Erika Jensen-Jarolim *Editor*

Comparative Medicine

Disorders Linking Humans with Their Animals

Springer

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To my family

Preface

Based on the great interest in our first book *Comparative Medicine: Anatomy and Physiology*, I felt encouraged to continue the enterprise. The present book is the result of our passionate research on selected diseases occuring in humans and their animals. In most cases, we found the common principles in disease mechanisms, diagnosis and therapy, among dogs, cats, horses, and humans, truly striking. The chapters on legal and ethical aspects of medicine with and for animals complete the picture.

With *Comparative Medicine: Disorders Linking Humans with Their Animals*, we thus aim to contribute to the indispensable dialogue among the medical disciplines. Our intention was to collect the latest state of the art for clinician and scientist to create a book useful for studying and teaching, but also enjoyable for interested lay people and animal owners.

Sincerely,

Vienna, Austria — Erika Jensen-Jarolim, MD

Acknowledgment

I would like to cordially acknowledge all authors of this book for their excellent contributions to comparative medicine teaching at the Interuniversity Messerli Research Institute in Vienna, Austria.

Warmest thanks also for constant support and encouragement to the board members of the Swiss Messerli Foundation, especially to Dr. Heinz Schweizer, Prof. Dr. Hans Hengartner, and Prof. Dr. Sabine Werner.

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Failure in Cardiac Action: Comparing Humans, Dogs, Cats, and Horses

1

Claudia Stöllberger and Mateo Markovic

Contents

Abstract

The heart is a central organ keeping the blood flow going, thereby providing oxygenation of peripheral tissues. An overview is given here on the most important heart diseases, comparing among humans and animals, especially cats and dogs. Whereas in humans cardiac diseases due to long-standing arterial hypertension and atherosclerosis represent the well-known most important death causes, in animals the disease is less recognized by the public and often occurs

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silent. In humans, the prevalence of diseases of the cardiovascular system varies according to the socioeconomic conditions of a geographic region: Whereas rheumatic heart diseases and cardiomyopathies due to infection and malnutrition are more prevalent in developing countries, arterial hypertension and atherosclerosis are more prevalent in industrial countries. In animals, the most frequent diseases of the cardiovascular system comprise chronic degenerative valve disease (endocardiosis), dilated cardiomyopathy in dogs, and hypertrophic cardiomyopathy in cats. In humans, arterial hypertension is frequently primary, whereas in animals secondary hypertension due to underlying systemic diseases is more frequent than primary hypertension.

1.1 Cardiac Disease: Number One of Death Causes in Humans

The most frequent cardiovascular diseases in humans (Fig. 1.1) comprise arterial hypertension, atherosclerotic vascular disease, cardiomyopathies, valvular heart disease, and Takotsubo cardiomyopathy. Since arterial hypertension is also one of the most important risk factors for the development of atherosclerosis, a high interdependence between these diseases exists. The most devastating manifestation of atherosclerosis is myocardial infarction, which may lead to stroke, potentially leading to lifelong disability or sudden death. Sedentary lifestyle, food composition, nicotine abuse, and environmental factors play a role in the development of hypertension and atherosclerosis. In the pathogenesis of cardiomyopathies, arterial hypertension but also genetic and metabolic factors, alcohol abuse, malnutrition, and infections may play a role in human patients. Valvular heart disease may either be congenital or caused by infections, rheumatic, autoimmune, or atherosclerotic mechanisms. Psychic as well as physic stress and emotional factors, which are difficult to measure and quantify, play an important role in the development of Takotsubo cardiomyopathy. These emotional factors, as well as sleep deprivation, are also assumed to be important risk factors for arterial hypertension and atherosclerosis.

The prevalence of the diseases of the cardiovascular system varies according to the socioeconomic conditions of a geographic region: Whereas rheumatic heart diseases and cardiomyopathies due to infection and malnutrition are more prevalent in developing countries, arterial hypertension and atherosclerosis are more prevalent in industrial countries.

1.1.1 Arterial Hypertension

The prevalence of elevated blood pressure, i.e., arterial hypertension in middle Europe is about 10–50 % of the human population with a steep increase with aging (Mancia et al. 2013). According to measurement results of the systolic and diastolic blood pressure, a classification has been established (Table 1.1).

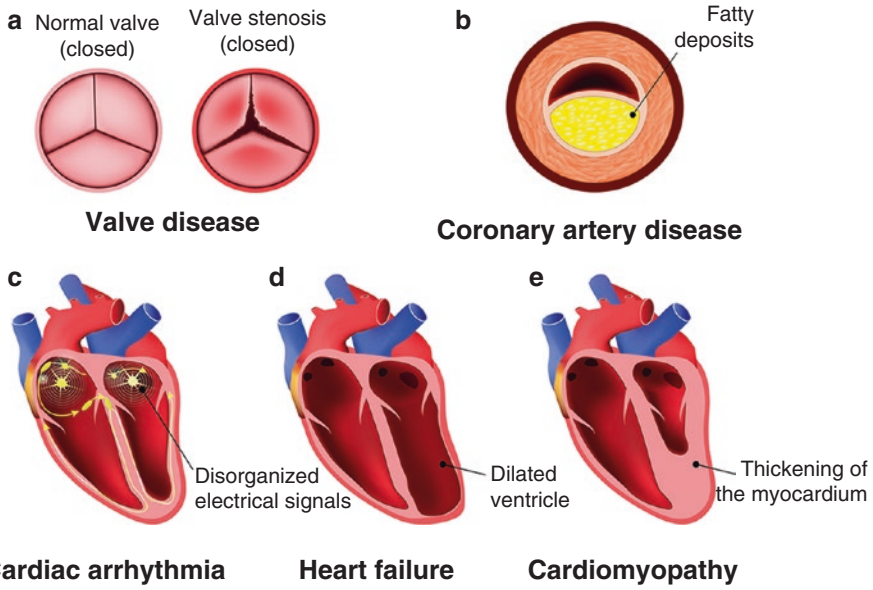

Fig. 1.1 Most frequent heart diseases in humans and animals. (**a**) Inflammation, for instance, through bacterial infections, may damage the ventile function of heart valves and result in functional insufficiency. Chronic degenerative valve disease (CDVD) is the most common acquired heart disease, for instance, in dogs, mostly affecting the *mitral valve* between the left atrium and ventricle; (**b**) arteriosclerosis, in contrast, is a disease mostly seen in humans associated with metabolic syndrome. Thereby, fatty deposits (plaques) are formed in endothelia, macrophages activated to phagocytose them. Coronary artery disease may present as reversible stenocardia or infarct. The latter occurs when an instabile plaque suddenly causes vessel obstruction. (**c**) Transient or stable obstructions of coronary arteries are typically associated with disorganized electric signals resulting in arrhythmia, which can be monitored in an electrocardiogram; (**d**) chronic insufficiency or inflammation of the heart muscle may lead to heart dilatation, associated with output failure due to decreased contractility; (**e**) cardiomyopathy as illustrated here is associated with thickening of the heart muscle, being, for instance, a consequence of constant overload in hypertonia (Fotolia.com-© designua)

Table 1.1 Classification of hypertension in humans

Classification	Systolic mmHg	Diastolic mmHg
Normal	90–119	60–79
Prehypertension	120–139	80–89
Stage 1 hypertension	140–159	90–99
Stage 2 hypertension	≥160	≥100
Isolated systolic hypertension	≥140	<90

In 90–95 % of humans with arterial hypertension, the cause remains unidentified, prompting the classification "primary arterial hypertension." The development of arterial hypertension is assumed to be a complex interaction between genetic,

environmental, and lifestyle factors, including sleep deprivation (Kohansieh and Makaryus 2015).

In contrast, in "secondary arterial hypertension," a specific cause can be identified. It may, for instance, occur in a minority of cases in patients with renal diseases, congenital vascular abnormalities, or hormonal diseases like Cushing syndrome, thyroid disorders, or pheochromocytoma.

Arterial hypertension is a silent offender. It causes no symptoms in the beginning of the disease, and blood pressure measurements are only occasionally performed in healthy adults. Hypertension, however, is the most important risk factor for atherosclerosis. When symptoms occur, hypertension has frequently already led to organ damage involving the brain, eye, heart, kidneys, and blood vessel walls.

The following lifestyle factors have been identified to lower the blood pressure: reduced salt intake, increased consumption of fruits, exercise, weight loss, and reduced alcohol intake (Mancia et al. 2013; Börjesson et al. 2016). When the blood pressure remains elevated despite modifications of lifestyle, long-term antihypertensive drug therapy is indicated.

1.1.2 Experimental Animal Models in Hypertension

An animal model for hypertension is sought-after in small animals, able to predict the potential antihypertensive properties of an agent, consume minimal quantities of compounds, simple to perform and uniformly reproducible, and comparable to some form of human hypertension. Unfortunately, there are no adequate models for primary hypertension (Dornas and Silva 2011). The animal models of hypertension are mainly models for secondary hypertension, which is rare among humans (Table 1.2). Natural history of the disease and observations of therapeutic effects can be only translated with caution from animal experiments to humans. Therefore, the concept of comparative medicine, i.e., comparison of human disease to veterinary patients, is highly attractive. Intriguingly, animals and owners share many of the environmental and lifestyle factors.

1.1.3 Atherosclerosis

Atherosclerosis is characterized by an accumulation of deposits of lipid, fibrous tissue, and calcium in the arterial walls, which eventually results in luminal narrowing. Atherosclerosis is induced by chronic endothelial injury. Several factors like arterial hypertension, nicotine abuse, hyperlipidemia, diabetes mellitus, and toxic, inflammatory, and immunologic reactions are involved in the process of endothelial injury.

Table 1.2 Experimental animal models of genetic hypertension

Phenotype driven	Genotype driven
Spontaneously hypertensive rat (SHR)	Renin-angiotensin system
SHR stroke prone	Sympathetic nervous system
Dahl salt-sensitive rat	Atrial natriuretic peptide
Genetically hypertensive rat	Nitric oxide
Sabra model	Endothelin
Lyon hypertensive rat	Neuropeptide Y
Obesity related	Vasopressin
	Prostaglandin
	Kallikrein-kinin

Clinical consequences of atherosclerosis depend on the affected arteries. When the coronary arteries are affected, atherosclerosis might cause angina pectoris. In the case atherosclerosis leads to an occlusion of a coronary artery, myocardial infarction may occur. Affection of the cerebral arteries may lead to stroke, and of the peripheral arteries to intermittent claudication and gangrene.

Acute myocardial infarction is characterized by oppressing chest pain lasting >30 min, dyspnea, nausea, vomiting, palpitations, sweating, and anxiety. If one or more of these symptoms occur, the emergency service should be called immediately since life-threatening arrhythmias like ventricular fibrillation are frequent. Ventricular fibrillation will lead to cerebral hypoxia and, if untreated, to the patient's death. Ventricular fibrillation can be treated by defibrillators. Furthermore, the patient with myocardial infarction should be transported to the hospital as soon as possible for acute intervention to open the occluded coronary artery (Fig. 1.2). The shorter the artery remains occluded, the lower will be the consecutive damage of the myocardium. Reperfusion therapy by acute coronary intervention in patients with acute myocardial infarction saves life and the myocardium (Bainey and Armstrong 2016).

1.1.4 Experimental Models in Atherosclerosis

Atherosclerosis rarely occurs in animals. Atherosclerotic lesions have been detected in aged pigs and parrots and in dogs with hypothyroidism and diabetes mellitus. Animals used for atherosclerosis research comprise rabbits, mice, rats, guinea pigs, hamsters, swine, and nonhuman primates (Fuster et al. 2012). In these animals, atherosclerosis is induced by either cholesterol feeding or mechanical endothelial injury. Furthermore, genetically engineered mice are used.

Pigs, rabbits, and chicken are susceptible to the experimental disease produced by feeding of a high-cholesterol diet, whereas dogs, cats, cows, goats, mice, and rats are resistant. Rabbit models of atherosclerosis have the advantages that they are easy to maintain and handle and have low costs, that the animals are highly

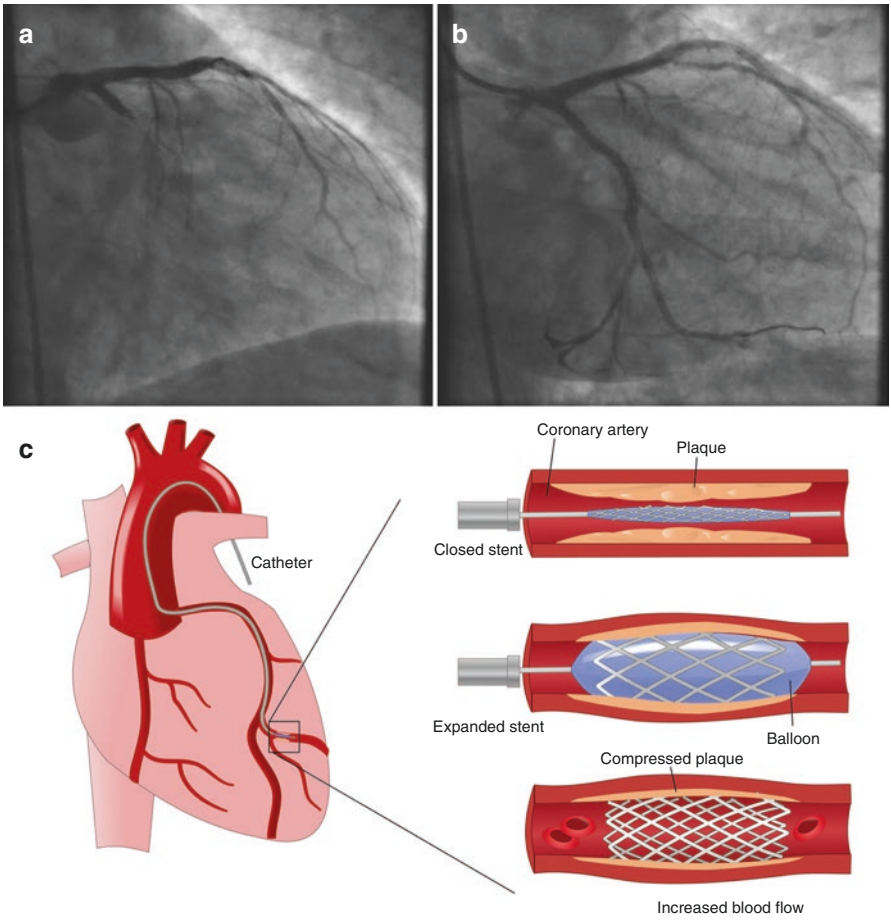

Fig. 1.2 Acute myocardial infarction in a human patient and stent therapy. (**a**) Acute coronary angiography showing an occlusion of the circumflex branch of the left coronary artery in a patient with acute myocardial infarction; (**b**) after recanalization and implantation of a stent within the narrowed coronary artery, a normal blood flow is seen in the artery, which had been occluded in the beginning of the procedure; (**c**) in case of blockage of the arteria coronaria, e.g., by arteriosclerotic plaques, via cardiac catheterization a ballon stent can be introduced that enlarges the diameter of the vessel by compressing the plaque. The balloon is removed after the manipulation, whereas the stent remains (Fotolia.com-©ellepigrafica)

available, that their lipoprotein metabolism is similar to humans, and that they show a good response to dietary cholesterol. Disadvantages of rabbits in animal experiments of atherosclerosis are the highly abnormal diet and that long-term cholesterol feeding induces hepatic toxicity and massive inflammation. Pig models of atherosclerosis have the following advantages: cardiovascular anatomy similar to humans, spontaneous formation of atherosclerotic lesions, morphology of lesions, and lipoprotein metabolism similar to humans. Disadvantages of pig models are high cost of purchase and maintenance, difficulty in handling, and that atheroma formation

requires time. Advantages of nonhuman primates as animal model for atherosclerosis are that they are phylogenetically close to humans and show spontaneous formation of atherosclerotic lesions and that these vascular lesions are similar to humans. Disadvantages of nonhuman primate models are high costs of purchase and maintenance, limited availability, requirement of special animal facilities, and ethical concerns. Mouse models of atherosclerosis have the advantages of easy breeding and handling, short generation time, well-defined genetics, and well-established protocols for genetic manipulation. Disadvantages of mouse models are a high resistance to atherosclerosis development in wild-type mice, a plasma lipid profile different to humans, differences in the morphology of vessel wall, and the absence of plaque rupture and luminal thrombosis.

1.2 Cardiac Diseases in Dog and Cat Patients

The common cardiovascular diseases in veterinary medicine include chronic degenerative valve disease (endocardiosis), dilated cardiomyopathy in dogs, hypertrophic cardiomyopathy in cats, and systemic hypertension.

Acute myocardial infarctions are uncommon in veterinary medicine and are most commonly associated with concurrent systemic or cardiac disease that leads to a thromboembolic state. Endocarditis, neoplasia, renal disease, immune-mediated hemolytic anemia, and pancreatic disease are the most frequent conditions. In human medicine there is a high incidence of infarcts associated with atherosclerosis, whereas in veterinary medicine, the patients with infarcts are very rarely diagnosed with atherosclerosis (Meurs 2010).

1.2.1 Chronic Degenerative Valve Disease (CDVD) Mostly Affects *Mitral Valve*

Endocardiosis, also known as chronic degenerative valve disease (CDVD), is the most common acquired heart disease of dogs accounting for 75–80 % of all cases. The mitral valve is the one most commonly affected, but the tricuspid valve may be affected concurrently and/or preferentially in individuals. The disease is characterized by the accumulation of glycosaminoglycans (myxomatous proliferation) within the spongiosa and fibrosa layers creating a vegetative nodular appearance. Small breed dogs such as Cavalier King Charles spaniel, Chihuahua, dachshund, poodle, and papillon are frequently affected (Egenvall et al. 2006). The disease is uncommon in young dogs. In older dogs the condition is frequent, with a prevalence >90 % in dogs over 10 years of age.

CDVD has a strong resemblance to primary mitral valve prolapse (MVP) in humans. Knowledge about the canine disease may thus help to increase the understanding of the disease in humans.

The disease appears to be inherited in the dog as well as in man. It is known that most dogs develop myxomatous mitral valve disease with age and this disease is very similar macroscopically as well as microscopically to primary MVP in humans

(Pomerance 1981). In affected patients of both species, the most frequent macroscopic changes are enlarged, thickened leaflets, interchordal hooding, and elongated chordae tendineae. Furthermore, the involvement of the tricuspid valve, secondary leaflet fibrosis, ruptured chordae tendineae, jet lesions, dilatation of the left ventricle, left atrium, and mitral annulus can develop (Pomerance 1981; Kogure 1980).

A major difference in pathological findings among human and canine species is the risk for endocarditis. Whereas humans are more prone to develop endocarditis, in dogs this condition occurs rarely only, and large breed dogs, which typically have no risk for CDVD, are more affected (Calvert 1982).

It is speculated that primary MVP is a part of a generalized connective tissue abnormality. Similarly, the dogs predisposed to CDVD have a risk of developing connective tissue disorders such as intervertebral disk disease, collapsing trachea, and ruptured cruciate ligaments.

The myxomatous changes start along the line of apposition of the leaflets and progress in severity with advancing age. One study including 190 clinically healthy dachshunds showed a positive correlation of an increase of valvular changes (with positively correlation) with age. Dachshunds are often affected, and 50 % typically develop a regurgitation murmur before 10 years of age (Olsen et al. 1999).

The prevalence and severity are clearly age dependent in dogs suffering from CDVD and in humans with MVP (Whitney 1974; Davies et al. 1978).

In some dog breeds, almost all dogs are affected. For example, in Cavalier King Charles (CKC) spaniels, a typical murmur of mitral regurgitation can be found in 50 % of dogs at the age of 5–6 years and in all dogs at 10 years of age. Most of the dogs of this breed showed a typical MVP in the ultrasound (Häggström et al. 1992).

In both species the myxomatous mitral valve disease is a slowly progressing disease which in most cases has a benign course, and the severe form usually develops in old age (Pedersen et al. 1999; Häggström et al. 1992).

According to the studies in humans with MVP following for a mean period of 6–13 years, 5–10 % of the patients developed severe mitral regurgitation requiring surgery (Duren et al. 1988). Another study followed 250 patients for an average period of 40 years have found that after the age of 50, about one fourth will undergo some form of surgical therapy (Chapman 1994). In CKC spaniels aged less than 10 years, the mitral regurgitation becomes severe enough leading to the spontaneous death or to the euthanasia in 15–20 % of cases (Häggström 1996).

In dogs, as well as in humans, males have almost twice the risk to develop a severe disease in old age (Agozzino et al. 1992; Swenson et al. 1996).

A strong positive correlation exists between the murmur intensity and the degree of mitral regurgitation in patient affected by MVP in both species (Pedersen et al. 1999; Häggström 1996). In mild disease a heart murmur with short duration can be detected, and in cases with severe mitral regurgitation, a holosystolic murmur can be heard. Mild mitral regurgitation in dogs is characterized by typically early systolic murmurs, and seldom is a late systolic murmur found. In contrast, in humans with mild mitral regurgitation, short murmurs appear mostly late systolic (Ranganathan et al. 1976; Pedersen et al. 1999).

Echocardiography is the method of choice to diagnose and assess the degree of mitral regurgitation. Using this procedure a different 2D-Echo test changes

including leaflet thickness, degree of leaflet protrusion, and recognition of regurgitation jet on spectral or color flow Doppler can be detected.

Typical signs of remodeling secondary to mitral regurgitation involve progressively increasing dimensions of the left atrium and ventricle (Brown et al. 2007). The systolic left ventricle diameter is initially preserved, and its increase in late stage disease has been interpreted as a sign of myocardial dysfunction.

1.2.2 Systemic Hypertension: An Increasing Problem in Dogs and Cats

Persistent elevation of systemic blood pressure is increasingly recognized in dogs and cats. In most cases it presents a complication of other systemic diseases and is defined as a secondary hypertension.

If the cause for systemic hypertension cannot be found, the condition is classified as a primary hypertension. Secondary hypertension occurs more often than a primary (idiopathic, i.e., without detectable reason) hypertension, accounting for approximately 18–20 % cases in cats (Brown et al. 2007).

Systemic hypertension may be recognized in animals with systemic disease associated with the development of hypertension. The other scenario could occur when blood pressure measurement is performed in patients with clinical signs of hypertension-related target organ damage.

Stress-induced systemic hypertension should be excluded, and single value cannot be used for the diagnosis of elevated blood pressure in the absence of other clinical data.

According to current recommendations, a systolic blood pressure exceeding 160 mmHg indicates hypertension, and over a long term, a persistent damage of the effector organs such as the eyes, the central nervous system (CNS), the heart, and kidneys is expected.

Systemic hypertension in animals is often clinically silent. The most common signs are the ophthalmologic changes with a prevalence rate nearly 100 %. They include intraocular hemorrhage, hypertensive retinopathy, hypertensive choroidopathy, and hypertensive optic neuropathy. Dogs and cats may be presented due to an acute onset of blindness from complete, bilateral exudative retinal detachment. Antihypertensive treatment can lead to retinal reattachment, but the restoration of vision generally occurs seldom (Magio et al. 2000).

Hypertensive encephalopathy has been reported in dogs (Jakob et al. 2006), in cats (Magio et al. 2000), as well as in people (Kletzmayr et al. 2003). CNS signs have been reported in 29 % (Magio et al. 2000) and 46 % of hypertensive cats (Littman 1994) and include seizures, vascular accident, and changes in mentation. Other central nervous system abnormalities, including hemorrhage and infarction, which accompany chronic hypertension in people (Manolio et al. 2003), are also observed in dogs and cats.

The most reported cardiac changes following systemic hypertension are cardiac gallop, systolic heart murmurs, and left ventricular hypertrophy. In contrast to humans, the congestive heart failure secondary to hypertension occurs seldom. The

hypertensive cats may be less tolerant to fluid administration, and in rare cases, the intensive fluid therapy can lead to congestive heart failure.

As a consequence of systemic hypertension, an epistaxis (bleeding from the nose) can occur due to hypertension-induced vascular abnormalities (Brown et al. 2007).

1.2.3 Frequent Cardiac Problems in Horses

Mitral regurgitation, atrial fibrillation, aortic regurgitation, and tricuspid regurgitation are commonly reported cardiac disease in horses, whereas pulmonary regurgitation, ventricular arrhythmia, ventricular septal defect, and congestive heart failure are less frequently reported.

Mitral regurgitation is the most common cardiac disease with a prevalence of 4.4 %. In this setting, the cardiac output with oxygenized blood is reduced due to insufficiency of the mitral valve between the left atrium and left ventricle of the heart (Fig. 1.1). atrial fibrillation has a prevalence of 2.3 % and in some studies is considered as only lone or paroxysmal atrial fibrillation (Reef et al. 1998). According to another study, atrial fibrillation is most often occurring secondary to underlying cardiac disease like mitral regurgitation, tricuspid regurgitation (affecting the valve between the right atrium and ventricle, before the pulmonary passage of the venous blood), and pulmonary regurgitation. Moreover, the authors report that heavier and larger horses have a higher risk of developing atrial fibrillation and thus arrhythmia. In accordance with previous studies, in horses with a larger left atrium, the reflux of blood occurs easier and represents an underlying mechanism of atrial fibrillation. Whereas horses, large breed dogs (i.e., Irish wolfhound), and humans predominantly suffer from primary atrial fibrillation without atrial enlargement, small dogs and cats develop atrial fibrillation secondary to severe atrial (mostly left atrium) enlargement. The prevalence of atrial fibrillation is 2.1 % in horses with a cutoff level of 13.5 years of age from which on horses have a higher risk to develop atrial fibrillation. The prevalence for tricuspid regurgitation is 1.7 %. Congestive heart failure has a prevalence of 1 %. Taken together, atrial fibrillation and all valvular regurgitation except aortic regurgitation are risk factors for congestive heart failure in horses, whereas the body weight and age are not (Leroux et al. 2013).

1.3 Synopsis

An overview is given on the most important cardiovascular diseases. In humans, cardiac diseases due to long-standing arterial hypertension and atherosclerosis are frequent. In animals, the most frequent diseases of the cardiovascular system comprise chronic degenerative valve disease (endocardiosis), dilated cardiomyopathy in dogs, and hypertrophic cardiomyopathy in cats. In humans, arterial hypertension is frequently primary, whereas in animals, secondary hypertension due to underlying systemic diseases is more frequent than primary hypertension.

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Epilepsy in Humans and Animals: From Patients to Disease Models

2

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Contents

Abstract

Epilepsy occurs in humans and animals. It is one of the most prevalent neurological disorders worldwide, but with the availability of modern diagnostic tools and therapeutic measures the outcome has been increasingly improved during the last years. Disregarding these achievements, about one third of the epilepsies remains refractory to treatment and requires special attention of treating epileptologists and basic scientists. Refractory epilepsy is associated with a high prevalence of complications such as sudden unexplained death in epilepsy, poor adherence,

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cognitive decline, long-term side effects, and a poor outcome. In case of refractory epilepsy, alternative therapies to antiepileptic drugs should be considered which include ketogenic diet, hypothermia, steroids, intravenous immunoglobulins, propofol, ketamine, inhalative anesthetics, electroconvulsive therapy, transcranial magnetic stimulation, vagal nerve stimulation, or epilepsy surgery. Particularly status epilepticus, which may be convulsive or nonconvulsive, requires aggressive emergency treatment. With the invention and approval of new therapeutic agents, it should be possible to further narrow the segment of intractable epilepsies, not only in human but also in veterinary patients. Experimental and spontaneous animal models are used to find clinically useful therapies for symptomatic management of epilepsies and to understand the basic pathomechanism of novel forms of epilepsies.

2.1 Introduction

Epilepsy is one of the most prevalent central nervous system (CNS) disorders in humans worldwide. The incidence of epilepsy varies between countries considerably and has been reported to range from 26 to 92/100,000/year (Banerjee 2011). The prevalence of epilepsy ranges between 3.6 and 41.3/100,000 (Banerjee 2011). Epilepsy prevalence is age dependent and shows a bimodal distribution with a peak in childhood and a second peak after 50 years of age. Epilepsy is diagnosed if there are at least two unprovoked or reflex (photosensitive, hot water, reading, startle) seizures occurring >24 h apart, if there is one unprovoked seizure and a probability of at least 60 % for a further seizure over the next 10 years, or if there is an epilepsy syndrome (Table 2.1) (Fisher et al. 2014). The risk to experience another seizure after the first unprovoked seizure is 33 % in adults and 42–54 % in children. Risk factors for recurrence of seizures are epileptiform discharges on electroencephalography (EEG) or structural lesions on imaging. A seizure is defined as the clinical manifestation of hypersynchronous discharges of cortical neurons, and symptoms of a seizure comply with the physiological function of the affected cortical area. Clinical manifestations of seizures may mimic any neurological abnormality, but most frequently seizures manifest as focal or generalized cramping, focal or generalized myoclonia, impaired consciousness, impaired sensation, confusion, coma, tongue bte, secessus, fall, or cries.

2.2 Seizure Types and Triggers of Seizures

Seizures are generally classified as focal (seizure activity originates from part of the cortex), generalized (seizure activity originates from the entire cortex), or nonclassified (Davidson 2015). Focal seizures are further subdivided into simple focal seizures, complex focal seizures, and focal seizures with secondary generalization. Generalized seizures are further divided into absences (typical or atypical),

Table 2.1 Epilepsy syndromes

Angelman syndrome
Benign Rolandic epilepsy
CDKL5 disorder
Childhood and juvenile absence epilepsy
Dravet syndrome
Frontal lobe epilepsy
Glut1 deficiency syndrome
Hypothalamic hamartoma
Infantile spasms (West syndrome)
Juvenile myoclonic epilepsy
Landau–Kleffner syndrome
Lennox–Gastaut syndrome
Epilepsy with myoclonic absences
Ohtahara syndrome
Panayiotopoulos syndrome
PCDH19 epilepsy
Progressive myoclonic epilepsies
Rasmussen's syndrome
Ring chromosome 20 syndrome
Reflex epilepsies
Temporal lobe epilepsy

myoclonic seizures, clonic seizures, tonic seizures, tonic–clonic seizures, or atonic seizures. Triggers of seizures may be either central nervous system (CNS) disorders (e.g., stroke, bleeding, migraine, stroke-like episode, immune encephalopathies), systemic disease (e.g., metabolic dysfunction), or external factors such as flashing light, hyperventilation, missed antiepileptic drug (AED) intake, stress, anxiety, hormonal dysregulation, alcohol, withdrawal, sleep deprivation, fatigue, drugs, overheating, overexertion, and missed meals.

2.3 Classification of Epilepsy

Epilepsy may be classified according to the seizure type, according to the etiology, or according to age (congenital, infancy, childhood, adolescence, senescence). According to the seizure type, focal or generalized epilepsy may be differentiated (Rudzinski 2011). According to the etiology, idiopathic, symptomatic, acquired, provoked, and cryptogenic epilepsy is differentiated (Shorvon 2011). Idiopathic epilepsies are those due to a single gene mutation or pure epilepsies with complex inheritance (Shorvon 2011). Symptomatic epilepsies are predominantly genetic or developmental and include childhood epilepsy syndromes (Table 2.1), progressive myoclonic epilepsies, neurocutaneous syndromes, chromosomal defects, or developmental abnormalities (Shorvon 2011). Acquired epilepsies include epilepsy due to hippocampal sclerosis, perinatal or infantile damage, traumatic brain injury,

Table 2.2 *Operational dimensions in status epilepticus*, with time points t1, indicating the need for emergency treatment, and t2 indicating the beginning of long-term consequences

Type of status epilepticus	t1 (min)	t2 (min)
Tonic–clonic	5	30
Focal with impaired consciousness	10	>60
Nonconvulsive	10–15	Unknown

Modified according to Trinka et al. (2015)

cerebral tumor, cerebral infection, cerebrovascular disease, immunological CNS disorders, or degenerative disorders (Shorvon 2011). Provoked epilepsies are those unequivocally triggered by provocation such as fever, menstruation, sleep deprivation, metabolic or endocrinologic disorders, drugs, alcohol, or other toxins (Shorvon 2011). If seizures last for >5 min, a status epilepticus is present. A status epilepticus may be classified according to the four axis paradigms (semiology (phenomenology), etiology (known, unknown), EEG (no evidence-based EEG criteria available), age (neonatal, infancy, childhood, adolescence, adulthood, senescence)). Phenomenologically, the status epilepticus may be accompanied by motor manifestations (convulsive, myoclonic, focal motor, tonic, hyperkinetic) or without motor symptoms (nonconvulsive status epilepticus with or without coma) (Table 2.2) (Trinka et al. 2015). According to the new classification of status epilepticus, two operational time points (time point 1 – a seizure is likely to be prolonged leading to continuous seizure activity and time point 2 – seizure activity may have long-term consequences, such as neuronal injury or death, impairment of neuronal networks, or functional deficits) are determined to facilitate the management (Table 2.2) (Trinka et al. 2015). In two thirds of the cases, epilepsy is classified as idiopathic or cryptogenic. In one fifth of the patients, epilepsy is acquired. Epilepsy syndromes are listed in Table 2.1.

2.4 Diagnosis

The diagnosis of epilepsy is based on the individual and family history, the clinical exam, the EEG, and imaging studies. Supportive measures can be blood chemical investigations, cerebrospinal fluid investigations, MR spectroscopy, SPECT, magnetic resonance encephalography, invasive EEG, or video-EEG.

Diagnosing epilepsy can be challenging if seizures are unwitnessed, in case of amnesia for the event; if seizures last only for a few seconds; if the EEG is noninformative; or if the clinical manifestations are minimal or unusual (e.g., gelastic epilepsy). The diagnosis may be difficult if other causes could be responsible for the clinical manifestations as well (e.g., syncopes, psychosis, confusion). Concerning the individual history, it is important to ask for possible triggers and predisposing factors (e.g., pregnancy, birth trauma, developmental disturbance, febrile seizures, meningitis, encephalitis, traumatic brain injury), prodrome, aura, the seizure type, and the postictal condition (coma, confusion, agitation, muscle aching).

2.5 The Electroencephalogram (EEG)

The most reliable investigation to diagnose epilepsy is the EEG. By means of the EEG, epileptiform discharges (e.g., spikes, sharp waves, spike–wave complexes) can be recorded, psychogenic seizures can be differentiated from epileptic seizures, an epileptic focus can be localized, and the assignment to an epilepsy syndrome becomes possible (Fig. 2.1). The sensitivity of the EEG is highest 12–24 h after a seizure. The first EEG after a seizure shows epileptiform discharges in only 30–50 % of the cases. If serial EEGs are recorded, 80–90 % of the patients have epileptiform discharges. In 10 % of the epilepsy patients, epileptiform discharges are never recorded. If routine EEGs are normal, postprandial EEGs, EEGs after sleep deprivation, or video-EEGs may discover epileptiform discharges. The specificity of the EEG is high since 90 % of those with spikes have epilepsy. In addition to epilepsy, the EEG may be abnormal in numerous other conditions (e.g., migraine, dementia, stroke-like episodes, Creutzfeldt–Jakob disease). To localize the epileptic focus in case of intractable focal epilepsy, invasive recordings may be necessary to decide which part of the cerebrum should be removed by epilepsy surgery.

2.6 Treatment

As soon as epilepsy is diagnosed, treatment is indicated. The goal of treatment is to stop seizures, to prevent recurrence of seizures, and to reduce the seizure frequency (optimal seizure control). Treatment also aims at avoiding side effects and achieving good AED tolerance, allowing simple handling, providing co-treatment for eventually present associated disorders (e.g., mood disorders, psychosis, sleep disorder), and meeting special needs (e.g., kids, pregnancy, elderly, retarded, females in childbearing age). The most common type of epilepsy treatment includes application of old or new AEDs (Table 2.3). In case of refractory seizures or superrefractory status, epilepticus application of ketogenic diet, hypothermia, steroids, intravenous immunoglobulins, propofol, ketamine, inhalative anesthetics, electroconvulsive therapy, transcranial magnetic stimulation, vagal nerve stimulation, or epilepsy surgery may be necessary (Bayrlee et al. 2015). The choice of any of these treatment options for a given patient is based on his clinical presentation, imaging, and the EEG (Bayrlee et al. 2015). Epilepsy treatment is dependent on the classification of epilepsy and of seizures. Focal seizures respond to other AEDs than generalized seizures, and an epilepsy syndrome may require other AEDs than idiopathic epilepsy. Epilepsy treatment is also different for an acutely ongoing seizure or seizures only on the history. Treatment also depends on the severity of a seizure. Self-limiting seizures are treated differentially than a status epilepticus. AEDs currently used in the daily routine are listed in Table 2.3. In case the patient is unable to take the AED orally, intravenous application is indicated. AEDs, which can be given intravenously, include lorazepam (LZP), valproic acid (VPA), phenytoin (PHT), levetiracetam (LEV), lacosamide (LAC), phenobarbital (PB), ketamine, and propofol. Treatment of choice for discontinuing ongoing seizures is LZP intravenously. If

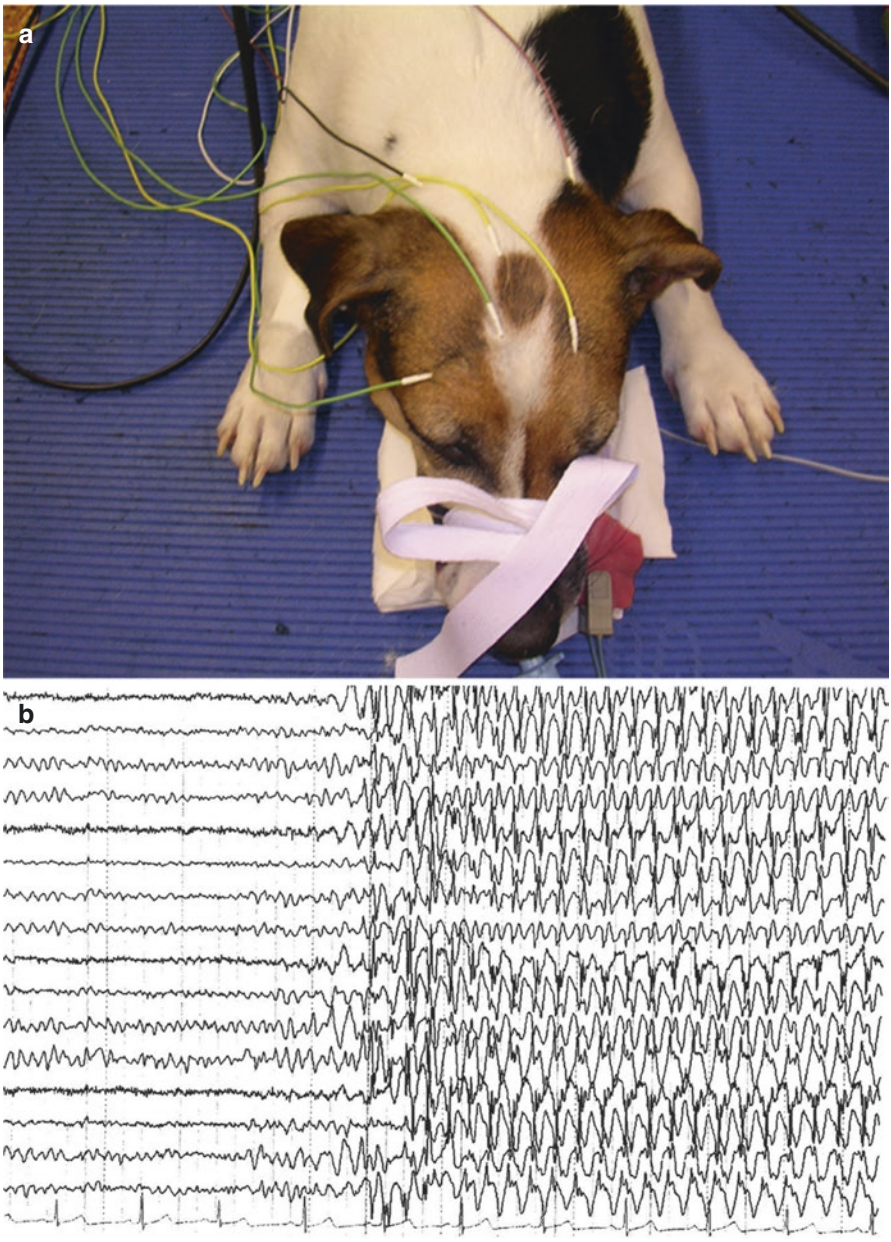

Fig. 2.1 The electric electroencephalogram is a diagnostic method applied in humans and animals. (**a**) The electroencephalographic recording in an epileptic dog, which actually does not belong yet to routine diagnostic work-up in veterinary patients but in some cases may support diagnosis. (**b**) Scalp electroencephalogram showing normal background activity and intermittently generalized polyspike waves followed by generalized 3 Hz spike–wave and slow–wave discharges deduced from a child with absence epilepsy

Table 2.3 Currently used antiepileptic drugs (AEDs)

Abbreviation	Generic name
Standard AEDs	
CBZ	Carbamazepine
CLB	Clobazam
ETX	Ethosuximide
LZP	Lorazepam
PB	Phenobarbital
PHT	Phenytoin
PRM	Primidone
VPA	Valproic acid
New AEDs	
ESL	Eslicarbazepine
GBP	Gabapentin
LAC	Lacosamide
LEV	Levetiracetam
LTG	Lamotrigine
OXC	Oxcarbazepine
PER	Perampanel
PGB	Pregabalin
RUF	Rufinamide
TGB	Tiagabine
TPM	Topiramate
VGB	Vigabatrin
ZNS	Zonisamide

seizure control cannot be achieved despite application of several dosages, VPA should be given as a bolus followed by continuous intravenous application. If VPA is ineffective, LEV, LAC, or PHT may be added. If seizures are still intractable, measures as outlined above should be considered. Intravenous application of AEDs is also indicated in case seizure control can be achieved but the patient does not regain consciousness. If the patient awakes but it remains unclear which AEDs should be given on the long run, clobazam (CLB) can be given during the interval.

2.7 Epilepsy Surgery

About two thirds of the epilepsy patients become seizure-free after application of one or two AEDs. In one third of the epilepsy patients, however, AEDs remain ineffective. For intractable epilepsy (e.g., epilepsia partialis continua, status epilepticus), alternative therapies can be optionally applied (see above). In case intractable epilepsy presents with focal seizures, thorough evaluation and localization of the epileptic focus is indicated. Localization of such a focus can be challenging and may require application of invasive EEG recordings. In case an epileptic focus can be identified, and AEDs are ineffective, removal of the focus by epilepsy surgery

should be considered. The focal epilepsy which responds best to epilepsy surgery is mesial temporal lobe epilepsy. It is characterized by a history of febrile seizures with an onset <5 years, followed by a seizure-free interval, followed by seizures without fever between age 5–10 years. These seizures respond favorably to established AEDs. At age >10 years, temporal lobe epilepsy becomes refractory to AEDs in 70–80 % of the cases. If refractory to AEDs, a mesial hippocampectomy can be performed, the procedure most frequently carried out in epilepsy surgery. Other cerebral regions are also accessible to epilepsy surgery.

2.8 Outcome

Seizures have to be regarded as an emergency condition which requires immediate and usually also long-term treatment. The outcome is better the the earlier treatment is initiated and the more effective it is at onset. The optimal outcome is achieved if the patient becomes seizure-free. A beneficial effect of an AED treatment is defined if seizure frequency can be reduced by >50 %. Epilepsy is regarded as resolved if patients are past the applicable age of an age-dependent epilepsy syndrome or if they are seizure-free for at least 10 years and off AEDs since at least 5 years (Fisher et al. 2014). If patients are seizure-free for at least 2 years and if the EEG and imaging are normal, discontinuation of AEDs can be considered, if the patient agrees. The outcome of epilepsy surgery is dependent on the location of the lesion, the type and severity of epilepsy, the peri-interventional risk, and the amount of tissue resected.

2.9 From Animal Models to Polar Bear Knut

Animal models are used to find clinically useful therapies for symptomatic management of epilepsies and for improving quality of life of affected individuals (Barker-Haliski et al. 2015). It is common knowledge that also pets may develop epilepsy. Among dogs especially the following popular breeds are prone to develop epilepsy (alphabetical order): beagles, collies, dachshunds, German shepherds, golden retrievers and Labrador retrievers, Irish setters, and poodles. Epilepsy is also common in client-owned cats, and there are laboratory cats with familial spontaneous epilepsy supporting that hereditary epilepsy occurs in that species as well (Hasegawa et al. 2014). Estimated 0.5–3.5 % of feline patients visiting the University of Veterinary Medicine Vienna are affected.

Therefore, animal models for disease are not only relevant for the human but also veterinary neurological patients. Epilepsy can be basically induced in each animal also without genetic predisposition. As models preferentially animals with defined onset of seizures are used, particularly in case seizures can be evoked by easy means such as exposure to light, touch, sound, or vestibular stress (De Sarro et al. 2015). This type of condition is called reflex epilepsy, and probably the most famous examples for it are the dilute brown agouti coat color (DBA)/2 mouse and the genetically

epilepsy-prone (GEP) rat. When DBA/2 mice or GEP rats are startled by a sudden loud sound (e.g., the ringing of a doorbell), they develop seizures. The easy seizure inducibility in these animals provides an ideal situation for the search for antiepileptic drugs, since the suppression of sound-induced seizures by the tested substances provides an easy readout system for their efficacy (De Sarro et al. 2015). Another animal model of single, acute seizures is induced in mice upon intravenous or subcutaneous injection of pentylenetetrazol, and the success of antiepileptic treatment with test compounds is measured according to its effect on onset latency/severity and duration of the seizure response (Mandhane et al. 2007). Such models of epilepsy not only helped to develop a large number of clinically important therapies for human patients but also led to a novel treatment for epileptic dogs (Tipold et al. 2015). Cumulatively, the discovery of antiepileptic therapies using animal models was and currently still is a story of success. Unfortunately, the situation is completely different for antiepileptogenic therapies. For example, ~40 % of epilepsy patients developed this condition after experiencing traumatic brain injury, stroke, tumor, or infection, and such risk patients would enormously benefit from therapies which could prevent epileptogenesis (i.e., the development and progression of an epileptic condition) (Barker-Haliski et al. 2015). There are many animal models for epileptogenesis – mostly induced in mice and rats – for example:

(a) Kindled seizure models. Such animal models are induced by kindling (e.g., the repeated stimulation of limbic brain structures via implanted electrodes, which leads to a progressively more intense brain excitability and to an establishment of a permanent epileptic focus in the stimulated region) (McIntyre et al. 2002).
(b) Post-status epilepticus models. The status epilepticus is defined as a seizure of very long duration or a series of acute seizure episodes with very short intervals, and it can be induced in experimental models by systemic or local administration of chemoconvulsants like kainic acid or pilocarpine, or by electrical stimulation (Sharma et al. 2007).
(c) Models of hyperthermia, which are induced in pups to reflect febrile convulsions of children (Sharma et al. 2007).
(d) Models for infection-induced epilepsy. Here, viral encephalitis-induced epilepsy in patients is reflected in Theiler's murine encephalomyelitis virus-infected mice (Barker-Haliski et al. 2015).

Most of these models reproduce the typical pathological changes observed in epilepsy patients, i.e., neurodegeneration and reactive gliosis (Barker-Haliski et al. 2015). In spite of these striking similarities, however, not a single one of these models could be successfully used to date to identify a clinically validated antiepileptogenic agent (Barker-Haliski et al. 2015).

Over the last years, there is increasing evidence for an association of seizures with antibodies against ligand-gated or voltage-gated ion channels in a subset of human epilepsy patients (Vincent et al. 2011). Under normal circumstances, serum antibodies are shielded from brain and spinal cord by the blood–brain barrier, which is formed by vascular endothelial cells connected to each other by tight junctions.

This barrier profoundly restricts the entry of immunoglobulins to only very small amounts, which in general are insufficient to induce immune-mediated tissue injury. Some brain areas like the circumventricular regions and the choroid plexus do not have tight junctions in endothelial cells but have tight junctions between astrocytes and plexus epithelial cells instead, which also restrict the diffusion of proteins into the CNS parenchyma. However, in the course of CNS inflammation, activated T cells open the blood–brain barrier for the entry of cellular and humoral immune mediators. Under these conditions, antibodies against ligand-gated or voltage-gated ion channels can easily find their targets in the CNS and bind to it, with striking consequences for affected patients.

For example, when antibodies against ligand-gated ion channels target N-methyl-D-aspartate (NMDA) receptors, they frequently recognize and bind to the extracellular domain of the NR1 subunit of the NMDA receptor (Dalmau et al. 2008). This leads to destabilization of NMDA receptors and subsequently to a reduction of NMDA receptor clusters in the hippocampus. Affected patients present first with neuropsychiatric disturbance and seizures and often progress to bizarre movement disorders. Although this type of epilepsy can occur at any age, a large number of young children with this condition have been described. Patients with NMDA receptor antibody encephalitis only partially respond to treatment with antiepileptic drugs, but do respond to immunotherapies suppressing NMDA receptor antibody levels (Vincent et al. 2011). Just recently, spontaneous anti-NMDA receptor encephalitis has been described in a captive polar bear (*Ursus maritimus*). Shortly after birth in 2006, this animal had been rejected by his mother and was raised by his keepers. Termed Knut, he became an extremely popular animal in the Berlin Zoological Garden until 2011, when he suffered epileptic seizures, fell into the enclosure's pool, and drowned. A detailed pathological examination of this animal, using the same diagnostic criteria also applied to human patients, revealed that Knut had high concentrations of antibodies against the NR1 subunit of the NMDA receptor in his cerebrospinal fluid, infiltrating immune cells in his brain, but only minimal neuronal loss in the affected brain areas (Pruss et al. 2015) suggesting that similar to the situation in affected human patients, also Knut's NMDA receptor-specific autoantibodies interfered with neuronal function rather than with neuronal viability.

Another possible target for antibodies could be voltage-gated potassium channels (VGKC). The VGKC complex is a multimolecular complex of proteins which includes leucine-rich glioma-inactivated 1 (LGI1), contactin-2, and contactin-associated protein 2 (CASPR2). Any of these proteins could be targeted by autoantibodies (Vincent et al. 2011). For example, patients with limbic encephalitis frequently have antibodies against LGI1 (Bien et al. 2012), which gain access to the brain in the course of CNS inflammation and modify neuronal activity upon binding to LGI1. Consequently, affected patients present with seizures, but also with amnesia, confusion, and other psychological disturbances (Vincent et al. 2011). Similarly, addition of LGI1 antibodies to hippocampal slice cultures in vitro induces seizure-like activity (Lalic et al. 2011), and mutations or deletions of LGI1 in humans or mice, respectively, cause epilepsy (Chabrol et al. 2010; Morante-Redolat et al. 2002). Patients with LGI1 antibody-associated limbic encephalitis only partially

respond to antiepileptic drugs but respond very well to immunotherapies reducing their anti-LGI1 antibody levels. In some patients, however, long-term effect of these antibodies may additionally include antibody- and complement-mediated neuronal damage causing permanent deficits (Bien et al. 2012).

For this disease, a spontaneous animal model has been recently discovered in Austria in client-owned cats with partial epileptic seizures (typical ictal clinical signs include episodic orofacial automatism with salivation, chewing, licking, facial twitching, motor arrest, vocalization, and mydriasis/feline complex partial seizure with orofacial involvement (FEPSO)). Increased concentrations of antibodies against VGKC and LGI1 were detected in the acute stage of the disease in 5 out of 14 (36 %) cats, but neither in cats in remission nor in healthy control cats. It was concluded that spontaneous autoimmune limbic encephalitis is common in cats and that the target of the immunoreaction is the VGKC complex associated with LGI1. CASPR2 and GAD antibodies could not be detected (Pakozdy et al. 2013). Histologically mild T-cell infiltration and strong complement (C9neo) deposition and IgG infiltration were found. The presence of complement strongly resembles human VGKC encephalitis (Klang et al. 2014). The response to immunotherapy is currently under investigation. In conclusion FEPSO can be elicited by limbic encephalitis; however not all FEPSO cases have an inflammatory etiology. The most likely explanation is that FEPSO generally is a clinical characteristic of feline temporal lobe epilepsy (fTLE) (Sato 1975; Wada and Sata 1974), which can be elicited by vascular, toxic, developmental, and neoplastic causes and limbic encephalitis.

The feline limbic encephalitis of the cat represents a spontaneous animal model of an antibody-mediated epilepsy, with variable age at onset, and it is an ideal tool to study pathological changes in LGI1 antibody-mediated epilepsy. In this animal model, antibodies are produced spontaneously in affected cats and are proven pathogenic. The situation is completely different for other forms of epilepsy, in which patients have autoantibodies against neuronal proteins in the serum. This list of patients is still growing, but when novel antibody associations are detected, it is initially unclear whether the antibodies can access their target, interfere with its function, and are hence pathogenic or whether they are unable to do so and are just harmless bystanders of the disease. To discriminate between these two possibilities and to learn whether affected epilepsy patients would benefit from lowering their immunoglobulin levels in the serum, the antibodies in question are purified and transferred to rats and mice for further detailed analysis of any resulting clinical, physiological, or histological consequences. Clearly, such animal models are used to understand the basic pathomechanism of novel forms of antibody-associated epilepsies.

2.10 Synopsis

Epilepsy is one of the most important neurological disorders worldwide and does not only affect humans but also animals. Although the outcome of this disease has improved during the last years, due to the availability of modern diagnostic tools

and therapeutic measures, about one third of the epilepsies remains refractory to treatment and requires special attention of treating epileptologists and basic scientists. To further narrow the segment of intractable epilepsies, the basic pathomechanisms of novel forms of epilepsies have to be discovered, in order to develop and approve new therapeutic agents which could benefit human and animal patients alike.

