietrobelli et al. 1996). It is based on the fact that the emitted energy is absorbed at different degrees relative to the type of tissue it encounters, thus enabling clear imagining of

Table 8.3 Pros and cons of the most regularly used methods for body condition measurements

Type	Method	Humans		Dogs	
		Pros	Cons	Pros	Cons
Complex	DEXA	Accurate determination of the body fat, lean mass, and bone content (Rothney et al. 2009; Shiel et al. 2017)	Expensive, not practical or applicable in routine analysis (Laflamme 1997; Munguia-Izquierdo et al. 2018)	Accurate for assessing body fat and lean mass, small radiation dose, fast (Speakman et al. 2001)	Requires sedation or anesthesia, expensive, depends on lean tissue, hydration status, positioning accuracy, and patient position (Speakman et al. 2001; Santarossa et al. 2017)
	D_2O	Accurate estimation of total body weight, acceptable in all age groups (Wells and Fewtrell 2006)	Delayed results, inaccurate in diseases that affect hydration status (Wells and Fewtrell 2006)	Noninvasive, accurate estimation of BF indirectly (Burkholder and Thatcher 1998)	Depends on hydration status (Burkholder and Thatcher 1998)
	BIA	Noninvasive, simple and practical (Kopelman 2000)	Reliability of this method could be affected by the quality of electrodes used, intra-variability, body temperature, fasting, bladder condition, body positioning, menstrual cycle, etc. (Sergi et al. 2017)	Noninvasive, correlates with DEXA (German et al. 2010)	Poor precision and accuracy in dogs with high percentage of body fat; depends on hydration status (German et al. 2010)
Simple	Body weight	Objective, repeatable, can identify changes overtime	Does not quantify fat versus lean mass	Easy to perform, can indicate weight change (German and Morgan 2008)	Does not quantify fat versus lean mass, scales not calibrated (German and Morgan 2008)

(continued)

Table 8.3 (continued)

Type	Method	Humans		Dogs	
		Pros	Cons	Pros	Cons
	Anthropometric measures	Easy to use, correlates strongly with densitometry estimation of body fat percentage (Kopelman 2000)	Subjective, does not discriminate body fat from lean mass (Kopelman 2000)	Easy to use, estimates body fat percentage (Witzel et al. 2014)	Subjectivity in each determination (Witzel et al. 2014)
	Morphologic scales	Not used	Not used	Easy to use, conveys degree of body fat (Laflamme 1997; Witzel et al. 2014)	Highly subjectivity (Laflamme 1997; Witzel et al. 2014)

DEXA Dual-energy x-ray absorptiometry, *D_2O* Deuterium oxide dilution, *BIA* Bioelectrical impedance analysis

different tissues (fat mass (FM), LMB, and bone) (Rothney et al. 2009). This technique is considered the most extended system for determination of the body composition in humans (Shiel et al. 2017). In a similar manner, DEXA is a criterion-referenced method for determining bone mineral content and body composition in vivo in dogs (Speakman et al. 2001).

8.3.1.2 Deuterium Oxide Dilution (D_2O)

The fundament of this method is that body water is predominantly associated with nonfat tissue; therefore, a measure of total body water provides an indirect measure of fat-free mass. In humans, the D_2O is administrated, and, following equilibrium, the enrichment of the body water pool is measured in samples of either saliva, urine, or blood (Davies and Wells 1994). This is the best option to use in populations, where the normal hydration is known or can be assumed, being simple to carry out (Wells and Fewtrell 2006).

In dogs, D_2O dilution technique for the estimation of the body composition has been validated by Burkholder and Thatcher (1998) and is considered as a referenced method. In this species, the D_2O content is determined in serum samples obtained 2–4 hours after its administration, which then is used in an equation to calculate total body water, LBM, and FM (assuming that adipose tissue has minimal water content and that lean BM consists of 73% water) (Zanghi et al. 2013).

8.3.1.3 Bioelectrical Impedance

Bioelectrical impedance analysis (BIA) has emerged as a valid alternative for the assessment of body composition, given that it is relatively fast and inexpensive as compared to DEXA or D_2O, or even advanced technique images (such as magnetic resonance) (Sergi et al. 2017). Since it does not expose subjects to radiation risks, it can be repeated safely during the follow-up. Furthermore, because of its fast turn-around time, a large number of individuals can be examined in a short period of time (Böhm and Heitmann 2013). However, like other techniques, BIA represents some disadvantages when estimating body composition. These include factors that can influence the reliability of the measure: instrument-related factors (quality of electrodes, intra-variability), technician-related factors, subject-related factors (body temperature, fasting, bladder condition, body positioning, menstrual cycle, etc.), and environmental-related factors (temperature) (Sergi et al. 2017).

BIA has been used in dogs as a safe, noninvasive, and rapid method for assessing body composition (Santarossa et al. 2017); however, further studies are needed to validate this method for use in dogs since it was shown that BF can vary depending on electrode positioning, dog skin, different breed conformation, air temperature, etc., leading to both BF underestimation and overestimation (German et al. 2010).

8.3.2 Simple Body Condition Measurement Methods

8.3.2.1 Body Weight

Determination of body weight (BW) consists in an objective measure that is repeatable and capable of identifying changes over time. This tool exemplifies a simple technique, which is costless and, therefore, of open access for all people, although it is not capable of measuring the BF and LBM. In a similar manner, in dogs, the BW relative to the ideal BW or breed standard is used as a defining criterion for obesity (Burkholder 2001). However, the problems of using BW scale in dogs is that BW varies within breeds, and even more difficulties appear when the dog is mongrel, making it impossible to determine the body composition by this way alone (Burkholder 2001).

8.3.2.2 Anthropometric Measures

The most popular anthropometric measurements for body condition determination are the calculation of body mass index (BMI), the waist circumference (WC), and the skinfold thickness.

Body Mass Index

The BMI was firstly described by Quetelec in the nineteenth century and is the most widely applied system to study the overweight/obesity epidemiology in humans (Kopelman 2000). The BMI is calculated following the equation (Eq. 8.1):

$$BMI = \frac{Body\ weight\left(kg\right)}{\left[Height\left(m\right)\right]^2} \tag{8.1}$$

BMI individuals are categorized as underweight (BMI <18.5), normal weight (BMI 18.5–25.0), overweight (BMI 25.0–30.0), obese (BMI 30.0–40.0), and morbidly obese (BMI >40) (Kopelman 2000). This method correlates strongly with BF% through densitometry measurements, but it fails to discriminate between BF% and LBM (Kopelman 2000; Romero-Corral et al. 2008).

In the case of dogs, the attempt to calculate the BMI was made by different authors following the formula (Burkholder and Toll 2000) (Equation 8.2):

$$Canine\ BMI = \frac{Height\ at\ shoulder\left(cm\right)}{Height\ at\ shoulder\left(cm\right) * Body\ length\left(cm\right)} \tag{8.2}$$

where body length is the distance from the occipital protuberance to the base of the tail.

Furthermore, sex-specific morphometric measurement-based equations for BF percentage have been published (Equations 8.3 and 8.4):

$$Male\ BF\% = \left(-1.4 * TS\left(cm\right)\right) + \left(0.77\ XPC\left(cm\right)\right) + 4 \tag{8.3}$$

$$Female\ BF\% = \left(-1.7 * TS\left(cm\right)\right) + \left(0.93 * PC\left(cm\right)\right) + 5 \tag{8.4}$$

where TS is the distance from the tibio-tarsal joint to the stifle joint in cm and PC is the pelvic circumference at the level of the flank in cm. These sex-specific equations for BF percentage showed a good correlation (r = 0.948) with DEXA (Mawby et al. 2004).

The morphometric measurements in dogs have some limitations that should be taken into consideration. These include variability associated to the different conformation and size of different dogs (Witzel et al. 2014). In addition, this technique requires up to 10 min to accurately obtain the measurements, and the identification of the anatomical landmarks, and, therefore, results can differ among investigators resulting in bias (Burkholder 2001).

Waist Circumference

The waist circumference provides a simple and practical anthropometric measure for assessing central adiposity (Wei et al. 2006; Wells and Fewtrell 2006) and is determined by measuring at midpoint between lower border of ribs and upper border of the pelvis (in cm) in human beings (Kopelman 2000). Actually, an increasing

Table 8.4 Waist circumference action levels related to metabolic complication in humans

Level	Description	Men	Women
1	Increased risk	≥94 cm	≥88 cm
2	Substantially increased risk	≥102 cm	≥88 cm

Adapted from Kopelman (2000)

number of studies are reporting strong associations between the waist circumference, visceral adipose tissue, and obesity-related health risks (Hill et al. 1999; Sidney et al. 1999). In addition, it has been reported that the waist circumference is a better predictor of metabolic abnormalities than percent BF measured by bioimpedance method (Sidney et al. 1999) and correlates with metabolic syndrome indicators better than BF(%) determined by DEXA or BMI (Wei et al. 2006). Furthermore, different authors have suggested that the waist circumference, either singly or in combination with BMI, may have a stronger relation to some health outcomes than BMI alone (Han and Lean 1998; Janssen et al. 2004). Waist circumference action levels related to the metabolic complications are shown in Table 8.4. Nevertheless, using this system, the intra-abdominal (visceral) and intramuscular fat cannot be determined.

In dogs, although the waist circumference is considered for the BMI calculation (Formulas 8.3 and 8.4), this measurement alone is not used for overweight/obesity determination and/or monitoring.

8.3.2.3 Morphologic Scales

Morphologic scales are based on observation and palpation, and, unlike humans, they are commonly used in dogs due to its simplicity and fast performance. These methods include body condition score (BCS) and body fat index (BFI), which are currently the most used methods for body condition determination in dogs in investigation and clinics.

BCS is a subjective, semi-quantitative assessment of body composition that uses the body frame independent of BW (Burkholder 2001) with a range of categories from cachectic to severely obese (Mawby et al. 2004). This system consists in visual and palpation assessment of BF at various locations in the body (German et al. 2010) such as rib cage, pelvic bones, lumbar area, abdomen, and waist (Laflamme 1997). Different charts exist for BCS determination in dogs; the most frequently used ones are 9-point and 5-point scales and a BFI, which complements the first two (Table 8.5). All of them are based on palpable and visual characteristics. The 9-point scoring system and BFI were shown to present a strong correlation with DEXA in dogs (Laflamme 1997), while the 5-point scoring system was validated with D_2O (Paetau-Robinson et al. 2017). BFI is a relatively new method for BF determination and was designed to complement the BCS scales showing more accurate results when estimating dogs with BF up to 65% (Witzel et al. 2014).

Table 8.5 Different body composition scales to estimate the body fat percentage in dogs

	Corporal body fat	9-point BCS (Laflamme 1997)	5-point BCS (Laflamme 1997)	BFI (Witzel et al. 2014)	Description (Freeman et al. 2011)
Underweight	<5%	1/9	1/5		Ribs, lumbar vertebrae, pelvic bones, and all bony prominences evident from a distance. No discernible body fat
	5–9%	2/9			Ribs, lumbar vertebrae, and pelvic bones easily visible. No palpable fat. Some evidence of other bony prominence
	10–14%	3/9	2/5		Ribs easily palpated. Tops of lumbar vertebrae visible. Pelvic bones becoming prominent. Obvious waist and abdominal tuck
Normal	15–19%	4/9			Ribs easily palpable, with minimal fat covering. Waist easily noted (from above). Abdominal tuck evident
	20–24%	5/9	3/5		Ribs palpable without excess fat covering. Waist observed behind ribs when viewed from above. Abdomen tucked up when viewed from side
Overweight	25–29%	6/9	4/5	30	Ribs palpable with slight excess fat covering. Waist is discernible viewed from above but is not prominent. Abdominal tuck apparent
	30–34%	7/9			Ribs palpable with difficulty; heavy fat cover. Noticeable fat deposits over lumbar area and base of tail. Waist absent. No abdominal tuck. Obvious abdominal distention may be present
Obese	35–39%	8/9	5/5	40	Ribs not palpable under very heavy fat cover, or palpable only with significant pressure. Heavy fat deposits over lumbar area and base of tail. Waist absent. No abdominal tuck
	40–45%	9/9			Massive fat deposits over thorax, spine, and base of tail. Waist and abdominal tuck absent. Fat deposits on neck and limbs. Obvious abdominal distention
	46–55%			50	
	56–65%			60	
	>65%			70	

BCS body condition score, *BFI* body fat index

8.4 Analytical Alterations

8.4.1 Lipids

Many studies in humans and dogs have shown that obesity is associated with dyslipidemia. Dyslipidemia is an abnormal amount of lipids, such as cholesterol and triglycerides, in the blood and is a widely accepted risk factor for cardiovascular disease in humans. In humans, obesity-related dyslipidemia is primarily characterized by increased levels of plasma free fatty acids and triglycerides, decreased levels of high-density lipoprotein (HDL), and abnormal low-density lipoprotein (LDL) composition (Jung and Choi 2014). In obese individuals, adipose tissue (mainly intra-abdominal) releases increased amounts of non-esterified fatty acids (NEFAs) that provide substrate for triglyceride synthesis in the liver and for triglyceride-rich very low-density lipoprotein assembly and secretion (Bailhache et al. 2003). At the same time, increased hepatic lipase activity (usually found in the state of insulin resistance) reduces HDL cholesterol levels (Bailhache et al. 2003).

In canine obesity, modifications of the lipoprotein profile are also common changes. However, the alterations observed differ from those in humans since the circulating concentrations of both LDL and HDL-cholesterol are usually increased in obese dogs (Tvarijonaviciute et al. 2010b; Tribuddharatana et al. 2011). Although some speculations exist, it is not clearly the exact explanation for these differences between dog and human lipid metabolism.

8.4.2 Adipokines

Adipokines are the cytokines produced and secreted by white adipose tissue, which directly or indirectly modulate different metabolic processes in the organism (Osto and Lutz 2015). Although numerous adipokines have been described, the most studied ones are leptin and adiponectin (Clark and Hoenig 2016).

Leptin is abundantly expressed in adipose tissue and is involved in the regulation of energy homeostasis (Friedman and Halaas 1998). It inhibits appetite and food intake and stimulates energy expenditure (Friedman and Halaas 1998). However, circulating leptin levels are increased in obese humans and dogs probably due to the existence of leptin resistance (Considine et al. 1996; Friedman and Halaas 1998; Hoenig 2014). This resistance and the concurrent reduction of the body's energy metabolism may contribute to further weight gain in obese subjects because the brain is unable to adequately "measure" the body's adipose tissue reservoirs (Osto and Lutz 2015).

Leptin is positively correlated with fat mass in humans and dogs (Considine et al. 1996) being considered as a marker of adiposity in both species (Ricci and Bevilacqua 2012). It increases and decreases in response to weight gain and weight loss, respectively, but also increases more acutely in response to food intake (Weigle

et al. 1997; Romon et al. 1999; Ishioka et al. 2005; Jeusette et al. 2005, 2006). It is important to be cautious with the interpretation of plasma leptin concentrations because of the influence that several conditions could have on their concentrations. Thereby factors such as fasting (24–72 hours) (Kolaczynski et al. 1996), emotional stress (Otsuka et al. 2006), physical exercise (Hickey et al. 1997), testosterone and estrogen levels (Ahima and Flier 2000), or dexamethasone administration (Considine et al. 1997) have been shown to effect circulating leptin values. Similarly, circadian rhythm, feeding state, treatments, and thyroid gland activity (Ricci and Bevilacqua 2012) have been reported to influence leptin concentrations in dogs. Particular attention should be paid with regard to feeding state since serum leptin concentrations increase up to 5 folds in response to food intake (Ishioka et al. 2005). Thus, concentrations of this adipokine should be strictly determined in fasting, treatment, and, if possible, stress-free patients.

Adiponectin is an adipokine synthesized and secreted mainly by the adipose tissue, but recently other tissues, although in a lower level, were shown to secrete this protein (Katsiougiannis et al. 2006). Adiponectin in blood circulates in three different forms: trimer, hexamer (which is also called low-molecular-weight oligomer (LMW)), and high-molecular-weight multimers (HMW). All forms have different biological activities, and HMW is thought to be the most active form (Sinha et al. 2007). Several studies have shown that obese humans have significantly lower adiponectin concentrations (mainly HMW) than non-obese subjects and that adiponectin negatively correlates with body fat accumulation in both men and women (Arita et al. 1999). Hypoadiponectinemia in obese people is a consistent finding that results in loss of protective insulin sensitization, enhanced fatty acid metabolism, and anti-inflammatory actions of adiponectin (Ouchi et al. 2011). However, studies in dogs show variable results for adiponectin concentrations in obese dogs. Some studies, in a similar way to what occurs in humans, describe lower circulating adiponectin concentrations in obese dogs and detect negative correlation between this adipokine and fat mass, while others do not detect significant differences (Ishioka et al. 2006; Tvarijonaviciute et al. 2010a; Verkest et al. 2011b). Discrepancies also exist with regard to adiponectin concentrations after weight loss since some studies reported an increase in circulating adiponectin (Tvarijonaviciute et al. 2012a, b) where others found no effect in dogs (Wakshlag et al. 2011; Bastien et al. 2015). The possible explanation for all these discrepancies may be related with (1) the studied populations, since adiponectin presents high inter-individual variability, degree of obesity, and weight loss "amount" (as in humans, the decrease of a minimum of 10% of weight was shown to be necessary to detect improvement in adiponectin concentrations), and with (2) the different methods used for its determination in terms of the assay sensitivity and performance (Verkest et al. 2011b; Tvarijonaviciute et al. 2012a, c; Clark and Hoenig 2016). Furthermore, adiponectin decreases in inflammation and is affected by sex hormone changes in female dogs (Tvarijonaviciute et al. 2011a, 2013a). On the other hand, contrarily to leptin, adiponectin concentrations do not appear to be influenced by diurnal, fasting-eating/feeding cycles in humans or dogs (Merl et al. 2005; Tvarijonaviciute et al. 2012c).

It must be pointed out that although initially restricted to metabolic activities (regulation of glucose and lipid metabolism), adipokines currently represent a new family of proteins that can be considered key players in the complex network of soluble mediators involved in the pathophysiology of immune/inflammatory diseases (Francisco et al. 2018). Thus, in order to evaluate concentrations of adipokines, including adiponectin and leptin, in obesity, the inflammatory status of a patient should be taken into account in both humans and dogs (Yilmaz et al. 2008; Francisco et al. 2018).

8.4.3 Inflammatory Biomarkers

Obesity is associated with pathological changes in adipose tissue morphology, including infiltration of immune cells, and obese individuals have higher circulating levels of inflammatory markers than lean individuals (Herder et al. 2005, 2006).

The inflammatory process that occurs in obese people differs from the classical inflammatory response in certain aspects. This inflammatory process manifests itself systemically and is characterized by a chronic low-intensity reaction. In this context, the Toll-like receptor (TLR4) signaling pathway has been recognized as one of the main triggers in increasing the obesity-induced inflammatory response. This pathway responds to the increased exposure to saturated fatty acids and to LPS. Both of these are relevant in the context of obesity, with saturated fatty acids arising from within the adipose tissue triglyceride stores and the LPS arising from increased intestinal permeability perhaps due to an altered gut microbiota (Rogero and Calder 2018; Stephens et al. 2018).

Adipose tissue contains adipocytes (see above) and infiltrated macrophages, both of which release a spectrum of inflammatory mediators, including acute-phase proteins (like PAI-1), cytokines (like IL-6, TNFα), and chemokines (like MCP1). Consequently, circulating levels of inflammatory markers are elevated in human obese subjects and associate with obesity-related parameters (Weisberg et al. 2003; Kim et al. 2006; Maury et al. 2009; Maury and Brichard 2010).

Unlike humans, the involvement of inflammatory markers in canine obesity and weight loss is less clear. There is one report which showed that the cytokines and chemokines IL-6, MCP-1, and TNFα are expressed in adipose tissue depots of dogs (Ryan et al. 2010). Recently, concentrations of interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1), but not IL-8, were found to be increased in overweight dogs (Frank et al. 2015), whereas another study found decreasing concentrations of IL-8 and other interleukins with weight loss in dogs (Bastien et al. 2015). C-reactive protein (CRP) and MCP-1 have been reported to decrease with weight loss in some but not in other studies (German et al. 2009; Wakshlag et al. 2011; Tvarijonaviciute et al. 2012b; Bastien et al. 2015). Evidences exist that acute weight gain does not produce significant changes in positive acute-phase proteins in the dog (Tvarijonaviciute et al. 2011b). However, chronic obesity could predispose the development of inflammation (Tilg and Wolf 2005; Tvarijonaviciute et al. 2012a).

8.4.4 *Insulin Resistance, Hyperinsulinemia, and Glucose Concentrations. Relationship with Diabetes Mellitus*

One of the most well-recognized changes that occur with excess of the adiposity is insulin resistance, or a diminished cellular response to a given plasma insulin concentration (Saltiel and Kahn 2001). In humans, it has been shown that obesity leads to insulin resistance in all three major metabolic organs (adipose tissue, muscle, and the liver) (Conte et al. 2012). That is, in obese individuals, the responses to insulin in these organs are blunted, and higher concentrations of insulin are needed to keep plasma glucose and free fatty acid concentrations within normal limits. During everyday conditions, these higher concentrations of insulin are supplied endogenously: plasma insulin concentrations in obese humans are 20–50% greater than in lean humans (Conte et al. 2012).

Insulin sensitivity and resistance are classically assessed by the euglycemic–hyperinsulinemic clamp (EHC), in which insulin is infused at a variable rate to keep blood glucose within a set of predetermined parameters. The infusion rate necessary to accomplish this is an indicator of the response of peripheral tissues to insulin, that is, the lesser insulin necessary to control blood glucose, the more insulin-sensitive the individual (Clark and Hoenig 2016).

Insulin resistance with elevated fasting plasma insulin concentration and insulin to glucose ratio has also been demonstrated in obese dogs (Gayet et al. 2004; German et al. 2009). In addition, an improvement of insulin sensitivity after successful weight loss has been reported (German et al. 2009). Traditionally, compensatory hyperinsulinemia has been thought to result from an increase in beta cell mass (Weir et al. 2001; Saisho et al. 2013). However, decreased insulin clearance has been shown in obese, hyperinsulinemic dogs (Ader et al. 2014). Insulin resistance has also been demonstrated in obese dogs using EHC (Mattheeuws 1984), and decreased glucose clearance in obese dogs, starting at gain of 40% over lean body weight, has been shown using intravenous glucose tolerance tests (Bailhache et al. 2003).

Despite peripheral insulin resistance, obese dogs are able to maintain normal plasma glucose concentrations for extended periods of time. In addition, plasma glucose concentrations do not necessarily rise with obesity or decline with weight loss in dogs (Mattheeuws 1984; German et al. 2009; Tvarijonaviciute et al. 2012a) since although one study reported fasting hyperglycemia in some obese dogs (Tvarijonaviciute et al. 2012a), others did not find this (Verkest et al. 2010, 2011a, 2012).

Obesity is a known risk factor for type 2 diabetes mellitus in humans. Insulin resistance is thought to be involved in this predisposition, and it has been postulated that the increased secretory demand associated with obesity-induced insulin resistance eventually leads to depletion of pancreatic insulin stores and beta-cell exhaustion (Prentki and Nolan 2006). However, the latter mechanism has been demonstrated primarily in situations of preexisting beta-cell compromise, and many humans never develop diabetes mellitus, despite years of insulin resistance. Therefore, long-term

peripheral insulin resistance is not a sole prerequisite for the progression to a diabetic state, and concurrent beta-cell functional compromise must be present.

The relationship between obesity and diabetes mellitus in dogs is less clear; dogs might be protected from the development of type 2 diabetes by either compensating adequately for obesity-induced insulin resistance or by additional factors. Obese dogs appear to compensate for years of insulin resistance by maintaining high fasting insulin concentrations and an increased first-phase insulin secretion during glucose tolerance tests (Verkest et al. 2012). Other factors may also protect dogs from obesity-induced diabetes, i.e., factors that are involved in the pathophysiology of β-cell failure in humans and cats but not in dogs. The most important of these factors may be the lack of amylin-derived islet amyloid in canine diabetes since contrarily to humans and cats, canine amylin does not aggregate and does not form pancreatic islet amyloid in diabetes. Therefore, because islet amyloid is absent in obese and diabetic dogs, this species may be protected from the development of diabetes mellitus in obesity (O'Brien et al. 1990; Jordan et al. 1990).

8.4.5 Other Biomarkers

8.4.5.1 Cholinesterases (ChEs)

Butyrylcholinesterase (BChE, EC 3.1.1.8) is a non-specific ChE since although it hydrolyzes butyrylcholine at a higher rate, it also hydrolyzes acetylcholine and propionylcholine. BChE presents a significant positive correlation with the BW, BMI, and different serum analytes related to adiposity, such as triglycerides and total cholesterol (Randell et al. 2005; Calderon-Margalit et al. 2006; Iwasaki et al. 2007). Similarly, a correlation between BChE and the cholesterol profile and triglycerides has been reported in canine obesity (Tvarijonaviciute et al. 2010b, 2013b). In dogs, and experimentally induced overweight/obesity models, it was associated with the increased activity of BChE in serum (Tvarijonaviciute et al. 2010b) decreasing after successful weight loss (Tvarijonaviciute et al. 2013b). As a mechanism, it has been proposed that an incremental flux of free fatty acids from adipose tissue to the liver might stimulate the hepatic synthesis of plasmatic BChE (Cucuianu et al. 2002). On the other hand, increased activity of BChE was related to reduced levels of acetylcholine, an anti-inflammatory molecule, predisposing overweight in humans and dogs to local and systemic inflammatory disease (Das 2007). Thus, increased activity of BChE could be considered another factor responsible for the low-grade inflammatory status development in chronic overweight/obesity in both humans and dogs.

8.4.5.2 Ghrelin

Ghrelin is a peptide that has a unique structure with 28 amino acids secreted mainly in the stomach and is the only known circulating appetite-stimulating (orexigenic) factor being a potent stimulator of food intake and growth hormone secretion

(Álvarez-Castro et al. 2013; Dodds 2017). Ghrelin circulates in the bloodstream in two different forms: acylated and desacylated. It was thought that acylated ghrelin was the only active form of ghrelin, but some studies showed that desacylated ghrelin has also multiple biologic activities and can even counteract some of the metabolic responses of the acylated ghrelin (Gauna et al. 2005; Liu et al. 2008). Therefore, it was suggested that measuring acylated and desacylated, and total ghrelin separately, might provide further information on the role of ghrelin in the regulation of glucose homeostasis (Ukkola 2011).

Blood levels of ghrelin rise during fasting and fall rapidly after a meal consumption, indicating that ghrelin output is regulated by caloric intake (Klok et al. 2007). In humans, plasma levels of this peptide were inversely correlated with body weight being reduced in obesity possibly due to its hyposecretion (Álvarez-Castro et al. 2013). Similarly to that described in humans, obese dogs presented lower plasma ghrelin, while weight loss results in an increase in its plasma concentrations (Jeusette et al. 2005). In addition, studies in dogs suggested that ghrelin is more influenced by BW than by food consumption in this species (Jeusette et al. 2005).

It is important to highlight that care must be taken when measuring and interpreting ghrelin concentrations since their levels are altered by the fast/meal (Klok et al. 2007; Álvarez-Castro et al. 2013) and acylated ghrelin is highly unstable in both humans and dogs (Kanamoto et al. 2001).

8.5 Consequences

8.5.1 Health

Obesity was associated with the negative health outcomes in both humans and dogs resulting in decreased health-related quality of life (HRQoL) (German et al. 2012; Olszanecka-Glinianowicz et al. 2014; Yam et al. 2016) (Muñoz-Prieto et al. 2018).

The main mechanisms that lead obesity to cause divergent diseases can be grouped into two main groups:

1. *Mechanical*. Increasing body mass due to increasing fat is accompanied by excessive load to the joints, resulting in orthopedic diseases, restriction of the respiratory execution resulting in altered respiratory function, increased pressure due to visceral fat that worsens incontinence, and alterations in total blood volume leading to cardiac dysfunction (Kopelman 2000; German 2006; Raffan 2013).
2. *Metabolic*. Adipose tissue is an active endocrine organ that secretes a number of metabolically active analytes. Chronic excess of white adipose tissue results in an imbalance in the insulin-metabolism-related and pro- and anti-inflammatory molecules (see Sect. 8.4 for more details) leading to insulin resistance in both humans and dogs (Kopelman 2000; German 2006; Raffan 2013).

Table 8.6 Obesity-related pathologies in humans and dogs

Obesity-related disorders
Metabolic abnormalities
Endocrinopathies
Orthopedic disorders
Cardiovascular disease
Respiratory system dysfunction
Urogenital system
Neoplasia
Decreased life span

In the long run, the interaction of the two mechanisms, mechanical and metabolic, results in the impairment of different organ systems increasing the probability to suffer obesity-related diseases and decreased life span of both obese humans and dogs (Table 8.6) (Kealy et al. 2002; Weeth 2016; Fruh 2017). It is important to highlight that approximately 20% of all cancer cases in humans, including those of gastrointestinal and urinary tract among others, were attributed to the excess of weight (Wolin et al. 2010; Boutari and Mantzoros 2018). Although, to date, the mechanisms linking obesity and cancer were not fully elucidated, evidences exist that chronic insulin resistance, inflammation, and increased growth factor production due to increased adiposity could be the main mechanisms (Donohoe et al. 2017; Boutari and Mantzoros 2018; Stone et al. 2018).

8.5.2 Economic

A number of studies in different countries were performed to estimate the economic consequences of human obesity (van Baal et al. 2008; Swinburn et al. 2011; Cawley and Meyerhoefer 2012; Cawley 2015; Kim and Basu 2016; Biener et al. 2017; Fallah-Fini et al. 2017), while information about the health-care costs of obese dogs is very scarce (Bomberg et al. 2017). According to Biener et al. (2017), the estimates of the obesity-related medical costs are important because they are necessary (1) to calculate the cost-effectiveness of obesity prevention programs, treatments, and policies; (2) to target weight loss programs; and (3) to correct public policy making.

In humans, the medical costs in US health-care system rise highly in obese individual, in average $3429 per year (in 2013 dollars) (Biener et al. 2017). Overall, it was estimated that 20.6% of US national health expenditures are spent for obesity-related disease treatment (Cawley and Meyerhoefer 2012). However, it is important to highlight that expenditures related to BMI are not linear, but are J-shaped (Fig. 8.1). This means that expenditures are almost equal for normal weight and overweight persons and rise rapidly for the BMI range 35–40 and especially for those with BMI >40 (Biener et al. 2017). The McKinsey Global Institute (2014) estimated that the global impact of obesity is about $2.0 trillion (2.8% of

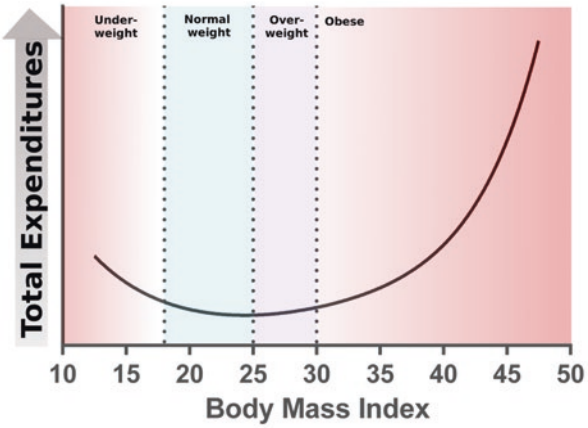

Fig. 8.1 Predicted relationship between body mass index and annual medical care expenditures for adults in the USA. (Adapted from Biener et al. (2017))

worldwide GDP), an amount almost equal to that of smoking and war, violence, and terrorism. Furthermore, the increased morbidity of the obese persons results in higher absenteeism rates, or workers who still assist to work are less productive, the fact that was estimated to have additional $1.1 trillion per year economic impact (Witters and Agrawal 2011).

In canine obesity, in a similar manner as described in humans, the average medical costs were 17% higher than of normal weight dogs (Banfield Applied Research and Knowledge. 2015). Bomberg et al. (2017) estimated that in the USA, obesity-related costs in dogs could reach $76.68 billion.

8.6 Conclusions

In this chapter, we have discussed the main causes of obesity in human and dogs, and we have highlighted the important problems that are associated with this condition. There are many links between human and canine obesity both in causes and consequences and in physiopathological mechanisms. In particular, the dog ownership constitutes a very interesting model to study the relations between human and canine obesity. One of the factors to combat obesity and increase well-being was reported to own a dog (Christian et al. 2013; Mubanga et al. 2017). However, it is also true that not in all cases this works, and furthermore, the presence of obesity in dogs due to the owner behavior with respect to dog routine care was reported, and the possible relationship between owner and dog obesity was suggested (Muñoz-Prieto et al. 2018). Further efforts in the prevention of human and canine obesity should be performed with a special focus in the benefits that the relationship human-dog can have in decreasing obesity and improving lifestyle in both humans and dogs.

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Chapter 9
Canine and Feline Spontaneous Mammary Tumours as Models of Human Breast Cancer

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Abstract The frequency of cancer presents an increasing trend in humans and companion animals, and despite recent advances in diagnosis and treatment, it remains a major cause of morbidity and mortality in human and veterinary medicine. The epidemiological and clinicopathological similarities between spontaneous tumours of companion animals and their human counterparts make them suitable natural models for human cancer research. Moreover, the faster progression of cancer in dogs and cats in comparison with humans, associated with the shorter life span of companion animals, enables faster data retrieval than in human malignancies. Furthermore, the health effects associated with exposure to environmental hazardous materials, including cancer, occur similarly in companion animals and humans; consequently, in an epidemiological context, dogs and cats can also be useful as sentinels of human malignancies. For these reasons, comparative oncology, which can be defined as the study of spontaneous cancers in animals as models for human disease, has gained increasing importance over the last decades. Breast cancer represents the most prevalent cancer among women worldwide and the leading cause of cancer-related mortality in women. Mammary gland tumours are

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also among the most frequent tumours in female dogs and cats. Canine and feline mammary tumours present similar incidence, relative age of onset, risk factors, biological behaviour, metastatic pattern, histological, molecular, and genetic features, and response to therapy to human breast cancer; thus, they are recognized as suitable natural models for human breast cancer studies. The comparative "One Health" approach allows advances in knowledge of the diseases in order to obtain an improvement in clinical outcomes for affected humans and animals.

Keywords Breast cancer · Canine · Comparative oncology · Feline · Mammary tumours · Natural animal models · One Health

9.1 Introduction

Domestic animals develop several spontaneous diseases, including cancer, that in many aspects parallel human morbidities; hence, they are considered appropriate natural models of human diseases (MacEwen 1990; Porrello et al. 2006; Roman et al. 2013). These spontaneous models of cancer have several advantages over the classic in vitro tumour cell lines and the in vivo xenograft models, namely, the evaluation of the animal's immune response to the tumour, the ability to reproduce interactions between the neoplastic cells and the microenvironment, and the capacity to reproduce the metastatic behaviour of the neoplasm (Vargo-Gogola and Rosen 2007; Pinho et al. 2012; Nguyen et al. 2018). Moreover, the shorter life span and faster progression of cancer in dogs and cats allow an earlier data collection than in human malignancies (Cannon 2015). Furthermore, companion animals share the same environment as humans, and the health effects associated with exposure to hazardous materials, such as cancer, might also be detected in animals; consequently, in an epidemiological context, animals can act as sentinels of human

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malignancies (Misdorp 1996; Porrello et al. 2006). Changes in the canine cancer incidence ratios, and probably also in feline tumours, were described to precede by 2 years similar changes in human incidence rates, which might be useful for predicting changes in cancer patterns in humans (Garbe 1988).

Spontaneous canine and feline mammary tumours share several similarities with their human counterpart, including incidence, relative age of onset, risk factors, biological behaviour, metastatic pattern, histological, molecular, and genetic features, and response to therapy; thus, they are recognized as suitable natural models for human breast cancer studies (Vail and MacEwen 2000; Zappulli et al. 2005; Nguyen et al. 2018; Abadie et al. 2018).

9.2 Epidemiology and Risk Factors

Breast cancer represents the most prevalent cancer among women worldwide, and despite the recent advances in diagnosis and treatment, it remains the leading cause of cancer-related mortality in women (Ferlay et al. 2013, 2015; Ghoncheh et al. 2016). Furthermore, a trend for an increase in breast cancer incidence is observed worldwide (Glass et al. 2007; Arnold et al. 2015). According to the World Health Organization, 2.1 million women are diagnosed with breast cancer every year, with an estimation of 627,000 breast cancer-related deaths in 2018, corresponding to approximately 15% of all cancer-related deaths in women (WHO 2018). The incidence of breast cancer increases in women over 40 years of age, with a median age at diagnosis of approximately 50–60 years, depending on geographical location and tumour type (Bray et al. 2004; Song et al. 2014; Corbex et al. 2014; Monticciolo et al. 2017). Breast cancer also occurs in men; however, male breast cancer is considered a rare disease, corresponding to 1% of all breast cancer diagnoses and approximately to 0.1% of all male cancer-related deaths (Fentiman 2016; Ferzoco and Ruddy 2016). Nonetheless, a trend to an increase in incidence has also been observed in the last years (Giordano et al. 2014; Howlader et al. 2017). Several differences in epidemiology and clinical features of male and female human breast cancer have been described (Fentiman 2016; Deb et al. 2016).

Mammary tumours are also among the most frequent neoplasias in female dogs and cats (Vascellari et al. 2009; Egenvall et al. 2010; Grüntzig et al. 2016; Baioni et al. 2017). Canine mammary gland tumours represent more than 50% of all tumours in female dogs, with an estimated incidence of approximately 100–250 cases per 100,000 dogs per year (Dobson et al. 2002; Vascellari et al. 2009, 2016; Grüntzig et al. 2016; Baioni et al. 2017). Feline mammary tumours account for 17% of all tumours in female cats, with an incidence of approximately 25 cases per 100,000 female cats per year (Morris 2013). However, the prevalence and incidence of mammary tumours in companion animals vary geographically, being lower in areas where females are routinely neutered at younger ages (Beauvais et al. 2012; Salas et al. 2015). As in humans, canine and feline mammary tumours are rare in males. Female dogs present a predisposition 62 times higher than males to develop

mammary tumours, and most tumours in males are benign (Euler 2010; Bearss et al. 2012). In felines, approximately 1% of all mammary tumours occur in tomcats, with no sex-related differences of biologic behaviour or clinical signs (Hayes Jr et al. 1981; Skorupski et al. 2005; Gregório et al. 2012).

The relative ages of female dogs and cats with mammary tumours are similar to those described for women with breast cancer (Metzger 2005). Mammary tumours occur mainly in middle-aged to older bitches and are rare, namely, the malignant tumours, in dogs under 5 years of age; the mean age at diagnosis of malignant mammary tumours is of 9–11 years and of benign neoplasms of 7–9 years (Sorenmo et al. 2009, 2013). As in women, the incidence increases with age, with a peak at 11–13 years of age (Schneider 1970; Egenvall et al. 2005). In queens, the incidence of mammary tumours also increases with age and also occurs mainly in middle-aged to older queens, with a mean age at diagnosis of 10–12 years (Millanta et al. 2006; Morris 2013; Figueira et al. 2015).

Hormonal influence is another common feature of humans' and companion animals' mammary gland tumours (Schneider et al. 1969; Overley et al. 2005; Farhat et al. 2013; Finlay-Schultz and Sartorius 2015). The endocrine environment, defined by the length of exposure to the sex hormones oestrogen and progesterone, has been suggested to have a role in the development of canine and feline mammary carcinomas (Rutteman and Misdorp 1993; Overley et al. 2005; Queiroga et al. 2015). Evidences indicate that the steroid hormones act at the early stages of tumour development and that oestrogen receptor and progesterone receptor levels are decreased in carcinomas when compared to benign tumours, which may indicate a hormone-independent growth at the advanced stages of malignancy (Rutteman et al. 1991; Rutteman and Misdorp 1993; Martín De Las Mulas et al. 2000; Millanta et al. 2005b). In female dogs, the risk to develop mammary tumours is reduced to 0.5%, 8%, and 26% if the ovariohysterectomy or ovariectomy is performed before the first, before the second, or after the second estrus, respectively, with no risk reduction if performed after the second estrus (Schneider et al. 1969). Queens neutered before six months and one year of age are reported to have a 91% and 86% reduction risk, respectively, for the development of the disease when compared to intact queens (Overley et al. 2005). The administration of progestogens to prevent estrus increases the risk of mammary tumour development by a dose-related carcinogenic effect (Misdorp 1991; Misdorp et al. 1991; Rutteman and Misdorp 1993). This outcome appears to be more evident if these drugs are given regularly for long periods of time rather than intermittently (Misdorp et al. 1991), and male cats present a similar risk if treated with progestogens (Jacobs et al. 2010).

Besides the ovarian hormones, also the pituitary hormones prolactin and growth hormone have been associated with carcinogenesis of human breast cancer and canine and feline mammary tumours (Mol et al. 1995; van Garderen et al. 1997; Queiroga et al. 2014; Wang et al. 2016; Subramani et al. 2017).

Although any pure-breed or cross-breed dog or cat can develop mammary tumours, a genetic predisposition has been suggested in some canine and feline breeds. In dogs, mammary tumours are more frequent in pure-breed than in cross-breed dogs, and small and medium breeds are more commonly affected than

large and giant breeds (Moe 2001; Egenvall et al. 2005; Sorenmo et al. 2013; Salas et al. 2015; Grüntzig et al. 2016; Baioni et al. 2017). Moreover, different predispositions to mammary tumours have been described in dogs from the same breed but from different lineages, reinforcing the genetic influence in disease development (Schafer et al. 1998). A genetic predisposition is also suspected in cats, with Siamese, Oriental, and Domestic shorthair breeds appearing to be associated with a higher risk for the development of mammary neoplasias (Hayes Jr et al. 1981; Novosad 2003; Sorenmo et al. 2013). A familial genetic predisposition for breast cancer development is well established in human medicine, with different genes and gene mutations being associated with an increased risk for the disease (Lalloo and Evans 2012; Adank et al. 2013; Brewer et al. 2017). Similar genetic basis has been described in human and in companion animal breast cancer (Im et al. 2013; Enginler et al. 2014; Canadas et al. 2018b, c) and will be discussed later in this chapter.

Overweight and obesity are associated with a higher risk for human breast cancer, and among affected women, associated with more aggressive tumours and with a worst prognosis (Carmichael and Bates 2004; Jiralerspong and Goodwin 2016). Obesity has also been associated with canine mammary tumour development, mainly juvenile obesity, with female dogs with overweight or obesity at 9–12 months presenting a higher risk of mammary tumour development (Sonnenschein et al. 1991; Pérez Alenza et al. 1998). Obesity at 1 year before diagnosis of mammary masses was also associated with a higher prevalence of canine mammary tumours and dysplasias (Pérez Alenza et al. 1998). Moreover, as in women, overweight or obese diseased bitches tended to have more aggressive tumours than lean or ideal weight dogs (Lim et al. 2015a, b). Furthermore, the ingestion of homemade meals, namely, with a high content of red meat, was also associated with a higher risk for mammary tumours (Pérez Alenza et al. 1998).

9.3 Clinical Course of Disease

Approximately 50–75% of mammary tumours in bitches are malignant, and 25–50% are benign (Hellmén et al. 1993; Salas et al. 2015; Rasotto et al. 2017; Canadas et al. 2018a). At presentation, approximately 20–30% of malignant cases present regional lymph node metastases, and although less frequently, distant metastases might also be present, mainly in the lungs, but also in the liver, bone, and other organs (Sorenmo et al. 2013; Santos et al. 2013a; Gundim et al. 2016; Canadas et al. 2018a). Recent studies reported an overall median survival time of 11 months after mastectomy, that approximately 30% of cases developed local recurrence and/or distant metastases and that 25–40% of dogs died or were euthanized within two years after diagnosis due to disease progression, and a two-year overall survival rates ranging from 36.4% to 48% (Santos et al. 2013a; Nguyen et al. 2018; Canadas et al. 2018a). However, the clinical course of the disease varies significantly according to different clinical and tumour features, including clinical staging, tumour histological type and grade, mode of growth, immunophenotype, and molecular and genetic features

(Yamagami et al. 1996; Santos et al. 2013a; Nguyen et al. 2018; Abadie et al. 2018; Canadas et al. 2018c; Canadas et al. 2018a).

A characteristic feature of mammary gland tumours in dogs is the common presence of multiple nodules at diagnosis, with benign and malignant tumours coexisting in the same patient (Santos et al. 2010a, b; Vascellari et al. 2016). This fact suggests that benign and malignant mammary tumours might not be separate entities; instead they may be part of a continuum process in which the malignant invasive carcinomas correspond to the advanced stages of the process. In this sense, canine mammary cancer provides an adequate model to study mammary gland carcinogenesis and progression, with direct application in human breast cancer research (Sorenmo et al. 2013).

In cats, approximately 80–90% of mammary tumours are malignant, and most of these present an aggressive behaviour and a poor prognosis (Hayes Jr et al. 1981; Ito et al. 1996; Millanta et al. 2002; Figueira et al. 2014). Feline mammary carcinomas are usually characterized by rapidly growing, highly infiltrative, and invasive nodules, with extensive necrotic areas, skin ulceration, and metastases (Misdorp and Weijer 1980; Martín De Las Mulas and Reymundo 2000), features associated with a poor prognosis (Weijer and Hart 1983; Amorim et al. 2006). At the time of diagnosis, approximately 25% of the cats with mammary carcinomas present neoplastic vascular invasion, and distant metastases are also often detected, ultimately leading to high morbidity and mortality rates (Misdorp and Weijer 1980; Zappulli et al. 2005; Sorenmo et al. 2013). The most common sites of metastization are the regional lymph nodes (83%), lungs (83%), pleura (22%), and liver (25%), and although less frequently, metastases to the adrenal glands, diaphragm, and kidneys are also described (Hayes Jr et al. 1981; Weijer and Hart 1983; Hahn et al. 1994). Feline malignant mammary tumours are generally more aggressive than canine mammary neoplasms, with reported survival times ranging from a few months to a few years; the main prognostic factors of canine malignancies act in a similar way in feline mammary cancer (Morris 2013).