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Chronic Kidney Failure Affects Humans and Other Mammalians

3

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Contents

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Abstract

CKD, both in animals and in humans, is a structural and/or functional impairment of one or both kidneys that has been present for more than 3 months, is normally irreversible, and gradually progresses, over time, to end-stage renal disease (ESRD). It is not a disease in itself but describes the stages of renal failure and their consequences caused by a multitude of underlying disorders. CKD in human is mainly caused by diabetes and hypertension and is, with an estimated prevalence of 8–13 %, a common disorder and a worldwide public health problem. The underlying causes in dogs and cats are largely not identifiable; however, CKD is also a common disease mainly in older dogs and cats, and it affects every third cat and 10 % of dogs older than 15 years. This chapter highlights the common disease mechanisms and often similar treatment strategies for CDK in dogs, cats, or horses and their owners.

3.1 Introduction

3.1.1 Comparison of Animal Kidney Disease to Human Disease

The definition and classification of chronic kidney disease (CKD) were introduced by the National Kidney Foundation (NKF) Kidney Disease Outcomes Quality Initiative (KDOQI) only as recently as 2002 (National Kidney Foundation 2002) and were subsequently adopted by the international guideline group Kidney Disease Improving Global Outcomes (KDIGO) with minor modifications in 2004 (Levey et al. 2005). The guidelines introduced the now generally accepted concept that CKD is not an uncommon life-threatening but a common condition with a range of causes and presentations.

CKD, both in animals and in humans, is a structural and/or functional impairment of one or both kidneys that has been present for more than 3 months, is normally irreversible, and gradually progresses, over time, to end-stage renal disease (ESRD) (Table 3.1). If untreated, ESRD causes death due to the retention of fluid and metabolic waste products (uremia or uremic syndrome) and therefore requires renal replacement therapy. CDK is not a disease in itself but describes the stages of renal failure and their consequences caused by a multitude of underlying disorders.

CKD, with an estimated prevalence of 8–13 %, is a common disorder and a worldwide public health problem that had moved in 2013 from rank 36 in 1990 to rank 19 on the worldwide list of global years of life lost, surpassing colorectal cancer on place 27 (McCullough et al. 2012; Jha et al. 2013). There is a rise due to aging population and the diseases responsible for chronic renal injury, hypertension, and diabetes mellitus.

Patients suffering from CKD consume a disproportionate share of healthcare resources although the exact costs of CKD and ESRD can only be estimated. Thus, spending for patients with CKD aged 65 and older exceeded $50 billion in 2013, representing 20 % of all Medicare spending in this age group (United States Renal Data System 2015).

Table 3.1 *Stages of CKD renal impairment* are defined by a GFR below 60 ml/min/1.73 m^2 for more than 3 months or when a patient's urine albumin-to-creatinine ratio is over 30 mg of albumin for each gram (g) of creatinine (30 mg/g)

Stage	GFR (normalized to an average surface area of 1.73 m^2)	Description	Treatment
1	>90	Normal kidney function but urine findings or structural abnormalities or genetic trait point to kidney disease	Observation, control of blood pressure
2	60–89	Mildly reduced kidney function, and other findings (as for stage 1) point to kidney disease	Observation, control of blood pressure and risk factors
3A 3B	*45–59* *30–44*	Moderately reduced kidney function	Observation, control of blood pressure and risk factors
4	*15–29*	Severely reduced kidney function	Planning for end-stage renal failure
5	*<15 or on dialysis*	Very severe or *end-stage* (established) kidney (renal) failure	Renal replacement therapy

Adapted from http://www.renal.org/information-resources/the-uk-eckd-guide/ckd-stages#sthash.0j9hk4eS.QEQpBK8q.dpbs. See more at http://www.renal.org/information-resources/the-uk-eckd-guide/stage-3-ckd#sthash.Ff8ewt2c.dpuf

Chronic kidney disease is also present if there are pathological abnormalities in blood or urine tests or imaging studies. The presence of chronic kidney disease should be established, based on the presence of kidney damage and level of kidney function (glomerular filtration rate [GFR]), irrespective of diagnosis

CKD is also a common disease in dogs and cats. The age of affected animals ranges from 9 months to 22 years, and the prevalence in general cat and dog populations is 1–3 % and 0.5–1 %, respectively. The overall incidence is not high, but increases with age, and it is common in old dogs and cats. Over 53 % of the cats with CKD are older than 7 years, and every third cat and 10 % of dogs older than 15 years suffer from CKD. In horse the prevalence in the general population is 0.12 % and increases to 0.26 % in old horses (0.51 % in old male horses) (Schott 2007).

3.1.2 Etiology and Pathogenesis

As mentioned above, CKD is not a disease but the consequence of a variety of disorders injurious to the kidney, and we have to distinguish underlying cause from accelerating (risk) factors and consequences or symptoms resulting from it. The cause of CKD differs around the globe: in high-income countries, diabetes and hypertension remain, with an age- and ethnicity-dependent variation, the most common causes of CKD and ESRD. Although generally rare, they are followed by glomerular diseases as the third leading cause, whereas HIV, hepatitis B and C, GN, and exposure to environmental toxins are common causes in low-income countries (Remuzzi et al. 2013).

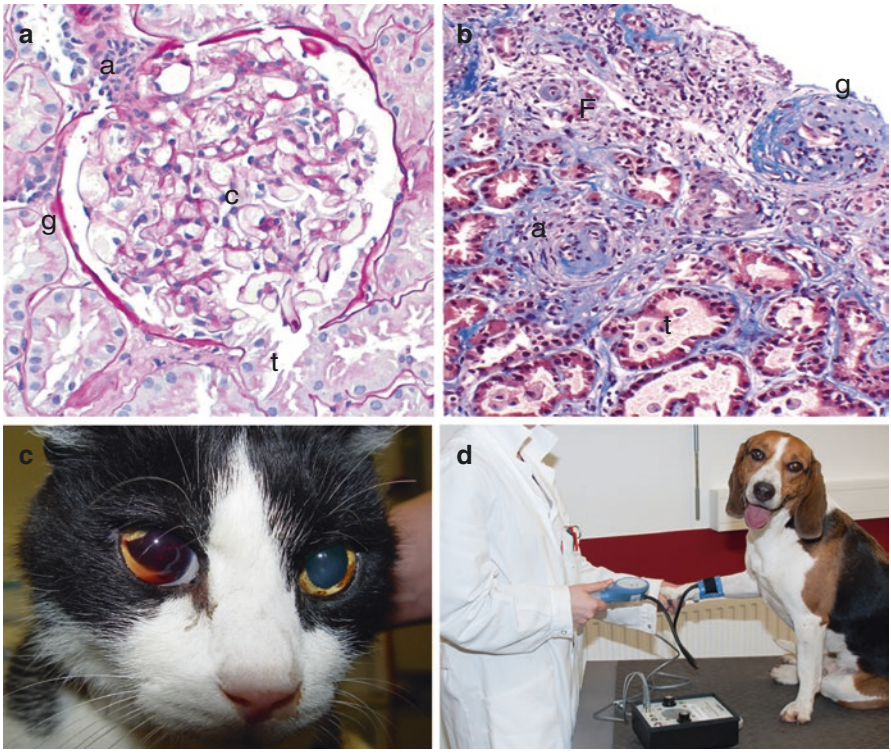

Fig. 3.1 The microanatomy changes in the kidney and clinical symptoms during CDK. (**a**) Normal kidney tissue with intact filter unit for urine production, the glomerulus. Glomerulus (*g*) with small artery (*a*) feeding into the capillary tuft (*c*) into which the urine is filtered from the blood and directed into the tubuli (*t*). (**b**) The microanatomic structure of the kidney is severely affected in CKD and results in loss of function. CKD due to hypertension is shown, leading to narrowing of the small arteries (*a*), increased fibrosis (*F*, blue stain), and reduced numbers to tubuli (*t*). Glomeruli (*g*) undergo scarring, called sclerosis. (**c**) Cat with hyphema (blood in the anterior chamber of the eye) due to systemic hypertension. (**d**) Blood pressure measurement in a Beagle dog

Mammalian kidneys are highly complex organs. They consist of arteries that transport the oxygenized blood to the kidney and veins that transport the oxygen-poor blood back to the heart. A specialized structure of the blood vessels are the glomeruli which consist of a convolute of small thin capillaries that filter the primary urine which is then concentrated in specialized epithelial tubes, the tubuli, before reaching the urinary bladder via the renal pelvis and the ureters (Fig. 3.1a). One glomerulus and the tubuli originating from it form the smallest renal unit called nephron. Pathological changes can involve all three compartments; however, usually one compartment is preferentially affected. Thus, we speak of primarily glomerular, tubulointerstitial, and vascular diseases.

Inciting causes for CKD are broadly divided into two groups, congenital and acquired. With the exception of diseases that are caused by mutations in a single gene (monogenic disorders), renal diseases in human often cannot be classified

into either category. Among genetic disorders, the most common inherited diseases are polycystic kidney disease and some forms of glomerulonephritis. Renal diseases are more often caused by a combination of genetic predisposition involving several genes (polygenic) and acquired factors. Thus, the most common causes of CKD in human, hypertension and diabetes mellitus, can have either a predominantly genetic or acquired cause however most often represent a combination of both. This is also true for most forms of glomerular diseases, commonly called glomerulonephritis, in which the genetic background predisposes for the mainly immunologically determined pathogenesis. These include diseases like focal segmental sclerosis (FSGS), the most common cause of nephrotic syndrome due to a multitude and combination of genetic, immunological, and environmental factors. Also membranous nephropathy (MN), IgA glomerulonephritis (IgA-GN), and the renal involvement in systemic lupus erythematodes are included in this group (McGrogan et al. 2011). Acquired tubulointerstitial diseases are predominantly caused by infections (ascending urinary tract infections or pyelonephritis), drugs (i.e. antibiotics), or renal toxicity of ingested substances like polyethylene glycol or mushroom poisoning.

Some common causes in cats and dogs, like kidney stones, are not a major contributor to CKD in human.

The most common congenital diseases in cats and dogs are polycystic kidney disease, renal amyloidosis, glomerular disease, Fanconi syndrome, and juvenile renal dysplasia. Polycystic kidney disease affects up to 38 % of the Persian cats, but is sinking due to early detection and breeding selections. In horses unilateral anomalies in the renal development leading to CKD play a more important role than in cats and dogs. Acquired diseases play an important role as a cause of CKD in cats, dogs, and humans. While in most canine, feline, and equine CKD patients this underlying cause is non-determinable at the time of diagnosis, in humans 75 % of adult cases are caused by diabetes mellitus, systemic hypertension, and glomerulonephritis. Kidney stones are found in up to one-third of cats with CKD and calcium imbalance; as consequence CKD predisposes to stone formation, the contribution of urolithiasis to the development of ESRD in human is much less clear. In equines immune-mediated disorders leading to glomerulonephritis and environmental toxins, like heavy metals, also can lead to the development of CKD.

The major difference between humans and cats, dogs, or horses affected by renal disease is that the underlying cause in human can be diagnosed by renal biopsy. This allows early therapeutic intervention, for example, using immunosuppression in immune-mediated disorders or treating symptoms and concomitant disorders like hypertension early to slow progression.

3.1.3 Pathophysiology of CKD

Most factors that initiate kidney damage leading to CKD remain unknown in dogs and cats, but it is hypothesized that the general pathophysiological process is similar to that described in human or other species. The self-perpetuating theory of CKD

states that one or more initial insults lead to the loss of nephrons. When the number of intact nephrons declines below a critical number, the remaining nephrons undergo a series of compensatory changes to maintain homeostasis. Unfortunately these changes cause additional tissue damage that ultimately results in inadequate excretory, regulatory, and endocrine function of the kidneys leading to the typical clinical signs and laboratory changes associated with CKD after two-thirds to four-fifths of all nephrons are lost. The data available indicate that, after the initial phase of injury and loss of the critical mass of functional tissue, the final pathway of compensation-driven progressive changes is the same in all mammalian species. In addition, this ongoing progression is largely due to factors that are independent of the primary kidney disease. In the end this self-perpetuating vicious cycle leads to renal failure, whether or not the inciting kidney disease is effectively treated.

The first step in the final pathway of maladaptive changes is an increased glomerular filtration rate (GFR, glomerular hyperfiltration) in the remaining glomeruli to compensate for reduced numbers of nephrons. This is achieved by increasing the blood flow in the remaining glomeruli (increased single-nephron GFR) accompanied by increased blood pressure and volume in the glomeruli (local glomerular hypertension) causing further glomerular damage. In step two an increased loss of normally in the blood retained high-molecular-weight proteins and cytokines, is observed in the urine (proteinuria). The reabsorption of these proteins from the primary urine in the tubular system leads to an inflammatory response in the tubulointerstitial space (step 3) that in step 4 of this process initiates progressive scarring. Interstitial mesenchymal cells, the fibroblasts, synthesize a new and disordered collagenous matrix that disrupts the small blood vessels (increasing oxidative stress, a result of chronic hypoxia that is likely to be important in the pathogenesis of feline CKD) and remaining tubules (step 5: tubulointerstitial fibrosis). In the final stage, step 6, surviving cells are isolated from local survival factors that provided a supportive microenvironment leading to cell death and formation of an acellular scar.

The loss of functional tissue below the critical mass and compensatory mechanism lead to impaired renal function resulting in typical clinical signs and complications of CKD. The kidney is a main contributor in the homeostatic regulation of electrolytes, maintenance of acid-base balance, regulation of blood pressure, regulation of extracellular fluid volume, maintenance of salt and water balance, excretion of waste products and production of red blood cells.

When GFR declines, the metabolic waste products, normally eliminated with the urine, accumulate, and the body is overexposed to these uremic toxins. Different clinical signs are caused or aggravated by these toxins like loss of appetite, gastritis with vomiting, and enteritis with diarrhea leading to weight loss and muscle wasting. The increase of the blood levels of creatinine and urea is inversely correlated with GFR, and therefore these markers are called indirect markers of GFR.

Diagnosis of CRF in the earliest periods of the disease is problematic because the ongoing loss of nephrons is generally non-detectable during the subclinical phase; also, gross clinical signs do not always correlate with laboratory changes in later stages of the disease. Identifying the initiating cause(s) is often impossible by the time the diagnosis of CRF is made because of the dissociation or non-detection of events with time.

Table 3.2 Consequences and symptoms of CKD

Function	Malfunction	Consequences	Symptoms
Excretion of fluid	Increased urine volume (lack of ability to concentrate urine)	Polyuria (increased volume of urine)	Loss of fluid (dehydration) Polydipsia
	Decreased urine volume	Oliguria (decreased volume of urine)	Retention of fluid (edema)
	No urine excretion	Anuria (little or no urine)	Retention of fluid (edema)
Excretion of metabolic waste or ions	Retention	Creatinine raised Urea (raised BUN) Electrolytes raised (i.e., hyperkalemia, hyperphosphatemia)	Azotemia or uremic syndrome: fatigue, somnolence, headache, itching, vomiting, inflammation of inner organs and mucous membranes, anemia caused by toxicity of waste products retained
Retention of substances normally not excreted	Loss of proteins or blood cells	Proteins (albumin): proteinuria Leukocytes (leukocyturia) Red blood cells (hematuria)	Hypoproteinemia Nephrotic syndrome (>3.5 g proteinuria/1.73 m^2 body surface area/day: edema, hyperlipidemia, hypertension) Nephritic syndrome (leukocyturia, hematuria, hypertension) Malnutrition, weight loss Anemia, risk for infections
Blood pressure regulation	Loss of renal tissue leads to activation of RAAS	Hypertension	Increased blood pressure
Erythropoietin	Decrease or loss of production of Erythropoietin	Failure to produce red blood cells	Anemia (fatigue, tiredness)
Vitamin D	Decreased renal synthesis of 1,25-(OH)2-vitamin D	Decreased 25-(OH)-vitamin D [25(OH)D],	Kidney-related bone disease

Uremia is the pathological manifestations of severe azotemia that refers to high levels of urea in the blood
BUN blood urea nitrogen

3.1.4 Symptoms of CKD in Humans and Animals

The signs and symptoms of renal disease are a result of the kidneys' loss of normal functions, summarized in Table 3.2. They are however not specific for the underlying disease and may include some symptoms also seen in other diseases, like nausea, vomiting, loss of appetite, fatigue, weakness, sleep problems, dizziness,

swelling of feet and ankles, and itching. In human, clinical signs become apparent early in acutely manifesting renal diseases, like some forms of glomerulonephritis. However, if kidney damage progresses slowly, they only develop over time and may become apparent as late as during stage 3 or 4 of CKD.

Major clinical signs of feline and canine CKD are, in the early course of disease, nonspecific and manifest late in the disease process, and some owners may recognize first symptoms not before stage 3. Polyuria and polydipsia are the earliest and most common clinical symptoms of CKD. Polydipsia, a compensatory mechanism to the renal water loss caused by the renal inability to adequately concentrate the urine (polyuria), is easily recognized, but not always recognized as a disease process by owners. In advanced stages, polyuria results in dehydration as fluid loss from the body exceeds fluid intake. Selective appetite, anorexia, and vomiting (gastrointestinal signs) leading to weight loss are manifestations of uremia and typical reasons for the owner to consult a veterinarian. Hypergastrinemia, reported in cats with CKD but not in dogs and humans, may induce gastric hyperacidity aggravating uremic gastritis, gastrointestinal bleeding, anorexia, and vomiting. Other reported clinical signs and complications are lethargy, weakness, depression, periodontal disease, gingivitis, small irregular kidneys, anemia, hypothermia, neurological signs (tremor, myoclonus, seizures), myopathies, uremic pericarditis and pneumonitis, hypothermia, and renal osteodystrophy. These disorders are multifactorial and their pathogenesis has not generally been investigated in cats. Constipation, due to dehydration and treatment with intestinal phosphate-binding agents, is more common than diarrhea in cats with CKD.

Hypertension is commonly associated with CKD and the main cause of ESRD in human. In turn, CKD is a major contributor to systemic hypertension in human, dogs, and cats. Spontaneous or experimental renal injury often leads to activation of the renin-angiotensin-aldosterone system (RAAS), a main regulator of the systemic blood pressure. Activation of RAAS results in increased levels of angiotensin II, which induces vasoconstriction of the vessels emerging from the renal glomeruli (efferent arteriole) causing increased pressure inside the glomerulum and consequently glomerular hypertension. The intrarenal renin-angiotensin system is correlated with the severity of kidney disease, but the underlying mechanism differs between dogs and cats (Mitani et al. 2013). Blocking the production of angiotensin II in cats with CKD using angiotensin-converting enzyme (ACE) inhibitors causes a decline in glomerular capillary pressure, the ratio of efferent to afferent arteriolar vascular resistance and proteinuria, but histopathological improvement or increased survival has not been shown. The application of angiotensin receptor blockers (ARBs) in cats reduces systemic blood pressure and proteinuria in cats with CKD. But this effect is only advantageous with hypertension and proteinuria. Cats with CKD without hypertension and proteinuria seem not to benefit from early treatment with ARBs despite RAAS activation. The influence of aldosterone and fibroblastic growth factor 23 could also contribute to the development of CKD via several pathogenic mechanisms.

High blood pressure progressively damages the blood vessels in the kidneys, narrowing them and thus driving chronic renal injury (Fig. 3.1c). This process is

however not confined to the kidney but affects all organs and reduces blood flow through them (ischemia). Sometimes ischemic organ damage like retinal changes (acute onset blindness or intraocular bleeding) is the first recognized symptoms of CKD (Fig. 3.1d) highlighting the necessity for blood pressure control as early as possible (Fig. 3.1e).

Proteinuria is a sign of kidney damage and a strong indicator for progression of CKD (Syme et al. 2006) and, independent of its underlying cause, associated with decreased survival time. Medium- to big-sized proteins are normally not filtered in the glomerulum, and filtered small-sized proteins are reabsorbed in the proximal tubule. Loss of proteins above the threshold of 3.5 g per 1.73 m^2 body surface area per day leads to decreased oncotic pressure in the blood system and to edema, an increase of the extracellular water. This characteristically manifests in swelling of the face (eye lids and lips) and the lower limbs.

Proteinuria and malnutrition often seen in patients suffering from CKD also result in a decrease of lipoproteins that transport cholesterol and other lipids in the circulation which leads to hyperlipidemia (or hypercholesterolemia).

Glomerular or tubular dysfunction leading to proteinuria causes renal inflammation and fibrosis accelerating the progression of CKD. Increasing proteinuria is positively correlated with intraglomerular pressure, serum creatinine concentration, and systolic blood pressure and a predictor of future progression, but the impact of proteinuria on progression of CKD in cats and dogs still has to be proven. At the moment it remains unknown whether proteinuria, like in human, is contributing to CKD progression or whether proteinuric CKD is intrinsically more rapidly progressive in cats and dogs.

Renal bone disease In human, CKD mineral and bone disorder (CKD-MBD) is a complex syndrome of bone and mineral metabolism caused by hormonal and metabolic abnormalities as a result of the declining renal function. It involves abnormalities of calcium, phosphorus, parathyroid hormone (PTH), and vitamin D metabolism that result in abnormal bone turnover, mineralization, mass and strength with increased risk of fractures. The most severe form of CKD-MBD is renal osteodystrophy (ROD) that is seen in patients with advanced CKD.

CKD induces phosphate retention, due to a decreased GFR, and causes reduced renal production of calcitriol, the active form of vitamin D, leading to a misbalanced calcium homeostasis. As a direct effect calcium absorption in the gastrointestinal tract is decreased. Indirectly hyperphosphatemia and low calcitriol level impair the action of parathyroid hormone [PTH] on calcium release from skeletal bone and renal calcium reabsorption resulting in hyperphosphatemia and hypocalcemia. Hypocalcemia, calcitriol deficiency, and hyperphosphatemia stimulate production of PTH by the parathyroid and cause parathyroid cell proliferation causing secondary renal hyperparathyroidism (srHPTH). Increased levels of PTH increase bone resorption and mobilize calcium from the bone which changes bone volume and microarchitecture and mineralization and sometimes results in pathological soft tissue (muscle or fat) and organ calcification or the formation of kidney stones.

Hypokalemia CKD-associated hypokalemia is often multifactorial and can cause mild to severe complications from muscle weakness to kidney dysfunction and cardiac arrhythmia. Therefore, CKD-associated potassium depletion could in turn contribute to progression of the renal disease and worsening of the clinical condition. Hyperkalemia is normally only a finding of stage 4 (end-stage) CKD.

Renal anemia CKD-induced anemia mainly results from impaired erythropoietin production of peritubular interstitial cells leading to reduced erythropoiesis (hypoproliferative anemia), malnutrition (iron deficiency), metabolic alterations, and uremic toxins that negatively affect red blood cell life span and promote gastrointestinal blood loss. Anemia might contribute to renal tissue hypoxia and therefore promote progression of CKD. Treatment of CKD-induced anemia is composed of application of erythropoietin, iron, gastric protection, a change in diet, and if necessary correction of metabolic acidosis.

Metabolic acidosis due to low plasma bicarbonate with high anion gap and low chloride concentrations has in feline CKD a prevalence of 22–88 % and is increasing with the severity of CKD. Contributing factors could be potassium depletion and impairment of renal ammoniagenesis leading to decreased urinary hydrogen ion excretion and decreased excretion of nonvolatile acids. Metabolic acidosis promotes many adverse clinical effects in the renal patient, including lethargy, anorexia, malnutrition, weakness, and vomiting.

3.1.5 Risk Factors of CKD Progression

The risk factors and comorbidities contribute, irrespective of the underlying cause, to the progression of end-stage renal failure (ESRD). As outlined above, in human they include first and foremost hypertension; however, consequences of renal injury, like proteinuria, and metabolic disorders, diet and lifestyle are recognized as major contributors to chronic disease progression. Thus, obesity seems to be a very strong risk factor for future risk of ESRD when present during young adulthood, while dieting has been shown to increase GFR in patients with BMI over 27 kg/m^2 (Tirosh et al. 2013).

The role of systemic hypertension and proteinuria in the development of CKD in cats and dogs remains unclear. Normal dog kidneys are not susceptible to induced systemic hypertension, and systolic blood pressure (SBP) has been reported to be minimally affected by aging in cats. The urine protein-creatinine ratio (UPC) is associated with the development of azotemia in cats, but aging in cats with CKD is not a risk factor for increasing proteinuria. However, in dog models, it has been proven that the renal autoregulatory mechanism, a critical function mainly of the glomerular afferent arteriole, is impaired in induced azotemia in dogs and that the degree of impairment is directly related to the severity of renal dysfunction (Brown et al. 1995). Therefore, systemic hypertension in dogs with existing CKD could cause barotrauma in the renal microcirculation contributing to renal injury and progression of CKD.

Proteinuria is a predictor of future progression of CKD confirmed in spontaneous CKD in cats, dogs, and humans. Therapeutic interventions that reduce

intraglomerular pressure and proteinuria, for instance, with angiotensin receptor blockers or angiotensin-converting enzyme (ACE) inhibitors, are renoprotective and therefore a cornerstone in the treatment of proteinuric CKD.

3.1.6 Diagnosis of CKD

CKD diagnosis relies on clinical and laboratory investigations including blood pressure, chemical blood, and urine profile that measure the levels of metabolites such as creatinine and blood urea nitrogen (BUN). A complete blood count indicates signs of anemia (Table 3.2). X-ray and ultrasound imaging are used to observe the size and shape of the kidneys as indicator of the stage of CKD. Kidney biopsy, as mentioned above, is the gold standard to evaluate renal pathology in human.

3.2 CKD in Humans

3.2.1 Clinical Course

Causes and risk factors for CKD progression have been outlined above. Symptoms of CKD do not vary from those observed in cats, dogs, or horses as they are determined by the consequences of loss of renal function. One major difference to the disease in animals is that in human the underlying pathology is established by morphological examination. Renal needle biopsy is today a safe way to a diagnosis early, at the first signs of kidney disease. The investigation of renal tissue by light microscopy, using special stains, by immunohistochemistry that detects the deposition of pathological immunoglobulins or proteins of the complement system and transmission electron microscopy (TEM) that allows to visualize pathological abnormalities at ultrastructural level, is supplemented by clinical examination, laboratory investigations, and genetic testing. These investigations have become the gold standard and are in most cases able to establish a diagnosis that guides subsequent treatment. Thus, we can distinguish whether a primary glomerular, tubulointerstitial, or vascular disease is present and whether the cause can be classified as inherited or acquired, immunologically or metabolically (i.e., diabetes mellitus) mediated, due to hypertension, infection (i.e., bacterial), or medication (e.g., due to analgesics). Morphology is also able to inform about the stage of acute and chronic renal injury.

3.2.2 Treatment

The therapy of CKD in humans aims at first identifying the underlying cause, if possible eliminating it and if not possible alleviating the resulting symptoms. Thus, immunologically mediated disorders are treated with immunosuppressant therapy; bacterial infections are treated with antibiotics or suspected drugs causing renal

failure eliminated. Treatment of underlying disease like diabetes mellitus includes blood sugar-lowering drugs or insulin. A cornerstone of CKD therapy is at the control of blood pressure which in itself is an accelerating factor of its progression. This is accompanied by dietary measures and change of lifestyle like exercise that is increasingly recognized as beneficial in CKD irrespective of the underlying cause. Measures to ensure slowing of CKD progression also include accompanying complex treatment strategies to correct ion and fluid balance or treat renal bone disease. Symptomatic intervention in CKD progressing to ESRD is generally an intermediate measure until renal replacement therapy becomes necessary. Unlike animals, humans do not usually die of uremia but are treated with hemodialysis (HD) which removes metabolic waste products from the circulation. A significant change in ESRD therapy is today the increasing numbers of patients who undergo peritoneal dialysis (PD) until renal transplantation, able to almost fully restore renal function, is performed. This allows patients – with the restrictions of immunsuppression – to follow an almost the normal lifestyle.

3.3 CKD in Dogs

3.3.1 Clinical Course

Much of our knowledge about CKD is derived from renal remnant kidney models in dogs that demonstrated the adaptive responses of nephrons were similar whether the reduction in functional renal mass occurred by surgical removal, infection, inflammation, or chemical injury and that adaptations were the same whether the primary disease affected the renal vasculature, tubulointerstitium, or glomeruli.

Diagnosis of CKD in the earliest periods of the disease is problematic because the ongoing loss of nephrons is generally non-detectable during the subclinical phase; also, gross clinical signs do not always correlate with laboratory changes in later stages of the disease. Identifying the initiating cause(s) is often impossible by the time the diagnosis of CRF is made because of the dissociation or non-detection of events with time.

Most common causes of CKD can include obstruction of the urinary tract or of the ureters, drugs, malignancies like lymphoma, diabetes mellitus, and genetic (hereditary) factors.

Typical clinical signs of renal failure in dogs are polyuria and polydipsia, weight loss, selective appetite, vomiting, diarrhea, lesions of the oral mucosa, uremic fetor, hypertension, and anemia (all symptoms together in the terminal stage of kidney failure are called uremia or uremic syndrome). Typical laboratory changes are isosthenuria (decreased urine concentrating ability), proteinuria (abnormal loss of proteins with the urine), azotemia (abnormally high levels of nitrogen-containing compounds such as urea and creatinine in the blood), hyperphosphatemia, and anemia. Diagnosis of CKD in the earliest periods of the disease is problematic because the ongoing functional loss is due to the compensatory mechanism generally non-detectable during the subclinical phase.

3.3.2 Treatment

CKD is a chronic disease and requires frequent checkups and monitoring of progression. There is currently no cure or measures to prevent it. Therefore, therapy of CKD in dogs, as in cats, is symptomatic and consists mainly of dietary measures and maintenance of the fluid balance. Adequate amounts of clean water ensure hydration to replenish depleted body fluid levels. In case of severe dehydration or refusal to drink, supplemental fluids may be given intravenously or subcutaneously.

Dietary protein intake may be restricted using specially formulated (commercially available) kidney diets to minimize the symptoms and slow the progression of the disease. Also diet low in phosphorus, calcium, and sodium and higher in potassium and polyunsaturated fatty acids (omega 6 and omega 3 fatty acids) may be beneficial to the kidneys.

Phosphorus binders and vitamin D supplements are given to improve calcium and phosphorus balance. H2 receptor blockers, or other medications to treat gastritis and gastric ulcers, can be helpful as they increase a dog's appetite. Depending on the symptoms and conditions, ACE inhibitors or ARBs and erythropoietin are prescribed to alleviate hypertension and anemia.

3.4 CKD in Cats

3.4.1 Clinical Course

Upper *urinary tract uroliths* (kidney or ureteral stones) were found in 15–29 % of cats with CKD in two studies, and azotemia and hyperphosphatemia are frequent findings in cats with ureteral calculi. In three-fourths of the cats with unilateral ureteral calculi, azotemia can be found, and in one-half of these, the other kidney is smaller than normal, indicating that a preexisting renal disease is common in cats with ureterolithiasis. After treatment of the urolithiasis, kidney function remains impaired in half of the cats, but it is difficult to differentiate whether ureterolithiasis is the cause or the consequence of CKD (Kyles et al. 2005).

Neoplastic diseases, as renal lymphoma, are with 16 % the most common primary renal neoplasm in cats and may cause CKD. Most cats with renal lymphoma are azotemic, but renal lymphoma might only be an additional disease in cats with unrecognized chronic kidney diseases and can also cause acute renal failure.

Hyperthyroidism, the most common endocrine disease of aged cats (>13 years), can be observed concurrently with CKD. The prevalence of preexisting CKD in hyperthyroid cats is similar to aged cats without hyperthyroidism and ranges from 14 to 40 %. Studies measuring urinary parameters of kidney function suggest that cats with hyperthyroidism have increased tubular lesions and dysfunction, and therefore hyperthyroidism may induce kidney damage. Problematic is that mild to moderate CKD is often undetected in cats with untreated hyperthyroidism as typical clinical symptoms like weight loss, vomiting, polyuria, polydipsia, and some parameters of

urine examination (proteinuria and low urine-specific gravity) are frequently encountered in both diseases. During hyperthyroidism, the main blood parameters for diagnosing CKD may falsely be normal, masking the CKD. Therefore, renal disease becomes apparent in 15–39 % of cats after treatment of hyperthyroidism.

Infections might play a causal role in the development of CKD, and *urinary tract infections* (UTIs) are considered a risk factor for the development of CKD. In cats there is some evidence of a link between the feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) infections and the development of CKD. Not all cats with CKD are infected with one of these viruses, but 9 % of FIV-infected cats have CKD, and FIV infection induces renal disease experimentally. The feline morbillivirus was also shown to be associated with tubulointerstitial nephritis.

Experimentally induced UTI can lead to chronic pyelonephritis, nephritis, and renal fibrosis in cats. In feline CKD, the prevalence of UTI is reported to be 17–33 % and that of chronic pyelonephritis has been estimated at 10–42 %, which might be due to decreased local and systemic defense mechanisms, urodynamic alterations, and changes in the composition of urine in CKD. In cats older than 10 years with UTI, two-thirds are affected by CKD, more than in the general population, but there is no association between UTI and markedly elevated serum creatinine concentration or other urine parameters. Periodontal disease has been identified as a clinical risk factor for CKD in dogs and cats.

Nephrotoxic drugs, including some anti-inflammatory drugs, or poisoning (e.g., lily poisoning) can cause acute kidney injury (AKI) that may be only subclinical. Incomplete renal recovery after AKI could contribute to CKD.

3.4.2 Risk Factors

Aging is considered a risk factor for the development of CKD in cats, and CKD is more frequently diagnosed in older cats (Piyarungsri and Pusoonthornthum 2016). The mortality rate due to CKD is also higher in senior and geriatric cats over 9 years old. Pathologic examinations in cats that die due to nonrenal causes often reveal clinically non-detected renal lesions (mainly tubulointerstitial lesions). The severity of these lesions increases with age, but only 30 % of geriatric cats develop azotemia, suggesting that aging per se is not the primary cause of development of azotemia. *Breed* appears to be a potential risk factor in cats, because Siamese, Persian, Maine Coon, Burmese, and Abyssinian cats are reported to be more likely affected. *Gender* is not a risk factor for the development of CKD.

Systemic hypertension is diagnosed in 20–65 % of cats with CKD, and azotemia is observed in 61 % of hypertensive cats (Jepson 2011). Therefore, CKD is supposed to be the main cause of hypertension in cats. But the relationship between hypertension and severity of renal interstitial fibrosis or inflammation, and therefore the role of hypertension as an independent factor of progression, remains unproven in cats. As in humans, CKD causing hypertension leads to the development of proteinuria, but proteinuria in cats is not predictive of survival. Nevertheless, it is assumed that untreated hypertension might lead to more severe renal lesions, and in cats receiving antihypertensive therapy with amlodipine, this is proven.

Cardiovascular disease resulting from, or independent of, systemic hypertension has been reported in cats with CKD. The prevalence of left ventricular hypertrophy has been estimated to be 46.6 % in cats with CKD. About 59 % of cats with hypertrophic cardiomyopathy (HCM) are azotemic and 12.7 % have CKD. Systolic arterial pressure is also higher in azotemic than in non-azotemic cats with HCM. Investigations are needed to document the pathophysiology of concomitant CKD and cardiac disease (Gouni et al. 2008).

Experimentally induced short but severe *hypoperfusion* of one kidney with oxygen undersupply and acute tissue damage develops in the long term to pathohistological changes similar to the pattern of CKD. This implicates that drugs and diseases that cause transient renal hypoperfusion might cause mild kidney damage that contributes to the development of CKD (Brown et al. 2016).

In cats with *primary hyperaldosteronism*, mild azotemia is often found beside hypokalemia and systemic hypertension, and a gradual increase in azotemia and the development of CKD have been observed in such patients. Furthermore, CKD was reported as a cause of euthanasia in cats with primary hyperaldosteronism. The histopathological renal changes were similar to those observed in humans with primary hyperaldosteronism and might also be a mediator of CKD in cats.

While exclusive feeding of an imbalanced protein-rich, acid-rich, and potassium-depleted diet over a long time period was shown to induce CKD in healthy cats, ad libitum feeding and increased ash intake (the nonorganic mineral elements in food) might only be associated with an increased risk of CKD. The negative effect of diets containing increased amounts of salt on blood pressure and renal function was not confirmed in a study of aged cats. In general it seems that not individual nutrients might be the import factors but the patterns of usual dietary intake.

A recent study identified that risk factors for developing CKD in cats are male sex and outdoor lifestyle. Commercial dry cat food and an indoor lifestyle were associated with a decreased risk. In this study tap water increased and filtered water decreased the risk, but this might not be true for all regions and depends on the tap water quality.

Clinical signs in the early course of disease are nonspecific and manifest late in the disease process. Some owners may recognize first symptoms not only before stage 3, and the frequency of clinical signs is similar in stages 2 and 3 as defined by the International Renal Interest Society (IRIS). Polyuria and polydipsia are the earliest and most common clinical symptoms of CKD. Polydipsia, a compensatory mechanism to the renal water loss caused by the renal inability to adequately concentrate the urine (polyuria), is easily recognized by owners, but not always recognized as a disease process. In advanced stages polyuria results in dehydration as fluid loss from the body exceeds fluid intake. Selective appetite, anorexia, and vomiting (gastrointestinal signs) leading to weight loss are manifestations of uremia and typical reason for the owner to consult a veterinarian (Fig. 3.2b). Hypergastrinemia, reported in cats with CKD but not in dogs and humans, may induce gastric hyperacidity aggravating uremic gastritis, gastrointestinal bleeding, anorexia, and vomiting. Other reported clinical signs and complications are lethargy, weakness, depression, periodontal disease, gingivitis, small irregular kidneys, anemia, hypothermia, neurological signs (tremor, myoclonus, seizures), myopathies, uremic

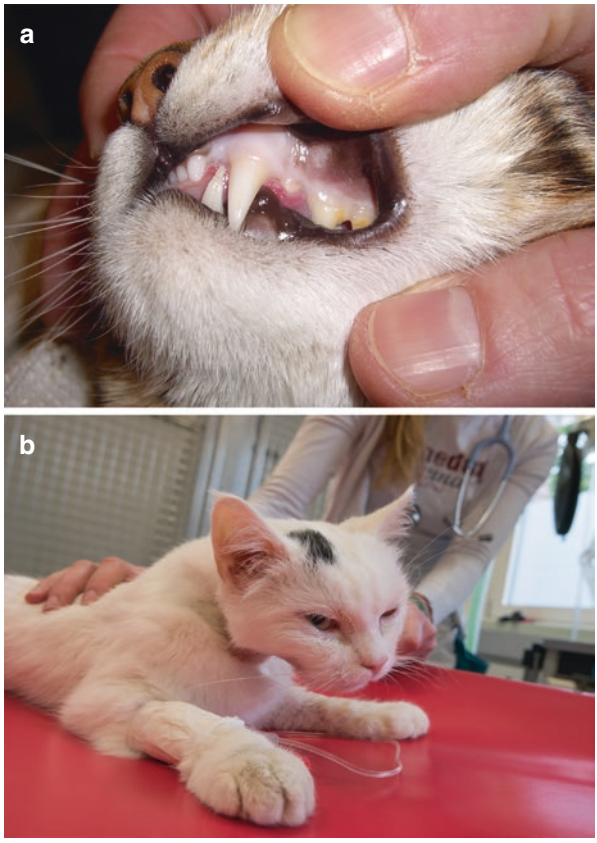

Fig. 3.2 Clinical symptoms in CKD in cats. (**a**) Pale mucous membranes in an anemic cat with CKD. (**b**) Cat suffering from end-stage chronic renal disease

pericarditis and pneumonitis, hypothermia, and renal osteodystrophy. These disorders are multifactorial, and their pathogenesis has not generally been investigated in cats.

Thirty to sixty-five percent of cats with CKD develop anemia which is a frequent finding in cats with end-stage CKD and also adversely affects the quality of life of cats with CKD.

Constipation, due to dehydration and treatment with intestinal phosphate-binding agents, is more common than diarrhea in cats with CKD.

Elevated levels of some of cytokines indicating progressive CKD, as outlined above, can be found in the urine of cats with CKD. Proteinuria in cats with naturally occurring CKD is generally mild compared with their canine and human counterparts with 90 and 49 % of cats with CKD having a UPC of <1.0 and <0.25, respectively. Proteinuria in azotemic cats and dogs with CKD stages 2–4 is treated when the urin protein/creatinin (UP/C) ratio is ≥0.4 or ≥0.5, respectively. While in non-azotemic CKD (stage 1), treatment is only initiated with a UP/C level >1 in cats and >2 in dogs. Treatment consists of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers together with dietary protein restriction.

Hypertensive retinopathy is often the first symptom of CKD. The pathogenesis is multifactorial including RAAS activation, impaired excretion of sodium,

stimulation of the sympathetic nervous system, arterial structural changes, endothelial dysfunction, and oxidative stress. Hypertensive organ damage increases morbidity and mortality in cats and dogs with CKD, but it also adversely affects renal function by inducing or worsening of glomerular hypertension and proteinuria (Finco 2004).

Hyperphosphatemia and increased plasma parathyroid hormone (PTH) concentrations are observed in 13–100 % and 47–100 %, respectively, of cats with CKD. With increasing severity of CKD, the srHPTH increases reaching 100 % in end-stage renal failure (King et al. 2007). Fibroblast growth factor 23 (FGF-23) is a phosphaturic hormone involved in the pathogenesis of RHPTH in humans and can also be used as a marker for CKD. In cats with CKD, plasma FGF-23 is strongly correlated with plasma creatinine concentration and PTH, showing an inverse relationship with GFR, but no correlation with the plasma phosphorus concentration (Finch et al. 2013). Clinical signs resulting from calcium phosphate precipitation induced by srHPTH into tissues are uncommon in cats with CKD, but metastatic paw calcification and osteoporosis have been reported.

Hypokalemia is a common laboratory finding in cats with CKD stages 2 and 3 (18–30 %) and is the most common cause of hypokalemia in cats.

Tubulointerstitial fibrosis appears to be the common final outcome of most feline CKD. Primary glomerular diseases are rare and develop commonly secondary to systemic disease, specifically neoplastic (e.g., leukemia, lymphoma), infectious, and noninfectious inflammatory disorders (e.g., pancreatitis, immune-mediated diseases). The presence of interstitial fibrosis is the strongest histomorphometric predictor of plasma creatinine concentration. Tubular damage occurs early in the course of disease, before elevations in serum urea and creatinine concentrations. Although cats are able to maintain urine concentrating ability after subtotal nephrectomy, urine-specific gravity decreases as plasma creatinine increases in cats with CKD, suggesting a close relationship between glomerular and tubular function during the progression of feline CKD.

3.4.3 Treatment

As mentioned above, CKD is diagnosed and the symptoms treated, irrespective of the underlying disorder. Treatment is in most cases symptomatic and relies on dietary measures, blood pressure control with ACE inhibitors and ABRs. Treatment of urolithiasis relies on surgical intervention. If necessary, diabetes mellitus is controlled with insulin. There is currently no means of preventing CDK.

3.5 CKD in Horses

3.5.1 Clinical Course

The most common symptoms of CKD recognized by owners are weight loss (86 %), polyuria and polydipsia (56 %), and ventral edema (42 %). Other symptoms are decreased appetite, rough hair coat, lethargy, and poor athletic performance.

Identifying renal disease, like in human and other species, is based on a thorough history, physical examination, complete blood count, serum biochemistry, and urinalysis. The presence of serum concentrations of blood urea nitrogen and creatinine above the upper limit of the reference range (azotemia) in conjunction with not adequately concentrated urine based on urine-specific gravity (USG) is, like in other species, the hallmark of renal disease (Divers 2009). Other laboratory findings that may be associated with chronic kidney disease are mild anemia (40%), proteinuria, hypoalbuminemia (86%), hyperkalemia (56%), hyponatremia (65%), hematuria, and metabolic acidosis in end-stage cases. Unlike in cats and dogs, hypercalcemia (56–67%) with or without hypophosphatemia (47%) is a common finding in horses with CRF.

While it is suspected that the pathophysiological mechanisms leading to weight loss, polyuria and polydipsia, isosthenuria, anemia, proteinuria, and most other abnormalities are the same as in humans and dogs or cats, the high rate of hypercalcemia is special for horses and ponies (LeRoy et al. 2011). In healthy horses 1α-hydroxylase was not detected in the kidneys, and it may be speculated that vitamin D-mediated control of serum calcium concentration must differ in the horse. Serum PTH concentrations in horses with CKD were very low, suggesting that CKD does not cause hyperparathyroidism in horses (Estepa et al. 2003). Some authors believe that hypercalcemia in equine renal failure is simply the result of ongoing intestinal absorption with impaired renal tubular excretion. It is also believed that the self-perpetuating theory of CKD can be applied to equine CKD, as equine patients progress to end-stage renal disease like dogs and cats.

3.5.2 Treatment

Treatment of horses with CKD is focused on the maintenance of a good body condition. Therefore, a high-quality diet, rich in carbohydrates with a moderate protein content, additional oil supplementation (e.g., omega 3 fatty acid-rich fish oils), and adjusted calcium content, is fed. Infusions are useless in most cases. Corticosteroids are only used in selected cases with glomerulonephritis and proteinuria. Until now there are no published studies about the use and possible benefits of angiotensin-converting enzyme inhibitors in horses with CKD and hypertension or proteinuria.

3.6 Synopsis

CKD describes the state of renal excretory function and the pathophysiological consequences caused by it. It is not a disease in itself but the common pathway of chronic renal injury, irrespective of the original cause, that ultimately leads to ESRD. CKD is often diagnosed late in dogs, cats, and horses, and the underlying renal diseases are unknown. The natural disease and its course in these animals can therefore not serve as models to study or compare the etiology and course of renal disease more generally.

Most of our knowledge about the pathomechanisms of diseases underlying the development of CKD in human is derived from renal biopsies, genetic investigations in humans, and rodent models of renal injury. Naturally occurring genetic disorders in cats and dogs could however provide suitable models to study the corresponding diseases, like polycystic kidney disease and amyloidosis, in human. This said, much of our knowledge about the pathophysiology and consequences of the loss of renal tissue is derived from now abandoned studies in dogs and cats. These demonstrated that mechanisms of CKD and progression to ESRD are not significantly different in those animals and human.

Consequently, treatment strategies to alleviate symptoms of loss of renal function and comorbidities, like hypertension or ROD, are strikingly similar in dogs, cats, or horses and their owners. These rely mostly on drugs developed for use in human (like ACE inhibitors, ARBs, insulin); however, we can assume that investigation of their mode of action in cats or dogs, monitored in controlled clinical studies, would augment our knowledge and thus profit human therapy.

One major difference between human CKD and that in animals is the availability of long-term renal replacement therapy once ESRD is reached in human patients. Peritoneal and hemodialysis or renal transplantation replace or restore renal function in human while treatment of animals remains symptomatic and aims at controlling factors that accelerate or drive progression of CKD.

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Inflammatory Bowel Disease in Humans, Pets, and Horses

4

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Contents

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Abstract

Inflammatory bowel disease (IBD) is a group of chronic inflammatory conditions of the gastrointestinal tract. In humans, two major types exist: Crohn's disease and ulcerative colitis. Similarly, also pets can suffer from IBD having predominantly lymphocytic-plasmacytic enteritis/colitis, but also eosinophilic enteritis/colitis is common. Accumulating evidence suggest the induction of an inappropriate inflammatory response to intestinal microbes in a genetically susceptible host as well as a dysregulation of the intestinal microbiota.

Symptoms of IBD are similar in humans and pets and may include diarrhea, vomiting, weight loss, anemia, as well as extraintestinal manifestations affecting the eye (uveitis), skin (rash), and joints (arthritis). Diagnosis of IBD requires a number of tests with endoscopy being the best tool to determine IBD. Biopsies of the mucosa are taken for differential diagnosis of IBD. As the pathogenesis of IBD is still unresolved, but implicates genetic factors, microbes, diet, and an aberrant immune response, so far treatment regiments only aim to control the disease activity by immunosuppressive medications like glucocorticoids. However, novel treatment options are underway that aim to reset the microbiota and showing promising efficacy.

There are thus many similarities in the clinical picture of IBD affecting humans, horses, and dogs, which not only give us great insight in the pathogenesis of IBD, but enable the pursuit of novel treatment options.

4.1 Introduction

Inflammatory bowel disease (IBD) describes conditions with a chronic or recurring immune response and inflammation of the gastrointestinal tract. The two most common entities of IBD in humans are ulcerative colitis (UC) and Crohn's disease (CD). IBD is caused by a combination of environmental, immune, and bacterial factors in genetically susceptible individuals (Fig. 4.1).

Peak onset of IBD in humans is usually between 15 and 30 years of age, but can occur at any age. There seems to be no gender bias for the occurrence of IBD as men and women are equally affected.

Inflammatory bowel disease resulted in 34,000 human deaths in 2010 (GBD 2013 Mortality and Causes of Death Collaborators 2015) and slightly reduces life expectancy.

4.1.1 Genes and Environment

In humans, the familial risk to develop IBD is tenfold higher and strongly suggests that these disorders have a genetic cause (Orholm et al. 1991).

Both genetic and environmental factors may influence the prevalence of IBD in the general population, as well as the degree of familial aggregation of IBD. The

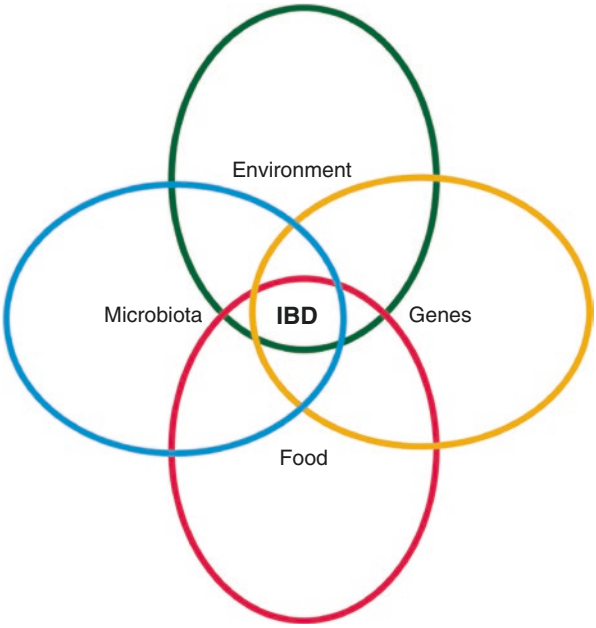

Fig. 4.1 The IBD pathogenesis is driven by the genetic background, environment, microbial flora, and the immune system

relative importance of these factors in causing familial aggregation may differ between low- and high-prevalence areas.

Worldwide, an increasing incidence and prevalence of IBD is observed with similar high-prevalence rates reported for Europe and North America. Based on the estimates, approximately 0.6 % of the Canadian population suffers from IBD, with UC being more prevalent than CD (Molodecky et al. 2012). Caucasians and people of Jewish descent also seem to have an increased risk, though it also seems to depend on the westernized lifestyle, as individuals emigrating from low-prevalent regions to higher prevalent countries (e.g., England) acquire increased risk for developing IBD (Molodecky et al. 2012).

Genome-wide association studies identified susceptibility loci that – triggered by environmental factors – result in a disturbed innate (e.g., disturbed mucosal barrier structure and function, impaired recognition of microbes) and adaptive (e.g., imbalance in regulatory and effector T cells) immune response toward a diminished diversity of commensal microbiota (Baumgart and Sandborn 2012).

There exist differences in genetic backgrounds for IBD, e.g., the NOD2 mutation is associated with CD in Western countries, but not in Japan, China, or Korea. Despite the discrepancies in genetics, familial aggregation is still observed to a similar degree in Asian and Western countries. Genes associated with IBD are involved in mucosal immunity and include roles in barrier function and microbe recognition (Park et al. 2006). In canine IBD patients, certain breeds as Soft-coated Wheaten Terriers, Lundehunds, or German Shepherds seem to be overrepresented in veterinary

literature (Littman et al. 2000) suggesting that also here the genetic background increases the risk of IBD. Recent genetic studies support this concept as certain polymorphisms in the Toll-like receptor-4 (TLR4) and TLR5 genes were significantly associated with inflammatory bowel disease in German shepherd dogs (Kathrani et al. 2010). In horses, genetic studies are ongoing. Granulomatous enteritis, a form of IBD with histological similarities to human Crohn's disease, shows a familial predisposition in Standardbred and Thoroughbred horses suggesting a genetic component (Lindberg 1984). No data exist regarding the prevalence of eosinophilic IBD in horses, although their incidence rate seems to rise. Also human patients have been reported to be afflicted in very rare cases with eosinophilic esophagitis, gastroenteritis, or colitis (Cianferoni and Spergel 2015). A possible relationship to a decreased level of intestinal parasitic infections is under discussion. The prevalence of lymphocytic-plasmacytic enterocolitis in horses was stated as 0.02 % (Kemper et al. 2000), but van der Kolk et al. (2012) noticed much higher prevalence especially in dressage horses. Though genetics set the ground for human and animal IBD, environmental and psychological factors are able to modulate and trigger the disease (Fig. 4.1).

4.1.1.1 Food and IBD

What animals and humans consume also exerts an influence on the development of IBD. Whereas high intakes of total fats, polyunsaturated fatty acids, omega-6 fatty acids, and meat are associated with increased risk of human IBD in general, high vegetable intake is associated with a decreased risk of UC, and intake of fibers and fruits is associated with a reduced risk of CD (Hou et al. 2011). Specialized dietary formulations (elemental, semi-elemental, and polymeric diets) have been studied in the treatment of pediatric CD, and although the exact mechanism is not fully understood, they reduce disease activity and symptoms (Levine and Wine 2013). There exists also a link between vitamin D and IBD, with low vitamin D levels associated with an exacerbation of the disease (Vatn and Sandvik 2015). Also a high-iron content in drinking water has been associated with an increased risk of developing IBD in Norway (Aamodt et al. 2008).