9.4 Histopathological and Molecular Features of Mammary Tumours

9.4.1 Canine Mammary Tumours

Histopathology constitutes the gold standard method for mammary tumour diagnosis (Sorenmo et al. 2011; Rasotto et al. 2012; Goldschmidt et al. 2017). It is usually impossible to distinguish between benign and malignant mammary neoplasia at the clinical setting, and the accuracy of cytological differentiation is relatively low in canine mammary tumours; therefore, histopathology plays a central role in providing an accurate tumour diagnosis, as well as prognostic information (Goldschmidt et al. 2017).

The mammary gland is a modified apocrine sweat gland, histologically characterized by a tubuloalveolar structure (Sorenmo et al. 2011). The epithelium is composed by a dual-cell population of luminal epithelial and basal myoepithelial cells, juxtaposed to a basement membrane (Sorenmo et al. 2011). The gland is a hormone-dependent organ, and physiological changes are histologically identified throughout the distinct phases of the estrous cycle (Rehm et al. 2007; Santos et al. 2010b).

Routine histopathology allows recognition and distinction of a myriad of proliferative entities in the mammary gland, from hyperplasia to benign or malignant tumour lesions. Histological classification systems for canine mammary tumours published by the World Health Organization (WHO) were primarily based on descriptive morphology and to a lesser degree on prognosis (Hampe and Misdorp 1974; Misdorp et al. 1999; Misdorp 2002). In 2011, a revised classification has been proposed, based on morphological and prognostic features, incorporating several new histological subtypes (Goldschmidt et al. 2011; Rasotto et al. 2012). This new WHO classification subdivided the proliferative alterations of the canine mammary gland into eight distinct groups: hyperplasias/dysplasias; benign neoplasms; malignant epithelial neoplasms; malignant epithelial neoplasms, special types; malignant mesenchymal neoplasms (sarcomas); carcinosarcoma, malignant mixed mammary tumour; neoplasms of the nipple; and hyperplasia/dysplasia of the nipple (Goldschmidt et al. 2011). A complete description of the histological types is beyond the scope of this chapter, and a comprehensive review can be found in references (Misdorp et al. 1999; Goldschmidt et al. 2011).

Canine mammary neoplasms are characterized by a diverse morphology, originating from the proliferation of epithelial, myoepithelial, and/or mesenchymal cells (Misdorp et al. 1999; Goldschmidt et al. 2011). Myoepithelial cell proliferation constitutes one of the most distinctive features of canine mammary tumours, being frequently observed both in benign and malignant lesions. For tumour nomenclature purposes, simple type refers to the proliferation of one epithelial cell type (luminal epithelial or myoepithelial cells) and complex type to the proliferation of two epithelial cell types (luminal epithelial and myoepithelial cells) (Misdorp et al. 1999; Misdorp 2002; Goldschmidt et al. 2011, 2017).

Complex adenoma and benign mixed tumour represent the predominant benign tumour histotypes; both are characterized by the proliferation of luminal and myoepithelial cells, with benign mixed tumour being associated with the presence of metaplastic elements, such as bone and/or cartilage (Fig. 9.1a, b) (Misdorp et al. 1999; Goldschmidt et al. 2011).

The majority of malignant tumours have epithelial origin (carcinoma), with different morphological types identified – tubular, tubulopapillary, solid, and anaplastic (Fig. 9.1c) (Misdorp 2002; Sorenmo 2003; Goldschmidt et al. 2011; Sleeckx et al. 2011). The current proposed classification includes several new morphological subtypes, such as micropapillary invasive carcinoma, comedocarcinoma, ductal carcinoma, intraductal papillary carcinoma, and carcinoma and malignant myoepithelioma (Fig. 9.1d) (Goldschmidt et al. 2011; Rasotto et al. 2012). The application of this new modified classification revealed that it is a valuable tool for predicting the metastatic potential of canine mammary carcinomas (Rasotto et al. 2012).

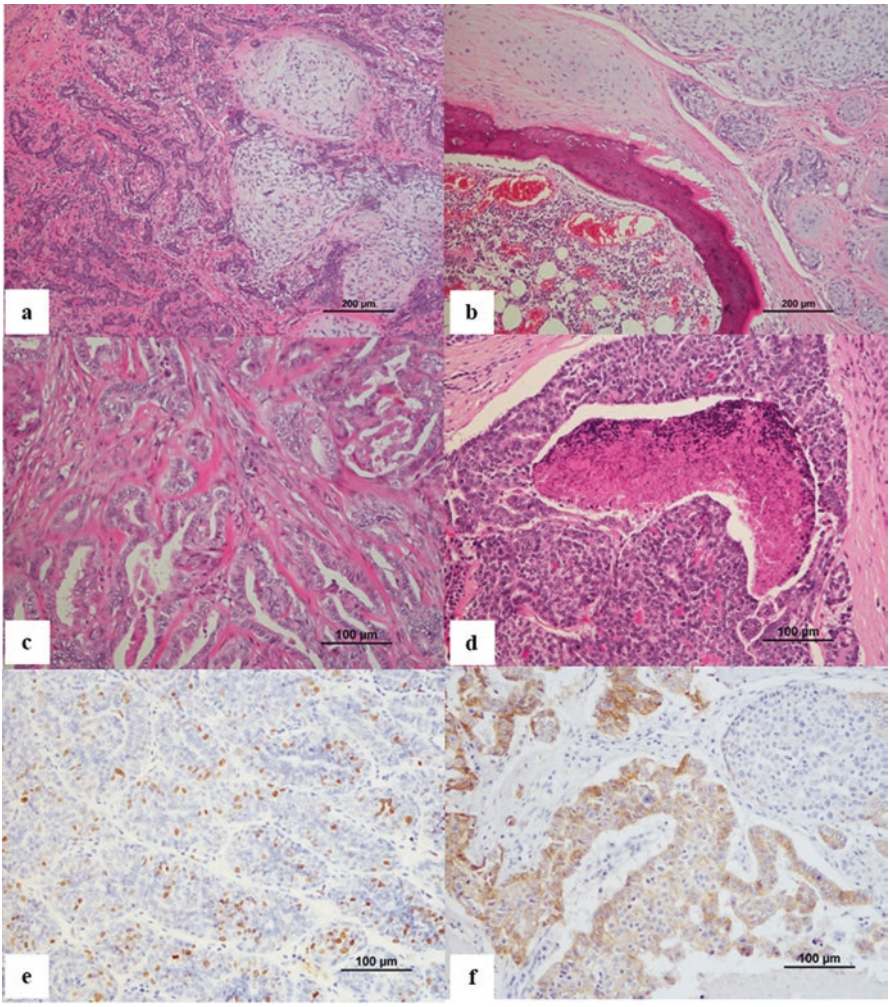

Fig. 9.1 Canine mammary gland tumours: (**a**) complex adenoma, benign proliferation of epithelial and myoepithelial cells. Myoepithelial cells present a fusiform to stellate form and are surrounded by a basophilic mucinous matrix (10×); (**b**) benign mixed tumour, proliferation of epithelial and myoepithelial cells associated with osseous differentiation. Note the presence of bone marrow (10×); (**c**) tubulopapillary carcinoma, neoplastic epithelial cells arranged in a tubular and papillary pattern (20×); (**d**) comedocarcinoma, neoplastic epithelial cells showing a central area of necrosis (20×); (**e**) solid carcinoma showing nuclear Ki-67 positive immunostaining (20×); (**f**) tubulopapillary carcinoma with reduced membrane expression of E-cadherin (20×); (**a**–**d**) hematoxylin and eosin; (**e**–**f**) streptavidin–biotin complex method. (Gill's hematoxylin counterstain)

With regard to simple carcinomas, an increase in the metastatic potential was observed from tubular to tubulopapillary, to solid, to anaplastic carcinoma (Rasotto et al. 2012), corroborating previous studies (Bostock 1975; Misdorp et al. 1999; Chang et al. 2005). Micropapillary invasive carcinoma, comedocarcinoma, and carcinoma and malignant myoepithelioma subtypes were also recognized as having significant metastatic potential (Gama et al. 2008a; Rasotto et al. 2012). In contrast to carcinoma and malignant myoepithelioma (characterized by the proliferation of malignant luminal epithelial and myoepithelial cells) (Rasotto et al. 2012; Goldschmidt et al. 2011), complex carcinomas (characterized by the proliferation of malignant luminal and benign myoepithelial cells) are commonly associated with a better prognosis (Misdorp et al. 1999; Misdorp 2002; Goldschmidt et al. 2011).

Special types of malignant epithelial neoplasms are less frequent, including squamous cell carcinomas, adenosquamous carcinomas, mucinous carcinomas, lipid-rich carcinomas, and spindle cell carcinomas (malignant myoepithelioma, squamous cell carcinoma–spindle cell variant, and carcinoma–spindle cell variant) (Goldschmidt et al. 2011, 2017).

Mesenchymal malignant tumours are unusual, but several sarcoma types are described, including osteosarcoma, chondrosarcoma, and fibrosarcoma, among others. Osteosarcoma is by far the most commonly diagnosed, being associated with a poor prognosis (Goldschmidt et al. 2011). Malignant mixed mammary tumour (known as carcinosarcoma) is uncommon, being characterized both by a carcinomatous and sarcomatous component, frequently associated with metastatic spread (Misdorp 2002; Goldschmidt et al. 2011).

With regard to human counterpart, WHO released a new and updated classification of breast tumours in 2012 (Lakhani et al. 2012). Fibroadenoma represents the most common benign breast tumour type, usually diagnosed in younger women (Yang et al. 2014); the most frequent type of breast cancer is the invasive carcinoma of no special type (IC-NST) (previously known as invasive ductal carcinoma not otherwise specified, NOS), which is a diagnosis of exclusion as it includes a heterogeneous group of carcinomas that fail to exhibit sufficient features to achieve classification as a specific histological type of carcinoma, such as lobular or tubular carcinoma (Lakhani et al. 2012).

Special types of human breast cancer have distinctive morphological characteristics and account for up to 25 % of all invasive breast cancers (Horlings et al. 2013); human classification includes several specific entities, namely, invasive lobular (5–15%), tubular (2%), cribriform (0.3–0.8%), metaplastic (0.2–5%), medullary (less than 1%), papillary (1–2%), and micropapillary (0.9–2%) carcinomas (Lakhani et al. 2012). Differing from canine mammary gland, lesions showing myoepithelial differentiation are uncommon in human breast; myoepithelial lesions are characterized by a varied morphology, including adenomyoepithelioma, myoepithelial carcinoma (malignant myoepithelioma), and epithelial–myoepithelial carcinoma (Lakhani et al. 2012). The prognosis for patients with myoepithelial neoplasia is usually good, with the exception of myoepithelial carcinoma (Foschini and Eusebi 1998; Rakha et al. 2006; Buza et al. 2010). As in canine species, this less aggressive nature of neoplasms with myoepithelial differentiation might be justified by the

tumour-suppressive properties of normal myoepithelial cells (Sternlicht et al. 1997; Jones et al. 2003; Reis-Filho et al. 2006).

Besides histological type, the histopathology report includes additional information relevant for prognosis such as the histological grade (Rasotto et al. 2012; Ehrhart et al. 2013). Several systems have been proposed for the grading of canine mammary tumours (Misdorp 2002; Clemente et al. 2010; Karayannopoulou et al. 2005; Goldschmidt et al. 2011), mainly based on the Elston and Ellis system for human breast invasive carcinomas (Elston and Ellis 1991). In women, histological grade is a powerful prognostic factor, and invasive breast carcinomas are routinely graded applying Elston and Ellis grading system (Lakhani et al. 2012). This numeric system is based on the assessment of tubule formation, nuclear pleomorphism, and mitotic counts, classifying carcinomas in grade 1 (well-differentiated), grade 2 (moderately differentiated), and grade 3 (poorly differentiated) (Elston and Ellis 1991; Lakhani et al. 2012). Recently, Peña et al. (2013) adapted Elston and Ellis system to canine mammary cancer, taking into account their heterogeneity, as well as the assessment of the frequent myoepithelial and mixed lesions; a prospective prognostic study revealed that this updated system constitutes a useful tool for predicting prognosis (Peña et al. 2013).

In addition to histological type and grading, the presence of stromal infiltration (Rasotto et al. 2012), lymphovascular invasion, and lymph node status have been found to be of prognostic significance (Kurzman and Gilbertson 1986; Sarli et al. 2002; Chang et al. 2005).

Although most mammary neoplastic lesions can be diagnosed by routine histopathology alone, some cases require the application of immunohistochemistry (IHC) to reach a definitive diagnosis; common scenarios that demand the use of immunohistochemical diagnostic markers both in human and canine settings include the identification of specific histological subtypes, the assessment of invasion, or the detection of lymph node micrometastases (Hicks 2011; Goldschmidt et al. 2011; Sorenmo et al. 2011; Liu 2014; Peña et al. 2014).

Carcinoma and malignant myoepithelioma and myoepithelial carcinoma diagnosis require immunohistochemistry to confirm the presence of myoepithelial cell proliferation, given that they usually lack their classic morphological appearance (Rasotto et al. 2012; Peña et al. 2014). Similarly, immunohistochemical cell differentiation markers are useful to classify unusual woman breast lesions, namely, adenomyoepithelial cell tumours, to differentiate radial scars from tubular carcinomas and for the diagnosis of breast papillary lesions (Dewar et al. 2011; Hicks 2011; Walker et al. 2012).

In human breast pathology, IHC is also routinely used in invasive carcinomas to assist in prognosis and to direct to specific treatments, through the evaluation of oestrogen (ER) and progesterone (PR) receptors and epidermal growth factor receptor 2 (HER2), which constitute targets and/or biomarkers of effective therapies (Payne et al. 2008; Lakhani et al. 2012).

Microarray-based gene expression studies revealed that human breast cancer encompasses a heterogeneous group of diseases, characterized by distinct molecular features (Badve et al. 2011; Guiu et al. 2012). Different breast cancer “intrinsic”

subtypes were identified (luminal A and B, basal-like, HER2 overexpressing, normal-like), resulting in a molecular taxonomy with prognostic significance (Perou et al. 2000; Sorlie et al. 2001, 2003). Surrogate immunohistochemical panels have been used to identify these subgroups, including hormone receptors, HER2, and proliferative and basal cell differentiation markers, with triple-negative (hormone receptor and HER2 negative) and basal-like (triple-negative positive for basal cell differentiation markers) carcinomas being associated with poor prognosis (Nielsen et al. 2004; Matos et al. 2005; Cheang et al. 2009; Blows et al. 2010). Although an IHC panel was adopted by the St. Gallen Consensus Committee for early breast cancer molecular subtyping leading to therapeutic and prognostic stratification (Goldhirsch et al. 2011, 2013; Nielsen and Perou 2015), controversies on the definition of IHC-defined taxonomy still prevail (Guiu et al. 2012). A current challenge is the distinction between luminal A and luminal B (HER2-negative) carcinomas, which has therapeutic implications (Goldhirsch et al. 2011). The value of using Ki-67 labelling index (Fig. 9.1e) for subgrouping these tumours has been questioned, due to the high degree of inter-laboratory variation, and experts have recently recommended the use of multi-gene expression assays (if available) to define high-risk signatures in ER-positive and HER2-negative carcinomas (Goldhirsch et al. 2013).

In the canine species, several studies applied the human molecular classification, with contradictory results (Gama et al. 2008c; Sassi et al. 2010; Kim et al. 2013; Im et al. 2014; Abadie et al. 2018), probably associated with differences in immunohistochemical cell markers, criteria, and sample selection (Peña et al. 2014). Even so, basal-like and triple-negative mammary carcinomas were frequently identified in the female dog, usually associated with an aggressive phenotype (Gama et al. 2008c; Im et al. 2014) and lower survival rates (Gama et al. 2008c; Kim et al. 2013; Abadie et al. 2018); these findings suggest canine mammary carcinomas as natural models for the study of triple negative and human basal-like breast carcinomas (Gama et al. 2008c; Abadie et al. 2018).

The use of IHC in canine mammary cancer has increased tremendously in the last decades in the search for relevant prognostic markers. Besides hormone receptors (Geraldes et al. 2000; Nieto et al. 2000; Martin et al. 2005), HER2 (Rungsipipat et al. 1999; Martin et al. 2003; Dutra et al. 2004; Hsu et al. 2009) and cell proliferation markers (Peña et al. 1998; Sarli et al. 2002; Matos et al. 2006), other molecular markers have been investigated, such as adhesion molecules (Brunetti et al. 2005; Matos et al. 2006; Gama et al. 2008), among others (Pinho et al. 2007).

The acquisition of an invasive epithelial phenotype has long been associated with functional loss or downregulation of epithelial (E-) cadherin-mediated adhesion, which is considered a hallmark of epithelial to mesenchymal transition (EMT) (Fig. 9.1f) (Cano et al. 2000). Both in canine and human breast cancer, numerous studies have focused on E-cadherin expression (Gamallo et al. 1993; Oka et al. 1993; Siitonen et al. 1996; Brunetti et al. 2005; Matos et al. 2006; Gama et al. 2008). In canine mammary carcinomas, E-cadherin loss or reduced expression was frequently associated with poor differentiation (Reis et al. 2003; Gama et al. 2008), invasion (Sarli et al. 2004; Brunetti et al. 2005; Matos et al. 2006, 2007; Gama et al.

2008), lymph node metastasis (Matos et al. 2007; Gama et al. 2008), or prognosis (Gama et al. 2008), corroborating human findings (Gamallo et al. 1993; Oka et al. 1993; Siitonen et al. 1996; Yoshida et al. 2001). However, results are not consensual in both cancer models, and the proposed tumour-suppressive role of E-cadherin in human breast cancer has been questioned (Hugo et al. 2017). In addition to E-cadherin, several studies highlighted the importance of associated catenins, such as β-catenin; its reduced expression in canine mammary carcinomas was found to be associated with high grade and invasion by some authors (Brunetti et al. 2005; Gama et al. 2008).

P-cadherin is a cell–cell adhesion molecule associated with tumour-promoting effects in human breast (Vieira and Paredes 2015); P-cadherin overexpression is found in a subset of human breast carcinomas, being associated with aggressive biological behaviour and poor outcome (Palacios et al. 1995; Peralta Soler et al. 1999; Paredes et al. 2002, 2005). In the female dog, P-cadherin is also frequently overexpressed in mammary carcinomas, being associated with tumour cell invasion (Gama et al. 2008). As in human breast cancer (Matos et al. 2005; Paredes et al. 2007), P-cadherin expression is primarily found in the basal-like subtype of canine mammary carcinomas, which is associated with poor prognostic features (Gama et al. 2008c; Gama and Schmitt 2012).

At present, no prognostic/predictive immunohistochemical marker is recommended for routine diagnosis of canine mammary cancer due to the absence of marker-associated therapies, contradictory results on biomarker prognostic value, and lack of standardized methodologies (Peña et al. 2014; Goldschmidt et al. 2017). Recently, supportive guidelines on the most useful immunohistochemical markers for canine mammary tumours have been provided, in an attempt to standardize their use and interpretation, ultimately leading to accurate and reproducible results (Peña et al. 2014).

9.4.2 Feline Mammary Tumours

The most widely accepted system for histological classification of feline mammary tumours is the WHO classification (Misdorp et al. 1999). More recently, an updated version has been proposed, including some new morphological subtypes (Goldschmidt et al. 2017). Hyperplastic/dysplastic mammary lesions are common in feline species and comprise several specific categories, while benign neoplasms are not frequent in queens. Most feline mammary tumours (80–90%) are rapidly growing malignant lesions, with an aggressive biological behaviour. Definition of the histological subtype of feline mammary carcinomas is based mainly on the cell types involved (luminal epithelial and/or myoepithelial cells) and on the arrangements adopted by the neoplastic cells – tubular, papillary, cystic, cribriform, and/or solid (Fig. 9.2a–c). As in women, malignant mammary tumours in queens usually encompass only luminal epithelium, being thus classified as simple carcinomas; myoepithelial involvement and metaplastic features (chondroid or osseous ele-

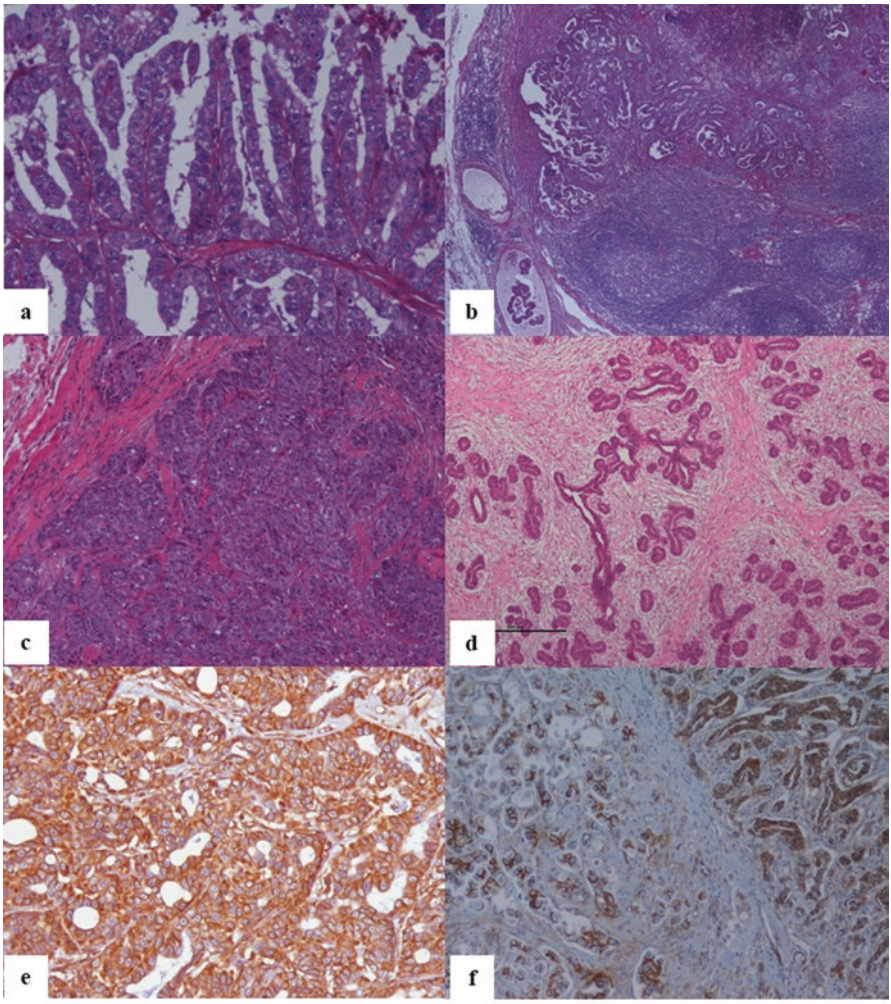

Fig. 9.2 Feline mammary gland tumours: (**a**) tubulopapillary carcinoma, neoplastic epithelial cells arranged in a tubular and papillary pattern (40×); (**b**) lymph node metastasis and intravascular neoplastic emboli of a tubulopapillary carcinoma (same case of fig. **a**, 4×); (**c**) solid carcinoma, neoplastic epithelial cells arranged in a solid pattern (40×); (**d**) fibroadenomatous change, benign ductular proliferation surrounded by an extensive stroma rich in mucin and collagen fibres. (4×); (**e**) tubulopapillary carcinoma showing vimentin positive immunoexpression (20×); (**f**) tubulopapillary carcinoma with reduced membrane expression of β-catenin (10×); (**a**–**d**) hematoxylin and eosin; (**e**–**f**) polymer-based system. (Mayer's hematoxylin counterstain)

ments) are uncommon findings. Generally, neoplastic epithelial cells exhibit a large nucleus, with prominent nucleoli and numerous mitotic figures (Misdorp et al. 1999; Goldschmidt et al. 2017). Histological grading of feline mammary carcinomas is established according to the same morphological criteria as for human and canine breast cancer, namely, tubular differentiation, nuclear pleomorphism, and mitotic

counting (Elston and Ellis 1991). Similarly to humans, histological grade is considered a valuable prognostic factor for feline mammary carcinomas, constituting a good independent predictor of disease-free interval and overall survival (Castagnaro et al. 1998; Seixas et al. 2011; Hughes and Dobson 2012; Zappulli et al. 2015). Recently, lymphovascular invasion and nuclear form were proposed as additional histological features in feline mammary carcinoma grading (Mills et al. 2015).

The histological presentation of several feline mammary hyperplasic, benign, and malignant lesions closely parallels those of human breast disease, enhancing the value of this animal species as a model for the study of their analogous lesions in women. That is the case of fibroadenomatous change and fibroadenoma, (Goldschmidt et al. 2017). Fibroadenomatous change is a large, rapidly growing, hormone-dependent, hyperplasic lesion. It is typical of young intact queens and can be also found during pregnancy. Microscopically, fibroadenomatous change is characterized by ductular proliferation surrounded by an extensive stroma rich in mucin and collagen fibres (Fig. 9.2d). Fibroadenoma is a benign neoplasm consisting of multiple tubules lined by a cuboidal/columnar epithelium, surrounded by an exuberant stroma of loose connective tissue (Misdorp et al. 1999; Goldschmidt et al. 2017). Both lesions share several morphological features with benign fibroepithelial tumours of the human breast, particularly with fibroadenoma, which comprises the vast majority of benign breast tumours, usually occurring in young women (Yang et al. 2014; Tan and Tan 2018). Histologically, human fibroadenoma is characterized by biphasic proliferation of both epithelial and stromal elements, closely resembling feline fibroepithelial lesions.

As previously performed in human breast cancer (Park et al. 2012; Goldhirsch et al. 2013), recent immunohistochemical studies allowed the establishment of a molecular-based classification for feline mammary carcinomas, with several recognized subtypes, namely, luminal A, luminal B, HER-2 overexpressing, and triple-negative basal-like and triple-negative normal-like carcinomas, some of which clearly mimic their human counterparts. This molecular-based categorization, which relies on the assessment of the immunoexpression of hormonal receptors, HER-2, luminal epithelial/basal markers, and proliferation markers, supports the identification and characterization of different feline mammary carcinoma subtypes, associated with specific clinicopathological features and with different clinical outcomes (Brunetti et al. 2013; Soares et al. 2016b).

Currently, the assessment of breast cancer molecular profile, namely, ER and PR status and HER-2 expression, is essential for diagnosis, classification, and treatment of the human disease (Perou et al. 2000; Peppercorn et al. 2008; Falck et al. 2013). Most human breast cancers are hormone receptor-positive carcinomas, of the luminal subtypes, that tend to respond well to endocrine therapy, presenting a good prognosis (Park et al. 2012; Goldhirsch et al. 2013). Other breast cancer subtypes (HER-2 overexpressing) benefit from treatment with humanized monoclonal antibodies, such as trastuzumab (Yin et al. 2011). However, part of breast cancer cases lack hormone receptors, are less endocrine sensitive lesions, miss other specific therapeutic targets, or develop resistance to endocrine/HER-2 targeted therapy. These breast cancer cases are usually characterized by a poorly differentiated,

highly aggressive phenotype and constitute a major clinical challenge, being associated with a worse prognosis (Bosch et al. 2010; Esteva et al. 2010; Toft and Cryns 2011; Elizalde et al. 2016; Liu et al. 2017).

The molecular-based classification of feline mammary carcinomas demonstrated their molecular heterogeneity, and several of the molecular subtypes identified present similarities with some aggressive forms of the human disease. Most feline mammary carcinomas are highly aggressive, hormone-independent tumours with an unfavourable clinical outcome. Several investigations have demonstrated a progressive decrease in hormonal receptor expression from feline normal mammary tissue to hyperplasic/dysplastic lesions and from benign to malignant tumours (Rutteman et al. 1991; Martín De Las Mulas et al. 2000; Cardazzo et al. 2005; Millanta et al. 2005b; Burrai et al. 2010; Caliari et al. 2014). This hormonal independence of feline mammary carcinomas has also been associated by some authors to high histological grade of carcinomas, vascular invasion, and lymph node metastases, being thus considered a reliable predictor of poor prognosis (Millanta et al. 2006; Soares et al. 2016c). Some reports have documented HER-2 overexpression in around 30–60% of FMC and associated this feature with poorly differentiated tumours, low disease-free survival, and short overall survival (De Maria et al. 2005; Millanta et al. 2005a; Brunetti et al. 2013; Soares et al. 2016b). Furthermore, Soares et al. (2016b) have recently demonstrated that triple-negative basal-like mammary carcinomas in cats are associated with large tumour size and vascular invasion, also showing the lowest overall survival and the shorter disease-free interval, clearly resembling their human counterpart. The correspondence observed between the molecular-based taxonomy of feline mammary carcinomas and human breast cancer emphasizes the potential comparative value of these lesions in the development of innovative and alternative therapeutic strategies for breast cancers unresponsive to conventional medical treatment and in predicting biological behaviour of mammary neoplasia.

More recently, a claudin (CLDN)-low molecular breast cancer subtype was reported; it is defined by an aggressive biological behaviour and high metastatic capacity, being frequently refractory to conventional chemotherapeutic protocols and associated with a bad prognosis (Kim et al. 2008; Prat et al. 2010; Lu et al. 2013). Similarly, a decreased expression of CLDN-1, CLDN-2, and CLDN-7 was also reported in feline mammary carcinomas, and CLDN-2 and CLDN-7 under-expression was significantly associated with metastization (Flores et al. 2014a, b). These findings clearly support the involvement of CLDN down-expression in mammary carcinogenesis and metastization in feline species and underline the importance of this animal species as a model in pursuing for new therapeutic regimens focused on this distinctive breast cancer subtype.

Feline mammary carcinomas are characterized by under-expression of low-molecular-weight cytokeratins (typical of well-differentiated epithelial tissues) and by the expression of basal high-molecular-weight cytokeratins (namely, CK5/6 and CK14) and vimentin (Fig. 9.2e) in neoplastic cells (de las Mulas et al. 1994; Espinosa et al. 1999; Peñafiel-Verdu et al. 2012; Brunetti et al. 2013; Caliari et al. 2014; Soares et al. 2016b). These features are more frequent in invasive carcinomas and have been significantly associated with a poor prognosis (Peñafiel-Verdu et al. 2012;

Soares et al. 2016b). Such findings resemble some aggressive forms of human breast cancer, which have been associated with invasive behaviour, metastasis, and increased drug resistance (Sommers et al. 1992; Gilles et al. 2003).

Like in human breast cancer, feline mammary carcinomas typically exhibit significant changes in mechanisms of cell adhesion, such as those involving the cadherin–catenin complex. E-cadherin down-expression was described by some authors in feline mammary carcinomas (Dias Pereira and Gärtner 2003; Zappulli et al. 2012; Figueira et al. 2014) and was associated with lymph node metastases (Peñafiel-Verdu et al. 2012). This feature was also reported in breast cancer cases, in which it is associated with high histological grade (Gamallo et al. 1993). On the other hand, P-cadherin overexpression was recently documented in feline mammary carcinomas, being related to high histological grade, infiltrative growth pattern, and vascular invasion (Figueira et al. 2012, 2014). Likewise, in human breast cancer, this immunostaining pattern is associated with recurrence and distant metastasis and with poor prognosis, namely, short overall survival and reduced disease-free interval (Liu et al. 2012). Furthermore, N-cadherin expression was also documented in mammary carcinomas in queens and associated with lymph node metastasis (Buendia et al. 2014), similarly to data from breast cancer (Bock et al. 2014). Feline mammary malignant tumours are also characterized by reduced membrane expression of α-, β-, and p120-catenin, as well as by abnormal subcellular localization of β- and p-120 catenin (Fig 9.2f) (Peñafiel-Verdu et al. 2012; Zappulli et al. 2012; Figueira et al. 2015). Peñafiel-Verdu et al. (2012) associated β-catenin under-expression to the development of metastases. Similarly, α-, β-, and p120-catenin abnormal/reduced expression in breast cancer was reported and related to a poor prognosis (Yoshida et al. 2001; Nakopoulou et al. 2002; Talvinen et al. 2010).

In addition, data from recent research demonstrated that feline mammary carcinomas emulate the EMT process, which has been described in human breast cancer and related to tumour invasiveness and metastatic capability (Fedele et al. 2017). Epithelial to mesenchymal transition is a program of phenotype transformation characterized by (1) loss of epithelial traits, in which neoplastic cells typically lose intercellular adhesion proteins, namely, E-cadherin, and exhibit downregulation of epithelial markers, such as low-molecular-weight cytokeratins, and (2) acquisition of mesenchymal-like features, with gain of several mesenchymal-associated markers, like vimentin and N-cadherin, and development of a fibroblast-like morphology through cytoskeleton reorganization. This process of epithelial plasticity leads to loss of intercellular contact and changes in cell shape and polarity, favouring the conversion of a stationary to a migratory phenotype, thus increasing the invasive potential of the neoplastic cells (Sarrió et al. 2008; Foroni et al. 2012; Wu et al. 2016). As described above, feline mammary carcinomas encompass a series of immunophenotypic changes (decreased E-cadherin and low-molecular-weight cytokeratin expression, along with vimentin, P-cadherin, N-cadherin, and basal high-molecular-weight cytokeratin overexpression) that clearly resemble EMT, endorsing the importance of this animal species in the study of tumour progression, invasion, and metastasis.

These extensive immunophenotypic changes that characterize feline mammary carcinomas and permit the definition of several different subtypes corresponding to distinct clinical entities (often mirroring their human counterparts) support the cat as an appropriate model for the study of some specific forms of aggressive breast cancer.

9.5 Genetics

Genetic homology between canine and feline mammary tumours and human breast cancer is well recognized and accepted (Lutful Kabir et al. 2015; Adega et al. 2016). Moreover, the remarkable progress in the development of molecular tools which allowed the coverage of the canine and feline genome, canine and feline DNA microarray use, and proteomic analyses is factual and in continuous evolution (Rivera and von Euler 2011; Thomas 2015). However, genetic basis of canine and feline mammary tumours remains poorly characterized when compared to its human counterpart. In fact, there is still a need to further understand canine and feline cancer genetics, in basic and clinical areas, in order to obtain robust models which could be reproducibly and effectively used to develop and test new therapeutic tools for humans and also for animals (Kim et al. 2004).

Recent molecular analyses have shown that rather than a single disease, breast cancer is a mixture of several diseases with different biological behaviours, which should direct to customized treatments for each patient (Verma 2012; Rivenbark et al. 2013). The understanding of individual genetic profiles in breast cancer enabled the implementation of guidelines and clinical practices. These are based on preventive measures such as prophylactic surgeries and family follow-up and specific or individual treatments, which are the core of the so-called personalized medicine (Sabatier et al. 2014; Stover and Wagle 2015). In women, almost 30% of breast cancer cases are considered hereditary, and up to 25% of these are due to a mutation in one of the few rare but highly penetrant identified genes, including BRCA1, BRCA2, PTEN, TP53, CDH1, and STK11 (Antoniou and Easton 2006; Walsh et al. 2006). An additional 2–3% of cases are due to a mutation in rare, moderate penetrant genes, such as CHEK2, BRIP1, ATM, and PALB2 (Shiovitz and Korde 2015). Several other candidate genes predisposing to breast cancer, such as FGFR2, LSP1, MAP3K1, and TOX3, have also been reported (Ripperger et al. 2009). Besides the susceptibility to develop breast cancer, the influence of genetic profiles in clinicopathological features is also well documented in numerous studies, including genome-wide association studies (Han et al. 2004; Long et al. 2007; Giess et al. 2010; Chan et al. 2012; Sirisena et al. 2018).

Being canine and feline mammary tumours a model for human breast cancer (Vail and MacEwen 2000; Burrai et al. 2010; Queiroga et al. 2011; Adega et al. 2016; Abdelmegeed and Mohammed 2018), the awareness of concrete genetic variations such as mutations, deletions, insertions, and genetic polymorphisms can be essential in canine and feline disease diagnosis, prognosis, and treatment and potentially lead to implementation of an individualized approach also in veterinary medicine (Lloyd et al. 2016; Pang and Argyle 2016).

9.5.1 Genetics of Canine Mammary Tumours

In 1989, a disease-causing mutation was identified for the first time in dogs (Evans et al. 1989), and 10 years later, the first germline mutation associated with canine mammary tumours was reported in the p53 tumour suppressor gene, which has long been classified as an important cancer catalyst in humans (Veldhoen et al. 1999). Since then, mutations have been described for over 130 diseases, and the vast majority of these (107) are inherited based on an autosomal recessive pattern (Slutsky et al. 2013). Furthermore, two of them have been successfully used for gene therapy in humans (Switonski 2014). Currently, over 130 molecular genetic tests are available for dogs, most of these being breed-specific mutations and single-nucleotide polymorphisms (SNPs), which emphasizes the nature of hereditary diseases in canine medicine (Slutsky et al. 2013). This great progress allowed the identification of genetic profiles of specific tumours, in addition to the recognition of clinically relevant constitutional genomic alterations in dogs. This recent evidence led to a new approach to mammary pathogenesis (Klopfleisch et al. 2011). The emerging studies show promising results regarding the significance of genetic profiles in cancer susceptibility and clinicopathological features.

Genetic approach to canine mammary tumours has been covering both somatic and germline genetic variations, alongside with the analyses of structural aberrations at the subchromosomal level, including interchromosomal rearrangements or chromosomal instability. The identification of genetic variants in tissue samples such as mutations and SNPs, among others, has been conducted by comparing normal and neoplastic mammary tissue or by comparing different histological types of tumours. In this field, several genetic variations have been identified, and homology has been studied and compared between canine and human genes, specially focusing both BRCA1 and BRCA2 genes (Szabo et al. 1996; Bignell et al. 1997; Ochiai et al. 2001; Yoshikawa et al. 2005; Goebel and Merner 2017).

Individual genetic background, emphasizing constitutional genetic variations, has also been the aim of recent studies. In canine species, the high incidence of mammary tumours in certain breeds suggests a genetic component effect, similar to familiar breast cancer. In fact, breeds showing the highest predisposition to develop mammary tumours are Poodles, Spaniels, Pulis, English Setters, German Shepherds, Yorkshire Terriers, and Doberman Pinschers (Egenvall et al. 2005). In 2009, Rivera et al. selected 10 canine orthologues of genes either known or predicted to increase the risk of human breast cancer, including BRCA1, BRCA2, CHEK2, ERBB2, FGFR2, LSP1, MAP3K1, RCAS1, TOX3, and TP53. Four to nine common SNPs were selected per gene, totalizing 63 genotyped SNPs. Haplotypes in BRCA1 and BRCA2 genes were significantly associated with an increased risk of developing mammary tumours (with a stronger association to BRCA1 in malignant cases) in English Springer Spaniel female dogs (Rivera et al. 2009). In 2011, Borge et al. included 64 SNPs from 11 candidate genes, namely, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EGFR, ESR1, HER2, PTEN, STK11, and TP53, from 8 different breeds, divided into “high risk” and “low risk” groups. The authors highlighted

potential pathogenic variants that appear to be associated with canine mammary tumours (Borge et al. 2011). Later, they also found a correlation with the ESR1 gene, revealing its influence in the susceptibility to mammary tumour development (Borge et al. 2013). In 2014, a case-control study surveyed female dogs from several breeds for genetic differences, specifically in BRCA1 and BRCA2. The latter was found to be associated with risk of mammary tumour development (Enginler et al. 2014). Recently, a study described the first canine mammary tumour genome-wide association study, including approximately 130,000 SNPs, comprising only English Springer Spaniel dogs (Melin et al. 2016). The authors revealed a new gene, namely, CDK5RAP2, involved in cell cycle regulation, with a possible key role in the development of mammary tumours (Melin et al. 2016). The most recent study, considering the risk of mammary tumour development, included 67 canine SNPs from 14 genes including HER2, EGFR, TP53, STK11, BRCA1, BRCA2, RAD51, CHECK2, PTEN, BRIP1, ESR1, PGR, PRLR, and COMT (Canadas et al. 2018b). From this study, RAD51 and STK11 genes emerged as being involved in the risk of mammary tumour development (Canadas et al. 2018b). Additionally, genetic variants, such as polymorphisms related to the hormonal environment, demonstrated to be pertinent in different clinicopathological features (Dias Pereira et al. 2008, 2009; Canadas et al. 2018c). In this context, an association between age at onset of mammary tumours and tumour recurrence has been reported (Dias Pereira et al. 2008, 2009). Later, prognostic features were found to be associated with the individual's genetic profile, specifically with histological grade and vascular invasion (Canadas et al. 2018c). These results emphasize the importance of genetic variations on recognized prognostic factors for mammary tumours in dogs.

Several features of human breast tumours involving genomic aberrations have been identified in canine mammary tumours (Beck et al. 2013; Liu et al. 2014; Borge et al. 2015; Santos et al. 2017). Beck et al. (2013) detected copy-number aberrations in five sequenced tumour genomes and analysed the representation of copy-number imbalances in the plasma cell-free DNA. A recurrently deleted region at the proximal end of chromosome 27 was found in four out of the five tumour genomes and was proven to be significantly related with higher Ki-67 scores (Beck et al. 2013). Liu et al. (2014) explored the genetic differences between simple and complex carcinomas. Their findings indicated that canine simple carcinomas probably arise from genomic aberrations, whereas complex carcinomas originate from epigenomic alterations. Aligned with Beck et al. (2013), Borge et al. (2015) also identified copy-number aberrations, fundamentally in PTEN and MYC genes that often occur during mammary tumour development, with increased frequency of aberrations and loss of heterozygosity being positively correlated with increased malignancy in terms of histopathological diagnosis. Lately, due to the known evidence on the heterogeneity in canine mammary tumours, a pilot study that included synchronous multiple tumours from the same animals reported different clonal genetic profiles between tumours, providing preliminary evidence for a probable independent pathogenesis of the different tumours of dogs presented with multiple mammary tumours (Santos et al. 2017).

9.5.2 Genetics of Feline Mammary Tumours

The study of the genetics of feline mammary tumours is of crucial importance allowing the data acquired to be effectively translated into the women's breast cancer. Indeed, the extraordinary homology between the human and the cat genomes allows the translation of the genetic data of the cat into the human counterpart, highlighting the use of the cat as a model of human breast cancer.

The genetic alterations that occur in cancer can serve as cancer biomarkers for diagnosis and prognosis and for choosing the adequate therapeutic program or also as markers to assess tumour response to therapy. As in the dog, also in the cat, the genes or chromosome alterations involved in mammary tumour carcinogenesis are far from being characterized.

The first published work regarding a cytogenetic study in feline mammary tumours goes back to 1991, where the loss of several chromosomes in two feline mammary cell lines were identified, namely, A3, B4, D2, F1, and F2, and also detected the gain of chromosome C2 (Minke et al. 1991). Further works were published, but most probably due to technical difficulties, several marker chromosomes were detected with the putative involvement of chromosomes B1, B2, and D4 (Mayr et al. 1995b, 1999; Santos et al. 2006). Numerous highly reshuffled karyotypes with recurrent losses of chromosome B2-material and E3-material were reported in feline mammary gland neoplasms (Mayr et al. 1999). More recently, several different aneuploidies in different passages of a feline mammary cancer cell line (FkMTp) were identified (Borges et al. 2016). These cell lines demonstrated to have a high degree of genome instability with several chromosome rearrangements involving different chromosomes (unpublished work). Interestingly, some of these chromosomes are the same as the ones involved in the previous cytogenetic works done by Minke et al. (1991) and Mayr et al. (1995b, 1999), demonstrating the recurrent use of these chromosomes in the tumorigenesis of feline mammary tumours. It is also important to highlight that some of these chromosomes, which seem to be involved in feline mammary tumours, are syntenic to human chromosome regions reported to be associated with the human breast cancer (Bièche et al. 1997; Popescu and Drazen 2002; Wessels et al. 2002; Bergamaschi et al. 2006; Korkola and Gray 2010).

Data regarding the key cancer-related genes that were associated with initiation or progression of feline mammary tumours was extensively reviewed by Adega et al. (2016). In this report, only the feline cancer genes that currently present more promising results to be used as models of human breast cancer in a near future will be focused. At the primary sequence DNA level, the genes that are being analysed more extensively in feline mammary tumours are the TP53 suppressor gene and the growth factor genes, such as the epidermal growth factor receptor, the EGFR family, mainly the erb-B2 receptor tyrosine kinase 2 (usually named ERBB2, EGFR2, HER2, or NEU) (Ignar-Trowbridge et al. 1992; Buerger et al. 2000; Santos et al. 2012a, 2013b).

The mutations detected in TP53 gene in human breast cancer, among other gene mutations, seem to be associated with the most aggressive triple-negative breast cancer (Walerych et al. 2012). Some mutations in this gene have also been reported

in cats with mammary tumours (Mayr et al. 1995a, 1998, 2000). In feline mammary carcinoma tissues, Nasir et al. (2000) detected a mutant p53 protein similar to what was reported for human breast cancer.

One of the most important genes for human breast cancer is the HER2 gene. In cats, HER2 gene at its DNA sequence was recently analysed for the first time (Santos et al. 2012a, 2013b) and was found that has no amplification with in situ hybridization techniques (Soares et al. 2013). The studies on HER2 expression in feline mammary tumours are more abundant (Adega et al. 2016). Some of these reports suggested that feline mammary tumours are a potential valuable model for HER2-negative human breast cancer, specifically those with a homologous gene behaviour and the recurrent occurrence of low HER2 expression levels in feline mammary tumours (Santos et al. 2013b). Moreover, it seems that feline mammary tumours can also be used as model for the HER2-positive human breast cancer (Soares et al. 2016a), since approximately 30% of feline mammary tumours test positive for the human epidermal growth factor receptor 2, which promotes the growth of cancer cells. However, Soares et al. (2016a) found that the HER2 protein in feline serum and in tumour tissue was associated with features of lower aggressiveness, contradicting what is described for humans. In fact, all these findings, together with the non-amplification of the HER2 gene (Soares et al. 2013), reinforce the need for more studies in order to clarify the biological role of this protein in feline mammary tumours.

Genetic investigation is quintessential and has been gradually increasing over the last years. The study of specific genetic variations in genes known to be involved in mammary carcinogenesis will undoubtedly contribute to a wider understanding of this complex disease. This subject holds great promise in human breast cancer and in canine and feline mammary tumours' clinical management because of its potential application in a preventive, diagnostic, and prognostic context and will certainly open new perspectives in determining potential targets for individual therapeutic approaches – the emerging trend called "theranostics" (Blomme and Spear 2010).

9.6 Conclusions

The "One Health" approach to oncologic diseases, including mammary tumours and other neoplasias, provides advances in the knowledge of malignancies and potentially an improvement in clinical outcomes for diseased humans and animals. The similar epidemiological, clinical, histological, molecular, and genetic features shared between human breast cancer and canine and feline mammary tumours described in this review represent important information to be used in research and in the clinical practice. However, despite the recent advances in diagnosis and treatment, breast cancer remains the leading cause of cancer-related mortality in women and also one of the most important causes of morbidity and mortality in canine and feline oncology. Future studies, using the "One Health" approach, might contribute to obtaining relevant knowledge in the clinical management of this disease.

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Chapter 10
Pets and Immunomodulation

Luis Taborda-Barata

Abstract The immune system is fundamental for survival. Its development and functions are modulated by various genetic and environmental factors. Pets are an important environmental factor, and pet ownership may have relevant immunomodulatory effects. Pets may induce immune modulation via changes induced in gut, cutaneous and respiratory microbiome in pet owners. Such immunomodulation-associated changes may have positive health outcomes. In fact, these may include a contribution towards reducing the risk of developing allergic diseases, if exposure to pets begins in early infancy. In addition, pet ownership may also have other beneficial health effects, namely, reduced psychological stress and depression, which, in turn, may be associated with positive immunomodulatory effects. Finally, pets may also stimulate their owners to have higher levels of regular physical activity and exercise, activities that also have potentially positive effects upon various functions of their owners' immune system.

Keywords Pets · Pet owners · Immune system · Immune modulation · Microbiome · Allergies · Stress · Depression · Physical activity

10.1 Introductory Note

It is generally accepted that keeping pets may have a positive influence upon health-related parameters. However, little is known regarding the effects pet ownership may have on the immune system. The objective of the current chapter is to attempt

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to clarify these aspects. However, before we can delve into what is known about possible pet-associated modulation of the immune system of pet owners, we first have to briefly analyse the principal features of how the human immune system works.

10.2 General Operational Features of the Human Immune System

The immune system is a complex network of cells and mediators that interact in order to perform an essential function: to discriminate between "self" (molecular sequences which are part of the organism) and "non-self" ("extraneous or foreign") molecules, in various settings (Burnet 1940; Billingham et al. 1956; Janeway 1992; Matzinger 1994).

The capacity which allows the immune system to mount an adequate response, in situations it identifies as those that involve "non-self" molecular sequences or "danger signals", is absolutely crucial. It distinguishes the immune system from all other body systems and makes it indispensable for survival, as demonstrated in HIV^+ patients with severe decreases in the numbers and function of $CD4^+$ T lymphocytes, who succumb to overwhelming infections and various forms of neoplasia. However, the immune system, as happens with all biological systems, is under complex functional control. This type of control is important and is certainly involved in an amazing property of the immune system: the capacity to adapt its responses to different substances it contacts with. This capacity is based upon two features that are crucial to a robust and adequate response: the specificity of the response and the capacity to generate immunological "memory". Thus, globally, the immune system has to perform a very complex and tailored balance between two principal objectives. The first one is to adequately respond to "non-self" molecular sequences that the body contacts with (microorganisms, certain chemical agents, etc.) or "danger signals/alarmins" released by trauma-, viral- or tumour-affected body cells; in this case, the immune system builds responses which lead to the elimination of those molecular sequences (Kang et al. 2015; Nie et al. 2016). The second objective is, in contrast, to develop responses that lead to immunological tolerance. Such tolerance applies to various "non-self" molecular sequences, namely, those that belong to certain microorganisms belonging to gut or respiratory microbiota or foods that are ingested. Such tolerance aims to avoid hypersensitivity reactions and promote tolerance to "self" molecular sequences, thereby averting inappropriate, deleterious responses against cells and mediators of the body the immune system belongs to and which might lead to situations of pathological autoimmunity and autoimmune diseases (Zharkova et al. 2017).

Thus the molecular ability for discrimination is the basis of the mechanisms carried out by the immune system, which contribute towards body homeostasis. However, it is necessary to artificially break down the immune system into two

components, in order to better understand how it operates. These two components are the innate and the acquired or adaptive branches of the immune system. Although they have their own mechanisms, it is important to acknowledge that these two branches work in collaboration.

The innate branch of the immune system includes a diverse array of cells and mediators, which constitute a first line of response against external ("non-self") agents or atypically expressed internal ("self") agents. In operational terms, innate immune responses are triggered by recognition of anomalous expression of "self" or "non-self" antigens, acting as a first line of response, and the mechanics of their action does not involve memory, significant specificity or significant improvement between the first and subsequent contacts with the same antigens. Apart from physical and chemical barriers, the innate immune system involves the actions of cells such as monocytes/macrophages, dendritic cells, natural killer (NK) cells, innate lymphoid cells (ILC), basophils, eosinophils and neutrophils, in various combinations (Mitchell and Isberg 2017).

In contrast to innate immunity, the adaptive or acquired branch of the immune system developed later in ontogenic evolution and is found only in vertebrates. It involves cells such as T lymphocytes (also known as T cells) and B lymphocytes (also known as B cells), as well as mediators known as immunoglobulins (antibodies of various isotypes: IgM, IgG, IgA, IgD and IgE). In particular, it is the adaptive immune system which ensures the previously mentioned essential features of specificity, adaptiveness, discrimination between "self" and "non-self", and memory (Chaplin 2010; den Haan et al. 2014; Pradeu and Du Pasquier 2018).

T cells, in particular, are absolutely essential to the induction and modulation of immune responses, namely, in terms of their specificity and memory (Chaplin 2010; Singh et al. 2017). In the body, they recirculate along various routes (tissues, lymph, blood), which allows them to take on a crucial role in immune surveillance against infectious microorganisms or tumour cells. These are the "effector" T cells, which can be globally subdivided into $CD4^+$ T cells and $CD8^+$ T cells. In addition, some T lymphocyte subsets have regulatory functions ("regulatory" T cells or Tregs) which they exert upon effector T cells in order to avoid excessive responses, namely, against self-antigens so that pathological autoimmune situations can be averted, or against external, non-self-antigens, with a view to avoiding excessive, hypersensitivity reactions, such as those that involve allergies (Sakaguchi et al. 2006; Mohr et al. 2018).

$CD8^+$ T cells essentially act through mechanisms involving direct cytolysis of target cells, whereas $CD4^+$ T cells preferentially operate via cytokine synthesis which allows them to stimulate and optimise functions of other cells they interact with, namely, macrophages and B cells (Chaplin 2010; Schmidt and Varga 2018). The interaction between T lymphocytes and other cells of the immune system is of paramount importance to the development and functions of not only the latter but also the former. In fact, in order to be fully functional, T lymphocytes need cells to present them stimulating peptide fragments (antigens), inserted into MHC (major histocompatibility complex) molecules expressed on the cell membrane of the antigen-presenting cell (Bustos-Morán et al. 2016). Cells with such a capacity are,

in fact, known as antigen-presenting cells (APC) and include macrophages, dendritic cells and B cells. On the other hand, once activated by antigen presentation, T cells also stimulate and improve the functions of APC. In this context, T cells induce macrophages and dendritic cells to become better at engulfing microorganisms and presenting antigens derived from them. In addition T cells also optimise antibody production by B cells and help them to become either full antibody-producing plasma cells or quiescent "memory" B cells, which are necessary for triggering adequate responses on a second contact with the same antigens (Ise 2016).

In practical terms, albeit in an incomplete fashion, immune responses can be broken down into two essential subtypes: those against "external" antigens and those against "internal" antigens.

10.2.1 Responses to "External" Antigens

If pathogenic bacteria enter the human body, the immune system can detect these bacterial cells as sources of foreign/"external" ("non-self") antigens since they express molecular patterns (pathogen-associated molecular patterns – PAMPs) that are not usually present in humans. Innate immune system cells that detect these foreign cells are usually resident in tissues and include APC such as macrophages and dendritic cells. For recognition of PAMPs, these cells use various families of receptors, broadly known as PRR (pathogen recognition receptors), among which Toll-like receptors (TLR) feature prominently. Recognition of PAMPs via PRR activates macrophages and dendritic cells, leading to the release of pro-inflammatory mediators such as prostaglandins, leukotrienes, bradykinin and other mediators (Rosenblat et al. 2014; Tartey and Takeuchi 2017). These molecules trigger a local inflammatory response which also includes the release of pro-inflammatory cytokines. These include IL-1β, IL-6 and tumour necrosis factor-alpha (TNF-α) which are released by dendritic cells and macrophages (Hodes et al. 2015; Rosenblat et al. 2014), thereby leading to the recruitment of immature monocytes as well as neutrophils from the blood into tissues where bacteria are present. Recruited monocytes can further differentiate into phagocytic macrophages to enhance inflammatory processes or promote resolution of inflammation (Ginhoux and Jung 2014; Shi and Pamer 2011). Apart from becoming activated due to the PAMP-PRR interaction, macrophages and dendritic cells, as well as incoming neutrophils, also use TLR and other membrane receptors to engulf the bacteria, through a process called phagocytosis, and also have internal microbicidal mechanisms that allow them, in most cases, to destroy these microorganisms. As a result of this process, bacterial-derived peptides are generated within macrophages and dendritic cells and are inserted into MHC molecules which are then expressed on the membrane of these APC. Since the expressed antigens are "external" (in this case, they stem from the engulfed bacteria), they are complexed together with MHC class II molecules, in the so-called endocytic pathway of antigen presentation (Blum et al. 2013). The involved APC then travel, via lymph, to the draining lymph nodes or, via blood, to the spleen,

where they show the membrane-located molecular complex of MHC class II $^{+}$ bacterial peptide/antigen to $CD4^{+}$ T cells. Those $CD4^{+}$ T cells that can specifically recognise this complex become activated and start producing a balanced set of cytokines (Th0-type cytokine pattern: IFN- γ, TNF-α, IL-2, IL-4 and IL-5, among others) which act upon the APC (Touzot et al. 2014). IFN- γ, for instance, can optimise phagocytic, microbicidal and antigen-presenting capacity of macrophages even further. In this context, it should be mentioned that some activated $CD4^{+}$ T cells, when continually activated, may preferentially start producing a biased set of cytokines, essentially involving IL-2 and IFN- γ (known as a Th1-type pattern), rather than a Th0-type (Maggi et al. 1988; Wierenga et al. 1991). In addition, specifically activated $CD4^{+}$ T cells also proliferate, thereby substantially increasing their relative numbers which allows a more robust response. Finally, most $CD4^{+}$ T cells also differentiate into effector T cells, although a small pool will become "memory" T cells, which will become rapidly activated in subsequent encounters with the same bacterial peptides.

Another cell can also present antigens to $CD4^{+}$ T cells: the B cell (Yuseff et al. 2013). In this case, most B cells, which are also located in the draining lymph nodes and in the spleen, can directly recognise bacterial antigens they are specific to and which reach them via the lymph or blood. These bacterial antigen-specific B cells then also act as APC and present these antigens via MHC class II molecules to specific $CD4^{+}$ T cells. Again, these T cells become activated, and both via direct cell-cell contact with the APC B cells and via production of cytokines, these $CD4^{+}$ T cells induce B cell proliferation and optimal production of antibodies against the bacterial peptides. In this context, IFN-γ produced by $CD4^{+}$ T cells interacting with these antigen-presenting B cells helps these B cells to undergo a process called isotype switching. This process allows B cells to stop producing IgM (which is a large molecular pentamer which works better in the blood) and start synthesising other isotypes such as IgG and IgA, which also work very well in mucosal surfaces and other peripheral tissues.

Effector $CD4^{+}$ T cells then exit the lymph nodes and recirculate via lymph and blood into target organs where bacteria are present and where T cells may more efficiently modulate the actions of innate immune cells in order to make them better at eliminating the involved microorganisms (Walling and Kim 2018). By the time these T cells reach the target organ, not only are macrophages and dendritic cells present in the vicinity of the bacteria but also recently migrated neutrophils are locally performing phagocytic activities. Finally, another component of the innate immune system also takes part in the elimination of bacteria: the complement system. Among other actions, the complement system can directly lyse bacteria.

As a result of the actions of the innate and the adaptive branches of the immune system, pathogenic bacteria can be eliminated, and both T and B "memory" cells persist in lymph nodes and/or the spleen, where they will become the source of rapid responses, in case of a future second contact with the same pathogenic bacteria.

The same type of general outline of immune response occurs with external "allergenic" proteins ("allergens"), since these are also taken up by macrophages and dendritic cells, which also present them to antigen-specific $CD4^{+}$ T cells. In

most cases, such antigen presentation leads to balanced cytokine production (a Th0 cytokine pattern, as previously mentioned) (Touzot et al. 2014). However, depending upon various factors, these $CD4^+$ T cells may be driven into producing high amounts of IL-4, IL-13 and IL-5 (known as a Th2-type pattern) (Maggi et al. 1988; Wierenga et al. 1991). These are cytokines that are associated with preferential production of IgE (via the actions of IL-4 and IL-13) and with the influx of eosinophils (due to the actions by IL-5) into peripheral tissues. These are the two main features of allergic responses, and Th2-type $CD4^+$ T cells are crucial determinants of such responses.

Before we move on to responses to "internal" antigens, it is necessary to mention that, in some cases, external antigens can form complexes not with MHC class II molecules but rather with MHC class I molecules. This means that, in this case, the usual endocytic pathway is not used but rather a pathway known as "cross-presentation" (Blum et al. 2013). Specific subsets of dendritic cells are very efficient at using this process, which is quite important to trigger responses to "external" antigens by $CD8^+$ T cells.

10.2.2 Responses to "Internal" Antigens

In contrast to responses generated against "external antigens", those that target "internal" antigens are mainly carried out not by $CD4^+$ T cells but by $CD8^+$ T cells. In this case, peptides are derived from proteins that are located in the cytoplasm of target cells. Responses to virus infection and to tumours are good examples of this type of immune response. When virus-infected cells are activated, they may transcribe and translate viral proteins. Some of these are transported into the proteasome which digests proteins into peptides. These viral peptides are then translocated into the endoplasmic reticulum where they are inserted into MHC class I molecules. Such MHC class I^+ viral peptide complexes are then transported to the cell membrane, where they are expressed (Blum et al. 2013). Circulating $CD8^+$ T cells that specifically recognise these antigens may then become activated, if antigen presentation occurs adequately. Activated $CD8^+$ T cells may subsequently become fully cytolytic and eventually lyse the infected cells which presented viral antigens to them (McBrien et al. 2018). In addition, viral antigen-specific $CD4^+$ T cells may also become activated if APC engulf infected cells which were destroyed by $CD8^+$ T cell-driven cytolysis. Activated $CD4^+$ T cells, which produce high levels of IL-2 (most frequently in a context of a Th1-type cytokine pattern), may also further contribute towards $CD8^+$ T cells becoming cytolytic, since IL-2 is a major inducer of cytolytic features in $CD8^+$ T cells. Again, each time a $CD4^+$ or $CD8^+$ T cell is activated, there is cell proliferation and differentiation with most cells becoming effector T cells and a minor proportion developing into "memory" T cells. Finally, viral antigen-specific B cells may also become activated and produce antibodies against those antigens, under the influence of viral antigen-specific $CD4^+$ T cells.

It is also important to highlight that, in some cases, both viruses and tumours downregulate expression of MHC class I on affected cells. This means that these

cells cannot activate $CD8^+$ T cells (since these cells only respond to MHC class I-mediated antigen presentation by cells) and, therefore, escape immune surveillance. However, one type of cell that belongs to the innate immune system – the natural killer cell (NK cell) – may become activated in this context of absence of expression of MHC class I molecules and lyse these virus-infected or tumour-affected cells (Kumar 2018).

10.2.3 Further Aspects Regarding Tregs

A subset of T cells, known as "regulatory" $CD4^+$ T cells (Tregs), control immune responses at various levels and use various mechanisms, namely, direct cell-cell contact inhibition or cytokine (IL-10 and/or transforming growth factor/TGF-)-driven inhibition. These cells include various subtypes (Mohr et al. 2018). It is currently known that when such cells are decreased in numbers or functionally deficient, various types of diseases may arise, depending upon the context. Examples include the development of Th1 T cell-driven autoimmune diseases or Th2 T cell-induced allergic diseases.

10.2.4 Development and Maturation of the Cells of the Immune System

All cells of the immune system originate in the bone marrow, via a process called haematopoiesis, which allows stem cells to differentiate into monocytes, T lymphocyte precursors, B lymphocytes, innate lymphoid cells, NK cells, eosinophils, basophils and neutrophils. T lymphocyte precursors, however, need to migrate to the thymus, where they mature into T lymphocytes. After leaving the bone marrow, B cells migrate to lymphoid organs such as the lymph nodes, the spleen and the gut-associated lymphoid tissue (GALT). The same happens to T cells, once they exit the thymus. In fact, GALT seems to be an extremely important lymphoid organ for the full development of the immune system (Lamichhane et al. 2014). In this context, immune cell trafficking is essential for immune surveillance and homeostasis (Takeda et al. 2017).

10.2.5 Factors Affecting the Immune System

Various environmental factors may affect the development, maturation or function of the human immune system. Such influences may be analysed independently, but one must not forget that they will most likely exert their modulating effects in clus-

ters of combinations. Since it is not possible to focus on all environmental factors in this chapter, we will essentially discuss pet ownership-associated factors that most likely influence the human immune system. Such influences include (a) exposure to non-pathogenic microorganisms, which may be associated with adequate maturation of the immune system, changes in human gut microbiota and protection against the development of allergies; (b) the psychological aspects of pet keeping, including a role in coping with stress, depression and anxiety; and (c) physical exercise.

10.3 Pet-Associated Factors with Immunomodulatory Capacity

Research involving the immunomodulatory role of keeping pets, particularly furry ones, is scarce. This review therefore concentrates on research that was carried out in the previously mentioned settings of human exposure to microbiomes; psychological stress, depression and anxiety; and physical exercise and then attempts to extrapolate such findings to the context of pet ownership. In addition, this review focuses on studies performed in humans, with just a few additional studies in murine models that may help to better understand immunomodulatory effects of the factors under study.

10.3.1 Exposure to Non-pathogenic Microorganisms, Modulation of Human Gut and Respiratory Microbiota and Risk of Developing Allergic Diseases

10.3.1.1 Gut Microbiota Modulate Immune Responses

The "hygiene hypothesis" applied to immune development and allergic disease by Strachan (Strachan 1989) and its subsequent adaptations and related hypotheses, such as the "microbiota hypothesis" or the "biota alteration or depletion theory" (Parker 2014), suggest that the lower the degree of exposure to microorganisms in childhood (with changes or depletion of biota, in post-industrial societies), the higher the probability of subsequent development of allergic diseases (and autoimmune diseases and even cancer, cardiovascular or neuropsychiatric diseases) (reviewed by Villeneuve et al. 2018).

Human exposure to microorganisms is a natural phenomenon which is fundamental to the colonisation of the gut, the skin and the respiratory mucosa by high amounts of diverse microorganisms that live in symbiosis with the human body – the microbiota (Eckburg et al. 2005). In ontogenic terms, microbiota are essential to the development and maintenance of adequate body homeostasis. In fact, microbiota have many positive effects, namely, the absorption of various nutrients from

foods, the synthesis of certain vitamins (O'Keefe et al. 2009; Scarpellini et al. 2015) or the metabolisation of undigested nutrients to produce short-chain fatty acids with potent anti-proliferative and anti-inflammatory properties (Pryde et al. 2002; West et al. 2014). However, in certain situations, microbiota that have somehow undergone dysbiotic processes may enhance disease pathogenesis via pro-inflammatory mechanisms (reviewed by Greer and O'Keefe 2011; Schippa and Conte 2014; West et al. 2014).

Colonisation of the gut by microbiota begins in early life, at birth (in utero exposure may be pathological), then undergoes various changes during the first year of life and subsequently remains relatively stable (Spor et al. 2011), although it can be modified by diet and some antibiotics (Abraham and Cho 2009; Cresci and Bawden 2015). Factors that contribute towards gut colonisation of the infant include (a) birth via vaginal delivery, which involves exposure to a mixture of gram-negative and gram-positive bacteria, aerobes and anaerobes (Dominguez-Bello et al. 2010; Makino et al. 2013); (b) breastfeeding (Perez et al. 2007), which is rich in prebiotics that promote the growth of intestinal microbes (Newburg and Walker 2007) and which also contains small amounts of *Bifidobacterium* (Martín et al. 2009); (c) diet, in later childhood (De Filippo et al. 2010; Yatsunenko et al. 2012); and (d) various environmental influences, namely, infections, exposure to aeroallergens or exposure to animals (West et al. 2014).

It should be stressed that colonisation of the human gut is not only important to the maintenance of homeostasis in the host but also in the immune system of the latter. In fact, diverse and rich gut microbiota modulate and train the immune system of the host, by contributing to its development and maturation, namely, in GALT (Mosconi et al. 2013; Macpherson and Harris 2004; Baptista et al. 2013; Geuking et al. 2011; Rakoff-Nahoum and Medzhitov 2008; reviewed by Gensollen et al. 2016 and by Zhao and Elson 2018). In particular, a critical time frame seems to exist from birth until the end of the first year. Such period constitutes a true "window of opportunity" for modulating the morphological and functional development of the immune system and, most importantly, for setting up mechanisms related to immune tolerance to gut microbiota (reviewed by Houghteling and Walker 2015), foods and other antigens (Spor et al. 2011). In fact, if colonisation does not occur during this "window of opportunity", problems of immune development in the gut or other secondary lymphoid tissues may be apparent in adults (El Aidy et al. 2013; Bauer et al. 1963; Gordon et al. 1966).

More specifically, gut microbiota influences the relative composition of intestinal mucosal T lymphocyte subsets with distinct effector functions. In this context, gut microbiota contributes to homeostasis by controlling the relative actions of pro-inflammatory Th1-type $CD4^+$ T cells that produce interferon-γ, Th17 cells (which produce pro-inflammatory cytokines IL-17 and IL-22) and some innate lymphoid cells, as well as the anti-inflammatory actions of $CD4^+$ Tregs. Furthermore, different types of bacteria may modulate the differentiation of different types of effector T cells. In the murine model of germ-free mice, which develop in a microbe-free environment, artificial gut colonisation with filamentous bacteria is associated with the preferential development of potentially pro-inflammatory Th17 cells as well as Th1

$CD4^+$ T cells, whereas other bacteria, such as *Clostridi*a or *Bacteroides fragilis*, favour the generation of anti-inflammatory, regulatory $CD4^+$ Tregs, which produce IL-10, inhibit the development of Th17 cells and provide help with gut homeostasis (Atarashi et al. 2011; Sefik et al. 2015), and contribute towards induction of tolerance to food antigens (Gaboriau-Routhiau et al. 2009; Ivanov et al. 2009). Another aspect must also be emphasised: a lack of $CD4^+$ Treg in germ-free mice is also associated with the development of clear Th2-type (rich in IL-4, IL-13 and IL-5) $CD4^+$ T cell effector responses, which are reflected in high serum IgE levels, due to the IgE isotype-switching effects of IL-4 and IL-13 in mucosal B cells. Again, this pattern can be reduced by gut colonisation with various bacterial types during early life (the window opportunity) but not thereafter (Mazmanian et al. 2005; Gaboriai-Routhiau 2009; Klaasen et al. 1993; Cahenzli et al. 2013).

It should be highlighted that microbiota colonisation of the gut of germ-free mice not only affects the T cell component of adaptive immunity but also B cells. In this case, there is an enrichment of the B cell repertoire and increased production of antibodies by B cells, particularly IgA, upon interaction with T cells (West et al. 2014).

A final aspect involves analysing whether optimal interaction between microbiota and the immune system can be protective against disease and also whether situations of dysbiosis may be associated with the development of diseases such as autoimmune diseases, cancer or allergies (reviewed by Greer and O'Keefe 2011; Schippa and Conte 2014). In fact, various studies have shown that children exposed to microbiota-rich environments, such as farms, from an early age, have a decreased risk of development of allergic diseases (Riedler et al. 2001; Schuijs et al. 2015). Curiously, the protective effect of farm and animal-rich environments may also be associated with increased Treg cell activity in the infant. Thus, although it is not firmly proven, one of the plausible explanations for the protective effect of early life farm exposure is the role of microbiota because individuals exposed to a farm environment possess different microbial diversities compared with other lifestyles (Dicksved et al. 2007). Overall, various studies have shown that reduced gut microbiota diversity during infancy is associated with allergic disease later in childhood (Kalliomaki et al. 2001; Penders et al. 2007a; Vael et al. 2011; Vebo et al. 2011; Bisgaard et al. 2011; Nakayama et al. 2011; Abrahamsson et al. 2012).

10.3.1.2 Pet Ownership Is Associated with Increased Diversity of Dust and Human Gut Microbiota

Having pets such as cats and dogs has been shown to make homes of their owners, namely, house dust, richer in bacterial products such as endotoxin and LPS (Heinrich et al. 2001). In fact, the microbiota in dust from households with cats or dogs is significantly richer and more diverse than that found in homes without pets, as shown in several cross-sectional studies (Dunn et al. 2013; Fujimura et al. 2010; Barberán et al. 2015; Dannemiller et al. 2016; Sitarik et al. 2018). As an example, a study of 746 infants from the Canadian Healthy Infant Longitudinal Development

Study (CHILD) cohort, in which over half of studied infants were exposed to at least one furry pet in the prenatal and/or postnatal periods, showed that pet exposure significantly enriched the abundance of *Oscillospira* and/or *Ruminococcus* bacteria (Tun et al. 2017). Curiously, these types of bacteria have been negatively associated with childhood atopy and obesity.

Furthermore, a recent, longitudinal study aimed to investigate whether introducing a dog into the home changes dust microbiota makeup in the home (Sitarik et al. 2018). Dust samples were collected on-site just before dogs moved into the homes as well as 12 months later. Microbiota composition was compared between homes that did and did not adopt a dog. This study clearly showed that the introduction of a dog into a home significantly resulted in establishment of greater microbiota diversity in that indoor environment. Another study examined a small set of house dust samples drawn from a birth cohort and revealed that dust in homes with dogs had higher relative abundance of specific *Treponema*, *Capnocytophata* and *Moraxella* taxa compared with dust from homes without dogs (Fujimura et al. 2010). Additionally, another study also demonstrated in a sample of approximately 1200 homes across the USA that house dust in homes with dogs had higher relative abundances of *Porphyromonas* and *Moraxella* bacteria, compared to house dust in homes without dogs (Barberán et al. 2015). Furthermore, many of the bacteria enriched in dog homes have previously been identified as common members of the canine oral microbiota (*Porphyromonas*, *Fusobacterium*, *Capnocytophaga* and *Moraxella*) (Sturgeon et al. 2013; Oh et al. 2015), as well as the canine gastrointestinal tract microbiota (*Fusobacterium*, *Prevotella* and *Streptococcus*) (Hand et al. 2013; Middelbos et al. 2010).

Since cats and dogs increase the amount and diversity of house dust microbiota, it is expected that ownership of these types of pets may also change the gut microbiota of their owners. In fact, dog owners tend to share many features of their microbiota, namely, bacterial diversity, with that of their dogs (Song et al. 2013). However, such interplay has not always been observed, and some studies have shown that greater microbe diversity in the environment may be associated with reduced diversity of the gut microbiome in humans (Dicksved et al. 2007). Thus, these aspects have to be further studied.

The precise mechanisms by which a child's gut microbiota can be influenced by their home dust and pet-derived microbiota are not specifically known. Additionally, it is not known whether a specific species or a network of species is necessary to impact the immune system's development. It is possible that many different combinations of bacteria in early life could yield better health in the child, but perhaps the optimal combinations depend on what the child has already been exposed to. In some cases, the prevalence or relative abundance of specific organisms has been associated with atopic diseases. For example, early life colonisation by *Clostridium difficile* reportedly increases the risk of childhood wheeze, eczema and asthma, whereas certain *Firmicutes*, *Bacteriodetes*, *Bifidobacterium* and *Lactobacillus* are regarded as protective (Kalliomaki et al. 2001; Bjorksten et al. 2001; Johansson et al. 2011; Murray et al. 2005; Lynch et al. 2014). A study which analysed the influence of pets and older siblings upon the microbiota of younger sibling showed that

these two traditionally protective "hygiene hypothesis" factors exert distinct effects on microbiota diversity (Azad et al. 2013). These results suggest that the "microflora hypothesis" of allergic disease is probably due to multidimensional changes in the composition of microbiota, rather than simplified variations in general microbiota diversity (Johnson and Ownby 2016).

Another important point should also be addressed. It is currently not clear whether gut microbial composition and immune function changes can be induced after the immune system has been educated in early life. This should be studied since elderly people shut up in care homes with little variety in human contact and little exposure to pets have diminished gut microbiota diversity that correlates with poor health outcomes and increased levels of biomarkers of inflammation such as IL-6 (Claessen et al. 2012).

10.3.1.3 Pet Ownership May Decrease the Risk of Developing Allergies

There have been various studies addressing the issue of whether having a dog or a cat at home in early childhood protects against or increases the possibility of developing allergic disease or respiratory symptoms. Most reports, but not all, have shown that children exposed to dogs (and, less significantly, those exposed to cats) since birth had fewer respiratory symptoms or infections (Hatakka et al. 2010 Grüber et al. 2008; Bergroth et al. 2012). These aspects are particularly relevant, since modern infants and children living in developed countries, particularly those who live in cities, tend to live isolated from contact with non-pathogenic, immune system-modifying bacteria, due to very high and stringent hygienic and disinfectant procedures frequently implemented in most homes. Such procedures lead to low environmental microbial load and reduced microbiota diversity. This may lead to less abundant and/or diverse gut and respiratory microbiota in these children. In this context, keeping a pet such as a dog may increase environmental microbiota diversity, as mentioned before. This may lead to exposure to more abundant and diverse microbiota, which tends to be associated with higher diversity in gut and respiratory diversity of microbiota of pet owners.

We should therefore first analyse results in terms of early exposure of children to microbial diversity. In this regard, the "microbiota hypothesis", a variant of the "hygiene hypothesis", states that environmental microbial diversity influences the developmental process of an infant's gut microbiota ecosystem which subsequently, together with exposure to allergens and microbes, influences the child's development of the immune system and lowers the risk of allergies and asthma (Penders et al. 2007a; Johnson and Ownby 2016; Johnson and Ownby 2017; Wegienka et al. 2010). Although one study showed that infants with eczema had higher faecal microbiota diversity than infants without eczema (Nylund et al. 2013) and a couple of other studies have shown no relationship, most studies have demonstrated that a higher level of diversity in gut microbiota tends to protect children from developing allergies, wheezing or asthma (Remes et al. 2001; Hagendorens et al. 2005; Penders et al. 2007a, b; Nermes et al. 2013; Abrahamsson et al. 2014; Sjögren et al. 2009;

Fujimura et al. 2016; Penders et al. 2013). Since being exposed to dogs and cats during infancy may increase a child's microbiome diversity, it is interesting to analyse whether such feature protects against allergic disease. Dogs, for instance, change home dust microbiota by increasing the types and relative abundances of specific genera (Sitarik et al. 2018). In fact work has shown that children with dogs in the home in the first year of life have greater microbial diversity in their stool (Levin et al. 2016).

Apart from pet exposure-associated child microbiome diversity, the overall relationship between being exposed to furry pets during infancy and protection against subsequent development of allergic disease should also be analysed globally. In this context, although results have not always been consistent, most epidemiological studies have shown that children with regular exposure to livestock and/or pets such as dogs in homes in early life have significantly higher home endotoxin levels and fewer cases of subsequently developed allergy and asthma (Hesselman et al. 1999; Litonjua et al. 2002; Burr et al. 1997; Ball et al. 2000; Ownby et al. 2002; Bufford et al. 2008; Pelucchi et al. 2013; Lodrup et al. 2012; Peters et al. 2015; Wegienka et al. 2011; Wegienka et al. 2010). As an example, the West Sweden Asthma Study (WSAS), which was a population-based study of 788 adults, showed that growing up with livestock or furred pets decreased the risk of sensitisation to various pollen aeroallergens (Bjerg et al. 2016). Furthermore, a meta-analysis of all relevant studies published between 1966 and 2008 assessed the real impact of these exposures on paediatric allergic risk (Tse and Horner 2008). This meta-analysis of 27 studies studying associations between pet ownership and the development of allergic manifestations clearly showed that pet ownership during childhood may lead to a 14% decrease in allergic risk, with dog ownership appearing to be more protective than cat ownership.

It is important to stress once again that previous studies, which showed that pet ownership protected against development of allergies, also showed that household pets increased home endotoxin levels. An interesting prospective study involving three European cohorts showed that the levels of exhaled breath nitrogen oxide levels (FeNO, which is an indirect indicator of eosinophil-rich, Th2-type allergic inflammation) were significantly lower in those children who had had higher endotoxin levels at an early phase of their lives (Casas et al. 2013). In addition, a meta-analysis showed that the risk of allergic manifestations was moderately but significantly reduced in children living in homes with higher endotoxin levels (Tse and Horner 2008). Furthermore, a study performed in the USA showed that the median levels of endotoxin were almost sevenfold higher in Amish rural homes involved in traditional animal-supported farming than in Hutterite rural homes using modern mechanised farming methods, and this was associated with less frequent asthma, lower total serum IgE levels, lower levels of allergen-specific IgE against common allergens and lower percentages of circulating eosinophils in Amish children (Stein et al. 2016). Although endotoxin is not the only microbial-derived product that may affect the immune system, it certainly is a very important one, and its effect may be mediated by Toll-like receptor 4 (TLR4) recognition by macrophages

and dendritic cells and subsequent stimulation of adequate immune responses (Horner 2006).

10.3.1.4 Immune Mechanisms Involved in Pet Ownership-Associated Decrease in the Risk of Developing Allergies

Let us stress again that the immune system needs to contact with microbiota in order to fully develop and acquire its adequate effector and homeostatic functions. Thus, having a furry pet at home increases, as mentioned before, the microbial load and diversity in the house dust. Both adults and children inhale and ingest dust (particularly children), which may be a relevant contributory way to enrich human respiratory and gut microbiota. Not only that, but animals also have their own cutaneous, oral, gut and respiratory microbiota, and interaction with pet owners by licking them or touching them may also play a part in making pet and pet owner microbiota becoming similar (Song et al. 2013).

So, why is interaction with pet-derived microbes immunomodulatory? Well, although very few data exist regarding this specific topic in terms of pet ownership, one can extrapolate information from the broad role that microbes play in shaping our immune system. In fact, when bacteria are present in our gut, they are detected by various receptors on macrophages and dendritic cells. These receptors as well as others that are also expressed on these innate immune system cells allow these cells to phagocytose the bacteria and destroy them by various microbicidal mechanisms. As previously mentioned, this allows presentation of bacterial antigens to specific $CD4^+$ T cells, which then become apt effector cells and, via production of IL-2 and IFN- γ (a controlled Th1-type cytokine pattern), allow macrophages to become even more efficient cells at engulfing and destroying bacteria as well as at presenting bacterial peptide via MHC class II to $CD4^+$ T cells. In addition, IFN- γ induces isotype switching from IgM to IgG in B cells that also present bacterial peptides to $CD4^+$ T cells. This change in isotype allows B cells to produce IgG antibodies that, unlike IgM, can very easily migrate into peripheral tissues in order to exert their functions. It is thus possible that exposure to a farming and animal-rich environment during pregnancy and early infancy modulates Th1-/Th2-type immune responses, as well as Treg responses, and thereby protects against subsequent development of respiratory diseases, namely, asthma in a mother's offspring (Schaub et al. 2009). In fact such an effect may be associated with the induction and maintenance of Th1-type T cell responses (Simpson 2010), rather than allergy-inducing Th2-type mechanisms against environmental antigens. A prospective cohort study involving 239 2-year-old children living in a rural environment in the USA, who had been followed up from birth, indeed showed that the percentage of peripheral blood Th2 cytokine-producing $CD4^+$ T cells was significantly higher in children with doctor-diagnosed asthma and children with wheezing at 2 years of age (Duramad et al. 2006). However, a multiple linear regression model showed that pet ownership and exclusive breastfeeding at 1 month were significantly associated with 35.3% and 34.5% increases in Th1 cytokine-producing $CD4^+$ T cells, respectively. Another, small, study compared production of IFN-γ by mitogen-activated mononuclear cells

from cord blood (CBMC – involving lymphocytes and monocytes) as well as from peripheral blood (PBMC) mononuclear cells, both at birth and at 3 months of age between children born on a farm and those who had not been born on a farm (Roponen et al. 2005). Although there were no differences in IFN-γ production at birth, at 3 months of age, mononuclear cells from children exposed to cats or dogs at home showed an enhanced IFN-γ response. Thus, accepting that there may be a pet-associated decrease in Th2-type $CD4^+$ T cell responses, one may expect levels of IgE to be lower in children who were exposed to pets in their early life. One large birth cohort study from the USA indeed showed that the presence of pets (either dogs or cats) in the home during pregnancy was associated with a lower mean cord IgE level at birth (Aichbhaumik et al. 2008). Similar results were observed in peripheral blood, in a study carried out in 6–7-year-old children who had been exposed to dogs in the first year, and showed that these children had reduced total and allergen-specific IgE levels (Ownby et al. 2002).

Thus, exposure to pets, at least in rural environments, and in infancy, may be associated with a shift from an unbalanced, allergy-associated Th2-type to a preferential Th1-type (or balanced Th2-/Th1-type) of $CD4^+$ T cell cytokine production. This is quite important also because induction and maintenance of Th1-type T cell responses inhibits the development of Th2-type (IL-4-, IL-13- and IL-5-rich responses) T cell responses, which are associated with allergic responses. Thus, exposure to bacteria at an early stage in life (possibly, to a lesser degree, even later on) may help to develop adequate responses against external ("non-self") and even internal ("self") antigens. As previously mentioned, early life exposure to diverse microbiota also allows the development of Tregs. This is most important since various types of $CD4^+$ (and $CD8^+$) Tregs (as well as regulatory B cells) may be crucial in avoiding exaggerated, hypersensitivity-type responses against external aeroallergens (Th2-type responses in allergies) and also exaggerated $CD4^+$ Th1-type and $CD8^+$ cytolytic responses against cells expressing "self" autoantigens (autoimmune diseases). In this context, a study in 285 infants showed that exposure to dogs at home was associated with higher levels of IL-10 (and IL-13) production by mitogen-stimulated PBMC from 1-year-old infants as well as with reduced allergen sensitisation (Gern et al. 2004). This suggests that a more "tolerogenic" profile, possibly due to Tregs, which produce high levels of IL-10, may be induced by regular early life exposure to pets.

10.4 Psychological Stress and the Immune System

10.4.1 Effects of Psychological Stress upon the Immune System

The effects of psychological stress upon immunity depend upon the intensity and duration of its causing agent (Padgett and Glaser 2003; Sorrells and Sapolsky 2007; Morey et al. 2015).

Acute stress is associated with the release of various "stress hormones and mediators", namely, adrenaline and noradrenaline, as well as cortisol, into the bloodstream, with rapid preparation of the body for "fight or flight" reactions. It should be borne in mind that various cells of the immune system express membrane receptors for these "stress hormones and mediators", which allows these haematopoietic cells to respond adequately to such stimuli. In practice, this is reflected in mobilisation of various cells of the immune system (e.g. neutrophils, monocytes, lymphocytes), in the blood, in order to optimise eventual responses to injury or infection (Segerstrom and Miller 2004; Dhabhar et al. 2012). In addition, this is also associated with activation of these cells, with production of pro-inflammatory cytokines, such as IL-6 and IL-1β, whose levels also increase in peripheral blood (reviewed in a meta-analysis by Steptoe et al. 2007). In fact, T lymphocytes may even change their responsiveness to those stress-related neurotransmitters and hormones in order to respond more robustly and rapidly to agents inducing acute stress (Rohleder 2012).

However, if stress becomes chronic, immunological responses become strained, and features associated with immune dysregulation may arise, namely, in terms of cell trafficking and activation in peripheral blood and various organs (McEwen 2012). Chronic stress is associated with the development of two detrimental features in the immune system. These features are, on the one hand, chronic inflammation, which involves higher levels of pro-inflammatory cytokines (Gouin et al. 2012), and, on the other hand, suppression of the innate and the adaptive branches of the immune system, at least in part, due to persistently elevated levels of glucocorticoids (Kiecolt-Glaser et al. 1991; Segerstrom and Miller 2004; Sorrells and Sapolsky 2007). Furthermore, these concurrent features are even more apparent in elderly individuals (Vitlic et al. 2014). Thus, chronic stress-associated systemic inflammation is a form of dysregulation of the immune system which increases the risk of development of chronic inflammatory diseases (Ershler 1993), cancer or autoimmune diseases, as well as the possibility of activation of latent viruses and subsequent infections (Pawelec et al. 2005; Cohen 2005).

In terms of the adaptive immune system, chronic stress may be associated with changes in cytokine production in T lymphocytes. Animal models have shown that such cytokine change involves a shift from a Th1-type to a Th2-type cytokine pattern. As mentioned previously, Th1-type cytokine production (high in IL-2 and IFN-γ) is very important in driving immune responses against both extracellular bacteria and virus infections, as well as against tumours. Thus, stress-induced suppression of Th1 cytokines may decrease responses against many kinds of infections and tumours. Furthermore, since Th1 and Th2 cytokine patterns inhibit each other, stress-associated inhibition of Th1-type T cell responses may lead to activation of Th2 cytokine production, which is a pattern involved in allergies (Marshall et al. 1998). Finally, chronic stress-associated immune dysregulation may also involve a decrease in the number of circulating T lymphocytes, as well as decreased proliferative responses to mitogen responses, both in mice (Dominguez-Gerpe and Rey-Mendéz 2001; Moroda et al. 1997) and in men (Kiecolt-Glaser et al. 1991), as well as changes in patterns of cytokine production, which may increase the possibility of development of autoimmune diseases (Stojanovich and Marisavljevich 2008).

10.4.2 Interaction with Pets May Lower Chronic Stress Levels

Pet ownership may have some beneficial effects in terms of reducing chronic psychological stress. Anti-stress effects of human-animal interactions have been reviewed by Beetz et al. (2012). Various studies have shown that interaction with friendly pets, particularly dogs, is associated with a reduction of stress-related hormonal responses, with a trend towards normalisation of peripheral blood levels of cortisol, adrenaline and noradrenaline. In one study in healthcare professionals, the effects of 20 minutes of quiet rest were compared with 5 and 20 min of interaction with a therapy dog. A significant reduction of reported stress, in association with lower serum and salivary cortisol levels, was found when these professionals interacted with the dogs (Barker et al. 2005), thereby suggesting that cortisol-related immune suppression might be reduced. Similar results showing reductions in plasma cortisol levels were also reported in other studies when adult dog owners were petting their own or an unfamiliar dog but not while quietly reading a book (Odendaal 2000; Odendaal and Meintjes 2003).

Similar results were found in children. One study analysed cortisol levels in children with autistic-spectrum disorder, and related stress, before and after the introduction of a dog into their homes as well as after the dog was removed for a short period of time (Viau et al. 2010). Although no changes in mean diurnal cortisol levels were seen with the introduction or removal of the dog, the magnitude of increase in cortisol levels after waking up (cortisol awakening response) dropped significantly in the morning when the dog was present in the family and increased again upon removal of the dog, thereby suggesting that the presence of the dog normalised the increment in morning peaks in cortisol levels. Another study compared the effect of the presence of a dog with that of a friendly human as social support during a social stress test, on the cortisol levels of children with insecure attachment representations (Beetz et al. 2011). Curiously, the presence of the friendly dog during the test was associated with significantly lower cortisol levels in the children than those observed when there was a friendly human during the test.

All of the above studies show that pet ownership may help to reduce chronic stress, and this may be associated with a decrease in corticoid levels, thereby suggesting that the immune system of pet owners may be less chronically inhibited and/or dysregulated by endogenous corticosteroids. This may, in fact, be suggested by a report which showed a significant increase in salivary immunoglobulin A (IgA) in psychologically stressed college students, after stroking a live dog in comparison with stroking a stuffed dog or sitting quietly for a while (Charnetski et al. 2004). This suggests that pet-associated reduction of psychological stress may contribute towards improved B cell function, as reflected in increased levels of antibody production, although this warrants further research since another study detected no differences between pet owners and non-pet owners before and after interaction with a dog (Krause-Parello et al. 2012).

However, apart from this report, there are hardly any other studies analysing the effects of pet-associated psychological stress reduction on human immunological function.

10.5 Depression, Anxiety and the Immune System

10.5.1 Effects of Depression and Anxiety upon the Immune System

Various clinical and epidemiological studies have shown that depression (and depression-associated psychological stress) is associated with effects on immune function in human adults (Musselman et al. 1998; Padget and Glaser 2003).

Just as happens with chronic psychological stress, these immunological changes are complex and may depend on the severity and chronicity of depression. Nevertheless, changes may involve both a pro-inflammatory component and functional deficits in innate and adaptive immune cells, as is also observed in patients with chronic stress. One meta-analysis showed that peripheral blood levels of the pro-inflammatory cytokines IL-6 and TNF-α were elevated in subjects with major depressive disorder compared with normal controls, thereby suggesting the presence of an inflammatory component in depression (Dowlati, et al. 2010). Another meta-analysis showed that additional immunological factors may also be altered in depression, pointing towards inflammation and cell-mediated immune activation features (Maes 2011). In this context, depression seems to be accompanied by indicators of activation of cellular immunity, namely, increased serum levels of the soluble IL-2 receptor (sIL-2R), as well as increased numbers and percentages of $CD25^+$ ($IL\text{-}2R^+$)-activated T cells. However, the same meta-analysis also showed the presence of glucocorticoid resistance in immune cells, which may contribute to a dysregulation involving inflammation but also immunodepression. In fact, other studies have suggested that depression may, in fact, suppress immune activation. Such suppression may involve reductions in T cell proliferative responses to mitogens and T cell responses to infectious agents, as well as decreases in NK cell activity (Irwin 2002; Irwin et al. 2011; Ford et al. 2018; Kronfol 1983). Furthermore, several meta-analyses, although essentially based on cross-sectional studies (Herbert and Cohen 1993; Weisse 1992; Zorrilla et al. 2001), have shown that depressive disorders are indeed associated with decreased numbers and function of NK cells and poorer T cell proliferative responses to mitogens. This was further shown in a prospective, 1-year-long follow-up study which demonstrated, in a group of 105 healthy individuals, that development of depression was associated with decreased numbers of peripheral blood NK cells (Nakata et al. 2011).