4.1.1.2 Smoking

Active and passive smoking significantly increase the risk to develop CD, even though apparently neither nicotine nor carbon monoxide is the cause (Baumgart and Sandborn 2012). In contrast, in UC smoking is protective and, after the onset of the disease smoking improves its course. Smoking cessation on the other hand aggravates UC (Lakatos et al. 2007).

4.1.1.3 Stress

Stress seems to increase the risk of symptoms in humans. Indeed, a high perception of stress correlates with an increase in symptoms. Although many individuals with active inflammation have active symptoms, there are also many individuals with active symptoms and no inflammation (Bernstein 2015). Also in cats and dogs, physical or psychological stress are likely to contribute in the etiology of IBD. As such, abnormal personality traits and potential environmental stress factors have been reported in 14 of 37 dogs (37.8 %), diagnosed with chronic idiopathic large bowel diarrhea (Leib 2000).

4.1.2 Immunobiology and the Microbiota

There exists an intricate balance between microflora, the intestinal barrier, and the immune system in the gut. Factors changing one of these three key players will have influence on the other two. Hence, changing the microflora does have an influence on the gut permeability and differentiation of intestinal epithelial cells and also changes the immune repertoire.

The microflora differs among human, dog, and horse (Kararli 1995). In humans, the stomach, duodenum, and proximal jejunum are predominantly colonized by aerobic organisms that include Streptococci and Lactobacilli with occasional *Candida* spp. (Balfour Sartor 2007). This changes in the distal ileum, where commensal bacteria take up an anaerobic predominance that more closely mimics the colon than the upper part of the small bowel. Dominant colonic organisms in humans include *Clostridium* spp., *Bacteroides*, and *Bifidobacterium* (Balfour Sartor 2007). Thus in human, *Firmicutes* and Proteobacteria phyla predominate in the duodenum, whereas in the distal colon *Firmicutes* and *Bacteroidetes* are the predominated phyla. Only few organisms are found in the upper gastrointestinal tract in humans. Indeed typical concentration of bacteria in the stomach, duodenum, and jejunum is 10^3–10^4 bacteria/ml content, which increases in the ileum to 10^8 bacteria/ml content. In the human colon, approximately 10^{11} bacteria/ml are measured; thus, approximately 3.9×10^{13} bacteria reside in the "standard" human colon (Sender et al. 2016). In contrast, in dogs a substantial microflora exists throughout the gastrointestinal tract with *Enterobacteriales* more common in the small bowel and *Clostridiales*, *Fusobacteriales*, *Bacteroidales*, and *Lactobacillales* present in the small intestine and the colon (Suchodolski et al. 2008). Thus, contrary to humans, horses, and chicken, *Fusobacteria* appear to be one of the major bacterial group (Suchodolski et al. 2008).

Commensal bacteria do not only help in digestion, but also promote epithelial cell growth and differentiation. Importantly, they also shape the immune repertoire, and as such germ-free mice are immunodeficient (Sansonetti 2004).

IBD seems to result from an impaired interaction of the intestinal commensal microbiota that is normally in a state of symbiotic mutualism with the human, canine, feline, or equine hosts. As such it is not an autoimmune disease, but rather seems to be an immunodeficient state (Bianco et al. 2015).

A number of specific pathogens have been incriminated in the development of IBD, though none have been confirmed as causal; rather, microbial antigens that are normally present in the intestinal lumen seem to drive inflammation in the gut (Abraham and Cho 2009).

4.1.2.1 Mucosal Immune System

In the intestine, innate immunity includes the epithelial barrier and phagocytic cells within the lamina propria (e.g., macrophages, dendritic cells, and neutrophils). The key population of the adaptive immunity arm is represented by T lymphocytes.

4.1.2.2 Epithelial Barrier

A single polarized epithelial layer covered by mucus film secreted from goblet cells represents the first-line defense of the mucosal immune system. Decreased expression of mucin as well as increased permeability and defective regulation of the tight junctions (Baumgart and Sandborn 2012) as well as defective sensing of microbial products is observed in IBD (Dahan et al. 2008). These abnormalities may be due to a primary defect in barrier function or may be the outcome of inflammation.

Macrophages and Dendritic cells

Macrophages in the gut mucosa display an anergic signature (are non-reactive) and do not produce inflammatory cytokines, but retain phagocytic and bactericidal activity. In IBD, however, a large number of macrophages recruited from the blood infiltrate the intestinal mucosa and secrete inflammatory cytokines (Smith et al. 2011; Kuhl et al. 2015).

4.1.2.3 T cells

T helper cell type 1 (Th1)-mediated immune responses are typically evoked in response to intracellular pathogen presented by antigen-presenting cells, with granuloma representing the hallmark of a Th1 response (Siegmund and Zeitz 2011). Th2 cells promote atopy, the induction of IgE responses, and eosinophil and mast cell activation. Th17 cells seem to specifically promote local tissue destruction. Last, there are also regulatory T cell populations that are responsible for the immunologically suppressive milieu in the intestinal mucosa.

The hallmark of active IBD in humans is a pronounced infiltration of innate immune cells (neutrophils, macrophages, dendritic cells, and natural killer T cells) and adaptive immune cells (B and T cells). The adaptive immune system in human IBD is now thought to mediate and perpetuate, but probably not start, intestinal inflammation. The disorder is characterized by an imbalance of effector T cells and regulatory T cells. Similarly as in humans, greater expression of Interleukin-17mRNA and Toll-like receptor 4 has been reported in horses with chronic active simple proctitis suggesting that Th17 cells are involved in active IBD in this species as well (Olofsson et al. 2015).

4.1.2.4 Eosinophils

Eosinophils are granulocytes that are associated with host defense against parasitic helminths, contribute in the pathology of allergic conditions, and also do play a role in immune regulation (Muniz et al. 2012). Eosinophils in human have been associated with fibrosis and strictures in humans with Crohn's disease (Masterson et al. 2015). In dogs, eosinophilic infiltration of the intestinal mucosa is considered as a subtype of IBD, whereas in cats the transition to a hypereosinophilic syndrome is less defined (Guilford 1996a, b). In horses, eosinophilic infiltrations are commonly detected in the rectal mucosa and submucosa of clinically normal horses suggesting that the presence of eosinophils in biopsies is no

proof of eosinophilic enteritis (Sloet van Oldruitenborgh-Oosterbaan and Grinwis 2014).

The IBD-associated dysbiosis might be due to the host's genotype that influences the composition of the microbiota, but also infections, antibiotics, drugs, and the diet are known contributors of a dysbiosis. Once this regulatory balance is disturbed, activation of leucocytes can lead to production and release of increased amounts of inflammatory molecules, which may lead to the development of chronic intestinal inflammation. Also in dogs and cats the enteric flora has an important impact on the etiogenesis of IBD. As such, significantly more mucosa-associated *Enterobacteriaceae* were reported in cats affected by IBD compared to healthy cats (Janeczko et al. 2008), and in dogs the number of clones belonging to the family of *Clostridiaceae* positively correlated with the clinical severity score (Xenoulis et al. 2008).

4.2 IBD in Humans

4.2.1 Clinical Picture

Primarily two types of chronic intestinal disorders exist in humans: Crohn's disease (CD) and ulcerative colitis (UC). Though similar symptoms like persistent diarrhea, abdominal pain, and cramping can be observed in both, they differ greatly in appearances and pathophysiology.

4.2.1.1 Crohn's Disease Versus Ulcerative Colitis

CD can affect any part of the gastrointestinal tract from the mouth to the anus, even though mostly the terminal ileum and the colon are affected, often discontinuously (Table 4.1). In contrast, inflammation in UC is continuously and restricted to the colon and rectum, seldom the anus (Fig. 4.2a, b). Also in CD, the inflammation is often transmural, and hence intestinal granulomas (Fig. 4.2b), strictures, and fistulas are common. Hence, chronic inflammation can make the inside of the intestine in CD so narrow that nothing can pass through. This is known as bowel obstruction, and it hinders digesting food and gas in the passage. The symptoms include severe cramping, nausea, vomiting, and a swollen belly. Bowel obstructions are treated in the hospital. If the obstruction does not clear on its own, surgery may be required. The transmural intestinal inflammation in CD may also generate deep ulcers with a pocket of pus, called an abscess.

Symptoms include fever, pain, and swelling. If an ulcer breaks through to an adjacent organ, it creates a tunnel called a fistula. A fistula between the colon and the vagina can allow bacteria into the vagina. A fistula to the bladder can cause chronic urinary tract infections. One that reaches the skin can create external sores. Fistulas and some abscesses are often treated with surgery.

In UC inflammation is restricted to the epithelial lining of the gut (Table 4.1 and Fig. 4.2a). While in UC pain associated food avoidance may lead to weight loss, in CD nutrient deficiencies are more common as it affects the small intestine, which is

Table 4.1 Important differences in Crohn's disease and ulcerative colitis

	Crohn's disease	Ulcerative colitis
Clinical picture	Affects any part of gastrointestinal tract from the mouth to the anus, mostly *terminal ileum*, rectum seldom, anus involvement common *Discontinuously*	Is restricted to the colon and *rectum*, seldom the anus *Continuously*
	Affects all bowel wall layers ("transmural lesions"), granuloma common → bowel perforation *Patchy* areas of inflammation *Nutrient deficiency*	Restricted to the mucosa (epithelial lining of the gut), granuloma seldom *Colon cancer risk*
Signs & symptoms	Diarrhea, abdominal pain, weight loss due to food avoidance and *malabsorption*	Diarrhea, abdominal pain Weight loss due to food avoidance
	Strictures, *perforations*	Rectal bleeding → *anemia*
Genes and environment	Runs in families, siblings 30 times more likely to develop CD than the general population 30 genes associated, most known NOD2 = CARD15 Associated with *Mycobacterium* and other pathogenic bacteria Higher risk for smokers	Aggregation in families, identical twins with concordance rate of 10 %, dizygotic twins with 3 % Ethnic differences 12 regions in genome are slightly linked Lower risk for smokers
	Majority of genes associated with IBD are involved in mucosal immunity, including roles in barrier function and microbe recognition	

responsible for nutrient absorption. The symptoms of IBD range from mild to severe and may return periodically over time. Most people have flare-ups folowed by longer asymptomatic periods, so-called remissions. Remissions can last for months or even years.

In UC, about 5–10 % of patients have symptoms all the time. The constant chronic inflammatory status of people affected with UC also makes them more susceptible for developing colon cancer. The risk is even greater when inflammation affects the entire colon.

Extraintestinal symptoms of IBD include inflammatory responses affecting the eye (uveitis), skin (rash), joints (arthritis), and bile ducts (cholangitis). IBD also increases the risk of deep venous thrombosis and autoimmune hemolytic anemia. Also clubbing, a deformity of the ends of the fingers, may be a result of IBD (Danese et al. 2005).

4.2.2 Pathophysiology

For the development of IBD, bacteria are decisive, and this is emphasized from animal studies in which IBD-susceptible mice (e.g., IL-10 knockout mice) do not develop IBD in the absence of a microbiota. Moreover, it is known that the diversity

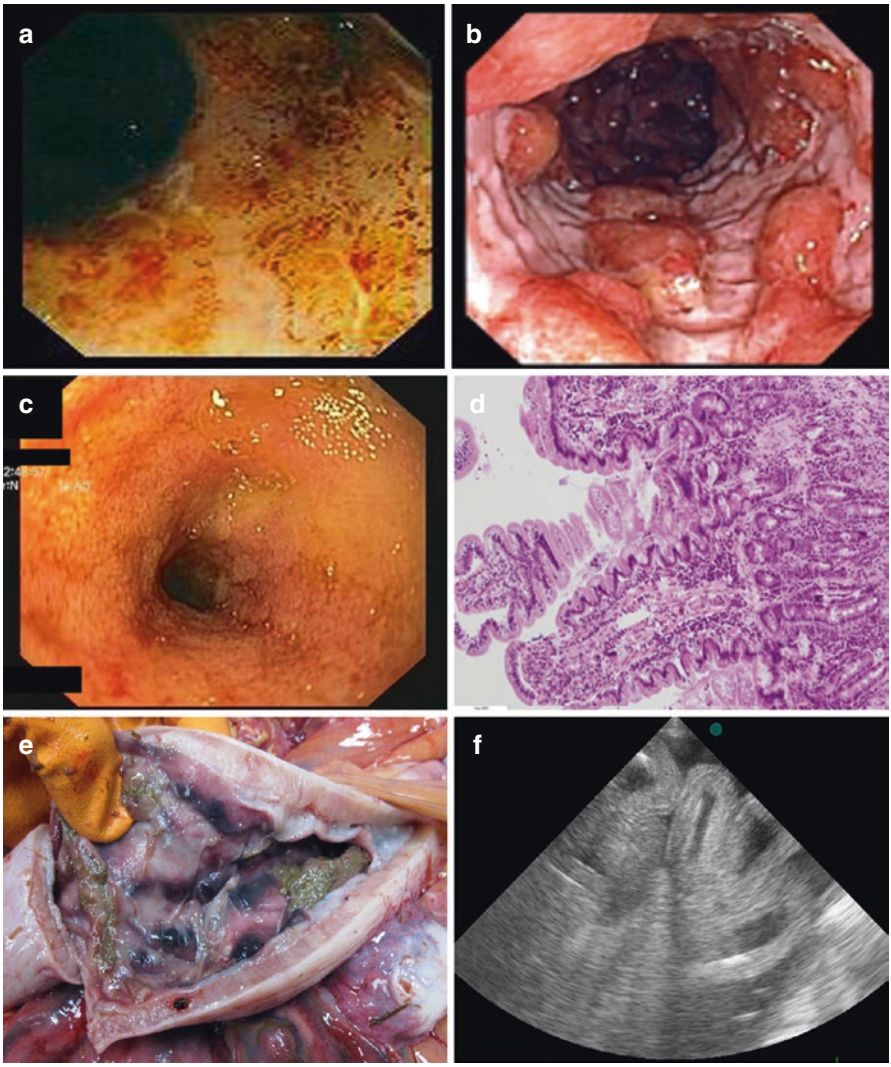

Fig. 4.2 Representative images from IBD lesions in different species. (**a**) Endoscopy in human ulcerative colitis with superficial ulceration, erythema, and friability of the mucosa. The internal surface of the colon appears blotchy and broken in places; (**b**) endoscopic image of human Crohn's disease showing a patchy, deep transmural inflammation (©David M Martin MD; www.EndoAtlas.com); (**c**) endoscopic appearance of the duodenum of canine IBD (note the flattened and glossy surface due to loss of folds of the absorptive mucosa as a result of inflammation); (**d**) histological appearance of canine IBD in courtesy of Barbara Richter (note the increased number of lymphocytes and plasma cells in the lamina propria of the duodenum as well as a low-grade infiltration with intraepithelial lymphocytes); (**e**) thickened jejunal wall and enlarged mucosal folds in a horse with LPE (equine lymphocytic-plasmacytic enterocolitis); (**f**) ultrasonographic appearance of thickened small intestinal walls in a horse with IBD

of the commensal microbiota is significantly reduced in IBD patient compared to control subjects with the phyla *Firmicutes* and *Bacteroidetes* being in particular affected (Frank et al. 2007). Also infections have been linked with the onset and exacerbation of IBD (Garcia Rodriguez et al. 2006).

An impaired acute inflammatory response by macrophages that leads to defective bacterial clearance has been implied for Crohn's disease. In contrast, macrophages of UC patients exuberantly respond toward bacteria and are also reflected by the formation of granulomas in CD and not UC (Kuhl et al. 2015). In CD predominantly Th1 and Th17 cells that promote local tissue destruction are observed. UC is thought to represent a Th2-driven disease. Recent data suggest that natural killer T cells (NKT) are the source of Th2 cytokines like IL-13 and target epithelial cells to become dysfunctional (Heller et al. 2005). Consequently, UC may be more of a superficial epithelial injury disorder.

4.2.3 Diagnosis

The gold standard for all patients with IBD is endoscopy (Baumgart and Sandborn 2012). Diagnosis of IBD can be challenging, with colonoscopy being the most effective for diagnosis. Usually a blood test to test for anemia or infections and a fecal occult blood test, followed by endoscopy, are performed. But also X-ray and computerized tomography (CT) scan for severe symptoms are performed to rule out perforation. Biopsies of the mucosa are taken to differentiate from UC and CD.

Moreover, urine analysis and stool culture, liver function test, and electrolyte studies may be necessary.

4.2.4 Treatment of human IBD

Despite the importance of gut flora in the pathogenesis of IBD, therapy has focused on suppressing the immune system rather than removing the agent that might be responsible for the aberrant response, by reestablishment of a normal healthy gut flora. Treatment options are mainly reduced to lifestyle alterations, if contributing factors are known, medical management of the symptoms, and surgical interventions.

4.2.4.1 Anti-inflammatory and Immunosuppressive Medications

5-Aminosalicylates are the standard treatment for active UC, in which a combination of oral and rectal therapy is better than oral or rectal therapy alone.

Also corticosteroids are effective for inducing remission in CD and UC. However, over time the response rate decreases, so there is no role for corticosteroids in maintaining remission in either CD or UC. Moreover, long-term

use goes along with serious side effects and increases the risk of infection in general (Bernstein 2015).

In humans, a seminal advance was the introduction of treatment with an anti-TNFα monoclonal antibody, which targets an inflammatory cytokine and is particularly effective in Crohn's disease. However, anti-TNF agents are not effective in up to one-third of individuals and are quite expensive. Moreover, loss of response or intolerance to anti-TNF therapy is observed among initial responders at a rate of 10 % per year.

4.2.4.2 Antibiotics

Antibiotic therapy may induce remission in active UC and CD and prevent relapse in patients with quiescent CD and support the thesis that altering gut microbial flora modulates IBD activity (Khan et al. 2011). However, overuse always bears the risk of generating resistant strains.

4.2.4.3 Surgery

There is no surgical procedure that can cure CD; however, in UC surgical removal of the large intestine (colectomy) cures the disease and is necessary in case of carcinoma, perforation, and exsanguinating hemorrhage.

4.2.4.4 Nutrition

Gradual loss of blood from the gastrointestinal tract, as well as chronic inflammation, often leads to anemia and is treated with iron supplements.

Enteral nutrition can induce remission in active disease (Lahad and Weiss 2015). Although the exact mechanism as to how enteral nutrition can reduce disease activity and symptoms is not fully defined, impacting on the gut microbiome and secondary effect on the epithelial barrier and immune response to gut microbes is very plausible (Bernstein 2015).

4.2.4.5 Probiotics

In clinical studies, probiotics such as *Escherichia coli* Nissle 1917 show promising result in maintenance of remission in UC, but there is no evidence to support the use of probiotics in CD (Bernstein 2015).

4.2.4.6 Fecal transplantation

As intestinal dysbiosis is important in the underlying pathophysiology of IBD and *Clostridium difficile* can be successfully treated with fecal transplantation, studies are underway investigating this approach in IBD. In contrast to *C. difficile*, in which the intestinal microbiome balance has been acutely disrupted, in IBD the intestinal microbiome is altered with more permanence. Thus, a more prolonged fecal transplant treatment seems to be necessary. Still, several case series exist, in which a healthy commensal flora with fecal transplantation by enema was reestablished (Borody et al. 2003).

4.3 IBD in Dogs

4.3.1 Clinical Problem

As in human patients, clinical signs associated with canine inflammatory bowel disease are primarily gastrointestinal symptoms. They comprise vomiting, small and large bowel diarrhea, anorexia, weight loss, flatulence, and borborygmus (rumbling sound caused by the movement of gas in the intestines), but also abdominal pain and "colic"-like signs are possible. Small bowel diarrhea includes signs as weight loss, vomiting, and loose-watery or black stool (digested blood, melena). Large bowel diarrhea is signed by tenesmus (ineffectual and painful straining), fresh blood and/or mucus in the stool, and urgency, and these patients can also suffer from vomiting and weight loss. The owners often are awake during the night, hearing their pets licking their lips, they hear stomach rumble and they sometimes have to walk their dogs. Respiratory signs develop, if the disease is accompanied with protein loss (protein-losing enteropathy, PLE) and hypoproteinemia. Therefore, ascites or pleural effusion can accumulate in their body cavities. Also peripheral edema (tissue swelling) is possible. Specially, PLE dogs are at risk of thromboembolism based on the hypercoagulability (Goodwin et al. 2011).

Not only the suffering of the patients but also the social incompetence and the difficult and expensive therapy of IBD dogs make this disease a severe problem in veterinary medicine.

In human patients, IBD can be subdivided into Crohn's disease and ulcerative colitis (UC) (Odze 2003). The two forms of the disease differ in their clinical picture as Crohn's disease is a marked transmural granulomatous process, which can affect any part of the gastrointestinal tract from the mouth to anus, whereas UC is a more superficial process, restricted to the colon (Odze 2003). In dogs, the two major forms of IBD cannot be distinguished, and IBD is common in both, the small and the large intestine (Fig. 4.2c, d) (Guilford 1996b). In contrast to human medicine where the two disease subtypes express different cytokine patterns (Sanchez-Munoz et al. 2008), a definitive inflammatory response or cytokine pattern could not be established in dogs (Tamura et al. 2014; Luckschander et al. 2009).

Extraintestinal signs of IBD play an increased role in human medicine. A variety of signs are described including musculoskeletal, dermatologic, hepatobiliary, ocular, renal, and skin diseases. Also pulmonary manifestations, including nonspecific lymphocytic infiltrations, organizing pneumonia, and noncaseating granulomas, are described (Majewski and Piotrowski 2015). In dogs, extraintestinal signs are not that often recognized, compared to human medicine. The most common concomitant problems in IBD dogs were pruritic skin diseases and otitis externa (Foster et al. 2003; Guilford 1996b). Also immune-mediated diseases as thrombocytopenia (Ridgway et al. 2001) and nonerosive polyarthritis are described in a few number of dogs (Pedersen et al. 1976).

4.3.2 Comparing Therapies

There is general agreement among human and veterinary investigators that IBD is a multifactorial disease. The external environment, the patient's genetic background, the intestinal microflora, and the immune system are involved in the generation of IBD (Scaldaferri and Fiocchi 2007) (Fig. 4.1).

When the diagnosis of IBD is established, client education and owner compliance is the key for a successful management of the IBD-affected pet. The owner has to be informed that IBD is a chronic disease, which can only be controlled, but not cured, and that relapses of the disease are possible. Canine IBD patients need an individualized therapy based on the severity of the disease, on the type of intestinal wall infiltration, and also on the environment and owner's commitment. A distinct step-by-step therapy in consent with the owner seems to be the best approach.

4.3.2.1 Nutritional Therapy: Dietary Trial

In contrast to human IBD, dietary management is the cornerstone of canine IBD patients. The diet has to fulfill the most important criteria as:

- The patient's nutrient requirements should be fulfilled.
- The patient should accept the diet.
- The owner's compliance should be achieved (should be able to feed or cook the diet).
- The diet should be highly digestible and fat restricted.

A novel diet should be introduced to the IBD patient. This can be as simple as switch to a new manufacturer, but usually an antigenic modification is used. This can be either the introduction of a novel single protein and carbohydrate source or the use of a protein hydrolysate (Simpson and Jergens 2011). In a hydrolyzed diet, the protein structure is disrupted, hereby preventing immune recognition by an already sensitized patient, but also preventing sensitization of a native individual (Cave 2006). A positive response to dietary trial implicates a food-responsive enteropathy (FRD), including food intolerance and food allergy (Simpson and Jergens 2011). The dietary trial should be continued for 8–10 weeks, although most canine patients have a positive response during the first 2 weeks (Luckschander et al. 2006).

4.3.2.2 Antimicrobial Therapy

For an antimicrobial trial, typically metronidazole and tylosin are orally given. Both medications have immunomodulatory characteristics, and they influence the intestinal flora by their antibacterial properties (Hall 2011). If the dog has a positive response, it is called antibiotic responsive (ARD) or tylosin-responsive diarrhea (TRD) (Kilpinen et al. 2015). Although metronidazole has antiparasitic, antibacterial, and immunomodulatory characteristics, it should be used with caution due to

its described potentially carcinogenic properties, especially after long-term use (Bendesky et al. 2002).

4.3.2.3 Anti-inflammatory and Immunosuppressive Medications

Corticosteroids are the most often prescribed immunosuppressant drug in canine IBD (Craven et al. 2004). Unfortunately, side effects are common, especially in large breed dogs and demand combination therapy with, for example, azathioprine or local active glucocorticoids (Budesonide®), although side effects cannot be excluded (Dye et al. 2013). If there is a poor response, additional immunosuppression as cyclosporine can be considered. In contrast to human medicine, less information exists concerning the use of anti-TNF alpha agents in canine IBD. As in human medicine, sulfasalazine is used for his anti-inflammatory property on colonic mucosa, but based on the various side effects in dogs, caution is warranted.

4.3.2.4 Probiotics

Although probiotics seem to have effects on the gastrointestinal flora in IBD dogs, the clinical efficacy is not proven (Rossi et al. 2014; Allenspach et al. 2006).

4.3.2.5 Fecal transplantation

Only few information are available about the use of fecal transplantation in IBD pets (Roman 2015).

4.3.2.6 Cobalamin

As in humans, cobalamin (vitamin B12) is receptor-mediated absorbed in the ileum. In humans the intrinsic factor, which plays an important role in the absorption of cobalamin, derives from the gastric parietal cells. In contrast, it is mainly produced in the pancreas of dogs and cats. It has been shown that IBD dogs with hypo-cobalaminemia have a higher risk for euthanasia, which can be prevented by subcutaneous administration of cobalamin in the treatment of IBD (Allenspach et al. 2007).

4.4 Feline IBD

4.4.1 Clinical Problem

Cats differ from dogs and humans, because they are true carnivores. Cats have low levels of amylase in the saliva (McGeachin and Akin 1979); they also have lower levels of enzymes for the breakdown of carbohydrates.

Cats differ in the anatomy of their intestinal tract, as the intestine is shorter and the pancreatic duct enters into the common bile duct, before it opens into the proximal duodenum marked by the papilla duodeni. The proximity of the bile duct system, the pancreas, and the intestine might contribute that feline IBD is often accompanied by cholangitis and/or pancreatitis, feline inflammatory

disease (FID), or triaditis (Jergens 2012). The clinical signs of the affected organs as jaundice or fever overlap with the clinical signs of IBD. Feline IBD can affect any age of cats and any breeds, although middle-aged cats and Asian breeds as Siamese cats are predisposed (Jergens et al. 1992). Similar to dogs and different to human patients, feline IBD is a diverse disease, which can affect the small and the large bowel. Feline IBD patients often are presented with vague clinical signs as weight loss, lack of appetite, and vomiting, also with signs of small and large bowel diarrhea. Additionally, extraintestinal manifestations involving the kidney (Weiss et al. 1996) and the skin are described in cats (Guilford et al. 2001). As in human or canine IBD patients, the course of feline IBD is cyclical and is characterized by spontaneous remissions and exacerbations (Jergens et al. 1992; Guilford et al. 2001). Specially in strict indoor cats, the diarrhea and defecating outside of the litter box put an emotional pressure on the owners of IBD cats.

4.4.2 Comparing Therapy

4.4.2.1 Nutritional Therapy

As in canine IBD, nutritional therapy is proven to be effective in the treatment of feline IBD. In one study, more than 50 % of cats with idiopathic IBD improved with elimination diet (Janeczko et al. 2008). Moreover, cats in this study improved quicker to dietary intervention (2–3 days) compared to canine IBD patients (10–14 days) (Luckschander et al. 2009).

4.4.2.2 Antimicrobial Therapy

Tylosin and metronidazole have been successfully used as a single agent or in combination with immunosuppressive drugs in the treatment of feline IBD (Allenspach et al. 2006). Metronidazole should not be used as a long-term treatment due to the possible carcinogenic side effects (Craven et al. 2004).

4.4.2.3 Immunosuppressive Medications

Glucocorticoids as a single agent or in combination with antibacterial therapy are the most often used therapy in feline IBD. Due to cat's sensitivity to salicylates, sulfasalazine should not be used in cats. Compared to canine IBD, only anecdotal reports exist concerning the use of cyclosporine in feline IBD (Jergens 2012; Allenspach et al. 2006).

4.4.2.4 Probiotics

Little information about the use of probiotics in feline IBD is known.

4.4.2.5 Cobalamin

Similar to dogs, the intrinsic factor for cobalamin (vitamin B12) resorption is mainly produced in the pancreas of cats. Low serum cobalamin concentrations are therefore an indicator for pancreatic diseases, but also for severe ileal

diseases, and additionally a negative prognostic factor in feline IBD patients (Ruaux et al. 2005). Various studies report the high efficacy of the parenteral supplementation of cobalamin in chronic enteropathy cats (Ruaux 2013).

4.5 Equine IBD

4.5.1 Clinical Problem

IBD in horses describes a group of gastrointestinal disorders characterized by cellular infiltration of the mucosa and submucosa with eosinophils, plasma cells, lymphocytes, basophils, macrophages, or epithelioid cells. The classification of equine IBD is based on the main histological cell types and includes:

- Granulomatous enteritis (GE), where the predominant cells are macrophages, giant cells, lymphocytes, and plasma cells forming circumscribed granulomas in the mucosa or submucosa. This form closely resembles Crohn's disease in humans with marked villous atrophy, especially in the ileum (Lindberg 1984). Pathophysiologically, abnormal reactions to intestinal bacteria, dietary agents, or aluminum exposure are discussed, but there is also some evidence of possible involvement of *Mycobacterium avium* (Lindberg 1984; Mönki et al. 2015). Young standardbreds are overrepresented, showing a familial predisposition (Lindberg 1984).
- Lymphocytic-plasmacytic enteritis (LPE), which is characterized by excessive infiltration of lymphocytes and plasma cells in the lamina propria of the gastrointestinal tract (Fig. 4.2e, f). There is no known sex, breed, or age predilection, although a recent study reported an increased incidence in dressage horses with possible gluten sensitivity as etiological factor (van der Kolk et al. 2012). LPE is discussed as a possible precursor of intestinal lymphoma (Kalck 2009). A certain degree of lymphoplasmacytic infiltration is common in intestinal biopsies; therefore, the histological results should be revised carefully (Kerbyson and Knottenbelt 2015).

Eosinophilic enteritis can be further subdivided in diffuse eosinophilic enteritis/enterocolitis (DEE), idiopathic focal eosinophilic enteritis (IFEE), and the multisystemic eosinophilic epitheliotropic disease (MEED). In DEE, infiltrations with eosinophils and lymphocytes can be detected in the mucosa and submucosa of small and/or large intestine. IFEE presents with single or multiple, focal, circumferential constricting, or plaque-like lesions with wall thickening mainly in the small colon and the left dorsal colon, where it is also termed segmental eosinophilic colitis (Makinen et al. 2008). MEED is chronic condition of unknown etiology with eosinophilic infiltration of multiple organs and the skin. As in GE, young standardbreds are most commonly affected by MEED, but the disease can occur in any breed, sex, or age (Kalck 2009).

As in human patients, horses with IBD present with a wide range of gastrointestinal signs that include mainly weight loss and also lethargy, poor appetite, intermittent fever, ventral edema, and diarrhea or free fecal water, if the large intestine is

affected (Kalck 2009). Abdominal pain can occur in all forms of IBD based on neuronal inflammation within the small intestinal walls or due to excessive gas production in the large intestine as a result of excessive amounts of undigested carbohydrates reaching the cecum and colon (Kalck 2009). Therefore, IBD poses a predisposition for displacement or volvulus of the large colon. In contrast to other forms of IBD, horses with IFEE often present with acute, sometimes severe, colic requiring surgical treatment (Kerbyson and Knottenbelt 2015).

Extraintestinal signs are not very common in horses, but can include hepatic and pulmonary granulomas in GE, exfoliative dermatitis in MEED and GE, or signs of multiple organ involvement in MEED as renal insufficiency, peripheral lymphadenopathy, respiratory problems, or ulceration of the tongue and mouth (Kerbyson and Knottenbelt 2015). Some horses present with pruritic skin diseases that resemble human dermatitis herpetiformis and could be associated with gluten sensitivity (van der Kolk et al. 2012).

Typical abnormalities in clinical pathology are hypoalbuminemia due to a protein-losing enteropathy, hypoproteinemia, anemia, and abnormal carbohydrate absorption tests. With liver involvement, elevated levels of gamma-glutamyl transferase are common (Kalck 2009; Kaikkonen et al. 2014).

4.5.2 Comparing Therapies

The treatment of horses with IBD is often unrewarding, and the prognosis is generally reported as poor, although a recent study stated an overall survival rate of 65 % (Kaikkonen et al. 2014).

4.5.2.1 Nutritional therapy

Similarly to dogs and to a smaller degree also as in human patients, dietary management is very essential for equine IBD patients. The patients should receive a highly digestible, well-balanced feed in small, but frequent meals. Usually, protein requirements are increased, and the rations should contain at least 14 % crude protein (House and Warren 2016). High-fiber diets minimize the involvement of the most common affected small intestine and provide energy through increased production of volatile fatty acids in the large intestine (e.g., addition of beet pulp) (Kalck 2009). Supplemental fat (corn oil, rice bran) is used to increase the energy content of the diet (House and Warren 2016). It is also recommended to place the horse on a mono-diet to eliminate possible dietary antigens. The preferred ration consists of grass hay and oats that are considered to be gluten poor (van der Kolk et al. 2012). A case report demonstrated clinical improvement, reduction of antibody levels, and increases of duodenal villus length after 6 months on this diet (van der Kolk et al. 2012).

4.5.2.2 Immunosuppressive medications

As in dogs, corticosteroids are the most often used drugs in horses in order to decrease the intestinal inflammation. A prolonged and tapering course is necessary. Due to the poor absorptive capacity of the intestine leading to decreased absorption

of oral medications, parenteral routes of administration are preferred initially (Kalck 2009), although some authors reported a fair to moderate outcome with oral prednisolone (Kaikkonen et al. 2014). In this study, the overall survival rate was significantly higher in horses that responded to the initial treatment (Kaikkonen et al. 2014).

Anecdotal evidence speaks of azathioprine as a useful adjunct in the treatment of IBD (Divers 2010), but evidence-based proof of efficacy is lacking.

Sulfasalazine is sometimes used in horses with acute colitis for its anti-inflammatory effects in the large colon due to the inhibition of eicosanoid metabolites and the interaction of 5-aminosalicylic acid with oxygen-derived free radicals, but side effects as keratoconjunctivitis sicca or thrombocytopenia are possible (Divers 2010). As such, the application in equine IBD patients is not described.

4.5.2.3 Probiotics and fecal transplant

Despite widespread use and promising results in vitro, in vivo there is still no convincing evidence for successful use of probiotics (Schoster et al. 2014). Effects of fecal transfaunation in equine IBD are not described yet.

4.5.2.4 Antibiotics

Similarly to human medicine, antibiotics are not regularly recommended in equine IBD, although metronidazole can be considered for its antimicrobial and anti-inflammatory effects (Kalck 2009). Other authors consider treatment with macrolides and rifampin in horses with GE due to a possible etiological involvement of *Mycobacterium avium* (Mönki et al. 2015).

4.5.2.5 Antihelminthics

Administration of larvicidal antihelminthics is recommended even in cases with proper deworming regimes and negative fecal flotation tests due to the difficulties to diagnose larval cyathostomiasis and in face of a possible link between parasite-induced inflammation and the development of IBD (Kaikkonen et al. 2014).

4.6 Synopsis

Taken together, humans, dogs, horses, and cats do suffer from IBD with a very similar pathophysiology and disease course, strongly determined by their anatomy and genetic background.

It is striking to note, when comparing human and pets, that in particular dogs share a similar physiology and clinical picture. As in humans, an underlying immunodeficiency (genetic predisposition) with a misbalanced microbiota that is reinforced by environmental factors (nutrition, smoking habit) results in the disease. However, unlike in humans in whom IBD comprises two entities (UC and CD), in pets this is not observed as the inflammation usually include the small and the large intestine. IBD also differ in horses as it affects primarily the small intestine and not

the colon. This difference might be explained by their exclusive herbal diet. Moreover, the observed discrepancies between human and pets are very likely due to the differences in the microbiota distribution along the gut. In human very few organisms are residing in the upper bowel, whereas in dogs – though a gradient is also present – much more organisms reside in the upper regions of the small intestine. Also differences in the main bacterial strains exist in human and pets, e.g., *Fusobacteria* in dogs are underrepresented in humans. In contrast, IBD in cats differ, which can be explained by their meat diet and anatomical features predisposing them for concurrent inflammation of the liver, the pancreas, and the intestines (triaditis).

The observed differences however broaden our understanding on the pathophysiology of IBD, and this has implication in the therapy choice for human and pets, e.g. for the treatment the site of application (oral versus rectal by enema) might be relevant to reach the site of inflammation (oral for small bowel, enema for large bowel).

Though, standard therapy still usually includes the use of immunosuppressive medication to diminish the symptoms, causative treatments, that aim to re-establish a healthy gut microbiota, are underway. In particular, a sustained change in diet, food supplementations as well as fecal transplantation show promising efficacies in human, but also pets.

It has to be emphasized that IBD in pets, and in particular canine IBD, represent a natural model of IBD and thus studies with canine patients are highly indicative for a therapeutic success as they do not rely on an artificial system. As so far biologics are usually tested and approved for human use and secondarily are transferred to the veterinarian patients, comparison of the similar pathophysiology suggest that the exchange of therapeutic strategies may be done bi-directionally in the future.

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Out of Breath: Asthma in Humans and Their Animals

5

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Abstract

Asthma is a common chronic inflammatory disease of the airways characterized by reversible bronchoconstriction and airflow obstruction due to mucus hypersecretion, inflammatory infiltrates, and edema. Common symptoms are coughing, wheezing, and respiratory distress. The disease seems to be caused by a combination of genetic and environmental interactions. This short characterization of bronchial asthma does not only apply to humans but similarly to domestic animals, even though the pathophysiology as well as nomenclature may differ. Feline asthma (FA) is one of the most important syndromes out of a spectrum of chronic inflammatory airway diseases in cats. In dogs a syndrome termed eosinophilic bronchopneumopathy (EBP) exists, which shares some similarities with pulmonary eosinophilic syndromes in humans. Recurrent airway obstruction (RAO) or "heaves" is described as a naturally occurring asthma-like disease in horses. Therapeutic efforts in both human and animal patients target the underlying inflammatory response of the disease by administration of anti-inflammatory glucocorticoids. Bronchoconstriction can be prevented or eased by administration of anti-obstructive ß2-sympathomimetics. The only curative treatment in allergic or IgE-mediated asthma is allergen-specific immunotherapy, which is readily applied in human asthmatic patients. In domestic animals, this therapeutic option is used only in experimental settings because successful identification of causative allergens is often difficult.

5.1 Introduction

Asthma, derived from the Greek *aazein*, which means 'to pant', is a chronic lung disease that inflames and in the attack also significantly narrows the airways. It is caused by a combination of environmental and genetic factors. This statement does not only apply to asthma in humans, but also different domestic animal species such as cats ("feline asthma"; FA), dogs ("eosinophilic bronchopneumopathy"; EBP), or horses ("recurrent airway obstruction"; RAO) develop symptoms, comparable at least in part to the situation in humans.

The prevalence for asthma in humans varies worldwide, but about 5 % of any investigated population suffer from this disease. It is more common in developed than in developing countries. In the USA and UK, the prevalence is 7 %, in Australia and New Zealand, 14 %. No reliable data exist concerning the prevalence of FA in cats or of EBP in dogs. For adult horses, an estimated RAO prevalence of 14 % has been reported in the UK which seems to be representative of many Northern Hemisphere countries and relates to the prevalence in the human population.

Many people with asthma are atopic which means they have a genetic and hereditary predisposition to develop certain allergic diseases like eczema, allergic rhinitis, and allergic asthma. In domestic animals the observed breed associations mirror a strong genetic predisposition for susceptibility to inflammatory airway diseases (cats, Siamese; dogs, Siberian Huskies and Malamutes; horses, Thoroughbreds) (Noli et al. 2014; Akdis and Agache 2013).

5.1.1 Risk Factors

The most common risk factors associated with human asthma development are viral respiratory infections in childhood, early and continuing exposure to indoor allergens (house dust mite, animal dander, molds) and outdoor pollutants/allergens (pollen, molds, ozone, particulate matter), tobacco smoke, obesity, and low socioeconomic status (Toskala and Kennedy 2015). Also horses have a higher risk to develop RAO when they were affected by viral infections under the age of 5 years (Pirie 2014). No such trend could be found concerning cats; here it seems that from all factors mentioned above the most important risk factor for inducing feline asthma are allergens. Experimental studies have shown that cats respond to the same allergens as humans do, such as house dust mite, tree or grass pollen, and molds (Reinero 2011; Jensen-Jarolim et al. 2015). This is in part also correct for horses, as airborne organic dust from stabling containing molds and pollen seems to play a major role in eliciting asthmatic symptoms (Mueller et al. 2016). For dogs the definitive cause of the disease is not known, but it is believed to be caused by hypersensitivity to probably respiratory allergens (Clercx et al. 2000). Therefore environmental and lifestyle conditions which we share with our domestic animals as they are exposed to the same environment, seem to be major risk factors for asthma development. Interestingly, recent research focuses on allergenic molecules such as lipocalins occurring in many mammalian species that show high structural similarities to the human counterparts (Jensen-Jarolim et al. 2016). These allergenic molecules are released via saliva, urine, or shed skin into the environment, making a bidirectional exchange of allergens between pets and their owners likely. However, it has not been demonstrated yet whether human lipocalins can act as allergens for pet animals.

The "hygiene hypothesis" attempts to explain the increase in asthma prevalence worldwide linking changes in the human lifestyle and the impact of these changes on the immune system: reduced exposure to nonpathogenic bacteria and viruses during childhood, increased cleanliness, decreased family size, and highly processed diet could support asthma development, while a rural farming environment seems to have a protective effect. Some elements of the "hygiene hypothesis" (clean environment, processed diet, endoparasite control) might play a role for domestic animals kept indoors and their prevalence to develop asthmatic symptoms (Akdis and Agache 2013; Noli et al. 2014).

5.2 Asthma in Humans

5.2.1 The Clinical Problem

Asthma is a common chronic inflammatory disease of the airways. It is characterized through variable airflow limitation. The main symptoms are dyspnea (shortness of breath), wheezing, chronic cough, and chest tightness. Edema and mucus in the bronchi enable air to enter into the lungs, whereas it is difficult to exhale again against the obstruction. This phenomenon is called "air-trapping": the lungs are

fixed in the state of inspiration, whereas expiration is hindered and prolonged. Exacerbation of the condition compensatory leads to tachypnea (abnormally rapid breathing; ventilation rate >20 breaths/min) and in severe cases to cyanosis (bluish discoloration of skin and mucous membranes due to low oxygen saturation of the tissues). Asthma mortality rates rise rapidly with age and are higher in males, although since 1990 mortality rates from asthma have declined worldwide due to improved diagnosis and medication (Akdis and Agache 2013).

5.2.2 Causes and Mechanisms of Asthma

Two major phenotypes of asthma can be differentiated. (1) 70 % of cases can be classified as *extrinsic asthma* caused by specific immunoglobulin E (IgE)-mediated bronchial hyperreactivity. (2) 30 % of cases are *intrinsic asthma*, non-immunologically triggered by viral or bacterial infections, pharmacological substances, exercise, or physical factors.

5.2.2.1 Extrinsic Asthma

Patients suffering from *extrinsic asthma* are in most cases allergic patients that bronchially hyperreact to normally innocuous environmental proteins, i.e., allergens. Prominent sources of allergens are pollen from trees, grass, or weeds (seasonal allergens), or animal dander, excretions of house dust mites, molds (perennial allergens), insect stings, drugs, or food.

In a first sensitization phase, inhaled allergens via the mucosal barrier of the lung reach B lymphocytes which recognize them by their specific IgM, an isotype that is expressed on the surface of all naive and immature B lymphocytes. These B cells, besides dendritic cells and others, then act as antigen-presenting cells: They phagocytose the allergen often in context with a typical allergen-related danger signal, for instance, coming along with the pollen grains, and present digested allergen peptides again on their surface. This step is important to also activate allergen-specific T lymphocytes, that transform to Th2 cells, which typically secrete mediator substances which force the B lymphocytes to immunoglobulin isotype switch toward IgE. This IgE then sticks to specific receptors on inflammatory cells. In any subsequent allergen encounter, the IgE gets cross-linked by the allergen and within seconds releases inflammatory mediators. In the lung they cause immediate spasm of bronchial smooth muscles (bronchoconstriction) and mucus secretion: the allergic asthma attack! Altogether, the allergens have thus achieved a Th2 shift of the immune response in the patient and sensitization has occurred.

Re-exposure to allergens, recruitment of inflammatory cells, and mediator release are responsible for an *immediate*, followed by a *late phase*, allergic response. The *immediate* allergic response develops within minutes after contact with allergens, as IgE-sensitized inflammatory cells degranulate and release histamine, leukotrienes, and cytokines. These substances recruit granulocytes as eosinophils and basophils, and T lymphocytes, which all contribute to the *late phase* allergic response. This may cause a second weaker asthma attack in the patients up to 8 h after allergen exposure.

Asthma may become chronic when allergen exposure continues to last. The chronic allergic inflammation is characterized by reversible to irreversible accumulation of inflammatory cells and mediators (prostaglandins, leukotrienes) leading to production of more and thicker mucus, tissue edema and fibrosis, smooth muscle cell hyperplasia, and narrowing of airway lumen ("airway remodeling") (Abbas et al. 2012; Holgate and Polosa 2008).

5.2.2.2 Intrinsic Asthma

Intrinsic asthma can be caused by pharmacological substances such as analgetics (aspirin). In aspirin-hypersensitive patients inhibition of the enzyme cyclooxygenase (COX) leads to increased production of leukotrienes from arachidonic acid, which induce bronchospasm. Another trigger for asthma is exercise ("exercise-induced asthma"). Here, physical activity synergizes with a preexisting allergy, often to food molecules such as ω5-gliadin of wheat or beta-conglycinin of soybean. Stimulation of the vagal nerves during the activity leads then to enforced spastic contractions of smooth muscle in the bronchi of the hypersensitive patients. Moreover, asthma can be triggered by extreme electrophysical, often meteorological conditions such as thunderstorms. In this case changes in barometric pressure accompanied by wind, high levels of humidity, and high pollen load in the air can lead to asthmatic symptoms. The pathophysiologic process of airway constriction in intrinsic-triggered asthma is similar to IgE-mediated asthma suggesting that alternative mechanisms of mast cell degranulation (i.e., local production of neurotransmitters) may underlie the disease (Akdis and Agache 2013).

5.2.3 Pathophysiology

The main pathophysiological features of asthma are (1) spasm of smooth muscle in the bronchi leading to bronchoconstriction, (2) enhanced mucus production (goblet cell hyperplasia), (3) infiltration of the airways with inflammatory cells (eosinophils, macrophages, lymphocytes), and (4) increased vascular permeability and interstitial edema. These inflammatory conditions are associated with cellular and structural changes that result in thickening of the basal membrane and subepithelial fibrosis ("airway remodeling") thereby leading to airway narrowing and airflow limitation (Galli et al. 2008).

5.2.4 Asthma Diagnosis in Humans

The diagnosis of asthma is based on identifying both a characteristic respiratory symptom (wheezing, dyspnea, chest tightness, cough) and variable expiratory airflow limitation.

The latter is measured by means of spirometry, which is a pulmonary function test (Fig. 5.1a–c). Spirometry allows measurement of lung function, especially the amount (volume) and/or speed (flow) of air that can be inhaled and exhaled again.

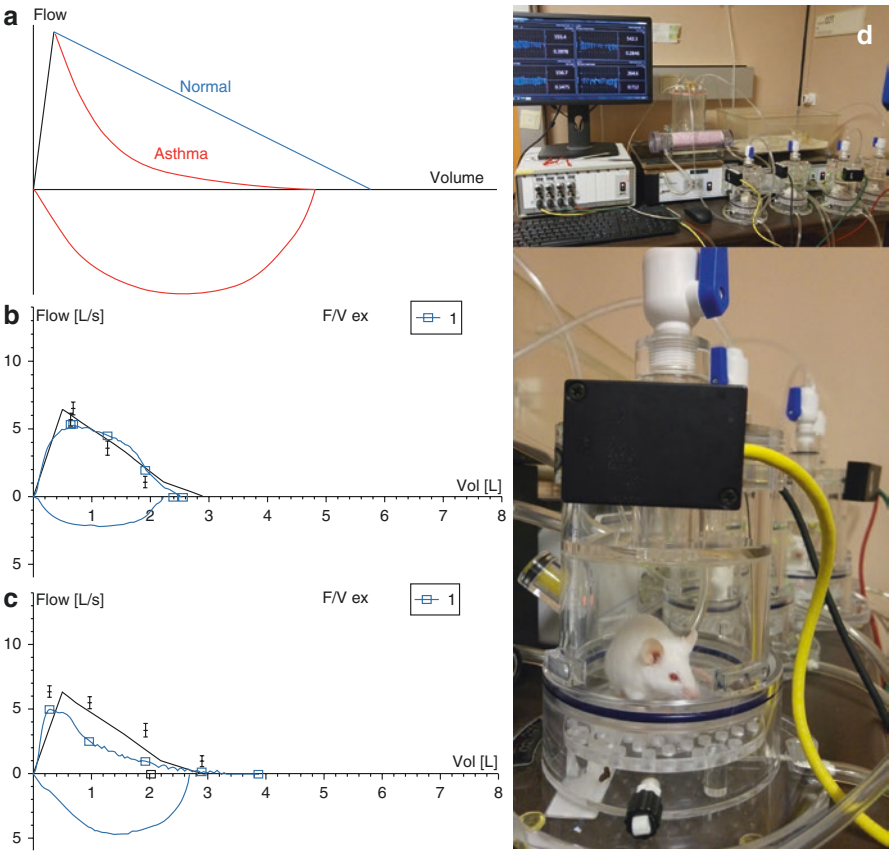

Fig. 5.1 Spirometry: principle and typical test results. (**a**) Diagram showing the changes in airflow in healthy (*blue*) and asthmatic condition (*red*) (Adapted from the GINA guidelines Reddel et al. (2015)). (**b**) Original spirometry test results of a human healthy patient; (**c**) of a patient suffering from bronchial asthma. Note the reduced airflow (*upper blue line*) due to bronchoconstriction above the *x*-axis in (**c**), as compared to normal lung function in (**b**); *Y*-axis: forced expiratory flow (FEF) in L/s; *x*-axis: expired air volume in liters; (**d**) Whole-body plethysmography in mouse studies for asthma research (*top*, series of measurements in mice; *bottom*, single tested mouse). The experimental principle is identical to the human diagnostic test.

For "small lung function tests," handheld spirometers are used; for more precise "big lung function tests," so-called body phlethysmographs are used, i.e., closed chambers able to house the whole body during measurements. These are also dependent on the cooperation of the patient. Body phlethysmograph devices are also used in mouse studies (Fig. 5.1d). In general, a patient is asked to take the deepest breath he or she can manage and then exhale through the mouth into a sensor as intense as possible, for as long as possible. The result is represented in a volume-time curve, showing volume (liters) along the *Y*-axis and time (seconds) along the *X*-axis. The most common parameter measured in spirometry is the forced expiratory volume in

1 s (FEV1). FEV1 is the volume of air that, following full inspiration, can forcibly be exhaled in one second. Average values for FEV1 in healthy people depend mainly on sex and age, and values between 80 and 120 % of the average value are considered normal. In obstructive diseases like asthma, the FEV1 is diminished because of increased airway resistance to expiratory flow (intermittent and mild persistent asthma >80 %; moderate persistent asthma 60–80 %; severe persistent asthma <60 % of the predicted value). Another similar measurement is the peak expiratory flow (PEF), which is the maximal flow (or speed) achieved during the maximally forced expiration initiated at full inspiration (liters per minute). Narrowing of the airways is indicated by slowing down of the speed of air coming out of the lungs. Furthermore, the peak flow meter is a small device that measures the peak flow and by doing so allows patients to self-monitor the disease (Reddel et al. 2015).

Once the diagnosis of asthma is made, the specific asthma phenotype (i.e., extrinsic, intrinsic, other forms) must be determined according to the patient's history and additional investigations, as this has impact on the therapy. This usually includes an allergologic evaluation since the most common cause of asthma is allergies: IgE-mediated allergic reactions can be diagnosed by a skin prick test inducing a so-called "wheal and flare" reaction. For this purpose, drops of different allergen extracts are put on the inner forearm (see also Chap. 8 and 9). The skin is then pricked through each drop using a lancet. In response to allergen-stimulated release of mast cell mediators (i.e. histamine), local blood vessels dilate and become leaky to fluid which produces redness and local swelling (wheal). Simultaneous dilation of capillaries around the wheal produces the appearance of a red radial flare reaction. This "wheal and flare" reaction happens within 15 min after allergen contact. Of equal importance, determination of the levels of allergen-specific IgE to allergen extracts or molecules in a serum laboratory test helps to identify the causative allergen (Abbas et al. 2012).

5.2.5 Therapy of human Asthma

Symptomatic treatment Antihistamines and mast cell stabilizers are used to prevent the effects of histamine in allergic asthma. Antihistamines work by attaching to the same receptors that histamine uses to cause allergic symptoms. By occupying these sites, they effectively block the histamine -mediated symptoms. Mast cell stabilizers block calcium channels essential for mast cell degranulation, stabilizing the cell and thereby preventing the release of histamine.

Acute symptomatic treatment and long-term control medications consist of two classes of medication. On the one hand, "controllers" such as glucocorticoids and leukotriene antagonists have an anti-inflammatory effect and reduce the severity of airway inflammation. The so called "relievers" act anti-obstructive and belong to the ß2-sympathomimetics (ß-agonists). There are short-acting ß-agonists (SABAs) that relax airway muscles to give prompt relief of symptoms and long-acting ß-agonists (LABAs) for relief and prevention of bronchospasm. The latter are mostly used in combination

with inhaled glucocorticoids, especially in moderate to severe persistent asthma stages with a FEV1 lower than 80 % (Aalbers et al. 2016). The intensity of treatment depends on the severity of disease. According to the GINA (Global Initiative for Asthma, www.ginasthma.org) guidelines, the pharmacologic therapy corresponds to a stepwise therapy depending on the severity of disease. In mild cases, the initial therapy is 1–2 puffs of a short-acting ß-agonist (Fig. 5.2a); however, in severe cases according to the stepwise approach, the inhalative therapy may need to be complemented by a systemic corticosteroid therapy. Other therapeutic options in selected patients with persistent allergic asthma include specific anti-IgE therapies with monoclonal anti-IgE antibodies. The goals of asthma management are to achieve a good control of symptoms and maintain normal daily activity levels, to minimize future risks by reducing the risk of flare-up, to maintain lung function, and to minimize medication side effects (Reddel et al. 2015).

Fig. 5.2 Symptomatic treatment of asthma. Inhaled formulations of bronchodilators and glucocorticoids can be administered to (**a**) children (Fotolia.com©pololia), (**b**) cats, and (**c**) dogs via a spacer (holding chamber) and a tightly fitting facemask

Curative treatment Allergen-specific immunotherapy (AIT) is the only effective curative treatment in IgE-mediated allergic asthma, which has the potential to change the course of the disease. AIT involves the repeated administration of allergen preparations in order to induce clinical and immunologic tolerance to the offending allergen thereby leading to suppression of allergic inflammation in the affected tissue. The two most commonly prescribed routes for AIT are subcutaneous (SCIT) and sublingual (SLIT). SCIT protocols generally involve weekly injections during a build-up phase, followed by monthly maintenance injections for a period of 3–5 years. SLIT involves daily drops or a tablet of allergen extract under the tongue from where the extract is quickly taken up via the oral mucosa (Jutel 2014).

5.3 Asthma-Related Syndromes in Companion Animals

5.3.1 Feline Asthma

Feline asthma represents one of the most common lower airway diseases in cats. No reliable data exist regarding the incidence of this syndrome, although a prevalence of 2–5 % has been proposed by some. Cats of any age may be affected, but first signs are observed frequently in younger animals. Siamese cats appear to be overrepresented and may be affected more intensively, which is in support of a genetic predisposition. FA exhibits numerous parallels to allergic asthma in humans, with aeroallergens considered as the main inciting cause (Reinero 2011). Intrinsic asthma triggers like in humans (e.g., exercise, nonsteroidal drugs) have not been specifically recognized in cats.

The proposed mechanism of asthma in cats is repeated allergen exposition, which leads to chronic airway alteration and inflammation (epithelial and goblet cell hypertrophy, hyperplasia of sub-epithelial mucus glands resulting in excessive mucus production, edema and inflammatory bronchial wall infiltrates, hypertrophy and hyper-contractility of bronchial smooth muscles). Allergen contact or nonspecific stimulation leads to paroxysmal and reversible bronchoconstriction, further narrowing the airway lumen. Experimental studies in cats reproducing the clinical signs and airway changes of spontaneously occurring disease are in support of an underlying allergic background of FA (Norris Reinero et al. 2004). Sensitization and challenge with relevant inhalational allergens (e.g., house dust mite, Bermuda grass) resulted in an eosinophilic airway inflammation, airway hyperresponsiveness, Th2-dominated cytokine pattern in peripheral blood, and bronchoalveolar lavage fluid (BALF) as well as production of allergen-specific IgE. Interestingly, experimental studies in cats have demonstrated that neonatal allergen exposure seems to be protective against sensitization later in life.

Clinical signs in affected cats are variable, and some individuals experience symptom-free intervals. In many animals, chronic productive cough predominates. Commonly, an increased expiratory effort with engagement of abdominal wall muscles is observed. Some cats present with acute severe, sometimes life-threatening

respiratory distress as the first clinical sign. Sometimes, the owners report on inciting events for acute episodes (e.g., contact to perfumes, household detergents, cat litter dust, cigarette smoke, and others).

Typical findings on physical examination may include tracheal sensitivity; hyperinflated chest; prolonged, "active" expiration; predominantly expiratory wheezing; and sometimes crackles on auscultation. Cats with acute bronchoconstriction may exhibit severe expiratory respiratory distress, open-mouth breathing, and cyanosis. With severe lung overinflation ("air trapping"), also inspiratory respiratory distress may be observed.

Hematology and blood chemistry usually are not specifically helpful. Currently, no biomarkers or simple blood tests are available to support the diagnosis. A rapid response to short-acting bronchodilators is indicative of bronchoconstriction in cats with acute respiratory distress. Diagnosis is based on thoracic radiography, exclusion of underlying parasitic disease, and demonstration of eosinophilic airway inflammation on BALF cytology.

Most of the pulmonary function tests which are well established in human medicine cannot be performed in cats (and dogs) due to lack of active patient cooperation. Barometric whole-body plethysmography (BWBP) as a noninvasive function test allows for the detection and quantitation of airflow limitation due to bronchoconstriction during acute episodes and the differentiation from other common causes of respiratory distress (heart failure, pleural effusion) in the nonsedated or anesthetized cat (Hoffman et al. 1999). As shown in Fig. 5.1, it is also used in experimental laboratory animals in asthma research. Furthermore, the response to therapeutic intervention (e.g., β2-agonistic bronchodilators) can be measured (Rozanski and Hoffman 1999). A positive response to bronchodilators further differentiates asthma from chronic bronchitis. Finally, testing of nonspecific airway responsiveness (the ease of induction of bronchoconstriction with nonspecific, e.g., pharmacologic stimuli) can be performed with BWBP (Hirt et al. 2011).

Based on findings in experimentally induced asthma, the definitive identification of inciting allergens is problematic. In experimental studies, allergen-specific serum IgE was associated with a very low sensitivity (around 20%). Furthermore, depending on the test system used, also false positive allergens may be detected, against which cats are not sensitized (Lee-Fowler et al. 2009). Generally, skin tests in cats have resulted in a high number of false-positive tests. Due to the current problems associated with the definitive allergen identification, allergen-specific immunotherapy in spontaneous disease is not feasible, although it has been demonstrated to reduce airway inflammation and clinical signs in experimental feline asthma (Reinero et al. 2006). In addition, avoidance of allergen exposure is seldom practical.

Similar to the therapeutic recommendations in humans, feline asthma is treated with glucocorticoids and bronchodilators (β2- receptor agonists or methylxanthines). During the last 15 years, inhaled drugs (mainly glucocorticoids and β2-agonists) administered as metered dose inhalers with spacers (e.g., Aerokat®, Trudell, Canada) have experienced an increase in popularity, thereby avoiding the side effects and complications of systemic administration, e.g., diabetes mellitus, skin atrophy, and immunosuppression following oral glucocorticoid treatment

(Fig. 5.2b) (Galler et al. 2013). Systemic glucocorticoids may be restricted to initial therapy in severe cases and during asthma exacerbations. The short-acting β2-receptor agonist salbutamol administered by inhalation should be given only during acute bronchoconstriction, since with chronic use, the S-enantiomer of this racemic drug will accumulate in the body, which has no bronchodilator effect but triggers an inflammatory response. This has been demonstrated in humans as well as in cats (Dhand et al. 1999; Reinero et al. 2009). No such effect is known for the weaker, but long-acting β2-agonist salmeterol, which therefore can be administered by inhalation on the long term. Studies have shown that leukotrienes have no role as mediators in FA (Norris et al. 2003), and drugs targeting leukotrienes (e.g. zafirlukast) are not effective. Antihistamines such as cetirizine and serotonin antagonists (cyproheptadine) also failed to be effective in experimental feline asthma models (Schooley et al. 2007).

5.3.2 Eosinophilic Bronchopneumopathy in Dogs

Eosinophilic bronchopneumopathy (EBP), formerly known as "pulmonary infiltrates with eosinophils," is characterized by eosinophilic infiltration of bronchial walls and pulmonary parenchyma with variable contribution and occurs predominantly in young to middle-aged dogs (Clercx et al. 2000). A breed predisposition exists for Siberian Huskies, Malamutes, and other Nordic breeds as well as Rottweilers. Females are affected more frequently. Although the definitive cause of the disease is not known, it is believed to represent a hypersensitivity reaction to (probably inhaled) allergens (e.g., molds). Investigations in dogs with EBP have revealed an increase in CD4+ and a decrease of CD8+ T cells in BALF, as well as a cytokine pattern consistent with a Th2 immune response (Peeters et al. 2005, 2006). Other causes of eosinophilic airway inflammation (e.g., endoparasites) should be excluded with appropriate testing.

Clinical signs of EBP are variable. Almost all dogs have a productive cough and oftentimes also a decreased endurance. Furthermore, respiratory distress may be observed. On auscultation, wheezing and crackles may be heard. In severe cases, the general condition may be poor and the animal may be depressed. Changing appetite may lead to weight loss. In only about 50 % of affected dogs, a peripheral blood eosinophilia is found. Radiographs reveal a bronchointerstitial lung pattern with peribronchial infiltrates. In some patients small patchy alveolar densities or tracheobronchial lymphadenomegaly (enlarged lymph nodes) may be detected. With severe, long-standing disease, irreversible dilation of the bronchi with accumulation of thickened airway secretions and inflammatory cells, called bronchiectasis, may be found with endoscopy, radiography, or computed tomography (CT).

Age, breed, history, clinical signs, radiographic changes, and blood eosinophilia (if present) may raise the suspicion of EBP. Definitive diagnosis requires demonstration of eosinophilic inflammation on cytology of BALF samples. Typical bronchoscopic findings include copious amounts of yellow-greenish mucopurulent secretions; thickened, irregular mucosal surfaces with sometimes polypoid

appearance; and occasional expiratory bronchial collapse. In severe cases, bronchiectasis may be detected.

The mainstay of treatment is glucocorticoid (prednisolone) administration initially for 1 week until attenuation of clinical signs, followed by successive dose reduction to effect, establishing the minimal effective maintenance dose. Given the side effects of systemic glucocorticoids, which are more pronounced in dogs than in cats, therapy with inhaled glucocorticoids appears even more attractive in dogs (Fig. 5.2c) (Bexfield et al. 2006; Hirt et al. 2008). In severe cases, initially combining inhalation and oral glucocorticoids may be required. With drug discontinuation, recurrence of clinical signs is common. Although some authors advocate the use of bronchodilators such as ß2-agonists or methylxanthines, evidence of effectiveness in dogs is lacking. The probable explanation is that dogs do not develop spontaneous bronchoconstriction. Other suggested effects of these drugs (e.g., strengthening of respiratory muscles, synergism with glucocorticoids, increased mucociliary clearance – i.e., clearance of mucus from bronchial lumen) are outweighed by side effects (e.g., gastrointestinal upset, nervousness, tachycardia).

5.3.3 Recurrent Airway Obstruction, an Asthma-Like Disease in Horses

An often-described clinical picture in horses is recurrent airway obstruction (RAO), also termed "heaves." RAO is described as a hypersensitivity reaction to inhaled allergens. It is one of the most common causes for coughing in horses, sharing many characteristics of asthma. An increased RAO risk has been described in association with age (mature horses >4 years), breed (Thoroughbreds), and season (winter, spring).

Similar clinical signs as in asthmatic humans are chronic cough and respiratory effort, especially at rest. Other symptoms include flared nostrils, nasal discharge, exercise intolerance, and a heave line (hypertrophy of abdominal muscles assisting with expiration). The symptoms arise because RAO-affected horses show excessive accumulation of mucus in the lower airways, bronchospasm, bronchial hyperreactivity, and airway remodeling. These pathophysiological features are very similar to the human situation in asthma. A major difference can be found in the underlying inflammatory response with mainly T lymphocytes (CD4+) and neutrophils that accumulate in the airways but less eosinophils than in asthmatic humans. In an experimental setup, horses affected by RAO showed no immediate phase reaction after controlled allergen challenge, no immediate bronchospasm, and no elevated histamine levels in lung fluid. Therefore the role of IgE and mast cells is still controversial; some studies found elevated IgE levels in serum of RAO horses, particularly against mold allergens, while others could not find a connection with IgE-mediated allergic responses. RAO in horses is at present characterized by a delayed hyperreactivity response occurring 6–8 h after allergen encounter involving neutrophils and T lymphocytes, similar to the delayed hyperreactivity in human asthma (Leclere et al. 2011; Pirie 2014).

The disease eliciting stable dust from hay and straw bedding includes bacterial endotoxins, molds, proteases, microbial toxins, mites, plant debris, and inorganic dust. The high amount of pro-inflammatory agents (allergic and nonallergic) could in part explain the differences to the human situation. Especially the high endotoxin concentration in organic dust could play an important role in neutrophil recruitment and potentiate the reaction to mold allergens.

Therapeutic efforts start at the level of environmental management. This means maintaining the horse at pasture and the use of low dust bedding at the stable to minimize the exposure to stable dust (similar to allergen avoidance in humans). Symptomatic treatment is in line with treatment in human patients: horses with RAO receive bronchodilators in the form of ß-agonists for relief of airway constriction and glucocorticoids as an anti-inflammatory medication either systemically or via inhalers. Leukotriene antagonists were reported to be ineffective suggesting that leukotrienes are not important mediators of bronchoconstriction in RAO. Due to the very heterogeneous nature of stable and hay dust, successful identification of causative allergens is very difficult. Therefore curative treatment of RAO with allergen-specific immunotherapy is not common in horses (Noli et al. 2014).

Clinically related diseases are summer pasture associated-RAO (SPA-RAO) and inflammatory airway disease (IAD) which require diagnostic differentiation from RAO. SPA-RAO has an identical disease phenotype to RAO with airway obstruction and neutrophilic airway inflammation. It affects mature horses at pasture throughout summer with high load of grass pollen and fungal spores in the air. Therefore the main difference between these conditions seems to relate to the inciting inhaled allergen. Horses affected with IAD show neutrophilic airway inflammation as in RAO, although there have been reports on eosinophilic inflammatory responses. In contrast to RAO, IAD can affect horses of any age, including young racehorses, and these horses show no increased respiratory effort at rest. The actual cause of IAD remains unknown, but both infectious and noninfectious environmental causes have been proposed. Therapy in both SPA-RAO- and IAD-affected horses involves administration of glucocorticoids and bronchodilators similar to treatment of RAO (Pirie 2014).

5.4 Synopsis

Recurrent airway obstruction in horses and feline asthma in cats are two naturally occurring asthma-like conditions that affect domestic animals. The pathophysiological features in both animal species include bronchoconstriction and inflammation of the airways, characteristics that are comparable with the situation in humans. Both cats and horses also share environmental exposure to aeroallergens or air pollutants with humans. Especially cats are exposed to the same indoor conditions as their owners are. Horses with their relatively long lifespan may suffer from the disease for long periods of time, even decades, which is similar to human asthma. Less is known about the role of (aero)allergens as a potential cause of eosinophilic bronchopneumopathy in dogs. The aforementioned factors allow for the conclusion

that domestic animals (especially cats and horses) could serve as good models for the comparable human disease. Further understanding of causes and mechanisms of FA in cats or RAO in horses could give insight in the environmentally mediated human disease.

Horses with RAO could, moreover, serve as a model to study the contribution of neutrophils to the asthmatic phenotype or give insight into the possible reversibility of airway remodeling, which cannot be easily conducted in other animal models (rodents) or humans due to technical or ethical reasons. Although some of the mechanisms (especially concerning the involved inflammatory mediators) may differ between cats and humans, there has been (and will be) a lot of information gained from experimental feline asthma models. This holds true especially with regard to new therapeutic options. Among veterinarians, attempts have been made to increase the communication between human and animal health professionals using collaborative clinical practice protocols. These would involve veterinarians who treat cats with respiratory problems to discover if humans in the same household also experience respiratory problems, providing information about shared environmental exposure risks. Similarly, human health clinicians could inquire about health problems in companion animals. These measures could help to improve detection, prevention, and understanding of asthma in several species.

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Comparing Two Major Bone Pathologies in Humans and Companion Animals: Osteoporosis and Hyperparathyroidism

6

Wolfgang Sipos, Ursula Föger-Samwald, and Peter Pietschmann

Contents

Abstract

Metabolic/endocrinological diseases of bone are a major topic in human medicine, but are not that frequent in veterinary medicine. We will focus on two major bone diseases of human medicine, namely, osteoporosis and hyperparathyroidism, and compare their expression and pathophysiology in companion animals.

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In humans, postmenopausal osteoporosis is the most frequent and economically most important bone disease, but interestingly has no direct equivalent in veterinary medicine. This is also due to the fact that osteoporosis is not only a matter of low bone mineral density but also of altered bone (micro)structure. Also, hypoestrogenism does not seem to be of that clinical importance in our domestic mammals. On the other hand, hyperparathyroidism, which is frequently diagnosed in humans as well, resembles a clinical problem also the veterinarian has to deal with.

6.1 Introduction

6.1.1 Bone Diseases in Humans and Companion Animals

Diseases of bone are heterogeneous and affect humans as well as animals. They may be of sole physical origin, leading to different forms of fractures in case of trauma or exostoses (osteophytes) in case of chronic irritation, but may also have endocrinological, immunological, and/or oncological backgrounds, which in many cases are not clearly definable and go hand in hand. Especially immune cells and other cell types capable of producing cytokines, chemokines, and related mediators influencing the immune system interact with different types of bone cells and thus may promote bone formation and/or bone resorption. In the light of comparative medicine focusing on humans and companion animals (horse, dog, cat), endocrinological bone diseases seem of highest relevance. In humans, postmenopausal osteoporosis is the most frequent and economically most important bone disease, but interestingly has no direct equivalent in veterinary medicine. For some reason, hypoestrogenism does not seem to have a major clinical impact on bone in companion animals. On the other hand, hyperparathyroidism, which is frequently diagnosed in humans as well, resembles a clinical problem also the veterinarian has to deal with.

6.2 Osteoporosis in Humans

The National Institute of Health defined osteoporosis “as a skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture. Bone strength reflects the integration of two main features: bone density and bone quality.” Whereas in clinical routine bone mineral density (BMD) can be easily measured, many determinants of bone quality such as bone microarchitecture, bone geometry, mineralization, and bone matrix properties can only be assessed in highly specialized research laboratories (Fig. 6.1). In general, osteoporosis results from a mismatch between the activity of osteoblasts (bone-forming cells) and that of osteoclasts (bone-resorbing cells) with a preponderance of bone resorption. Nevertheless, osteoporosis is a heterogeneous disease with a strong genetic component.

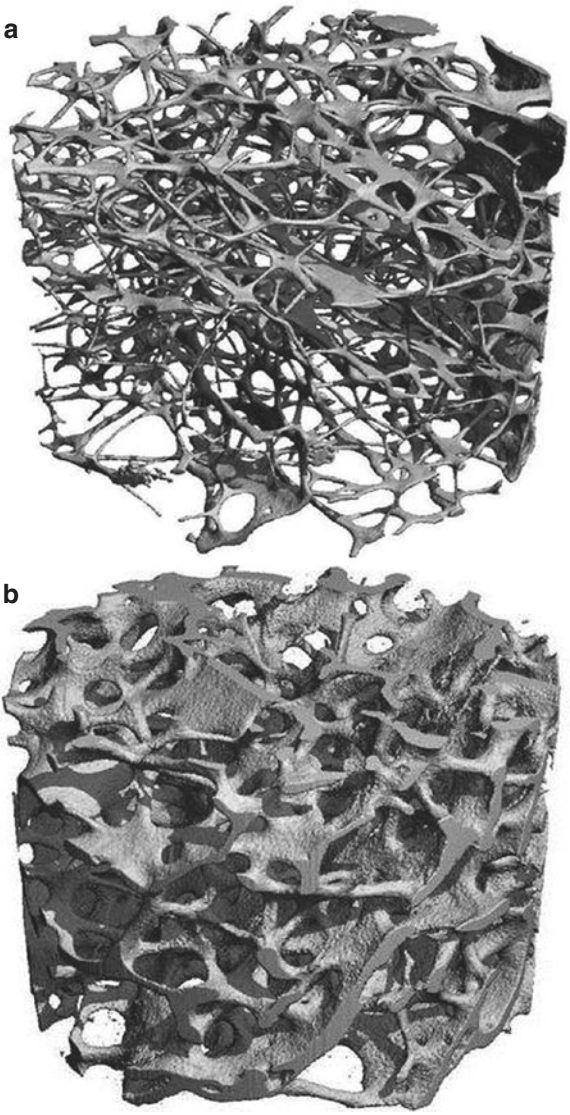

Fig. 6.1 Trabecular microstructure of the femoral head of a woman with an osteoporotic hip fracture (**a**) and a healthy control woman (**b**) assessed by micro-computed tomography (μ-CT)

In osteoporosis impaired bone strength results in an increased risk of fragility fractures, i.e., fractures resulting from a trauma that would not cause a fracture in healthy bone. There are several fracture locations that are particularly frequent in osteoporosis, namely, vertebral fractures, fractures of the distal radius, and hip fractures. Especially in postmenopausal women, osteoporosis is extremely frequent. It has been estimated that the lifetime risk of a 50-year-old (Caucasian) woman is 40 % (the corresponding risk of a 50-year-old man is 15–20 %).

6.2.1 Classification: Primary or Secondary Osteoporosis

It is common clinical practice to distinguish *primary* and *secondary osteoporosis*. The term primary osteoporosis comprises idiopathic juvenile osteoporosis, idiopathic osteoporosis in young adults, postmenopausal osteoporosis, and age-related osteoporosis. In this chapter postmenopausal and age-related forms of osteoporosis, which are the most frequent types of osteoporosis, will be described in more detail. Secondary osteoporosis results from specific medical conditions, such as rheumatoid arthritis, or treatments, such as long-term glucocorticoid applications, that impair bone strength and structure.

In Fig. 6.2 the course of BMD in humans over a lifetime is shown. During puberty BMD markedly increases; the maximal BMD ("peak bone mass") normally is attained in early adulthood. Although BMD starts to decline some years before menopause, in most women a rapid phase of bone loss is evident 5–10 years after menopause. The rapid phase of bone loss is followed by a continuous and lifelong slow phase of bone loss. In contrast to women, men normally do not experience a rapid phase of bone loss. This could explain at least in part why clinically relevant osteoporosis is more common in women than in men. Traditionally, among the primary forms of osteoporosis, type 1 (*postmenopausal*) and type 2 (*senile*) osteoporosis are distinguished. Type 1 osteoporosis is related to the rapid phase of bone loss, whereas type 2 osteoporosis is linked to the slow phase.

It is well established that the rapid phase of bone loss predominantly is due to *estrogen deficiency*. In a relatively short time period during menopausal transition,

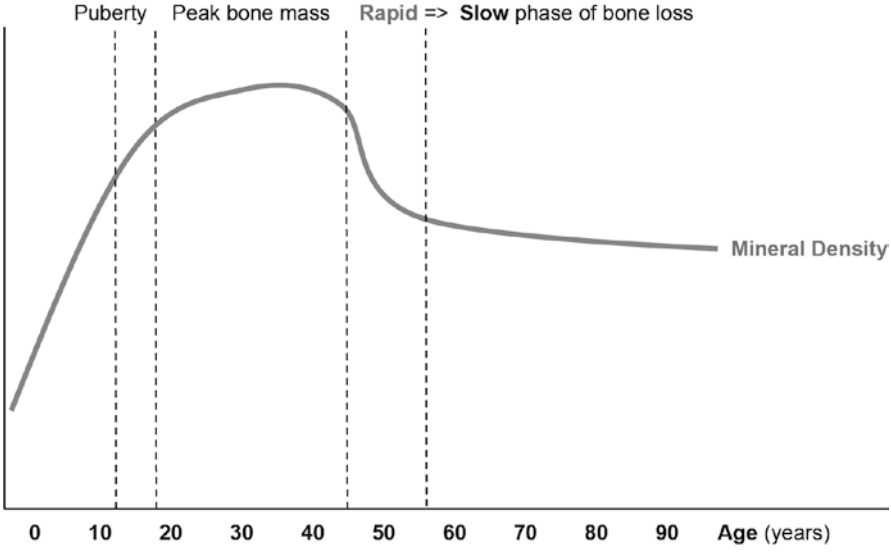

Fig. 6.2 Schematic representation of the course of bone mineral density in a female human throughout life (Modified from Kleerekoper (2013))

estrogen levels decline by 80–90 %. As a consequence, the production of RANKL (receptor activator of nuclear factor-κB ligand) and other cytokines such as interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α increases, and the expression of osteoprotegerin, which is a decoy receptor for RANKL, decreases. This leads to an increased generation and activity of osteoclasts. Due to the "coupling phenomenon," also bone formation increases ("high bone remodeling"). Nevertheless, since bone resorption increases to a higher extent than bone formation, BMD and bone strength decline.

Vitamin D_3 ($VitD_3$) deficiency is an important pathogenic factor of *senile osteoporosis*. $VitD_3$ deficiency impairs intestinal calcium absorption, decreases serum calcium levels, and thereby induces a secondary increase of parathyroid hormone (PTH) levels. Elevated PTH levels, increased concentrations of proinflammatory cytokines such as TNF-α in the context of "inflammaging," and persistent sex hormone deficiency contribute to an increase of bone resorption. Bone formation is decreased relative to bone resorption in age-related osteoporosis. Low bone formation in senescence is a consequence of decreased expression of osteoblast transcription factors such as runx2. In elderly subjects not only bone mass but also muscle mass declines. Sarcopenia is an important determinant of falls and consequent fracture risk.

6.3 Osteoporosis in Companion Animals

As outlined in the introduction part, a pathologic condition identical to human osteoporosis, i.e., a systemic disease based on hypoestrogenism going ahead with osteopenia and a high incidence of pathologic fractures due to bone mass deficiencies and failures in bone microarchitecture, does not occur in companion animals. Even in scientific veterinary literature, the term "osteoporosis" is often used for *osteopenic* situations, but one has to keep in mind that this pathologic entity is not associated with hypoestrogenism. Female dogs in general have very low estradiol titers throughout most of the year (<5 pg/ml) with the exception of the period of heat; therefore they appear "asexual" most of the time. *Equine bone fragility syndrome* (BFS) also is not related to estrogen deficiency, despite some similarities to human osteoporosis are evident. These are prominent osteopenia and a disturbed microstructure of trabecular bone, resulting in spontaneous bone fractures, which predominantly affect the scapulae, ribs, pelvis, and vertebrae. However, BFS is not correlated with advanced age and may have different reasons including hyperparathyroidism (see below). Also, pulmonary silicosis, a chronic inflammatory lung disease, is discussed of being associated with BFS. Nevertheless, there exist rodent (and macaque) models, which quite closely resemble the situation in humans and are used in biomedical research for preclinical testing of anti-osteoporotic drugs and immunotherapeutics. Also, in laying hens, there exists a pathologic condition resembling osteoporosis to some extent. However, this is a multifactorial disease, and the pathophysiology behind it is not fully understood.

6.4 Hyperparathyroidism in Humans

The term "hyperparathyroidism" refers to an increased secretion of PTH. Three different forms of hyperparathyroidism can be distinguished:

- *Primary hyperparathyroidism*: excessive secretion of PTH by a parathyroid adenoma, parathyroid carcinoma, or hyperplasia of the parathyroid gland
- *Secondary hyperparathyroidism*: (physiologically) increased secretion of PTH as a consequence of hypocalcemia or hyperphosphatemia
- *Tertiary hyperparathyroidism*: development of a parathyroid autonomy in the setting of (mostly long-standing) secondary hyperparathyroidism

6.4.1 Primary Hyperparathyroidism

Primary hyperparathyroidism is the third most common endocrine disease with a prevalence of approximately one percent. Over the years the clinical presentation of primary hyperparathyroidism has changed significantly. Today most patients with primary hyperparathyroidism are asymptomatic and are identified through routine blood chemistry (hypercalcemia) or bone densitometry. In the past, patients often were highly symptomatic and presented with bone pain, kidney stones, and gastrointestinal as well as neuromuscular complaints.

Excessive secretion of PTH results in the retention of calcium by the kidneys, increases the activity of osteoclasts, and consequently mobilizes calcium from bone. As a result of the action of PTH on kidneys and bone, blood calcium levels in primary hyperparathyroidism are elevated. Since PTH inhibits renal phosphate reabsorption (and thus leads to an increased excretion of phosphate in the urine) in primary hyperparathyroidism, blood phosphate concentrations typically are low (hypophosphatemia). Most of the clinical manifestations of primary hyperparathyroidism can be explained by the action of PTH on bone remodeling and hypercalcemia. The classical bone manifestation of the disease, *osteitis fibrosa cystica*, is seen only in a small fraction of the patients today. As a result of massively increased bone degradation, radiologic signs of subperiosteal bone resorption and bone cysts develop. In very high concentrations, PTH also stimulates fibroblast activity. Bleeding in bone cysts may give rise to *brown tumors*. The term "tumor" is misleading, as brown tumors are not neoplasms, but mainly consist of fibrous tissue and blood vessels. As already mentioned, today only few patients present with fibrous cystic osteitis. Nevertheless, in many patients BMD is low (especially in the forearm) and it is well established that fracture risk in primary hyperparathyroidism is increased. Hypercalcemia often results in hypercalciuria and thus may lead to kidney stones. Hypercalcemia may also explain neuromuscular symptoms (muscle weakness, fatigue, depression) and cardiovascular manifestations such as valvular, myocardial, and endothelial calcifications. Gastrointestinal manifestations of classical primary hyperparathyroidism include peptic ulcers and pancreatitis. In severe cases of primary hyperparathyroidism, a life-threatening hypercalcemic crisis may develop.

6.4.2 Secondary Hyperparathyroidism

As mentioned above, *secondary hyperparathyroidism* develops as a physiologic reaction of the parathyroid gland to either hypocalcemia or hyperphosphatemia. Important causes of secondary hyperparathyroidism are $VitD_3$ deficiency and chronic renal failure. In the latter condition hypocalcemia results from an impaired production of $1,25(OH)_2VitD_3$ (calcitriol) and a decreased excretion of phosphate leading to hyperphosphatemia.

6.4.3 Tertiary Hyperparathyroidism

Tertiary hyperparathyroidism may occur in patients with chronic renal failure or after renal transplantation.

6.5 Hyperparathyroidism in Domestic Animals

Whereas in humans hyperparathyroidism frequently is diagnosed already in an asymptomatic stage, this endocrine malfunction often leads to *osteodystrophia fibrosa* (ODF) in animals, a pathologic condition characterized by osteoclastic degradation of bone and substitution with fibrous connective tissue. Trabecular thickness is decreased but at the same time a massively increased osteoblast proliferation with simultaneously decreased osteoid production can be observed. Also cortical bone is affected with osteoclastic bone resorption starting on the endosteal surface and finally involving the total compacta, which now becomes flexible. The fibrous proliferation simultaneously may lead to an increase of total bone volume, which is most prominent at the maxilla and the mandibula. Hyperparathyroidism is classified as primary, secondary (alimentary and renal), and paraneoplastic (pseudohyperparathyroidism) in veterinary medicine. Hyperparathyroid conditions have been described in all classes of tetrapod vertebrates including mammals, birds, reptiles, and amphibians and are often associated with inaccurate husbandry. As the main focus of this book is on companion animals, we will concentrate on these species.

6.5.1 Primary Hyperparathyroidism in Animals

Primary hyperparathyroidism is caused mainly by adenomas of the parathyroid gland and is found mainly in elderly dogs. ODF leads to pathologic fractures (i.e., fractures of weakened bone, which would not occur under physiologic conditions) often affecting vertebrae with consecutive neurological signs through compression of the spinal cord. Facial hyperostosis with loss of teeth may also be observed. Clinical signs of hypercalcemia include generalized muscle weakness and depression, anorexia, vomitus, obstipation, polydipsia, and polyuria. Due to increased renal secretion of calcium and phosphate, also nephrocalcinosis and urolithiasis may be observed.

6.5.2 Secondary Hyperparathyroidism in Animals

The *secondary alimentary hyperparathyroidism* is the most frequent entity of this complex being diagnosed in our companion animals and affects mainly growing individuals. Relative (or absolute) alimentary calcium deficiency and/or nutritive phosphorus overload as is triggered by sole meat feed in dogs (especially in large breeds) and cats as well as bran feed in horses leads to a pathologic activation of the parathyroid gland. A similar situation is seen in growing pigs fed with inadequate grain mixtures without calcium supplementation. On the other hand, adult pigs (multiparous, non-lactating sows) seem to be quite resistant to prolonged alimentary calcium deprivation. Besides, pigs and horses have relatively high physiologic total serum calcium levels (2.5–3.5 mmol/l) when compared to dogs and cats (2.3–3.0 mmol/l), which is important when interpreting blood chemistry results. Alimentary hyperparathyroidism in dogs can be diagnosed easily by radiography of the long bones and the axial skeleton through typical alterations such as thinned cortical bone (including changes in shape of the bones due to muscular forces) and compression fractures. Diagnosis can be completed by investigation of PTH and calcitriol levels. Therapy of nutritive secondary hyperparathyroidism includes a decrease of phosphorus intake (as high serum phosphate levels decrease ionized calcium) and aims at maintaining a finely tuned balance of calcium, phosphorus, and $VitD_3$ in the feed. This is especially important for growing dogs of large breeds, as also hypercalcemic conditions or hypervitaminosis D_3 can cause a multifactorial pathologic condition termed *osteochondrosis* (OC).

Chronic renal diseases with reduced glomerular filtration rates cause hyperphosphatemia due to phosphate retention on the one hand and decreased activity of 1α-hydroxylase with consecutively diminished renal production of the active form of $VitD_3$. Dogs suffering secondary renal hyperparathyroidism present with ODF most frequently associated with flexible mandibulae (“rubber jaws”) and loss of proper fixation of teeth in alveolae due to demineralization (Fig. 6.3). Additionally, elevated PTH levels themselves are regarded as nephrotoxic. This so-called *osteorenal syndrome* is associated with bone pain, lameness, renal acidosis, and metastatic calcium phosphate deposition. As in nutritive hyperparathyroidism, meat (protein) ratio in canine nutrition should be lowered, which serves primarily to attenuate acidosis. Chronic renal insufficiency with consecutive hyperparathyroidism is also a major problem in the old cat. Therefore, attention should be given to an adequate diet supporting renal function especially in the geriatric felines.

6.5.3 Paraneoplastic Syndrome or Pseudohyperparathyroidism

The entity of hypercalcemia due to malignant conditions is termed *paraneoplastic syndrome* or *pseudohyperparathyroidism*. In veterinary medicine increased bone resorption due to bone metastases, an increased secretion of parathormone-related peptide (PTH-rP) in adenocarcinomas of the apocrine cells in the anal glands of female dogs, and systemic malignant entities of the hematopoietic system are the

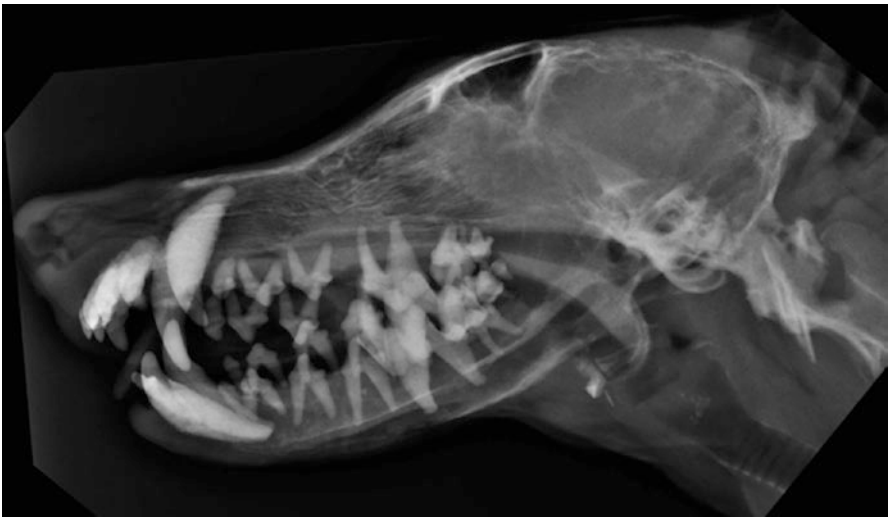

Fig. 6.3 Radiograph of the head of a female fox terrier, age 19 years, with secondary hyperparathyroidism due to renal insufficiency (osteorenal syndrome), lateral projection. Note the generalized osteopenia of the bones of the head with a lacelike trabecular pattern at the level of the frontal bone. The teeth appear to be of increased opacity (Courtesy from the Division of Diagnostic Imaging, University of Veterinary Medicine Vienna)

most frequent causes of hypercalcemia. Clinical signs of hypercalcemia are associated with gastrointestinal (vomitus), neuromuscular (weakness), cardiovascular (bradycardia), and renal (acidosis) symptoms.

Interestingly, domestic mammals with an intense milk production, which is a very calcium-consuming process, such as cows or sows, easily develop a pathologic entity termed "milk fever" (hypocalcemia in the peripartal period) under specific conditions, but are more or less prone to noteworthy bone resorption despite increased PTH values. It is speculated that increased levels of calcitonin may lead to an ineffectiveness of PTH to mobilize calcium out of bone.

6.6 Synopsis

Postmenopausal osteoporosis is the most frequent bone disease in humans associated with a high economic burden. A corresponding disease developing from estrogen deficiency leading to compromised bone strength and eventually to an increased risk of fractures is not known for companion animals, which might be explained by a different sexual endocrinological situation and/or different skeletal responses to estrogen deficiency in diverse animal species. Conversely, hyperparathyroidism is frequently diagnosed in humans as well as in companion animals. Clinical manifestations of primary hyperparathyroidism include neuromuscular, cardiovascular, gastrointestinal, and renal symptoms and are very similar in humans and animals.

However, due to routine blood screenings, the clinical presentation in humans has changed significantly, and classical bone manifestations such as osteitis fibrosa cystica and brown tumors are seen only in a small fraction of patients, whereas osteodystrophia fibrosa, the corresponding pathologic condition in animals, presents more frequently. The major causes of secondary hyperparathyroidism in humans are $VitD_3$ deficiency and chronic renal failure. In companion animals, the major cause of secondary hyperparathyroidism is alimentary calcium deficiency/nutritive phosphorus overload.

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Life Out of Balance: Stress-Related Disorders in Animals and Humans

7

Lisa Maria Glenk and Oswald David Kothgassner

Contents

Abstract

Stress is a complex phenomenon and commonly referred to as a range of bodily reactions toward a potentially harmful stimulus that may disturb homeostasis. It is important to understand that stress impacts psychological, physiological, immunological, and behavioral functions that not only require individual coping strategies but affect disease onset and/or progression in both humans and animals.

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Integrative studies have demonstrated significant consequences of stress on metabolic, cardiovascular, mental, and reproductive health as well as individual fitness and immune modulation. It is apparent that there exist many similarities between humans and animals encountering a stressful stimulus. Individual variation in stress susceptibility is related to genetic predisposition and early experience. This chapter provides an overview on stress concepts, physiological cascades, and stress-related diseases.

7.1 Introduction

Like humans, animals need to keep their body in biological and psychological balance to maintain health. The ability to react to changes and challenges of the internal and external milieu is essential to keep bodily parameters within acceptable ranges. Stress is a complex phenomenon with wide-ranging effects on the organism. Throughout the history of stress research, different concepts and definitions of stress have been used by scientists to describe the cascade of bodily responses to arousal. Generally, these physiological efforts are regarded as adaptive in the short term but maladaptive under long-term conditions. Defining stress, most people refer to a situation in which an individual is confronted with a potential or actual threat. Cortisol and adrenaline are frequently labeled as "the" stress hormones, highlighting the key role of the autonomic nervous system and activation of the adrenal glands during a stressful experience. But also other systems of the body respond to stress in the dynamic regulation of metabolism, reproduction, growth, and immune function. These bodily systems are driven by neurobiological pathways that interact and communicate via the nervous system and neurochemicals (i.e., hormones, neurotransmitters, peptides) to help the organism adapt to challenge. All neurobiological pathways that respond to stress are not solely stress-associated systems but do serve other functions in the maintenance of homeostasis.

7.1.1 Evolutionary Aspects

Comparative vertebrate studies highlight that the stress response is almost identical across species, facilitating a context-appropriate behavioral response to overcome an imminent threat. Stress responses are merely stereotypic, innate reactions that have evolved to guarantee survivals and maintain homeostasis. According to the "polyvagal theory," the phylogenetic stage of the autonomics predicts the behavioral, physiological, and affective features of stress reactivity, based on three distinct systems. The first system depends on the unmyelinated *nervus vagus*, regulates immobilization behavior (death feigning, avoidance, freeze), and lacks direct neural innervation. Thus, there is limited regulatory ability to calm down or self-sooth after cardiac excitation. It refers to jawless and cartilaginous fish, in which cardiac output

is regulated by circulating catecholamines. Driven by phylogenetic development and increasing neural complexity, the second system relies on the sympathetic nervous system mediating mobilization (fight-or-flight behavior that has an acute preservative impact on survival). It refers to bony fish, amphibians, and reptiles. Alongside with the highest cortical development, the mammalian myelinated *vagus* system has evolved to inhibit sympathetic activation and allows functional modulation of heart rate via vagal efferent pathways ("vagal brake"), coordinating mobilization and communication. However, during danger and threat, there is a greater dependence on sympathetic excitation, involving the evolutionarily more primitive systems with reduced regulation of cardiac and metabolic output (Porges 2001).

7.1.2 Stressor

Stress responses vary with type, magnitude, and duration of a stressor. Stressors are actual or perceived threats to health, survival, and/or individual fitness that provoke sympathetic and adrenal activation (see Table 7.1).

In laboratory animal model research, stress protocols commonly feature psychosocial stressors including neonatal isolation, circadian rhythm changes, and predator/conspecific confrontation. Common physical stressors are restraint, immobilization, temperature variation, and electric foot shock (Campos et al. 2013). In contrast, human stress research focuses on different experimental paradigms: cognitive tasks under judgment (Trier Social Stress Test; Kirschbaum et al. 1993), degradation (Montreal Imaging Stress Test; Dedovic et al. 2005), or interpersonal stressors using exclusion through verbal and nonverbal exclusion or rejection in computer games (Williams and Jarvis 2006; Kothgassner et al. 2014). Analysis of salivary biomarkers has significantly advanced stress research over the past decades. Saliva can be collected noninvasively with commercial devices in both humans and animals (Fig. 7.1).

Stress is a transactional phenomenon that primarily depends on the meaning of the stimulus (i.e., stressor) to the perceiver (Lazarus 1966). Moreover, individual coping strategies and resources determine the subsequent response to stress. Incoming information is processed through neural pathways and shaped by subjective experience and genetic predisposition, resulting in a psychophysiological and behavioral output.

Stress systems are temporally activated during various intra- and interspecies interactions such as mating or prey hunting that many people would describe as

Table 7.1 Different types of stressors (three examples are given for each category)

Physical	Psychological	Physiological	Chemical	Social
Noise	Fear	Pain	Toxins	Separation
Temperature	Aggression	Hunger	Drugs	Competition
Light	Self-esteem	Pathogens	Pollution	Isolation
…	…	…	…	…

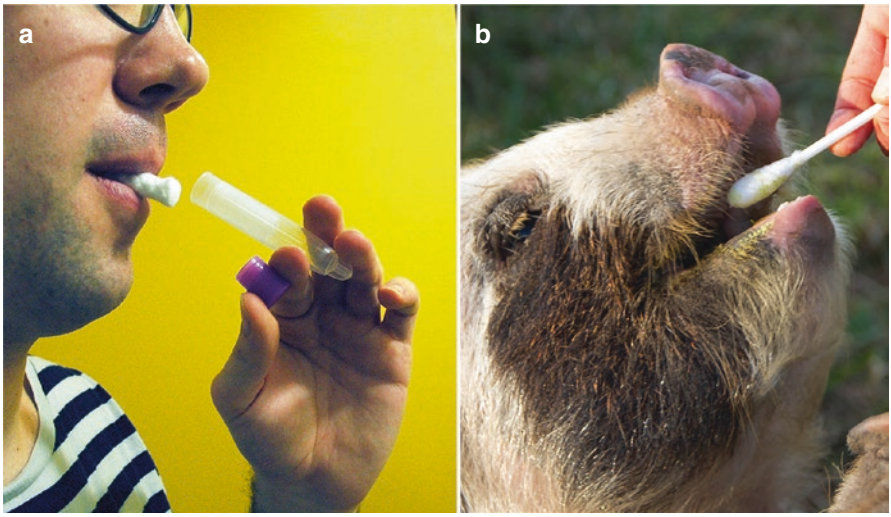

Fig. 7.1 Saliva collection for analysis of stress-related biomarkers; (**a**) a human volunteer collects saliva in specially designed tubes (Salivette®); (**b**) saliva can be collected with cotton swabs in trained pig "Rudi" (pig: ©A. Veit)

stimulating rather than stressful. Selye in 1974 differentiated between eu- and distress. Eustress refers to a state in which the individual mobilizes energy through positive stimulation. In contrast, distress involves challenges that potentially or actually overtax an organism's coping mechanisms and, thus, is more relevant with regard to onset and progression of stress-related diseases.

7.1.3 Stress Hormone Pathways

The adrenal glands are of central importance in the neuroendocrine response to stress (Fig. 7.2). In the brain, neurons in the paraventricular nucleus of the hypothalamus promote secretion of corticotropin-releasing hormone (CRH) that stimulates the secretion of adrenocorticotropic hormone (ACTH) from the pituitary, which then results in glucocorticoid (GC) release from the adrenal cortex, forming the hypothalamic-pituitary-adrenal (HPA) axis. The main GC in humans and most other mammals is cortisol, while in birds and most rodents it is corticosterone. To avoid excessive release of stress hormones, the HPA axis operates on negative feedback loops. Increasing circulating GCs inhibit the secretion of both CRH and ACTH. Particularly in species with distinct diurnal or nocturnal behavior patterns, GC secretion is characterized by a circadian rhythm. In humans and primates, GC levels peak in the morning and decrease toward the evening. However, potent stressors can cause afternoon levels to rise above morning values. GC receptors on leukocytes modulate a variety of functions, including cell proliferation and cytokine production.

Under acute stress, the CRH-activated *locus coeruleus* (LC) in the brain stem releases noradrenaline (NA) to rapidly activate sympathetic fibers (LC/NA

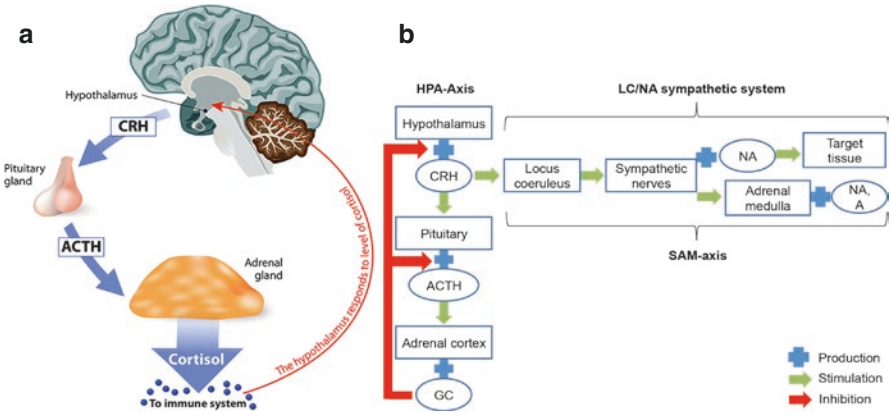

Fig. 7.2 Central and peripheral components of the hypothalamus-pituitary-adrenal (HPA) axis. (**a**) Illustration of the central role of the hypothalamus in the brain from where corticotropic hormone (CRH) is released and stimulates the neighboring pituitary gland from where adrenocorticotropics hormone (ACTH) is released. This leads to a stimulation of the cortex of the adrenal gland to synthesis and release of cortisol. Cortisol as end product also has immunomodulatory potency and provides a negative feedback to the hypothalamus (Fotolia.com©designua); (**b**) diagram showing the hierarchy in the HPA axis, triggering at various steps the sympathetic system, or the SAM axis. Various positive and negative feedback mechanisms regulate the stress hormone production circuits

sympathetic system) and secrete the adrenomedullary catecholamines NA and adrenaline (A), which correspond to the sympathetic-adrenal-medullary (SAM) axis. Immunoregulatory functions of catecholamines are primarily mediated by β-adrenoreceptors on peripheral blood cells and splenocytes. The autonomic response to stress occurs on a faster timescale than the HPA axis, contributing directly to the fight-or-flight response (Chrousos and Gold 1992; Chrousos 2009). Sympathetic activation increases heart rate, blood pressure, and respiration rates. Neuroendocrine responses to stress cause restrained growth, reproduction, and digestion to preserve energy resources. Moreover, inflammatory immune responses are suppressed. Metabolic adaptations involve gluconeogenesis, lipolysis, and redirection of oxygen and nutrients to the central nervous system. Facilitation of adaptive behavior leads to increased arousal, anxiety, and vigilance. In addition, endogenous opioids contribute to stress-induced resistance to pain (Chrousos and Gold 1992).

7.2 Stress and Disease

7.2.1 Reproduction

In utero stress exposure may predispose the development of stress-associated diseases. Rodent studies have shown that maternal stress during pregnancy has several negative consequences for the offspring. Prenatal stress has been associated with lower birth weight, impaired cognitive function and development of the

brain, as well as reduced cardiovascular health and fertility (Harris and Seckl 2011). Moreover, GCs have organizing effects during sensitive phases in development and can induce the demasculinization of male offspring, or masculinization of female offspring, with severe consequences for reproductive success (Kaiser and Sachser 2005).

In mammals, secretion of the neuropeptide hormone oxytocin is strongly linked to parturition and lactation. Breast-feeding women and lactating rodents are less susceptible to stress-induced physiological arousal. Lactation modulates female stress reactivity by decreasing circulating GCs and catecholamines. These effects have been attributed to the release of oxytocin as injected oxytocin can inhibit the HPA axis on the level of ACTH and cortisol secretion. However, human and animal studies indicate that acute maternal stress can impair milk ejection by reducing the oxytocin pulse in the first place (Dewey 2001). The brain of chronically stressed dams exhibits lower oxytocin mRNA expression in the medial amygdala, increased expressions of CRH-mRNA in the central nucleus of the amygdala, and increased GC receptor mRNA in the paraventricular nucleus (Murgatroyd et al. 2015).

7.2.2 Maternal and Social Isolation Effects

The impact of maternal and social deprivation on emotional stability and stress susceptibility has been elucidated by behavioral psychologist Harry Harlow in the 1930s. In his controversial but widely recognized studies, infant rhesus monkeys were raised in isolation to develop symptoms of depression. Follow-up research revealed that behavioral symptoms of depression were accompanied by structural and functional aberration in the hippocampus. Similarly, reduced hippocampus volume and less synaptic density have also been reported in humans (Heath 1972; Teicher et al. 2006). These data are supported by the fact that individual differences in behavioral stress responses are associated with early-life maternal care. Maternal nurturing behavior can decrease anxiety in rats: early-isolated rats showed less exploration behavior as well as enhanced persistence and reduced extinction of conditioned fear as adults (Caldji et al. 2000; Callaghan and Richardson 2013).

7.2.3 Memory Consolidation

Prolonged exposure to high concentrations of endogenous GCs has been linked with both impaired memory and decreased volume of the hippocampus. Increased HPA activity is more prevalent in aged rats with spatial memory deficits than in cognitively unimpaired aged rats (Lupien et al. 2005). More specifically, high levels of GCs seem to impair neuronal cell metabolism and eventually lead to stress-induced dendritic atrophy in the hippocampus, especially affecting CA1 and CA3 pyramidal neurons (Kim and Diamond 2002). In guinea pigs, separation of cohabitated individuals has been associated with impaired spatial memory and elevated cortisol levels (Machatschke et al. 2011).

7.2.4 Depression, Anxiety, and Post-traumatic Stress Disorder

Early experiments by Weiss (1972) showed that the predictability of stress shapes the stress response. The length of gastric ulcer lesions in rats that were given a signaled electric shock was significantly smaller than those in rats that were confronted with an unsignaled electric stimulus. Moreover, if rats were provided with warning signals, they had also lower concentrations of corticosterone. Seligman (1975) demonstrated that dogs, if given electroshocks without the possibility to avoid the aversive stimulation, developed behavioral abnormalities including lethargy and lack of motivation to escape. His conclusion was that unpredictable environments impose adverse effects upon individuals and induce learned helplessness – a causal factor in the development of depression. These findings have been replicated during mild stress experiments in rats, where depressive symptoms and HPA hyperactivity were found in individuals subjected to instable social hierarchies and unpredictable environments (Herzog et al. 2009). Also, male tree shrews (*Tupaia glis*) developed depressive-like symptoms during confrontation experiments with limited possibilities to escape from a dominant conspecific (Fuchs and Flügge 2002). In humans, anxiety disorders including the social anxiety disorder (SAD) and general anxiety disorder (GAD) represent the group with highest prevalence in psychopathology. Besides pre- and postnatal stress, the development of anxiety disorders is highly associated with conditioning processes and is regulated by serotonin, benzodiazepines, and GABA neurotransmission. Many anxiety disorders in humans refer to early-life stress and have been related to decreased sensitivity of benzodiazepine receptors as in rats and primates that were raised in maternal separation and exhibit novelty-induced suppression of appetitive behavior and exploration (Caldji et al. 2000).