B cell function may also be affected in chronic depression, at least in terms of antibody production. A study performed in measles-vaccinated individuals showed that adolescent and adult individuals with current major depressive disorder had

significantly lower levels of anti-measles IgG antibodies which made them less likely to test seropositive for measles than normal controls (Ford et al. 2018). Thus, this study showed that individuals with major depression were at greater risk of measles infection and severity possibly due to impaired maintenance of vaccine-related protection from measles. Similar results were observed in elderly individuals, in a study which showed that *Varicella zoster* virus (VZV)-specific cell-mediated immunity and VZV-specific $CD4^+$ T cells were significantly lower in the depressed group than in the controls (Irwin et al. 2011). Furthermore, there was a trend for depressive symptom severity to be associated with lower production of IFN-γ.

Various related studies assessed various aspects of immune function in 101 hip fracture patients 6 weeks and 6 months after injury and in 43 healthy age-matched controls (Duggal et al. 2013, 2014a). Thirty-eight of the hip fracture group patients were found to be depressed. There was a significant reduction of superoxide production in response to *Escherichia coli* in the monocytes of these depressed patients compared with nondepressed hip fracture patients or healthy controls. Thus, depressive symptoms may be associated with impaired function, reflected in reduced microbicidal mechanisms in monocytes and neutrophils.

Other studies by the same group, using a similar sample of patients, showed that depressed patients had altered T cell phenotypes, with an increase in activated, senescent $CD4^+$ and $CD8^+$ T cells and augmented production of pro-inflammatory cytokines (TNF-α) (Duggal et al. 2014b). Finally, the frequency of regulatory T cells ($CD4^+$ $CD25^+$ $Foxp3^+$ Tregs) and IL-10 production by $CD4^+$ T cells with regulatory properties and the frequency and IL-10 production by regulatory B cells (Bregs) were also studied in a similar sample of hip fracture patients and healthy age-matched controls (Duggal et al. 2016). A significant reduction in the frequency of Bregs was observed in patients who developed depression compared with nondepressed patients or healthy controls. Bregs also showed a significant decline in IL-10 production in depressed hip fracture patients compared with controls and nondepressed patients. In contrast, there was an increase in IL-10 production by $CD4^+$ T cells in hip fracture patients with new-onset depression compared to hip fracture patients without depression and healthy controls. This study suggests that patients with new-onset depression may have heightened Treg function, with inhibition of various immune functions by these cells, which may contribute to reduced microbicidal function observed in monocytes and neutrophils in these patients.

Interestingly, immune dysregulation in depressed patients may also affect relative expression of Th1/Th2 cytokine patterns in T lymphocytes. In this context, a study showed that parent-reported perceived stress and depressive symptoms in their children were associated with increased levels of the T helper cell type 2 (Th2) markers IL-4 and eosinophilic cationic protein in the latter (Wolf et al. 2008).

Overall, the apparently contradictory findings between inflammation and cellular immune activation and immune depression may have to do with the different study populations and levels of depression in the studied patients. Nevertheless, it is clear that depression changes various functional aspects of the immune system, and such changes may explain the observed higher frequency of infections, autoimmune diseases and cancer in chronically depressed patients.

10.5.2 Pet Ownership May Decrease Depression and Anxiety Levels

Pet owners may be less likely to suffer depression episodes, although the magnitude of this effect clearly varies depending upon the type and age of the animal, as well as the age and health status of the owner.

In a study which analysed depression levels in elderly adults who had been admitted to a rehabilitation unit, those that had been allocated a companion bird during their stay at the ward showed a decrease in depression levels (Jenssen et al. 1996). Another study, carried out in adult psychiatric patients, which compared 15 minutes of reading with 15 minutes of interactions with animals before applying a stressor agent showed that interaction with the animals significantly reduced anxiety levels (Barker et al. 2003). Another study, again in adult psychiatric patients also showed that a 12-week interaction with farm animals was associated with lower state anxiety at 6-month follow-up in the intervention group (Berget and Braastad 2011). Similar results were seen in adults hospitalised with heart failure (Cole 2007). In this study, one group of patients received a 12-minute visit from a volunteer with a therapy dog, whereas another group received a 12-minute visit by a volunteer and the control group just received usual care. When compared with controls, the group who had been visited by the volunteer and therapy dog had significantly greater decreases in anxiety levels, systolic pulmonary artery pressure and pulmonary capillary wedge pressure during and after the intervention. These changes were also associated with significantly greater decreases in epinephrine and in norepinephrine levels during and after the intervention. Thus, this study showed that dog-assisted therapy may decrease anxiety levels and improves cardiopulmonary pressures and neurohormone levels in patients hospitalised with heart failure.

Hardly any studies have addressed the issue of whether pet ownership improves immunological parameters in depressed patients. However, just as occurred with stress, pets also improve depressive symptoms, and this may be associated with improved immune function, although studies are clearly needed to ascertain this.

10.6 Physical Activity and the Immune System

10.6.1 Regular Physical Activity Can Boost the Immune System

Various studies have shown that regular physical activity and exercise training may reduce the risk of diseases such as hypertension and other cardiovascular diseases (Mora et al. 2007; Szostak and Laurant 2011; Ekblom-Bak et al. 2014) or type 2 diabetes and metabolic syndrome (Gaesser 2007; Fleg et al. 2015; Shephard and Balady 1999). In addition, higher levels of physical activity and regular exercise are

associated with reduced risks of all-cause mortality (Blair et al. 1995; Zhao et al. 2015).

In general, regular, moderately intense physical exercise has been shown to have antioxidant and anti-inflammatory action in various tissues, by modulating the ratio between anti-inflammatory and pro-inflammatory cytokine profiles, as well as by interfering with the antioxidant/pro-oxidant enzyme balance (reviewed by Sallam and Laher 2016). Overall, such actions underlie the most frequently observed anti-inflammatory effects of regular physical activity training (Nimmo et al. 2013). Furthermore, regular physical exercise also stimulates functions of the immune system (Turner and Brum 2017). However, one should be aware that the type and intensity of physical activity performed clearly influence inflammatory or immunological outcomes. In this context, low-intensity physical activities such as quiet walking or household tasks may not be sufficient to significantly decrease inflammatory parameters. But even so, low-intensity but regular physical exercise may still have a positive effect on the immune system. In a study involving 17 sedentary individuals who started an 8-week-long low-intensity exercise programme, chronic exercise was associated with upregulation of M2 macrophage response markers (CD14 and mannose receptor), which are activated by Th2-type responses, and downregulation of M1 macrophage markers (MCP-1), which are active in response to infections or tissue injury (Yakeu et al. 2010). The relevance of these changes needs to be ascertained. However, quite interestingly, low-intensity chronic exercise was also associated with the development of an anti-inflammatory profile, with an increase in plasma concentration of anti-inflammatory cytokines such as IL-10 and a decrease in IL-6 and TNF-α levels after exercise (Yakeu et al. 2010). Finally, regular physical exercise of moderate intensity has been shown to optimise NK cell numbers and function (reviewed by Bigley and Simpson 2015).

In contrast, evidence demonstrating that regular physical activity of moderate intensity has an anti-inflammatory potential is more robust (Nimmo et al. 2013; Sallam and Laher 2016). In addition, immunostimulatory effects may also be more pronounced. Furthermore, regular physical exercise may also reduce the relative proportion of pro-inflammatory macrophages (reviewed by Walsh et al. 2011), as well as the rate of T cell immunosenescence in elderly individuals (reviewed by Turner and Brum 2017), even though not all studies have shown that regular exercise can affect T or B cell function. A possible, practical reflection of such an effect can be observed in various cross-sectional as well as in randomised controlled studies which demonstrated that regular physical activity of moderate intensity can result in stimulation of higher levels of antibody responses to vaccination, particularly in elderly but also in non-elderly adults (Kohut et al. 2002; Schuler et al. 2003; Smith et al. 2004; Keylock et al. 2007).

Curiously, recent studies suggest that regular physical exercise may increase human gut microbiota volume and diversity (Clarke et al. 2014; reviewed by Monda et al. 2017). All these effects are beneficial for the host, improving one's health status, namely, in terms of homeostasis (Bermon et al. 2015).

10.6.2 Regular Pet-Associated Physical Exercise May Boost the Immune System

Having certain types of pets at home, such as dogs, may drive owners to be physically more active. In fact, dogs need to be walked and enjoy being involved in physical games outside. In some cases, dog owners only marginally, but significantly, walk longer per week than non-dog owners (Bauman et al. 2001). However, most studies addressing this issue have shown that dog owners are more likely to be physically active compared with non-owners (Anderson et al. 1992; Dembicki and Anderson 1996; Bauman et al. 2001; Parslow and Jorm 2003; Thorpe et al. 2006; Cutt et al. 2008; Shibata et al. 2012), although dog ownership on its own does not necessarily imply that dogs are walked by their owners.

Regular dog walking has been shown to be associated with positive health effects, namely, in terms of dyslipidaemia (Dembicki and Anderson 1996) or control of glycaemia in type 2 diabetes mellitus (Peel et al. 2010). However, there appear to be no studies in the literature that have addressed the benefits of pet walking on the immune system. Thus, one has to postulate that if pet owners perform regular physical exercise of the correct intensity (e.g. daily walking the dog), they may achieve some beneficial effects upon the immune system that have been previously described in non-pet owners who exercise regularly. However, such a possibility needs to be confirmed by adequately designed studies.

10.7 Conclusions

Pet ownership, particularly dogs (with which evidence is more robust), may have relevant immunomodulatory effects with associated positive health outcomes (summarised in Table 10.1). Immune modulation may be due to pet-induced changes in gut, cutaneous and respiratory microbiome. These changes may even contribute towards reducing the risk of developing allergic diseases, if exposure to pets started during infancy. In addition, pet ownership may also have other effects which involve reduced psychological stress and depression as well as increased levels of regular physical activity and exercise. Potentially, these effects may also modulate the immune system of pet owners. However, overall, very few or no studies have focused on such relationships and clearly further studies are warranted. In the meantime, I am very glad that I have a dog at home.

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Table 10.1 Synopsis of pet ownership-associated actual and potential modulatory effects upon owners' immune system

		Innate immune system		Adaptive immune system	
	General immunological features	Monocytes/macrophages	Other cells	T lymphocytes	B lymphocytes
Exposure to pet-derived microbiota	Adequate development of gut-related immune system[a] Avoidance of immune dysregulation[a]	Adequate development of gut-related macrophage role in antigen presentation[a]	Adequate development of neutrophil microbicidal roles[a]	Decrease in bias towards Th2-type T cell responses (IL-4; IL-5) Increased Th1-type T cell responses (IFN-γ) Increase in regulatory $CD4^+$ T cells (Tregs)[a] Decrease in pro-inflammatory $CD4^+$ Th17 cells in gut?[a]	Decreased production of total and allergen-specific IgE levels Enrichment of B cell repertoire[a] Adequate production of IgA and IgG antibodies[a]
Psychological stress	Decrease in chronic inflammation[a] Decrease in dysregulation of immune system[a]	Normalisation of impaired microbicidal mechanisms[a]	Normalisation in NK cell functions[a] Normalisation of peripheral blood NK cell numbers[a] Normalisation of impaired microbicidal mechanisms in neutrophils[a]	Decrease in bias towards Th2-type T cell responses (IL-4; IL-5)[a] Increased Th1-type T cell responses (IFN- γ)[a] Normalisation of T cell mitogen-driven proliferative responses[a]	Adequate production of IgA and IgG antibodies[a]

(continued)

Table 10.1 (continued)

		Innate immune system		Adaptive immune system	
	General immunological features	Monocytes/macrophages	Other cells	T lymphocytes	B lymphocytes
Depression and anxiety	Decrease in chronic inflammation (IL-6, TNF-α)[a] Decrease in dysregulation of immune system[a]	Normalisation of impaired microbicidal mechanisms[a]	Normalisation in NK cell functions[a] Normalisation of peripheral blood NK cell numbers[a] Normalisation of impaired microbicidal mechanisms in neutrophils[a]	Decrease in non-specific $CD4^+$ and $CD8^+$ T cell activation[a] Normalisation of antigen-driven T cell proliferative responses[a] Decrease in T cell immune senescence[a] Decrease in bias towards a Th2-type T cell profile[a] Normalisation of $CD4^+$ Treg activity[a]	Increase in numbers and function of Bregs[a]
Physical exercise	Decrease in chronic inflammation (IL-6, TNF-α)[a] Increase in human gut microbiota volume and diversity[a]	Changes in inflammatory / non-inflammatory macrophage balance[a]	Optimisation of NK cell numbers and function[a]	Increase in $CD4^+$ Treg activity (increase in IL-10 production)[a] Decrease in T cell immune senescence[a]	Normalisation of antibody response to vaccination (particularly in elderly patients)[a]

[a]No pet-related studies have been reported. Results are extrapolated from other studies in humans

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Chapter 11
Pets as a Novel Microbiome-Based Therapy

Mariana C. Salas Garcia, Ashley R. Schorr, Wyatt Arnold, Na Fei, and Jack A. Gilbert

Abstract While genomics can be used to determine genetic susceptibility to certain illnesses, genetic-based approaches alone are rarely capable of predicting the onset of a disease. Environmental factors, such as microbial exposure, often play a significant role in determining human susceptibility to illness, which impacts the genetic predictability of a disease in many cases. While humans are exposed to microbes in almost every facet of their daily lives, one vector has become of particular interest as of late: pets. In addition to the mental and physical benefits conferred by pets unto their owners, it is thought that human exposure to animal-associated microbes can play a significant role in bolstering human health. In response to this, a new treatment that leverages exposure to pet-associated microbes is being proposed for diseases such as asthma, atopic dermatitis, rhinitis, cardiovascular disease, obesity, and even depression. Emergent treatments like these, which have grown out of the human microbiome study, have begun to open up new frontiers in the field of personalized medicine. Microbial therapies such as probiotics, fecal microbiome transplants, and personalized diets are already having a substantial impact on patient care and are heralding in a new vision of precision medicine. Microbiome-based therapeutics involving microbial exposure in homes have been increasingly investigated for their potential to prevent and treat chronic diseases. This chapter explores the evidence that symbioses between humans and their cohabiting pets shape the interaction between microbes, host, and the environment and how that interaction affects human health and disease.

Keywords Pet · Microbiome · Human disease · Allergies · Therapy

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11.1 Introduction

Research into the human microbiome has shown that long-term antibiotic usage can lead to the development of antibiotic-resistant microbial strains and (Jernberg et al. 2010) a disruption of the microbial ecosystem within the human gut (Blaser 2017). While antibiotic use may provide the clearest picture of how external factors can affect the human microbiome, it has also emerged that the use of probiotics, fecal microbiota transplants, and personalized diets can redefine the human microbiome and impact health (Mimee et al. 2016). Recent developments in microbiome-based therapeutics have shown profound potential for the prevention and treatment of chronic diseases such as asthma, atopic dermatitis, rhinitis, cardiovascular disease, obesity, and even depression (McCloskey et al. 2017). Perhaps one of the most intriguing remediation treatments, which may also have one of the most lasting impacts, is the exposure to pets within a household.

While the figure varies from person to person, it is not uncommon for individuals in the modern era to spend over 90% of the time indoors (Klepeis et al. 2001), and this shapes the microbial world that they are exposed to. Moreover, an explosion in the growth of the world's cities has meant that more people are now growing up in urban environments than ever before (Wang et al. 2017). Apart from some managed green spaces, many urban dwellers receive little or no exposure to more biodiverse, microbially complex outdoor environments (Lehtimäki et al. 2017). Yet while more and more people see their contact with nature dwindle, they may still maintain some connection to the outdoors in the form of domestic animals (Fig. 11.1) While in some cases, this connection can result in the transmission of disease, it also facilitates

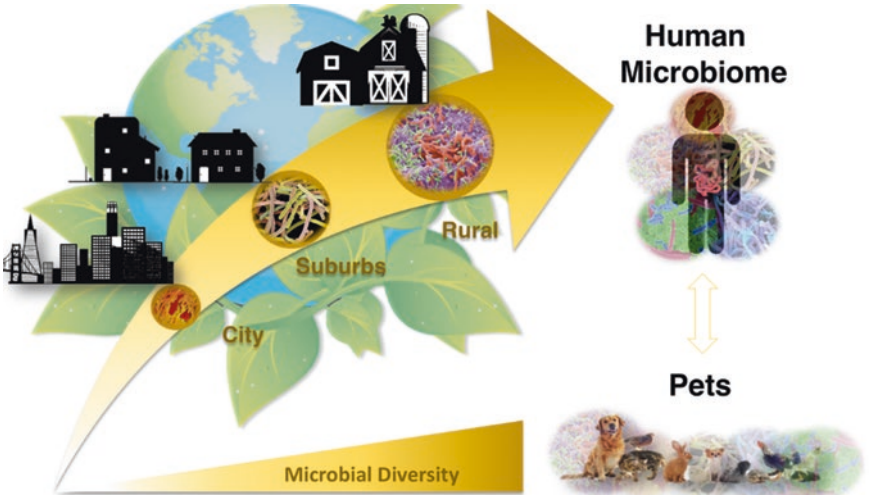

Fig. 11.1 Regardless of where people grow up in the world, they are almost certainly exposed to plethora of flora and fauna in one way or another. As a result, humans' microbial diversity increases over time, which leads to positive effects on human health

an exposure to animal-associated microbes that might beneficially influence immune development and encourage suppression of chronic diseases (von Mutius 2016).

Of the 82.5 million households with pets, roughly 40% own dogs, while 33% own cats and around 4% own other mammals (Steneroden et al. 2011). Pets are an increasingly integral part of society, and many of those households owning pets even consider them a member of their family (Carr and Rockett 2017). In addition to family pets, there are over 20,000 service dogs serving in the United States, according to the American Humane Association (Canines and Childhood Cancer n.d.). Indeed, living and existing day to day in such close proximity to pets, many of which are constantly entering and exiting the home, can create a variety of disadvantages as far as keeping the house clean and probably concerns of pathogens. However, the argument is becoming ever stronger that the benefits of coexisting with pets by increasing microbial exposure may outweigh the drawbacks in regard to human health.

Historically, pets have undoubtedly provided emotional companionship to humans (Sable 1995), yet as of late it has begun to emerge that pets may also aid humans physiologically by acting as a route of exposure to a more diverse microbial community (McCune et al. 2014). Studies surrounding the microbiome of the built environment suggest that humans who spend a majority of their time in a pet-driven indoor environment are exposed to saprophytic soil organisms with immunomodulatory potential (Hoisington et al. 2015). This chapter explores, holistically, the impact that pets may have upon human health and well-being and examines whether or not pets may be able to function as a new microbiome-based therapy.

11.2 Characterization of the Human Microbiome

Our microbiome consists of approximately 30–40 trillion cells, a number that rivals the amount of human cells within the body (Hoffmann et al. 2016). To make sense of this massive cohort of microbes, research has generally turned to the era of target gene sequencing, which allows for microbes to be accurately and easily identified from a sample, like stool (Karst et al. 2018). However, if the functional potential of the microbiome is what's of interest, metagenomics, which involves sequencing the genome of a taxon rather than a single gene, is used to characterize the microbiome (Shah et al. 2010). While these methods can determine which taxa are present, and potentially what they can do, it's still not sufficient to determine whether the microbes are detrimental or beneficial, as the virulence potential of a microbe is nearly always context dependent (Campanaro et al. 2018). Hence, just knowing that *Escherichia coli* exists in the stool samples using 16S rRNA sequencing, and even the evidence of antibiotic-resistant *Clostridium difficile*, it does not mean that you have an active infection (Zeng et al. 2017). Therefore, other approaches are required to characterize the functional microbes in the host, such as culturing, or enriching communities of microbes, and applying them to animal models to determine true virulence potential (Sabat et al. 2017). This caveat is extremely important, as it is all

too easy to falsely associate certain suspect taxa with particular health outcomes. Determining, accurately, which microbes are potentially beneficial or detrimental is of great interest as we move toward interventional studies, which strives to develop bacteria-based therapies to improve human health.

11.3 Colonization and Development of the Human Microbiome

Colonization of the microbiome begins at birth and develops significantly over time. Factors such as mode of delivery and the choice of feeding method are some of the first definitive events that can significantly impact the biodiversity of an infant's gut microbiome during development (Dominguez-Bello et al. 2016). Some studies showed that the infant could also obtain bacteria from the mother's amniotic fluids, but the evidence for these events is still extremely sparse and by no means proven (Neu 2015). Instead, for instance, the gut bacterial diversity is greater in a vaginally delivered infant compared to cesarean section delivery, likely due to exposure to vaginal and fecal microbes during delivery (Perez-Muñoz et al. 2017). Breast milk feeding has also been shown to result in greater gut bacterial diversity, when compared to formula feeding (Al-Shehri et al. 2015). The microbiome of infants is highly dynamic and does not really settle down to a more stable composition and structure until the infant starts on solid food (Bäckhed et al. 2015). Accumulating evidence shows that a more diverse microbiome can stimulate and "strengthen" the immune system, thereby reducing the likelihood of developing allergies, asthma, and other autoimmune conditions; the converse is also hypothesized (Zeevi et al. 2016).

Environmental factors, such as antibiotic, pets, and lifestyle, affect significantly human microbiome development in later life. Traditional standards hold that a household with pets might be "dirtier," while perspectives on hygiene are changing as research has revealed the importance of microbes in our lives (Martinez 2014). The once popular hygiene hypothesis, which focused on antibacterial hygiene, indicated that a decrease in pathogenic microbes would lead to a lower likelihood of getting ill. However, this hypothesis neglected to take into account the variety of helpful microbes that are being killed off along with the pathogenic microorganisms (Nermes et al. 2013). Research on the microbiome has revealed the significant differences between controlled hygiene ecosystems in comparison with ecosystems containing naturally occurring microbes (Bloomfield et al. 2016). Due to the essential roles of certain microbes in humans that have been discovered, people are able to use them as a medium to fight all these diseases instead of wiping them away with "antibacterial" products (Hartmann et al. 2016). There is a need to abandon this hypothesis, starting with children (Lambrecht and Hammad 2017). Although parental instincts may cause people to be more protective in the first months during the early life, interaction with environmental sources including pets, dirt, plants,

and even other humans is in fact proving to be beneficial to infant health. While understanding the importance of avoiding pathogenic diseases through a healthy level of hygiene is essential, the challenge is finding what extent of hygiene is most beneficial to human health (Bisgaard et al. 2011).

11.4 Microbiome-Immune System Axis

Study of the human microbiome has begun to transform how modern medicine responds to certain diseases and conditions, as a linkage between the microbiome and immune system. Already there are a multitude of ways to manipulate the immune system in medicine, such as suppressing unwanted responses to transplant rejection, autoimmunity, and allergies or stimulating protective responses against pathogens (Charnetski et al. 2004). During the first Next Gen Immunology—From Host Genome to the Microbiome: Immunity in the Genomic Era conference, at the Weizmann Institute of Science in Israel in 2016, many microbiologists and immunologists collaborated to conclude that, after years of research, there exists an intrabody relationship of the immune system with the microbiome. In addition, they concluded that the microbiome plays a role in many immune pathways, such as inflammasomes, innate immune system, and regulatory T cells (Oh et al. 2015). Research also explained how the neonate immunity is shaped from the microbiota in pregnancy and how the mother transfers innate immunity to offspring. As a result of the exchange of microbiota from mother to offspring through T-cell differentiation, antibodies, which often times are needed, are stimulated or blocked, such as with autism spectrum disorder (ASD) (Zeevi et al. 2016). Therefore, the microbiota as an immunomodulator could serve as new therapy to treat diseases (Fig. 11.2).

The dynamic microbial ecology in early life may play a role in helping children to adapt to their environment and build a mature immune system. In *Gut Microbes May Drive Evolution,* the author suggests that subsisting with other species such as dogs or cats may increase our microbial diversity and help the immune system development, thereby shaping our evolution through natural selection (Arnold 2012). Therefore, our symbiotic relationship with the trillions of microbes in our body and in the environments may profoundly shape our health (Costello et al. 2009). Understanding the relationships between the microbiome and immune system, especially across different life stages, is extremely important to optimize human health (Hooda et al. 2012).

Maintenance of a healthy microbiome beginning in early development is critical throughout life. A major component of the ongoing discussion of "Pets as a novel microbiome-based therapy" is the argument that pets are a potential protective mechanism during early childhood to combat the development of atopic disorders later in life (Bisgaard et al. 2011). A study demonstrated the interrelationship between pets and allergic diseases in children 5–14 years old, based on cross-sectional surveys between 1992 and 1999 from 5630 children in East Germany. Eighty-five percent of the children were discovered to be reactive to common

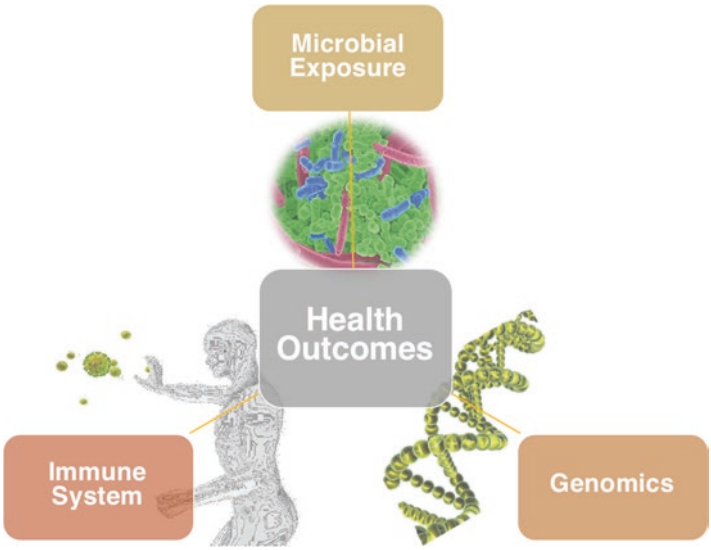

Fig. 11.2 Schematic diagram of the relationship between genetic background and microbial exposure as a determinant of immune system response. These three factors in combination are capable of holistically influencing human health outcomes

aeroallergens such as grass, mites, cats, dogs, and other pets, according to the IgA concentration testing. However, kids in contact with dogs in the first years exhibited a noticeable decrease in IgA concentration (Goldstein and Abrahamian 2015). Particularly noteworthy were the children experiencing this lower IgA concentration despite having one or more atopic parents, which increases their genetic probability of displaying phenotypes such as asthma, eczema, itchy rash, and pollen sensitization (Hölscher et al. 2002). Additionally, researchers have hypothesized that environmental exposures impact the immune system (Knight 2018). Thus, identification of the sources (pets) and how they interact with humans is crucial in order to understand human health outcomes and for the creation of therapies to combat chronic disease.

In an innovative study to understand the impact of pets on secretory immunoglobulin A (IgA) levels, Carl J. Charnetski et al. in 2014 observed and analyzed 55 college students assigned in 3 groups, each with varying levels of exposure to dogs for 18 min. Group 1 petted a live dog, group 2 petted a stuffed dog, and group 3 just sat on a couch, and pre- and post-test saliva swabs revealed that group 1 experienced a significant increase in IgA. This protein is essential in blood, and sufficient levels of it play a key role in the prevention of many diseases. And the study further suggests that the increased levels of IgA could be caused by the microbiome changes due to relaxation after exposure to the dog (Charnetski et al. 2004). These results demonstrate one example of how pets could modulate the structure of the microbiome, which may act as a novel immunomodulator (Hoffmann et al. 2014).

11.5 Sources of Microbial Exposure

11.5.1 Pets as Carriers

While it is important to maintain a certain amount of hygiene when cohabiting with pets, dogs and cats who are outdoor and indoor dwellers introduce microbially rich detritus from the outdoors that can play an important role in the health of the occupants. This effect is supported by a study in which the microbial communities of various residences, possessing either a dog, a cat, or no pets, were documented. The dogs, being both outdoor and indoor pets, exhibit reduced Th2 cytokine production, fewer activated T cells, and a distinct gut microbiome composition (Miletto and Lindow 2015). This composition is rich in *Lactobacillus johnsonii*, a microbe that can facilitate airway protection when orally supplemented as a single species (Fujimura et al. 2010). Conversely, cats are more likely to be exclusively indoor (Hedlin et al. 1986), which may contribute to the fact that samples from cat homes did not differ significantly from those of non-pet homes. These results indicate that animal activity can influence the levels and diversity of bacteria introduced to homes. Therefore, humans interacting with animals which carry allergic or infectious airway diseases will experience an increased diversity in the gastrointestinal microbiome and in turn will be less susceptible to a negative immune response of developing allergic atopy (Azad et al. 2013). Hygiene is key to maintain, but the microbial interaction that occurs due to the exposure to dogs or any other pets can radically and beneficially change the human microbiome in the first stages of life, proposing pets as a new microbiome-based therapy (Fig. 11.3).

In order to compare the effect of both dogs and cats on the microbial environment of homes, Fujimura et al. in 2010 used high-density phylogenetic microarray to test five dog homes, five cat homes, and five no pet homes for bacterial community richness, evenness, and diversity. The results implied that dog ownership increased the house dust diversity, caused by the introduction of many types of bacteria, and increased bacterial richness. A hierarchical cluster analysis was completed, revealing two main clusters. In the first cluster, which contained 100% of the no pet homes, the bacterial community was less diverse, rich, and even. A majority of the dog homes, and some of the cat homes, were in the second cluster, all of which contained a higher microbial diversity, richness, and evenness.

In consideration of common behaviors in pets, cats are generally more likely to be exclusively indoor pets, while dogs are both outdoor and indoor pets. Naturally, dogs most likely introduce environmental air-, water-, or soilborne bacteria that cats might not have the same level of exposure to if they live solely inside of the home (Fujimura et al. 2010). The study also found an inverse relationship between bacterial diversity and fungal richness, while many of the fungi included were known to cause lung inflammation and hyperreactivity. The implication of this outcome is that increasing the diversity of our microbiome may also aid in decreasing harmful fungi and viruses from our environment (Rosario et al. 2018). Reese et al. in 2007 found that chitin, which is a key component of fungi cell walls, induces an airway

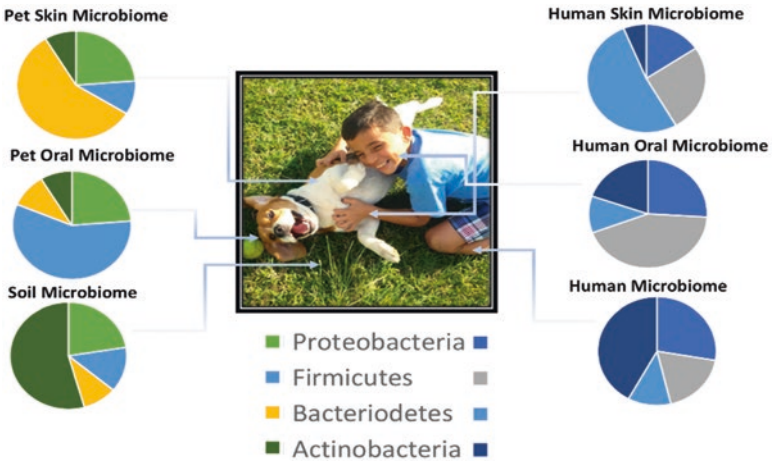

Fig. 11.3 Cohabiting with pets during early development represents the psychological and physiological symbiosis that can occur between the two species. Here a child is playing with a dog outdoors and cat indoor, increasing the diversity of microbes he is exposed to, which in turn transforms the microbial environment indoors and sustains increased long-term microbial diversity. The figure above visualizes an example scenario of shifting microbial abundances through interaction between humans and their pets

eosinophil and basophil influx in challenged mice similarly to asthmatic airways. Moreover, Fujimura et al.'s conclusions in 2010 indicate that exposure to a less diverse bacterial community, in combination with an increased exposure to chitin-containing microorganisms, could lead to the development of an allergic phenotype (Reese et al. 2007).

11.5.2 Pets as Microbial Transformers

There are multiple methods for testing the microbiome of an individual, including oral, skin/fur, and fecal sampling, all of which vary depending on the organism and differences of exposure for each part of the body. In a study conducted in 2014, Rodrigues Hoffmann et al. explored the skin microbial composition of healthy and allergic canines, while a similar study was completed by Older et al. in 2017 regarding the skin microbiome of healthy and allergic felines. Rodrigues Hoffmann et al. found that the order of prominence in microbial composition in dogs was *Proteobacteria*, *Firmicutes*, *Fusobacterium*, *Bacteroides*, and *Actinobacteria*. The same phyla were found in the feline microbiome, but in a different order: *Proteobacteria*, *Bacteroides*, *Firmicutes*, *Actinobacteria*, and *Fusobacterium*. It was discovered that *Bacteroides*, which is found abundantly in the oral microbiome of both dogs and cats, was significantly more present throughout the cats' microbiome than in the dogs', which may be attributed to their grooming behaviors.

Ironically, the body site with the most significant level (>15%) of *Bacteroides* in the canines was the dorsal perianal location (useful information for those people who may let their K9 friends share their oral microbes more than others) (Older et al. 2017; Hoffmann et al. 2014).

In the human microbiome, most of the bacteria are found in the gut, with the skin being a less hospitable, dry, and cool environment. There are various moist microenvironments in the skin, as well as dryer areas, with each skin type being a specialized niche with differing taxa. *Actinobacteria* and *Firmicutes* colonize it most prevalently, along with a fungal genus *Malassezia*. Canine skin, on the other hand, is more uniform across all skin sites with haired, sebaceous skin containing apocrine glands. Thus, it was discovered that the individual, rather than the skin site, was the main driving force in skin microbiome composition and diversity of canines (Anandan et al. 2016). Concerning fungal species, allergic canines were found to have a lower richness and diversity than those without allergies, unlike allergic felines who did not experience a decrease in richness or diversity. In both dogs and cats, *Dothideomycetes* predominated in allergic individuals, and the abundance of *Staphylococcus* increased significantly (Noli 2017).

Considering the differences in microbial composition for both humans and dogs, the interaction of different external sites between humans and their dogs has varying effects. In 2013, Se Jin Song et al. researched the level at which cohabiting family members share their microbiota with each other and with their dogs (Song et al. 2013). The results indicated that the two dogs to human site relationships with the highest number of shared microbial phylotypes are paw to palm and forehead to palm, followed by forehead to forehead and paw to forehead. Of the three groups tested in all of these areas, adult couples who owned dogs shared the most phylotypes with their dogs, followed by other dog owners. In addition, partners owning dogs share more phylotypes with other dog-owning couples than non-dog-owning adults share with their own cohabiting partner. Dog owners share as much microbial diversity with each other as cohabiting partners share together, even if the two dog owners do not cohabit. These results suggest that cohabitation with dog creates a more diverse microbial environment than cohabiting with a human (Dannemiller et al. 2016).

The possibility of transferring pathogens from pets to humans has been a concern for years. In 2012, researchers discovered that oral periodontopathic bacterial species could be transmitted between dogs and humans (Yamasaki et al. 2012). In this study, PCR and gel electrophoresis were used to detect pathogens. It revealed that of 11 periodontopathic species that were primed for, 3 of the species were present in both humans and their dogs. *Porphyromonas gulae, Tannerella forsythia,* and *Campylobacter rectus* were present in 71.2%, 77.3%, and 66.7%, respectively, of the dogs tested and present in 16%, 30.9%, and 21% of their human counterparts. This outcome indicated the possibility of cross contamination of certain periodontopathic species between humans and dogs, although the prevalence was much lower in the humans than the dogs. In 2012, Dewhirst et al. explained, based on a 16S rRNA-gene sequence comparison, that 16.4% of oral bacterial taxa were shared between dogs and humans. Wanting to dive more deeply into the commonalities

between microbial composition of dogs and their owners, researchers completed another study using next-generation sequencing technology. The results differed slightly from the previous study, revealing that humans and dogs only shared 4.9% of the identified operational taxonomic units (OTUs). These results suggest that bacterial dissemination between dogs and humans is not common. However, the study indicated that precautions should still be taken to minimize the possibility of contamination by pathogens from pets (Dewhirst et al. 2012).

11.6 Pet-Human Interaction and Health Outcomes

As a vast majority of health responses to disease are dictated by the host's immune response, factors which promote immune health, like an exposure to microbial diversity through pets, may be important when thinking about how health is approached. Pet-associated bacteria have been shown to have a positive influence on health outcomes in animal models and thus may serve as a novel preventative treatment for a number of human diseases. For example, the murine model has shown that mice that have been exposed to dog-associated house dust microbial environments are protected against airway allergens (Kau et al. 2011).

11.6.1 Allergic Rhinitis

Allergic rhinitis, also commonly known as hay fever, is a condition that affects over 40 million Americans. Sufferers of hay fever are likely to experience symptoms such as skin rashes, runny nose, itchy eyes/mouth, fatigue, and other symptoms. Hay fever comes in two forms, the first being seasonal (spring, summer, and early fall), which is caused by significant sensitivity to airborne mold spores or to pollens from weeds and grass. The perennial form is caused by year-round sensitivity to many allergens, even including other human beings. Hay fever not only has physical effects, but it can also affect daily life by causing irritability, sleep disorders, and fatigue and can decrease one's ability to focus. Individuals have even been known to miss a day of school or work and succumb to injuries at school or work, and even motor vehicle accidents have occurred at the hands of hay fever (Tran et al. 2011). Therefore, the discovery of new methods of prevention and treatment is urgent in order to decrease the prevalence of allergic rhinitis cases. Yet, drastic climate changes that negatively impact the environment, increasing airborne dust, pollen, and humidity, can make hay fever seem almost unavoidable (Wheatley and Togias 2015).

Regardless, all living places, including rural, urban, and suburban, obviously have their advantages and disadvantages. In Austria, allergic atopic disease prevalence in children was compared, revealing the immense difference between kids living on farms and those living in non-farming environments. Perhaps the most

surprising outcome was that factors such as genetic background, diet, and housing did not have an effect on allergic sensitization. Instead, only children who were in regular contact with animals experienced a significant difference compared to kids who lacked consistent animal exposure. The results demonstrated an association between regular contact with farm animals and a reduced risk of atopic sensitization (Riedler 2000). Moreover, this outcome can possibly be accredited to the suppression of TH2 cells and stimulation of TH1 cells through the development of immunotolerance to microbial antigens during animal exposure in early childhood development.

Dunedin Multidisciplinary Health and Development selected random members of a birth cohort born between April 1972 and March 1973 in New Zealand, for a longitudinal investigation of health and behavior. A series of skin pricks, which tested for common allergens, was performed on the cohort of 1037 at 13 years old as well as 32 years old, in order to observe any linkage between exposure to pets and a lower risk of developing allergies. While exposure to one pet or the other led to less allergic sensitization, amazingly, being exposed to both dogs and cats had the greatest impact in lessening the chance of developing allergic atopy at 13 years and even at 32 years old. Thus, combining the microbiome of these two pets has the potential to influence humans' own microbiome positively (Mandhane et al. 2009).

11.6.2 Atopic Dermatitis

Exposure to pets may have the potential to decrease the severity and prevalence of atopic dermatitis (AD), also known as eczema, which produces weeping, oozing plaques of very itchy skin, and is more common in infants and children up to 10 years old (Byrd et al. 2018). Further, dermatologists point out that those adults experiencing symptoms are most likely experiencing them in combination with or related to seasonal hay fever, asthma, and other diseases (Guo et al. 2016). Currently, the prevalence of AD is increasing due to factors including environmental changes, reduced microbial diversity, reduced family sizes, urbanization, and pollution (Craig 2016). Predisposition of atopic dermatitis through a variety of factors is possible as explained by epigenetics, the study of gene makeup. However, although genetic studies can reveal a phylogenetic predisposition, it is far from explaining the precise cause of AD and other diseases. Moreover, the common saying of "we are what we eat," lifestyle and the species that people cohabit with can all offer reasoning behind some individuals' misfortune of experiencing these atopic diseases (D. Ownby and Johnson 2016). In one investigation, parents of 1-year-olds were asked how many pets were in the home from birth to 1-year-old, and the children were then tested throughout childhood until the age of 6–7 years for both pet and non-pet allergies. As the number of dogs/cats increased, the development of atopy to indoor or outdoor allergens decreased. Exposure to pets at 6 or 7 years old had no effect on allergies (D. R. Ownby et al. 2002).

An unhealthy microbiome has shown to be an influence in the increase of intestinal permeability, bowel and small intestinal bacterial overgrowth, inflammatory bowel disease, and a myriad of other conditions. All of these diseases also have a repercussion of atopic dermatitis, and in this way, a gut-skin connection exists (Leickly 2003). The changes in the skin can indicate that this is a systematic problem rather than just an external rash, which may suggest an immune response for dysbiosis of the microbiome. Therefore, based solely on the more active lifestyle that generally comes with being a dog owner, dogs are having a positive impact on microbial diversity and in turn contributing to an overall healthier environment for humans.

11.6.3 Asthma

Asthma is defined as a respiratory disease causing a tight feeling in the chest, shortness of breath, coughing, or wheezing and presents a profound adversity for those who suffer from its symptoms. In particular, asthma sufferers who desire an active lifestyle may be discouraged to partake in physical activity due to the difficulty caused by the asthma symptoms. Unfortunately, problems resulting from a decrease in physical activity include weight gain, depression, poor dietary choices, insomnia, and fatigue (Dannemiller et al. 2016). Thus, it is critical to treat asthma as a disease, rather than just as allergy symptoms that can be controlled with prescription or over-the-counter medicine. While no one truly knows the cause of asthma, there are factors that influence this disease such as genomics, allergic atopy, respiratory infections, and environment (Bonamichi-Santos et al. 2015).

Genomics tells us that if asthma tends to run in your family, you have a greater likelihood of developing it at some point in your life (Johnson and Ownby 2017). Therefore, considering that atopic allergies come from genetics, if one of your parents possess the genes for allergic atopy, your susceptibility also increases (Ober and Yao 2011). In Indiana, asthma prevalence was compared between Amish and Hutterite children, two groups with strikingly similar genetics and origins. While their lifestyles are comparable in many ways, the major difference between the groups is the environment in which the children develop, with the Amish practicing traditional farming and the Hutterites using industrialized farming practices (Nermes et al. 2015). Asthma was four times less prevalent and allergic sensitization six times less prevalent in Amish children than those from the Hutterite group. There were also remarkable variances in the house dust compositions of the homes of the different populations. House dust extracts from both groups were intranasally injected in mice, with the dust from Amish homes specifically inhibiting airway hyperreactivity and eosinophilia. These results indicate the protective element of animal interaction in the sense that they significantly influence the microbial community in which the children are developing. This conclusion in turn highlights the profound effect of the built environment on human health, in some cases even

being more impactful than genomics regarding a person's inclination of developing allergic sensitization and asthma (Stokholm et al. 2016).

Historically, pet ownership has been viewed with concern for allergen exposure provoking sensitization. However, rural lifestyle that comes with a higher level of contact with livestock and animals has demonstrated to be protective against asthma and allergies due to increased microbial diversity. A randomized, placebo-controlled study with women experiencing atopic sensitization/allergies and their infants compared families with pets and without pets, testing for *Bifidobacterium* microbiota. A larger percentage of pet-exposed infants harbored animal-specific *B. pseudolongum* than non-exposed controls, which indicates a more diverse microbiome as a result of cohabiting with pets (Nermes et al. 2015).

11.6.4 Obesity

Progress of research into the mutual benefits of human-animal interaction in recent years has contributed to rapid growth in our understanding of the benefits of pet ownership (McCune et al. 2014). Pets play a crucial role not only in dictating human response to, and development of, allergic disease but also in cardiovascular health (Huang and Boushey 2014). According to the American Pet Products Association, approximately 68% of households in the United States have a pet present, which totals 82.5 million homes. Along with cohabiting pets comes the plethora of bacteria that they have to offer to humans' microbial communities. While there has been a variety of research in recent years concerning the effect of our built environment on human health, not nearly as much effort has been invested in similar studies regarding the canine microbiome.

In 2015, H.J. Park et al. explored the role of gut bacteria in dog obesity, using 14 healthy beagles. Half of the dogs were fed commercial food for 6 months, while the other half were fed a restricted amount of the same food for the same amount of time. Fecal samples were taken at the end of the 6 months, revealing that the second group had greater microbial diversity of bacteria than that of the first group. Once again, *Firmicutes* was the predominant group of bacteria in the lean group of dogs, while gram-negative *Proteobacteria* were more prevalent in the obese group. Due to lipopolysaccharide being a major component of the gram-negative bacterial cell wall, extensive presence of these *Proteobacteria* can lead to an increase in LPS levels in the intestines of dogs, which may then in turn be associated with chronic inflammation in obese dogs (Park et al. 2015).

According to research published by Nestle Purina Petcare Company, the ratio of proteins and carbohydrates in a dog's diet influences the balance of microbes found in the gut (Li et al. 2017). Sixty-four beagles and golden retrievers were subjected to the same diet for 4 weeks, and then for the second 4 weeks, half of the individuals were fed a high-protein, low-carbohydrate diet, while the other half were fed a high-carbohydrate, low-protein diet. The study found that a high-protein, low-carb diet decreased the ratio of *Bacteroidetes* to *Firmicutes* bacteria in the gut microbiome of

pets. In addition, the study group that was fed a high-protein, low-carbohydrate diet had an increase in the microbial gene network, which has also been associated with human weight loss (Wexler 2007). *Bacteroidetes* has also been known to be more prevalent in obese individuals than those of a healthy weight (Turnbaugh et al. 2006).

Despite some individuals' busy days at school or work, pets also come in handy in the sense that they encourage a more active lifestyle when people need to take them out, walk them, and play with them. In fact, dog owners are 34% more likely than non-dog owners to walk at least 150 minutes per week, and dog owners who regularly walk their dogs are 69% more likely to participate in leisure time physical activity (Reeves et al. 2011). These outcomes resulting from cohabitation with dogs create a healthier environment for both dogs and their owners by increasing the level of exercise as well as increasing the microbial diversity for both species. Improvement of these conditions has the potential to decrease levels of obesity in both humans and their canine companions.