Post-traumatic stress disorder (PTSD) refers to an anxiety disorder after exposure to a critical life event or severe trauma that is commonly perceived as life-threatening. In humans, it can be regarded as a complex chronic stress phenomenon that is linked to intrusive thoughts, hyperarousal, avoidance, and dissociation. In PTSD, a prolonged continuation of biological responses following stress has been associated with blunted cortisol secretion (Yehuda 2000) and higher levels of catecholamines (Young and Breslau 2004). Studies comparing human PTSD with animal models show striking similarities regarding ineffective stress coping (Cohen and Zohar 2004) as well as hippocampal rearrangement and dysfunction (Goswami et al. 2013). The type II GC receptor can be modulated by antidepressants and mood stabilizers, restoring effective GC function (Marques et al. 2009).

7.2.5 Stress Modulates the Immune System

Some decades ago, it was generally assumed that stress affects the immune system solely via suppression, accounting for the increased susceptibility to develop infectious diseases in chronically stressed individuals. More recent research has, however, indicated that stress may also exacerbate medical conditions that are associated

with an activation of the immune system. Hence, it may be more appropriate to shift to an immunomodulatory model of stress.

An important component in the regulation of immune responses is the secretion of cytokines. T helper (Th)1 cytokines play an important role in cellular immunity, that is, combating intracellular pathogens by activating NK cells and cytotoxic T cells. In humoral immunity, Th2 cytokines fight extracellular pathogens by promoting antibody production and activation of B cells. A growing body of research has demonstrated a stress-related shift in cytokine secretion profiles as well as decreased T cell proliferation and NK cell cytotoxicity in response to acute (time-limited) naturalistic stress (Segerstrom and Miller 2004). Thus, short-term exposure to naturalistic stressors exerts a shift away from Th1 cytokines toward Th2 cytokines. Decreased cellular immunity function during acute stress may increase the vulnerability for invading pathogens such as bacteria and viruses (Murali et al. 2007).

The acute-phase response is a bodily reaction of the innate immune system to restore health if an organism is confronted with infection, inflammation, stress, or tissue damage. The subsequent synthesis of acute-phase proteins (APPs) is stimulated by endogenous GCs and pro-inflammatory cytokines. In animal models, both the administration of adrenaline and exposure to stress lead to higher plasma levels of cytokine interleukin (IL)-6. Also, anxiety and depression can trigger the secretion of IL-6 that induces C-reactive protein (CRP), an APP that has a major role in cardiovascular diseases. Elevated IL-6 and CRP during inflammatory processes have been related to insulin resistance, non-insulin-dependent diabetes mellitus type II, metabolic syndrome, hypertension, and arterial diseases (Black 2003; Glaser and Kiecolt-Glaser 2005).

It has been suggested that increasing levels of NK cells in peripheral blood are an adaptive feature of the fight-or-flight reaction. Negative thoughts and anticipatory threat have been suspected to blunt the stress-related regulation of NK cells. In a laboratory experiment, where phobic individuals were confronted with a fearful stimulus, lower numbers of NK cells were secreted by people who scored high on worries (Segerstrom and Miller 2004).

Chronic stress exposure may have an even stronger impact on the onset and progression of diseases than acute challenges. If individuals experience stress for a prolonged period of time, Th1 cytokines significantly decrease, and in some studies, a reduction of Th2 cytokines has also been reported. GCs have a dominant role in chronic stress conditions. Chronic inflammatory diseases such as asthma and rheumatoid arthritis have been associated with blunted cortisol secretion in response to stress (Murali et al. 2007).

7.2.6 Stress and Cancer

In lung cancer patients, a flattened diurnal cortisol slope has been linked with low total and cytotoxic T-lymphocyte counts and decreased survival. Hence, circadian disruption may accelerate tumor progression, and an effective diurnal slope could be a significant, independent predictor of survival (Sephton et al. 2013). Perceived

stress and mood in breast cancer patients modulate NK and T cell counts and effectiveness (Thornton et al. 2007). Furthermore, animal model studies provide evidence that higher levels of catecholamines and GCs under chronic stress conditions (i.e., restraint stress and repeated social defeat) support tumor growth, angiogenesis, and metastasis (Xie et al. 2015; Wu et al. 2015).

7.2.7 Glucocorticoid Resistance in Chronic Stress

In chronically stressed individuals, some studies suggest heightened GC reactivity, while other research points at blunted responses. The "glucocorticoid-resistance model" proposes that chronic stress alters the ability of GC to orchestrate immunity. Thus, hyperactivity of the stress axis over a prolonged period of time elevates the secretion of GCs, requiring an adaptation of immune cells to downregulate their GC receptors. As a consequence, immune cells fail to react adequately to GC signals, for example, by inducing pro- instead of anti-inflammatory pathways. Under persisting stress, GC levels may rebound to normal or lower, resulting in hypoactive stress coping. This theory is also partially supported by Selye's "general adaptation syndrome" (Selye 1950), describing three stages of coping with a stressor that involve adaption, resistance, and exhaustion. First, an organism adapts and mobilizes energy in response to challenge. The phase of resistance is characterized by steady consumption of resources. Eventually, depletion of resources and accumulating pathological changes may lead to system imbalance, breakdown, and/or death.

7.3 Synopsis

In both humans and animals, stress affects physical and psychological health in manifold ways. Its general mechanisms are highly conserved among vertebrate species. Through the action of stress hormones, actual and perceived threats to health can severely disrupt proper immune function. Acute stress results in mobilization of bodily resources to maintain inner balance. Individual coping strategies and differences in stress responsiveness have been attributed to disease susceptibility. If experienced over a prolonged period of time, stress facilitates the onset and progression of various diseases including cancer. Thus, the understanding of how stress orchestrates immune responses is crucial in human and veterinary medicine.

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Allergies, with Focus on Food Allergies, in Humans and Their Animals

8

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Abstract

Hypersensitivity reactions to respiratory, ingested, percutaneously encountered, or injected allergens are classified according to different pathophysiological mechanisms. In the case that food causes the adverse reactions, most typically symptoms along the digestive route (oral allergy syndrome, angioedema, stomachache, vomiting, diarrhea) but also systemic reactions (urticaria/hives, asthma, up to life-threatening anaphylaxis) may occur. On the contrary, food intolerance reactions are disagreeable but do not elicit dangerous systemic reactions. Therefore, it is important to diagnostically differentiate between immune-mediated hypersensitivities and the more harmless food intolerances. Principally, food adverse reactions may occur in all mammalian species.

To single out the suspected food in children and animal patients the allergist is much dependent on collaboration with parents or owners, respectively. For diagnosis of food allergies in humans and animals, evaluation of the allergen-specific serum IgE levels, skin tests, and sometimes elimination diets and oral provocation tests are performed. Intolerances are diagnosed via hydrogen breath test or blood glucose test, in addition to elimination diets.

The offending food allergen must be avoided. Clinical tolerization strategies and experimental immunotherapies have shown promising results. Symptomatic treatment may include the prescription of emergency self-medication in patients at risk for anaphylaxis.

Whereas mostly murine models are used for developing more effective diagnostic and treatment options for food allergies, we propose the systematic inclusion of companion animals as spontaneous food allergy models in examination and diagnosis of allergy.

Abbreviations

ADHD	Attention-deficit/hyperactivity disorder
AFR	Adverse food reaction
APC	Antigen-presenting cell
DBPCFC	Double-blind placebo-controlled food challenge
FcεRI	High-affinity IgE receptor
FcεRII	Low-affinity IgE receptor
FIAD	Food-induced atopic dermatitis
Ig	Immunoglobulin

8.1 Introduction

8.1.1 Prevalence of Allergies and Intolerances

Allergic diseases have been described in humans, rodents, nonhuman primates, avian species, and in all domestic animals; among them, also food allergies can occur in humans, dogs, horses, and several other species; and all of these species share the ability to develop anaphylactic shock (Gershwin 2015). But according to anecdotal reports, also more exotic animals show adverse food reactions, such as a baby walrus reacting allergic to cow's milk formula diet with eczematous skin, disorders of the mucosal membranes, and severe diarrhea (Schroeder 1933). About 15–25 % of the adult human as well as canine population are affected by some type of allergy. These numbers are on the rise in humans, where, for instance, food allergies have increased especially in children aged 0–17 years from 3.4 % in 1997–1999 to 5.1 % in 2009–2011 (Jackson et al. 2013). In summary, food allergy today affects about 2 % of the adult population and about 3–7 % of children, although the majority of children outgrow food allergies by the time they start school. Allergies also increase in veterinary patients, where the percentage of dogs for instance sensitized to grass pollen increased from 14.4 % from 1999 to 27.7 % in 2010 (Roussel et al. 2013). In contrast to the low numbers of immunologically mediated "true" food allergies, in total about 20–25 % of the general adult population of Western countries seem to be affected by some type of food intolerance, like intolerance against fructose (15–25 %), lactose (7–20 %), sorbitol (8–12 %) (summarized in (Raithel et al. 2013)), or histamine (1–3 %) (Jarisch 2013).

8.1.2 Geographical Differences in Humans

The prevalence of allergic diseases seems to differ among countries. For instance, regarding self-reported food hypersensitivity reactions (including allergies and intolerances), data from about 17,280 young adults from 34 centers in 15 countries have been compared. Within this cohort, 12.2 % reported symptoms after a

particular food; however, this percentage ranged from 4.6 % in Spain to 19.1 % in Australia (Woods et al. 2001). Reasons can be differences in cross-sensitivity between pollen and aeroallergens ('cross-reactivity') or in dietary habits. A well-known example is peanut allergy, which is more common in the USA than in other countries due to its extensive use in food products.

Differences between geographical regions suggest the influence of environmental factors, such as status of hygiene. For instance, worm infestations and bacterial exposure are regarded as being protective against allergies. On the other hand, the load of diesel exhaust particles due to heavy traffic in industrial countries alters the permeability of epithelial cells at the skin and mucosa (Fukuoka et al. 2016). Accordingly, there are more allergies in developed than in developing countries (Rottem et al. 2015). For instance, significantly more people younger than 45 years had a positive allergy test in West as compared to East Germany (Nicolai et al. 1997); however, prevalence of allergic diseases in East Germany reached West German numbers shortly after reunification. This observation allowed for detection of possible underlying environmental factors: the steepest increase occurred in hay fever and allergy to birch pollen in East Germany, where single-room heating with fossil fuels and living as only child in a family explained up to 23.5 % of the excess trend in East Germany (Kramer et al. 2015). Differences do not only occur between countries or continents but also between rural and urban regions. The protective effect of living on a farm, including the consumption of raw milk, has been revealed several years ago; the responsible mechanisms are currently being investigated (Schuijs et al. 2015).

8.1.3 Genetic Risk for Human and Animal Atopy

A genetic predisposition with a higher genetic risk to develop allergies is called atopy. Atopy may occur in humans, dogs, horses, and cats (Gershwin 2015; Roudebush et al. 2010). In humans, the genetic influence is underlined by the elevated risk for allergies among children with a history of allergy in the family: in the case neither parent is allergic, the risk for allergy in the child is 5–16 % compared to 50–80 % when both parents suffer from allergy (Litonjua et al. 1998). For dogs, differences in atopy can be found among the different breeds, e.g., Labrador retrievers and setters have a higher risk for allergies (reviewed in (Jensen-Jarolim et al. 2015)), and among cats, the Siamese is more affected by adverse food reactions.

8.2 Basic Mechanisms of Allergic Diseases and Intolerances

Allergy is classified as an immunological hypersensitivity disorder and occurs when a patient's immune system reacts with an exaggerated response to normally harmless environmental substances. Although immunopathological disorders have been classified into seven categories before (Sell 1996), the long-standing classification

by Gell and Coombs from 1963, which divides allergies into four types, is still much more common and will be used in this chapter.

8.2.1 Type I Allergy: IgE-Mediated Immediate Reaction to Allergens

Type I allergy is also called immediate-type allergy due to the rapid onset of symptoms (several minutes) after allergen contact. During the sensitization phase, the antigen is taken up at mucosal or skin surfaces by potent antigen-presenting cells (APC, e.g., dendritic cells, B cells), where innate immune receptors or immunoglobulins can be involved in antigen recognition. The APCs may transport the allergen to specific lymph nodes and directly present them to B lymphocytes to stimulate immunoglobulin production. Alternatively, these APC may also digest the antigen and present its peptides to antigen-specific, naive T cells. In context with an allergen-associated danger signal, the T cells become T-helper cells 2 (Th2), which release cytokines like IL-4 and IL-13. IL-4 leads to immunoglobulin class switching and IgE synthesis in specific B cells. During this silent, asymptomatic sensitization process, specific memory B cells and T cells are generated. Moreover, secreted IgE binds via specific receptors (FcεRI; FcεRII) onto a range of effector cells, including mast cells, basophils, monocytes, and dendritic cells. A subsequent allergen contact then induces not only production and secretion of even more allergen-specific IgE antibodies by the B cells but also reaches the sensitized effector cells. In this effector phase of allergy, cross-linking of the receptor-bound IgE antibodies by allergens leads to cell degranulation and release of mediator substances (e.g., histamine, leukotrienes), which are responsible for the immediate allergy symptoms. A late-phase reaction may occur 6–8 h later due to released leukotrienes and cellular infiltrate. From the pathophysiological mechanism, it is obvious that IgE antibodies (and in mice also IgG1) play a critical role in type I allergy. IgG and IgE have not been identified in reptiles, amphibians, or birds, suggesting they are unique for mammals (Warr et al. 1995). Normally, IgE plasma levels are low but vary among species: nonallergic humans generally have between 10 and 400 ng/ml serum, and it was observed that the probability for allergy is very high when the IgE level is above 200 kU/L (i.e., 480 ng/ml) (Carosso et al. 2007); domestic canines have IgE levels 10–100 times higher than laboratory mice and humans (500 ng to 50 μg/ml), whereas IgE in a wild Scandinavian wolf population was twice as high as in dogs, namely, 67 μg/ml in the mean (Ledin et al. 2008); and horses have total IgE around 1000-fold higher in normal horse than in normal human serum (Wagner 2009). Typically type I allergies in humans can be elicited by various allergens from sources like pollens, animal dander, house dust mite, molds, insect venoms, drugs, or food. In dogs, IgE-mediated allergies mainly present with itchy inflammatory skin symptoms (Mueller et al. 2016a) and can be attributed to pollen, house dust mites, drugs, food, or insects like fleas. Paradoxically, like humans may react to pet dander (Morris 2010), domestic animals may develop allergies to human dander of their owner (Sture et al. 1995).

8.2.2 Pathophysiology of Type II Allergy

For type II allergies, IgG or IgM antibodies, complexing with antigens fixed on patient's cells (e.g., drugs like penicillin) or directed against cell membrane components (e.g., to the high-affinity IgE receptor FcεRI-alpha chain), activate the complement system, leading to cytotoxic reactions. The clinical picture depends on the destroyed target cells and may, for instance, result in hemolysis when erythrocytes are destroyed or to thrombotic thrombocytopenic purpura when thrombocytes are targeted. If the alpha chain of FcεRI is targeted on human mast cells, this can lead to chronic urticaria. Furthermore, cross-reactive antibodies can develop during infections and may then bind to normal tissue antigens, resulting in antibody-mediated cytotoxicity. For example, horses with streptococcal infection can develop a cross-reaction between *Streptococcus equi* and vascular basement membranes, leading to *purpura hemorrhagica*.

8.2.3 Pathophysiology of Type III Allergy

Excessive soluble antigen induces IgG, IgA, and IgM production and then forms blood-borne immune complexes when binding to these antibodies. Immune complexes are normally cleared by innate immune cells, especially in the spleen. In malfunction of this clearing system, a relative overload of immune complexes occurs. They are deposited at certain body sites and result in complement activation with inflammatory responses and fever. Pathological changes typically occur after 7–12 days, encompassing endocarditis and arthritis in rheumatic fever or in alternate examples such as vasculitis, glomerulonephritis, or enteritis. In humans and domestic animals, type III allergic reactions, for instance, contribute to celiac disease, where IgG, IgA, and IgM antibodies against gliadin from gluten in different cereals are formed and cause duodenal enteritis. In dogs, the term "wheat-sensitive enteropathy" is used (Marietta et al. 2011; Batt et al. 1987). Farmer's lung arises from inhalation of high loads of fungal spores from moldy hay, pigeon fancier's lung from proteins from powdery pigeon dung, humidifier fever from normally harmless protozoans (like fungi of the species *Aspergillus*, *Cladosporium*, *Penicillium*, etc.) in air-conditioning units. In domestic animals, type III hypersensitivity affecting lung tissues is termed hypersensitivity pneumonitis. It is most common in large animals, where spores of thermophilic *actinomycetes* from moldy hay are an important antigen source. The chronic inhalation of these spores causes a condition similar to farmer's lung disease, e.g., in cattle.

8.2.4 Pathophysiology of Type IV Allergy

These reactions are mediated by specific T cells, the recruitment of which needs time (24–48 h) and therefore are called "delayed-type hypersensitivity" (DTH) reactions. T cells can either elicit direct toxic/cytolytic effects or release cytokines,

which activate and recruit inflammatory cells such as eosinophils, monocytes and macrophages, neutrophils, or natural killer cells, which altogether form an inflammatory infiltrate. Typical examples in humans are contact allergies (e.g., against nickel and other inorganic molecules) or drug allergies. In the canine patient, contact allergies are also quite common and contribute to flea allergy as well as to hypersensitivity against several types of ectoparasites (e.g., scabies via *Sarcoptes* mites or demodicosis caused by the mite *Demodex canis*). In both humans and dogs, besides the IgE-mediated mechanisms, also T cells contribute to the chronic phase of the eczematous reaction in atopic dermatitis.

8.2.5 Pathophysiology of Food Intolerances

In contrast to allergies, the immune system does not take part in food intolerance reactions, and there is no immunological memory either. The underlying mechanisms can be malfunctions or lack of enzymes important for metabolism of different substances (e.g., lactase for metabolizing milk sugar, diamin oxidase for histamine, acetaldehyde dehydrogenase for alcohol, phenylalanine hydroxylase for phenylalanine) or defects in transporter molecules (GLUT-5 for fructose uptake).

8.2.6 Experimental Models of Food Allergy

To study the pathophysiological mechanisms and the development of novel therapeutics for food allergy, as well as the allergenic potential of novel food sources like algae or insects, suitable animal models are inevitable, because protein homology to known allergens, stability to gastric in vitro digestion, or in silico analysis of proteins is not sufficient. Several food allergy animal models have already been established; however, there are some drawbacks, recently comprehensively reviewed by Bøgh et al. (2016).

Most commonly, the mouse is used for food allergy studies. The outcome very much depends on the strain, where the BALB/c might be best suited. Also, Brown Norway rats have been used. Furthermore, the dog has a long-standing history in allergy research, because it develops allergies spontaneously, with the disadvantages that there are great interindividual variations and that immunological reagents are lacking. The same is true for the swine as food allergy model. Other animals are guinea pigs and rabbits; however, guinea pigs do not synthesize IgE antibodies, and rabbits are poorly characterized, at least in terms of allergy induction.

For all the mentioned models, the outcome of the allergic response also depends on the route of sensitization, where oral exposure of course best resembles the physiological situation. However, often adjuvants (like cholera toxin, aluminum hydroxide, *Staphylococcus aureus* enterotoxin B (SEB), medium-chain triglycerides (MCT), or endotoxin) have to be used to break or prevent tolerance. Also, epicutaneous sensitization is possible, even without adjuvant, when the skin is abraded.

Further considerations are the dose of the antigen; the processing of the antigen prior to application (e.g., heating, hydrolysis, pH treatment, pressure); the food matrix, which may show a dampening buffer effect; or the presence of protease inhibitors or competitive proteins for digestion proteins.

Environmental factors, which could influence sensitization in the animal models, are unintentional dietary exposure before the experiment, bioactive lipids or nondigestible fiber in the animal diet, environmental pollution, interference with gastric digestion by acid-suppressing medication (Pali-Scholl and Jensen-Jarolim 2011), or the composition of the gut microbiome.

Readouts in these models are clinical signs like diarrhea, decrease of physical activity and of body temperature (Manzano-Szalai et al. 2016), and piloerection. In addition, IgE levels in serum and for degranulation studies on basophils or mast cells, as well as cytokine production from spleen cells, serum mast cell protease mMCP-1 and histamine, airway hyperreactivity, passive cutaneous anaphylaxis, and immediate- and delayed-type hypersensitivity measurement in earlobes and the skin can be performed.

In summary, the ideal animal model for defining the allergenic potential of proteins or for comparison of results among different study groups is not established yet.

For investigating the mechanisms and novel treatment options of food allergy, we propose animals, which spontaneously develop allergies (like dogs) and live in the same environment like humans as ideal patient models.

8.3 Human Food Allergy and Intolerances

8.3.1 Clinical Problem

Food allergies can, in principal, manifest as symptoms along the digestive tract, from oral allergy syndrome, and angioedema (Fig. 8.1a) to colic and diarrhea, or also evoke systemic reactions, from skin disorders, runny nose, coughing, and wheezing to a life-threatening anaphylactic shock. The outcome of an allergic reaction might depend on the antigen dose, its stability against digestion, or the inflammatory status of the patient affecting mucosal barrier function. In comparison, intolerance reactions rather remain restricted to the digestive tract (stomachache, flatulence, diarrhea) but may include flush, headache, angioedema, heart palpations, and psychiatric disturbances (e.g., ADHD). Intolerance reactions are evoked by relatively higher doses than allergies, where minute amounts of the food allergen may trigger the reaction.

8.3.2 Sensitization

In principle, there are several possible sensitization routes for food allergy in humans. First, direct sensitization via the digestive tract can occur. Here, the stability of the allergen to digestion seems to play a critical role. Second, sensitization via

Fig. 8.1 Typical symptoms of allergy or food allergy in human, cat, dog, and horse. (**a**) Angioedema of the lower lip in a human caused by food allergy to crustaceans (With kind permission of Brunello Wüthrich and the Swiss Medical Forum). Published in *Schweiz Med Forum* 2012;12(7):138–143. www.medicalforum.ch; (**b**) head and neck dermatitis with excoriations due to adverse food reaction in European shorthair cat; (**c**) atopic inflammation around the mouth and neck (With chronic pyoderma and *Malassezia* dermatitis) in an English bulldog (By courtesy of Lucia Panakova, Univ. of Veterinary Medicine Vienna, Austria); (**d**) hives in a horse due to allergy (With kind permission from Rene van den Hoven, University Equine Clinic, Section Equine Internal Medicine, Univ. of Veterinary Medicine Vienna, Austria)

aeroallergens, followed by symptoms due to cross-reactive food allergens, is possible. An example is birch pollen allergy that is often complicated by food-related cross-reactivity, such as to apple, hazelnut, or carrot. This process is relevant for stable as well as digestion-labile proteins. However, if proper gastric digestion is impaired, e.g., during application of acid-neutralizing medication (antacids, proton-pump inhibitors, H2-receptor blockers), also digestion-labile proteins can directly induce type I allergy (Pali-Scholl and Jensen-Jarolim 2011). Another disputed topic is sensitization via the skin route (Lack 2008).

8.3.3 Characteristics of Allergens

Allergens eliciting type I allergies in humans are mainly small proteins or glycoproteins. Very often they possess a certain biological function that supports sensitization, like enzymatic activity (Stremnitzer et al. 2014). Some molecules form pockets (e.g., lipocalins) and may transport immune-modulatory ligands. Actually, quite a number of allergens eliciting symptoms in humans, structurally and functionally represent lipocalins, which act as transporter molecules for fat-soluble, iron-binding, or odorant substances (Jensen-Jarolim et al. 2016). They play an important role as animal-derived allergens, as they are secreted via the skin and liberated by the animal dander or are secreted into the urine or saliva. Interestingly, these pocket-containing molecules rather evoke Th2 responses in the unloaded state (Roth-Walter et al. 2014a, b). Moreover, it has been shown that aggregated allergens, as well as natural dimeric and multimeric allergens, have a higher sensitizing potential (Scholl et al. 2005; Niemi et al. 2015). The three-dimensional and quaternary structures are thus important for allergenic proteins during sensitization as well as in the effector phase. (Pali-Schöll and Jensen-Jarolim, 2016)

For food allergens, a pivotal criterion is their digestion stability. For instance, the major allergenic proteins from peanut are stable against gastric digestion. Furthermore, food allergens that are heat stable can also trigger symptoms in cooked state (e.g., celery proteins, some egg allergens). Last not least, the modifications of food constituents during food processing, such as pasteurization or roasting, enhances the allergenic potential (Roth-Walter et al. 2008; Kim et al. 2013).

8.3.4 Food Allergens

The list of foods responsible for allergic reactions in humans is rather short (Table 8.1), with 14 foods and food groups being responsible for over 90 % of food adverse reactions, including IgE- and immune complex-mediated, delayed-type allergies and intolerances: cereals containing gluten, crustaceans, eggs, fish, peanuts, soybeans, milk and lactose, celery, mustard, sesame, sulfur dioxide and sulfites, and lupin and mollusks. All of them have to be listed on prepacked food, and information about their content has to be given for non-prepacked food (e.g., in restaurants) according to the updated EU labeling directive (European and Council of the European Union 2011).

Children below school age mainly respond to milk, egg, wheat, nuts, and legumes, whereas adults rather react to fruits and vegetables via cross-reactivity to respiratory allergens and furthermore to egg, milk, nuts, fish, and crustacean. Some of these allergens, like milk and wheat, are also elicitors of food hypersensitivity in domestic animals (see below).

8.3.5 Diagnosis

An important part of allergy diagnosis in humans is covered by the patient's history. Diagnostic tools include the measurement of total and specific IgE levels in patients'

Table 8.1 Examples of confirmed food allergens for human and veterinary patients

Human	Dog	Cat	Horses
Adults:	Beef	Beef	Oats
Nuts	Dairy products	Fish	Wheat
Peanuts	Chicken	Chicken	Corn
Fish	Wheat	Dairy products	Barley
Shellfish	Lamb	Wheat	Soy
Soybeans	Soy	Corn	Peanut
Wheat	Corn	Egg	(Dry) garlic
Egg	Egg	Barley	Lucerne
Milk	Pork	Rabbit	Alfalfa
Sesame	Fish (tuna, herring, cod, salmon)	Lamb	Malt
Mollusks	(Brown)	Sardines	Bran
Children:	Rice		Buckwheat
Egg	Rabbit meat		Potatoes
Cow's milk	Chocolate		Beet pulp
Peanut	Kidney bean		Clover
Wheat	Tomato		Chicory
	Turkey		
	Brewer's yeast		
	Venison		
	Duck		

This table lists the allergen food sources most important for human patients in Western countries (Sampson et al. 2014) and their companion animals, dogs, cats (Mueller et al. 2016b), and horses (Fadok 2013; Marsella 2013), excluding cross-reactive foods and cereals responsible for celiac disease

serum via fluorescence- or radio-allergo-sorbent tests. In human food allergy, IgE diagnosis is important and, on a molecular basis, predicts the risk for systemic reactions (Canonica et al. 2013). Furthermore, immediate- or delayed-type skin tests (prick, prick-to-prick, intradermal, epicutaneous) can be performed. For food allergies, also oral provocation tests and elimination-reintroduction trials (diet free of allergen, followed by exposure to the suspected allergen) are applied under physician's supervision by specialized clinics. The gold standard today still is the double-blind placebo-controlled food challenge, DBPCFC, wherein neither the patient nor the allergist knows which food is tested to avoid psychological bias. Lactose and fructose intolerances on the other hand are diagnosed via the hydrogen breath test, where non-digested sugar is fermented by intestinal bacteria that produce H2. The relatively higher H2 amounts in exhaled air can then be determined by the examiner and lead to the diagnosis. Genetic mutations of the milk sugar-fermenting enzyme lactase can be determined by molecular biology methods. There are many more specific tests available. In addition, an elimination diet will help to identify the offending food.

8.3.6 Therapy

When diagnosis could confirm food hypersensitivity or intolerance, the first therapeutic step in both diseases is the avoidance of the offending substance. For this, the patient or parents, respectively, need to be educated regarding avoidance strategies

accompanied by substitution of essential nutrients. Otherwise, during allergen-free diets, malnutrition could become a serious concern, especially for small children. Furthermore, the ingredients of all consumed products need to be screened carefully. The labeling of food constituents in prepacked and loose food is an important help for the allergic consumer. The treatment of symptoms of allergic diseases is performed with antihistamines, corticosteroids, decongestants, and/or leukotriene inhibitors. In addition, epinephrine, an important emergency medication in anaphylaxis, has to be carried precautionary by the patient in case of a history of severe reactions. The only causal treatment of allergy can be reached by allergen immunotherapy (desensitization, hyposensitization), either by subcutaneous or sublingual route, but these treatments have not been adopted into daily food allergy management. Here, clinical trials with sublingual (SLIT) and epicutaneous (EPIT) treatment (increasing doses of the offending food are applied) show promising effects in children for milk, egg, and peanut (Sindher et al. 2016; Arasi et al. 2016). Patients with food intolerance should find the tolerable dose of the offending food and include it in their diet further on. In addition, they have the possibility to replace enzymes (e.g., lactase, diamin oxidase) or convert the carbohydrate before ingestion (fructose) or buy food free of the offending ingredient (lactose, histamine, gluten).

8.4 Canine Food Allergy

8.4.1 Clinical Problem

Also among canine adverse food reactions (AFR), food allergy, but not food intolerance, has an underlying immunological mechanism. In contrast to the human situation, in dogs, the limited antigen elimination diet is the only gold standard of diagnostic approaches due to a lack of reliable direct testing methods. However, as this method does not allow distinguishing between food allergy or food intolerance, the true hypersensitivity mechanisms in AFR are hardly documented. In this chapter, we will therefore refer in general to AFR.

AFR commonly affects the gastrointestinal organs and/or the skin of dogs. The main symptoms are nonseasonal pruritus and skin lesions (Fig. 8.1c), which can mimic canine atopic dermatitis; therefore the disease is also called food-induced atopic dermatitis (FIAD). Additionally, erythema and papules can be detected on various body sites, in some cases similar to canine atopic dermatitis and in others just in a specific region, e.g., face, perianal, or pinnae. Also gastrointestinal signs, like increased bowel movements, soft stool, vomiting, and diarrhea, are common (20–30 %) but are mostly mild and often not even recognized by the owner. Beyond that AFR can also result in atypical presentation like urticaria and angioedema, pyotraumatic dermatitis, otitis, claw disease, perianal fistulae, and pruritis (Favrot et al. 2010; Hillier and Griffin 2001).

Onset can occur at a very young age. In a study, 83 % of affected dogs showed clinical symptoms before the age of 3 years, and 48 % of dogs were even younger

than one year (Picco et al. 2008). On the other hand, dogs of any age can be affected by AFR (Proverbio et al. 2010).

Some breeds such as boxer, German shepherd, West Highland white terrier, pugs, and Labrador retrievers seem to be genetically predisposed for AFR (Picco et al. 2008).

8.4.2 Pathogenesis

It has been presumed that food allergy in dogs, besides type I hypersensitivity reactions, also involves type III and IV reactions. Some cases of atypical presentation, e.g., vasculitis, claw diseases, and sebaceous adenitis, have been associated with food, however, without revealing the pathogenesis. Cutaneous type I reactions are associated with immediate and late-phase reactions in dogs. Moreover, food allergy appears to have a delayed component, as patch testing correlated better with symptoms than skin or blood testing for detecting food allergies (Bethlehem et al. 2012).

Although dogs with inflammatory bowel disease and idiopathic antibiotic-responsive diarrhea showed more mixed immune cell infiltrates, there was no significant infiltrate found in the intestine of dogs suffering from AFR compared to control dogs (German et al. 2001). Interestingly, an allergic reaction of the gastric mucosa was found in endoscopy, revealing erythema and mucosal swelling 2 min after application of the food allergen (Guilford et al. 1994). This resembled a true immediate-type reaction in this small number of dogs, but this method is certainly not useful in daily practice.

Regarding the antibody response in food-allergic dogs, there are inconsistent findings, like increase in IgG but not in food-specific IgE, or increase of allergen-specific IgE; however, this IgE was not only directed against the offending food. Furthermore, an interlaboratory study revealed discrepancies among several commercially available allergen-specific IgE and IgG tests (Hardy et al. 2014). Despite those apparent differences in the course of the natural disease, the dog has often been used as a model for human food allergy (Buchanan and Frick 2002).

8.4.3 Food Allergens

The most frequently reported food allergens involved in canine AFRs (Table 8.1) were beef (34 %), dairy products (17 %), chicken (15 %), wheat (13 %), and lamb (14.5 %). Other less commonly reported offending food were soy (6 %), corn (4 %), egg (4 %), pork (2 %), fish, and rice (2 %). Also rabbit meat, chocolate, kidney bean, and tomato were reported as food allergens in single cases (Olivry et al. 2015). Among 101 canine atopic dermatitis patients, the most common food allergens were chicken (60.4 %), followed by turkey (57.3 %), brown rice (42.7 %), brewer's yeast (41.7 %), and soybeans (36.6 %). The sensitization rates for rabbit, venison, duck, and tuna were lower compared to those of the other allergens (less than 10 %) (Kang et al. 2014).

The major allergens recognized by specific IgE of 10 dogs allergic to lamb, milk, and beef had molecular masses between 51 and 58 kDa, which were identified as phosphoglucomutase and the IgG heavy chain. The smallest protein that elicited the IgE response had a molecular mass of 27 kDa. Hydrolyzed diets do not seem to be always effective, suggesting that small proteins are involved, or the hydrolyzed proteins are still large enough to function as allergen (Martín et al. 2004). Hydrolyzed formulas may, moreover, still trigger T-cell-driven delayed-type inflammation. Although food additives such as flavoring substances or colors are often blamed by the public, these substances are not documented causing food hypersensitivity in dogs.

In humans, the oral allergy syndrome secondary to pollen allergy is a well known problem of crossreactivity. In comparison, this syndrome is documented so far in just one dog, allergic to Japanese cedar pollen and cross-reacting to tomato, but the overall importance in dogs is unclear (Fujimura et al. 2002).

8.4.4 Diagnosis and Therapy

The diagnosis of AFR in dogs is based on a compatible history and clinical signs. Due to a highly variable clinical presentation, all possible differential diagnoses should be ruled out first, and afterward, an elimination diet should be started.

For example, in the case of food-induced atopic dermatitis, other pruritic conditions, e.g., ectoparasites, atopic dermatitis and secondary bacterial or yeast infections, should be excluded, and the dog food should be changed to a novel protein and carbohydrate source (Picco et al. 2008). During the elimination diet, the clinical signs should resolve, and no further treatment should be necessary. The strict diet trial should last at least 8 weeks to detect over 90 % of affected dogs. During this time, there is the possibility to use an allergen-limited homemade diet or to feed a hydrolyzed commercial food. The challenge after the trial is important to affirm diagnosis: by feeding the suspected food, clinical signs will be reevoked in true AFR. Any dermal or intradermal skin tests with food allergens are, like in human food allergies, not reliable because of frequent false-positive results. Furthermore, various studies documented a low accuracy of antigen-specific IgE or IgG testing in serology (Zimmer et al. 2011). This may generally indicate that there is a need for improving canine allergy diagnosis.

If the dog is in remission, a balanced non-offending diet can prevent the disease, and further food sources can be introduced as long as no symptoms are appearing. Unfortunately, there is still the possibility that the dog starts to react against previously non-offending food over time. Probiotics have shown some useful effects in 21 dogs with diarrhea, which were hyperresponsive to their diet, but no further attempts were made to confirm these data (Sauter et al. 2006).

AFR can become quite frustrating for the dogs, but also for their owners, especially if the dog takes up food anywhere. The diagnosis and treatment of AFR can take a certain time, and it can be challenging and demoralizing for the owner. A better understanding of this disease would probably help to invent more promising diagnostic tools and new treatment options.

8.5 Feline Food Allergy

8.5.1 Clinical Problem

Most cats having food allergy or food hypersensitivity develop dermatological signs including nonseasonal pruritus of varying severity. Dermatological signs (Fig. 8.1b) include miliary dermatitis, eosinophilic dermatitis, self-induced alopecia, head and neck excoriations, pyotraumatic dermatitis, or scaling dermatoses (Roudebush et al. 2010; Hobi et al. 2011). Nevertheless, none of these reaction patterns is pathognomonic for allergic dermatitis. Also clinical signs like angioedema, urticaria, or conjunctivitis may occur. Gastrointestinal disturbances occurred in 21 % of cats with cutaneous manifestations of food hypersensitivity (Hobi et al. 2011). Vomitus, diarrhea, lymphoplasmacytic enteritis, and colitis are also common signs. Among 55 cats with chronic idiopathic gastrointestinal problems, 16 (29 %) were diagnosed with food sensitivity by elimination-challenge tests (Roudebush et al. 2010). If both organs, the skin as well as the gastrointestinal tract, are affected, food hypersensitivity is very likely the reason.

In a colony of 26 cats, vomiting with increasing frequency and squamous dermatitis in eight cats was observed (Hirt and Iben 1998). Differentials showed significant eosinophilia (mean = 1.3×10^9/l) and a mild lymphocytosis in five cats as well. Feeding a hypoallergenic diet for 12 weeks resulted in a dramatic reduction of eosinophilia (mean = 0.6×10^9/l, s = 0.27) as well as of clinical signs. In two of the five cats with lymphocytosis, the number of lymphocytes decreased to normal values. After 16 weeks of dietary rechallenge, seven of the eight cats demonstrated significant eosinophilia again. A recurrence of clinical signs could be observed in four of the cats. Lymphocytosis was found in the same five cats again. Interestingly, histology of the endoscopically obtained gastric and duodenal specimens revealed a moderate eosinophilic infiltration of the duodenum in only two cats; another two cats had a mild eosinophilic infiltrate in the stomach. According to Scott et al. (2001), absolute peripheral eosinophilia occurs in 20–50 % of feline cases. Hobi et al. found in 502 pruritic cats that in 12 %, food hypersensitivity was the cause for pruritus (Hobi et al. 2011).

Chronic intestinal inflammatory diseases may be preceded by the development of dietary hypersensitivity due to a possibly defective digestion, mucosal lesions, and therefore the absorption of proteins with higher molecular weight.

The onset of food allergy in feline patients ranges from an age of 3 months to 11 years with a mean age of 4–5 years (Bryan and Frank 2010).

Siamese or Siamese cross cats seem to be at increased risk for developing AFR, as they accounted for nearly one third of food allergy cases (Roudebush et al. 2010).

8.5.2 Feline Food Allergens

The most common food allergens for cats (Table 8.1) are beef, fish, chicken, and dairy products (Mueller et al. 2016a; Roudebush et al. 2010). Wheat, corn, egg,

barley, rabbit, and lamb were also reported (Mueller et al. 2016b). To the authors' knowledge, there are no data about cross-reactivity in cats.

8.5.3 Diagnosis

Evaluating allergen-specific IgE levels is no reliable diagnostic tool for hypersensitivity to food or environmental allergens in cats but can be successfully used for diagnosing fleabite hypersensitivity.

Ishida et al. conducted a lymphocyte stimulation test in three cats suffering from AFR; in total, 12 allergenic food ingredients were identified by oral food provocation tests in at least one of the three cats (Ishida et al. 2012). Nine of those food antigens were shown to be positive in a lymphocyte stimulation test, but none of them were positive in antigen-specific IgE testing. Four food antigens were positive in intradermal testing (beef, corn, tuna, cod). The cats that were fed elimination diets showed decreased lymphocyte stimulation in tests.

Like in dogs, a differentiation between an immune-mediated reaction against food and food intolerance of non-immunologic origin is not common in practice due to the lack of proper diagnostic tools. After exclusion of all other diseases that can elicit dermatological symptoms, e.g., ectoparasites and some metabolic problems, feeding an elimination diet followed by a challenge test is recognized as the most reliable diagnostic procedure in cats as well. However, palatability and client compliance can each be a problem; specifically, many owners are unwilling to perform a provocation challenge, which is required to confirm a suspected food allergy. Additionally, in some cases, cats may have concurrent allergic conditions. Finally, it has to be mentioned that the elimination-challenge test confirms or rules out adverse food reactions but, in many cases, does not reveal the underlying mechanism (Roudebush et al. 2010).

8.6 Equine Food Allergy

8.6.1 Clinical Problem

Although food allergy is believed to occur in horses, it is very rare, and no reliable scientifically supported literature exists about its prevalence, its causes, or pathogenesis. Furthermore, it is not known whether food intolerance exists in horses. All skin reactions to food substances are therefore considered to be related to food allergy. The most common clinical signs are seasonal or nonseasonal pruritus and urticaria (Fig. 8.1d). Self-mutilation can occur because of the pruritus (Harris et al. 2013). Signs of described food allergy were reported to include recurrent urticaria, pruritic skin disease, and anal pruritus (Fadok 2013). A specific form of food reaction in horses is named protein bumps, oat bumps, or alternatively sweet feed bumps, which manifest as small 1–3 mm skin bumps often with little crusts, and resolve after a low-protein diet. Whether this represents a certain form of food

intolerance or hypersensitivity is not known. By decreasing the protein concentration in the diet, the bumps obviously disappear (Harris et al. 2013).

8.6.2 Equine Allergens

Foods incriminated in equine skin efflorescences (Table 8.1) were sweet feed, oats, corn, other grains, and alfalfa (Fadok 2013). There is one case of urticaria reported in literature, which was caused by garlic feeding (Miyazawa et al. 1991). There were no other clinical findings except wheals distributed over the skin of the whole body. The authors conducted an Ouchterlony double immunodiffusion test between the extracts of all food components and the serum of the horse. A precipitation line was found against garlic, indicating the presence of garlic-specific IgG antibodies. When garlic was excluded from the diet, the wheals disappeared within 13 days.

8.6.3 Diagnosis

Commercial IgE-based tests are available for diagnosis of food allergies and are commonly used in equine practice. Dupont et al. evaluated an IgE test for hoses by sending blinded samples of 17 healthy ponies to a laboratory for screening of common food allergens (Dupont et al. 2016). Only seven ponies were negative on the IgE-based test at the two chosen time points, three had positive results twice, but only one tested positive twice for the same food allergen. The results show that the available IgE-based tests are not reliable to screen for equine food hypersensitivity.

As for dogs and cats, an elimination-challenge trial is the only diagnostic tool, although diet trials in horses are more difficult to conduct than in dogs and cats (Marsella 2013).

8.7 Synopsis

The clinical pictures of food allergy and intolerances are different between humans and their companion dogs, cats, and horses. Whereas well defined in humans, the underlying pathophysiological mechanisms are less understood in the veterinary patients. Consequently, the diagnosis of food allergies are more sophisticated in human patients, whereas in veterinary patients, elimination-reintroduction trials lead to diagnosis. For improving the understanding and therapy of food allergies, most often mouse models with experimentally induced food allergies are used, whereas companion animals suffering from spontaneous diseases would be more ideal models. In addition, there are several animal species for which allergy and intolerances are not yet in the focus of medical interest, for instance, farm and production animals, but clearly should be for their welfare as patients and improvement of holding conditions. In conclusion, revelation of mechanisms and development of

improved diagnosis and treatment options could benefit from comparing humans with different species and exchanging knowledge between experts in both fields.

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Allergic and Atopic Eczema in Humans and Their Animals

9

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Abstract

The encounter of high levels of allergens via the skin, such as from house dust mite, may induce allergic dermatitis. Atopic individuals, both human and animal, are genetically predisposed for a deficient skin barrier function and have an inborn higher jeopardy for percutaneous allergy and infections. The atopic phenotype also has typically a higher risk for environmental allergies which is in humans termed the atopic march.

Atopic dermatitis (AD) or eczema is clinically associated with chronic or recurrent, often persistent skin inflammation at typical body sites: head and face, neck, intertrigo areas, and bend and hollow sites of arms and legs. It often occurs from early age, may persist lifelong, and is complexed by the associated itch. The clinics in humans and animals are comparable in terms of the pruritic inflammation, and scratching bears a high risk for superinfections. Further symptoms may be thickening of the keratocyte layer associated with overall atrophy of the skin (lichenification); more rarely severe systemic courses may take place.

The diagnostic criteria are the eczematous skin appearance, elevated total IgE levels, and occurrence of allergen-specific IgE associated with hay fever, asthma, and food allergies. Treatments of AD are based on skin repair and anti-inflammatory and immunomodulatory drugs, mostly local, in severe cases systemically. All treatments today are symptomatic.

The great homology of human and veterinary AD should be recognized to speed up the understanding of the underlying pathophysiology and result in development of an improved generation of drugs with true healing potency.

9.1 Introduction

Whereas allergic dermatitis may be caused by high exposure levels to skin allergens with an aggressive potency, for instance enzymes released from house dust mites, the atopic dermatitis (AD) is associated with a genetically predisposed skin and mucosal barrier weakness. Many of the physiologic features of the skin are disturbed in

allergic skin inflammation and become especially severe and chronic on an atopic genetic background. Atopy also leads to a generally higher likelihood for sensitization to allergens. Atopy also means that individuals from birth have an insufficient skin barrier function and early onset of disease, often rendering lifelong acute and chronic inflammation. Fair phenotypes are more prone to AD, but on the other hand, in humans, AD can also be observed in the black population. The inflammation in AD is characterized by a Th2 lymphocyte-dominated immune response. A prominent result is the elevation of IgE immunoglobulins that accelerate the disease and cause acute as well as chronic symptoms. The Th2 response and elevated IgE results from the alarm signals derived from disturbed skin and immune cells.

9.1.1 Skin Function in Health and AD

The skin is the outer barrier to the environment of humans and animals. It is constituted by a multilayer protective sheet. The epidermis with its keratinocytes is mostly responsible for mechanical defense, which is reinforced by cutaneous extensions such as hairs or as feathers. These extensions are, together with fat deposited in the deeper subcutis layer of the skin, responsible for thermoregulation. Most of the epidermal function also relies on a turnover of layers from the bottom to the top. With this process of releasing cuticles, the body also gets rid of parasites, bacteria, and other potentially harmful agents. In an aged individual, the turnover process gets slower, enhancing the likelihood for aberrant ectodermal colonization. Especially, the lower layers of the epidermal cells are tightly fixed to each other by various junctions that minimize water loss via the skin. The "tight junction" is constituted by several molecules that extend into the wall of the neighboring cells. The keratinocytes are also cemented to each other by fatty glue containing ceramide that is produced from shingomyelin (Pullmannova et al. 2014). The epidermal filaggrin molecule is, together with the lipids, contributing to the skin barrier function. The integrity of the keratinocyte layer is therefore disturbed through detergents and fat-solubilizing substances, e.g., contained in shampoos. The overuse of such cosmetics may also lead to a disturbance of the pH milieu of the skin which is usually in the area of 4.0, but by alkaline substances may be elevated. A recent study using an atopic dermatitis mouse model proved that "alkalinization" of the skin in disease-free NC/Tnd mice activates a cascade of inflammatory events that finally lead to skin inflammation, similar to what is known in atopic dermatitis (Jang et al. 2016).

9.1.2 The Skin Has a Microflora

There is also a microflora on each healthy skin that is dependent on and also contributing to the skin's low pH. The resident microflora may normally be regarded as harmless as long as the skin barrier function is intact. Constituents of the healthy skin comprise, e.g., *Malassezia* yeast species (*Pityrosporon ovale*) or bacteria as *Staphylococcus aureus* in humans and, e.g., *Staphylococcus pseudintermedius* in dogs. The flora of the healthy skin is sometimes comparable among humans and for

instance sheep (Haarstad et al. 2014) or horses (Shokri 2016), depending on the breed. In dogs, *Proteobacteria* and *Oxalobacteraceae* play a role in the skin microbiome, and it was found that the spectrum of colonization slightly varied among the individuals (Rodrigues Hoffmann et al. 2014). However, generally it is reported that in healthy skin of humans and animals, there is a higher phylogenetic variety than in atopic individuals which tend to overgrow certain species, consequently dominating the flora (Hoffmann et al. 2016; Salava and Lauerma 2014). The situation gets more complex even, considering that the microbiome can be transferred from humans to their pets and vice versa (Song et al. 2013).

The microflora is tolerated in healthy individuals by the intact skin but may upon barrier disruption become pathogenic and induce inflammation. In atopic individuals it is not so clear yet whether the observed modified skin colonization is the cause or the reason for this skin disease.

9.1.3 Factors Affecting Skin Barrier Function

Generally, skin barrier disruptions of any kind produce danger signals to the body. First, keratinocytes release antibacterial peptides to the surface when stressed. The keratinocytes do also release danger signals that stimulate the immune defense system, including cytokines, such as IL-8 that attracts neutrophilic granulocytes or thymic stromal lymphopoietin (TSLP) that promotes a Th2-dominated immune response. Also alkalinization results in TSLP production (Jang et al. 2016).

The so-called Th2-type immune response is characterized by the recruition and activation of T-helper-2 lymphocytes that are a prominent source of cytokines IL-4 and IL-13. These cytokines shape the typical Th2 response including an overwhelming switch of immunoglobulin production to the IgE isotype. Total IgE levels in atopic dermatitis are in fact extremely elevated in the periphery and also in the skin. The high IgE levels induce the expression of the high-affinity IgE receptor FcεRI on various effector cells that release inflammatory mediators when its receptor-bound IgE is cross-linked. Th2 cells are also a source of IL-5 which induces eosinophilic inflammation. Notably, all attracted inflammatory cells are again a source of Th2 cytokines and aggravate the pathophysiology of the eczematous reaction.

From the above it is clear that the primary event in atopic dermatitis is an inborn barrier deficiency, for instance, by mutations in the filaggrin and many other genes (Elias and Wakefield 2014). The leaky skin results in lower ceramide levels, elevated skin pH, transepidermal water loss, and malcolonization of the skin by dominating *Staphylococcus aureus* and *Malassezia* species that induce more inflammation. Inflammation is again disturbing the barrier, all resulting in a *circulus vitiosus*.

The atopic skin by its barrier deficiency is also able to more easily take up environmental allergens. The atopic human patient is therefore characterized by elevated IgE levels, atopic eczema, and a tendency to more environmental allergies and asthma. In atopic canine and equine patients, typically pruritic dermatitis with classical distribution is seen, whereas total IgE levels are *a priori* higher than in humans. Environmental allergens *per se* harbor different mechanisms to stimulate allergic

inflammation. For instance, some house dust mite allergens act as enzymes and actively open up the tight junctions (Stremnitzer et al. 2015). Some other house dust mite allergens interact and stimulate Toll-like receptor 4 that is involved in Th2-type immune responses (Trompette et al. 2009).

Also food and pollen allergens can be taken up by the skin and lead to sensitization more easily in the atopic individual.

9.2 AD in Human Patients

9.2.1 Clinical Problem

Atopic dermatitis manifests mostly in early childhood, when it may also be known as neurodermitis. The cumulative incidence according to the German Sk2 guidelines in Europe lies between 11 and 21 % (Werfel et al. 2016). The infants develop eczematous skin, starting from the head and neck area, the flexural sites of arms and legs, and intertrigo areas (Fig. 9.1d, e). Sometimes, the lesions spread over the body and may in severe cases lead to significant disease including erythema. The course may be chronic or chronic relapsing. The eczema-associated itch prompts the patient to scratching which secondarily leads to bacterial or viral superinfections at the affected sites. Allergies may complicate the disease and must be diagnosed. Besides the elevated IgE levels that indicate the atopic state, also specific IgE may therefore be found directed against typical environmental allergens (pollen, house

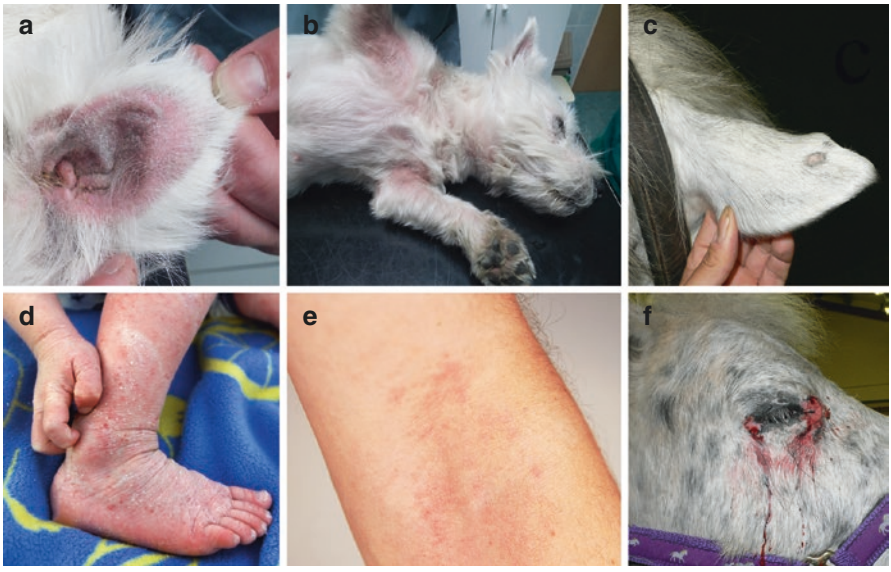

Fig. 9.1 Typical atopic dermatitis lesions. In a Maltese dog's ear (**a**) and subaxillary (**b**); itchy AD (neurodermitis) in a child (**d**) and flexural site in a human adult (**e**); on ear and around the eye of a horse (**c**, **f**)

dust mites), as well as to foods (most typically milk, egg, and wheat besides others). The diagnosis can be made by skin prick testing outside lesional skin (Fig. 9.2a). Some allergens like house dust mite may due to their immunostimulatory (Stremnitzer et al. 2014), irritating, or enzymatic properties (Stremnitzer et al. 2015) give false-positive results in atopic skin and must be approved by serum IgE testing. Besides ImmunoCAP also component-resolved tests are available and practiced by the experienced experts as first-line diagnosis (Canonica et al. 2013) and showed a high predictive value in AD patients recently (Choi et al. 2014).