11.6.5 Depression

The increased level of exercise that comes with owning dogs as emotional companions has shown to combat not only obesity but also depression, as represented in a study indicating that humans experience higher levels of oxytocin during interactions with dogs (Nagasawa et al. 2009). In addition to the physical impacts the human microbiome has on human health, many studies have shown it to have a tremendous impact on mental and psychological health, as well. Major depressive disorder (MDD) is emerging as the leading cause of disability on a worldwide scale (Zheng et al. 2016). Moreover, the gut microbiome is becoming an increased topic of discussion as an environmental factor capable of shaping the brain over time. Fecal microbiota transplants involving germ-free mice were used as a mechanism for exploring the effect of the microbiome on depression. When mice were colonized with microbiota from individuals experiencing MDD, they expressed more depressive-like behaviors than those mice who were colonized with microbiota from healthy individuals (Zheng et al. 2016). Mice with depression exhibited disturbances of microbial genes and host metabolites involved in carbohydrate and amino acid metabolism. Therefore, these results indicate that dysbiosis of the gut microbiome may have a causal role in the development of depressive-like behaviors. The microbiome-gut-brain axis is thought to play a substantial role in changing mood and behavior, with unhealthy diet emerging as a key contributor to and risk factor for depression (Dash et al. 2015). Further studies indicated that gut permeability and related bacteria translocation may contribute to inflammation in individuals experiencing symptoms of MDD. There were also found to be increased levels of IgM and IgA against the lipopolysaccharides (LPS) of the gut in depressed patients (Maes et al. 2008).

11.7 Microbiome-Based Therapy

11.7.1 Prebiotics, Probiotics, and FMT

As there can be over 40 trillion bacterial cells existing in the human body, a more comprehensive understanding of the human microbiome is still needed before probiotic therapies can truly become effective. Similarly, comprehending human immunological pathways remains a cumbersome obstacle, yet one that must be overcome on route to microbiome-based therapeutics (Fuller 1991). The studies identifying these pathways can reveal the manner in which pets may provide increased microbial diversity for humans, thus affecting the immune system (Anandharaj et al. 2014). That all being said, looking at how applications of prebiotics, probiotics, and FMTs have impacted the human body is an excellent place to start.

Every aspect of diet has an impact on the microfloral composition of the gastrointestinal tract, and thus the value of healthy eating should be emphasized to avoid susceptibility to, or development of, food-related illness (Roberfroid 2007). The most common bacterial groups in humans are bifidobacteria and lactic acid bacteria, both of which confer largely beneficial impacts on humans. Research has shown how these bacteria improve digestion, particularly mineral absorption (Milani et al. 2015). These bacteria, which can play a beneficial role in the human gut, are just a few of a growing number of potentially beneficial taxa which have become the focus of prebiotic and probiotic research as of late. The word probiotic comes from the Greek etymology indicating *pro* (favoring) and *biotic* (with life) (Salminen and van Loveren 2012). The Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO) define probiotics as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host." These symbiotic and mutualistic groups function as a nontrivial component of the human microbiome (Prince et al. 2015).

Unlike antibiotics, probiotics play a key role in maintaining and replenishing the friendly flora of the digestive tract (Fuller 1991). As the impacts of many drugs are complex and two-sided, recent studies have increasingly revealed inconsistencies in the benefits conferred by certain uses of antibiotics. Antibiotics have been criticized for their ability to knock out the flora of our gut with excessive use over time, an effect many patients in the past can attest to (Thomason et al. 2017). As there are a multitude of generic probiotics on the market already, research is striving not only to make them more effective but also to understand the appropriate dosage for each patient depending on their particular microbiome (Rauch and Lynch 2010). Probiotics have explicitly and inadvertently been used for centuries through food products and more recently have shown potential as medical therapy. The manner in which probiotics are having the greatest impact is through their employment in treating medical conditions affecting, or pertaining to, the gut microbiome. This is

due to the gut microbiome's role in supporting the immune system by improving resistance to allergens, destroying toxins, and acting as a natural "antibiotic" by killing negative pathogens through the growth of positive and healthy bacteria (Jernberg et al. 2010).

Initial health trials of probiotics have shown promise in treating various diseases, including inflammatory bowel disease (Crohn's disease and ulcerative colitis), diarrhea, gastroenteritis, inadequate lactase digestion, infant allergies, depressed immune functions, failure to thrive, *Helicobacter pylori* infections, genitourinary tract infections, and hyperlipidemia (Sherman et al. 2009). While many reasonable questions persist about the safety of probiotics, there is currently little evidence that research-based treatments pose any kind of health risk. Due to the fact that the majority of potentially impactful probiotics are still in the early stages of development, the public remains skeptical toward probiotics, as the majority of current products on the market are more homeopathic. Hopefully, however, researcher's ever-expanding insights into the human microbiome can dictate a new era of treatments (Doron and Snydman 2015).

The human body is composed of many systems, chemical and living, all of which together determine function and health (Gismondo et al. 1999). As the human body is full of microorganisms, many of which originate from environmental origins, it stands to reason that human health responds to microbes and viruses in the environment. For this reason, the human body, and particularly the immune system, must constantly adapt in reaction to various environmental agents and diseases (Singhi and Kumar 2016). Commonly, adaptation is key for the strain to function, and a sufficient count of forming units (CFU) is essential in order to make it through the digestive tract. Many food probiotics have a primary focus on the quantity of CFUs for the same reason; however, the variety of bacterial strains is also extremely important. The quantity and variety of bacterial strains are both crucial for a healthy digestive system, which in the end will contribute to many other benefits in all of the systems in human body (Reid et al. 2010). Certain bacteria and fungi can be helpful to compete with and inhibit organisms, such as candida, which allows individuals to combat a wide variety of viruses and foodborne illnesses.

In addition to prebiotics and probiotics, fecal microbiota transplants (FMT) are a rapidly growing microbiome-based therapy being implemented by many health professionals. According to the Fecal Transplant Foundation, a fecal microbiota transplant is defined as "a procedure in which fecal matter, or stool, is collected from a tested donor, mixed with saline or other solution, strained, and placed in a patient, by colonoscopy, endoscopy, sigmoidoscopy, or enema" (What is FMT? n.d.). In recent years, the use of FMT has increased significantly due to epidemics in *Clostridium difficile* infection (CDI) throughout the United States and Europe and FMT's high success rate in curing CDI and accompanying symptoms (Borody and Khoruts 2012). With growing popularity in the microbial health, an increase in the number of patients requesting such treatment has led to a rise in research into FMT and its growing number of potential benefits. Not only is FMT used as a microbiome-based therapy for humans, it is also being implemented in certain animals such as canines and felines (Garcia-Mazcorro et al. 2016). Specifically, dogs suffering from diseases

including idiopathic IBD (inflammatory bowel disease), chronic enteropathy, chronic colitis, and gastrointestinal infections caused by *Clostridium perfringens* have experienced positive results after consistent fecal microbial transplantation (Bottero et al. 2017).While FMT is still in clinical trials for both human and canine/feline patients, the results thus far are promising as doctors increasingly pursue the path of microbiome-based therapeutics as a means of treatment for various diseases.

11.7.2 Pets as a Novel Microbiome-Based Therapy

Simply cohabiting with pets in the early stages of development introduces a child to a great diversity and abundance of bacteria, many of which have the ability to help train the immune system to combat a variety of chronic diseases. Even though the study of the immune benefits of pet ownership is still in the early stages of research, early results indicate that there are indeed immunological benefits to cohabitation with pets. Continued investigation on the ability of pet exposure to decrease problematic immune responses will likely have important implications for chronic diseases such as asthma.

In addition to the psychological and emotional benefits conferred by companion animals onto their owners, research on the microbiome is increasingly pointing to the existence of a newly discovered physiological benefit of these human-animal relationships. Environmental exposures to microbes, which increasingly takes place within the confines of the built environment, can have a profound effect on health and predisposition to disease. The discovery that exposure to pets throughout life may increase microbial diversity supports the notion that human-animal interactions can have a positive impact on human health. If a mother is sharing a microbial community with an animal throughout her pregnancy, the moment that the child is born, he or she is already exposed to a significantly higher diversity of microbes compared with a child born to a mother without pets (Levin et al. 2016). Further, as the child develops in his or her early years, he or she is at an advantage over a child developing in a home absent of animals. This increased diversity specifically has a tremendous outcome in the protection against the development of asthma and allergic atopy.

Although historically there has been discussion surrounding the myth of pets being a leading cause of allergies, this compelling research is pointing to the revolutionary idea that pets are quite the opposite. Understanding the effects that pets have on the development of hay fever, atopic dermatitis, and allergic asthma indicates the possibility of pets as immunomodulators assisting in prevention of these diseases during early development. Dogs and cats, particularly those pets who live both outdoor and indoor, introduce an array of bacteria into our homes that do not exist in non-pet-owning homes (Lax et al. 2014). As the microbial diversity increases with the exposure to animals, humans' immune systems become more capable of combating the pathogens that cause allergic diseases. While there is more research on the dog microbiome, dogs and cats both introduce individual microbial communities

that can be beneficial to our health. Not only is cohabiting with dogs and cats helpful in that they introduce a variety of microbes that humans might not otherwise be exposed to, but pets also actively assist in the transformation of our microbiome by encouraging an overall healthier and more active lifestyle. Dog owners are more likely to walk a greater distance per week and at a higher speed than people who do not own a dog. Increased cardiovascular activity alone has been proven to adjust our microbial makeup in such a way that improves our immune health. This elevation in exercise can decrease our susceptibility to diseases such as diabetes, obesity, cardiovascular disease, and in some cases depression. Therefore, pets undoubtedly play a key role in healthy human development due to the impact they have on people's lifestyle and built environment (Hoisington et al. 2015).

Humans and their pets have coexisted for thousands of years, with obvious benefits for both sides. Pets benefit by having a secure source of food and a sustainable, safe shelter with their owners, and the emotional and psychological impact of pets on human health has been researched for years. More recent research has made it clear that pets create positive outcomes in the physiological health, as well. As a result, people have the opportunity to have a healthier environment in their own homes simply by cohabiting with dogs and cats. By increasing the microbial diversity in our homes where we spend most of our time and raise our children, pets are acting as a new microbiome-based therapy, decreasing children's likelihood of developing asthma and allergic diseases (Sanz 2011) and contributing profoundly to a healthier built microbial environment for humans throughout their lives.

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Chapter 12
Neurological Perspectives on Pets and the Elderly: The Truth About Cats, Dogs and Grandparents

Maria Vaz Patto

Abstract The last century and the beginning of the current one have seen an increase in the number of elderly people in society. Ageing is generally associated with an increase in dependency, multimorbidity and social isolation, but old people with a healthy ageing process are able to fully operate in society, by being important contributors to several processes and serving as mentors to the younger generation. Does owning a pet have any advantage for an elderly individual? Pets are helpful in terms of social, emotional, cognitive and motor capacities of their elderly owners, but they also can be a source of trouble. The benefits and hazards of having a pet for an elderly population are reviewed in the light of the more frequent neurological changes presented after 65 years of age. In spite of some very interesting studies about pet ownership in the elderly, there are still several questions to be answered. Pet ownership can be, together with changes in mentality and changes in political and social issues, a positive factor for a healthy ageing process in the elderly, as can be seen when we review and evaluate data obtained in various studies, so far.

Keywords Pet ownership · Therapy dogs · Elderly · Ageing · Disablement

12.1 Introduction

The last century and the beginning of the current one have seen an increase in the number of elderly people in society, due to several reasons, namely, reduction in death rates at younger ages and medical improvements, which have contributed

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towards an increased survival after age 65. Around 80% of life years generally lost because of death due to preventable medical causes were eliminated, and world life expectancy more than doubled over the past 200 years (WHO 2015). Average life span has risen from 47 to 73 years in the last century, and this led to an increase in the number of elderly people living in the community (Fries 1980). These changes, together with the observed decrease in birth rates as well as migrant mobilizations particularly in the Old Continent, placed ageing as an important problem of the political agenda. Currently, only Japan has a high percentage of elderly population, reaching 30%, but, in the middle of the twenty-first century, this proportion will also be reached in various countries in Europe, North America, South America and Asia (WHO 2015; Fries 1980; Engelman et al. 2010).

Ageing is generally associated with an increase in dependency, multimorbidity and social isolation. All of these factors will contribute towards a dependency effect of the elderly population, which, if occurring, will push social security to its limits in the countries where it occurs (Raleigh 1999). An increase in labour years from 65 to 67 years in some countries was implemented in order to help with social security burden, and increases in retirement age to 70, or even 74 years, are currently being debated in Britain and Japan (Manton et al. 1993, 1995). The ageing effect is not only a social problem, but it is also a real economic problem: Ikegami and Campbell in 1995 suggested that in 2025, the economic growth in Japan could be compromised by the number of elderly people living in the country. Currently, Japanese life expectancy continues to be the world's highest, with a mean value of 83.7 years of life (WHO 2015).

The association of ageing societies with economic and social problems is related to old people being perceived as a dependent, frail group with multimorbidity. However, retrospective studies suggest that a 70-year-old person in 2005 had a better health status than a person of the same age in 1970, and a similar decline in multimorbidity and dependence has been observed in several retrospective and prospective studies (Vetter 2010). This trend may allow elderly people to live autonomously in the community and enable them to be economically relevant to society, by pursuing their previous work, carrying out voluntary work or conducting important work in the community through political and social intervention. Reduction in multimorbidity and dependence in elderly people will result from multiple actions in prevention and construction of a healthy ageing process, in accordance with various indications from the World Health Organization and other international and national health agencies. Societies which are able to implement a prevention system that generates healthy elders may allow old individuals to live healthier lives, with all the social and economic dividends that are obtained with that scenario (WHO 2015).

Ageing well depends upon the creation of opportunities for good health, in order to allow elderly people to take an integrative part in the society and enjoy independent and high quality of life. Old people with a healthy ageing process are able to fully operate in society, by being important contributors to several processes and serving as mentors to the younger generation (EuroHealthNet). This implies political and environmental changes allowing elderly people to have access to (a) education and lifelong learning processes; (b) regular physical activity; (c) various

support services; (d) a healthy environment with accessibility for elderly people with disabilities; (e) new technologies; (f) adequate, healthy diet and nutrition habits; (g) long-term care; (h) employment and volunteering opportunities; and (i) social inclusion and participation in society.

Pet ownership can be, together with changes in mentality and changes in political and social issues, a positive factor for a healthy ageing process in the elderly, as can be seen when we review and evaluate data obtained in various studies, so far.

12.2 The Importance of Pets for Reaching Healthy Physical and Social Old Age: My Pet, My Doctor

The importance of pet ownership around the world is significant: 83% of Australians have had a pet somewhere in their lives; 68% of New Zealanders have a pet, the highest percentage in the world. The number of pets, particularly in urban areas, is increasing: pet ownership has more than tripled in the USA since the 1970s, and, currently, around 69 million Americans have dogs, and 74 million Americans have cats, while 191 million Brazilians have birds (PetFoodIndustry 2018). A study performed by the American Veterinary Medical Foundation showed that 36.5% of households in the USA had dogs, 30.4% had cats, 3.1% had birds and 1.5% had horses, with some owners owning more than one pet (AVMA 2012). More interesting is to know that 36% of American pet owners give their dog birthday presents, 9 out of 10 American pet owners consider their pet to be part of the family and 50% of pet owners in the USA admit to talking to their pet (Pet Secure 2018). From a previous utilitarian position as guardians against mice or burglars, dogs and cats have somehow climbed up the social ladder and become a part of our lives. What can their importance be when we reach the final decades of our lives and become old?

12.2.1 Granny and Her Pet: A Health Advantage or Another Brick in the Wall?

Elderly pet owners are special. Ageing is a time of harvesting all the good and bad things life gave us, but it is also a time of loss: loss of work and social position, loss of friends and family and loss of health. Loss creates fear, sadness and depression, as well as the feeling of being unable to be in control of our own life. A pet, as a form of a permanent friendship and a family member, can be a constant element in the life of an elderly person and an important contributor to good physical, mental and social health. Social and environmental factors are important issues in old people's health as described by some authors (Cassel 1976; Pohnert 2010), and pet ownership, while contributing to social and environmental stability, can be an important part of elderly life *Satisfaction*.

Physical health is defined not only by the absence of chronic conditions or symptoms but also by health practices including regular physical activity, sufficient hours of sleep, regularity of meals and avoidance of smoking and drinking. Physical health, however, is not the only aspect of what is called successful ageing or the ability to achieve old age with the capacity and the will to enjoy it. Old people's definitions of successful ageing include among other learning new things; sense of humour; spirituality; mental, psychological, physical and social health; life satisfaction; and having a sense of purpose (Bowling 2005). In some aspects, pet ownership can be an important way of attaining successful ageing because of pet effects upon physical and social health and life satisfaction while giving a sense of purpose to their owners' lives.

Some reports suggest that pet ownership is important to elderly people because it may promote more physical activity, and hence healthier habits in elderly patients, with measurable results. Pet owners may indeed have more regular physical activity than non-pet owners, according to some authors (Oka and Shibata 2009; Ruzić et al. 2011; Kuban et al. 2016). For instance, in the study by Kuban et al. (2016) involving 270 haemodialysed individuals with a mean age of 62.7 years, 43% had a dog at home, and these were more frequently out for a walk than non-dog owners, even when they had a longer time of haemodialysis than non-dog owners. The authors concluded that a dog could be an important factor for physical activity in these dog owners. Ruzić et al. (2011) evaluated elderly patients during the first year after myocardial infarction: the group that performed a regular dog walking three times daily had a better cardiac performance after 1 year than the group of non-dog owners. Another study in 177 men and 174 women who replied to mailed enquiries, aged between 20 and 80 years, suggested that dog owners walked significantly more time per week than non-dog owners (Brown and Rhodes 2006). This study also showed that dog ownership, due to the obligation of caring and pampering the dog, was a major factor contributing to the increase in walking and exercise time (Brown and Rhodes 2006), giving the owners a sense of purpose. Oka et al. also concluded that dog owners reported having more physical activity than other pet (such as cat) owners or non-pet owners in a study involving 5253 Japanese adults who replied to an online questionnaire (Oka and Shibata 2009). More recently, a survey of people with social, economic and health problems found that, in this social group, owning a pet was associated with increased physical activity (Nagelhout et al. 2017). Furthermore, a study which used an activity monitor in elderly volunteers – activPAL accelerometer – showed that an independent old person owning a pet dog had an increase in time spent walking when compared to non-dog owners of the same age (Dall et al. 2017). In this study, pet owners walked for more minutes at a moderate pace and presented fewer sitting events. This allowed the authors to conclude about a possible positive effect upon pet owners' activity profile and health, when compared to non-pet owner-matched counterparts (Dall et al. 2017). Thorpe et al. showed that elderly dog owners were more likely than non-pet owners to engage in non-exercise-related pet walking, but did not differ from non-pet owners in walking for exercise

or any other physical activity (Thorpe Jr et al. 2006). This would show the effect of owning a pet upon physical activity, particularly in elderly people, since a large number of older adults do not engage easily in physical activity. These results were also consolidated by a meta-analysis performed with 29 published articles, which suggested that there is in fact a positive relationship between dog ownership and pet-related physical activity (Christian et al. 2013). In this context, a recent study, which observed a sequential number of subjects with coronary disease, reported that having a pet was associated with decreased coronary artery risk, and this was more apparent in dog owners than in cat owners. Furthermore, the number of years of keeping a pet, walking and playing with it, seems to be related to a reduced coronary risk in pet owners (Xie et al. 2017).

Pets may increase their owners' physical activity levels by getting them to play, walk and generally move more in order to attend to their needs, since a pet is mostly dependent upon their owner. And this involves not only walking the dog daily but also a vast amount of other types of mobility actions related to pet ownership. Even for elderly people without mobility problems, pets can still help to improve mobility. In an interview-based study of 23 rural elderly people, Scheibeck et al. (2011) showed that dog owners walked every day, thereby doing regular physical activity. The same was observed by Yabroff et al. (2008), who evaluated a large number of old adults in California and found a moderate relationship between owning a pet and performing physical exercise, if the pet evaluated was a dog. In general, research studies agree that elderly dog owners are physically more active than non-dog owners (Christian et al. 2013), which may help to prevent motor disability or compensate for acquired motor disability over the years.

In contrast, other authors claimed that there was no significant effect of pet ownership on pet owners' health, after controlling for several factors such as health habits, human social support and owners' attachment to the pet (Winefield et al. 2008). A report using data from the English Longitudinal Study did not find any relationship between pet ownership and lung function, grip strength, chair raising time, balance and memory, among other markers of ageing, in a population of around 9000 individuals in which one third of them owned a pet (Batty et al. 2017). In addition, a survey based on the Nord-Trøndelag Health Study (The HUNT Study) found no evidence of any association between improved health (measured by all-cause mortality) and physical activity levels associated with dog ownership (Torske et al. 2017).

One should take into account that, besides important differences in the populations under analysis as well as in research methodologies between the previous studies, apparent discrepancies in the association between pet ownership and health parameters may also suggest that physical activity in the elderly is not the only marker or the only aspect of good health that can be supported by a pet. In fact, some other aspects are also important: pet ownership is directly associated with mental health changes due to love and companionship obtained from the human-animal interaction, but also from social interchanges with neighbours as well as from intergenerational contacts obtained through the same pet.

12.2.2 A Pet as a Stress-Reducing Factor: Are We What We Play?

Apart from the unconditional love that we obtain from pets, they like to play and have attention given to them, and this will force their owners to play with them and respond to their attention needs. Playing with pets increases owners' exercise levels and changes their mood, decreasing the influence of the autonomous sympathetic system, which can contribute towards increasing survival after myocardial infarction, as has been shown in some studies (Friedmann et al. 2003; La Rovere and Pinna 2014). For some owners, the anti-stress factor that owning a pet represents translates into decreased hypertension levels, decreased heart rate and reduced cholesterol plasma levels (Baun et al. 1984; Allen et al. 2002; Virués-Ortega and Buela-Casal 2006). Elderly subjects who owned dogs and who performed regular physical activity with their pets more frequently had serum triglyceride levels in the normal range than those who did not own dogs Dembicki et al. This aspect may be reflected in other health effects, namely, in terms of risk of cardiovascular disease. Thus, in spite of gaps in knowledge, research seems to suggest that elderly pet owners have better health, less depression and a much higher rate of survival 1 year after a heart attack and are less likely to have a heart attack than non-pet owners (Friedmann et al. 2003; Müllersdorf et al. 2010). This may be due to the fact that having a pet decreases the sympathetic system arousal, as previously mentioned, and also reduces anxiety and depression at the same time. Aiba et al. (2012) demonstrated that owning a pet was an independent and a positive factor for reducing cardiac autonomic imbalance in 191 patients with a mean age of 69 ± 8 years and who had diabetes, high blood pressure and hyperlipidaemia. Other authors showed that even simple and passive processes such as watching an aquarium may have soothing effects, decrease pulse rate, increase skin temperature and decrease muscle tension in elderly people (DeSchriver and Riddick 1990). In fact, humoural chemical changes such as decreases in cortisol and increases in dopamine and endorphin serum levels have been shown to be related to times playing and petting dogs (Odendaal and Meintjes 2003). Finally, the American Heart Association suggests that pet ownership may contribute towards a reduction of cardiac disease, since improved outcomes in relation to hypertension, obesity, diabetes, hyperlipidaemia, autonomic function, cardiovascular reactivity and survival have been reported for individuals with cardiovascular disease (Levine et al. 2013). Other studies dispute these findings. Parker et al. (2010) analysed 424 pet owners in a cardiac unit who replied to a questionnaire: pet owners, when compared to non-pet owners, were more frequently readmitted or died after hospitalization due to a cardiac event, and this was worse for cat owners. Again, it is difficult to compare specific populations, such as the ones attending cardiac units, with the general population evaluated in the majority of studies. All published results need to take features of the populations under study into account, because these features, by themselves, may explain differences observed.

12.2.3 Health Services: A Pet a Day Keeps the Doctor Away?

For elderly people, who are sometimes in vulnerable situations, either due to social isolation or health problems, a pet may significantly contribute to their quality of life (Heuberger 2017; White et al. 2017; Beck and Meyers 1996; but see also Winefield et al. 2008). In older adults, the importance of good health is related to their ability to maintain autonomy and stay in their homes and to use social and health services less frequently. Health-care use has become a measure that reflects outcomes of physical health, mental health and mortality in elderly people (Seeman and Crimmins 2001). Due to their fragility in terms of health but also in terms of economic and social difficulties, older people will use health services not only due to their health problems but for other reasons as well: need of social interaction, psychosomatic symptoms related with fear and anxiety and need of reassurance from their doctor about their health. In this regard, Siegel et al. (1990) surveyed 938 subjects for 12 months and found that pet owners were less frequently going to the doctor, both in normal and also in stressful times: again, dog ownership showed a more significant impact than cat ownership or other pets. Similarly, a telephonic survey in Australia, in which 60% of the individuals had a pet, also suggested that elderly pet owners had fewer doctor visits, less medication usage and fewer chronic health issues than non-pet owners (McHarg et al. 1995). A study using data from the Canadian Health Ministry database showed a significant difference in the use of medical services between pet owners and non-pet owners, with longer hospital stays for non-pet owners: in general, pet owners seemed to be significantly more autonomous in their daily living, better able to solve problems and happier with their everyday life (Raina et al. 1999). In addition, a longitudinal study carried out both in Germany and Australia, in which a population of health responders was followed up from 1996 to 2001, demonstrated in both countries that people that had always owned a pet had significantly fewer doctor visits than those who had ceased to have a pet or had "never" had one. Health gains were also seen in people who had acquired a pet in the last years of the study. In this study, the impact of pet ownership was reduced when controlled for other confounding variables, but the impact was still important (Headey and Grabka 2007).

12.2.4 Boris and My Friends: The Social Role of Pets?

Studies in elderly people also provide another clue to the effect of pets on human life. In a very aged population, the presence of an animal provides a sensation of companionship, so many times lost after the bereavement of a spouse, a sister or a son and a feeling of validation when one wakes up and has to interact with a being that depends upon you and which is seriously happy when you return home. Probably also because of pet ownership, pet owners are able to overcome more stress and have more psychological health than non-pet owners, and, when victims

of violence, pet owners mentioned their pets as an important source of emotional support (Siegel 1990; McNicholas et al. 2005; Krause-Parello 2012; Stanley et al. 2014).

For an isolated elder, with loss of social contacts, due to retirement, lack of family support and death of spouse and friends, a pet can be an object of love and affection: pet owners have a specific attachment to animals as described in a study with functional MRI by Hayama et al. in 2016. In this study, pet owners showed greater cerebral activity than non-pet owners while watching photographs of pets, and such activity involved visual attention networks but also the insula, which is a brain structure that plays a role in compassion and empathy. In fact, other authors even show that dog owners are emotionally attached to their pets and their family, at similar levels (Flynn 2000; Cooke 2013; Walsh 2009). In addition, social isolation associated with old age can also be reduced by the presence of a pet. Walking a dog or a cat is a social adventure: people ask questions about the dog, how it behaves and how nice it is (a friendly dog is a natural conversation starter). This promotes social bridges between people of similar ages as well as across different generations and even facilitates conversation for shy people. For instance, pet owners are more likely to talk to their neighbours while walking their dog (Hara and Shimada 2007; Tissot 2011). Thus, the decrease in social networks that takes place due to professional retirement as well as due to the loss of friends and family may be compensated by new acquaintances, which are encouraged through pet ownership. This may give elderly people a new sense of community and belonging, as well as the social interactions that are necessary to feel fulfilled. In a community study based upon telephone surveys, Wood et al. (2015) were able to show the integrative role of pets in a community, with an increased interaction between pet owners, but also with non-pet owners, who often interact with pet owners through the pets of the latter.

The most widely used definition of health is that by the World Health Organization, which regards health as a "complete state of physical, social and mental well-being and not merely the absence of disease or infirmity" (WHO 2014). Pets, either because they seem to improve health indicators and help with loneliness and depression or because they are part of the family and an important member of one's social life (for pet owners at least), seem to be an active contributor towards an improvement of an elderly pet owners' health status.

12.3 Neurological Changes with Ageing

Since the ability to cope with stressors decreases with advancing age, age-related chronic diseases are frequent and a source of disablement among the elderly. Apart from normal ageing-related acquired limitations, such as changes in vision or audition, changes in mobility and changes in cognition, all of which have a certain degree of variability, a large number of elderly people are affected by disablement, defined as the limiting consequences of chronic health conditions. In a UK-based study, the prevalence of disablement increased in a step-by-step

manner, over the years, from 7% in those aged 40–49 years to 68% for those aged 80 years and above (Stuck et al. 1999). Seeman et al. demonstrated, in 2010, that older Americans faced progressive disability even in recent generations. The WHO report on disablement shows the presence of a disproportionate representation of older people in the group of disabled persons, with rates of disability much higher in elderly persons above 80 years of age (WHO 2011). Even if, in recent years, there has been an improvement in disability levels across generations, due to elders possibly being increasingly healthier (due to better nutrition, better health care and better avoidance of disease), there is still physical deterioration that is perceived in everyday life (WHO 2015).

Neurological changes associated with ageing are some of the most important changes a person can suffer, because they significantly interfere with functionality and quality of life. We are born more or less the same way, but we will age differently, according to genetics, environment and our personal history.

12.3.1 The Special Ones – I: Guide Pets for Sensorial Deprivation?

As we get older, even without any pathological issues involved, we will present changes in sensorial systems, namely, visual difficulties, due to changes in the capacity to react and accommodate to light and darkness, insufficiency of convergence, restricted range of eye movements and changes in visual fields. Difficulties in hearing are also frequently reported by the elderly population, due to progressive hearing loss, which most frequently involves difficulty in perception of the human voice. A reduction of the sense of taste may induce changes in food habits, and a reduced sense of smell may increase the possibility of eating rotten food or being unable to feel poisonous gas, for instance (Adams 2005). Pets, trained for special functions, can be of great help: a trained dog may be able to show a different behaviour if confronted with a noxious smell, for instance, and can be helpful in drawing attention to it – this is being increasingly used in some pathological situations, such as diabetes and cancer (Horvath et al; Hardin et al. 2015).

In addition, pets can also be an important asset in terms of hearing loss if they are trained to warn their owners about incoming phone calls, various different noises around or even those made by an intruder. Trained hearing dogs are able to alert their owners to specific sounds such as doorbells, smoke alarms, telephone ringing or people calling their own name. They are also able to help in managing sounds from outside the house (Guest 2006). Apart from their utility, these pets create other significant changes in their owners' lives. In a longitudinal study carried out by the Hearing Dogs for Deaf People organization, which followed up 51 recipients of a hearing dog, such recipients reported significant improvement in independence, social involvement and in response to sounds from the environment after receiving a hearing dog, which was also associated with lower levels of tension, depression and anxiety (Valentine et al. 1993).

Guide dogs for people with vision problems are also an obvious choice: dogs can guide people outside the house, can help people go to the supermarket and can help people maintain one's routine and autonomy. Guide dogs follow a course of basic obedience and are mostly trained to bring their human owners through a series of hazards and obstacles and to ignore any commands that will endanger the life of the human who holds the leather handle attached to the dog (Prestrude and O´Schea 1996). Loneliness and social exclusion are difficult problems that elderly people with eyesight difficulties frequently perceive. Thus, the maintenance of a social routine is very important, and, in this context, the effects pets have on social life of their owners, apart from the effects as guides, are also quite important. Cats, as well as other animals which cannot be used as guide pets, are less interesting to elders who cannot see because they are of no use in guidance outside the house. However, one cannot forget that even such pets help to keep company and, being a part of the family, also help to reduce the sense of isolation.

In the elderly population, a hearing or visual guide dog, apart from the independence that it provides, can also bring a new interest to life, by providing a bond with another living being. White et al. (2017) analysed the quality of life and attachment styles of 73 owners of service dogs and showed that the owner-service dog relationship is unique and interdependent and enhanced the quality of life of the owner.

12.3.2 The Special Ones – II: Pets to Improve Mobility and Promote Independency

Risk factors associated with a functional decline in elderly people were identified by Stuck et al. and included (1) poor self-perceived health, (2) smoking, (3) vision impairment, (4) low frequency of social contacts, (5) low level of physical activity, (6) no alcohol use compared with moderate use, (7) increased and decreased body mass index, (8) lower-extremity functional limitation, (9) cognitive impairment, (10) depression and (11) disease burden (comorbidity) (Stuck et al. 1999). An increase in years of life will bring changes that are not pathological but that are going to impact upon everyday life of a person. Ageing is associated with reduced speed in walking and performing daily activities, decreased coordination and reduced muscular power, due to a progressive decrease in the amount of nervous system cells that coordinate the process, as well as in the muscular and tendinous cells involved in motor function (Adams 2005). Sensitivity is also decreased, and even without pathological features such as neuropathies and movement disorders, old people will fall more frequently than younger ones, which increases their comorbidities with fractures and immobility, which in turn leads to more reduced mobility and further falls. When there is a high degree of dependency and mobility difficulties, assistance dogs can be helpful: they are important to the mobility of dependent elders, by contributing to an increase in freedom and independence. Assistance dogs are helpful in catching things, opening doors and fetching the phone and, in addition, are able, as seen before, to improve the social life of their owners, by getting frequent gestures of friendliness from strangers and by

increasing social interactions of their owners (Hart 1998; Eddy et al. 1988). Also, service dogs for people with mobility problems can alleviate the mental burden of daily activities and have been shown to subjectively improve physical functioning of their owners. This has been confirmed in a comparative study between service dog owners with mobility problems and those who, in spite of similar problems, had not yet been able to obtain a service dog (Shintani et al. 2010).

In fact, in terms of mobility, there is an almost linear dose-response relationship between health status and physical exercise, mostly if individuals were previously sedentary, particularly in older adults (Hills et al. 2015). Older people perceive their health-related status in terms of the amount of physical exercise they can perform (Eifert et al. 2014) although very few are able to exercise in the way needed to achieve health results. Mobility decline can be prevented with exercise, and exercise in itself is related to improvement in several health problems in the elderly (Frankel et al. 2006; Montero-Fernández and Serra-Rexach 2013; Chen et al. 2014; VanSwearingen and Studenski 2014). Walking is a very affordable exercise, which allows people to go out of their homes, get information about their environment and also socialize. In elderly subjects, a walking training programme resulted in improvements in mobility, lower limb strength and aerobic endurance as compared with a control group, which had not been enrolled in such a programme (Magistro et al. 2014). Other studies also showed that regular walking can be a very important part of a programme to maintain elderly people healthy and autonomous (Mosallanezhad et al. 2014; Forte et al. 2015). Thus, physical activity is one of the key points in disability prevention, and low levels of physical activity are associated with several health problems in the elderly (Bastone et al. 2015).

It should be borne in mind that the effect of a dog on physical activity will be more apparent in people who do not generally exercise, who are not fond of exercise (and who, without a pet, would not leave the sofa, etc.) or who have very few incentives to do physical exercise. Thus, positive effects of pet-related physical exercise may be less noticeable in cultures such as those from northern Europe or in adolescents and young adults, who are generally more active and more prone to doing regular physical exercise. For elderly people with walking limitations due to osteoarticular degeneration or balance problems, walking the dog may be the sole incentive to perform some physical exercise (Cutt et al. 2008). Again, the type of pet may be important. In a recent case-control study in older adults, which compared pairs of cat or dog owners with non-pet owners, pet owners had fewer health conditions, but cat owners had a higher body mass index and less activity per day, than dog owners (Heuberger 2017).

When there is an effective relationship between a pet and its owner, pets can change one's health by influencing behavioural factors and everyday life patterns. Cats move around and like to play; horses need to be walked and pampered. Dogs need to be played with and walked, and responsible pet owners will exercise with them even in bad weather, also because their owners derive positive outcomes from the perception that their pets are enjoying the experience (Westgarth et al. 2017). Dog owners do feel an obligation towards their dogs, in this regard, either because of its health or because the pet feels happy when outside, and this happiness is perceived by the owner. Happiness observed in a pet helps to create such a sense

of obligation and a social impact in their lives; in addition, having a routine is important for elderly owners. Activity-related benefits of pet ownership in older adults seemed to be limited to dog owners, who engaged in the greater overall physical activity (Campbell et al. 2017). Even in situations in which walking the dog can vary between 1 hour every day and 1 hour every week, dog owners are in general still more active than non-dog owners (Bauman et al. 2001; Cutt et al. 2007; Suminski et al. 2005). In contrast, physical activity levels of cat owners do not increase or at least not significantly, as reported by several authors (Dembicki and Anderson 1996; Oka and Shibata 2009; Thorpe Jr et al. 2006; Yabroff et al. 2008). All the activity provided by a pet, and the sense of obligation associated with being a responsible pet owner, generates a sense of importance and reduced feelings of loneliness in an elderly person, who is generally retired and with little social life (Ong et al. 2016; Boss et al. 2015; Dury 2014) So, even if there were no advantages in terms of physical exercise, the feelings of belonging and importance that the pet keeping "job" implies will be very important to the mental health of the elderly owner of a pet.

Horses are special types of companion animals, considering how difficult it is to call them pets. Horse owners claim a different type of relationship with their pet, which allows an active interaction and a different sort of companionship than the one present with smaller animals (Endenburg 1999; Robinson 1999). Horse riding has been shown to be associated with improvement in balance and stability in elderly subjects from the community (Homnick et al. 2015; Aranda-García et al. 2015; Kang 2015). Furthermore, horse riding was also associated with improvement in lower limb strength and whole body reaction in elderly subjects, probably due to the continuous movement of the horse, generating an increase in muscle tonus due to the constant muscular contraction obtained in response to the animal movement (Yu et al. 2014). Using a horse riding simulator, an improvement in balance and functional capacity was found in elderly patients with osteoarthritis of the knee (Kim 2016). Horse riding for prolonged time allows greater overall postural stability, thereby allowing a better coordination of vestibular and somesthetic information in elderly people (Olivier et al. 2017). Horse riding effects were observed not only in the locomotor system: Kim et al. (2015) showed an improvement in the EEG responses in elderly people after horseback riding for 8 weeks, when compared to a control group, suggesting that horseback was also able to improve cerebral functions in this group.

12.3.3 The Special Ones – III: Pets that Have Emotional and Cognition Effects (My Pet, My Psychiatrist)

Being a result of progress and civilization, old age should be a time of joy and happiness, with a fulfilled life, and family and friends around. However, as previously mentioned, old age is more frequently an age of loss: loss of work due to retirement or to poor health, loss of family and friends that moved away or died,

loss of economic capacities and loss of environmental clues to changes. It is difficult to deal with these changes, and feelings of sadness, depression and loneliness may be present in elderly people (Boss et al. 2015; Dury 2014). Animals by themselves and without any training can be of therapeutic benefit due to their presence and their companionship. Pets seem to be able to give love and affection without asking for anything in return, and this can compensate for several losses present in elderly people's lives. In a survey of 5210 men and women from the English Longitudinal Study of Ageing, pet ownership was associated with loneliness in elderly women, possibly because having a pet was used as a compensatory factor (Pikhartova et al. 2014). Loneliness and depression in the elderly are related to progressive poor health, loss of socialization, loss of mobilization and presence of physical disease. In this context, the presence of a pet, as reported in another survey of 830 primary care patients who lived alone, was associated with a happy mood and a decreased report of loneliness (Stanley et al. 2014). Even in experimental conditions, pets are able to reduce anxiety and fear in their owners. For instance a decrease in anxiety levels was seen in a controlled experiment which involved exposure to a tarantula: subjects who were holding and playing with a real pet at the time they were put in contact with the tarantula showed fewer anxiety effects than subjects who did not interact with pets during the experiment (Shiloh et al. 2003).

Saunders et al. (2017) reviewed the possible mechanisms whereby pets may contribute to human psychological well-being and health in general. Two theories were reported: the "main effects hypothesis", which suggests that pets have diffuse effects on human lives, and the "buffering hypothesis" which puts forward that pets are helpful mainly when stressful factors are present – as seen before. Some research with stressors in laboratory suggests that this effect is important (Shiloh et al. 2003; Allen et al. 2002). Beetz et al. (2012) suggested a possible role for oxytocin in order to integrate both hypotheses and explain several effects regarding human-animal relationships.

Oxytocin is related to love and companionship and is one of the hormones associated with a feel-good factor, to which both animals and humans respond and which induces anti-stress effects, namely, decreased cortisol levels and blood pressure (Uvnäs-Moberg 1998), as well as increased pain threshold (Petersson et al. 2017). Oxytocin also increases trust (Kosfeld et al. 2005) and causes anxiolytic effects (Heinrichs et al. 2003). Nagasawa et al. (2015) showed increased levels of oxytocin in owners visually interacting with dogs, and vice versa, which suggests that the mechanism that improves health in pet owners is the same mechanism that allows the formation of a human-animal bond. Oxytocin is also present in high quantities when a positive human-dog relationship is present, a situation which is also associated with lower cortisol levels (Schreiner 2016). However, the bond established between owner and pet is different from person to person and depends on the link reached between them, on the personality of the owner and of the pet and also on the owner's experience, among other factors (Calvo et al. 2016a; Calvo et al. 2016b; Cline 2010). Maybe that is the reason why different studies of the effect of having a pet upon psychological and mental distress have shown varying results. For instance, Winefield et al. (2008) interviewed 314 elderly subjects who owned

pets and found no effect of their ownership upon their health and well-being. In contrast, Enmarker et al. (2015) studied 12,093 elderly people and reported that non-pet owners had lower levels of anxiety and fewer depression feelings than pet owners and also that male cat owners were less depressive than female cat owners: health status was not related with pet ownership in this group. Another study in 2551 older adults found that pet owners had higher levels of psychoticism and more depressive symptoms and female pet owners had poorer health (Parslow et al. 2005). We could, of course, argue that owning a pet would contribute to maintaining a certain balance, and that might be the reason for having one if you suffer from mental health problems. That might account for the results from the previous studies. In this context, a study involving a convenience sample of 169 elderly female pet owners who replied to psychological profile questionnaires found a significant relationship between loneliness, depressed mood and pet attachment support (Krause-Parello 2012), suggesting that the relationship with pets had a very special support role in the owners' lives. This also may explain the results of the Nord-Trøndelag Health Study, which suggested that non-pet owners had the lowest scores for depression in an evaluation of 12,093 subjects (Enmarker et al. 2015).

More positive results were seen for pet ownership in terms of reducing anxiety and depression symptoms, in the work of Garrity et al. (1989): these authors used a phone survey in a sample of elderly Americans and showed that pet attachment significantly predicted depression but not illness experience, and in the particularly stressed bereaved subjects, pet ownership and strong attachment were significantly associated with lower levels of depression. This is in line with the findings from the previously mentioned study by Krause-Parello (2012) in older women, which showed that pet ownership and pet attachment were positively related as a form of support for loneliness and depression feelings. In fact, animal-assisted therapy in nursing home residents has been reported as positive, when compared with a control group with no animal-assisted therapy, both in terms of loneliness scores (Perelle and Granville 1983) and social scores (Banks and Banks 2002). Another study in 35 volunteers showed that being able to be with a pet dog while waiting for electroconvulsive therapy (ECT) treatments improved the levels of fear and anxiety (Barker et al. 2003). In addition, an Australian study of 68 nursing home residents showed that animal-assisted therapy was associated with lower levels of depression and fewer loneliness feelings, as well as with less fear and confusion: this was even more apparent when a resident dog was in scene, but also with a visiting dog, which allowed the authors to claim an important effect of resident dogs in nursing homes (Crowley-Robinson et al. 1996).

However, authors also disagree in this field. While elderly pet owners have a higher executive function than non-pet owners, their depressive score was identical to that of non-pet owners in the study designed by Branson et al. (2016). In a recent systematic review on patient-dog interactions, Lundqvist et al. (2017) suggested that there was a minor to moderate effect of dog-assisted therapy in psychiatric conditions. No association between pet ownership and relief of depressive symptoms was found in 8785 adults in the English National Study of Ageing (Batty et al. 2017), and Cherniack and Cherniack after revising the literature claimed that

"taken together, these studies imply a rather modest benefit at best for animals in depressed individuals" (Cherniack and Cherniack 2014).

12.3.4 Pathological Ageing and Assisted Animal Therapy

Ageing may be associated with neurodegenerative disorders, namely, dementia and movement disorders. There is a large group of studies relating the effect of pets and assisted animal therapy, mostly in nursing home residents, in the neurodegenerative field. Nursing home residents are a part of the elderly population in specific situations, mostly those with disabilities that prevent them from living at home, with those disabilities being not only cognitive but also frequently motor and sensorial, with depressive feelings and difficulties adjusting to an autonomous life. In a study using an animal-assisted therapy protocol that varied from no exposure to 1-week exposure or to 3-week exposure of assisting animals in a small number of elderly people, Banks and Banks (2002) were able to show that animal-assisted therapy was able to reduce the feelings of loneliness in the studied individuals. In residents with cognitive impairment, the presence of a dog reduced behavioural changes such as agitation and promoted social interaction among patients and staff by itself (Richeson 2003). In another study, the use of therapy dogs in a psychiatric ward was associated with decreased heart rates and a consistent reduction of agitation on the ward when the dogs were present (Walsh et al. 1995). Similarly, the presence of fish in aquariums in the dining room of a nursing care home for Alzheimer's patients was able to promote better appetite and an increase in weight in these patients, when compared with a control group. (Edwards and Beck 2002). Moretti et al. (2011) evaluated pet therapy in ten elderly patients with dementia and other psychiatric ailments: this study showed improvements after 6 months of pet-assisted therapy both in the geriatric depression scale and in cognitive scores (assessed using the Mini-Mental State Examination – MMSE), when compared with a control group, suggesting an effect not only on depression but also on the cognitive capacity of these patients. In another study, animal-assisted therapy was used for 6 months in a random group of Alzheimer's disease patients, and this approach was associated with an improvement in depression (assessed using the Geriatric Depression Scale – GDS) as well as in cognitive parameters (MMSE) (Menna et al. 2016). Furthermore, Swall et al. (2015) reported the use of animal-assisted therapy in demented patients with behavioural problems and concluded that the time spent with a dog allowed the patients to report memories and feelings, with pets creating an opportunity to reach the patient on a cognitive level. In another study, involving severely demented patients, no changes in cognition or neuropsychiatric symptoms were found with animal-assisted therapy (Mossello et al. 2011): however, this study reported a decrease in anxiety and sadness levels, as well as an increase in motor activity after animal-assisted therapy in these patients, when compared with control subjects. This also occurred in the study conducted by Kanamori et al. (2001) in patients with senile dementia: seven subjects subjected to animal-assisted therapy were analysed

with a cognitive scale (MMSE), a scale of daily living activities salivary cortisol levels, with all of these parameters showing improvements when compared with a control group. Thus, the presence of a dog seems to calm demented patients, allowing them to remember past pets, and encourages their mobility and their interpersonal contact, and, on the whole, in spite of the small numbers and very different methodologies of the existing studies, it seems to have a positive impact upon behaviour and, in some reports, upon cognition.

In a meta-analysis of papers reporting using animal-assisted therapy in elderly subjects up to January 2009, Virués-Ortega et al. (2012) found that this type of approach significantly improved social functioning of the nursing home residents, with a moderate effect upon depression and behavioural disturbances, particularly in patients with dementia. In fact, dementia patients seem to obtain some favourable results with animal-assisted therapy. Friedmann et al. (2015) studied elderly nursing home residents in whom a high percentage had dementia (60%) and/or depression (50%), 10% had behavioural problems and 5% had anxiety. All patients were on medication. Patients were subdivided into two groups; one of the groups was visited by animals, in the context of animal-assisted therapy, for 3 months, while the other group was the control group. At the end of the study, depression score values in the group visited by an animal were reduced, but no changes were seen in the use of medication.

However, not all studies have shown positive results with animal-assisted therapy. Phelps et al. (2008) found no improvement in depression, mood or levels of social interaction in elderly patients in a nursing home that received weekly dog visits. In addition, no differences were found in a study by Thodberg et al. (2016) between two groups of elderly patients in nursing homes, in which one group was submitted to biweekly sessions of dog therapy. Although their sleep hours improved, no changes were observed in cognition, weight, BMI, depression or daily life functions, in comparison with controls.

12.4 The New Age Pets

More recently, researchers started using robotic pets in elderly care, because such pets are easy to command, are predictable and are also cleaner than living pets, thereby avoiding some of the negative traits the latter can present. In an experience involving interaction of demented patients with “AIBO”, a metal robot that responds to spoken commands, Tamura et al. (2004) claimed that severely demented patients were able to increase communication, suggesting that robotic pets may be a tool to use in rehabilitation of these patients. of these patients. A randomised trial with the baby seal robot “PARO” showed that elderly subjects with dementia presented a long-term reduction effect in agitation and depression as compared with a control group with similar characteristics that did not interact with the robot (Jøranson et al. 2015). A similar study with the same robot, carried out in Japan in 2014 (Takayanagi et al. 2014), showed that elderly people with moderate to severe dementia had

increased positive responses to the robot pet when compared with a stuffed animal. Banks et al. (2008) compared the effects of a living dog and a robotic dog in a resident home for elderly people in order to assess differences in their ability to treat loneliness in the elderly population. Both types of animal-assisted therapy (AAT) showed improvements in the levels of loneliness of the residents, and although the robot animal group scored lower in some aspects of the scale used to assess attachment, both AAT showed a positive impact in terms of reducing loneliness in the elderly. Kramer et al. compared three different types of visits to a residential home of elderly people: a person visiting alone, a person visiting with a live dog and a person visiting with the metal pet robot AIBO. All three types of visits increased communication and interaction by the elderly, but both the living dog and AIBO were more effective than the living visitor, with AIBO provoking more attention and conversations than the live dog (Kramer et al. 2009).

12.5 Granny and Her Pet: The Bad Things

In every cloud there is a silver lining and vice versa: with all the positive aspects reported in the literature, having a pet should be a very positive experience. However, for elderly people, pets can be difficult to have and to keep. In fact, most of the times, elderly people will not have a pet in old age, more so if there was not a pet around when they were young.

Mobility difficulties associated with loss of equilibrium result in frequent falls, mostly nonfatal but specifically hazardous in an elderly population. The National Electronic Injury Surveillance System (NEISS) from the USA reported around 86,000 falls/year involving either a cat or a dog (2001–2006), with dogs being responsible around 88% of the times for the falls. These records showed that women tended to fall more than men, which can be more problematic since elderly women more frequently have osteoporosis and suffer worse consequences from a fall than men, namely, fractures. Pets were associated with falls in a predictive model for elderly people (Pluijm et al. 2006), and Kurrie et al. (2004) reported a series of elderly patients with fractures from falls due to their pets, most commonly dogs and cats but also birds, goats and even a donkey. This is even more important since, according to an official report, "falls are the leading cause of fatal injury and the most common cause of nonfatal trauma-related hospital admissions among older adults" (NCOA 2018).

Dogs can also be associated with human injuries related to walking a pet on a leash (Willmott et al. 2012), and both cats and dogs can cause lesions due to bites and scratches and car accidents (Blunck et al. 2013). And we must remember the fragility of the elderly population in relation to infections and contaminations through contact with animals (e.g. Simpson et al. 2000; Stull et al. 2012).

Apart from falls, taking care of a pet can be strenuous, when there is no correlation between the level of energy of the owner and that of the pet. The constant need for attention of a small furry friend can be difficult to compensate and may worsen the

feeling of incapacity, instead of improving the owner's mood (Graf 1999). Mobility difficulties may reduce activity outside the house and create the problem of "Who would walk the dog? Because I cannot walk by myself…". And, of course, pets are also expensive; food and toys and vaccines are important, but lots of elderly people do not have enough money to spend on them. In fact, sometimes, in order to care for their pets, elderly people even reduce the amount they spend on their own food or medication (Toohey and Rock 2018). Pets are also a source of worry – "Who will take care of it if I go first?" (Smith et al. 1992; Graf 1999). Family and friends may be of some help with that. I still remember the happiness of some owner when his pet friend, in his case a donkey, was cared for by one of his friends. Additionally, pets can be a source of grief: the kind of grief referred is comparable to the one felt when losing a close friend or family member, more so if you are older and your circle of friends is being reduced. For a lonely old person, a pet can offer distraction, can fight boredom and can give a purpose, and its death means an enormous empty space that can worsen other problems such as depression and cognitive problems (Clements et al. 2003).

12.6 Conclusions

Elderly pet owners are considered to be more confident, with fewer feelings of loneliness and less afraid of the world than non-pet owners. Pet ownership seems to ease depression and agitation in demented patients. However, some studies are contradictory. In spite of all these benefits, older persons are worried of having a pet, either because of their own health and fragility or because of financial concerns or their ability to care for the pet.

In spite of all the benefits we can see for pet ownership, it is difficult to convince an elderly person that never had pets around or that is very frail to have one. Pet ownership by the elderly needs to be adapted: a young and playful dog can be a disaster, a very lazy pet is not sufficiently demanding and an angry pet can make an elderly person's life a misery. More and more, we need to have pets and owners adapted to each other and suited for each other. It is a relationship for life, after all.

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Chapter 13
Companion Animals and Human Health: On the Need for a Comprehensive Research Agenda Toward Clinical Implementation

Karine Silva and Mariely Lima

Abstract The potential association between companion animal guardianship and human health has become a hot topic not only among researchers but also the media and the general public. Has research reached such a point to elevate these animals to the status of "clinical allies"? This chapter aims at providing an objective assessment of the literature allowing the reader to appreciate the distance between current understanding of the effects of companion animals on human health and its application in health promotion and healthcare. It is divided into two main sections. In the first section, evidence suggesting that companion animals may have a positive impact on human health is presented, followed by opposite findings. In the second section, attention is called upon the need for a comprehensive research approach (integrating confounding, mediating, and moderating variables) before we may attribute a "clinical role" to our companion animals.

Keywords Companion animals · Health promotion · Confounders · Mediators · Moderators

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13.1 Introduction

It has long been recognized that being integrated into close social relationships, or perceiving social support, is critically important to an individual's health and even survival (House et al. 1988). Numerous studies have established associations between measures of interpersonal relationships and mortality, psychiatric and physical morbidity, as well as adjustment to and recovery from chronic diseases, with effect sizes equaling or exceeding those of well-established behavioral factors including smoking cessation, sports, or absenteeism from alcohol (as reviewed in Cohen 2004). Holt-Lunstad et al. (2010), for example, reported data from 308,849 individuals followed for an average of 7.5 years and showed that individuals with adequate social relationships had a 50% greater likelihood of survival when compared to those with poor or insufficient social relationships. In line with this evidence, at the level of clinical practice, patients' social ecologies and, most particularly, the family have been assuming an increasing salience importance for their powerful effect on health. In this regard, McDaniel et al. (2005) presented a compelling case for family issues and family dynamics to be recognized in primary care, not only as potential influences on illness but also as powerful positive resources in providing quality primary healthcare. Interestingly, as this family-oriented clinical practice has been increasingly recognized, the family concept has been evolving to one that increasingly acknowledges the significant part that companion animals assume in people's lives.

A fundamental change in the way humans interact with companion animals has occurred over the last recent decades, likely derived (at least in part) from the improvements in veterinary advances regarding vaccination and parasite control. The social structure of families in modern western societies may have also contributed to the way we now interact with our companion animals. Family sizes tend to be smaller, with larger numbers of people living alone. Animal companions, therefore, are increasingly fulfilling various emotional needs, amazingly sharing our lifestyles, our homes, our bedrooms, and even our beds (Archer 1997).

Increasingly, research has been exploring the new way people relate to companion animals, notably regarding its potential impact on health. The proposition has even emerged that if relationships with companion animals confer the same benefits as close human relationships, then a biopsychosocial model of care is not complete unless it also considers the companion animals in patients' lives (Jennings 1997; Hodgson and Darling 2011; Smith 2012). Accordingly, the One Health initiative, which was primarily developed to prevent the spread of disease from animals into human populations, has now evolved to account for the human-animal bond and its potential benefits to human health (Gibbs 2014; Rabinowitz et al. 2017). A term was coined to describe this "positive inverse of zoonosis," "Zooeyia" (Hodgson and Darling 2011), and it was suggested that effective health promotion and healthcare strategies could be planned based on the potential benefits to human health that could be derived from leveraging the human-companion animal bond (Bartges et al. 2017). More specifically, it was proposed that companion animals could benefit patients as builders of social capital and motivators of healthy behavior change and

as adjuncts to therapy with the aims to facilitate recovery from disease and reduce the use of a medication (Hodgson et al. 2015). Following from this, the present chapter aims at providing a global view of the literature exploring the effects of companion animal guardianship on human health and discusses the need for a comprehensive research agenda, addressing potential confounding, mediating, and moderating variables, before application in clinical settings.

13.2 Is Human Health Affected by Animal Companion Guardianship?

13.2.1 Reports Suggesting Benefits on Human Health

Evidence of an association between companion animals and improved health comes mostly from two types of quasi-experimental or observational studies: (i) studies assessing changes in health parameters in new companion animal guardians following adoption of a companion animal and (ii) studies comparing health parameters in companion animal guardians and non-guardians. We will here refer separately to some evidence provided by each of these two types of studies, without however attempting a systematic review of the literature.

13.2.1.1 Studies Exploring the Adoption of a Companion Animal

Studies exploring the adoption of a companion animal have found evidence suggesting that companion animals may have a positive impact on health over relatively long periods of time, either in general population (Serpell 1991) or in particular groups (Allen et al. 2001; Wright et al. 2015). In a 10-month prospective study, Serpell (1991) examined changes in physical activity and health status among 71 adult individuals following the acquisition of a new companion animal (either dogs or cats). As compared to a control group of 26 individuals, new companion animal guardians showed a significant decrease in minor health problems during the first month following the acquisition of the animal, an effect that was sustained in new dog guardians through to 10 months. Also, new guardians showed improvements in general health over the first 6 months, and in dog guardians, this improvement was maintained for 10 months. According to Serpell (1991), at the basis of such differences between dog owners and cat owners may be increased physical exercise in the form of dog walking.

In another study – the closest to a randomized controlled trial – Allen et al. (2001) randomly assigned 48 hypertensive adult individuals starting lisinopril (an angiotensin-converting enzyme (ACE) inhibitor) to a group adopting a companion animal (dog or cat) or to a wait-list control group for 6 months. They assessed participants' physiological responses (blood pressure, heart rate, and plasma renin activity) to mental stress before and during ACE inhibitor therapy, with results showing that before drug therapy, mean responses to mental stress did not differ

significantly between experimental and control groups. Lisinopril therapy lowered resting blood pressure in both groups, but responses to mental stress were significantly lower among animal guardians compared to those who only received lisinopril. The authors concluded that ACE inhibitor therapy alone lowered resting blood pressure, while increased social support through companion animal guardianship lowered blood pressure response to mental stress. Finally, Wright et al. (2015) assessed the impact of the acquisition of a companion dog on the stress levels of caregivers of children with autism spectrum disorder. Stress levels of 38 individuals acquiring a dog and 24 controls not acquiring a dog were sampled at "pre-intervention" (17 weeks before acquiring a dog), "post-intervention" (3–10 weeks after acquisition), and "follow-up" (25–40 weeks after acquisition). Results showed a significant improvement in stress levels in individuals who acquired a dog, as compared to controls. Of notice, a significant number of those parents moved from "clinically high" to "normal" levels of parental distress.

13.2.1.2 Studies Comparing Guardians and Non-guardians

General Health Indicators

Numerous cross-sectional and longitudinal observational studies have reported significant differences in general indicators of health between companion animal guardians and non-guardians. Siegel (1990), for example, surveyed the physician utilization behavior of 938 elderly individuals (Medicare enrollees in a health maintenance organization) for a 1-year period and found that individuals living with a companion animal reported fewer doctor contacts than those who did not. In addition, they found evidence suggesting that companion animals helped their owners in times of stress: pre-baseline stressful life events were associated with increased doctor contacts during the study year but only for participants without companion animals. In another study involving a large sample of older adults (baseline $n = 1054$; follow-up $n = 995$), Raina et al. (1999) found that the ability to complete activities of daily living decreased more in 1 year for people who did not live with companion animals than for people who did. Headey and Grabka (2007) confirmed in a study with 10,960 adult individuals what was previously reported in cross-sectional studies (e.g., Headey 1999). They concluded that people who continuously lived with a companion animal were the healthiest group (as evidenced by fewer annual doctor visits). People who ceased to have an animal or never had one were less healthy.

Cardiovascular Risk Factors

Some of the earliest reports relating to associations between companion animal guardianship and human health focused on individuals who survived coronary heart disease. Friedmann et al. (1980) looked at 92 survivors of myocardial infarction or angina pectoris and found that among those living with companion animals, the

1-year survival was 94% as compared to 72% among those who did not. This study was replicated and extended to a larger number of participants by Friedmann and Thomas (1995). One-year survival data was obtained from 369 patients with results showing that high social support and owning a companion animal tended to predict survival independent of physiologic severity, demographic, and other psychosocial factors. Dog guardians, in particular, were significantly less likely to die within 1 year than non-guardians. Data has since accumulated showing seemingly beneficial associations between companion animal guardianship and cardiovascular health. Accordingly, in 2013, the American Heart Association published a scientific statement about animal companionship and cardiovascular risk. This report concluded that "pet ownership, particularly dog ownership, is probably associated with decreased cardiovascular disease risk" and that "pet ownership, particularly dog ownership, may have some causal role in reducing cardiovascular disease risk" (Levine et al. 2013). More recent studies seem to support these conclusions. Mubanga et al. (2017) studied incident cardiovascular disease and death in over more than three million adults followed through nationwide register linkage over a 12-year period. They found that dog guardianship was associated with a lower risk of incident cardiovascular disease in single-person households and with lower cardiovascular and all-cause mortality in the general population. Also, recently, Chowdhury et al. (2017) assessed the association of companion animal guardianship and all-cause and cardiovascular mortality over a long-term follow-up among 4039 elderly treated hypertensive individuals. Results showed a 22 and 26% reduction in cardiovascular mortality observed among previous and current animal guardians, respectively, compared with those who were never companion animal guardians. Recent evidence also has been found suggesting that companion animal guardianship may also be beneficial to children's cardiovascular health. Xu et al. (2017) explored the association between exposure to companion animals and blood pressure in 9354 children and found that keeping dogs in the home was related to a significantly lower prevalence of hypertension.

Mental Health

Some studies in general populations have found a positive association between companion animal guardianship and mental health, as estimated by components of well-being (such as subjective happiness and life satisfaction) and related constructs (such as self-esteem, depression, and loneliness). McConnell et al. (2011), for example, compared common well-being measures in a community sample of 217 adults and found that animal guardians not only had greater self-esteem but also tended to be less lonely than non-guardians. Other studies have found evidence suggesting mental health benefits of companion animals also for individuals at increased risk for psychological problems (Siegel et al. 1999; Stanley et al. 2014; Lem et al. 2016; Muldoon et al. 2017). Siegel et al. (1999) explored data from 1872 participants, 38% of which were HIV-positive and 11% had AIDS. These authors found that being an animal guardian reduced the likelihood of depression in men diagnosed with AIDS and more

particularly in those individuals with lower social support (i.e., few confidants). Similarly, Muldoon et al. (2017) examined the relationship between dog guardianship and depression in a sample of 199 adults living with HIV and found that non-dog guardians had three times higher odds of depression when compared to dog guardians. In another study, Lem et al. (2016) showed an association between companion animal guardianship and depression among 189 street-involved youths, with the odds of being depressed being three times greater for youths who were not animal guardians. Stanley et al. (2014) utilized survey data from a sample of 830 older adult primary care patients and found evidence suggesting that companion animal guardianship may attenuate feelings of loneliness and its related sequelae: animal guardians were 36% less likely than non-guardians to report loneliness.

Studies in children and adolescents have also found differences in mental health among companion animal guardians and non-guardians, pointing to a positive effect of these animals (Black 2012; Reis et al. 2017). Black (2012) explored the relationship between loneliness and companion animal guardianship among 293 rural adolescents. Animal guardians reported significantly lower levels of loneliness than non-guardians. Also, attachment to the animals was inversely related to loneliness levels. Reis et al. (2017) tested in a sample of 6026 adolescents what kinds of feelings animal companions provided and whether well-being, life satisfaction, and psychological symptoms in adolescents were associated with having a companion animal. Obtained data showed that companion animal always or almost always provided the participants with feelings of happiness, companionship, nurturing, tranquility, security, and responsibility. Also, results showed that having a dog was associated with better perception of well-being, more life satisfaction, and less psychological symptoms. Finally, positive associations between companion animal guardianship and psychological health have also been found in studies focusing on individuals with mental health conditions (Fritz et al. 1995; Zimolag and Krupa 2009). Fritz et al. (1995) studied 64 Alzheimer's patients to explore the potential effect of companion animals on the progression of cognitive decline and the manifestation of concomitant noncognitive symptoms. Prevalence of episodes of verbal aggression and anxiety was reported less frequently in 34 patients who had contact with companion animals compared with patients who had not. Zimolag and Krupa (2009) examined the association between companion animal guardianship and engagement in meaningful activity as well as community integration in 204 service recipients diagnosed with serious mental illnesses. These authors found that individuals who had contact with companion animals tended to score higher on engagement in meaningful activities as well as on social and psychological community integration.

Social Health

Studies comparing companion animal guardians and non-guardians also suggest that companion animal companionship may have a positive impact on people's social capital, hence social health (Hyde et al. 1983; Wood et al. 2005). Hyde et al. (1983) administered measures of self-esteem, interpersonal trust,

and social sensitivity to 60 companion animal guardians and 60 non-guardian undergraduates. Results showed that animal guardians tended to score higher than non-guardians on both social sensitivity and interpersonal trust. In another study, results from a random survey of 339 adults showed that companion animal guardianship was positively associated with some forms of social contact and interaction, as well as with perceptions of neighborhood friendliness (Wood et al. 2005).

13.2.2 Reports Suggesting Null or Detrimental Effects on Human Health

The data reported above can indeed be exciting from the health promotion and healthcare perspectives. Notwithstanding, research exploring the relationship between companion animals and human health – either exploring the adoption of a companion animal or comparing companion animal guardians and non-guardians – has not always produced positive results. For example, Paul and Serpell (1996) investigated the impact of adopting a dog on the lives of 27 middle childhood children and found that children who adopted a dog experienced increases in the number of ill health symptoms they suffered by the 12-month follow-up (although, as highlighted by the authors, there were no differences between the groups at 1- and 6-month follow-ups). Results also showed that children who were more attached to their newly adopted animals were more likely to show an increase in illness symptoms (than those who were less attached to the animals) at the 1-month follow-up. Importantly, and as also highlighted by the authors, there were no particular types of illness that were especially associated with these observations; instead, it was a "generalized rise in miscellaneous symptoms." These findings highlight the possibility that for children (if not for adults), some allergies and/or zoonoses may result from adopting a dog (at least in the first year following animal adoption). In another study, Gilbey et al. (2007) ran a longitudinal test of the belief that companion animal ownership reduces loneliness. They collected data from 59 participants seeking to acquire a companion animal and found no evidence that adoption of an animal reduced levels of loneliness, irrespective of the type of companion animal adopted.

In some studies, comparing companion animal guardians and non-guardians, animal companion guardianship was found to be associated with poorer mental and physical health (Parslow and Jorm 2003; Parslow et al. 2005; Rijken and van Beek 2011).

Parslow and Jorm (2003) found no differences in mental and physical health profiles or use of general practitioner services between companion animal guardians and non-guardians among a sample of 2530 middle-aged adults. Animal guardians, however, used pain relief medications more frequently than non-guardians. Similar observations have been reported for 2551 elderly adults by Parslow et al. (2005). These authors compared sociodemographic attributes along with mental and physi-

cal health measures between companion animal guardians and non-guardians and found that companion animal guardianship exhibited more symptoms of depression, poorer physical health, and higher rates of use of pain relief medication.

Negative associations between companion animal guardianship and cardiovascular health have also been found. Parket et al. (Parker et al. 2010) assessed the rates of cardiac death and readmission among 424 patients admitted to a cardiac unit with an acute coronary syndrome, 1 year following hospitalization. These authors showed that animal guardians, when compared to non-guardians, were more likely to experience death or readmission following their hospitalization. In turn, Parslow and Jorm (2003) found that, in a sample of 5079 adult individuals, companion animal guardians had similar levels of systolic blood pressure with non-guardians, but significantly higher diastolic blood pressure, as well as higher BMI, and a greater likelihood of being smokers.

More recently, in a nationally representative study including data from 8785 elderly adults, Batty et al. (2017) explored variously established biomarkers of aging in the domains of physical, immunological, and psychological function but found no evidence of a clear association of any type between companion animal guardianship and health. Similarly, Torske et al. (2017) analyzed data from 53,418 participants and found no evidence for an association between the presence of a companion animal in the household and all-cause mortality. Also, no difference was observed in the incidence of being overweight between companion animal guardians and non-guardians. The positive associations between companion animal guardianship and child/adolescent health have been also put into question in a study by Mathers et al. (2010) referring to a sample of 928 participants.

13.3 Addressing the Unknown

For companion animals to be effectively integrated into health promotion and healthcare strategies, healthcare professionals and policymakers should be able to extract from the literature clear answers to some major questions. First, whether companion animals can indeed cause better – or poorer – health. Second, what may be the mechanisms underlying the potential effects of companion animals on human health, and how to influence those mechanisms so to reach desired health outcomes in patients? Third, when are people most likely to benefit (or suffer) from interacting with companion animals, or, in other words, to whom should health promotion and healthcare strategies be directed? It seems to us that the literature does not yet provide conclusive answers to these questions, the reason being that these questions refer to variables which have been neglected or poorly explored in most studies up to this point. Specifically, the first question refers to confounding variables, the second question refers to mediating variables, and the third question refers to moderating variables. Following definitions presented in Bauman et al. (2002), confounding variables are variables that are associated with the “outcome” (in this case human health) but also with exposure to “intervention” (in this case companion

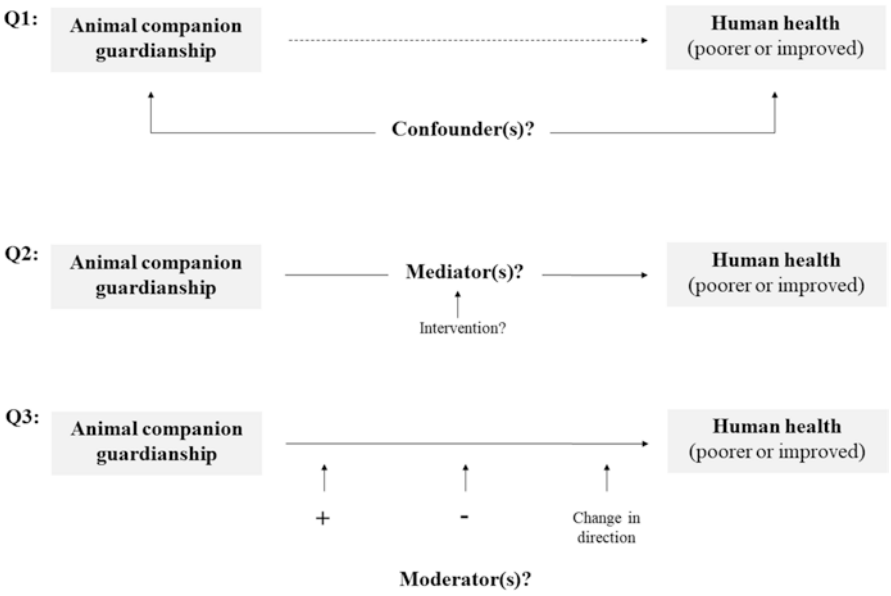

Fig. 13.1 Major open questions (Q1, Q2, Q3) in research exploring the effects of companion animal guardianship on human health and related variables (confounders, mediators, and moderators)

animal guardianship) (Q1 in Fig. 13.1). These variables can distort the association between "outcome" and "intervention." Mediating variables, or intervening causal variables, are variables that are necessary to complete the cause-effect link between, in this case, "companion animal guardianship" and "human health" (Q2 in Fig. 13.1). Moderating variables, or effect modifiers, are variables that affect the direction, strength, or both, of the relationship between companion animal guardianship and mediating variable(s) or mediating variable(s) and human health (Q3 in Fig. 13.1). We will here refer separately to each of these variables in the context of the literature presented in the above section of this chapter.

13.3.1 Confounding Variables

The discipline of modern epidemiology has set criteria for assessing evidence of a causal relationship, the most important being study design (Rothman and Greenland 2005). In the hierarchy of study designs, data coming from randomized, controlled trials are given the highest scientific weigh. To our best knowledge, there is no such study exploring the effects of companion animals on human health – likely because of practical and ethical difficulties since people cannot be randomly assigned to adopt a companion animal. In the absence of randomized controlled studies, quasi-experimental studies assessing changes in health in new (self-selected) animal

guardians provide evidence of causality from the highest grade. With such a design, one should be able to specify changes in health relative to the timing of the baseline assessment (i.e., preadoption proceedings) as well as the timing of the follow-up. Available studies, however, are only but a few (we could find only four studies; see above). Moreover, they used very limited samples (from 27 participants in Paul and Serpell 1996 to 71 participants in Serpell 1991), and some of them did not include observations before the adoption of the companion animal. This is important to note, as ideally, the baseline should be established over multiple assessments before the adoption of the animal (Nelson et al. 2014). In line with this, a recent systematic review of the evidence on the association between companion animal guardianship and child/adolescent development highlighted the shortage of high-quality longitudinal studies (Purewal et al. 2017).

Studies comparing animal guardians and non-guardians, using either longitudinal or cross-sectional designs, are far more numerous, and some of them report data from nationally representative surveys. Notwithstanding, and as repeatedly highlighted in the literature, these studies cannot establish whether observed health differences between animal guardians and non-guardians are caused by the presence of the companion animals or rather by factors – confounding factors – impacting on the decision to have a companion animal, such as socioeconomic status (Herzog 2011). In this respect, it seems important here to refer to a recent study by Saunders et al. (2017) comparing companion animal guardians and non-guardians across a variety of sociodemographic and health measures that could act as confounders in observational studies exploring the association between companion animal guardianship and human health. These authors collected data from 42,044 adult individuals and found that animal guardians significantly differed from non-guardians across several variables that can potentially impact health. Thus, it is possible that some of the health differences observed in previous studies between companion animal guardians and non-guardians might have been over- or underestimated due to differences in those variables and not necessarily (or solely) differences in companion animal guardianship patterns. Accordingly, Saunders et al. (2017) highlighted the importance for studies using quasi-experimental or observational approaches to investigate the relationship between animal companionship and human health to consider confounding variables in data collection plan and to deal with them using appropriate methods such as propensity score matching utilizing boosted regression. Most studies reported up to Saunders et al.'s study, however, did not. One exception was found: Headey and Grabka (2007) accounted for differences in potential confounders using propensity score matching. Interestingly, when they did so, the association between animal companionship and doctor visits, while remaining significant, diminished to half its size. Interestingly, in a more recent study, Miles et al. (2017) used double robust regression analyses to examine the association between living with a dog or cat and children's health outcomes, while accounting for confounding factors. Unadjusted analyses found that children living with companion animals were significantly healthier than children not living with an animal. However, when estimates were adjusted for confounders, the effects were smaller and no longer statistically significant. Thus, the benefits of companion animals

observed in this study were largely explained by confounding factors. Additional studies are needed with large samples and better methodological quality before any conclusion can be drawn on the causal effect of companion animal guardianship on human health. Importantly, at this point, the possibility that there is a noncausal association between companion animal guardianship and human health must be acknowledged.

13.3.2 Mediating Variables

The association between companion animal guardianship and human health is likely to involve a complex combination of mechanisms (Luhmann and Kalitzki 2018). Among these mechanisms, attachment and perceived social support from the animal – notably to engage in physical activity – have received considerable research attention and will here be presented in detail.

13.3.2.1 Attachment

It is known that attachment bonds can contribute to improved psychological health, which in turn may bolster physical health. Interestingly, data from several sources suggest that the relationship (some) individuals establish with their companion animals, and with dogs most particularly, does resemble an attachment bond (Archer 1997) and even seems to share striking neurophysiological features with the mother-child bond. Stoeckel et al. (2014) examined functional magnetic resonance imaging brain activation patterns as mothers viewed images of their child and dog and an unfamiliar child and dog. They found that there was a common network of brain regions involved in emotion, reward, affiliation, visual processing, and social cognition when mothers viewed images of both their child and dog (which elicited greater positive emotional responses than unfamiliar children and dogs). Also, participants in this study reported similar pleasantness (valence) and excitement (arousal) ratings for their child and dog with a larger difference in the own vs unfamiliar child compared to the own vs unfamiliar dog comparisons for arousal. Accordingly, in another line of investigation, some authors have found that petting or merely engaging in eye contact with one's dog can lead to an increase in neurotransmitters and neurochemicals associated with well-being and emotional attachment, such as dopamine, endorphin, and oxytocin. Such a direct effect of dogs on these health-related parameters was first shown by Odendaal (2000) and Odendaal and Meintjes (2003). These authors assessed changes in plasma levels of b-endorphin, oxytocin, prolactin, b-phenylethylamine, and dopamine in adult dog owners when petting their own, or an unfamiliar dog, or when quietly reading a book. Both humans and dogs in this study showed significant increases in plasma b-endorphin, oxytocin, prolactin, phenylacetic acid, and dopamine. In humans, there was also a significant increase in prolactin. Quiet book-reading produced similar changes as positive

human-dog interaction, but increases of b-endorphin, oxytocin, and prolactin were higher during interaction with the dog. Increases in oxytocin levels in adult individuals following interaction with one's dog were also reported by Miller et al. (2009), Nagasawa et al. (2009), Handlin et al. (2012), as well as Nagasawa et al. (2015).

Overall, the above findings give weight to attachment to companion animals as a mechanism underlying some of the reported positive associations between animal guardianship and human health – particularly if one considers the recognized health-promoting effects of oxytocin (reviewed by Beetz et al. 2012). Other studies, however, suggest that the attachment bond people develop with companion animals may negatively mediate the association between animal companions and human health, so that detrimental effects are observed. It is known that animal guardians often treat their companion animals like children and are therefore similarly concerned about their health and security (Archer 1997). Also, it is recognized that the threat of separation from a close companion animal may lead to noncompliance with medical advice (McNicholas et al. 2005) and failure to leave inferior but animal accommodating housing conditions (Morley and Fook 2005). Of notice, and because of limited research in this area, it is not yet well understood how often people prioritize their relationships with their companion animals before that of their well-being (Chur-Hansen 2010), which, as highlighted in Wells (2009), is, clearly, an issue for concern (Winefield et al. 2008). For example, according to McNicholas et al. (2005), advice to get rid of a companion animal due to allergies or immunocompromising conditions may be disregarded by up to 70% of owners.

13.3.2.2 Perceived Social Support from the Animal

Some authors have suggested that, to better understand how people may derive health benefits from animal companionship, researchers may benefit more from looking at the supportive functions of person-animal companion relationships, rather than attachment (Collis and McNicholas 1998; see also Wells 2009). Following Cobb (1976), social support can be described as information leading to the belief that one is cared for, loved, esteemed, and part of a network of mutual obligation. In some respects, animal companions may meet many components of social support. They are typically perceived as nonjudgmental, as noncritical, and to be there in times of trouble; the behaviors they display (e.g., the greeting rituals exhibited by dogs upon their owners' return to the house) create the impression of unconditional love, faithfulness, and dependability (as previously highlighted in Wells 2009).

Two models have been proposed to account for how perceived social support (including from companion animals) may influence health: the "stress-buffering" model and the "main effects" model (Cohen et al. 2000). While the "stress-buffering" model posits that social support may provide informational, emotional, and/or tangible resources promoting adaptive behavioral and/or neuroendocrine responses to acute or chronic stressors, the "main effects" model describes a direct positive effect of support irrespective of stressors.

Interestingly, research exploring direct, short-term effects of interactions with companion animals suggests that the perceived social support from companion animals may influence animal caregivers' health via both these pathways. Indeed, and in line with the "main effects" model, experimental studies have shown that stroking or petting one's companion dog, under resting conditions, can lead to transient decreases in cardiovascular parameters, such as blood pressure and heart rate (Baun et al. 1984; Jenkins 1986; Handlin et al. 2011), and also in hormonal indicators and neurotransmitters, such as cortisol, epinephrine, and norepinephrine (Odendaal and Meintjes 2003). In another line of investigation, experimental studies have shown that being in the presence of a familiar companion animal, during a stressful task, can reduce psychophysiological stress responding in both adults (Allen et al. 1991; Allen et al. 2002; Zilcha-Mano et al. 2012) and children (Kertes et al. 2017; Kerns et al. 2018), thus giving weight to the "stress-buffering" model. In one of the most cited experiments, Allen et al. (2002) investigated the effect of stressors in married couples, either in the presence of own companion animal (dog or cat), a friend, or the spouse. Non-animal guardians had the lowest reactivity (i.e., lowest increases in heart rate and blood pressure) when they performed mental arithmetic alone and the highest reactivity in the presence of their spouses. Animal guardians had the highest reactivity with their spouses present, but their lowest reactivity occurred in the presence of their companion animal. Similarly, Kertes et al. (2017), who investigated the effect of the Trier Social Stress Test for children, either in the presence of their companion dog, a parent, or with no support figure, showed that the presence of the animal significantly buffered perceived stress response in comparison to that observed in the other two conditions.

Importantly, it has been proposed that perceived social support from animals may also, sometimes, indirectly affect health by impacting on health-related behavior and most particularly physical activity. Accordingly, findings from several longitudinal observational studies suggest that dog adoption may result in people walking more (for a review see Christian et al. 2013). Cutt et al. (2008b), for example, found that, after 12 months of follow-up, people who became dog guardians accumulated approximately 48 minutes per week of recreational walking (compared with just 12 minutes per week in people who remained non-dog guardians). Also, data has been reported showing that dog owners tend to engage in more physical activity than non-owners and are more likely to exercise at the recommended level of 150 minutes per week (Epping 2011; Thorpe et al. 2006).

As recognized by a number of authors (Chur-Hansen et al. 2009; Peacock et al. 2012), despite evidence suggesting that attachment to and perceived social support from companion animals may have a significant role in the causal chain between animal companionship and human health, both have been "largely ignored or poorly controlled for" in most of the quasi-experimental or observational studies reported to date. Indeed, most studies have predominantly focused on companion animal guardianship rather than attachment to or perceived social support from the animal. Such studies, therefore, have not accounted for an important source of variation

across participants in the ways (positive and negative) their health may or may not be affected by animal companionship, which may contribute to explain the reported conflicting results among studies.

From a health promotion and healthcare perspective, studies have yet to identify the key factors of the mechanism underlying the associations between companion animal guardianship and human health. For example, in respect to the direct, short-term, psychophysiological effects of interactions with companion animals (likely derived from attachment to and perceived social support from the animal), several questions were put forward that remain unanswered. For example, it is not known whether the mere presence of a companion animal is sufficient or optimal for responses to occur, or if such responses need a particular type of physical interaction, with a specific duration and intensity (Friedmann and Gee 2017; Beetz et al. 2012). Also, it is not known if the reported physiological effects can extend beyond the time when the companion animal is present, and if so, how long do such effects last before a new "inoculation" is necessary (Friedmann and Gee 2017). This is worth noting here since only with a thorough understanding of the mechanisms through which animal companion guardianship may affect human health one may be able to effectively translate findings from quasi-experimental or observational studies into broader health promotion and healthcare strategies (as also highlighted in Saunders et al. 2017). Not surprisingly, given the current state of the art, health promotion interventions exploring the role of dog walking as a potential means of increasing motivation for the practice of physical activity have yet not been particularly successful (Cutt et al. 2008a, b). Indeed, numerous factors are likely impacting on how dogs affect the promotion or change of behavior (Cutt et al. 2008a, b).

13.3.3 Moderating Variables

Evidence suggests that the strength or even the direction of the association between companion animal guardianship and human health may vary according to various factors and that a comprehensive understanding of this association cannot be complete without considering these moderating variables or effect modifiers (Amiot and Bastian 2015; Amiot et al. 2016). Miller et al. (2009), for example, showed gender-based differences in hormonal responses to interaction with one's companion dog. These authors assessed changes in oxytocin levels in adult participants in response to interaction with their dog after a day at work (as compared to a reading control condition in the absence of the animal). They found that oxytocin levels increased significantly more in the dog condition than in the reading condition, but only in women. In fact, male oxytocin levels decreased in both the dog and the reading conditions. Gender-based differences were also reported by Tower and Nokota (2006) who showed that unmarried women living with a companion animal had the fewest depressive symptoms, while unmarried men living with a companion animal had the most. Accordingly, these authors suggested that single women may benefit from companion animal guardianship, whereas single men may be burdened by it

(see also Ogechi et al. 2016). Life conditions have also been pointed as a potential moderating variable, with studies suggesting that the presence of a companion animal may be particularly beneficial for individuals who live under great stressful conditions, who have limited access to human social support, or who live alone (Amiot et al. 2016). Garrity et al. (1989), for example, showed that, in a group enduring great distress (the bereaved), companion animal guardianship and attachment to companion animals were significantly associated with less depression but only when the number of available human confidants was minimal. Also, the type of the companion animal was suggested to impact the strength of the association between companion animal guardianship and human health. Accordingly, Serpell (1991) found significant reductions in the frequency of minor physical ailments for both dog and cat guardians 1 month following animal adoption, but while dog guardians maintained this decrease in health problems 10 months later, cat guardians did not. Other potential moderating variables may include the nature of our association with companion animals, notably the degree of compatibility between person and animal. In this regard, Budge et al. (1998) reported that the more animal guardians reported a high degree of behavioral compatibility between their companion animals and themselves, the higher the attachment to their companion animal, and the more likely they were to report positive overall mental health.

Clearly, companion animals may not have a general effect but rather different effects for different groups and under different conditions. Thus, it is not surprising that research exploring a general effect of companion animals on human health has produced conflicting results. Indeed, most studies in the field have not introduced moderating analyses. This means that, from a clinical perspective, currently, it is yet not possible to identify specific groups of patients for whom companion animals may provide the strongest benefits or may pose increased risks to health.

13.4 Recommendations

More in-depth information can be extracted from research studies exploring the potential effects of companion animal guardianship on human health if measures of confounding, mediating, and moderating variables are taken into consideration. Researchers embracing such a challenge might consider the following steps: (i) identify potential variables that may exert confounding, mediating, and/or moderating effects on the relationship between companion animal guardianship and human health; (ii) provide a rationale for those effects; (iii) plan for adequate data collection; and (iv) use appropriate statistical analysis to test for the hypothesized effects.

Given possible difficulties in discriminating between moderators and mediators, researchers might consider some guidelines presented in the literature. Bennett (2000), for example, proposes that if a particular relationship between companion animal guardianship and human health appears strong – and has been controlled for confounders – researchers might be interested in finding the mediator(s) that explains how or why that relationship occurs. On the other hand, if a particular

relationship between companion animal guardianship and human health appears weak or inconsistent, researchers might consider turning their attentions to (a) moderator(s), the levels of which could explain the circumstances that affect the association (strengthening it, weakening it, or even changing its direction) (as depicted in Fig. 13.1, Q3). Researchers need to be well familiar with the proper statistical analyses to test for confounding, moderating, and mediating effects. Such analyses are thoroughly discussed in the literature and will not be presented here as they are out of the scope of this chapter. Crucially, researchers need also to be familiar with available instruments measuring important variables of interest such as attachment to, and perceived social support from, companion animals. Some examples include the Lexington Attachment to Pets Scale (LAPS; Johnson, Garrity, & Stallones, 1992) and the Monash Dog Owner Relationship Scale (MDORS; Dwyer et al., 2006) which, importantly, measures both perceived costs and perceived benefits of having a dog. Alternatively, researchers may consider developing and validating their own instruments.

13.5 Conclusions

Surveys indicate that it is a widely held belief among the general public that there are health benefits of companion animal guardianship regardless of whether there is demonstrable proof. This belief, according, for example, to Herzog (2011), might well have been fueled by media reports and books extolling the "healing power of companion animals" as if it was a fact. Negative or null effects, in contrast, and as also highlighted in Herzog (2011), never make it to headlines, showing a significant bias toward reporting only positive outcomes in the media. Companion animal adoption campaigns have even built on this idea that animal guardianship is good for our health (e.g., "All the Ways a Puppy Can Make Your Life Better Right Now"; Anonymous 2018a). One such adoption campaign in Portugal has come to the point of showing people getting puppies, instead of medication, at a pharmacy (Anonymous 2018b). In light of this general belief, it is likely that people, in general, might happily accept the integration of companion animals into health promotion and healthcare strategies, notably within clinical settings. In line with this idea, Hodgson et al. (2017) showed that asking about companion animals may enable primary healthcare providers to better access relevant information on patients while also strengthening the therapeutic alliance. Notwithstanding, the integration of companion animals into health promotion and healthcare strategies does not yet seem to fit into the practice of evidence-based medicine, and defending this idea might even do a disservice to patient care. Primary healthcare providers, however, are in a privileged position to inform patients about current state of the art and alert about inaccurate and misleading information disseminated in the media. Also, primary healthcare providers are in a privileged position to collaborate with researchers in practice-based investigations. Special attention to the issues noted in this chapter may make the difference between a frustrating hit-and-miss practice and a productive and

upper-level research experience. Specifically, primary healthcare providers and researchers should acknowledge that, and up to this point, the actual effects of companion animal guardianship on human health – either for better or for worse – are yet to be established. In this respect, confounders need to be properly addressed in future studies. Also, future research should aim at more than to just exploring a potential direct effect of companion animal guardianship and human health. It should account for potential mediator and moderating effects so that insightful information can be obtained about why this phenomenon might occur and under which circumstances interventions might be more effective in minimizing the risks or leveraging the benefits of companion animals to human health.

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Part III
Psychosocial and Psychophysiological Effects of Human-Animal Interactions

Chapter 14
Phonetic Variation in Cat–Human Communication

Susanne Schötz

Abstract In this chapter, the phonetic variation of the vocal communication between domestic cats and humans is summarised and described, based on previous research as well as more recent studies and observations. Emphasis lies on classifying and describing the different vocalisation types of the cat using phonetic methods and terminology. The articulation, phonetic transcription and acoustic patterns of the most common vocalisation types are described. In addition, the segments (vowel and consonants), the prosody (the tone, intonation, rhythm and dynamics) of cat sounds as well as human perception of cat vocalisations is summarised.

Keywords Cat–human communication · Cat vocalisations · Phonetic description and transcription of cat sounds

14.1 Introduction

The domestic cat (*Felis silvestris catus*) is one of the world's most popular pets. Generally, pets can positively influence the wellbeing and health of humans in several ways (Beetz 2017), and are often kept as companions.

However, the vocal behaviour of cats is still poorly understood. Not only is their vocal repertoire more complex than in many other mammalians, it is also characterised by "an indefinitely wide variation of sound and of patterning" (Moelk 1944). Therefore, by learning more about the main phonetic features of the vocal signals of cats, we may be able to interpret them better. This may not only benefit cat owners in general, but also people working with these animals professionally, as in veterinary medicine, breeding, sheltering, animal training and animal-assisted therapy.

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Despite a number of efforts to classify and describe the different vocalisation types of the cat, no robust and standardised classification system seems to be available. Lately, some zoologists have moved away from the traditional onomatopoetic names for vocalisation types, like hiss and meow in favour of a more neutral terminology (Owens et al. 2017). However, by applying a phonetic approach and by using a well-known terminology, we can describe the different vocalisation types to laymen more easily and still be able to classify the sounds in a robust and reliable way based on some basic articulatory, acoustic and auditory features.

When studying cat–human communication using phonetic methods, cat vocalisations are in many ways compared and related to human speech. Humans have a very complex vocal apparatus, with many important organs, including the lungs, the larynx, the jaw, the tongue and the lips. Despite some differences (mainly the size and shape of the tongue, vocal tract and lips as well as the position of the larynx), the vocal organs of the cat are similar to ours in many respects. Both human and cat sounds are produced with an airflow (e.g. from the lungs) which is obstructed between the vocal folds and/or further along the vocal tract (e.g. when the tongue is raised towards the palate or when the lower lip comes into contact with the upper lip) to create a sound wave, which is modified by resonance, depending on the shape and size of the vocal tract. For instance, the consonant m and the vowels eow in a typical domestic cat "meow" [miau] are probably articulated quite similar to how humans would produce these sounds.

A large part of the research on animal vocalisations is carried out within the field of bioacoustics. However, by relying not only on acoustic features when classifying and analysing different call types, but by also studying the articulatory gestures and perceptual (auditory) cues, we may be able to get a more comprehensive picture of the vocal interaction between cats and humans. A deeper understanding of the phonetic characteristics of cat vocalisations may improve our inter-species communication and may also increase the wellbeing of our own animals—and domestic cats in general.