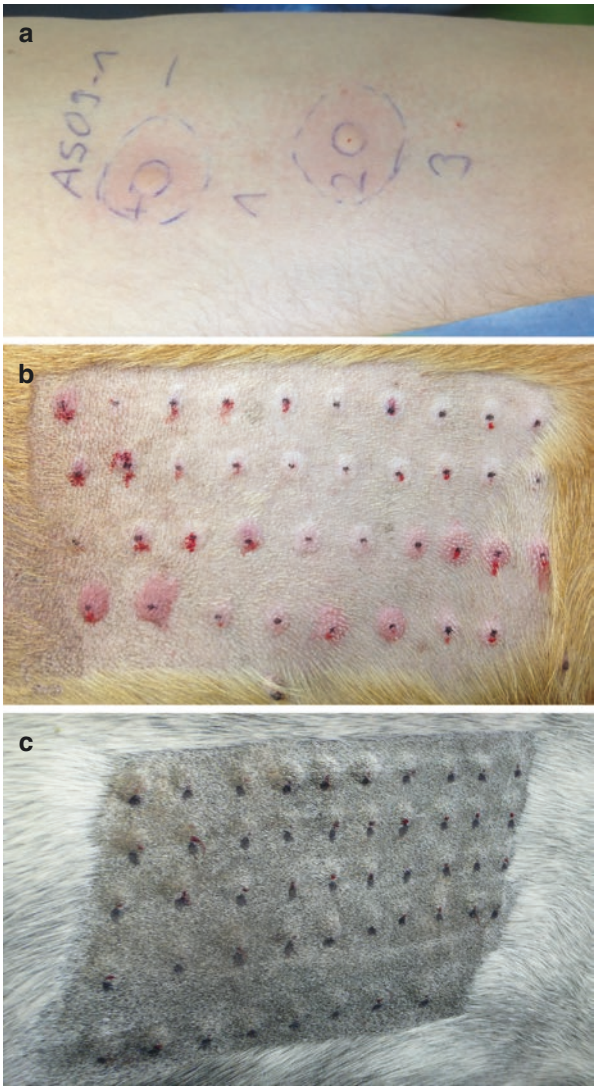

Fig. 9.2 *Results of allergy testing in atopic human, dog and horse*. (**a**) Skin prick test result in a human atopic patient indicating specific sensitization to grass pollen allergens (+, histamine prick as positive control; −, 0.9 % NaCl solution as negative control; 1, birch pollen extract, 2, grass pollen extract, 3, house dust mite extracts); (**b**) intradermal test in dog with 40 allergens; (**c**) intradermal test in horse with 50 allergens

The clinical symptoms develop with distinct dynamics and allowed the identification of four patient types (Lee et al. 2016): (1) 26 % with early onset of AD and low atopy; (2) 48 % with early onset but high atopy, high eosinophil numbers, and persistence of disease; (3) 10 % with late onset and low atopy; and (4) 15 % with late onset, high atopy but normal eosinophil counts. Especially, the children of group 1 have a higher risk to develop the atopic march, characterized by hay fever, bronchial hyperrreactivities, and asthma, as well as food allergies (Lee et al. 2016).

To objectivize the disease severity, two parameters have been introduced, Eczema Area and Severity Index (EASI) and Scoring Atopic Dermatitis (SCORAD) (Schmitt et al. 2013).

9.2.2 Therapy of AD

Due to the generally chronic disease, patient education within a team of pediatricians, dermatologists, dieticians, and nurses is very important (Barbarot et al. 2013). The interdisciplinary team should also include psychologists as atopic dermatitis patients have a lower stress threshold level. Interestingly, the disease itself may be determined in children through stress of the mother during pregnancy (Andersson et al. 2016). Dietary measures may be helpful and in case of specific sensitization the prevention of "atopic foods" egg, milk, and others. Additionally, probiotics may be protective, but the evidence for their therapeutic potency is still weak in humans (Powers et al. 2015) in contrast to canine studies (Kim et al. 2015).

It is recommended to treat the atopic dermatitis as skin disease and simultaneously the comorbidities. A major goal is the establishment and repair of the barrier function of the skin by bland oily ointments and cleansing, astringent treatments, and baths (tannin, zinc) even in the absence of inflammation. There are increasing efforts to also imprint textiles with astringent substances as silver nitrate. Antipruritic therapy may be needed, too, and can be accomplished by antihistamines, especially when allergic sensitization is documented. To downregulate inflammation local glucocorticoids are used especially in the acute phase sometimes under occlusion. Tacrolimus is an alternative topical medication that may be used from the age of 3. In babies the enhanced resorptive capacity of the skin must be considered and potential systemic undesired side effects including potential negative effects on growth by the glucocorticoids (Baum et al. 2002). This was confirmed for intranasal glucocorticoids treatment in atopics (Singh et al. 2013) but is possibly less relevant for the topical atopic eczema with glucocorticoids and calcineurin inhibitor tacrolimus (Gradman and Wolthers 2007) but must be considered in oral glucocorticoid therapy in severe cases. The immunosuppressive drugs cyclosporine in adulthood and azathioprine (classically in Anglo-American area) or methotrexate are indicated in chronic, severe cases.

To prevent superinfections patients should participate in regular vaccine programs according to the German vaccine standing committee (Werfel et al. 2016). Superinfections may be treated by systemic and local antibiotics (incl. fusidic acid) or in case of yeast colonization antifungal therapy. In refractory cases cyclosporine

can be used topically. Innovative approaches include the downregulation of IgE by the anti-IgE antibody omalizumab, possibly in combination with IgE immunoabsorption (Zink et al. 2016). The lowered IgE levels are followed by a downregulation of its high-affinity receptor FcεRI on inflammatory cells. Reduction of IgE levels therefore interferes with the T-cellular aspect of the atopic inflammation, to reduce the IgE-mediated antigen uptake and presentation to Th1 and Th2 lymphocytes. Other biologicals are in the pipeline for human AD patients, among them anti-IL-4R antibody dupilumab which has shown clinical efficacy in severe AD, which is not approved to this end (Beck et al. 2014).

Last but not least, studies are ongoing to demonstrate the successful treatment of secondary allergies by allergen immunotherapy including sublingual immunotherapy (SLIT) (Arasi et al. 2016). Although human guidelines are hesitating to provide clear recommendations (Pfaar et al. 2014; Jutel et al. 2015), SLIT against house dust mite allergens has recently been successfully applied in atopic dogs (DeBoer et al. 2016).

9.3 Canine Atopic Dermatitis (CAD)

9.3.1 Clinical Problem

In dogs the skin represents the most important anatomic site where allergic disorders become manifested and visible. Due to similarities between the clinical signs of atopic dogs and atopic humans and comparable immunological pathways, as far as identified in dogs, the naturally occurring disease of our pets may help to increase the knowledge about the pathogenesis of atopic dermatitis in both species (Olivry 2012).

Canine atopic dermatitis (CAD) is per definition a genetically predisposed inflammatory and pruritic allergic skin disease (Fig. 9.1a, b) with characteristic clinical features associated with IgE antibodies most commonly directed against environmental allergens (Halliwell 2006).

There is a discussion ongoing whether food allergy might be the same disease. This is due to similar clinical signs as seen in CAD, only caused by food (Fig. 8.1c). As a result of this discussion, the term food-induced atopic dermatitis (FIAD) was introduced for canine food allergy (see Chap. 9). In this chapter we focus on the classical CAD caused by environmental substances.

CAD is one of the most common skin diseases of dogs, with a prevalence of 3–15 % in the general dog population (Hillier and Griffin 2001). It is a usually a lifelong disease that can be controlled but rarely cured (Saridomichelakis and Olivry 2016). The age of onset is typically very young including puppies from 6 months to dogs up to 3 years (Picco et al. 2008). Various studies document that West Highland white terrier, Labrador retriever, golden retriever, boxer, French bulldog, German shepherd, and cocker spaniel dogs represented the most commonly affected breeds, whereas there are geographical differences in the breed predisposition (Bizikova et al. 2015b). The main feature of CAD is pruritus and skin lesions like erythema, macules, and papules in specific body sites, e.g., on the face, the paws, the belly, the

ears, and the flexural areas of the legs, although there are slight breed-specific differences in the presentation of CAD signs (Jaeger et al. 2010; Wilhem et al. 2011). The clinical appearance may change with a more chronic character of the disease, and lesions like pustules, crusts, self-induced alopecia, and lichenification are present due to secondary infections and excoriations. Secondary infection and/or otitis with bacteria or yeast is commonly seen in these dogs (DeBoer and Griffin 2001). Atopic conjunctivitis is reported in 21–30 % and rhinitis in around 7 % of CAD dogs as a non-cutaneous condition (Picco et al. 2008; Wilhem et al. 2011; Favrot et al. 2010). One of the most commonly detected allergen in sensitized dogs is house dust mite, and it is considered to be clinical highly important, although also seasonal pollens play a role in the disease (Jensen-Jarolim et al. 2015; Mueller et al. 2015). As in humans atopic dogs have in general a higher risk of sensitization to allergens; therefore also some dogs represent with more than one allergic disease. Common and difficult to diagnose are combinations of CAD + FIAD and CAD + flea allergy dermatitis or even a combination of all three allergies (Loeffler et al. 2006; Sousa and Halliwell 2001).

9.3.2 Pathogenesis of CAD

As already mentioned in the introduction, the pathogenesis of AD is multifactorial and involves a complex genetic background, an immune system dysfunction, and an impaired skin barrier in humans, and this is also true for dogs. However, clinical manifestation is only expressed in some individuals with these predisposing factors where different triggers and underlying causes are combined in an optimal, not yet completely understood, way.

In dogs AD is a heritable disease influenced by other factors such as environmental components (Wilhem et al. 2011; Picco et al. 2008). Various trials attempting detection of responsible genes associated with the development of CAD revealed inconsistent results (Tengvall et al. 2013; Roque et al. 2012; Salzmann et al. 2011). The loss of function mutation in the gene encoding the filaggrin protein is increasing the risk of AD in humans; however this could not be detected in the breed of West Highland white terriers, boxer, German shepherd dog, golden retriever, Shiba Inu, shih tzu, and pit bull (Salzmann et al. 2011; Barros Roque et al. 2009; Wood et al. 2009). Different canine studies agree that a breed-specific genetic background is highly likely, but also regional differences in gene mutations should be considered (Bizikova et al. 2015b). The skin barrier impairment in dogs is comparable with data from the human field regarding an increase of transepidermal water loss (TEWL) and decrease of ceramide levels in atopic dog skin (Marsella et al. 2011; Shimada et al. 2009; Bizikova et al. 2015a). As above-quoted also the microbiome of the canine skin came in the focus of researchers, and the first results confirmed the data found in children that the diversity of the microbiota in diseased atopic dogs was decreased compared to healthy dogs (Kong et al. 2012; Rodrigues Hoffmann et al. 2014). The uptake of allergens in dogs is mainly via cutaneous exposure predisposing the dogs with an impaired skin barrier of higher allergen burden. Environmental allergens can be captured by Langerhans cells and are presented to naive CD4+ T lymphocytes.

CD4+ T cells differentiation to the T-helper type 2 phenotype stimulates the maturation of B cells and production of IgE. This allergen-specific IgE binds to the surface of mast cells, through their expression of the high-affinity IgE receptor (FcεRI). After second allergen contact, the allergen cross-links the IgE receptors, and the degranulation of mast cells leads to the allergy symptoms (Pucheu-Haston et al. 2008). All these factors play a role in the disease and are interrelated, but for many of them, it is still unclear whether they represent a primary cause or a secondary phenomenon in CAD (Saridomichelakis and Olivry 2016).

9.3.3 Diagnosis by Exclusion

CAD is a clinical diagnosis, and the disease is considered after a diagnostic approach to exclude other pruritic disorders. There is no test so far that can directly distinguish between healthy or atopic individuals using total or allergen-specific serum IgE (Egli et al. 2002; Lauber et al. 2012; Koebrich et al. 2012).

Important differential diagnoses that must be considered/excluded first are scabies and other pruritic ectoparasitic disorders, food-induced atopic dermatitis, flea allergy dermatitis, and bacterial and/or yeast skin infections. Therefore, antiparasitic trials controls, antimicrobial treatment, and food-restriction diets are performed before diagnosing CAD.

9.3.4 Therapy of CAD

Allergen testing either with specific IgE or intradermal skin test is only done if a causative therapy with an allergen-specific immunotherapy (ASIT) is planned. In contrast to human AD, ASIT is commonly prescribed in dogs with CAD with a moderate-to-high success rate.

The other treatment option is the symptomatic treatment with an individual patient-based combination therapy consisting of, e.g., immunosuppressive drugs, in topically or systemic formulation, skin barrier care products, essential fatty acids supplement, and avoidance of flare factors (Saridomichelakis and Olivry 2016).

After all it is important to find the most effective treatment with the lowest risk of side effects convenient for each individual dog and owner. For the future it is necessary to improve our understanding of the pathogenesis of the disease in order to develop new generations of targeted therapies.

9.4 Feline Atopic Dermatitis (FAD)

9.4.1 Clinical Problem

Clinical signs of allergies in cats differ from that in humans or dogs. Allergic cats are usually presented with pruritus (head and neck pruritus or generalized pruritus), specific skin lesions like miliary dermatitis and eosinophilic granuloma complex, or

occasionally otitis externa. Rarely cats present with additional respiratory problems attributable to feline asthma (Noli and Cena 2015; Ravens et al. 2014).

Symptoms associated with cutaneous allergies are not specific for allergic disease in cats. Uncertainty exists not only among the pathogenesis but also among the nomenclature of allergies in cats (Noli and Cena 2015). Flea, environmental, and food antigens are the main allergens in feline cutaneous allergy (Favrot et al. 2012). This type of allergies may coexist in the individual patient.

Although, based on clinical signs, differentiation between cutaneous food allergy and atopic dermatitis (environmental allergen caused allergy) is not possible, some of the clinical signs are more often seen with some particular underlying hypersensitivities in cats (Hobi et al. 2011). Clinical signs in affected cats are variable, and some individuals even experience symptom-free intervals. Two scoring systems, SCORFAD and FEDESI, evaluating severity and extent of skin changes in atopic cats were introduced (Steffan et al. 2012; Noli and Cena 2015). Cats show nonseasonal or seasonal symptoms, according to the allergens involved, geographic area, and lifestyle. Hematology and blood chemistry are not helpful in diagnosing allergies, and no biomarkers or simple blood tests will support the diagnosis of feline atopic dermatitis, food, or flea allergy. Allergic cats present with one to several symptoms, sometimes even at the same time (Favrot et al. 2012).

9.4.2 Clinical Appearance of FAD

Miliary dermatitis refers to a dermatitis with papules and crusts that usually develops on the back. Primary lesions consist of small papules topped by yellow crust. The lesions may be difficult to see but are easily palpated.

Head and neck pruritus is usually a severely itchy dermatitis of the head and of the neck with secondary lesions (self-induced alopecia, erosions, ulcerations, and excoriations) as well as papular and crusted dermatitis. The lesions are often complicated by secondary bacterial and less commonly yeasts infections (see also Fig. 8.1b).

Self-induced hair loss (alopecia) is characterized by usually symmetrical noninflammatory hair loss mostly on the flanks and abdomen caused by excessive licking; alopecic skin is not inflamed, and the hair tips in and around the lesions are broken.

Eosinophilic granuloma complex consists of inflammatory plaques or granulomas with the predominant cell type of eosinophils and/or indolent ulcer:

- The indolent (eosinophilic) ulcer is an erosion or ulceration of the upper lips, as the name says, typically being devoid of pain.
- Eosinophilic granulomas may present as usually non-itchy bumps firm lesions occurring mostly in the oral cavity and interdigital areas at the paws, chin, and limbs.
- Eosinophilic plaques are raised, red, exudative, and intensely itchy lesions usually localized on the abdomen and inguinal region. In most cases, secondary purulent infections (pyoderma) may complicate the primary lesion.

9.4.3 Diagnosis by Exclusion

Even though each of these patterns can be seen in association with numerous different causes, some of them are typical of specific etiologies. For example, involvement of the back is more often associated with flea hypersensitivity (Hobi et al. 2011). To make a specific diagnosis of feline atopic dermatitis in cats, patients must show one or more of the feline cutaneous patterns mentioned above, and other differential diagnoses must be ruled out first.

For example, in a cat with "head and neck pruritus," ectoparasites like ear mites, as well as inflammatory polyps from the ear canals or even some neurologic disorders should be ruled out first. Likewise in a cat with "symmetric self-induced alopecia," ectoparasitic infestation (e.g., *Demodex* or *Cheyletiella* mites, as well as fleas) but also other differentials (e.g., metabolic, internal) must be ruled out before diagnosing allergic dermatitis. In patients with signs of eosinophilic granuloma complex, skin infections and tumors are important differential diagnoses and are generally ruled out by cytology or histopathology. Afterward, the diagnostic approach to allergies should be attempted.

From allergic causes, flea allergy dermatitis should be always ruled out at the first place. This is performed by a treatment response, since neither fleas nor flea feces are necessarily discovered in patients with flea allergy dermatitis. In a cat with nonseasonal hypersensitivity, food allergy should be ruled out before diagnosing atopic dermatitis. The gold standard for ruling out food allergy is still performing proper elimination diet. After improvement of the skin condition on the elimination diet, re-challenge with the original food is suggested to finally prove the food allergy.

Owing to the many potential causes of pruritus, a stepwise approach to the pruritic cat is indicated. This can take time and requires a degree of patience from the client, but ultimately this provides the best patient care and is the most cost-effective.

After exclusion of other skin diseases, certain subset of cats with defined clinical signs, including chronic or recurrent pruritus that responds to corticosteroids or ciclosporin, have allergic dermatitis.

9.4.4 Identification of Allergens

Like in dogs, allergy testing in cats is performed for identifying causative allergens once a clinical diagnosis of atopic dermatitis (compatible history, clinical signs, and elimination of other pruritic dermatoses) was established. The main indication for such "allergy" testing is to identify relevant allergens for immunotherapy. Several studies demonstrated that even normal healthy cats can have "positive allergy test" results using commercially available *in vitro* tests (Diesel and DeBoer 2011). Two main testing types are available: intradermal tests and serum tests. Intradermal testing is useful, but the transient and often poor wheal formation is limiting its use (Fig. 9.2b). The use of fluorescein can significantly improve the ability to identify positive reactions.

Variable methodologies are used to measure serum IgE concentrations by commercial companies applying *in vitro* tests. Serum tests are easier to perform than intradermal tests. The most widely used and studied *in vitro* test is an ELISA that utilizes the cloned alpha chain of the human high-affinity IgE receptor (FcεRI). According to a retrospective study on atopic cats from Australia (Ravens et al. 2014), pollen and insect allergens elicited strongest reactions, in contrast to previous studies on FAD, showing that house dust mites were more frequently implicated.

9.4.5 Allergy Therapy

Immunotherapy (ASIT) can be formulated based on the results of intradermal or serum testing. ASIT offers an effective and safe treatment option for cats. Reported success rates range for 60–78 % in feline atopic patients. Additionally, the reported incidence of side effects in feline atopic patients undergoing ASIT is very low and mainly anecdotal. ASIT with a rush protocol was also performed on a small group of atopic cats (Trimmer et al. 2005).

Most allergic cats show good response and less side effects on glucocorticoids therapy in comparison to dogs. Topical, oral, or injectable glucocorticoids can be successfully administered in cats with allergies (Ganz et al. 2012). However, because of potential side effects like skin fragility syndrome or diabetes mellitus, long-lasting injectable glucocorticoids should be avoided, if possible. Other treatment options are symptomatic therapy with oral ciclosporin, oclacitinib, as well as antihistamines (Nuttall et al. 2012; Ortalda et al. 2015; Roberts et al. 2015; Wildermuth et al. 2013).

9.5 Equine Atopic Dermatitis (EAD)

9.5.1 Clinical Problem of EAD

Atopic dermatitis is not as well defined in horses as in other species, such as humans and dogs. Most prominent clinical sign of allergic skin disease in horses is itch, but urticaria may also be present without pruritus in horses (Fig. 8.1d). Typical localizations of lesions are head (Fig. 9.1c, f), distal limbs, trunk, or lesions may be generalized. In insect bite hypersensitivities (IBH), lesions are predominantly localized on dorsal or ventral midline, head, ears, base of mane, and tail. Initial signs include reddish skin, papules, and tufted hair. Pruritus-associated scratching and rubbing lead to skin damage that may be complicated by secondary bacterial infections presented as crusting superficial folliculitis or tail pyoderma (superficial, pruritic bacterial infection of the skin). Chronic cases are associated with more severe hair loss, fibrosis, hyperkeratosis, and lichenification (i.e., chronically affected and infected skin lesions, thickening of the skin, and formation of skin folds). In addition to skin symptoms, horses with IBH often have behavioral problems, such as anxiety, restlessness, and nervousness, which may interfere with normal feeding and result in loss of body condition and also may make the horse unfit for riding or other work.

9.5.2 Pathogenesis

Horses with skin allergies are most commonly sensitized to salivary antigens of biting insects or to environmental allergens, such as grasses, trees, mites, and molds. Dermatitis associated with food allergens has occurred in horses. Genetic predisposition to allergy (atopy) has been described in horses with skin allergies (IBH) and respiratory allergies (RAO). Evidence for skin barrier defect described in humans and dogs with AD has not been studied in horses; however, a recent case report showed ultrastructural changes of skin lamellae associated with AD (Marsella et al. 2014).

Most information about the pathogenesis of allergic skin diseases in horses derives from the study of IBH, which is the most frequent allergic skin disease in horses. Similar to human allergy, there is evidence of Th2 polarization and generation of allergen-specific IgE (Schaffartzik et al. 2012). Lesions of IBH are associated with increased expression of IL-13 and decreased expression of FoxP3 which is a transcription factor specific for regulatory T cells. Recurrent urticaria is also associated with expression of Th2 cytokines IL-4, IL-13, and TSLP in lesional skin (Hinden et al. 2012).

9.5.3 Diagnosis of EAD

Diagnosis is made based on history and clinical signs and may be supported by histology showing perivascular to diffuse infiltration of eosinophils and mononuclear cells. Differential diagnoses, such as ectoparasites or helminths have to be ruled out. Serology for allergen-specific IgE and intradermal testing can be used to identify the underlying allergens. However, neither intradermal tests (Fig. 9.2c) nor serum testing can reliably distinguish healthy from allergic horses (Lorch et al. 2001), but both can be used to identify allergens for immunotherapy. Current diagnostic tests are based on crude extracts, which are not standardized for allergen content. This results in poor sensitivity and specificity, with frequent false-positive reactions. In addition to allergen proteins, crude extracts contain a lot of other molecules, some of which may be irritant and result in positive skin test in some healthy horses. In case of IBH, several salivary allergens have been cloned and produced as recombinant proteins (Schaffartzik et al. 2011) with the potential for future use in component-resolved diagnosis and patient-tailored immunotherapy. Cellular *in vitro* test-based release of sulfidoleukotriene from basophils has been established in horses for the diagnosis of IBH with high sensitivity and specificity. The advantage of basophil activation tests, an in vitro allergy testing method, is that they identify sensitized horses also off-season when the clinical signs are absent, and antigen-specific IgE levels become undetectable (Baselgia et al. 2006).

9.5.4 Therapy of EAD

Control of allergic skin disease in horses is based on allergen avoidance, symptomatic anti-inflammatory treatment, and specific immunotherapy. Although allergen avoidance is still the best way to alleviate symptoms, it is often difficult to achieve

in horses. In IBH, contact with allergen can be reduced by stabling, wearing of face masks and protective blankets, and use of repellents and insecticides. Conversely, allergies involving dust, mite, or mold allergens can be improved by moving horses outdoors.

As in humans and other species, glucocorticoids are used as main symptomatic therapy for equine allergy. Topical administration has fewer toxic side effects, but severely affected horses may need systemic therapy. Induction, tapering, and maintenance dosage regimens are used in horses in a similar way to other species. Antihistamines and tricyclic antidepressants are commonly used in equine atopic dermatitis to alleviate symptoms and reduce use of glucocorticoids. However, clinical trials proving their efficacy are scarce. Although histamine release is involved in the pathogenesis of IBH, the use of antihistamines may not be sufficient in IBH as administration of cetirizine did not show any benefit in a placebo-controlled study (Olsen et al. 2011).

9.5.5 Allergen-specific Immunotherapy

Allergen-specific immunotherapy (ASIT) represents the only causative treatment for allergic diseases. In horses, like in other species, choice of allergens for immunotherapy is based on intradermal test or serum allergen-specific IgE. Reported success rates of immunotherapy in EAD are similar to other species, but scientific literature in this field is scarce and rather controversial. Retrospective study in EAD based on owner questionnaire showed overall 81 % response to ASIT (Stepnik et al. 2012). Prospective placebo-controlled studies are lacking. An early open study showed high efficacy of allergen-specific immunotherapy for IBH (Anderson et al. 1996), whereas two small-scale placebo-controlled studies of immunotherapy for IBH did not show any benefit over placebo (Barbet et al. 1990; Ginel et al. 2014). Reasons for these discrepancies are likely due to the lack of standardization in allergen extracts used in various studies. More controlled studies are needed to assess the efficacy of ASIT in EAD.

9.6 Synopsis

Taken together, humans, dogs, horses, and cats do suffer from atopic diseases with a very similar pathophysiology and disease course, strongly determined by the genetic background. The disease courses indicate a heterogeneity in affected patients and a multifaceted genetic imprint. In all these species, allergies and superinfections complicate the clinical course. Therapies are based on skin repair, anti-inflammatory, anti-infectious, antipruritic, and immunomodulatory strategies. Most AD therapies including biologics were tested and approved for human use and secondarily been transferred to the veterinary applications. However, the similar pathophysiology and increasing number of studies in veterinary trials suggest that the exchange of new drugs may be done bi-directionally in the future.

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Prophylactic Vaccination Against Papillomavirus-Induced Tumour Disease

10

Sabine Brandt and Edmund Hainisch

Contents

Abstract

The *Papillomaviridae* family comprises a large number of genetically heterogeneous papillomaviruses (PVs) that are the causative agents of benign lesions or cancer in humans and a wide range of animal species. Early research in animal PV systems has disclosed several important characteristics of PVs and led to the recognition of human papillomaviruses (HPVs) as carcinogenic viruses in 1995. One of the most crucial findings in animals was that in vitro generated PV major capsid proteins spontaneously self-assemble to empty viral capsids termed virus-like particles (VLPs) that are safe and highly immunogenic. This discovery paved the way for the establishment and commercial release of highly effective polyvalent VLP-based vaccines for the prevention of HPV-induced tumour disease in humans. In addition, it encouraged veterinary scientists to work on the establishment of analogous, VLP-based vaccines for the protection of horses and other equids from common PV-induced cutaneous and mucosal tumours that is bovine PV type 1/2 (BPV1/2)-associated sarcoids and equine PV type 2 (EcPV2)-induced squamous cell carcinomas (SCCs). So far, BPV1 and EcPV2 VLPs were

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shown to be safe and highly immunogenic in horses. Furthermore, immunisation of horses with BPV1 VLPs conferred complete protection from experimental BPV1 infection and associated pseudo-sarcoid formation and also elicited cross protection from BPV2 infection. Similarly, the protective potential of EcPV2 VLPs against experimental infection with EcPV2 pseudo-virions was shown in a murine model. Taken together, these findings indicate that BPV1 and EcPV2 VLPs are safe and highly effective in protecting equids from PV-induced sarcoids and SCCs.

10.1 Introduction

The *Papillomaviridae* family comprises a large number of human and animal viruses that are characterised by considerable genetic diversity yet adhere to common biological principles. Papillomaviruses (PVs) are relatively small non-enveloped viruses that consist of an icosahedral capsid harbouring a circular double-stranded DNA genome of up to 8 kbp in length. The capsid is composed of 72 L1 protein pentamers commonly termed capsomeres and 12 L2 protein monomers (Howley and Lowy 2001). The viral genome can be grossly divided into an early (E) and a late (L) coding region and a non-coding long control region (LCR). The early region codes for regulatory (E1, E2, E4) and transforming proteins (E5, E6 and E7), which are expressed early in the viral life cycle. The late region contains two genes encoding the major L1 and the minor L2 capsid proteins, which are not expressed until viral genome amplification has been completed. The LCR is essential in providing cis-responsive elements that are required for replication and transcription of the viral genome (Campo 2006b; Doorbar 2005).

PV virions cannot actively penetrate the skin or mucosa of their host. They gain access to basal epidermal cells through micro-abrasions. These stem cells provide the appropriate primary surface and secondary receptor molecules for virion attachment and uptake. There is evidence for surface heparan sulphate proteoglycans (HSPG) representing initial PV attachment sites (Giroglou et al. 2001; Joyce et al. 1999). Subsequent PV endocytosis possibly involves clathrin- and caveolin-mediated mechanisms (Day et al. 2003) and/or may necessitate the presence of tetraspanin-enriched microdomains (TEMs) (Spoden et al. 2008). The productive PV life cycle is described as being tightly linked to the differentiation process of keratinocytes. Following initial infection of basal cells, the early viral genes are expressed in the basal and suprabasal epithelial layers. The replication of the viral genome occurs in the differentiating cells of the spinous and the granular layers (Chow and Broker 2006). The late capsid genes are expressed in the final squamous layer, where new infectious virions are assembled and released via disintegration and shedding of dead squames (Graham 2006). Interestingly, PV virions are highly resistant to desiccation, thus opening the possibility of indirect transmission via fomites (Roden et al. 1997).

10.2 Human Papillomavirus-Induced Cancer Disease and Effective Prevention

An aetiological association of PV infection with tumour development was first established in rabbits (Shope and Hurst 1933). The observation that inoculation of cottontail and domestic rabbits with infectious wart extract induced papillomas that sometimes progressed to squamous cell carcinoma (SCC) led to cottontail rabbit papillomavirus (CRPV) becoming the first model for the study of PV infection-associated carcinogenesis (Rous and Beard 1935).

Experimental research in rabbits, cattle and dogs disclosed several fundamental characteristics of PVs, most notably their species specificity and their pronounced tropism for defined cellular environments, i.e. cutaneous or mucosal keratinocytes and, for some types, fibroblasts (Lowy 2010). Consequently, human papillomavirus (HPV) research had to rely on animal infection models for many decades (Campo 2002). This comparative approach brought together scientists from various fields of human and veterinary medicine. Investigations on CRPV, bovine papillomavirus types 1 and 4 (BPV1, BPV4) and canine oral papillomavirus (COPV) led to important insights into PV biology and pathogenicity (Campo 2002), thus paving the way for the official recognition of HPVs as carcinogenic viruses (IARC 1995).

Molecular biological methods and powerful in vitro and small animal systems established during the past 30 years led to a shift from animal PV to direct HPV research. To date, more than 200 HPV types have been identified. From these, about 15 types are carcinogenic and thus classified as high-risk (hr) HPV types. There is evidence for almost all diagnosed cervical cancers, 90 % of anal cancers, up to 50 % of genital tumours and 22 % of head and neck squamous cell carcinomas (HNSCC) being caused by hrHPV types (Dayyani et al. 2010; zur Hausen 1996, 2000, 2009). HPV oncoproteins E6 and E7 have been recognised as essential factors in HPV-induced carcinogenesis. Malignant transformation of infected epidermal cells is achieved by complex interactions of these oncoproteins with cellular factors involved in cell cycle regulation (Feller et al. 2010a, b). E5 has been shown to be likewise transforming and to downregulate major histocompatibility complex (MHC) class I cell surface expression, thus helping the virus to escape from immune surveillance and establish infection (Ashrafi et al. 2006). In conjunction with other carcinogenic factors such as UV-radiation, infection by cutaneous beta-HPV types may indirectly contribute to the development of cutaneous SCCs (Schiller and Buck 2011; Zur Hausen 1996, 2000, 2009).

One of the most crucial findings in animal PV models was that in vitro generated L1 capsid proteins spontaneously self-assemble into empty capsids termed virus-like particles (VLPs). The latter are morphologically and immunologically almost indistinguishable from wild-type virions in that they display conformation-dependent neutralisation epitopes and are able to induce high titres of type-restricted neutralising antibodies (Kirnbauer et al. 1992). Challenge studies conducted in rabbits and cows revealed that immunisation with homologous (i.e. CRPV and BPV4) but not heterologous VLPs conferred protection from experimental infection (Breitburd et al. 1995; Kirnbauer et al. 1996). Similarly, immunisation with COPV

VLPs induced protection from experimental COPV infection in dogs (Suzich et al. 1995). These and similar findings (Rose et al. 1993; Zhou et al. 1991) ultimately led to the establishment and commercial release of highly effective polyvalent VLP-based vaccines for the prevention of HPV-induced tumour disease in humans (Angioli et al. 2016; Villa et al. 2005).

10.3 Papillomavirus-Induced Tumours in Horses and Other Equids

10.3.1 Sarcoids

In cattle, bovine papillomaviruses of types 1 and 2 (BPV1; BPV2) are the causative agents of benign warts that usually regress spontaneously. Infection is productive, with cow warts harbouring millions of infectious particles (Campo 2006a). As the rare example of a cross-species infection, BPV1/2 can also infect equids, e.g. horses, donkeys, mules and zebras, and lead to the development of usually persistent, locally aggressive skin tumours termed sarcoids (Chambers et al. 2003a). The latter constitute the most commonly encountered tumour disease in horses, with a morbidity of 5.8 % in the UK (Ireland et al. 2013). Sarcoids are typically diagnosed in young adult individuals with a peak incidence at the age of seven. Depending on their gross appearance, sarcoids are classified as occult, verrucose (Fig. 10.1a), nodular, fibroblastic (Fig. 10.1b), mixed or malevolent (Knottenbelt 2005). Disease may present as single tumour or multiple lesions of various types at different sites of the body. Sarcoids have a high propensity to progress to a more severe and multiple form of disease, especially upon accidental or iatrogenic trauma, i.e. ineffective therapy (Hainisch and Brandt 2015). Disease may also impair the use of affected animals, entail considerable treatment costs and pronouncedly decrease the resale value of affected animals. As a consequence, sarcoids are the number one skin-related cause for euthanasia (Scott and Miller 2003a). Taking into consideration the high prevalence of the disease, the lack of universally effective therapeutic approaches and the fact that sarcoids affect relatively young horses, it is clear that equine sarcoids also have an important negative impact on the horse industry.

First evidence for an aetiological association of BPV1/2 with equine sarcoid disease has been obtained by inoculation experiments. In 1937, Montpellier et al. (Montpellier et al. 1937) reported the successful auto-transmission of sarcoids in a mule. In 1951 and 1969, two research groups succeeded in inducing sarcoid-like lesions by intradermal injection of horses with cow wart extract. Experimental lesions were morphologically and histologically indistinguishable from naturally acquired sarcoids, yet regressed spontaneously (Olson and Cook 1951; Ragland and Spencer 1969). Importantly, Voss was able to induce persistent sarcoids by inoculation of scarified skin with sarcoid extract, but not by intradermal injection of this inoculum (Voss 1969). The suspected causative involvement of BPV1/2 in sarcoid pathogenesis was further supported by in situ hybridization (ISH) experiments revealing the presence of viral DNA in the nuclei of tumour fibroblasts (Lancaster

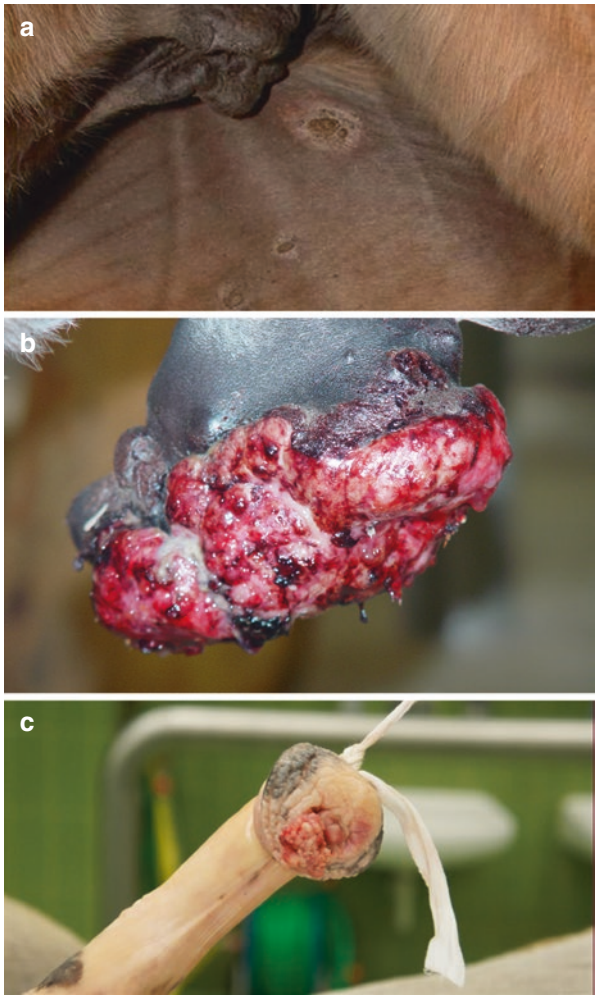

Fig. 10.1 Sarcoids in equine species: low- to high-grade lesions. (**a**) Sarcoids on the inside of the thigh of a horse. Example of low-grade lesions. Several sarcoids are present. The lesions are characterised by a hyperkeratotic, verrucose centre and a surrounding area of alopecia with mildly thickened skin; (**b**) example of a high-grade lesion in a donkey: fibroblastic sarcoid on the prepuce; (**c**) SCC on the penile glans of an aged gelding. The picture was taken immediately before surgery to amputate the distal 15 cm of the penis

et al. 1979). However, ISH failed to demonstrate BPV1/2 DNA in sarcoid epidermis, and no virion has been detected by electron microscopy at that time. Accordingly, BPV1/2 infection was assumed to be abortive in equids, with virus exclusively residing in sarcoid fibroblasts in an episomal form (Amtmann et al. 1980; Lancaster 1981). These experiments were the first in a long row of investigations leading to the recognition of BPV1 and BPV2 as the major causative agents of equine sarcoids along with trauma (Chambers et al. 2003a; Hainisch and Brandt 2015; Nasir and Reid 2006; Nasir and Brandt 2013).

With the advent of modern molecular biological and immunological methods, many important aspects of BPV1/2 infection in equids and associated tumour development have been elucidated (Hainisch and Brandt 2015; Nasir and Brandt 2013). However, it is still unclear how BPV1/2 is transmitted to equids. Infection

is still thought to be abortive, with sarcoid-affected animals thus representing a dead-end host. As a consequence, it has been assumed that infection is directly acquired from cow wart-affected bovines or, indirectly, from contaminated fomites or may be achieved by infected cells without the need of infectious virions (Bogaert et al. 2005; Chambers et al. 2003a). Contaminated fomites may include trees and fence posts on which horses scratch their body or tack and grooming kits. BPV1/2 DNA has also been found in insects caught in the vicinity of sarcoid-affected equids (stable flies, horse flies), leading to the theory that insect vectors may have a role in BPV1/2 transmission (Finlay et al. 2009; Kemp-Symonds 2000). This concept is substantiated by the fact that sarcoids often develop at insect-infested sites of the body such as the belly, the groin and the external genitals (Hainisch and Brandt 2015).

There are several lines of evidence that refute the theories of an abortive BPV1/2 infection in equids and the transmission of infection without virion. First, it has been demonstrated that intracranial injection of hamsters with BPV1 virion resulted in the development of sarcoid-like intracranial and cutaneous lesions, whereas injection of heat-denatured virion had no apparent effect (Robl et al. 1972). In analogy, intradermal inoculation of foals with BPV1 virion led to the formation of pseudo-sarcoids, whilst inoculation with naked BPV1 genome or primary sarcoid fibroblasts containing viral episomes produced no overt skin malignancies (Hainisch et al. 2012; Hartl et al. 2011). Gobeil et al. (2007) have likewise demonstrated that sarcoids are not inducible by an infectious cell line. Second, BPV1 L1 mRNA and capsid protein were shown to be intralesionally expressed (Brandt et al. 2011; Nasir and Reid 1999). Given that PV L1 expression and subsequent virion assembly is confined to the upper epidermal layer, this finding indicates that, contrary to previously reported data, BPV1 infection may also involve equine epidermis and be productive in this skin layer. Indeed, analysis of micro-dissected sarcoid epidermis revealed the presence of viral DNA and L1 protein in a subset of tumour samples (Bogaert et al. 2010; Brandt et al. 2011). Using an approach that combines an antibody capture step for selective virion isolation with highly sensitive BPV1/2 PCR, presence of L1 capsomeres in a complex with viral genome was shown for about 58 % of tested sarcoids with maximum concentrations of 125 complexes per 50 µl of cell-free sarcoid extract (Brandt et al. 2008). In accordance with this observation, Wilson et al. have visualised BPV1 virions in sarcoid sections by transmission electron microscopy (TEM) (Wilson et al. 2013). These laboratory findings are corroborated by a field study where co-stabling of sarcoid affected with healthy donkeys resulted in the latter developing sarcoids (Nasir and Campo 2008). In addition, sarcoids of donkeys and horses were shown to contain equid-specific variants of BPV1 that are not found in bovine warts (Brandt et al. 2008; Chambers et al. 2003b; Nasir et al. 2007; Trewby et al. 2014). Taken together, it appears more realistic that intact virions are needed for initial infection of equids, which is also productive, at least in some equids and/or at some stages of sarcoid disease.

In humans, many years can elapse between initial HPV infection and associated tumour development (Bosch et al. 2006). In equids, the time span between initial infection and sarcoid development appears to be relatively short, since BPV1/2

DNA is commonly detected in lesions, intact skin and peripheral blood mononuclear cells (PBMC) of sarcoid-bearing animals, but usually not in sarcoid-free individuals (Carr et al. 2001; Chambers et al. 2003a; Nasir and Brandt 2013). The high incidence of disease especially in younger horses further supports the assumption of a relatively short incubation period (Scott and Miller 2003a). Although cases of spontaneous tumour regression have been reported, sarcoids are usually persistent tumours because equids are unable to mount a measurable immune response upon natural BPV1/2 infection. The mechanisms underlying BPV1/2 immune escape are not yet understood. However, the E5 protein may provide a direct way of immune evasion by downregulation of MHC I, which in turn compromises viral antigen presentation by this complex to immune cells (Marchetti et al. 2009).

10.3.2 Squamous Cell Carcinoma

Squamous cell carcinomas (SCCs) represent the most common malignant epithelial tumour in equids (Scott and Miller 2003b). They can develop anywhere on the skin, yet predominate at mucocutaneous transitions, i.e. the ocular region and external genitalia (Scott and Miller 2003b; Sundberg et al. 1977). Given the invasiveness of SCCs, surgical excision is the current therapy of choice. In severe cases, this may necessitate the exenteration of affected eyes or the en bloc resection of affected external genitalia, which in turn may lead to postsurgical complications and euthanasia of the equid patient (Mair et al. 2000; van den Top et al. 2008).

Genital SCCs (gSCCs) account for 50–85 % of all genital tumours in hospital populations. The typical patient is a gelding older than 15 years. Lesions initially present as papillomatous plaques or papillomas on the penile glans or shaft. When left untreated, lesions progress to carcinoma in situ and SCC (Fig. 10.1c) that metastasises in about 12–15 % of cases. SCCs can spread through contact to the prepuce or via lymphatics to local lymph nodes and the abdomen and in rare cases to the vertebral bodies and lungs. Mares can be likewise affected, with disease mostly involving the clitoris or vulva (Scott and Miller 2003b; van den Top et al. 2008).

Over the past decade, evidence for an active involvement of papillomavirus infection in the development of equine SCCs has substantially increased. A novel PV termed equine papillomavirus type 2 (EcPV2) was identified from a case of genital SCC and its genome fully characterised (Scase et al. 2010). Subsequent screening of a series of genital and ocular SCCs/SCC precursor lesions (plaque, papilloma, carcinoma in situ) revealed the consistent presence of EcPV2 DNA and mRNA in the genital lesions, whilst SCCs of the nictitating membrane and conjunctiva scored negative for EcPV2 (Kainzbauer et al. 2012; Scase et al. 2010; Sykora et al. 2012). Screening of ocular and genital swabs as well as milk and semen from apparently healthy horses resulted in an EcPV2 DNA detection rate of 2.6 % (Sykora et al. 2012). Consistent presence of EcPV2 in genital SCC and the low incidence of infection in tumour-free individuals were confirmed by several independent studies (Bogaert et al. 2012; Fischer et al. 2014; Knight et al. 2013; Lange et al. 2013a). Taken together, this body of evidence indicates that EcPV2 infection is causally

associated with the development of genital plaques, papillomas, carcinoma in situ and SCC. EcPV2 DNA was also detected in a subset of equine oropharyngeal SCCs and ocular SCC metastases (Kainzbauer et al. 2012; Knight et al. 2013). The significance of these findings is subject to current investigations. Since the discovery of EcPV2 in 2010, five novel EcPV types termed EcPV3–7 have been identified from genital (EcPV4) and aural plaques (EcPV5–6) and penile lesions (EcPV3, 7). However, an aetiological association of these EcPV types with tumour disease remains to be established (Lange et al. 2013b; van den Top et al. 2015).

10.4 Protecting Horses from Papillomavirus-Induced Tumour Disease

Depending on the location and severity of the lesions, sarcoids and SCCs are treated by topical application of antiviral ointments or chemotherapeutics, cryo- and radiotherapy, total removal of the lesion by ligation or (laser) surgery or by combinations of these modalities. The earlier disease is diagnosed and treated, the better is the chance of successful therapy (Pascoe and Knottenbelt 1999). However, early detection of gSCCs and precursor lesions can be problematic, especially in geldings, where the development of penile lesions often remains unnoticed by the owner until they have progressed to massive malodorous bleeding masses, because the penis is usually retracted in the prepuce. At such a late stage, the prognosis is poor or hopeless (Hainisch and Brandt 2015; Pascoe and Knottenbelt 1999).

Given that sarcoids and gSCCs constitute highly relevant diseases in equids and that immunisation of humans with VLP-based vaccines has proven highly effective in preventing HPV-induced cancers, an attempt was made to establish a vaccine for protection of equids from sarcoids and gSCCs. Safety and immunogenicity of BPV1 L1 VLPs were assessed in a phase I dose-escalation trial, showing that intramuscular immunisation of horses with 50, 100 and 150 μg of BPV1 L1 VLP in alum was well tolerated and induced high titres of neutralising antibodies irrespective of the dose (Hainisch et al. 2012). On the basis of inoculation experiments conducted in the 1950s and 1960s (Olson and Cook 1951; Ragland and Spencer 1969; Voss 1969), four horses were intradermally inoculated with cow wart-derived BPV1 virions, naked BPV1 genome and sarcoid cells on the neck and then monitored (Fig. 10.2). Pseudo-sarcoids developed exclusively at sites inoculated with virions. Tumours became palpable 11–32 days after inoculation, reached maximum sizes of 2 cm in diameter and then resolved spontaneously within 6 months, although no neutralising anti-BPV1 serum antibodies were detectable throughout the trial. Interestingly, viral DNA and mRNA were not only detected from lesions but also from PBMCs already before lesions were first palpable. Immunofluorescent staining revealed the presence of the E5 protein in tumour fibroblasts, but not in the apparently normal epidermis overlying the lesions. Taken together, intradermal inoculation of horses with BPV1 virions reliably resulted in the formation of transient pseudo-sarcoids, thus constituting a robust challenge model (Hartl et al. 2011).

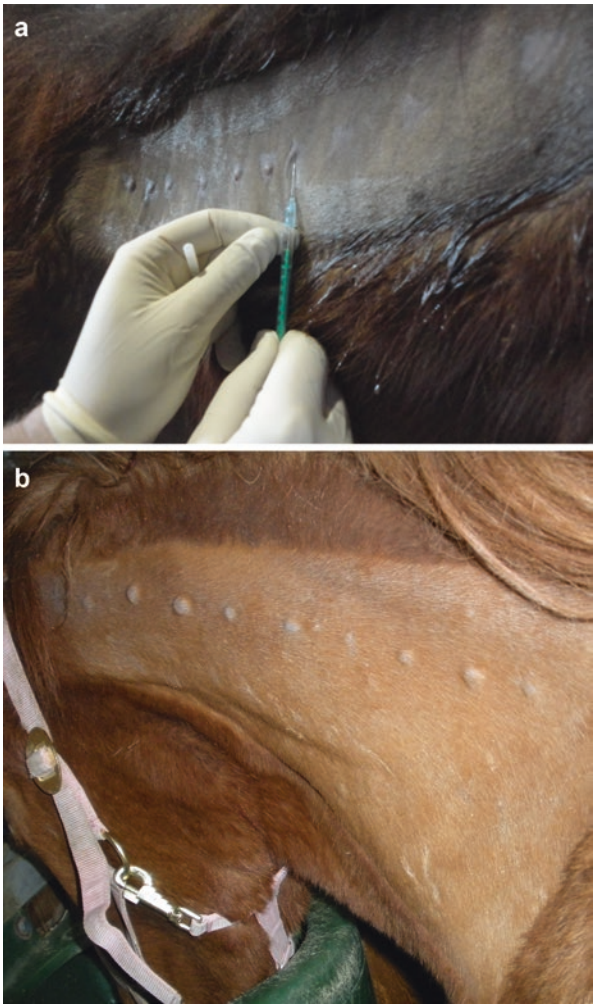

Fig. 10.2 Skin inoculation in horses with papillomaviruses. (**a**) Intradermal injection of cow wart extract in a horse. This is a robust in vivo method to test whether a papillomavirus causes infection. In this case the horse is inoculated with BPV1 to produce pseudo-sarcoids. Note the six sites to left of the needle which have already been injected. Intradermal injection results in a small wheal as fluid cannot readily disperse in the dermis. Subcutaneous injection does not result in wheal formation. Wheal formation is therefore a control for the proper use of the technique; (**b**) intradermal inoculation of the neck with BPV1 has resulted in the development of ten pseudo-sarcoids at all ten inoculation sites approximately 5 weeks after inoculation. The lesions at this point in time are at the peak of their growth and measure about 1 cm in diameter. Five months after inoculation, regression of all the lesions was complete in this horse

This model was used to address the protective potential of BPV1 L1 VLPs. To this aim, horses were immunised with BPV1 L1 VLPs or left unvaccinated and then challenged intradermally with BPV1 virions (Fig. 10.2a). Whilst

control horses developed pseudo-sarcoids at all inoculation sites (Fig. 10.2b), vaccinated horses showed complete protection from tumour formation. Because BPV1 and BPV2 were shown to be closely related serotypes (Shafti-Keramat et al. 2009), horses were vaccinated or not vaccinated with a bivalent BPV1/EcPV2 L1 VLP vaccine and then challenged with BPV2 virions. The rationale of this trial was to study the cross-protective potential of BPV1 L1 VLP-induced antibodies against BPV2 in vivo and to address the safety and immunogenicity of EcPV2 L1 VLPs. As anticipated, intramuscular administration of the bivalent vaccine was well tolerated and induced a robust antibody response that was however significantly lower than the response to the monovalent vaccine. As a conceivable consequence, vaccination resulted in incomplete protection from BPV2-induced pseudo-sarcoid formation. Given that extremely high virion concentrations were used for horse challenge, i.e. a minimum of 10^6 BPV2 virions per inoculation site, it can be speculated that BPV1 L1 VLPs as monovalent or component of a polyvalent vaccine will protect from natural BPV2 infection (Hainisch et al. 2015). Importantly, horses challenged with BPV1 more than 5 years after immunisation with three different doses of BPV1 L1 VLPs (Hainisch et al. 2012) were completely protected from infection. Surprisingly, protection did neither correlate with the vaccine dose nor with BPV1-neutralising serum antibody titres which were generally low and had dropped below detection level in one animal (Hainisch et al. 2015). Taken together, immunisation of horses with BPV1 L1 VLPs was safe, induced long-lasting protection from experimental BPV1 infection and confined partial protection from BPV2 challenge (Hainisch et al. 2015).

Immunisation of horses with BPV1/EcPV2 L1 VLPs proved safe and immunogenic. Therefore efforts were made to address the prophylactic potential of EcPV2 L1 VLPs in vivo. To this aim, rabbits were immunised with EcPV2 L1 or control VLPs, and then respective rabbit pre-immune or immune sera were transferred to mice. Subsequent intravaginal challenge of mice with EcPV2 L1 pseudo-virions (PsV), i.e. capsids harbouring a luciferase reporter plasmid, resulted in complete and exclusive protection from PsV infection in mice passively transferred with EcPV2 L1 VLP immune serum (Schellenbacher et al. 2015).

Provided that a causal association of EcPV2 infection with gSCCs and possibly SCC at other sites of the body can be conclusively demonstrated, these findings recommend EcPV2 L1 VLPs as prophylactic vaccine against EcPV2 infection and associated disease in equids.

10.5 Synopsis

Research in natural animal PV models including rabbits, dogs and cattle has chiefly contributed to today's knowledge regarding the mechanisms underlying PV infection and tumour formation. It has led to the recognition of HPVs as oncogenic viruses and ultimately to the establishment of effective vaccines for prevention of HPV-induced tumour disease in humans.

To date highly sophisticated molecular biologic and immunological methods and powerful in vitro and in vivo models are available for the direct study of HPV infection and associated human disease. This has led to important scientific and clinical insights, some of which have motivated veterinarians and virologists to attempt the establishment of vaccines for equid PV tumour prophylaxis and treatment. Whilst studies on sarcoid immunotherapy are still ongoing, a large body of evidence that BPV1 L1 and EcPV2 L1 VLPs constitute an effective vaccine for protection of equids from sarcoid and gSCC disease is available today and will be hopefully implemented into practice.

PV research in animals and humans, and particularly the establishment of PV VLPs as prophylactic vaccines, represents a good example for highly successful comparative research that merits to be encouraged for the benefit of the animal and the human patient.