In this chapter, I will describe and summarise the phonetic variation in the vocal communication between domestic cats and humans in the different sound segments (vowels and consonants) as well as in the prosody—especially in the melody (intonation)—based on previous research as well as my own studies and observations.

14.1.1 Human and Animal Vocal Communication

Humans mainly communicate using spoken, written or signed language. As children, we spend our first years learning this highly conventionalised code. Numerous studies have found that animal vocalisations are simpler and more limited than human speech. Animals seem to use communication mainly for a small set of basic needs: finding a partner, taking care of their offspring, warning against enemies and announcing when and where to go, and where to go to find food (Håkansson and Westander 2013:4), and they are often regarded as being unable to share more

abstract messages. In animal communication, a vocalisation roughly corresponds to a "word", which is related to a certain message in a certain behavioural context (which a human listener may interpret as a meaning). They seem to lack the need as well as the complex vocal apparatus required to develop communication codes with thousands of meaningful elements (morphemes). However, more recent research has suggested that many animal species may have some kind of "languageness", which does not share all the features of human language, but may still be very rich and complex (Kershenbaum 2017).

Despite the differences in our communicative codes, we are often able to communicate quite well with our pets. Some features seem to be universal. However, several differences exist which may lead to misunderstandings, unless we learn to interpret them correctly. By studying cat vocalisations, we may increase our interspecies understanding.

14.1.2 Human–Cat Communication

Humans mainly communicate using spoken, written or signed language. As children, cats and humans have developed a highly successful way of communicating with one another. Visual and tactile communication is also frequently used, but as most humans prefer speech and usually respond more quickly to sound, cats have learned that vocal signals generally work better. Some cat vocalisations seem especially effective in getting our attention, perhaps because we are biologically programmed to react instantly to when our children are in distress. Meowing and howling (yowling) are phonetically characterised by a high intensity and pitch as well as by a strained voice quality, which is very similar to the weeping and crying of human children.

Humans and cats living together often develop some common vocal habits or ingroup jargons or dialects. Cats can adapt to a specific environment, and they learn how to manipulate their surroundings in order to get what they want (Bradshaw 2013:144). For instance, by trying out different vocal patterns in their meowing when they are hungry, they learn which variations are the most successful ones in getting the attention—and the desired outcome—from their owners.

14.2 How Cats Communicate

Domestic cats are usually described as solitary animals, but they still exhibit a wide range of social relationships with other cats as well as with humans. They communicate using visual, tactile, olfactory as well as vocal (auditory, acoustic) signals either used alone or combined with other modalities in various behavioural contexts.

14.2.1 Visual Communication

Visual signals are used by cats when in visual range of its interlocutor(s). With body postures and movements, either of the entire body or of individual body parts, such as the tail, head, face, including the ears, eyes and whiskers, a cat can signal its intentions and mental or emotional state. For instance, cats raise their tails and hold them vertical to the ground—the "tail-up" signal—and turn their ears forward to express friendly intentions. Tail movements—with the whole tail or only the tip—can signal various degrees of excitement or arousal. Cats may try to warn or scare off an opponent by a number of ritualised signals, including stiffening the limbs and ears, piloerection, and staring. Contrarily, to signal non-aggression, a cat may crouch, turn its head away, lower its ears and lean back (Crowell-Davis et al. 2004). Visual signals are often used together with vocal or tactile ones, for instance, in agonistic contexts.

14.2.2 Tactile Communication

Tactile signals are primarily used in agonistic and intimate situations, for instance, during physical fighting, when mating and in friendly—including mother–offspring—interactions. Biting and claw scratching are used to attack or defend oneself. Nose touching and social rubbing (allorubbing) are common in affiliative contexts, for instance, when greeting a preferred associate (cat or human) to indicate peaceful intentions (Turner and Bateson 2000:86). Social grooming (allogrooming) is used by mothers to keep their kittens clean and also by befriended cats to clean areas which are hard to reach by oneself, such as the face, neck and ears. Moreover, cats may simply lie or sleep close together with a befriended cat or human to signal social affiliation (Crowell-Davis et al. 2004).

14.2.3 Olfactory Communication

Although cats' sense of smell is not as sophisticated as that of dogs, they are about five times better than humans at picking up scents (Bradshaw 2013). Olfactory communication is an important way of leaving more lasting messages. Urine spraying and faecal marking, as well as leaving scents via sebaceous glands by rubbing the chin, forehead or body against either a fellow family or colony member, or against an object in the environment, are olfactory signals. Sniffing the perianal region of another cat and allorubbing with other cats to distribute a "colony odour" are social behaviours, while urine and faecal marking and rubbing or scratching (there are skin glands between the digits) are probably used to communicate a number of messages, including the identity (e.g. "I am a female in heat"), location (e.g. "I was here") and mental and emotional state (e.g. "I am highly aroused") of the cat (Crowell-Davis et al. 2004).

14.2.4 Vocal (Auditory, Acoustic) Communication

As already mentioned, cats have a large and varied vocal repertoire. As sounds are instant and cursory, they have to be used in direct interactions with other individuals. Still, vocalisations can travel great distances, and they are frequently used by cats in heat to attract a mate or by kittens to call for their mother. Vocalisations are used in agonistic as well as affiliative contexts. Growling, howling (yowling), hissing, spitting, screaming and snarling may be used to warn or fend off an intruder or enemy, while trilling is common in friendly greeting, and purring may be used in intimate interactions. Although kittens mainly meow to get the attention or help from their mother, adult cats rarely meow towards other cats. Instead, meowing is one of the most common human-directed attention soliciting vocalisations. Prey-directed sounds—chirping and chattering—seem to be part of a hunting instinct where cats attempt to imitate the calls of the prey or practice the killing bite, for example, when a bird or an insect catches their attention.

In cat and other animal vocalisations, the context is often more important than in human speech. A meow uttered in one context (e.g. when greeting a friend) may signal something different than when uttered in another context (e.g. when in their transport box going to the vet clinic). When describing different cat vocalisation types, information about the context(s) where these sounds normally occur should be included.

To what extent cats use vocalisations to convey their physical, mental and emotional state is still not fully understood. Sick and emotionally or otherwise weakened cats avoid communicating their health state to prevent their natural enemies (predators such as foxes and racoons) from picking up these signals. On the other hand, kittens usually tell their mother when they are lost or in distress, sexual intents are usually signalled with sounds, and mothers tend to use different vocalisations and voice qualities when bringing up their offspring. Thus, befriended cats—and humans—should be able to detect the mental and emotional state of other cats by listening to specific patterns in their vocalisations.

14.3 Studying the Vocal Communication of Domestic Cats Using Phonetic Methods

Although most studies of domestic cat vocalisations have been carried out by non-linguists (e.g. Brown et al. 1978; Nicastro and Owren 2003; Yeon et al. 2011; Owens et al. 2017), investigations with phonetic methods may increase our knowledge of the different sound types. The variation in the articulatory, acoustic and perceptual features may be related to different mental states and behavioural contexts.

By observing the articulation—the movements of the lips, jaw and tongue—we can learn more about which vowel and consonant sounds cats are able to produce. For instance, cat hissing may be described as a voiceless egressive (produced with an exhalation airstream) dorsopalatal (the place of articulation is between the tongue

body and the hard palate) fricative (with a narrow constriction in the vocal tract creating a turbulent airstream and frication noise).

Acoustic analysis is frequently used in the study of human speech, and the same methods may be adopted to cat vocalisations. By measuring various acoustic features, we are able to analyse the segmental (i.e. the vowel and consonant sounds) as well as prosodic variation between or within certain vocalisation types. Such features include the duration, the sound pressure level (SPL, intensity), the fundamental frequency (F0, corresponding to the perceived pitch) and the spectral distribution of the sound energy (what we perceive as timbre or colour of the sound). A meow can, for instance, be pronounced as [mɪaʊ] or as [wɛːʊ] with a resulting difference in the spectral distribution and dominant frequencies (see, e.g. Schötz et al. 2017; Schötz 2017). Moreover, a meow is sometimes as short as 0.15 s and sometimes as long as 3 s, with an F0 between 200 and 1200 Hz and with a level, rising or falling intonation (Schötz and van de Weijer 2014; Schötz 2018). By relating different phonetic characteristics to specific behavioural contexts, we should be able to better interpret the vocal signals of the cat. A natural first step would be to identify the most common cat vocalisation categories, and the acoustic variation between and within the different types.

Auditory analysis may help relate the perceptual features of cat sounds—including different voice qualities and intonation patterns—to specific emotions or contexts. By listening carefully to various aspects of a cat vocalisation—sometimes with the additional aid of acoustic measurements and diagrams—we may describe the phonetic properties of a specific sound and more easily write it down using phonetic transcription. The International Phonetic Alphabet (IPA, see, e.g. International Phonetic Association 1999) was developed for human speech and includes symbols for all speech sounds found in human languages. In most cases, the same symbols can be used for cat vocalisations. For instance, hissing may be transcribed as [çː] or [ʝː], depending on the colour of the frication noise.

Phonetic methods can thus be used to describe and compare different cat vocalisations in terms of how they are produced, what their acoustic properties are and how they are perceived by the human ear.

14.4 Phonetic Characteristics of the Most Common Cat Vocalisations

Despite the vast individual, mental and context-related variation, cat vocalisations are usually classified into a small number of distinct types, based on their production (airstream, voice, and articulation) and whether they are produced in affiliative (friendly), competitive (agonistic) or prey (or hunting) contexts. The articulatory, acoustic and auditory characteristics of these categories are also generally—but not always—quite distinct. The following sections comprise phonetic descriptions of the most common vocalisation types and their subcategories, based on previous

descriptions (e.g. Moelk 1944; Leyhausen 2005; Bradshaw 2013) as well as my own studies and observations (Schötz and Eklund 2011; Schötz 2012, 2013, 2015, 2017; Schötz and van de Weijer 2014; Schötz et al. 2017). Whenever possible, I have included references to the probable emotional state the sound is signalling and also to the typical behavioural context. The accompanying phonetic transcriptions should be seen only as typical examples of what the vocalisations sound like (based on auditory and visual acoustic analysis), as it would be impossible to include the whole range of variation within each type.

14.4.1 Friendly and Affiliative Vocalisations

14.4.1.1 The Meow

As already mentioned, the meow is one of the most common human-directed vocalisations. It is normally produced with a gradually opening and then closing mouth resulting in a sound often—but not always—beginning with a labial consonant resembling an [m] or a [w] and followed by one or more vowels. Towards the end of the vocalisation, the mouth usually closes again, occasionally resulting in an additional consonant sound, often a [w]. Meows are voiced—and generally quite loud—sounds with varying intonation, typically with an arched (rising–falling, with the peak corresponding to the maximum mouth opening) intonation (Nicastro and Owren 2003), but several other tonal patterns—including level, rising, falling and falling–rising—have also been observed. The duration may vary substantially, but usually ranges between 0.5 and 1.5 seconds. Meowing is often used to solicit attention and to signal a number of physical, mental and emotional states in various contexts. Meows can be interpreted as assertive, plaintive, friendly, bold, welcoming, demanding, complaining or even sad, depending on the variation in vowel quality as well as in the F0 pattern. A tonal rise usually signals affiliation or a friendly request, while a falling intonation may be a sign of discomfort or stress. Due to the large phonetic variation, this category can be subdivided into several subtypes, including the mew, the squeak, the moan and the (typical) meow.

The mew is a high-pitched meow with an [i], [ɪ] or [e] vowel quality and may be transcribed phonetically as [mi], [wi] or [mɪu]. Kittens often mew to solicit their mother's attention, and adult cats may mew when they are in need of help.

The squeak is a hoarse and somewhat nasal, high-pitched and usually quite short mew-like call, often with an [ɛ] or [æ] vowel. It can be transcribed phonetically as [wæ], [mɛ] or [ɛʊ]. Contrary to the other subtypes, the squeak frequently ends with an open mouth. Adult cats may squeak to solicit attention from their owners, for instance, when they want to cuddle, play or want a treat.

The moan is produced with an [o] [u], or [ʊ] vowel quality, and is often used when cats are discontent, sad, frustrated or demanding, for instance, when they have been confined to their cat carrier and taken to the vet, e.g. [moʊ] or [wuæu].

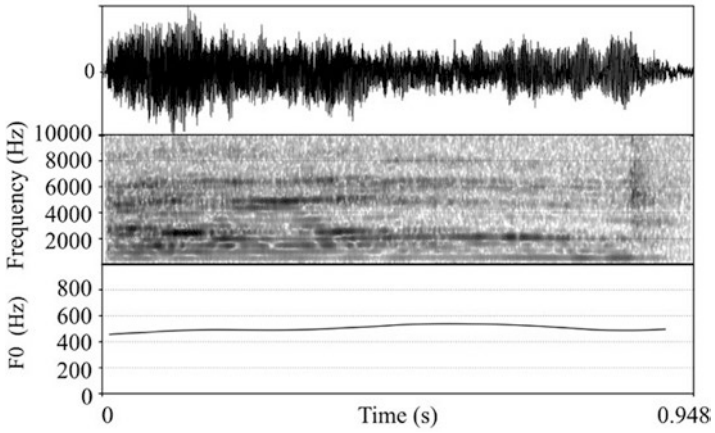

Fig. 14.1 Waveform (top), spectrogram (middle) and F0 contour (bottom) of a meow. (Adapted from Schötz (2016). Published with kind permission of © Susanne Schötz 2016)

The meow (or miaow) is produced with a combination of vowels resulting in the characteristic [iaʊ] sequence, as in [miau], [ɛau] or [wɑːʊ]. Meows are often used in cat–human communication to get our attention, e.g. to solicit food or to negotiate a barrier (e.g. a closed door or window).

Figure 14.1 depicts the waveform, spectrogram and F0 contour of a typical meow. The waveform shows that the amplitude is higher in the initial part, then gradually decreases and finally increases slightly towards the end. In the spectrogram, the grey horizontal lines represent the harmonics (the fundamental and the overtones) indicating that the sound is voiced. Due to resonance in the vocal cavities, a number of formants can often be observed in the spectrogram of a meow. These show the dominant frequency bands of higher amplitude with darker grey colour. This example meow has three formants: one around 2000–3000 Hz, one at about 4000–5000 Hz and one at 6000 Hz. The formant frequencies vary somewhat throughout the vocalisation, which is typically for a meow with several vowels. As shown in bottom pane of the figure, the F0 rises slightly from about 450 Hz to about 550 Hz and then falls to about 500 Hz towards the end, indicating the typical rising–falling intonation pattern of an attention-soliciting meow.

14.4.1.2 The Trill

A trill (chirr, chirrup, grunt, murmur) is a soft and often short vocalisation usually produced with a closed mouth resulting in a nasal sound (the sound wave escapes only through the nose). To the human ear, this sound resembles a human rolled or trilled, sometimes with a harsh voice quality, but is probably produced further back in the vocal tract or the larynx. Vocalisations belonging to this category can be subdivided into three main subtypes: the chirrup, the grunt, and the murmur.

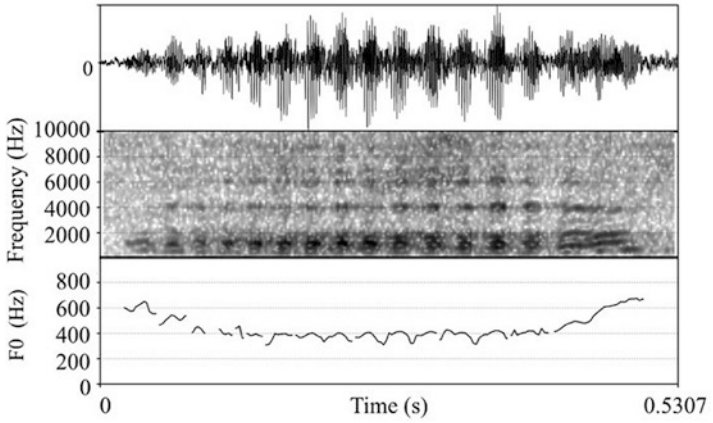

Fig. 14.2 Waveform (top), spectrogram (middle) and F0 contour (bottom) of a trill (chirrup). (Adapted from Schötz (2016). Published with kind permission of © Susanne Schötz 2016)

The chirrup (or chirr) is brighter and more high-pitched: [mr̃ːh] or [mːr̃ːt]—often with a rising intonation—and is often used during friendly approach and greeting, or to solicit food or play.

The grunt is darker, harsher and more low-pitched: [m̰ː] or [bɾ̃ː]—usually with a level or falling intonation. It is also used in affiliative greeting, but often also as a friendly confirmation ("Yes, I liked the treat you just gave me.").

The murmur (or coo) sounds more like a soft [m], as it is produced without any trilling.

The duration of trills is usually quite short (between 0.2 and 0.7 seconds), and the F0 of high-pitched chirrups is often between 500 and 900 Hz (some reach over 1000 Hz), while in lower grunts, it may be as low as under 100 Hz. Sometimes cats merge or combine trilling with meowing or purring, especially when soliciting food or a cuddle from their owners.

The waveform, spectrogram and F0 contour of a typical trill—in this case a brighter high-pitched chirrup—is shown in Fig. 14.2. In the waveform and spectrogram, clear pulses of sound energy (short periods of much energy followed by short periods of little or no energy) are shown throughout the vocalisation, which is rather short, around half a second. Most of the energy is in the low-frequency region (below 2000 Hz), indicating that trilling is a fairly dark and dampened (nasal) consonant-like sound. As frequently observed in chirrups, the F0 falls in the beginning and then rises again towards the end (from about 400 Hz to approximately 630 Hz).

14.4.1.3 The Trill–Meow

A trill–meow (or murmur–meow) is a very common human-directed sound. It is a complex vocalisation—a combination of a trill and a meow. Consequently, the duration is longer than for a simple trill or meow, often between 0.6 and 1.2 seconds.

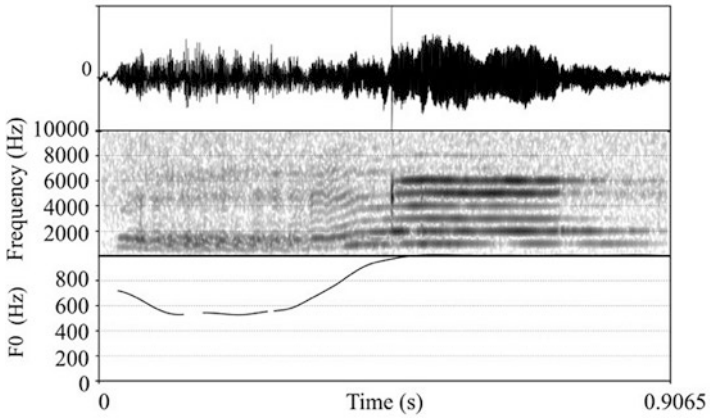

Fig. 14.3 Waveform (top), spectrogram (middle) and F0 contour (bottom) of a trill (chirrup). (Adapted from Schötz (2016). Published with kind permission of © Susanne Schötz 2016)

This combination gives the vocalisation type a distinct tonal pattern: a low F0 in the trill which rises in the transition to the meow and ends in a significantly higher F0. The mouth is closed during the trill and opens during the meow: [mrhiauw], [mhrŋ-au] or [whrːau]. Trill–meows are used in the same contexts as trills and meows and are mainly friendly attention-soliciting sounds when the cat is hungry, wants to play or be let out into the garden, and the rising pitch often attracts immediate attention.

Figure 14.3 shows the waveform, spectrogram and F0 contour of an example trill–meow. The first part of the vocalisation shows the pulsating sound energy in the low-frequency range (below 200 Hz) typical for nasal trills, while the last part is louder (higher amplitude in the waveform and spectrogram) with energy also in the region above 2000 Hz (up till about 6000 Hz). In the transition between the trill and the meow, the harmonics become increasingly visible in the spectrogram, and the meow displays a vowel-like pattern with formants (amplified frequency ranges) rendering the harmonics around 2000 Hz as well as the harmonics between about 5000 and 6000 Hz darker than the others. The F0 is lower in the trill (around 550 Hz), then rises throughout the transition into the meow part and ends in a very high plateau at about 1000 Hz.

14.4.1.4 The Purr

Purring is a very soft and regularly pulsating, extremely low-pitched and generally nasal sound produced "by aerodynamically driven vibrations of the vocal folds in the larynx" (Frazer Sissom et al. 1991) on an alternating (pulmonic) egressive and ingressive airstream. The mouth is usually closed, but may open slightly to generate a sound which is richer in overtones and thus more audible to the human ear. The fundamental frequency is so low—often between 20 and 30 Hz—that we perceive

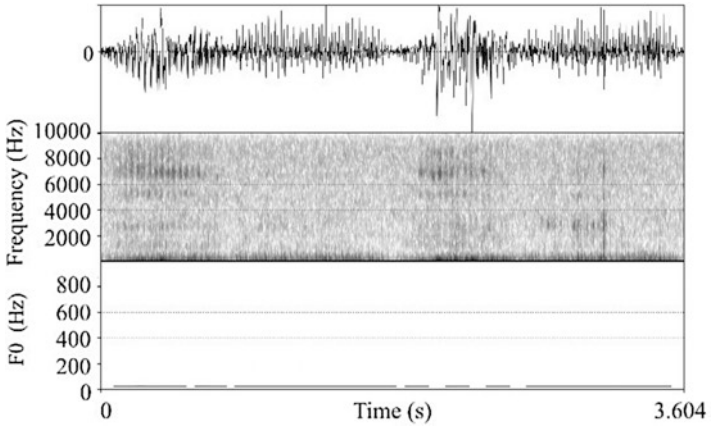

Fig. 14.4 Waveform, spectrogram and F0 contour of a purring sequence (two ingressive and two egressive phases). (Adapted from Schötz (2016). Published with kind permission of © Susanne Schötz 2016)

every pulse instead of hearing a tone. Purring may go on for minutes at a time: [↓hːr̃-↑r̃ːh-↓hːr̃-↑r̃ːh]. Cats purr when they are content, hungry, stressed, in pain, give birth or even when they are dying. The sound probably signals "I do not pose a threat" or "Keep on doing what you are doing". Sometimes purring is merged or combined with trilling or meowing, for instance, when cats are aroused or want to solicit food or a cuddle (McComb et al. 2009).

Figure 14.4 shows four phases of a purring sequence: two ingressive and two egressive phases. The waveform indicates that the sound energy (SPL) of each phase first increases and then decreases again before the next phase. In this example, the SPL is stronger in the ingressive phases (phases 1 and 3). Most of the energy is concentrated in the low-frequency area (below 500 Hz), but some strong overtones can also be found at higher frequencies, especially between 5000 and 8000 Hz. The regularly occurring vertical lines in the spectrogram—the "striped" pattern—represent the vibrations (or pulses). The F0 of these vibrations is extremely low, often around 20–25 Hz, which makes it possible to hear every beat.

14.4.2 Agonistic (Aggressive and Defensive) Vocalisations

Agonistic sounds come in a wide variety. Depending on the situation, cats may use one or several aggressive and defensive vocalisations to warn or to scare or fend off an (apparent) enemy. The sounds employed seem to depend on the distance between cats, as depicted in Fig. 14.5.

Growling, howling and combinations or variations with growling and howling are often common across longer distances. As the distance decreases, hissing and

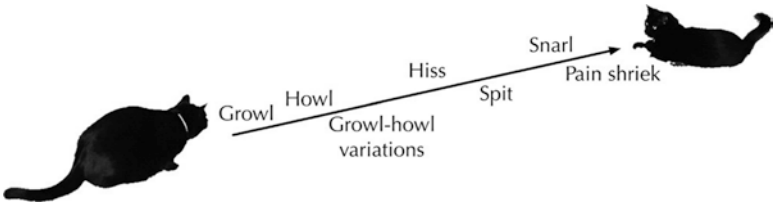

Fig. 14.5 Common cat vocalisations produced at various distances between opponent cats. (Adapted from Schötz (2018). Published with kind permission of © Susanne Schötz 2017)

spitting become more frequent. When the cats are within physical reach of one another, snarls, screams and pain shrieks become more common vocalisations. The different agonistic sound types are described in more detail in the following text.

14.4.2.1 The Howl (or Yowl)

Howling (yowls, moans, anger wails) is a loud and extended (often 2–8 seconds) vowel-like sound usually produced by gradually opening the mouth wider and closing it again. During a threatening situation, they are often merged or combined with growls in long sequences with slowly varying F0 and intensity (Brown et al. 1978; Eklund et al. 2012a). Moelk (1944) transcribed the anger wail as [wɑːouː] and pointed out that "[s]lighter wailing [...] occurs occasionally in connection with the growl in highly annoying situations which do not lead to fighting". Brown et al. (1978:566) found howling to be tonal sounds occurring in threatening or defensive responses with a wide variation in frequency distribution and modulation. Moans are described by McKinley (1982) as long, often slowly frequency-modulated vowel sounds occurring in the same situations as the growls. Bradshaw and Cameron-Beaumont (2000) distinguished howls from yowls in that howls are typically shorter in duration (howls: 0.8–1.5, yowls 3–10 seconds) and higher in F0 (howls: 700 Hz, yowls 200–600 Hz). Quite often, two opposing cats can be observed howling together as though in a duet: the dominant voice leads the melody up and down, and the other voice accompanies with weaker, brighter tones (Schötz 2015, 2018). This can go on for long periods of time (over 30 minutes is not uncommon).

A howl usually consists of two or more vowel (or approximant) sounds, including [ɪ], [i̞] and [j], sometimes with repeated diphthongs like [aʊ], [ɛʊ], [ɑʊ], [ɔɪ] or [ɑɔ], resulting in vocalisations like [awɔɪɛʊː], [jɪi̞ɛɑʊw], [ɔːɪɔɪɔɪɔɪɔɪɔːʊ] or [ɪːaʊaʊaʊaʊawawaw]. During an agonistic situation, howling can be combined and merged with growling in long sequences with slowly rising and falling intonation and intensity. This allows for the F0 to vary between about 100 and 900 Hz.

In Fig. 14.6, the waveform, spectrogram and F0 contour of a short howling sequence is displayed. The sound is over 1.5 seconds long and consists of a short and a long howl interrupted by a brief pause. The waveform shows that the sound pressure level (SPL) slowly increases and decreases throughout the vocalisation,

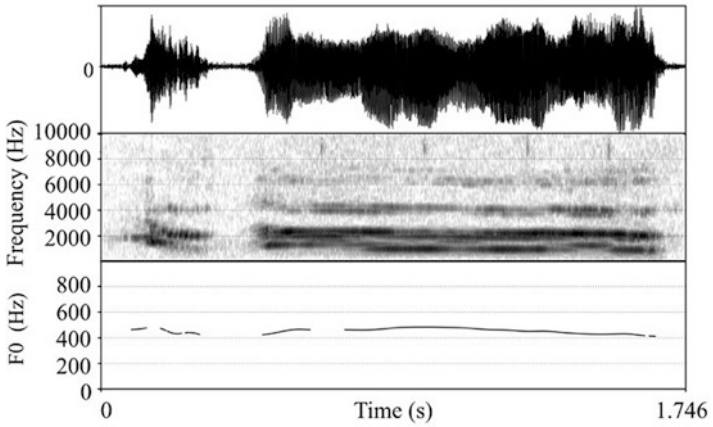

Fig. 14.6 Waveform (top), spectrogram (middle) and F0 contour (bottom) of a howl. (Adapted from Schötz (2016). Published with kind permission of © Susanne Schötz 2016)

creating a wave-like (rising–falling) intensity pattern. Both parts contain vowel-like sounds, with clear vowel-like formant patterns: one darker band between 1000 and 3000 Hz, another around 4000 Hz and a weaker dark band around 6000–7000 Hz. The F0 contour shows slight rises and falls between 400 and 500 Hz.

14.4.2.2 The Growl

The growl is a fairly weak, guttural, low-pitched (often 100–225 Hz) and regularly pulsating sound of usually long duration produced with the mouth held slightly open in the same position—sometimes with the lip curled up and exposed teeth—during a slow steady exhalation (Moelk 1944; McKinley 1982; Bradshaw and Cameron-Beaumont 2000; Eklund et al. 2012b; Bradshaw 2013). Brown et al. (1978, p. 556) describe growling as largely fricative and long in duration. It resembles a deep trilling consonant sound, sometimes with creaky voice quality, and can be transcribed as [gʀː], [ʀː] or [ɹ̰ː], occasionally beginning with an [m]. Growling is used to signal danger or to warn or scare off an opponent. The duration and F0 may vary, and falsetto growls are not uncommon. Often growling is intertwined with howling and hissing or merged with howling in order to produce an intermediate vocalisation between a growl and a howl. According to Houpt (2011), growls during a fight may vary between 400 and 800 Hz in F0, and this probably includes the intertwined howls, which are much higher in pitch.

Figure 14.7 shows the waveform, spectrogram and F0 contour of a growl. In the spectrogram, the sound energy is concentrated at frequencies below 1000 Hz. The F0 contour is also low, under 200 Hz in this case. The sound is pulsating (vibrating) quite regularly with a fairly steady SPL. The duration is quite long, over 2 seconds.

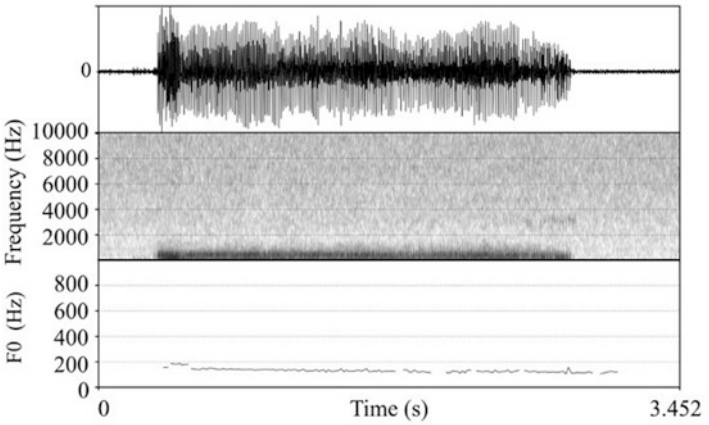

Fig. 14.7 Waveform (top), spectrogram (middle) and F0 contour (bottom) of a growl. (Adapted from Schötz (2016). Published with kind permission of © Susanne Schötz 2016)

14.4.2.3 The Howl–Growl

A howl–growl is a complex vocalisation consisting of one or more sequences where a howl slowly and often repeatedly turns into a growl or vice versa. Howl–growls are probably equally common as the individual call types howl and growl.

In Fig. 14.8, the waveform, spectrogram and F0 contour of an example howl–growl is displayed. Being a complex vocalisation, the duration is rather long, over 3.5 seconds. The sound begins with a howl with vowel-like formants in the spectrogram as well as a high rising–falling F0 pattern (peaking at over 800 Hz) and then slowly turns into a growl with a stable low F0 at about 200 Hz and the sound energy concentrated below 1000 Hz. Then the F0 rises again into a second (weaker) howl. Notice that the SPL varies greatly throughout the vocalisation.

14.4.2.4 The Snarl

A *snarl* (scream, cry, pain shriek) is a loud and usually harsh vowel sound produced just before or during active fighting (McKinley 1982, p. 13), often with [a] or [æ] vowel qualities: [æːo]. However, cats may also scream or cry when they are in pain and in heat or when they have captured a mouse or other prey. These vocalisations are then often longer in duration and not as harsh as the snarls produced in agonistic contexts. Snarls are used to startle or scare off an opponent. They are described by Moelk (1944) as "rapid inhalations harshly vocalised and marked by a heavy initial intake of breath and stopped suddenly with a slight [o] sound, [ˈœːo]". Pain shrieks are short, intense cries of tense vowels, often [æ], [ɛ] or [i], and are characterised by "great strain at mouth and throat and the force of breath" (Moelk 1944).

Figure 14.9 shows the waveform, spectrogram and F0 contour of an agonistic snarl. The acoustic-phonetic features include a short duration (under 0.5 seconds), a

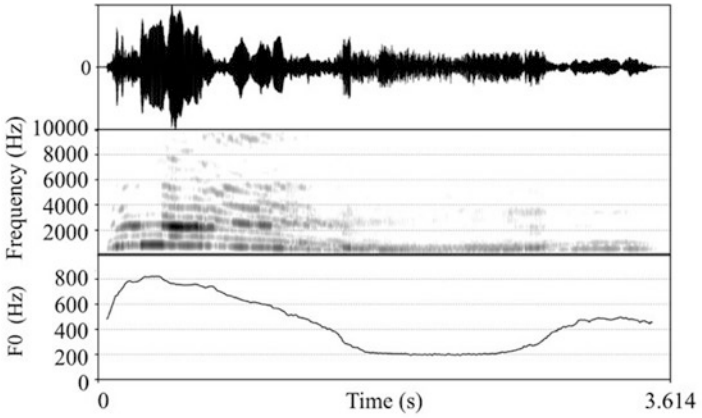

Fig. 14.8 Waveform (top), spectrogram (middle) and F0 contour (bottom) of a howl–growl. (Adapted from Schötz (2016). Published with kind permission of © Susanne Schötz 2016)

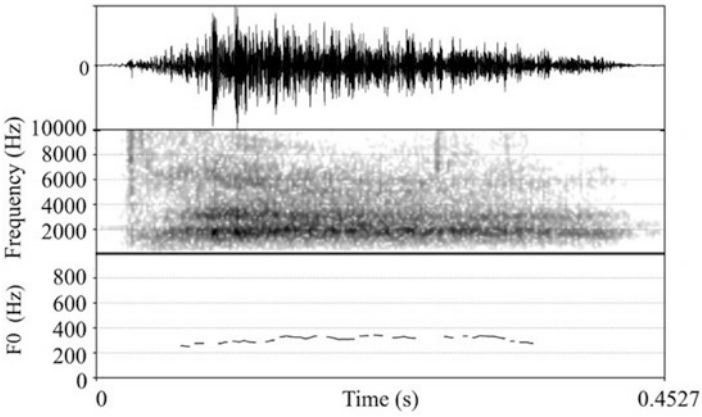

Fig. 14.9 Waveform (top), spectrogram (middle) and F0 contour (bottom) of a snarl (cry). (Adapted from Schötz (2016). Published with kind permission of © Susanne Schötz 2016)

high SPL which decreases throughout the vocalisation and a noisy vowel-like pattern with clear formants at around 2000 and 3500 Hz, as well as a weaker formant at around 6000 Hz. The F0 varies between 250 and 350 Hz and is somewhat irregular with several voice breaks due to the rough (harsh) voice quality.

14.4.2.5 The Hiss and Spit

Hissing and spitting are fricative warning signals produced with the mouth held tensely open, sometimes with the upper lip curled up to expose the teeth. These sounds can be uttered voluntary to warn or scare off an opponent, but may also be

involuntary reactions when surprised by an (apparent) enemy. The cat changes position with a startle, and breath is being forced rapidly through the slightly open mouth before stopping suddenly (Moelk 1944, p. 194). McKinley (1982) describes the hiss as an "agonistic vocalization given with the mouth wide open, teeth exposed, and sounding like a long exhalation", and the spit as "a very short explosive sound, given in agonistic situations frequently before or after a hiss". Hissing can be transcribed as [hː], [çː], [ʃː], [ɧː] or [ʂː]. Spitting is more intense, sometimes with initial k or t consonants: [t͡ʂː], [k͡hː] or [k͡ʃː].

The waveform and spectrogram of an example hiss are depicted in Fig.14.10. As shown in the waveform, the SPL increases until about three quarters into the vocalisation and then decreases again. The duration is rather short (0.8 s), and the noisy sound energy is spread out in the frequency domain. As hissing is voiceless, it has no F0.

Figure 14.11 shows the waveform and spectrogram of a spit. It is shorter in duration than the hiss and also begins with a pulse of higher intensity (SPL). The sound energy consists exclusively of frication noise, which is spread out similarly to the hiss, and there are no harmonic (vocalic) components—and thus no F0—as spitting is voiceless.

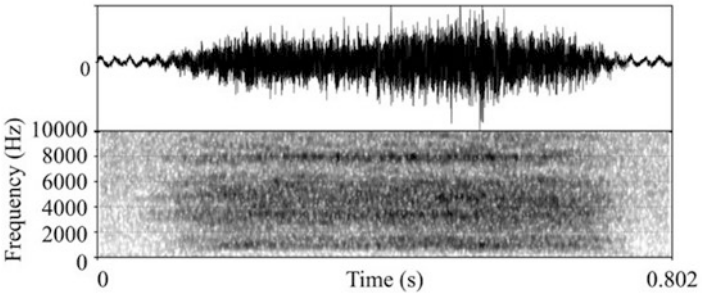

Fig. 14.10 Waveform, spectrogram and (lacking) F0 contour of a hiss. (Adapted from Schötz (2016). Published with kind permission of © Susanne Schötz 2016)

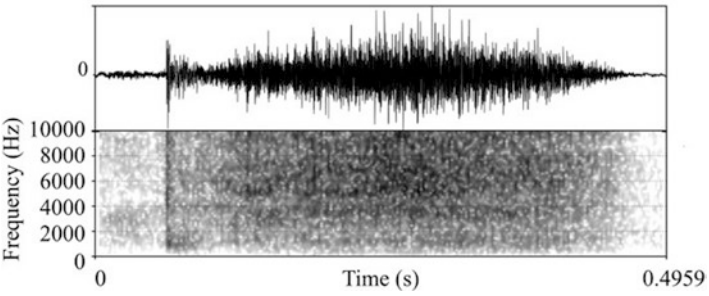

Fig. 14.11 Waveform, spectrogram and (lacking) F0 contour of a spit. (Adapted from Schötz (2016). Published with kind permission of © Susanne Schötz 2016)

14.4.3 Prey-Directed Vocalisations

14.4.3.1 The Chirp

Chirping and chattering are prey-directed vocalisations, usually caused by a hunting instinct, where the cat copies the calls of its prey, e.g. when a bird or insect catches its attention (by making a sound) and the cat becomes riveted to the prey and starts to chirp and chatter. These vocalisations may also be uttered as a displacement activity when the cat is frustrated, like when a bird is sitting just outside the window, or as a protest reaction when the cat is being told off by its owner. Chirps display a large phonetic variation, with several possible subtypes:

Typical chirps [ʔə] are short voiced calls consisting of a glottal stop [ʔ] followed by a vowel—usually [ɛ] or [ə]—said to be mimicking bird or rodent chirps. They are often reiterated in sequences [ʔɛʔɛʔɛ...].

Tweets are softer, weaker and somewhat longer than chirps, without an initial glottal stop [ʔ] and with more varying vowel qualities: [wi] or [ɦɛu].

Tweedles are prolonged chirps or tweets with clear voice modulation (tremor or quaver) resulting in much variation in F0 and SPL, e.g. [ʔəɛəɥə].

Figure 14.12 depicts the waveform, spectrogram and F0 contour of three chirps separated by two pauses. The phonetic characteristics of these sounds include a very short duration (often only 0.2 seconds), a clear vowel-like acoustic pattern in the spectrogram with at least one formant (around 2000 Hz) and a high F0 over 800 Hz.

14.4.3.2 The Chatter

Chatter (or teeth chattering) is a weak, usually unvoiced and quick clicking sequence of very short sounds made with juddering jaws—perhaps to practice the killing bite: [k̟̄ k̟̄ k̟̄ k̟̄ k̟̄ k̟̄]. Voiced variants have also been observed: [ḡ̟d̟̄ḡ̟d̟̄ḡ̟d].

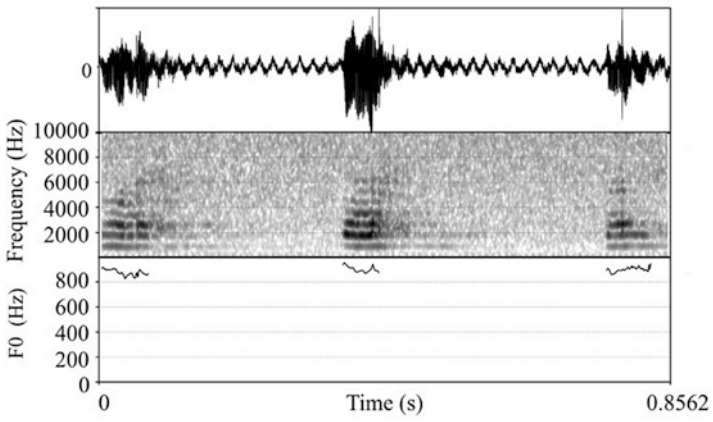

Fig. 14.12 Waveform (top), spectrogram (middle) and F0 contour (bottom) of three chirps. (Adapted from Schötz (2016). Published with kind permission of © Susanne Schötz 2016)

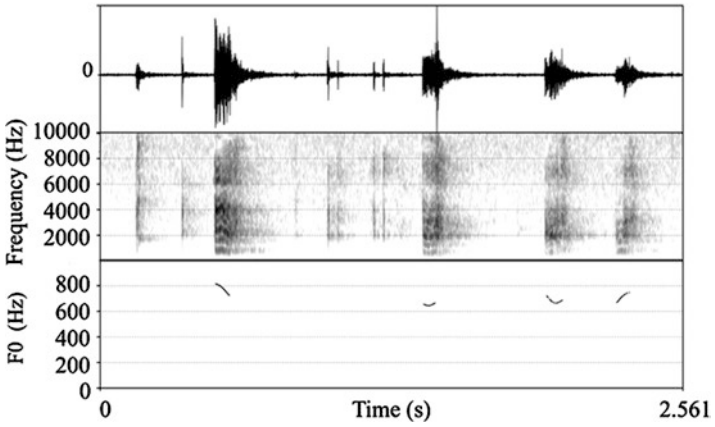

Fig. 14.13 Waveform (top), spectrogram (middle) and F0 contour (bottom) of a sequence of chatter and four chirps. (Adapted from Schötz (2016). Published with kind permission of © Susanne Schötz 2016)

In Fig. 14.13, the waveform, spectrogram and F0 contour are shown of a longer sequence containing chattering together with four chirps. The very thin vertical transients (spikes) in the waveform and the spectrogram depict instances of chatter. As they are voiceless, there is no F0 in the bottom pane. In this example, the sound energy is distributed in the frequency region of 1000 to 10,000 Hz, with two centres of gravity, one between 2000 and 4000 Hz and one between 6000 and 8000 H.

14.5 The Phonetic System of Cat Vocalisations

Phonetic analysis of the individual segments of the different cat vocalisation types enables us to identify the various vowel and consonant sounds that cats are able to produce. Even though cats do not use a human-like language when they communicate, it is still possible to order the different segments that they can produce into a phonetic-like system. The system presented here contains all the vowel and consonant sounds that I have identified in my studies of cat vocalisations as well as some prosodic features. Even though this is still work in progress—and far from complete—it contains the vowels, consonants and prosodic patterns that I have observed in different cat sounds.

Table 14.1 shows all the vocalisation types mentioned in this chapter together with their phonetic features, including articulation (location and movement of the mouth), voice (low pitched, high pitched, etc.), a short description of the phonetic category, the corresponding typical phonetic transcription as well as any additional comments—everything at a glance.

Table 14.1 Phonetic characteristics of the most common cat vocalisation types and their subcategories

Vocalisation type	Subcategory	Articulation (mouth)	Voice (pitch and quality)	Phonetic category	Typical phonetic transcription	Comments
Meow	Mew	Opening (open)	Voiced, very high pitched/ bright	High-pitched meow, often with [i], [ɪ], [e] and [u] vowel(s)	[wi], [miʊ] eller [meu]	Isolation or soliciting sound (often by kittens)
Meow	Squeak	Opening	Voiced, high pitched, bright, hoarse, raspy	Hoarse, raspy, nasal, bright often short mew-like sound with [ɛ] or [æ]	Often [wæ], [mɛ] or [ɛʊ]	Isolation or soliciting sound (often by adult cats)
Meow	Moan	Opening–closing	Voiced, often falling tone	Darker meow, often with [o] or [u]	Often [mou] or [wuæu]	Discontent or distressed sound
Meow	Meow	Opening–closing	Voiced, often rising–falling tone (but much variation)	Typical meow with vowel combinations like [iau] or [ɛau]	Often [miau], [ɛau] or [wɑːʊ]	The most common human-directed attention-soliciting sound
Trill–meow	Trill–meow	Closed–opening(–closing)	Voiced, rising tone	A trill directly followed by a meow	Often [bɾ̃iuw], [bɾ̃ːmiau], [mhɾ̃iauw] [mhɾ̃ŋ-au] or [whɾ̃ːau]	Common human-directed sound to solicit attention
Trill	Chirrup	Closed, airflow through the nose	Voiced, high-pitched, bright, rising tone	(nasal) trill like [r], but probably produced further back in the mouth	Often [mɾ̃ːh] or [mːɾ̃ːut]	*The* friendly greeting or calling sound
Trill	Grunt	Closed, airflow through the nose	Voiced, low-pitched dark, often level or falling tone	(nasal) often hoarse trill like [r] or [ʀ], probably produced further back in the mouth	Often [m̰ː] or [bɾ̃ː]	Friendly sound often used in greeting and confirmation contexts
Trill	Murmur, coo	Closed, airflow through the nose	Voiced, often high pitched	Nasal, often [m] (no trilling)	Often [m]	Friendly, like a meow without any vowels

(continued)

Table 14.1 (continued)

Vocalisation type	Subcategory	Articulation (mouth)	Voice (pitch and quality)	Phonetic category	Typical phonetic transcription	Comments
Growl	Growl, snarl	Slightly open	Voiced, very low	Very low extended trill, sometimes with initial creaky [m̰]	Often [ɡʀː], [ʀː] or creaky trilling [r̰ː], or [m̰ʀː]	Warning sound
Hiss	Hiss	Open	Voiceless	Dark (back) or bright (front) fricative (with audible friction)	Often [hː], [çː], [ʃː], [ɧ] or [ʂː]	Warning sound
Hiss	Spit	Open	Voiceless	Often dark (back) or bright (front) affricate	Often [t͡ʂː], [k͡hː] or [k͡ʃː]	Explosive warning sound
Howl	Howl, mating call	Open (slightly opening–closing)	Voiced, melody rises and falls in repeated patterns	Combination of extended vocalic sounds like ɪ], [ɨ], [j], [ɤ], [au], [ɛɔ], [aw], [oɪ] and [ɑo]	E.g. [awoɪːɛɔː], [jiɨɛɑw] or [ɪːauauauauauawawaw]	Similar to a child weeping or crying
Growl–howl	Growl–howl	Closed–opening–closing	Voiced, rising–falling (much variation)	Combination of growl and howl with substantial rises and falls in the melody	E.g. [ɡʀːawɪjɑoʀː]	Warning sound
Snarl (cry, scream)	Snarl (cry, scream)	Tense and open	Voiced, often hoarse and harsh, level or falling tone	Short, often loud vocalic sounds	Often [a], [æ], [aʊ] or [ɛo]	The sound of anger, pain or warning, used in fighting or when in pain
Mating call (mating cry)	Mating call (mating cry)	(closed–) opening–closing	Voiced, often final rise	Long stressed vowels, often begins with [w] or trilling consonant sound	Often sequences of [wɑ̃ːuw], [r̃ːɪːɑ̃ùː], [mhr̃ːwaːoːuːɪː] and [ʀ̃ːwːuːaːu]	Solo or duet with other cat, often lasting for hours, on spring nights

Purr	Purr	Closed, airflow mostly through the nose	Very low pitched (20 Hz), but regularly vibrating	Soft, extended, low, breathy vibrating sound, e.g. [r̃] or [r̃], often with soft [h], during both ingressive and egressive airflow	E.g. [↓hːr̃-↑r̃ ːh-↓hːr̃-↑r̃ːh]	Signals "I am content" or "I do not pose a threat", we still do not know exactly how or why cats purr
Chirp and chatter	Chatter, teeth chattering	Open	Voiceless	Sequences of consonants like [k] or [ʔ]	Often [ʔ ʔ ʔ ʔ] or [k̟⁼ k̟⁼ k̟⁼ k̟⁼ k̟⁼]	Often prey directed
Chirp and chatter	Chirp	Open	Voiced, often loud short sequences	Often initial glottal stop [ʔ] followed by short vowel, often [ɛ], [e] or [ə]	Often [ʔə], [k̟⁼e] or [ʔɛʔɛʔɛ]	Often prey directed
Chirp and chatter	Tweet	Open (slightly closing)	Voiced, often soft short sequences	Soft chirp without initial [ʔ], sometimes with initial [w], vowels often [i], [ɪ], [ɛ] or [u]	Often [wi] or [ɦɛu]	Often prey directed
Chirp and chatter	Tweedle	Open (closing–opening)	Voiced, soft longer sounds	Long extended chirp or tweet, with voice modulation	E.g. [waɛəɥə] or [ʔəɛəɥə]	Often prey directed

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14.5.1 Segmental Features in Cat Vocalisations

It is unlikely that cats are able to produce all sound segments that can be found in human speech. Similarly, we are unable to precisely imitate all cat vocalisations, like the purr, the trill, or the growl. The segments of cat sounds, i.e. the vowels and consonants that make up the building blocks, are thus sometimes quite similar to the ones produced by humans—and sometimes quite different. However, the vocal organs of the cat, although much smaller and often of different shape, resemble human organs and other structures in many ways. We all have vocal folds, jaws, lips, tongues, throats, teeth, oral and nasal cavities, and also lungs which produce the necessary airflow for sound production. Thus, we can use the same phonetic terminology, based on the anatomy of the vocal tract (place of articulation) as well as the type of constriction or closure, and the way the airstream is affected as it flows from the lungs and out through the mouth and nose (manner of articulation) when describing the segments of cat sounds.

In the following sections, I will describe the vowels, consonants, and the prosodic features (intonation, rhythm, dynamics) of cat vocalisations.

14.5.1.1 Vowel Sounds

In Fig. 14.14, depicting a vowel chart normally used for human vowels, the encircled vowels are the ones which I have identified in cat sounds so far. Based on articulation, cat vocalisations produced with an open mouth (with a lowered jaw) contain more open vowel sounds like [a], [ɑ] and [æ], while the vocalisations with

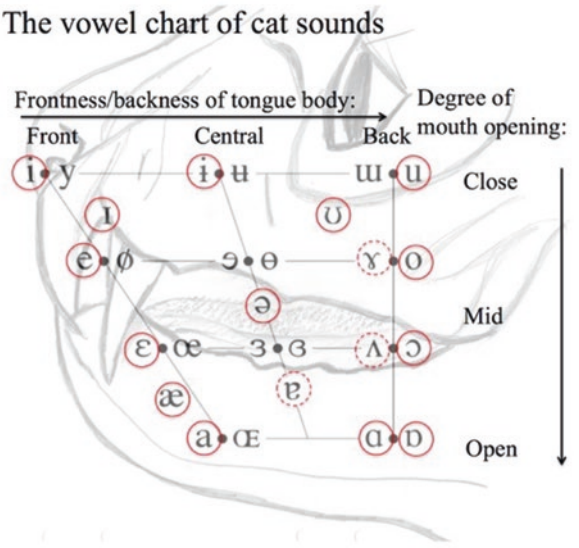

Fig. 14.14 The vowel chart of cat vocalisations. Solid circles represent vowel sounds found in cat vocalisations by the author; dashed circles represent vowel sounds which cats should be able to produce given their vocal anatomy. (Adapted from (Schötz 2018). Published with kind permission of © Susanne Schötz 2017)

a more closed mouth include more close vowels like [i], [e] and [u]. By ordering vowels in this type of vowel chart or space, where the horizontal position of the tongue and the degree of mouth opening (and thus the vertical position of the tongue) as well as the degree of lip rounding is shown, virtually all vowel sounds can be described phonetically. By listening carefully and by observing spectrograms of cat vocalisations and comparing them to human vowels, I have distinguished the front or near front vowels (from close to open) [i], [ɪ], [e], [ɛ] and [a]; the central vowels [ɨ] and [ə]; and the back or near back vowels [u], [ʊ], [o], [ɔ], [ɒ] and [ɑ]. Moreover, and partly as it has not yet been established to what degree cats are able to round their lips to produce rounded vowel sounds, it is possible that they would also be able to produce the vowels [ʌ], [ɤ] and [ɐ]. Although kittens probably learn to round their lips to be able to nurse, I have not yet observed a cat articulating a sound with clearly rounded or protruded lips. The phonetic symbols used here are thus mainly based on human perception, and not on whether they are articulated with or without lip rounding.

14.5.1.2 Consonant Sounds

I have observed the following phonetic consonant categories in cat vocalisations:

Stops are produced by first completely blocking of the vocal tract so that no air can escape from the lungs, causing a slight pressure difference to be built up behind the closure, and then by quickly opening the closure, causing the released airflow to make an audible sound (a short burst). In cat chatters, chirps and spits, I have identified the stops [t], [c], [k], [g] and [ʔ].

Fricatives are characterised by a turbulent airflow causing frication noise. They are produced by forcing air through a narrow constriction made by two articulators close together, e.g. the upper teeth against the lower lip as in [f]. Among the fricative sounds observed in cat hisses and spits are [ʃ], [ʂ], [ç], [ʝ] and [h].

Approximants are consonants produced by two articulators approaching each other closer than with vowels, but without causing any turbulent airflow. I have observed the approximants [w], [j] and [ɥ] in meow-like sounds and [ɹ] in trills.

Laterals are l-like consonants which are produced with the tongue against the middle of the palate, allowing the airflow to proceed along the sides. Cats should be able to produce laterals, but I have only observed a few instances of l consonants in vocalisations from very stressed or frightened cats and have not yet been able to record any such sounds myself.

Nasals are consonants produced by a closure in the oral cavity or at the lips, but with a lowered soft palate, allowing the sound wave to escape through the nose. Cat trills and meows sometimes begin with an [m], and in the transition from a trill to a meow, sometimes a [ŋ] can be observed.

Trills are produced with an airstream causing a small structure (e.g. the tongue tip) to vibrate, causing a regularly pulsating sound. Cat trills often contain [r] or [ʀ]-like consonants, which are usually nasalised [r̃] or [ʀ̃], as the mouth is closed.

Affricates are combinations of one stop consonant followed by a fricative produced with the same articulator. Cat spits are sometimes produced by an initial affricate, such as [t͡ʃ] or [k͡h].

Table 14.2 shows the consonants which I have observed in cat vocalisations, based on the assumption that cats are able to produce consonant sounds which resemble those of humans. The table rows contain the consonant categories described earlier, and each column corresponds to the specific place of articulation of the consonants:

Bilabial—with upper and lower lips (as in [m], [p] and [b])
Labiodental—with the lower lip against the upper front teeth
Dental—with the front of the tongue against the teeth
Alveolar—just behind the teeth
Postalveolar—somewhat further back than alveolar
Retroflex—further back between the alveolar ridge and the hard palate, sometimes with the tongue curled back
Palatal—with the tongue against the hard palate
Velar—further back with the tongue against the soft palate
Uvular—even further back with the back of the tongue against the uvula
Pharyngeal—with the root of the tongue against the throat (pharynx)
Glottal—all the way back with the vocal folds

In addition to the consonants depicted in Table 14.2, cats are probably able to produce the following consonants:

[w] (voiced labial-velar approximant)
[ɧ] (voiceless simultaneous postalveolar and velar fricative, produced with an open mouth and spread lips)
[ɥ] (voiced labial-palatal approximant)

14.5.1.3 Prosodic Features in Cat Vocalisations

Cat vocalisations are not just built from vowels and consonants, but also have other sound ingredients woven into them, such as pitch (F0) and intonation, loudness (intensity, SPL), length (duration) and voice quality. These phonetic characteristics—called prosodic features—are usually spread over syllables and larger units of a sound. In human speech, prosody is often used to signal para- or extralinguistic meaning. For instance, differences in speech rate, voice quality and varying dynamic patterns of intensity and intonation can be used to signal emotional state. Many of these patterns are likely to be universal, i.e. they are also understood and used by other animal (mammal) species. We still do not know much about the prosodic variation in cat sounds, but cats probably vary their voices and intonation in a similar way as humans and other animals do, and we know a bit about this universal variation.

If we relate cat vocalisations to the universal frequency code first described by John Ohala (1994), we may be able to interpret them better. According to this code, low-pitched and dark sounds signal "I am big, strong, dominant and ready to fight". Dark and low-pitched vocalisations are often used in agonistic situations to warn or

Table 14.2 Consonant table with phonetic characters for consonants observed in cat sounds. The "?" indicates that I assume that cats are able to produce a sound which I have not yet recorded

	Bilabial	Labiodental	Dental	Alveolar	Postalveolar	Retroflex	Palatal	Velar	Uvular	Pharyngeal	Glottal
Plosive				t			c	k, g			ʔ
Nasal	m							ŋ			
Trill				r					ʀ		
Fricative		f?			ʃ	ʂ	ç				h
Approximant				ɹ			j				
Lateral				l(?)							

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scare off an opponent. High-pitched and bright sounds are frequently used to signal the opposite, i.e. "I am small, weak, and harmless". There are many examples of this phenomenon. For instance, young animals and children have more high-pitched and light voices than adults, and dogs may whine and use other high-pitched sounds when they are anxious, and bark and growl with a low pitch when they feel threatened and upset—and humans are able to recognise this difference (see, e.g. Farago et al. 2014). Moreover, some vowel sounds—in human speech as well as in animal vocalisations—seem to signal large size (e.g. [o] and [ɑ]), while others are more likely to signal small size (like, for instance, [i] and [e]). This sound symbolism is quite often used in human languages to denote large and small objects using different vowels, e.g. large, tomcat, strong (big, dominant, aggressive) and teeny, kitten, and weak (small, friendly, peaceful) (Ohala 1994, pp. 335–336). Furthermore, the intonation or melody is highly related to the mental and emotional state or to the intention of the speaker or vocalising animal. Uncertainty is often accompanied by a rising intonation at the end of an utterance, while a falling intonation is used for certainty.

Human emotions are generally signalled with the voice. Regardless of the language, the intonation, intensity, timbre and quality of our voices are different depending on whether we are happy, angry, sad or afraid. Joy is often accompanied by a high-pitched voice with a full-bodied timbre and by numerous fast and large changes in the pitch. An angry voice is often fairly loud with a high pitch and abrupt changes in the voice (due to the increased muscle activity of the speech organs). Sometimes a more restrained anger can be signalled by a weak, tense voice with a slower speech rate. Sorrow is usually displayed with a slow and fairly low-pitched voice and a monotone (only small changes in the melody, due to the low muscle activity) pitch. A frightened voice is often characterised by long pauses between words, but with a high speech tempo and a large pitch range, sometimes accompanied by small irregularities in frequency as well as intensity.

Cats are able to vary not only the intonation, but also the length, intensity and voice quality in their vocalisations, and this may hold important information. For instance, meows uttered in a friendly feeding context more often have a rising intonation than meows produced in a stressed veterinary context (Schötz and van de Weijer 2014).

14.6 Human Perception of Cat Vocalisations

Many humans claim to understand the vocal signals of their cats very well. Perhaps this is not so surprising, as many cats and owners communicate with sounds several times every day. As previously mentioned, every human–cat pair seems to develop a unique communicative code and learns to relate a certain sound or tone of voice to a specific intention, need or desire. However, a number of studies have suggested that humans only perform modestly better than chance when given the task to judge cat meows from different behavioural contexts (Nicastro and Owren 2003; Schötz

and van de Weijer 2014). Although cat vocalisations seem to attract the attention of humans, some experience is needed to be able to correctly interpret the context where the sound was uttered. It is possible that prosodic patterns play an important role. For instance, Schötz and van de Weijer (2014) found that vocalisations with a larger F0 range were more often judged as food-related sounds than those with less tonal variation. Moreover, universal phonetic features, including the frequency code, may also influence our inter-species understanding, as low-pitched vocalisations are more likely to be associated with agonistic contexts than high-pitched sounds. This may sometimes lead to misunderstandings. For instance, inexperienced human listeners may mistake a friendly low-pitched trill or grunt for a hostile growl (Schötz 2014).

14.7 Human Speech in Human–Cat Communication

Although some humans talk to their cats in the same way that they talk to human adult friends or family members, many cat owners seem to apply a certain speaking style—pet-directed speech or pet talk—which shares several phonetic features of infant-directed speech (baby talk or motherese). This speaking style includes a higher overall pitch, with a larger intonational range and with longer and more distinctly pronounced (hyper-articulated) speech sounds (Burnham et al. 1998). Moreover, many humans interacting vocally with their cats seem to imitate the melody of the cat vocalisations, often resulting in a very high F0, and also tend to repeat the same word, phrase or utterance, such as "Are you a good boy? Yes, are you a good boy? Yes? Yes?" As far as I know, there have not yet been carried out any comparative studies of, e.g. cat-directed and dog-directed speech, but it is likely that we use a very similar speaking style when we talk to our pets, regardless of their species.

14.8 Concluding Remarks

As shown earlier, phonetic descriptions and transcriptions can be used to categorise and describe cat sounds. However, as phonetics was initially developed to study human speech, several questions remain. If we want to use phonetic methods to classify animal vocalisations, do we have to develop different species-specific phonetic systems or can we use the same one for all animals (including humans)? Should we extend the International Phonetic Alphabet (IPA) to include sounds that only non-humans can produce? Furthermore, several methods can be used to describe, transcribe and analyse the prosodic features of human speech. Which of these can best be adapted for intonation and voice quality in animal vocalisations? These questions and many others will have to be answered by linguists together with biologists and animal behaviourists before we can present a complete phonetic system of cat sounds.

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Chapter 15
A Dog's Perspective on Animal-Assisted Interventions

Lisa Maria Glenk

Abstract The practice of implementing dogs into therapeutic environments is an emerging field. Despite the increasingly growing scientific interest on human health outcomes, research efforts into the canine perspective of animal-assisted interventions (AAIs) have been scarce. The demands therapy dogs encounter during their performance in therapeutic environments however go beyond the challenge of accepting close social contact with strangers. Physiological and behavioral welfare indicators and dog handler surveys to identify stress related to AAIs have been used across the scientific literature. However, the current body of research presents a conflicting picture, making it difficult to generalize study results. Research indicates that frequency and duration of AAI sessions, novelty of the environment, controllability, age, and familiarity of recipients modulate animal welfare indicators. The biopsychosocial model of dog health in AAIs is proposed as a multidimensional framework of human–animal interaction effects on dogs. Moreover, training methods, attachment to handler, and inequity aversion in dogs are discussed as factors likely to affect welfare. This chapter highlights that clear conclusions on how the well-being of dogs is influenced by the performance in AAIs cannot be drawn due to the heterogeneity of programs, recipient and session characteristics, small dog sample sizes, and methodological limitations.

Keywords Dog · Animal welfare · Stress · Behavior · Cortisol · Animal-assisted intervention · Animal-assisted therapy · Animal-assisted activity

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15.1 Introduction

Animal-assisted interventions (AAIs) are commonly referred to as complementary and adjunctive initiatives that aim to positively affect human health by utilizing animals. In general, AAI programs seek to enhance quality of life variables of patients, clients, or residents and facilitate therapeutic progress. Thus, animals are integrated as a central part of a therapeutic or ameliorative process (Kruger and Serpell 2006).

In the literature, two other terms have been variously used and defined. Basically, in animal-assisted therapy (AAT), professionals engage in preventive, curative, promotional, or rehabilitative healthcare services. These are animal-supported and goal-centered programs, in which documentation and evaluation of therapeutic progress and outcomes is inevitable. Animal-assisted activity (AAA) refers to programs without a therapeutic aim, in which professionals or volunteers deliver interventions with spontaneous content that are neither concisely documented nor evaluated (Kruger and Serpell 2006).

As previously claimed by Palley et al. (2010), the scientific literature is characterized by an inconsistent use of terminology. The term AAIs may be used as an umbrella term to overcome this dilemma, but does not provide any further information on therapeutic content, if not otherwise specified. In the media, AAIs are also often related to as pet therapy.

15.1.1 Biopsychosocial Effects of Animal-Assisted Interventions

The biopsychosocial model of health provides a structural framework of individual and interactive dimensions of biological, psychological, and social health, integrated dynamic aspects. It has been introduced as a suitable model in understanding human–animal interaction effects on human health outcomes (Friedmann et al. 2010). One dimension affects the other two, underlining how unidimensional improvement or impairment may exert effects on systemic health.

A growing body of research into the human–dog relationship has highlighted that interaction with dogs may result in positive effects on human health. Psychological benefits may be derived indirectly via enhanced therapy motivation and facilitated relationships with psychotherapists and healthcare staff (Schneider and Harley 2006; Wesley et al. 2009; Wohlfarth et al. 2013). Direct psychological effects of AAIs include the reduction of depressive symptoms, negative mood, and anxiety (Crowley-Robinson et al. 1996; Cole et al. 2007; Lang et al. 2010). In general, dogs have been attributed a social lubricant function. Interpersonal interactions seem to be facilitated by the mere presence of a friendly dog. For instance, dog companionship increases human social attractiveness, stimulating smiles, conversations, and prosocial behavior from strangers (Eddy et al. 1988; Gueguen and Ciccotti

2008; Wells 2004). Positive effects have also been described for human physiological health parameters. Animals can act as a source of social support during cognitive tasks, leading to reduced endocrine and cardiovascular stress responses (Beetz et al. 2011; Allen et al. 2001). In addition, decreased perceptions of pain have been reported (Braun et al. 2009; Marcus et al. 2012; Ichitani and Cunha 2016).

15.1.2 Limitations in Research on Animal-Assisted Interventions

Research into the effects of AAIs on human health outcomes has been justifiably challenged because of methodological shortcomings that include lacking numbers of studied subjects, suitable control (non-treatment, alternative treatment) groups, randomization, or blinding (Stern and Chur-Hansen 2013). Accordingly, the majority of studies do not meet evidence-based medicine criteria. Especially for clinical populations, it has remained largely unclear whether the AAI treatment itself affects desirable patient outcomes or whether the results were modulated by other nonspecific factors (Anestis et al. 2014; Chur-Hansen et al. 2014). Another issue is that to date, the role of the animal as the outcome mediating factor in AAIs has remained intangible. Thus, it becomes apparent that there exists a discrepancy between the scientific justification of AAIs and lay public perceptions that are also manifest in the broad offer of AAI-related services. Accounting for the emerging popularity of AAI programs, animal welfare aspects need to be considered. Preliminary research has pointed out that dog welfare may be threatened by participation in AAIs, either via inappropriate handling by recipients or staff members (Hatch 2004), which warrants a closer look onto the dog perspective of AAIs.

15.1.3 Animal Welfare Recommendations

A comprehensive guideline for animal well-being in AAIs has been published by the International Association of Human-Animal Interaction Organizations (IAHAIO). According to the "IAHAIO White Paper," AAIs should only be performed with the support of animals that are in immaculate health, both physically and emotionally. Prior to their involvement in AAIs, individuals considered appropriate should be carefully evaluated via veterinary screening and temperament assessment by an expert in animal behavior. Such pre-selection procedures aim to identify animals with the proper disposition that most likely enjoy this type of human–animal interaction. Handlers and professionals working with animals are required to understand the fundamental, species-related, and individual needs of the animal so that its safety and comfort are guaranteed. Thus, any interactions involving inappropriate treatment of the animal, thereby putting recipients and the animal

at risk, are unacceptable. Animals must be cared for properly prior to, during, and after the sessions. Also overload associated with participation in AAIs must be avoided, and session durations should be time limited (IAHAIO 2014).

15.1.4 Dogs and Humans

Given the biological and psychological evidence for the extraordinary affinity of humans to companion dogs and vice versa, a strong interrelation between bio-psycho-social variables according to Fig. 15.1 across species is plausible.

Across the process of domestication, dogs have developed distinctive relationships with humans that facilitate integration into human societies. The fact that domestic dogs are highly sensitive to human communicative cues seems to have contributed to the wide distribution of the species that we see nowadays. Human gestures such as pointing and gazing are easily recognized already at puppy age (Ittyerah and Gaunet 2009; Zaine et al. 2015; Bhattacharjee et al. 2017). Dogs evidently outperform their wild ancestor, the wolf, and even chimpanzees with their rigorous capacity to understand human gestures (Udell et al. 2009).

In AAIs, dogs are commonly confronted with strangers in unfamiliar environments, which is a challenge per se because during the major part of the ongoing process of dog domestication and breeding, hunting and guarding have been desired skills. AAIs encompass a relatively novel area of working dog performance and have evolved only during a few decades, where the appreciation of close intimate contact with strangers became a desirable behavioral trait (Butler 2004).

Previous research on pet dogs has shown that dogs' social behavior strategies toward strangers were affected by the way the dogs were approached. For example, if family dogs were confronted with positive cues from unfamiliar humans including a friendly voice and face while being approached at a normal pace, the dogs exhibited high levels of contact seeking. In contrast, if approached by a stranger in a threatening manner including slow movements, staring eye contact, and a slightly bent upper body, the dogs avoided gaze, vocalized more often, and backed away (Vas et al. 2005; Györi et al. 2010).

Coordination of nonverbal behaviors between interactive partners takes place during the process of social synchronization in many mammalian species. The experience of synchrony roots in the mother–child relationship, and high levels of synchrony have been related to efficient bonding (Atzil et al. 2014; Leclère et al. 2014). Interestingly, dogs tend to automatically imitate their owners' behavior in a performance task (Range et al. 2011) and adjust their behavior to their owners' reactions toward an unfamiliar stimulus (Merola et al. 2012a, b). It has been suggested that referential communication may enhance behavioral organization during shared activities (Csányi 2000), but it may possibly also account for synchronization patterns during AAIs.

Previous research has examined whether dog owners recognize behavioral cues of discomfort in their pets. In a survey by Mariti et al. (2012), 60% of respondents

were able to provide a correct definition of stress and its impact on their dog's well-being. While intense behaviors like trembling, panting, and vocalizing were easily attributed to stress by more than half of the respondents, study participants failed to identify the more subtle behavioral signs of unease. These included behaviors like nose licking, yawning, paw lifting, and excessive food or water intake, which were only related to stress by less than 10% of the respondents.

These findings stress the importance of a broad dog handler education on dog ethology prior to participation in AAIs. This is particularly important in programs where volunteers with little or no previous experience with dogs may engage in AAIs.

15.1.5 Ethical Aspects

In 1991, Iannuzzi and Rowan conducted questionnaires and phone interviews to identify under which circumstances AAIs may raise ethical concerns for the animals involved. Study participants responded that particularly resident animals should be closely monitored for stress and fatigue and must have opportunities to withdraw and rest. In visitation programs, environmental conditions including high room temperatures in institutions and restricted access to water were the most frequently mentioned concerns. Working schedules should be limited to three sessions per week with an individual duration of no more than 60 minutes (Iannuzzi and Rowan 1991). According to Fejsáková et al. (2009), each animal should be provided a safe place within the working environment into which it can refuge when exhausted or stressed from overwhelming interactions. Zamir (2006) claims that the integration of animals into AAIs can be ethically justifiable only if also animals benefit from the interactions. Thus, this may refer to species that can establish close social relationships with humans like dogs, while non-domesticated species that generally exhibit a lower tolerance for stressful situations and stimuli should not be considered. Taylor et al. (2016) suggest that animals may benefit if people's attitudes and behaviors toward animals change for the better. Such changes in attitude are based on the acknowledgment of animal sentience and their role as a partner rather than tool during AAIs.

15.2 A Dog's Perspective: Review of the Literature

This chapter seeks to systematically review the current literature on the dog experience of AAIs (Sects. 15.2.1, 15.2.2, and 15.2.3). Moreover, factors that are likely to modulate therapy dog performance are discussed (Sects. 15.3.1, 15.3.2, and 15.3.3).

Scientific literature was identified from database keyword search and article reference sections. Inclusion criterion for reviewed literature was the publication of original research in a peer-reviewed scientific journal. Keyword search terms were

therapy dog, animal welfare, stress, arousal, behavior, AAI, AAT, and AAA. The literature search resulted in 13 relevant papers, further extending the body of knowledge presented in a recent review (Glenk 2017). The majority of studies in the present literature review focused on dogs in visitation programs where animals accompanied either their owners or handlers during visits in healthcare and educational settings (Parenti et al. 2013). Across studies, the dog experience of AAIs builds primarily on the assessment of behavioral (i.e., general activity and stress-related behaviors) and physiological variables (i.e., salivary cortisol or heart rate) and/or questionnaires designed to examine animal handlers' interpretations of their dogs' behavior. An overview on AAI program definitions, recipients, number of dogs, and welfare indicators is shown in Table 15.1. In addition, Table 15.2 exhibits AAI session characteristics including duration, arrangement of recipients (single or group intervention), between session intervals, and significant findings across the studies.

Two of the reviewed studies were case reports ($N = 1$) that followed one dog over time. In the other studies, the number of dogs varied between 4 and 47 (18.1 ± 12; Mn ± SD), indicating that existing research builds on a relatively small number of studied subjects. As previously reported, studies were carried out in multiple therapy sites including in-patient and out-patient facilities, schools, and university (Glenk 2017). The most common human–animal interactions during AAI sessions included verbal praise, petting, gentle scratching, brushing the dog's fur, walking the dog on- or off-lead, obedience commands, throwing or hiding dog toys, and mild exercise.

15.2.1 Case Studies

In a case study by Piva (2008), a shelter dog that was adopted and integrated as a resident dog in a nursing home for the elderly was observed over the course of 6 months. After being rehomed in, the dog was regularly enrolled in AAA group sessions. Welfare measures included clinical indicators, behavior, and cortisol levels. Behavioral disorders in shelter dogs are not uncommon, and also this particular dog exhibited a previous history of stereotypic autogrooming that had developed into an acral lick granuloma. Observations of the dog across three time points during the AAA program led to the conclusion that over time, the dog seemed to be more healthy, playful, and engaged in social interaction and exploration. Stress indicators such as hair cortisol, tachycardia, tachypnea, nose and lip licking, hypervigilance, walking-pacing, and the granuloma tended to decrease progressively over time, suggesting that the dog was successfully integrated in the new environment and participation in AAA did not impair its overall health and welfare.

The other case study by Palestrini et al. (2017) was carried out in a pediatric hospital where a dog-handler team was paired with a child during postoperative awakening, 2 hours after surgery. The dog was enrolled in 20 subsequent AAT sessions. Study outcomes were heart rate and analyses of the stress-related behavior,

Table 15.1 Overview on program definitions, therapeutic environment, recipients, sample of dogs, and welfare indicators

Reference	AAI type	Program type	Environment	Recipients	Dogs (*N*)	Welfare indicators
Haubenhofer and Kirchengast (2006, 2007)	AAA, AAT	Visitation	Hospitals, schools, rehabilitation centers, nursing homes	Adults, children	18	Salivary cortisol, emotions according to handler
Piva (2008)	AAA	Resident	Nursing home	Adults	1	Clinical protocol, behavior, fecal and hair cortisol
Marinelli et al. (2009)	AAA, AAT	Resident, visitation	Hospitals, clinics or rehabilitation centers, schools, nursing homes	Adults, children	18	Behavior, handler questionnaire
King et al. (2011)	AAT	Visitation	Hospital	Adults, children	21	Salivary cortisol, behavior, handler questionnaire
Glenk et al. (2013)	AAT	Visitation	In-patient mental Healthcare	Adults	21	Salivary cortisol
Glenk et al. (2014)	AAT	Visitation	In-patient substance abuse treatment	Adults	5	Salivary cortisol, behavior
Ng et al. (2014)	AAA	Visitation	University	Adults	15	Salivary cortisol, behavior
Koda et al. (2015)	AAT	Visitation	Prison	Adults	47	Salivary cortisol, handler questionnaire
Palestrini et al. (2017)	AAT	Visitation	Pediatric hospital	Children	1	Heart rate, behavior
Pirrone et al. (2017)	AAA	Visitation	Healthcare facility	Adults	4	Heart rate, behavior
McCullough et al. (2018)	AAT	Visitation	Pediatric hospital	Children	26	Salivary cortisol, behavior, handler questionnaire
Colussi et al. (2018)	AAA	Visitation	Kindergarten	Children	6	Salivary cortisol

Modified and extended from Glenk (2017)

exploration, passive behavior, environmental orientation, and interaction with children, animal handler, and other people (i.e., staff, parents). Heart rates did not vary whether or not children interacted with the dog during the sessions; neither did behavioral variables differ across the sessions. There were no incidences of the dog trying to withdraw from the intervention, and the high occurrence of panting was attributed to the relatively high room temperature. No acute concerns for the dog's welfare emerged during investigation of the program (Palestrini et al. 2017).

15.2.2 Original Research (N > 1)

Research by Haubenhofer and Kirchengast (2006, 2007) and Marinelli et al. (2009) exhibits a high variability of AAI settings referring to therapeutic environments and contents, the number and age of recipients, and session arrangements was found. Dogs' salivary cortisol concentrations were higher on days with AAIs if compared to a resting day according to Haubenhofer and Kirchengast (2006, 2007). In addition, the duration of sessions and the number of visits per week affected secretion of the glucocorticoid hormone. In their study, animal handlers reported that fewer breaks occurred during sessions between 1 and 3 hours and these were perceived to be more intense than longer sessions (up to 8 hours). Higher cortisol concentrations were also measured during shorter sessions. However, these results should be interpreted with caution as more recent recommendations demand a limitation of session duration (30 to 45 minutes) with respect to animal welfare (IAHAIO 2014). Handlers perceived their dogs to be more likely to be physically strained from therapeutic performance than they considered themselves (Haubenhofer and Kirchengast 2007). The results of higher cortisol levels associated with AAIs were confirmed by King et al. (2011) who measured enhanced salivary cortisol levels 1 hour after session begin in dogs that were involved in AAT in hospital environments. Stress-related behaviors observed during 1 minute after 2 hours of AAT included panting, pupillary dilation, yawning, whining, and air licking. Interestingly, the occurrence of these behaviors did not vary if dogs were subjected to 2 minutes of a quiet time-out after 60 minutes. Still, a correlation of stress behaviors and increases in salivary cortisol levels was found, and less behavioral signs of stress were observed if dogs had 2 years of experience in AAT or more and/or were older than 6 years (King et al. 2011).

The only study that evaluated data over a period of 3 years was by Marinelli et al. (2009) and analyzed handler reports on stress-related behaviors in dogs performing AAA/AAT and handlers' opinions on working conditions. According to animal handlers, both the frequency of sessions and the number of recipients increased for each dog with an overall lower perception of the quality of the intervention. An effect of recipient age was discovered to modulate the expression of stress-related behaviors, which were more frequently expressed when children under the age of 12 years participated in AAA/AAT sessions. Moreover, interferences, high temperatures, and lack of space were considered inappropriate for the maintenance of dog well-being (Marinelli et al. 2009).

Table 15.2 AAI session characteristics including duration, single or group intervention, between session intervals, and significant findings

Reference	Duration	Single/ group	Intervals	Significant findings
Haubenhofer and Kirchengast (2006, 2007)	1–8 hours	Not available	Differed from 9–50 sessions/3 months	↑ Salivary cortisol: on working days, during short sessions with high intensity, high frequency of sessions
Piva (2008)	20 min	Group	3–4 sessions/ week	↓ Stereotypic autogrooming; ↑play behavior, socialization; ↓ hair cortisol
Marinelli et al. (2009)	10–105 min	Single, group	Daily	↑ stress-related behavior if recipients were children <12 years; increase in the frequency of sessions and number of recipients across 3 years
King et al. (2011)	2 hours	Single	Biweekly	No effect of a short time-out session; ↑salivary cortisol after 60 minutes; ↑behavioral signs of stress in dogs <6 years and/or < 2 years of AAI experience
Glenk et al. (2013)	50–60 min	Group	Weekly	No difference in salivary cortisol between working and resting days; ↓ salivary cortisol in therapy dogs off-lead
Glenk et al. (2014)	55–60 min	Group	Weekly	↓ Salivary cortisol in sessions 4 and 5; no changes in behavior
Ng et al. (2014)	60 min	Group	Not available	No difference in salivary cortisol between working and resting days; ↑ salivary cortisol in novel environment
Koda et al. (2015)	70 min	Group	Weekly	No change in salivary cortisol from pre- to post-session in dogs rated as severely stressed by handlers; cortisol levels were significantly lower post session in dogs rated as minimally stressed
Palestrini et al. (2017)	20 min	Single	Not available	No changes in heart rate or behavior across 20 sessions
Pirrone et al. (2017)	55 min	Group	Weekly	No difference in salivary cortisol between working and resting days; ↑ joint attention and gaze synchrony during AAA; ↑ heart rate on working days
McCullough et al. (2018)	20 min	Single	Weekly	No difference in salivary cortisol between working and resting days; ↑ salivary cortisol levels related to ↑ stress behaviors and ↓ affiliative behaviors; ↓ affiliative behaviors in dogs with higher scores on stranger-directed fear
Colussi et al. (2018)	90 min	Group	Not available	↓ Salivary cortisol after AAA compared to before session and home levels

Modified and extended from Glenk (2017)

The results by Haubenhofer and Kirchengast (2006, 2007) and King et al. (2011) have been contrasted by Glenk et al. (2013, 2014) and Ng et al. (2014) who reported no differences in salivary cortisol concentrations when working days with AAI settings and resting days at home were compared. Research by Glenk et al. (2013, 2014) was conducted in in-patient healthcare facilities and used salivary cortisol and behavior to determine dog welfare. In the study published in 2013, experienced dogs that were kept off-lead and allowed to move freely during AAIs exhibited lower cortisol levels than experienced dogs on-lead and dogs in training that had not earned an AAI certificate at that time. The second study reported no significant changes in behavior over a period of 5 weeks, where the dogs were enrolled in AAIs weekly for approximately an hour. Salivary cortisol decreased significantly during the last two sessions, possibly due to habituation. These studies suggest decreasing levels of arousal in dogs that modulate closeness and distance themselves during human–animal interaction by moving freely in sessions with increasingly familiar recipients. A different setting was focused on in research by Ng et al. (2014) who monitored salivary cortisol and behavior during on-campus AAAs with university students as recipients. Salivary cortisol concentrations increased if dogs rested quietly with their handlers in an unfamiliar environment compared to when they were at home or involved in an AAA setting. No differences in the occurrence of stress-related behaviors were found between the three study conditions. Behavioral differences were only found for postural state, resulting in more standing and ambulating if stimulated by interaction with strangers during the AAA setting. Koda et al. (2015) used salivary cortisol and handler reports to assess dog welfare during an AAI program with prisoners. Dogs that were evaluated as showing severe stress did not exhibit significant changes in salivary cortisol from pre- to post-session. Dogs that were rated as minimally stressed had significantly lower cortisol levels post session. There was an effect of novelty, as a higher tendency in handlers to rate their dog as severely stressed was found in the first session of the 12-week program. Behavioral indicators of stress in animal handler protocols were however only based on occurrence, and therefore, information on frequency, duration, and intensity of behavior is lacking. A discrepancy between handler evaluation and dogs' salivary cortisol concentrations existed in 11% of cases, where dogs were rated as severely stressed in the absence of relevant changes in the glucocorticoid hormone (Koda et al. 2015).

Social synchronization patterns that have been previously measured between caregivers and children were studied in handlers and dogs in an exploratory study by Pirrone et al. (2017). Moreover, assessment of heart rate and salivary cortisol was carried out over the course of five subsequent AAA sessions with psychologically or physiologically disabled adults. Gaze synchrony, joint attention, and touch synchrony were registered before, during, and after the sessions. Social synchrony occurred prior to and during AAAs with joint attention being the most prevalent behavior. However, more gaze synchrony and joint attention were found during AAA performances than before. No differences in salivary cortisol levels were found except for individual differences between the dogs. Although heart rate was higher in dogs on working days with AAA sessions compared to control days, values remained within the common physiologic range, suggesting only minor

increased arousal. Individual preferences for physical contact with recipients were described with some dogs being more willingly to initiate contact with the patients than others.

McCullough et al. (2018) recently published their findings on salivary cortisol and behavior in dogs performing AAIs in pediatric oncology. Sessions were arranged in a manner that a dog-handler team was paired with a child, his or her parents, and hospital staff. No significant differences in dogs' salivary cortisol were detected when working concentrations were compared to pre-working levels at the hospital site or at home, paralleling previous data of Glenk et al. (2013, 2014), Ng et al. (2014), and Koda et al. (2015). However, during AAI sessions, higher salivary cortisol was associated with an increased frequency of stress behaviors and a reduced frequency of affiliative behaviors. In dogs that exhibited higher scores of stranger-directed fear in a behavior-centered questionnaire (i.e., C-BARQ), fewer affiliative behaviors were displayed in AAI sessions. The findings suggest that only mild expressions of distress in dogs were observed, but it is interesting that incidences of stress and affiliative behaviors were linked with certain activities. For instance, more stress-related behaviors were seen if the child put a bandanna on the dog, and fewer affiliative behaviors were found if the child used a stethoscope to listen to the dog's heartbeat, and the child played a game on the dog's vest or drew a picture of the dog (McCullough et al. 2018).

Colussi et al. (2018) carried out an exploratory study on dogs' salivary cortisol responsiveness during various cognitive and physical activities that included AAA as stimulus. In their study, dogs participated with their owners in group interventions in Kindergarten, where children had verbal and tactile contact with the dogs. To assess working concentrations of cortisol, a pre-session saliva specimen was collected and compared to a post-session sample at the end of the activity. In addition, home baseline samples were gathered. Results on working and resting salivary cortisol confirm previous findings by Glenk et al. (2013, 2014), Ng et al. (2014), Pirrone et al. (2017), and McCullough et al. (2018) in that no AAI-related increase was found. The authors stated that AAAs can be considered as low intensity exercises, and still dogs provide high psychological support to recipients. Significantly higher pre-session levels may be associated with anticipation stress or arousal during transportation to the facility, but a causal relationship cannot be inferred.

15.2.3 The Biopsychosocial Model of Dog Health in AAIs

The biopsychosocial model of health may not only refer to the human experience of AAIs but provides a comprehensive framework of categories for the canine perception as well. Research on social mammals has indicated that there exist common neural correlates that modulate social behaviors across species (Goodson 2005). Thus, effects of human–animal interaction during AAIs may influence the dog's biological, psychological, and/or social integrity in a similar way humans are affected. Figure 15.1 integrates significant common study outcomes that emerged from the literature review and are described in more detail in Table 15.2.

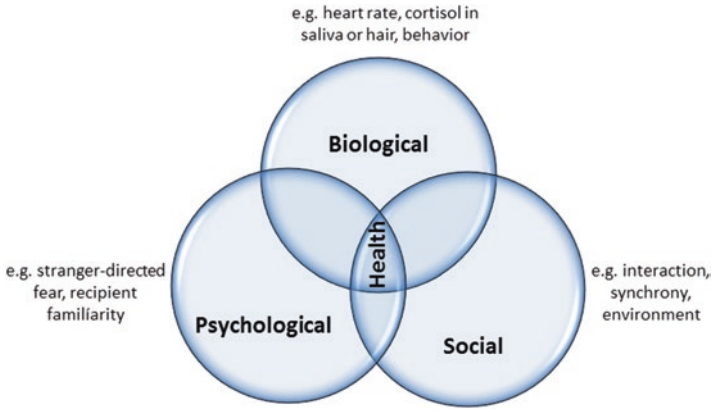

Fig. 15.1 The biopsychosocial model of dog health in AAIs as a multidimensional framework of human–animal interaction effects on dogs

15.3 Factors that Modulate Dog Welfare

15.3.1 Training Methods

In their research on training methods, problematic behaviors, and human–dog relationships, Hiby et al. (2004) found higher scores of obedience in dogs that were solely trained using reward-based methods. In comparison to punishment, specific tasks and behaviors were more easily learned if dogs were rewarded with positive praise, play, and treats. Study outcomes also indicate a causal relationship between punishment and problematic behavior, while no correlations were found between problematic behavior and reward-based training methods. The authors stressed that while positive reinforcement may improve human–dog relationships, punishment during training method may elicit anxiety in the dog which, in turn, is likely to impair dog welfare on health over time.

In a study by Deldalle and Gaunet (2014), dogs' behavioral responses to common human obedience commands were observed. In detail, the relationship between the frequency of stress signals was linked to whether the dogs were trained with positive (i.e., appearance of an appetitive stimulus like food or praise) or negative reinforcement (i.e., disappearance of an aversive stimulus like pressure or straining the lead). The study focus was set on two different popular training exercises: walking on-lead and responding the sit command. Dogs trained with negative reinforcement showed significantly more lip licking, when confronted with the sit command. Moreover, yawning, shaking, scratching, whining, and sniffing were exclusively seen in dogs that knew aversive training methods. During walking on-lead, dogs trained with positive reinforcement gazed significantly more toward their owner. Low posture (including tucked tail, ears back, and legs bent) and gaze avoidance

were more likely seen in negatively reinforced dogs during the sit command (Deldalle and Gaunet 2014).

The implications from these studies for dogs performing AAIs are obvious. Appropriate training via positive reinforcement will result in a more positive human–dog relationship and increased control of the owners over the dogs. Especially during interactions with strangers in unfamiliar environments, where the animal handler is urged to recognize subtle signs of discomfort immediately, dogs that seek eye contact may have a clear advantage.

15.3.2 Inequity Aversion

A phenomenon that has not yet been considered in the literature with regard to dog welfare in AAIs is inequity aversion, a sensitivity toward disadvantageous reward distribution. The pioneer work on inequity aversion in animals was carried out by Brosnan and De Waal (2003) and Brosnan et al. (2004) who investigated conditions under which capuchin monkeys and chimpanzees were willing to exchange a token with the experimenter for food. Study results indicate that the animals refused collaboration if they watched a conspecific obtain a more attractive food reward for equal or less effort. As demonstrated by Range et al. (2009), unequally rewarded dogs refuse participation in a paw lifting task earlier, hesitate longer to respond to human commands, and exhibit more stress behaviors. Bruck et al. (2016) replicated the results in a follow-up study, demonstrating that after the experiment, unequally rewarded dogs tended to avoid the experimenter and the conspecific dog in a neutral environment.

The prevalence of inequity aversion in dogs should be considered if multiple dogs participate simultaneously with multiple recipients in an AAI session, which is common in on-campus programs similar to the study by Ng et al. (2014) or in Kindergarten (Colussi et al. 2018) or in prison (Koda et al. 2015).

15.3.3 Attachment

An attachment refers to an intense, emotional relationship between two individuals. Previous studies have sought to attribute the dog–human relationship characteristics described for human caregiver–infant relationships. Thus, attachment has been associated also with the human–dog dyad and is characterized by behaviors including proximity seeking, exploration, and separation. Moreover, stressful experience may be buffered by the support of the human attachment figure (Payne et al. 2015). An experimental protocol to investigate attachment patterns explored whether aged dogs (7 years and older) reacted differently than adult dogs under 7 years. Attachment behaviors were similarly expressed between the groups, but the social challenge procedure led to an increase in salivary cortisol concentrations in older dogs

(Mongillo et al. 2013). These findings have important implications for dogs in AAIs as it appears that dogs are sensitive to separation from their handlers and that handler presence may help to attenuate a stressful event. Simultaneously, physiological correlates of separation distress are more prevalent in older dogs.

15.4 Future Directions and Summary

Similar to the limitations in research into how human can benefit from AAIs, the studies on dog welfare are characterized by small numbers of studied subjects, suitable control conditions and groups, and limited or lacking randomization. Moreover, as indicated by Glenk (2017), a researcher bias may exist if scientists are convinced of the positive effects of AAIs and may therefore be less willing to report unfavorable findings regarding therapy animals. Considering the emerging practice of dogs in therapeutic environments, standardized protocols for monitoring dog welfare in AAIs would be desirable. However, considering the large number of different types, therapeutic contents, and goals of AAIs, universal standardization of such protocols may not be feasible. More studies are needed that account for the heterogeneity in programs, patients, and dog characteristics. Factors that were described to have a modulating effect on the human–dog relationship (i.e., training methods, attachment) should be considered in future research on AAIs as it would be interesting whether these factors affect dogs' performance and perception.

In summary, as previously concluded by Glenk (2017), no acute manifestations of compromised dog welfare arose across the studies that would advise immediate prohibition or modification of AAI practices. Nevertheless, incidences of mild behavioral and physiological signs of stress warrant a closer inspection of the animal perspective of AAIs. Environmental factors such as temperature, familiarity of the surroundings and recipients, the presence of conspecifics, and the possibility to withdraw from unpleasant interactions can affect dog welfare and should therefore be carefully monitored. Considering these factors in combination with rigorous methodology will be valuable to researchers conducting both qualitative and quantitative studies on dog welfare in AAIs in the future.

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