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Tick Bites and Borrelia Infection: A Problem for Mammalian Species

11

Gerold Stanek

Contents

Abstract

Four species of the family Ixodidae vector the agents of Lyme borreliosis: *Ixodes persulcatus* and *I. ricinus* in Eurasia, *I. scapularis* and *I. pacificus* in North America. The ticks have three life cycle stages: larvae, nymphs and adult ticks (female and male). They are feeding once in each stage on a large variety of vertebrate animals. Rodents, hedgehogs and certain bird species are competent reservoirs for *Borrelia burgdorferi* sensu lato, the agents of Lyme borreliosis.

When humans expose to the natural habitats of ticks during the season (usually from March to October), they may be bitten by an infected tick. Every fifth person on average will develop Lyme borreliosis, most frequently the skin infection erythema migrans. Disseminated infection involves the nervous system, joints, heart and other organs.

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Domestic animals are regularly bitten when exposed to ticks. However, there is little evidence that Lyme borreliosis is responsible for frequent and significant morbidity in any domestic animals.

11.1 Introduction

Ticks are divided into two families, the soft ticks (Argasidae) and the hard ticks (Ixodidae). The noticeable difference of these tick families is shape (Fig. 11.1a, b) and that the hard ticks have mouthparts that project externally (as in larval soft ticks) and a dorsal plate, the scrotum, that is present in all stages.

Ixodes species are recognised as important vectors of agents of tick-borne bacterial disease, namely, Lyme borreliosis, anaplasmosis and rickettsioses; also tularaemia was identified as ixodid tick-borne disease in some cases. The relapsing fever *Borrelia* species are transmitted by soft ticks; however, some relapsing fever-like *Borrelia*, such as *B. myamotoi*, are now known to be transmitted by the hard ticks.

The life cycle of *Ixodes ricinu*s and of other important vectors is broadly similar. *Ixodes ricinus* is the most common tick in Northern and Central Europe. The life cycle takes approximately 3 years. Each life cycle stage (Fig. 11.1d) may take 1 year. Blood feeding is essential once in each stage and for a period of only a few days. Digestion of the blood meal and development to the next stage occur whilst hidden in the vegetation. The larvae hatch from an egg batch (Fig. 11.1c) of about 2000 and after a few days are ready to feed.

The six-legged larvae, which are about 1 mm long and just visible to the naked eye, climb the vegetation and wait for a passing host, usually a mouse or vole. When a host animal brushes past, the ticks attach to the skin with their mouthparts. After 2 or 3 days of feeding, during which they increase their weight 10–20 times, they drop off into the vegetation and commence development. After several months, the fed larva moults to an 8-legged 1.5–2 mm nymph that usually feeds in the following year for 4–5 days on a larger animal such as a bird or squirrel. Finally, the adult female ticks, about 4 mm long when unfed, feed on large animals such as deer or livestock for about 7 days, taking up to 5 ml of blood and growing to the size of small bean with a length of >1 cm. The male tick stays on the host for longer periods in order to mate with females. The larvae and nymphs can parasitise any animal but make most contact with hosts that move within the vegetation cover. The adults climb higher in the vegetation and usually only attack large animals from the size of a hare upwards. All three stages of development are known to bite humans, but nymphs are most commonly involved. Usually, *Ixodes ricinus* feeds on animals from March to October. The unfed ticks can survive for several weeks with sufficient humidity at the base of the vegetation. Transmission of pathogens can occur at any time during the warmer months of the year. Men will be attacked when intruding into tick habitats.

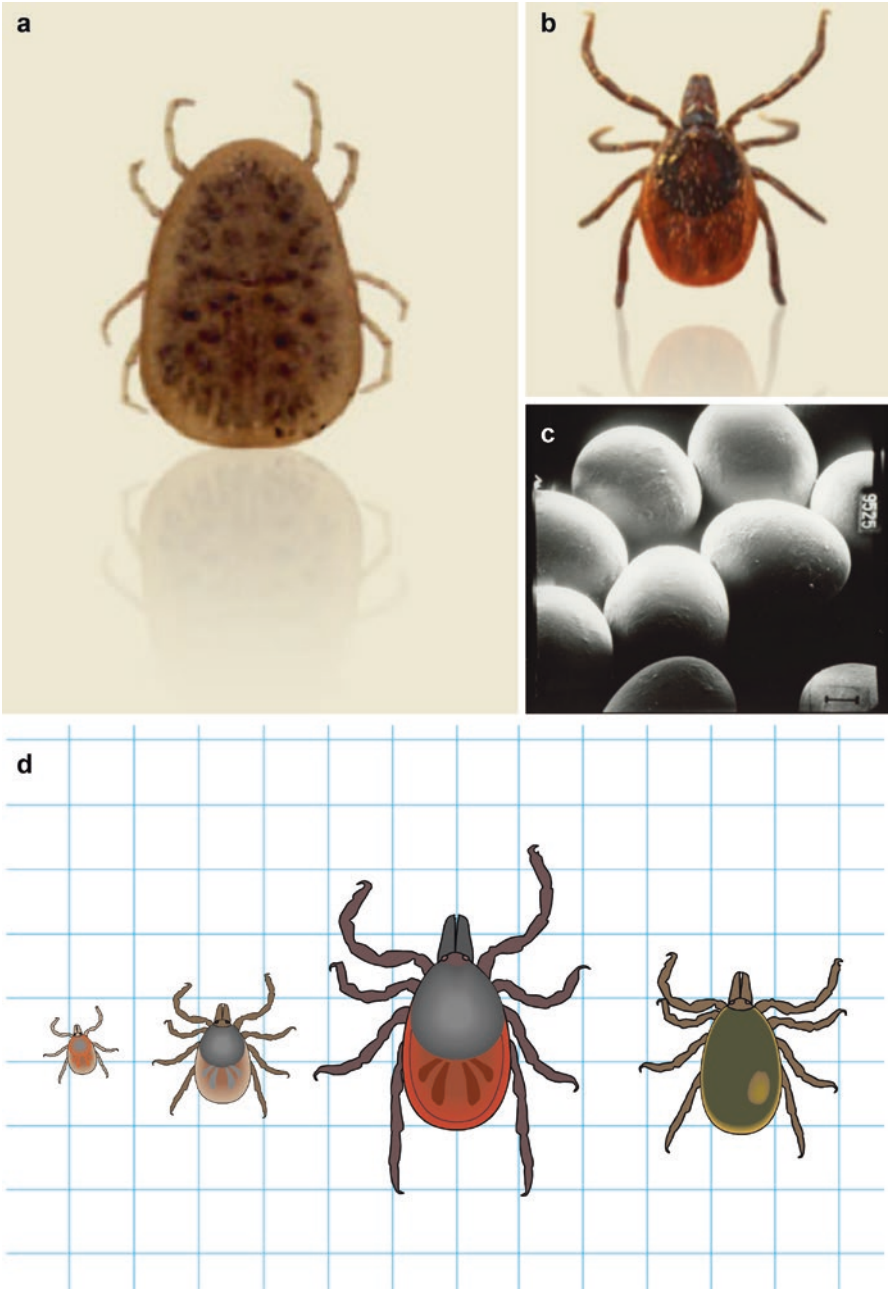

Fig. 11.1 Types and developmental stages of ticks. (**a**) The soft tick *Argas reflexus* (the pigeon tick) and (**b**) female *Ixodes ricinus*; body length of female *A. reflexus* is about 10 mm and female *I. ricinus* about 5 mm. Pictures: courtesy of www.zeckenwetter.de; (**c**) raster electron micrograph of an egg batch of *Ixodes ricinus*; bar indicates 50 µm; (**d**) life cycle stages of *Ixodes ricinus* – schematic. From left: six-legged larval tick, eight-legged nymphal tick, eight-legged adult female and male ticks; the scrotum in male ticks covers the entire dorsal side raster 1 mm

11.2 Lyme Borreliosis in Humans

Lyme borreliosis (LB) is the most commonly reported tick-borne infection in Europe and North America. The disease is a multisystem disorder which can affect a series of tissues including the skin, nervous system, joints, heart and rarely the eyes. The illness is caused by distinct species of the *Borrelia burgdorferi* sensu lato (sl) complex. Human pathogens in Europe are the genospecies *B. afzelii, B. bavariensis*, *B. garinii*, and *B. burgdorferi*. The number of genomic species is increasing (Stanek and Reiter 2011). The spirochaetes are tiny organisms with a diameter of ca. 0.3 μm and a length of 20–30 μm. Endoflagellae are inserted on each end in the protoplasmic cylinder. Both protoplasmic cylinder and endoflagellae are enveloped by a flexible outer cell wall. This architecture allows motility even in highly viscous media. A range of outer surface proteins are involved in the infectious cycle of the spirochaetes. *Borrelia* is transmitted during the blood feeding of ticks. Reservoir competent vertebrates are small rodents, squirrels, hedgehogs and certain birds species (Gern et al. 1998), and many of these have since been confirmed as hosts of particular genospecies in rodents and birds (Hanincová et al. 2003a, b).

Only hard ticks of the genus *Ixodes* transmit the agents of LB. Worldwide, four *Ixodes* species are recognised as important vectors of the agents of Lyme borreliosis: *Ixodes persulcatus* and *I. ricinus* in Eurasia, *I. pacificus* and *I. scapularis* in North America. Several other *Ixodes* species and also insects may carry *Borrelia*, but they are not capable of transmitting them.

The prefix ‘Lyme’ was first used following investigation into a geographical cluster of juvenile arthritis in the town of Old Lyme, Connecticut, USA, in the mid 1970s (Steere et al. 1977a). The aetiology of Lyme arthritis, later named Lyme disease when the clinical picture expanded (Steere et al. 1977b), was unknown until the discovery of spirochaetes in ticks. Subsequent studies led to the isolation of a spirochaete from the tick *Ixodes scapularis* (Burgdorfer et al. 1982), which was identified as a new *Borrelia* species and named *Borrelia burgdorferi* (Johnson et al. 1984).

The disease has, however, been known in Europe since the 1880s under a variety of names (including erythema chronicum migrans, lymphadenosis benigna cutis, acrodermatitis chronica atrophicans (Herxheimer and Hartmann 1902) tick-borne meningopolyneuritis [Garin-Bujadoux-Bannwarth]). In 1913, Benjamin Lipschütz in Vienna has first described an erythema expanding from the site of a tick bite as erythema chronicum migrans (Lipschütz 1913). In 1948, spirochaetes were observed in erythema migrans (EM) biopsies, and in 1951, Hollström successfully treated EM-infected patients with penicillin (Hollström 1951). Since the nosological entity was recognised, Lyme borreliosis is now the specific term for the multisystem disorder. It is now known that LB occurs as a significant disease in thousands of patients in the northern hemisphere every year.

11.2.1 Clinical Manifestations

Infection with *Borrelia burgdorferi s.l.* can be inapparent or have a range of clinical presentations, depending on the organ systems affected. It is not uncommon that

patients present with disseminated LB without having experienced early symptoms. The diagnosis must be made in the light of the medical findings, laboratory evidence and medical history (Table 11.1).

11.2.1.1 Early Localised Lyme Borreliosis

Erythema migrans is the most common clinical manifestation of LB (Fig. 11.2). The characteristic rash spreading from the site of a tick bite typically starts about 3–30 days after the tick bite and is the direct result of the spirochaete migrating through the skin. The rash can become relatively large sometimes with gradual clearing of the erythema in the centre. The patient may also experience other symptoms, including a mild 'flu-like' illness. Borrelial lymphocytoma is an uncommon form of early localised LB, which is usually seen on the earlobe (especially in children), nipple or scrotum appearing as an intense bluish-red localised painless swelling of the skin. The patient may not recall a tick bite. Histologic examination shows a very dense infiltrate of lymphocytes.

11.2.1.2 Early Disseminated Lyme Borreliosis

The *Borrelia* can spread through the bloodstream and lymphatics to other tissues, including other parts of the skin, nervous system, musculoskeletal system, heart, eyes and rarely other organs. Clinical features may present a few weeks to over a year after the initial infection. The features comprise multiple erythema migrans (additional lesions occur on various skin parts besides the initial lesion around the tick bite), neuroborreliosis, recurrent arthritis of one or more large joints (mono- or oligoarthritis), carditis with conduction defects and rarely others.

Lyme neuroborreliosis (LNB) is the second most common manifestation of LB – 5–10 % of all LB cases. LNB presents as facial palsy – unilateral or typical bilateral – other cranial nerve palsies (less common), aseptic meningitis, meningoradiculoneuritis Garin-Bujadoux-Bannwarth (Ackermann 1976), mild encephalitis and peripheral neuritis associated with acrodermatitis chronica atrophicans.

Facial palsy and mild aseptic meningitis are the most common features of LNB in children and painful meningoradiculoneuritis usually in adults. LNB manifests several weeks after initial infection.

Other rare manifestations have been reported, including cardiomyopathy, anterior and posterior uveitis, panophthalmitis, hepatitis, myositis and orchitis.

11.2.1.3 Late Lyme Borreliosis

Late LB presents several years after the initial infection and may involve the large joints, skin (acrodermatitis chronica atrophicans) or, rarely, chronic neurological syndromes.

11.2.2 Therapy

LB is treated with appropriate antibiotics. Duration of treatment is no longer than 2 weeks for early and disseminated infection and 4 weeks for chronic cases – the prognosis is excellent (Stanek et al. 2011, 2012).

Table 11.1 Clinical case definition for Lyme borreliosis

Term	Clinical case definition	Laboratory evidence: essential*	Laboratory/clinical evidence: supporting
Erythema migrans	With or without central clearing. Advancing edge typically distinct, often intensely coloured, not markedly elevated	None[a]	Detection of *Borrelia burgdorferi s.l.* by culture and/or PCR from skin biopsy
Borrelial lymphocytoma (rare)	Painless bluish-red nodule or plaque, usually on ear lobe, ear helix, nipple or scrotum; more frequent in children (especially on ear) than in adults	Seroconversion or positive serology[b]. Histology in unclear cases	Histology. Detection of *B. burgdorferi s.l.* by culture and/or PCR from skin biopsy Recent or concomitant EM
Acrodermatitis chronica atrophicans	Long-standing red or bluish-red lesions, usually on the extensor surfaces of extremities. Initial doughy swelling. Lesions eventually become atrophic. Possible skin induration and fibroid nodules over bony prominences	High level of specific serum IgG antibodies[b]	Histology Detection of *B. burgdorferi s.l.* by culture and/or PCR from skin biopsy
Lyme neuroborreliosis	In adults mainly meningo-radiculitis, meningitis, with or without facial palsy; rarely encephalitis, myelitis; very rarely cerebral vasculitis. In children mainly meningitis and facial palsy	Pleocytosis and demonstration of intrathecal specific antibody synthesis[c]	Detection of *B. burgdorferi s.l.* by culture and/or PCR from CSF. Intrathecal synthesis of total IgM and/or IgG and/or IgA. Specific serum antibodies. Recent or concomitant EM
Lyme arthritis	Recurrent attacks or persisting objective joint swelling in one or a few large joints. Alternative explanations must be excluded	Specific serum IgG antibodies, usually in high concentrations[b]	Synovial fluid analysis. Detection of *B. burgdorferi s.l.* by PCR and/or culture from synovial fluid and/or tissue
Lyme carditis (rare)	Acute onset of atrioventricular (I–III) conduction disturbances, rhythm disturbances, sometimes myocarditis or pancarditis. Alternative explanations must be excluded	Specific serum antibodies[b]	Detection of *B. burgdorferi s.l.* by culture and/or PCR from endomyocardial biopsy. Recent or concomitant erythema migrans and/or neurologic disorders

Table 11.1 (continued)

Term	Clinical case definition	Laboratory evidence: essential*	Laboratory/clinical evidence: supporting
Ocular manifestations (rare)	Conjunctivitis, uveitis, papillitis, episcleritis, keratitis	Specific serum antibodies[b]	Recent or concomitant Lyme borreliosis manifestations. Detection of *B. burgdorferi s.l.* by culture and/or PCR from ocular fluid

*Based on Stanek et al. 2011

If less than 5 cm in diameter, a history of tick bite, a delay in appearance (after the tick bite) of at least 2 days and an expanding rash at the site of the tick-bite are required

Specific antibody levels in serum may increase in response to progression of infection or may decrease due to abrogation of the infection process. Samples collected a minimum of 3 months apart may be required in order to detect a change in IgG levels; as a rule, initial and follow-up samples have to be tested in parallel in order to avoid changes by inter-assay variation

In early cases intrathecally produced specific antibodies may still be absent

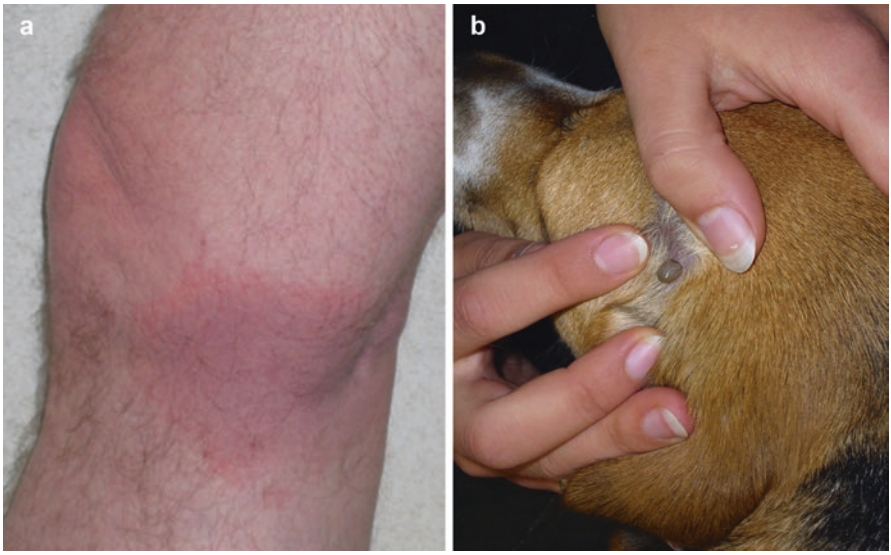

Fig. 11.2 Tick bites, a burden for humans and animals. (**a**) Erythema migrans on a man´s right knee, 3 weeks after tick bite in the knee pit; (**b**) *Ixodes ricinus* female tick on the head of a dog, almost fully engorged (Courtesy: Michael Leschnik, Univ. of Veterinary Medicine Vienna)

11.3 Lyme Borreliosis in Domestic Animals

The animal reservoir hosts of *B. burgdorferi s.l.* do not show signs of disease. However, domestic animals are said to show similar manifestations as seen in LB in humans. Serological studies suggest that infection with *B. burgdorferi s.l.* is

common, but at present there is little evidence that LB is responsible for frequent and significant morbidity in any domestic animals. Diagnosis of LB in domestic animals is particularly problematic because of the lack of clinical case definitions.

11.3.1 LB in Dogs and Cats

Joint involvement was described as a feature of LB in dogs (Kornblatt et al. 1985). Seroprevalence (i.e. the presence of specific immunoglobulins in serum after a tick bite) suggests widespread subclinical infection of dogs with *B. burgdorferi s.l.* (Leschnik et al. 2010). However, very few cases have been supported by isolation and identification of the pathogen. Krupka and Straubinger (2010) stated that 'because of the difficulties in finding sufficient indicative clinical signs, additional information (detailed case history, laboratory testing for antibodies) is especially important to make the clinical diagnosis of Lyme borreliosis in dogs and cats'. Despite the diagnostic difficulties and lack of good prevalence data, a commercial vaccine for canine LB is available in Europe. The significance of LB in cats is presently unclear because of lack of sufficient data.

11.3.2 LB in Horses

Horses in normal health condition were surveyed in two rounds for antibodies to *B. burgdorferi* sl. The age dependency analyses showed that the first infection with *B. burgdorferi s.l.* occurs in the first year of life. Continuously tick-exposed horses show stable seroprevalence. None of the 186 horses followed in this study developed clinical disease (Müller et al. 2002). As with dogs because of missing clinical case definitions and insufficient data, clinical cases of LB in horses appear to be uncommon.

11.4 Synopsis

Almost all vertebrate animals are blood hosts for hard ticks of the family Ixodidae. Small vertebrates like rodents and birds serve also as reservoir for a range of human pathogens including the agents of Lyme borreliosis. Human exposure to habitats of ixodid ticks may result in a bite by an infected tick. Every fifth person on average will develop Lyme borreliosis, most frequent the skin infection erythema migrans, then neuroborreliosis, arthritis and other rare manifestations. Animal exposure is regularly linked with tick bites; there is little evidence, however, that borrelial infection manifests in disease in any domestic animals.

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Parasitic Infections in Humans and Animals

12

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Abstract

Parasites are organisms that depend on a host for feeding and reproduction and belong to various unrelated taxa, primarily protozoa, helminths and arthropods. Complex life cycles have often lead to extreme adaptations; nevertheless parasites may harm their hosts and even cause serious disease and death. Transmission of parasite stages can be environmental, nutritional or vector-borne. Among the most important human protozoan parasites in a global context are *Leishmania*, *Plasmodium* (both transmitted by bloodsucking arthropods) and *Toxoplasma* which is soil- or food-borne. Parasitic worms (helminths) relevant for human health include *Echinococcus*, *Toxocara*, hookworms (all soil-borne) and *Dirofilaria* (transmitted by mosquitoes). Various arthropods (ticks, insects) are involved in the transmission of pathogens due to their blood-feeding behaviour. They are especially involved in transmission cycles between animals and humans (zoonotic infections). Research on parasites and their interactions with the host requires suitable animal models. For parasites with a wide host range or those naturally infecting rodent species available as laboratory animals, established models are available. Others, like the human malaria parasites, require sophisticated and often costly genetic manipulation to allow for infection in non-natural rodent hosts. Alternatively, surrogate models of closely related helminth species in rodent models are used to study human parasites.

12.1 Introduction

Parasitism is a lifestyle characterised by the dependency of the parasite on a host organism which has no benefit from its parasite. The most important groups are protozoa (i.e. unicellular eukaryotes from different taxa), helminths (i.e. parasitic worms from different taxa) and arthropods. Every organism may host several parasite species. Of all animals, humans have the greatest parasite diversity. While coevolution of the parasite with its host leads to adaptation limiting the damage to the host at the cost of parasite control, in a medical and veterinary context many parasites still cause considerable harm and, due to their infectious nature, can threaten human and animal health. Parasites often have complex life cycles which may involve stage conversion, metabolic and morphological changes during development and a switch from one host to another (heteroxenous development). Parasites frequently produce long-living stages that can persist in the environment for months and even years. While certain parasites are specialised on a single host species (stenoxeny), others are generalists (euryxeny) and can infect unrelated host species. Zoonotic infections are characterised by parasite transmission between humans and non-human vertebrates. In the following chapters, some examples of important parasitic infections are described with a focus on their relevance for human health, possible zoonotic transmission and animal models to study important human parasitic diseases.

12.2 Diseases Caused by Protozoa

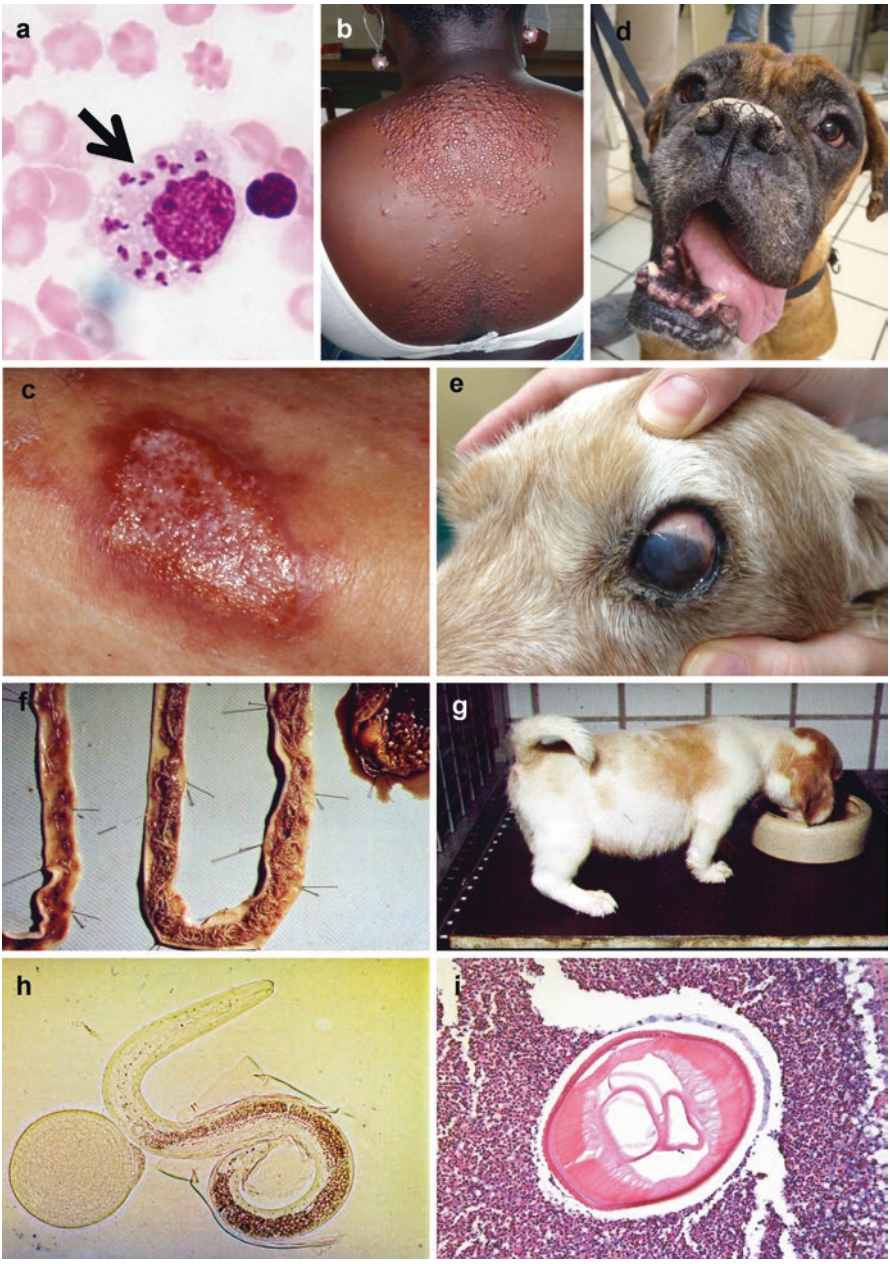
12.2.1 Leishmanioses

More than 30 *Leishmania* species are currently described, all exhibiting two different life cycle stages, one flagellated promastigote stage living extracellularly in the gut of the sandfly vector and one amastigote stage living intracellularly within macrophages and other cells of the reticuloendothelial system of the vertebrate host. Particularly dogs but also rodents play a significant role as reservoir hosts (Fig. 12.1a–e). Leishmaniae are transmitted by sandflies, *Phlebotomus* spp. in the Old World and *Lutzomyia* spp. in the New World.

Two different disease entities are caused by various *Leishmania* spp., the visceral leishmaniosis (VL) caused by representatives of the *L. donovani/L. infantum* complex and various forms of cutaneous leishmaniosis (CL) mainly caused by *L. tropica* and *L. major* in the Old World and representatives of the *L. mexicana* and *L. braziliensis* complexes in the New World. Worldwide around 12 million people are infected, and 350 million are assumed to be at risk of an infection. Approximately, 1.5 million new cases of CL and 500,000 new cases of VL occur every year, with 60,000 deaths annually. *Leishmania* infections are described from >90 countries, and the most affected are India, Nepal, Bangladesh, Sudan and Brazil for VL and Afghanistan, Iran, Saudi Arabia, Syria, Brazil and Peru for CL. The incubation period is highly variable, and infections may also remain without any symptoms. VL is characterised by diarrhoea, weight loss and high fever, children and immunocompromised individuals being at particular risk. Without therapy disease progression is usually fatal. CL can develop from a localised to a progredient form, depending on the parasite strain and the immune status of the patient. After an incubation period of several weeks or even months, a lesion around the site of infection is seen. While the localised form is usually self-limiting within less than one year, the disseminating mucocutaneous form, mainly caused by representatives of the *L. braziliensis* complex, leads to a gradual destruction of the skin, cartilage and even bone if left untreated.

Diagnosis relies on the examination of stained smears and tissue sections, respectively. In the recent past, PCR-based techniques have gained increasing importance for routine diagnostics. Alternatively, leishmanial antigens can be detected in clinical specimens, and a test for the detection of *Leishmania* antigens in urine is also commercially available. Serological tests are highly sensitive only for VL; a fast and easy strip test is available.

The most commonly used drugs are the pentavalent antimony compounds, but they can have severe side effects. Alternative drugs are amphotericin B, itraconazole and allopurinol for systemic and paromomycin for topical application. Miltefosine can be applied as an oral as well as a topical formulation. Generally it is assumed that even after successful therapy some leishmaniae may survive and persist within the host. Repellents and impregnated small-meshed mosquito nets help to avoid sandfly bites.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12.2.2 Malaria

Traditionally four *Plasmodium* spp. are recognised as human pathogens, namely, *Plasmodium falciparum, P. malariae*, *P. vivax* and *P. ovale*. However, in the recent past, an increasing number of human infections with the simian malaria parasite *P. knowlesi* have been reported. More than three billion people live in >100 countries with endemic malaria and are seasonally at risk of infection. Malaria is still one of the most important infectious diseases and the most important parasitic disease worldwide. An estimated 200 million cases of malaria occur every year, including at least 650,000 lethal cases. The vast majority of cases occur in Africa, and almost all fatal cases are caused by *P. falciparum*, the causative agent of malaria tropica. The population group at highest risk are children in sub-Saharan Africa under the age of 5 years. Historically, Europe was also endemic for malaria, mainly caused by *P. vivax*.

Malaria is transmitted by mosquitoes of the genus *Anopheles*. During the blood meal of the female mosquito, the so-called sporozoites are injected into the skin with the saliva. The parasites reach the bloodstream and undergo a first multiplication cycle in the liver. After approximately 1 week, they are released into the bloodstream where they infect the red blood cells and undergo successive cycles of multiplication. In infections with *P. vivax*, *P. ovale* and *P. malariae*, these cycles are synchronised, and every burst of red blood cells is accompanied by high fever (every 48 h in *P. vivax* and *P. ovale* and every 72 h in *P. malariae*). In *P. falciparum* malaria, the release of the parasites from the erythrocytes is not synchronised, more or less continuous or remittent fever occurs, which may be absent at the beginning of the infection.

Diagnosis still mainly relies on microscopic analysis of Giemsa-stained thick and thin smears of fresh finger-prick blood. In recent years, also rapid and simple dipstick tests have been developed. Due to the short incubation time, serodiagnosis only plays a role in advanced infections or in epidemiological studies, but not in the diagnosis of an acute malaria tropica.

Fig. 12.1 Zoonotic parasites can affect humans and animals in different ways. The protozoan *Leishmania* infects the macrophages: (**a**) microscopic view of an infected macrophage, arrow; it induces a variety of lesions; the location depends on the parasite and host species and the individual disposition. In humans it can cause cutaneous lesions, (**b**, **c**), whereas in dogs hyperkeratosis (**d**) and keratitis (**e**) are common. (**f**) The nematode *Toxocara* inhabits the small intestine of dogs; (**g**) in large numbers it impairs growth and leads to generally poor health of mostly puppies; (**h**) infection in humans takes place via ingestion of embryonated eggs from which a larva emerges and migrates through internal organs of the aberrant human host. In the case of the nematode *Dirofilaria repens* which is transmitted by mosquitoes, the larvae migrate into the tissue and are frequently enclosed in skin nodules in both the canine and the human host; (**i**) microscopic view of a nematode is enclosed in a skin nodule (Sources: **a**, **h**, **i**: Institute of Specific Prophylaxis and Tropical Medicine Medical University Vienna; b: by courtesy of Dr. R Moser, Eisenstadt, Austria; c: by courtesy of Prof W Bommer, Göttingen, Germany;**d**, **e**: by courtesy of Prof G Miró, Facultad de Veterinaria, Departamento de Sanidad Animal, UCM, Madrid, Spain; **f**, **g**: Institute for Parasitology, University of Veterinary Medicine Hannover, Hannover, Germany)

The most important drugs for the treatment of malaria are quinine, mefloquine, chloroquine, atovaquone/proguanil and artemether/lumefantrine, depending on the parasite species, the severity of the disease and the geographic region. In some African and Southeast Asian regions, resistance against several drugs is observed. An uncomplicated malaria which is promptly and appropriately treated has a very low mortality, but untreated severe malaria, particularly the cerebral form, is almost always fatal. Malaria is a preventable disease; mosquito nets and repellents can significantly reduce the risk of infection, and chemoprophylaxis with mefloquine and atoquavone/proguanil can prevent disease. Attempts to control and eradicate malaria include vector control, chemotherapy and the development of vaccines.

12.2.3 Toxoplasmosis

Toxoplasma gondii is one of the most prevalent human parasites worldwide; depending on the geographic region and age, up to 80 % of the population is infected. In Austria, infection rates are around 35 %. The only final hosts are cats and other felids which shed environmentally resistant oocysts with their faeces. Oocysts become infectious within a few days and can remain so for up to 1 year. Upon ingestion of these stages, sporozoites are released in the intestines and are spread in monocytes in the blood. After a phase of muliplication in various tissues and organs, the parasites form tissue cysts which can persist for years (probably for lifetime) and are infectious for other hosts after oral uptake.

Humans, other mammals or birds can become infected either by oral uptake of the oocysts or when ingesting undercooked meat from infected hosts. Transplacental transmission of circulating blood stages during gestation and infection of the foetus is possible and can lead to congenital toxoplasmosis with stillbirth, cerebral or ocular disease.

In the immunocompetent host, the infection usually remains without symptoms; however, the parasite survives within cysts in various organs, particularly in the brain, and remains viable throughout the host's entire life. In the immunocompromised patient, *T. gondii* can cause serious and also lethal diseases, mainly of the central nervous system and of the eye.

Diagnosis relies on serological tests detecting specific antibodies against *T. gondii* and PCR for direct detection of the parasite. A combination of serological tests is usually applied in order clarify the time point of infection. In immunocompromised individuals, diagnosis is achieved by PCR, and this technique can also be applied to umbilical cord blood to clarify the infection status of the foetus. Due to surveillance programs and adequate chemotherapy of infections during pregnancy, cases of congenital toxoplasmosis have been significantly reduced in most European countries.

Toxoplasmosis is usually treated with a combination of pyrimethamine and sulfadiazine plus folic acid. If a primary infection is diagnosed during pregnancy, the mother is treated in intervals until delivery. In the first trimenon, spiramycin is given as an alternative to the combination therapy. In AIDS patients, long-term treatment

may be necessary, at least during the phase of immunosuppression. Immunocompetent, nonpregnant patients do usually not require treatment. Complete prevention of infection is not possible, but pregnant women should avoid eating undercooked meat and raw vegetables possibly contaminated with oocysts, apply good hygiene when handling soil (during gardening) or raw meat and avoid contact with cat faeces.

12.3 Diseases Caused by Helminths

12.3.1 Alveolar Echinococcosis (AE)

AE is one of the most serious helminthic diseases of humans; it is caused by larval stages of *Echinococcus multilocularis*, the fox tapeworm.

The infection is acquired by oral ingestion of *Echinococcus* eggs excreted by foxes, dogs or (rarely) cats via contaminated vegetables, water, or the faecal-oral route upon direct contact with animal faeces. First larvae (oncospheres) hatch from the eggs in the small intestine of the intermediate host (humans and other mammals) and are transported with the blood stream to the liver where they develop to the metacestode stage infiltrating the liver parenchyma.

The incubation period varies from 5 to 25 years. Abdominal pain, hepatomegaly, malaise, icterus and fever are the most prominent clinical signs. AE is a chronic hepatic disease resembling hypertrophic liver cirrhosis or carcinomas of the liver or of the biliary system.

Diagnostic procedures consist of clinical and geographical anamnesis, imaging (e.g. ultrasound, CT or MRT scans) and – especially – of serological detection of specific antibodies. The stage of the disease (localisation and dimension of the parasitic lesions) can be categorised with the PMN system according to the WHO guidelines.

Treatment of choice is the surgical removal of the infiltrated liver parenchyma under pre- and postoperative anthelmintic therapy. In cases of inoperability, prolonged albendazole treatment for months, years or even lifelong are the alternatives, if albendazole is tolerated well.

AE has been known in Central Europe since the second half of the nineteenth century. In total 220 human AE cases have been diagnosed in Austria since 1897. The incidence of AE has increased significantly in recent years and now comprises about ten cases per year. The most affected provinces are Tyrol and Vorarlberg, but human infections could also be detected sporadically in all other provinces.

12.3.2 Toxocarosis (TC)

TC is a general term for different clinical manifestations caused by the nematode worms *Toxocara canis* and *T. cati* (large round worms of dogs and cats). *Toxocara* occurs worldwide in canids and felids.

Humans acquire the infection either by oral ingestion of infectious eggs via soil or hands, water or vegetables or by consumption of raw or undercooked meat from paratenic hosts (pig, poultry, rabbits, etc.) containing infectious larvae. After ingestion and passage of the portal venous system, larvae are distributed to nearly all organs via the blood stream. *Toxocara* larvae never become adult in humans but may have a life span of several years.

Most human infections may be clinically inapparent; however, severe clinical courses may occur (Fig. 12.1f–i). The most common forms are the visceral and ocular larva migrans (VLM, OLM) syndrome, covert (CT), general (GT), cerebral (NT) or cardiac toxocarosis (CT).

When clinical symptoms appear, diagnostic procedures consist of geographic and behavioural anamnesis, haematology (typical signs include eosinophilia, elevated IgE) and detection of antibodies by serological examination which has a high degree of sensitivity and also specificity. However, ocular or cerebral *Toxocara* infections are very often accompanied by low antibody levels. In general, asymptomatic infections require no treatment with anthelmintics. Patients suffering from VLM, CT and GT may receive albendazole, at least when eosinophilia is present or IgE is significantly elevated. OLM and NT have to be treated primarily with corticosteroids and afterwards or simultaneously with albendazole for 2–3 weeks.

Due to the fact that the knowledge about the epidemiology and nosology of human toxocarosis is rather limited in medical staff in Austria and other Central European countries, only very limited data are available on the prevalence and incidence of *Toxocara* infections and of human toxocarosis. However, several epidemiological studies in the past two decades revealed seroprevalences between 4 (average population) to 40 % (farmers). The incidence of toxocarosis comprises several hundred cases per year.

12.3.3 Subcutaneous Dirofilariosis (SD)

Dirofilaria repens is a filarial nematode of carnivores, particularly of dogs and cats, and is widely distributed in Southern and Eastern Europe and in Asia.

Humans can be accidental hosts when bitten by infected mosquitoes. Most transmitted larvae die off shortly after inoculation, but some may survive as immature worms. Maturation to the adult stage with production of microfilariae is seen very rarely.

SD manifests in skin nodules or as a migrating lump mostly on the head (eyes, orbita) and the upper thorax, less commonly the male genitalia (testes, epididymis). Diagnosis is mainly based upon histological and/or molecular biological examination of surgically removed nodules; serological tests are available in specialised laboratories. Resection of skin nodules is the therapy of choice. There is no experience with anthelminthic treatment (albendazole) of subcutaneous dirofilariosis so far.

SD is rarely seen in Austria. The first human case was observed in 1978, and between 1978 and 2014, 30 cases were registered in Austria; 29 infections were

imported from abroad (mainly in the Mediterranean region); one case was acquired autochthonously. Based on the fact that the incidence and prevalence of *D. repens* infections in animals and humans have increased in Hungary in recent years, it is likely that the incidence of subcutaneous dirofilariosis in humans will increase also in Austria in the future.

12.3.4 Larva Migrans Cutanea Syndrome (LMC)

LMC is an acute skin disease and is caused by larval stages of the animal hookworms *Ancylostoma braziliense* and *A. caninum,* which occur only in the (sub-) tropical regions. Humans acquire the infection by active penetration of the larvae during skin contact with faeces of infected definitive hosts. Neither species can mature in the human body nor can the larvae migrate subcutaneously in the erroneous human host. The clinical presentation (serpiginous, inflamed traces on the skin accompanied by intense pruritus) is pathognomonic. The therapy comprises symptomatic (antihistaminic, anti-inflammatory and antipruritic medication) as well as anthelmintic treatment (albendazole, tiabendazole). The incidence of LMC cases in Austria is low with no more than a few dozen cases per year.

12.4 Vectors

Two large groups of arthropods can serve as vectors for pathogens, the acari (mites and ticks) and the insects (e.g. bugs, lice, fleas or bloodsucking dipterans). Around 40 species of ticks are known to occur in Austria, belonging to two different families, the Ixodidae and the Argasidae. The so-called castor bean tick, *Ixodes ricinus*, is the most prevalent tick species and the most important vector of pathogens in Central Europe. It can transmit around 15 different pathogens, including tick-borne encephalitis (TBE) virus, various species of *Borrelia*, *Anaplasma phagocytophilum* and other bacteria as well as species of the protozoan genus *Babesia*. While it is assumed that only 1/1,000 ticks carries the TBE virus, infection rates in ticks with bacteria are usually much higher.

More than 50 species of mosquitoes (Culicidae), including also several species of *Anopheles*, are known to occur in Central Europe, and many of them also feed on human blood. Besides malaria, mosquitoes are the vectors of several nematodes (e.g. *Dirofilaria* spp.; see Sect. 12.3.3.) and a long list of viruses.

Sandflies (Phlebotominae) are vectors of *Leishmania* species and Phleboviruses. More than 800 sandfly species have been described, of which at least 80 are known to be vectors of *Leishmania* spp. Sandflies are widely distributed in the warmer regions of the world, including the Mediterranean countries, but in the recent past, small populations of sandflies have also been found to occur in Central Europe, including Austria. These populations may increase with global warming, but the emergence of *Leishmania* infections in Central Europe is mainly a result of globalisation.

The zoonotic transmission of pathogens by arthropod vectors is considered an emerging health problem since many arthropods display a low host specificity and feed on birds, mammals and humans.

12.5 Animal Models for Human Parasitic Diseases

Euryxenic protozoa, e.g. *T. gondii* or *Leishmania*, have been studied in great detail since they are able to naturally infect both humans and animals (including rodents). However, for most parasites, differences in susceptibility as well as size of, e.g. murine, models in comparison to humans often allow only for a part of the life cycle to be completed in a nontarget species. Nonpermissive models which do not support the completion of the full life cycle are useful when studying parasitic infections with humans as accidental (nonpermissive) hosts, such as larva migrans disease. The development of all stages, especially the long-lived adult metazoa, is however necessary to fully apprehend the course of disease and the host response to infection. To overcome the problem of host specificity of the target parasite, different options are available. One is the genetic manipulation of the parasite which is often technically not feasible since entry and development of a parasite in the host usually constitute a complex series of biological events that cannot easily be manipulated or mimicked. In a similar manner, well-defined murine models can be "humanised" to serve as suitable models as have been shown for human malaria. A third option is the use of surrogate models, i.e. closely related parasites infecting suitable animal models as natural hosts. This is often used for studies of helminths.

12.5.1 Models for *Toxoplasma gondii* Infection

The heteroxenic (two-host) development of *T. gondii* includes a natural prey–predator cycle involving the cat and its favourite prey, the mouse (as well as the rat). Different strains of mice display different susceptibilities to the parasite and this can be used to study clinical outcomes of infection, so this model allows for pathogenetic, immunological as well as genetic studies. *T. gondii* can be characterised on the subspecies level by genotyping using several PCR-RFLP genetic markers; these types vary in their virulence, host range and geographical distribution, and virulence in mice is one of the classical phenotypic traits by which such strains are characterised, which are recently complemented by genetic approaches.

12.5.2 Models for *Leishmania* Infection

Leishmania infantum (syn. *L. chagasi*) has a canine host which is considered the most important mammalian reservoir for this parasite. This has several implications

for human–animal interactions; firstly, dogs represent the reservoir host for this parasite and must be included in control measures (and large stray dog populations in many countries defy such measures); secondly, dogs maintain a synanthropic cycle of parasite transmission due to their close relationship with humans; and thirdly, dogs are important hosts that themselves may suffer from the disease. While rodent models for *Leishmania* infections have been used for a long time, and even formed the basis for the research on the Th1-Th2 paradigm in immunology, research on dogs as natural hosts for *Leishmania* is mainly driven by the requirement to develop a transmission-blocking vaccine for this host, as this may reduce the percentage of dogs infected in an endemic area and thus reduce the infection risk for humans; however, most studies on the immune response in dogs to infection or vaccination are restricted to (non-protective) antibody determination. Canine leishmaniosis due to *L. infantum* resembles human leishmaniasis in the development of progressive disease (often without clinical signs for long time periods), but the clinical signs differ between species. Dogs do not develop distinct pattern of cutaneous or visceral leishmaniosis; still they could serve as a model for human diseases as dogs also mirror the diverse genetic variability in natural populations. Mice, hamsters and monkeys are also used as models to study leishmaniosis.

12.5.3 Humanised Murine Models to Study Malaria

Besides the development of new drugs research on malaria control has mainly focused on the development of vaccines. Targets in malaria can be divided into three groups: (i) pre-erythrocytic stages, i.e. sporozoites and liver stages; (ii) erythrocytic stages in the red blood cells (merozoites and gametocytes); and (iii) sexual stages and ookinetes in the gut of the mosquito vector. One of the major obstacles in studying malaria is the high host specificity of most *Plasmodium* species; human malaria parasites cannot be transmitted to rodents, hampering both basic research on immunity and host-parasite interactions and preclinical testing of drugs. Surrogate models, i.e. rodent-specific *Plasmodium* species, such as *P. chabaudi*, *P. berghei* or *P. yoelii*, are used to investigate some aspects of infection and disease, e.g. immune responses in transgenic *P. berghei* expressing human malaria antigens, but the genetic differences between species are major and may account for human disease-specific traits, e.g. the development of cerebral symptoms upon infection with *P. falciparum*. Thus it is preferable to use human malaria species, and in order to be able to infect rodents, humanised models must be developed. Such models have been developed by using genetically manipulated mice that allow for the engraftment of human cells and tissue, both immune cells and, lately, also liver cells and erythrocytes which provide a localised “human environment” to study the development and immune response against the liver stages of *Plasmodium*. Correct expression of MHC I and MHC II is crucial in this model, and the successive replacement of murine tissue by human grafts is a technical challenge that is yet to be fully overcome; however, this technology provides new methods of studying vaccine efficacy before clinical trials.

12.5.4 Surrogate Rodent Models for Nematode Infections

Unlike the rarer "generalists" among the nematodes (e.g. *Trichinella spiralis* which naturally infects a range of mammalian hosts including rats and humans), most species are rather host specific and do not readily cross the species barrier and have so far defied attempts of genetic manipulation to achieve transfer of human parasites to murine models. In some cases, humans are aberrant hosts to migrating larvae of animal nematodes, and this can be mimicked in rodent models to a certain extent; however, the most important life history phase of a parasitic nematode is the development of fertile adults that can reproduce, if possible, for months to years. To investigate this stage, frequently surrogate models are used. These constitute infections of rodents with nematodes closely related to the respective human parasite. Humans have two anthroponotic species of hookworms, *Necator americanus* and *Ancylostoma duodenale*, but can also serve as final and aberrant hosts, respectively, for zoonotic species, including *A. ceylanicum*, *A. caninum* and *A. braziliense*. Due to the pathogenicity of these blood-feeding intestinal parasites and their worldwide distribution, attempts have been made to find adequate animal models of infection to support research on their control. Hamsters can be infected with *N. americanus*, and also with *A. ceylanicum*, dogs can harbour adult *A. ceylanicum* and *A. caninum*, and mice are hosts to the murine *Nippostrongylus brasiliensis*, all of which have a similar life cycle; however, the typical clinical feature of iron deficiency anaemia in human hookworm infection is not mimicked, and animals have a high rate of self-cure not mounted by humans – so the search for improved animal models is still ongoing.

12.6 Synopsis

Parasitic infections with protozoa, helminths or arthropods represent a considerable disease burden for humans and animals worldwide. Transmission to humans may be environmental, nutritional or via animals (zoonotic). Blood-feeding arthropods can serve as vectors of various pathogens including a number of parasites. Diseases caused by parasites may be chronic and debilitating or even acutely life-threatening. Research on them has focused on the interaction between host and parasite to improve current control measures. This involved the use of animal models which are sometimes challenging to obtain due to the specific requirements of parasitic life cycles.

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Comparing Human Breast Cancer with Canine Mammary Cancer

13

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Abstract

Tumors arising spontaneously in human and canine mammary gland tissue appear to have many common clinical features. This book chapter gives an overview of the incidence, risk factors, histological appearance, tumor genetics, biological behavior, molecular targets, and treatment responses in human breast cancer patients and dogs with mammary carcinomas. In both species, malignant tumors of the mammary gland are the most frequent neoplasms in females. Accordingly, the sexual steroid hormones, estrogen and progesterone, are considered not only to play a major role in the development of normal but also of neoplastic mammary gland tissue in both species. While most of the tumorigenic mutations arise during the life span of the affected individual, hereditary mutations like BRCA1 and BRCA2, p53, and PTEN are detectable in dogs and humans. Many diagnostic procedures, classification systems, and numerous prognostic features are similar in human and canine patients, though veterinarians tend not to have the same standardized procedures mostly due to the owners' financial limitations. Nonetheless, spontaneous mammary tumors in dogs provide important proof-of-concept models to support preclinical transgenic or xenograft rodent models in the development of new treatment strategies for human and consequently also canine patients.

13.1 Introduction

Human breast cancer (HBC) is a neoplastic disease of the epithelium of mammary glands that occurs mostly, though not only, in females. In this chapter, we will describe and compare the development, clinical symptoms, and treatment of breast cancer and mammary carcinoma in humans and dogs, respectively.

Taking into consideration that cancer is an acquired genetic disease, the decoding of the dog genome in 2005 and finding that the genome has high homology to its human counterpart were major breakthroughs for comparative oncology (Lindblad-Toh et al. 2005). In contrast to many chapters of this book, we will not include a separate chapter for horses, as mares do not tend to develop mammary carcinoma. Up to date, only few case reports on mammary carcinoma in horses have been published; therefore no epidemiologic or etiologic data is available. Similarly, data about cats with mammary carcinoma is rare to date, which makes the comparison with dogs and human mammary gland tumors inconclusive. However, the comparative analysis of the feline and canine genome, which was published just recently, will provide the key for researchers to explore and collect data on feline mammary tumors in the near future (Montague et al. 2014).

412,258 female patients suffered from breast cancer across Europe in 2012 (Ferlay et al. 2015), making this type of cancer the most common cancer in women. It is estimated that men have a 150-fold lower risk of developing breast cancer; thereby there were about 2500 men suffering from breast cancer in Europe in 2012. Similarly, mammary gland tumors are the most common neoplasms in intact female dogs, although reported data varies depending on the origin of the study. In general, open population-based or insurance-based studies may underestimate the natural

incidence of mammary carcinoma in dogs, although an open population-based study from the UK describes an annual incidence rate of 205 mammary tumors (malignant and benign) per 100,000 dogs (Dobson et al. 2002).

During the last decade, three main risk factors for canine mammary tumors (CMT) have been identified: breed, age, and hormone exposure (Perez Alenza et al. 2000; Sleeckx et al. 2011). Studies on mammary tumors have shown that certain breeds are more likely to develop mammary tumors than others, suggesting a genetic influence. Purebred and smaller dogs such as poodles, dachshunds, Maltese, Chihuahuas, Yorkshire terriers, and cocker spaniels are predisposed to mammary tumors (Schneider 1970). Nevertheless, larger dog breeds like boxers, English setters, pointers, Brittany and English springer spaniels, Dobermans, and German shepherds are also at an increased risk. While mutations in breast cancer genes (BRCA1) and (BRCA2) account for 5–10 % of all human breast cancers, data from canine BRCA mutation studies is limited at present (Rivera and von Euler 2011; Enginler et al. 2014). Interestingly, a recent study in English springer spaniels showed a significant correlation between germ line mutations in BRCA1 and BRCA2 and mammary carcinoma (Rivera et al. 2009). Usually, mammary tumors affect middle-aged and older dogs, the mean age for malignant mammary tumors being 9–11 years, while benign tumors occur at a mean age of 7–9 years. Though humans and dogs have different life expectancies, their biological age at onset of mammary tumors is approximately the same. Human patients have a reported peak incidence at the age of 50–58 years, which coincides with the age at peak incidence in dogs after converting "dog years" to "human years" by multiplying with an average factor of five.

The role of sexual steroid hormones has been well described, although a recent meta-analysis has found only a weak correlation between early castration and the development of mammary carcinoma (Beauvais et al. 2012). Nevertheless, the incidence of CMT in countries with the common practice of early castration of female dogs (before the first or second heat cycle), such as the USA and some countries in Western Europe, is significantly lower than it is in countries such as Spain, Italy, and Scandinavia, where most of the dogs are intact.

Interestingly, obesity increases the risk of developing mammary tumors and influences their behavior in humans and dogs alike. Studies in human and canine mammary cancer patients have identified hormones and cytokines and their receptors (insulin, aromatase, leptin, IL-6, insulin-like growth factor-1 (IGF-1), and its receptor (IGF-1R) secreted by the fatty tissue as potential risk factors (Lim et al. 2015).

13.2 Breast Cancer in Human Patients

13.2.1 Clinical Problem

Breast cancer, as the most common cancer in females in Western countries, represents a tough challenge for oncologists treating these neoplasms (Rahman and Hasan 2015). Nevertheless, during the last decades, significant progress was made

to establish improved diagnostic methods and therapies, followed by significant increase in patients' survival time after diagnosis. Today, diagnosis and therapy of breast cancer is a multidisciplinary issue involving gynecology, oncology, surgery, radiology, obstetric medicine, pathology, and several additional disciplines (Ueno and Mamounas 2016).

13.2.2 Pathophysiology

The human body consists of approximately 4×10^{13} cells (Bianconi et al. 2013). Various, entangled mechanisms are responsible for regulation of their growth and death, thereby preventing uncontrolled proliferation. If a cell escapes these regulating mechanisms due to changes in its genome, cancer occurs (Hanahan and Weinberg 2011). Cancer cells are characterized by longer survival, formation of new cancer cells, and invasion and destruction of other tissues (Hanahan and Weinberg 2011). They are even able to remodel surrounding tissue to ensure their nutrition by formation of additional blood vessels (Nishida et al. 2006). In the pathogenesis of breast cancer, where the epithelium of the mammary gland transforms into cancer cells, hormones like estrogen and progesterone play a crucial role. They are key regulators of physiological breast development and function. Therefore, breast cancer is a hormone-driven disease, and hormone levels are the major risk factor. Women without functional ovaria and estrogen substitution almost never develop breast cancer (Yager and Davidson 2006). Additional risk factors for developing breast cancer can be divided into genetic factors, ionizing radiation exposure, obesity, smoking, and alcoholism. Only 5–10 % of all breast cancers are caused by genetic mutations, which are present at birth. The probability for developing breast cancer is 65 % for women having the breast cancer gene mutation BRCA1 and 45 % for those women having the BRCA2 mutation (Antoniou et al. 2003). Other hereditary mutations, like abnormalities in the p53 tumor-suppressor protein (Li-Fraumeni syndrome), are also associated with a significantly higher occurrence of breast cancer, but are very rare in the general population. However, in about 40 % of women with breast cancers, mutations of p53 are found. In additional 10 % of cases, the PTEN gene that also acts as a tumor suppressor is mutated. Additional 25 % of cases express the epidermal growth factor receptor family member HER2. In this case the gene is not mutated; however, the resulting gene is overexpressed (Olayioye 2001). As with many other tumors, the occurrence rate of breast cancer correlates with increasing age; however, after the menopause, the rate is lower (Adami et al. 1985). So far, three periods in the life of a woman have been shown to have a high influence on breast cancer development: the time points of the menarche, of the menopause, and of the first pregnancy. Women with a later menarche (16 vs. 12 years), but earlier menopause (42 vs. 52 years) or pregnancy (under 18 years), have a 30–60 % lower risk of developing breast cancer. These factors, especially the age at first pregnancy, could explain the high country-dependent differences in breast cancer frequency (Adami et al. 1985). Women from North America have a nine times higher risk for developing breast cancer when compared to women in Asia

(Son et al. 2015). This correlates with lower estrogen levels in Asian women. These differences seem not to have a genetic background, as the estrogen levels of Asian women who immigrate to North America tend to adjust to those of Caucasian women born in North America. If the diet has an influence on the rate of breast cancer is currently discussed controversially (Lelievre and Weaver 2013). Studies have shown that a high calorie and fat intake increases the risk for breast cancer. However, so far, no correlation between the source of fats has been shown (Escrich et al. 2014). Tobacco and alcohol consumption are also associated with a higher breast cancer risk (Adami et al. 1985). Exogenous supply with hormones, for example, by hormonal birth control, seems to slightly increase the risk for premenopausal breast cancer. In contrast to hormonal birth control, the hormone replacement therapy, if given over a period of 6–7 years, doubles the risk for breast cancer. Due to studies performed by the Women's Health Initiative, the prescription rate of hormone replacement therapies has been reduced, followed by a reduction in breast cancer incidence (Collaborative Group on Epidemiological Studies of Ovarian Cancer et al. 2015).

Breast cancer is a very heterogeneous disease, ranging from indolent cancers that may never be diagnosed to malignant cancers that progress fast and have a grave prognosis. Due to improvements in screening procedures, the majority of breast cancers are detected in an early stage. This early diagnosis is the main reason for improved survival (Saadatmand et al. 2015).

13.2.3 Diagnosis

The definitive diagnosis of human breast cancer is usually made by a biopsy of the mammary tissue. Nevertheless, the routinely performed palpation of the breast is the first step in the diagnostic procedure. Some studies suggest that the self-examination does not increase survival in breast cancer and advise against it, as the false-positive rate is quite high. The German S3-guideline for breast cancer recommends that every woman should be aware of the changes of her body and that every woman above 30 should be offered with a manual examination of the breasts at least once per year (Albert 2008). In addition, not only the gynecologists but also general practitioners should perform breast examinations, as an early tumor recognition improves the rate of survival significantly. The procedure of the examination includes the palpation of the breast and of the armpit (axillary) region. Several methods for performing the examination are described, but a specific one is not recommended. It is helpful, if the same method is used all the time. Findings, like an irregularly defined, hard, and relocatable lump, can indicate a malignancy; however, the final diagnosis can only be made by a biopsy. If a woman performs self-examination regularly, she should do it always at same stage of the menstrual cycle in order to prevent hormonal influences to the texture of the tissue (Oeffinger et al. 2015).

According to the S3-guideline, women between the age of 50 and 70 should have a mammography every second year, as this is the only effective screening method

for early stage carcinoma. In the age group of 40–49, a mammography can increase the early recognition rate and survival; however, the false-positive rate is higher when compared to the age group of 50–70. Therefore, women younger than 50 years should undergo a routine mammography only if they have known risk factors for breast cancer (e.g., BRCA1 or BRCA2 mutations). Women with BRCA mutations or other risk factors should be examined in a center that specializes in breast cancer, as they need intensive, continuous monitoring starting at an earlier age (30 years). For those women with a high density of the breast, an additional sonography is recommended in order to increase the limited sensitivity of the mammography. Women above 70 can also be offered regular mammography screens depending on present risk factors, fitness, and life expectation. In women with a high risk for breast cancer development (e.g., BRCA1/BRCA2 mutations are present), contrast-enhanced magnetic resonance imaging should be used in addition to mammography. Despite the occurrence of false-positive and false-negative results, mammography is recommended as a tool for reducing mortality caused by breast cancer, and the benefits prevail the risks resulting from the exposition to radiation. Its sensitivity can be increased by up to 10 % if two radiologists analyze the same picture. However, in contrast to sensitivity, a second opinion does not increase the specificity. If a suspicious formation is present, several different imaging methods can be applied in order to improve the assessment of the risk for malignancy. They include magnified and spot-compressed views, oblique imaging, and sonography. If the risk of malignancy is still considered low (<3 %), a follow-up examination should be performed after 3–6 months. If the risk is higher than 3 %, additional examinations are necessary (Albert 2008).

The next step in the diagnostic procedure is a tissue biopsy. The method of the triple diagnosis, including palpation, mammography, and fine-needle aspiration, is not recommended, as the cytological examination requires extensive experience of the pathologist. The tissue biopsy, where not only cells themselves but also the coherent tissue is excised, obliterates the 1 % risk of malignancy that remains when fine-needle aspiration is used. Several methods of biopsy exist and are used depending of the position, size, texture, and chance of malignancy. Stereotactic biopsy, an image-guided intervention, is especially helpful if small, probably benign lesions are present. It is a less invasive method when compared to open excisional biopsy, which should be used only if an image-guided intervention is not possible. The benefit of the excisional biopsy is that the tumor is usually remove in total, and no further interventions are needed if tumor-free surgical margins have been achieved. In the presence of suspect lymph nodes within the axillary region, a biopsy of these nodes should be performed too. This can reduce unnecessary axillary surgeries (Albert 2008).

The biopsy material is evaluated for the type of cancer and for the maturity (differentiation) of the neoplastic cells. In general, better differentiated neoplastic cells tend to have a lower proliferation- and metastasis rate and thereby a better prognosis. Immunostaining for estrogen receptors, progesterone receptors, and HER2 is performed and provides a rationale for systemic treatment options. Molecular analysis for the presence of genetic aberrations also provides important information about prognosis and treatment options (Albert 2008).

A vital part of the diagnostic procedure in locally progressed carcinoma or suspicious clinical symptoms is the evaluation of the stage of progression. X-ray imaging of the chest and abdomen, sonography of the liver, scintigraphy of the bones, and additional imaging using computed tomography can reveal presence of metastasis and indicate for an additional, systemic therapy (Albert 2008).

Breast cancer staging uses the "TNM" system of the World Health Organization (WHO), where T refers to the primary tumor in the breast; N refers to progression into regional lymph nodes; and M refers to distant metastasis. A higher stage is associated with a less favorable prognosis. Lower stages as 0, I, and II, as well as many stage III cancers, are considered to be curable. Therefore, the primary treatment option is surgery. In higher stages, a systemic treatment is the first approach, followed by secondary surgery if reasonable (NCCN clinical practice guidelines in oncology: breast cancer).

13.2.4 Therapy in human breast cancer

As mentioned above, surgery is the method of choice for lower-stage carcinomas and can be curative. In smaller tumors, where at least 1 cm difference between the tumor and the healthy tissue can be achieved, a lumpectomy can be performed. This is a breast-conserving method, and it may be as effective as a complete removal of the breast (mastectomy) (Albert 2008). Due to cosmetic and psychological issues, this method is increasingly utilized. However, in large tumors, in low-differentiated tumors with a high risk of recurrence, or in presence of more than one tumor, a mastectomy may be indicated. The aim is to achieve tissue margins clear of tumor cells for complete tumor removal. In order to do so, sometimes additional tissues, like parts of breast muscle, have to be removed too. Additional tissues that are sometimes removed are the axillary lymph nodes (Albert 2008). Today, the so-called sentinel lymph node (SLN) dissection is used most often. In contrast to previous methods, which included removal of up to 40 axillary lymph nodes, the SLN dissection utilizes tracing of the specific lymph nodes responsible for tumor drainage by dye staining or radioactive tracing. Removal of only few lymph nodes can prevent problems with the lymph drainage of the affected arm with only low risk of increased recurrence and decreased survival (Kuehn et al. 2005).

Radiation therapy is frequently used in combination with surgery. It is primarily applied after a lumpectomy in order to remove eventual residual neoplastic cells from the breast tissue and prevent local recurrence of the tumor (Anderson et al. 2009). A lumpectomy combined with a radiation therapy has the same low recurrence rate as a radical mastectomy. However, radiation can be also used if the complete removal of the tumor by mastectomy was not possible. Radiation can be applied externally using high-energy X-rays or internally by placing the radiation source directly at the site of surgery (brachytherapy) (Albert 2008). Radiotherapy destroys both normal and tumor cells; therefore, it is usually given over a longer period (up to 10 weeks) of time, so that the normal tissue has the chance to recover, whereas tumor cells often lack the necessary repair mechanisms. Most common method of applying radiation is a linear accelerator, and the treatment is planned

using computed tomography. The radiation itself is performed from several angles, all focusing on the breast, so that the highest dose can be delivered to the former tumor site with reduced stress to the other organs. In patients with metastasized carcinoma, radiation therapy is applied only in a palliative setting in order to reduce pain and other symptoms (Albert 2008).

In patients with aggressive tumor types, HER2-positive tumors, with metastasizes or in young patients (<35), an additional systemic therapy is recommended. This systemic therapy can be divided in three different types: chemotherapy, immune therapy, and hormonal therapy (Albert 2008). An adjuvant (post-surgery) chemotherapy should contain a taxane and an anthracycline. The chemotherapy is cycle based, so that the normal cells have certain time periods for recovery. In case of HER2-overexpressing tumors, an immune therapy with trastuzumab (HER2-targeting antibody) should be started together with the adjuvant chemotherapy. After the completion of the chemotherapy, a hormonal therapy with tamoxifen should be given to patients with estrogen- and/or progesterone-sensitive tumors. If tamoxifen is given as a single drug, the hormonal therapy can be given over at least 5 years or until the tumor recidivates. In patients, where an adjuvant systemic therapy is indicated, it can also be given as a neoadjuvant therapy. The primary goal of a neoadjuvant therapy is to reduce the tumor size before a surgery can be conducted. However, in those tumors where surgery would be applicable as the first approach, there is no difference in survival regardless if the therapy is given pre- or post-surgery (Albert 2008).

In conclusion, many different drugs and drug combinations for treatment of breast cancer are available today. The suitable therapy is given depending on the grade and stage of the tumor, molecular and/or immune markers, age, fitness, and the hormonal status of the patient.

13.3 Tumors of the Mammary Gland in Dogs

13.3.1 Clinical Presentation

Female dogs usually have five pairs of mammary glands arranged in two mammary chains. Canine mammary tumors (CMT) are usually well palpable masses that are noticed by dog owners themselves. Alternatively, veterinarians may detect nodules in the mammary gland during a routine checkup. About half of the mammary tumors are benign and half are malignant and half of the latter metastasize to regional lymph nodes and/or lungs (Sleeckx et al. 2011). Tumors can arise in every mammary gland, though two most caudal mammary glands are most frequently affected. Whenever a dog is presented with a mammary tumor, thorough palpation of all mammary glands is recommended since 70 % of the intact dogs have more than one tumor at the time of diagnosis (Fig. 13.1). Dogs suffering from inflammatory mammary carcinoma (IMC), a rare tumor of the mammary gland, are often misdiagnosed as having mastitis due to the clinical appearance. In these cases, the entire mammary chain is swollen, warm, and painful. In most of the cases, affected patients have distant metastases and a very poor prognosis, similar to human patients with IMC (de M Souza et al. 2009).

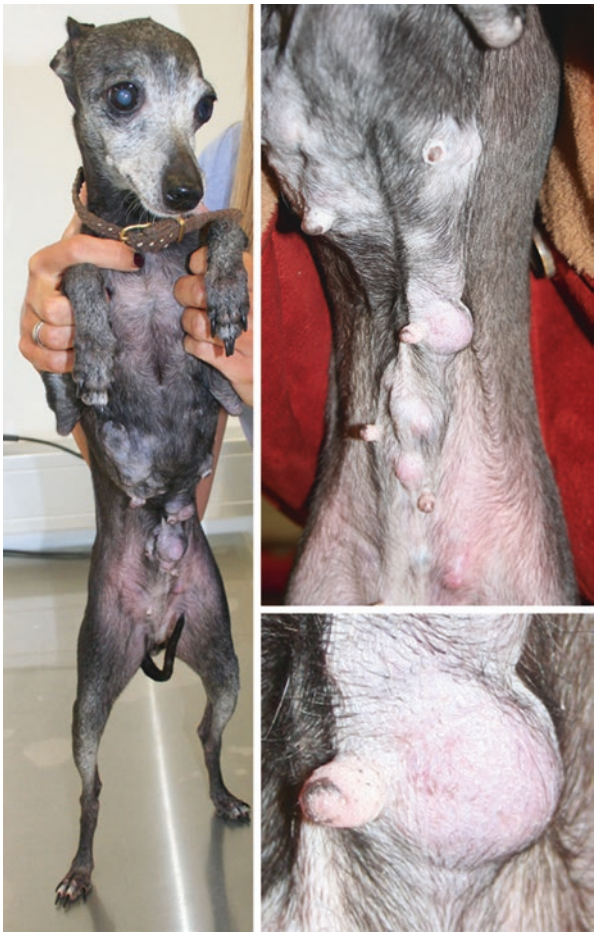

Fig. 13.1 Canine mammary cancer. Italian greyhound, female unspayed, 14 years of age with multiple tumors in both mammary chains

13.3.2 Pathophysiology

Hormone exposure is one of the main risk factors for mammary tumors in humans and dogs. Estrogens and progesterone are essential for normal mammary gland development and maturation. Historically, the tumorigenic effect of estrogens was attributed solely to the fact that their binding to the mammary gland receptors leads to increased growth factor production and therefore cell proliferation. More recent studies have detected that estrogens and their metabolites have direct genotoxic effects resulting in mutations and abnormal numbers of chromosomes in cells of the mammary gland (aneuploidy) as well. The tumorigenic effect of progesterone is caused by its stimulating of the production of growth hormone (GH) and the expression of growth hormone receptors, resulting in increased insulin-like growth factor-1 (IGF-1) levels in the mammary gland (Spoerri et al. 2015). The GH/IGF-1 axis has been identified as an important factor in tumor induction, and many studies have demonstrated elevated tissue concentrations of GH, IGF-1, progesterone, and

estrogen metabolites in malignant tumors. When comparing the hormonal etiology of CMT and HBC, the differing hormone cycles of women and female dogs as well as menopause, which dogs do not go through, must be taken into consideration.

Most dogs develop tumors at multiple sites along the mammary chains. Interestingly, tumors of different sizes and in different stages of progression – benign tumors, premalignant hyperplasia, carcinoma in situ, and malignant invasive tumors – may be found in one and the same patient (Sorenmo et al. 2009). This is called multistep carcinogenesis and is the result of the accumulation of genetic alterations like mutations in oncogenes and/or loss of tumor-suppressor genes in mammary gland cells.

Mammary cancer is a rather heterogeneous disease: mammary gland tumors may arise from epithelial, glandular, or mesenchymal tissue; some tumors remain benign, while others become malignant. However, similar to human breast cancer patients, most of the malignant tumors in canines are carcinomas of epithelial origin (Sorenmo 2003). Sarcomas are very rare tumors of the mammary gland, accounting for less than 5 % of all mammary tumors. The biological behavior of these neoplasms is highly aggressive and associated with a very grave prognosis.

The similar pathophysiology of canine and human mammary gland tumors provides unique opportunities to study risk factors, mammary carcinogenesis, and metastatic mechanisms in a comparative manner with direct implications for humans and dogs (Uva et al. 2009).

13.3.3 Diagnosis in canines

At present, the WHO international classification of mammary tumors in dogs combines their histogenetic classification, descriptive morphology, and prognostic elements (Misdorp 1976; Goldschmidt et al. 2011). While the cytological examination is a necessary initial diagnostic step to exclude differential diagnoses (e.g., abscess), it cannot be used to classify CMT. Classification and grading of the CMT must be based on the assessment of a biopsy sample or the surgically resected tumor itself (Goldschmidt et al. 2011).

In addition, 50–70 % of dogs with CMT have multiple tumors at presentation; therefore many veterinarians recommend bilateral mastectomies to remove the existing tumor and prevent new tumor formation in the remaining mammary glands. However, recent studies were not able to identify a significant difference between simple mastectomy (removal of one mammary gland) and chain mastectomy regarding disease-free interval and overall survival, but there are also studies available, which indicate that surgical extent is important. CMT are graded according to a specific scoring system from grade I (low grade) to grade III (high grade). The process of describing the severity of the patient's cancer based on the size of the tumor and whether or not cancer has spread in the body is called staging. As in human patients, CMT are staged according to the TNM system of the WHO, describing the size of the tumor as well as the presence or absence of local (lymph node) and distant metastasis (e.g. in the lungs). Staging includes a thorough history, physical

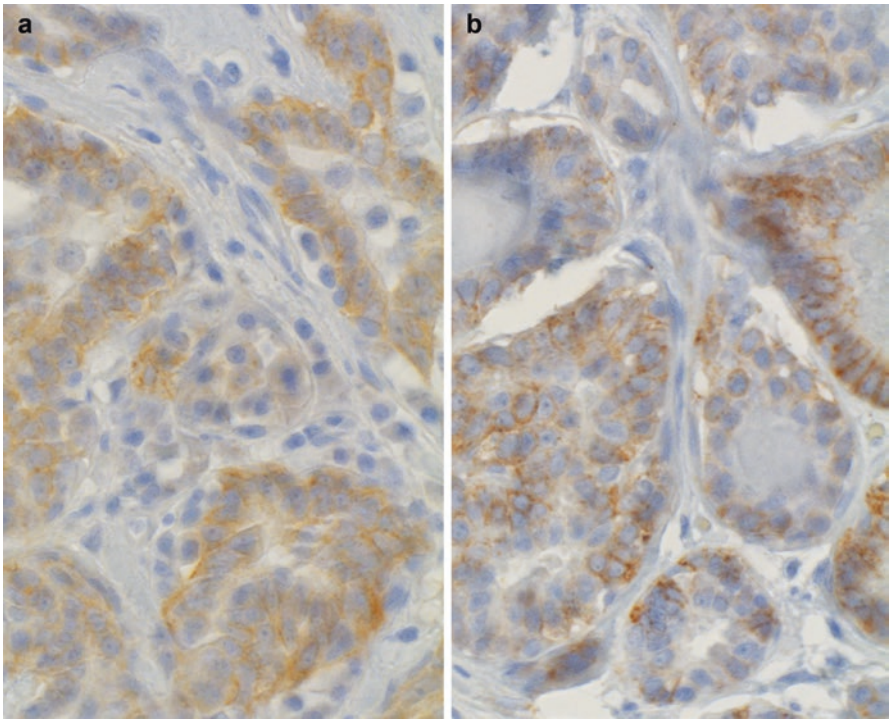

Fig. 13.2 Immunohistochemical staining of the HER2 equivalent in dogs with mammary cancer. (a) and (b) Two exemplary canine mammary carcinoma specimens showed positive staining with HercepTest™, as visible by the specific brownish color in the HER2 overexpressing cancer cells (staining kindly provided by Josef Singer, Institute uf Pathophysiology and Allergy Research, Medical University Vienna). HercepTest™ is the most routinely used immunohistochemical assay to determine HER2 protein overexpression in human breast cancer tissue. The molecular similarity (92 % amino acid sequence homology between human and canine HER2) makes the use of this test kit in canine samples thus feasible (Singer et al. 2012)

examination, complete blood work, thoracic radiographs, and abdominal ultrasound in cases of suspicious lymph node involvement. The use of additional imaging techniques like computed tomography (CT) or magnetic resonance imaging (MRI) has been evaluated in CMT, though these techniques, as well as positron emission tomography (PET), are not routinely applied in the diagnostic workup of dogs with mammary tumors. Nevertheless, a CT scan of the thoracic cavity is more sensitive for lung metastases than lung radiographs and will be increasingly established as part of routine tumor staging. As in humans, a higher stage is associated with a poorer prognosis, and the status of the local lymph nodes is highly prognostic. Additional markers, like hormone receptors (estrogen receptors (ER), progesterone receptors (PR), and other growth factors, e.g., human epithelial growth factor receptor-1 and -2; EGFR1/HER1 and EGFR2/HER2), are not part of the routine diagnostic procedures in dogs at present. Although ER and PR are usually detectable in

benign and low-grade carcinomas, high-grade carcinomas are more often negative for hormone receptors, as in humans with highly aggressive breast cancer. Interestingly, as shown in Fig. 13.2, HER2 overexpression has been detected in CMT using the same test as in human breast cancer diagnostics (Singer et al. 2012). Some studies have shown that, as in human breast cancer patients, HER2 staining significantly correlates with negative prognostic features and short survival time. However, there are also other studies with controversial results in regard to HER2 expression of CMT patients; therefore further research is warranted to confirm the species comparability in this concern (Pena et al. 2014). The p53 suppressor gene is a major regulator of cell growth after DNA damage and is able to stop tumor formation. When mutations appear in this gene, the tumor-preventive function is diminished and tumor formation and progression may occur. Mutations in this gene are detectable in about 20 % of dogs with mammary tumors and indicate a grave prognosis (Klopfleisch et al. 2011).

Many diagnostic procedures, the classification systems, and also numerous prognostic features are similar in human and canine patients; nevertheless, veterinarians do not use the same standardized procedures mostly due to financial limitations of the dog owners.

13.3.4 Therapy in Canine Cancer

Surgical excision is the therapy of choice in all dogs with mammary tumors except those with advanced metastatic stages and IMC. Dogs with benign mammary tumors and about half of the dogs with malignant tumors will be cured after surgical resection of the neoplasms. Whether radical mastectomy (surgical removal of both mammary chains) is indicated or partial resection might suffice continues to be controversially discussed. However, a recent prospective clinical study determined that 58 % of dogs undergoing only partial mastectomy developed a new mammary tumor after surgery (Stratmann et al. 2008). Therefore, these authors recommended a more aggressive surgical approach. Furthermore, resection of the inguinal lymph nodes is indicated in all dogs due to their proximity to the caudal mammary glands. Controversy also persists regarding gonadectomy after CMT resection. Whether late castration of female dogs with CMT influences survival time is still under investigation, and further investigations are warranted. However, there is one recent report that shows a significantly longer survival time in dogs with CMT that underwent ovariectomy or ovariohysterectomy after CMT surgery (Sorenmo et al. 2000). Since the most common hormone therapy in HBC patients with ER-positive tumors, the antiestrogen drug tamoxifen is not recommended in canine mammary cancer due to its severe hormone-related side effects; gonadectomy seems to be the most practical approach in dogs.

In the last decade, clinical trials have been performed demonstrating the effectiveness of radiation therapy, hormone therapies, chemotherapy, and treatment with COX-2 inhibitors and angiogenesis inhibitors. Nevertheless, there are no established guidelines regarding additional therapies after surgical excision of CMT. For

example, while radiation therapy has long been established in HBC therapy to control local recurrence, the benefit of radiation therapy has not yet been evaluated in the course of treatment of CMT.

The efficacy of numerous cytotoxic drugs like 5-fluorouracil, doxorubicin, cyclophosphamide, paclitaxel, and gemcitabine has been evaluated in CMT patients, demonstrating clinical potency, but broader clinical trials are warranted to support evidence-based guidelines in dogs (Simon et al. 2006). Despite this uncertainty, chemotherapy is recommended for highly malignant tumors in dogs. Similarly, antiangiogenic drugs should be effective as there is strong evidence that CMT have a higher vessel density than mammary gland tissue (Madej et al. 2013). However, these drugs are very expensive and therefore not used in veterinary medicine at present. Immunotherapy against overexpressed growth factors like EGFR2 (HER2) with monoclonal antibodies is established in HBC, and there is strong evidence that this treatment may benefit dogs with HER2-positive CMT as well (Singer et al. 2012). Unfortunately, however, these antibodies are humanized and cannot be applied in dogs to date, though efforts are being made to develop caninized monoclonal antibodies against tumor-associated proteins for the treatment of CMT (Singer et al. 2014).

Mammary gland tumors are one of the major health problems in women and female dogs alike. Exposure to similar risk factors due to shared environment and life style, the fact that humans and dogs have similar genomes and gene mutations, and the high incidence of CMT in female dogs make spontaneously occurring CMT a valuable model for oncological research (Queiroga et al. 2011). As a result, canine patients benefit from new diagnostic procedures and treatment options developed for HBC patients. The one-health concept is internationally accepted in the field of comparative oncology, making many novel and highly effective targeted drugs available to canine cancer patients as well.

13.4 Synopsis

In this chapter, the incidence, risk factors, histological appearance, tumor genetics, biological behavior, molecular targets, and treatment responses of malignant neoplasms of the mammary glands are compared in humans and dogs. While breast cancer is a very heterogeneous disease, there are many similarities regarding clinical appearance, diagnostic procedure, therapeutic options, and prognostic features. Therefore, a one-medicine concept to develop new treatment strategies can benefit both human and canine mammary cancer patients.

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Regulatory Animal Testing for the Development of Medicines

14

Günter Waxenecker and Regina Binder

Contents

Abstract

European pharmaceutical legislation requires a valid marketing authorisation for human and veterinary medicinal products before they can be placed on the market to ensure that the benefit of the drug outweighs its risks. In order to obtain such an authorisation, European legislation relating to medicinal

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products for human use (Directive 2001/83/EC) and to veterinary medicinal products (2001/82/EC) requires a dossier containing pharmaceutical and clinical as well as non-clinical safety data, frequently based on results of *in vivo* studies. Drug safety testing often requires a large number of animals and may cause them considerable pain and distress. In 2010 European laboratory animal legislation was renewed by the adoption of Directive 2010/63/EU, reinforcing that *in vivo* testing methods have to be refined and alternative testing strategies need to be favoured. Among other institutions OECD and EURL-ECVAM have been contributing to the implementation of the 3Rs in regulatory testing by incorporating refinement techniques into standard *in vivo* protocols and by establishing and validating alternative approaches, i.e. assays and methods replacing the use of live animals. In the following chapter, the impact of recent legal and scientific measures on drug development, affecting non-clinical safety testing as well as quality testing (i.e. pharmacopoeia) standards in Europe fostering the application of 3R principles, is discussed.

14.1 Introduction

In the Western world, widespread use of drugs and other medicinal products is part of everyday life. Governments bear responsibility for ensuring that these products are sufficiently safe and effective when entering the marketplace. Thus, legal regulations have to ensure that products are appropriately tested and that manufacturers are held responsible for their safety. Among other products pharmaceuticals require premarket regulatory approval to ensure that the benefit of a drug outweighs its risks. Drug safety testing is a staggered process from bench to bedside, frequently requiring adaptations and reiterations to improve the benefit-risk profile; it often requires a large number of animals and frequently causes them considerable pain and distress.

Institutions such as OECD (Organisation for Economic Co-operation and Development) and EURL-ECVAM (European Union Reference Laboratory for alternatives to animal testing) have been contributing to the implementation of the 3Rs by incorporating refinement techniques into standard *in vivo* protocols and by establishing and validating the so-called alternative approaches, i.e. assays and methods replacing the use of live animals. Aiming for more stringent and transparent measures in the field of animal experimentation, the EU updated and replaced laboratory animal legislation in place since 1986 (Directive 86/609/EEC) by adopting Directive 2010/63/EU, being effective since 9 November 2010. These measures should help to increase the use of alternative approaches and eliminate unnecessary duplication of testing.

The impact of these measures on drug development, affecting non-clinical safety testing as well as quality testing (i.e. pharmacopoeia) standards in Europe fostering the application of 3R principles, is discussed in this chapter.

14.2 Regulatory Background on Marketing Authorisation of Medicinal Products

European legislation requires a valid marketing authorisation (MA) for human or veterinary medicinal products (HMPs, VMPs) before they can be placed on the market.

Fundamental criteria and procedures of the European pharmaceutical legislation are provided by the Community code relating to medicinal products for human use (European Directive 2001/83/EC) and with the Community code relating to veterinary medicinal products (Directive 2001/82/EC).

14.2.1 Legal Types of MA Applications

Both directives provide sufficient flexibility to submit MA applications with limited or even without *in-vivo* data generated by the applicant, if the product candidate belongs to the category of "abridged applications". For HMPs those are based on Article 10 of Directive 2001/83/EC, relating to generic, "hybrid" and "biosimilar" medicinal products: the general and common concept of these applications is that reference is made to a product which is or has been authorised in the European Union (Reference Medicinal Product, RMP) to avoid the unnecessary repetition of tests and studies if a marketing authorisation has already been achieved for the same or a biosimilar product. Several other particularities for this type of MAs are to be considered, which would go beyond the scope of this book.

In absence of RMPs, the category of a "full application" (according to Art. 8 (3) of Directive 2001/83/EC) is applicable. For new entities the dossier is expected to be based on data generated by the applicant, supported with relevant published literature ("stand alone applications"). When there is sufficient well-documented experience to establish all aspects of clinical efficacy and safety for a (i.e. known) compound, the dossier may be presented in the form of "mixed applications" based on published literature, and separate non-clinical investigations are not required. In case of safety concerns, or if particularly reproductive toxicity, genotoxicity or carcinogenicity poses a safety concern, non-clinical investigations are necessary, as such effects are difficult or impossible to be revealed clinically (EMA 2005).

14.2.2 Scientific and Technical Requirements for the Content of the MAA

Besides standard MA dossier requirements set out in Part I of Annex I to Directive 2001/83/EC (cf. Table 14.1), Parts II, III and IV provide adapted requirements for specific MAs (e.g. well-established medicinal use, essentially similar medicinal products), "particular medicinal products" (e.g. biologicals, herbals) and "advanced therapy medicinal products" (gene therapy medicinal product, somatic cell therapy medicinal products, tissue engineered products), respectively. By this means the legal requirements take the specific aspects of various different product classes into consideration.

Table 14.1 Non-clinical content of a standardised marketing authorisation dossier

Non-clinical reports		
Pharmacology	Non-clinical pharmacokinetics and metabolism	Toxicology
Primary pharmacodynamics	Analytical methods and validation reports	Single-dose toxicity
Secondary pharmacodynamics	Absorption	Repeat-dose toxicity
Safety pharmacology	Distribution	Genotoxicity/mutagenicity
Pharmacodynamic interactions	Metabolism	Carcinogenicity
	Excretion	Reproductive and developmental toxicity
	Pharmacokinetic interactions	Local tolerance
	Other pharmacokinetic studies	Other toxicity studies (e.g. antigenicity, immunotoxicity)

Annex I of Community code relating to VMPs (Directive 2001/82/EC) defines "chemical, pharmaceutical and analytical standards, safety and residue tests, preclinical and clinical trials in respect of testing of veterinary medicinal products". Special rules are applied to ensure consumer protection on residue limits from pharmacologically active substances used in food-producing animals. Safety assessment of VMPs needs to cover the user, consumer (of food-producing animals), target animal species and environment. In contrast to HMPs, environmental concerns may constitute a ground for refusal of marketing authorisation for VMPs.[1]

14.2.3 Good Laboratory Practice (GLP)

Experimental testing frauds generated the GLP reforms which are meanwhile applicable to a broad range of different products (e.g. chemicals, medicinal products, cosmetics; WHO 2009).

GLP is a managerial concept covering the organisational process promoting the quality and validity of non-clinical health and environmental safety studies. Essential elements are, for instance, the role of the Study Director (OECD GLP 1999) and the Mutual Acceptance of Data (MAD): test data generated in any member state in accordance with OECD Test Guidelines and Principles of GLP shall be accepted in other member states for assessment purposes. This avoids duplicative testing, is beneficial to animal welfare and reduces costs for industry and governments. Moreover,

[1] A revision of the legal framework for VMPs will replace Directive 2001/82/EC by a regulation, as such will be directly binding for all member states; cf. http://europa.eu/rapid/press-release_MEMO-14-522_en.htm, http://ec.europa.eu/health/files/veterinary/vet_2014-09/regulation/reg_part1_en.pdf. Accessed 10 Jan 2016.

common principles for GLP facilitate the exchange of information and prevent the emergence of non-tariff barriers to trade, while contributing to the protection of human health and environment (Directives 2004/9/EC and 2004/10/EC).

In practical terms this means that GLP compliance is required for toxicology and safety pharmacology studies, with a potential extension to pharmacokinetics and bioavailability (WHO 2009; ICH Topic S7A).

Same as with human pharmaceuticals applies also for VMPs: applications submitted to demonstrate safety to man and the environment (*pharmacological*, *toxicological*, *residue and safety tests*) must be conducted and reported in accordance with GLP.

There might be the impression that the transition from discovery to preclinical development is more like a continuum, whereas the boundary between preclinical development and clinical trial is sharply defined, at least in human medicine (Fürdös et al. 2015). For instance, results of GLP-compliant, definitive toxicity studies – in most cases conducted in one rodent and one non-rodent species – are required before first administration to human is accepted, but in fact scientific and reporting integrity is expected for any study aiming appropriate drug product labelling (ICH topic M3(R2); Baldrick 2014).

14.3 Regulatory Animal Testing in Laboratory Animal Law

14.3.1 Definition of "Regulatory Testing"

The legislation of the Union defines that substances and products can be marketed only after appropriate safety, and efficacy data have been submitted in order to manage risks to human and animal health and the environment. Although recital 10 of Directive 2010/63/EU states that it is desirable to replace the use of live animals in procedures by alternative methods, it also confirms that the use of live animals continues to be necessary to protect human and animal health and the environment. With regard to regulatory animal testing, recital 42 of the Directive affirms that some of the safety and efficacy requirements can be fulfilled only by resorting to animal testing.

Testing of new medicinal products for safety, quality and efficacy reasons is usually carried out in line with standardised protocols as defined, for example, by the European Pharmacopoeia (*Ph. Eur*). For this subset of experiments, which may be mandatory according to diverse legal (i.e. pharmaceutical) regulations and is referred to as "regulatory testing", Directive 2010/63/EU sets out specific rules, mainly relating to application and authorisation.

According to current EU legislation, the principles of the 3Rs do not apply to regulatory testing of VMPs as these tests are explicitly exempted from the scope of Directive 2010/63/EU, and Directive 2001/82/EC does not make sure that the 3Rs are adequately implemented in the field of veterinary clinical trials. This shortcoming will, however, be eliminated by the new regulation which is to replace Directive 2001/82/EC.[2] Making use of the option to maintain more extensive provisions to

[2] Cf. recitals 20 and 21 of the proposal for a regulation of the European Parliament and of the Council on veterinary medicinal products, 10.9.2014 COM(2014) 558 final http://ec.europa.eu/

protect laboratory animals, which had been in force on 9 November 2010, the Austrian Act on Animal Experiments 2012 (*Tierversuchsgesetz 2012 – TVG 2012*) sticks to its former definition of the term "animal experiment" ruling that laboratory animal law does also apply to clinical trials of VMPs, which therefore have to comply with all the requirements set out by the Directive 2010/63/EU.

14.3.2 Special Authorisation Provisions on Regulatory Testing

In sharp contrast to basic and applied research (discovery), the second (non-clinical) step of the drug life cycle is indicated by standardised protocols, aiming to satisfy regulatory requirements of safety testing (WHO 2009). As regulatory testing shares elements of routine or repetitive nature, the Directive 2010/63/EU provides the opportunity for the member states to introduce a "reduced application" and a "simplified administrative procedure" for the evaluation of projects containing regulatory procedures, provided certain requirements laid down in the Directive are complied with.

The Austrian Act of Animal Experiments 2012 makes use of this statutory possibility, introducing a "reduced application" and a "simplified administrative procedure" for projects meeting the requirements defined by Article 40 of the Directive. In line with § 26 (3) *TVG 2012*, a "reduced application" is sufficient for specific projects which are defined by purpose/method, severity category and the species of animals involved in the procedures (cf. Table 14.2).

There are, however, only minor differences between the two types of applications and administrative procedures, respectively (cf. Table 14.3).

14.4 Directive 2010/63/EU and Its Influence on Pharmaceutical Development

Although pharmaceutical legislation clarifies which safety aspects need to be covered, the unreflected application of certain protocols (e.g. OECD) is not supported for drug development. Instead, the various non-clinical safety guidelines spur for a "science-based risk assessment", preventing a "tick box approach" (Committee on Improving Risk Analysis Approaches Used by the U.S. EPA 2009; Committee on Toxicity Testing and Assessment of Environmental Agents 2007). Adapting existing protocols to the specific needs of the compound under question or the use of newer alternative nonanimal methods for safety assessment resulted in an explosion of initiatives by numerous organisations (especially OECD, EPAA, EURL-ECVAM), which are not yet sufficiently coordinated.[3] In 2010 the European Medicines Agency's Management Board endorsed therefore the formation of an ad hoc expert working group on the application of 3Rs in the development of medicinal

health/files/veterinary/vet_2014-09/regulation/reg_part1_en.pdf. Accessed 10 Jan 2016.

[3] http://www.hesiglobal.org/files/Tab%203%20-%20Emerging%20Issues%20Session(1).pdf. Accessed 10 Jan 2016.

Table 14.2 Correlation between severity category and type of application/administrative procedure

Severity category	Procedural consequences
Non-recovery	"Reduced application" and "simplified administrative" procedure applicable to Two types of testing Regulatory testing Use animals for production or diagnostic purposes with established methods If no nonhuman primates are involved
Mild	
Moderate	
Severe	Regular application and administrative procedure (irrespective of purpose)

Table 14.3 Regular and reduced applications versus regular and simplified administrative procedures

Regular applications and regular administrative procedure	Reduced applications and simplified administrative procedure
Content of project application	
Non-technical project summary Obligatory	Non-technical project summary *Not* obligatory
–	Group authorisation possible if multiple generic projects are carried out by the same user
Period for decision by competent authority	
40 working days	40 working days
+15 working days[a]	
Maximum authorisation period	
5 years	

With regard to complex or multidisciplinary projects (Art. 41/2 Dir. 2010/63/EU)

products representing all relevant disciplines to foster the application of 3Rs in the regulatory testing of medicinal products throughout their life cycle.[4]

Cross-company reviews in the pharmaceutical regulatory field aim to identify various options to reduce animal use in practice while maintaining the scientific objectives (Sewell et al. 2014). The European Directorate for the Quality of Medicines & HealthCare (EDQM) organises likewise large collaborative studies on alternative methods, in the framework of the Biological Standardisation Programme under the aegis of the European Commission and the Council of Europe, involving official medicines control and industry laboratories from different countries to develop and validate alternatives to compendial methods (e.g. validation of serological methods for diphtheria, tetanus, pertussis vaccines).[5]

It is expected that the adoption of Directive 2010/63/EU further increases the regulatory acceptance of alternative test methods for development of

[4] http://www.ema.europa.eu/docs/en_GB/document_library/Other/2011/10/WC500115626.pdf. Accessed on 21 Jan 2016.

[5] https://www.edqm.eu/en/BSP-programme-for-3Rs-1534.html. Accessed 10 Jan 2016.

pharmaceuticals (Hartung 2010).[6] Following its implementation it has been considered appropriate to carry out a general review of the EMA guidance documents aiming to ensure best practice in the implementation of the 3R strategy in the regulatory testing of medicinal products. As the availability of new methodologies and retrospective reviews continuously stipulates updates of EMA guidelines to reflect changes in scientific development, the number of guidelines affected will be relatively small (EMA 2014/1). For instance, EURL-ECVAM recommended two cell-based assays (Direct Peptide Reactivity Assay and KeratinoSens™) to be used as part of an integrated testing strategy for testing skin sensitisation potency without animal testing (OECD 2012).[7] Nevertheless, the absence of a process supporting the post-validation and implementation of new methods that would help to ensure the rapid and widespread uptake of new alternative methods was recognised. A draft guideline has been issued on scientific and technical criteria for regulatory acceptance of 3R testing approaches, including a process for collection of real-life data, based on a safe harbour concept (EMA 2014/2). It also clarifies the difference between scientific validation (usually a prerequisite for regulatory acceptance) and regulatory acceptance process, as the validation protocol may not encompass the specific needs of the product to achieve regulatory acceptance (e.g. due to limited validation parameters).[8]

Another concept paper elaborates on the regulatory uptake of alternative methods already validated in collaborative trials (EMA 2014/3). Article 13 of Directive 2010/63/EU includes the request that "the competent authorities responsible for granting approval of animal testing will request the more animal friendly Ph.Eur. method" (EMA 2012). Moreover further evolution of the "consistency approach" is expected, which is repeatedly discussed in quality control of vaccines: a set of process- and product-specific tests could enhance the level of quality control throughout the manufacturing process, which would facilitate the implementation of *in vitro* methods instead of continuing *in vivo* tests (De Mattia et al. 2011; Duchow 2012).

14.5 Synopsis

The development of medicinal products is subject to a complex set of regulations pursuing the objective to protect human and animal health, as well as the environment. In the context of regulatory testing, the development, validation and application of alternative methods are especially important. Thus, Article 47 of Directive 2010/63/EU regulates that the commission as well as the member states shall

[6] http://ec.europa.eu/environment/chemicals/lab_animals/3r/alternatives_information_en.htm. Accessed 10 Jan 2016.

[7] https://eurl-ecvam.jrc.ec.europa.eu/validation-regulatory-acceptance/topical-toxicity/skin-sensitisation. Accessed 22 Jan 2016.

[8] http://ec.europa.eu/environment/chemicals/lab_animals/3r/regulatory_en.htm. Accessed 10 Jan 2016.

contribute to the development and validation of alternative approaches which could provide the same or higher levels of information as those obtained in procedures using animals, but which do not involve the use of animals or use fewer animals or which entail less painful procedures, and they shall take such other steps as they consider appropriate to encourage research in this field. There are various independent projects pursuing this objective, which are, however, not yet sufficiently coordinated. A broadened agreement on objectives for determining the credibility of nonanimal testing may finally improve the translational value of data and thereby increase generation and regulatory uptake of alternative methods.

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One Health: Many Patients? A Short Theory on What Makes an Animal a Patient

15

Herwig Grimm and Martin Huth

Contents

Abstract

The holistic understanding of health is a crucial idea of *One Health* and *Comparative Medicine*. Both concepts aim at bridging human and veterinary medicine and the transfer of medical knowledge. The aim of this paper is to analyze the possibility to transfer knowledge from human biomedical ethics to veterinary ethics. Based on the concept of *patient* in human medicine and its normative implications, the concept of *animal patients* in veterinary medicine will be analyzed. As we will argue, the crucial similarity is to aim at health-related interests in both fields. Focusing on such interests seems to be the unquestionable goal in human medical contexts. However, since these interests are not always the end of veterinary action, criteria will be explicated that allow to judge whether an animal can rightly be referred to as patient. In a last section moral implications and the limits of transferring the concept of patient to animals will be investigated. Therefore, the famous four principles of biomedical ethics by Beauchamp and Childress will be used. The transfer of the *non-maleficence* and

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the *beneficence* principle is widely uncontroversial, whereas the principles of *justice* and *autonomy* open a number of moral questions regarding the concept of *animal patients* that will be addressed.

15.1 Introduction: One Health, Comparative Medicine, and the Question of Animal Patients

In the recent past, the concept of "One Health" (OH) has gained prominence (cf. Sandøe et al. 2014, 610; cf. Stärk et al. 2015, 127). Defined as "any added value in terms of health of humans and animals, financial savings or environmental services achievable by the cooperation of human and veterinary medicine when compared to the two medicines working separately" (Zinsstag et al. 2015, 18), the term OH refers to the idea of bridging various fields of health care and gaining added value by linking human and veterinary medicine (Bresalier et al. 2015; Zinsstag et al. 2015). The main premises of OH are that knowledge can be meaningfully transferred from animals to humans and vice versa and that this has significant consequences for human and animal health (cf. Bresalier et al. 2015; Nieuwland et al. 2015, 132f). To give an example, animal research is one of the most prominent fields demonstrating the possibility of transferring knowledge from one field to the other (within limits). The majority of experiments are justified with reference to the idea that knowledge gained with animal *models* can be meaningfully transferred to humans and can benefit them. However, the knowledge gained and its use to establish clinical treatments in human medicine allow also for the transfer back into clinical veterinary medicine, if the demand for advanced medical treatment of animals is generated (Gardiner 2006a, b; Sandøe et al. 2016, 26f). In the view of Nieuwland et al. (2015, 132), such instances reflect a holistic understanding of health. This holistic concept leads to an approximation in methods, concepts, paradigms, and treatments of humans as well as animals and promises to benefit both. However, according to Sandøe et al. (2014), the initial aim of developing the OH concept was to fight against zoonosis, meaning infectious diseases of animals that can be transmitted to humans, and was therefore focused on human health. Similar to OH the use of the term "Comparative Medicine" (CM) is rather new in the debate, although its subject is probably as old as medicine itself (cf. Jensen-Jarolim 2014, 3; Bresalier et al. 2015). CM shares the idea with OH that human medicine can benefit from veterinary medicine and vice versa. Hence, both fields make use of the approximation of methods, concepts, and paradigms for the treatment of humans and animals.

In this article we will follow this idea. In particular, we will draw from debates and knowledge established in the field of human medical ethics and transfer it to veterinary medical ethics. The central question is: Can a holistic concept of "patient" that comprises both human and animal patients be developed and defended? In recent times, "patient" has increasingly become a central term in veterinary medicine (Jones 2003; Gardiner 2009). Hence, the question arises whether "patient" in

"human patient" and "animal patient" reflects similar or different ideas. Approaching this subject is the main aim of this article. We are going to argue that the concept of "patient" illustrates what it means to deal with both humans and animals in ways that are directed toward their health and well-being in clinical contexts. As it will turn out, this idea can be paralleled with the principle of respecting the moral status of humans and animals in medical practice.

Since we will argue that the idea of animal patients can only plausibly be defended if the moral status of animals is acknowledged, we start with some thoughts about the moral consideration of animals in general. Building upon these clarifications, we are going to identify criteria for what makes an animal a patient with moral status and then specify the corresponding obligations for veterinarians. We will draw from the concept of human patients in order to transfer criteria from the human field to animals. In this section we are going to follow the idea of a holistic concept of health that can provide a reasonable background to approximate the concept of "human patient" and "animal patient." In the subsequent part of the article, we will elaborate on the practical consequences of acknowledging animals as patients. In this regard, we will apply the principles of biomedical ethics by Beauchamp and Childress in order to sketch normative implications of treating animals as patients.

15.2 Extending the Moral Community

The arguments to extend moral concern and apply moral principles to animals have a long, complex, and not very uniform tradition. From ancient philosophers like the Pythagoreans to thinkers like Michelle de Montaigne, Arthur Schopenhauer, and Jeremy Bentham in modernity to present animal ethicists, a great variety of arguments have been put forward for acknowledging the moral significance of animals (cf. Grimm et al. 2016b). Nowadays, the dominant approach to this question is *moral individualism* (cf. Grimm et al. 2016a, c; Rachels 1990). Thinkers who follow this idea justify the moral status of animals on the basis of individual capacities, such as the ability to experience positive and negative states (i.e., being sentient). McReynolds (2004), for instance, presents a well-reasoned argument that similarities between humans and animals guide and support the idea of extending the moral community in animal ethics. The argument proceeds from a core group of individuals of moral significance (humans) and is then extended to others (at least some animals) with similar morally relevant capacities: "[…] structural feature: whenever moral standing is *extended* to a new group, it is granted to the new group *to the extent of and on the basis of their similarity to* members of the old group" (McReynolds 2004, 64). Such extensions have an obvious impact on veterinary medicine, since also medical treatments in humans and animals are parallel if animals are to be considered morally relevant. Although the dominating theory of moral individualism can be put in doubt (cf. Grimm et al.2016b, d), we will address and analyze the outlined question of the status of animals as patients in this

philosophical framework. We are certain that other academic approaches that have a different understanding of ethics can also lead to success in the formulation of a theory of animal patients. However, in this context, we think that a moral individualistic approach can highlight important aspects and open the doors for further debates by linking questions regarding the concept of "animal patient" to well-established theories and traditions in medical ethics.

To illustrate what moral individualism is all about, we refer to Peter Singer. He formulated a prominent and influential individualistic theory in animal ethics. Singer takes sentience as the individuals' characteristic that gives us sufficient reason to integrate animals into the moral community (Singer 2011, 50). If a being is sentient and has a sufficiently high degree of (self-) consciousness, it will have *an interest* in experiencing pleasure and avoiding pain and suffering, his argument goes. As long as we have reason to think that these interests are similar and comparable with human interests that we consider morally relevant, these comparable interests of nonhuman beings have to be taken into account in our moral life. In other words, we have reason to extend the moral community and consequently treat these animals with moral respect. They are receiving ends of our moral duties, which prohibit, for example, harm without justifying reasons. Taking the well-being (e.g., satisfaction of interests) of beings into account for their own sake is a clear sign of moral status or, in other words, of being a member of the moral community (DeGrazia 2002; Gruen 2014; Grimm et al. 2016b).

The acknowledgment of animals as members of the moral community is not only obvious in the academic field, but it is also increasingly part of our commonsense morality (cf. Rollin 2006, 7–41). In the following section, we are going to argue that the concept of the "animal patient" is directly linked to the idea of moral status in the context of veterinary medicine. If an animal's health is cared for in medical contexts *for the animal's sake*, its moral status is respected. In contrast, if an animal's health is cared for merely because of ends other than the animal's good, such as the owner's interests, gaining knowledge in experiments, or public health like in the case of zoonosis, the animal should not be referred to as a patient. As we will demonstrate, only when the animal's health-related interests are the end of a treatment carried out by a veterinarian can we rightly speak of the animal as a patient.

Let us now focus on this idea by utilizing arguments from a debate on what have been coined "marginal cases" in ethics. As the term indicates, "marginal cases" refers to beings that are at the margin of the moral community and not undoubtedly members. In order to clarify whether they are members from a moral individualistic point of view, the following question needs to be answered: are their capacities sufficient to include them in the moral community? In human medical ethics, children, severely impaired humans, fetuses, and others are considered "marginal cases." Some 20 years ago, a debate was started on which human marginal cases could be considered patients in the full sense in clinical practice. We will use some insights from this debate in human medicine for our question of animal patients.

15.3 What Makes a Being a Patient?

For centuries, the starting point for what morally ought to be in clinical practice has been the obligation to protect and promote the interests of the patient (Chervenak et al. 1996, 115). This general obligation plays a major role in the context of medicine, understood as a particular practice that is centered on the patient. As a starting point, we follow Chervenak et al. (1996) and use the term "medicine" in the following way: "On the basis of scientific knowledge, shared clinical experience, and a careful, unbiased evaluation of the patient, the physician identifies clinical strategies that will likely protect and promote the health-related interests of the patient and those that will not. The health-related interests of the patient include preventing premature death and preventing, curing, or at least managing disease, injury, handicap or unnecessary pain and suffering" (ibid., 115). In this context, the *principles of beneficence and of non-maleficence* direct the clinical perspective to the interests of the patient, and it obligates the physician to seek the greater balance of goods over harms for the patient (ibid., 116). Therefore, the clinical perspective on the patient's goods and harms has to be complemented with the *perspective of the patient herself*, represented, e.g., in the concept of informed consent or role taking if the patient cannot consent herself. If the health-related interests are not the end of medical treatment, it is not very plausible to talk about a patient. Against this background, Chervenak et al. give an answer to the question whether human fetuses are patients (cf. ibid., McCullough et al. 1994). Their argument is illustrative and helpful in order to formulate a crucial component of an ethical theory of the patient. They argue in favor of a dependent moral status of the fetus:

> Instead [of having an independent moral status; H.G./M.H], being a patient means that one can benefit from the application of the clinical skills of the physician. Put more precisely, a human being without independent moral status should be regarded as a patient when two conditions are met: 1) when that human being is presented to the physician and 2) when there exist clinical interventions that are reliably expected to be efficacious, in that they are reliably expected to result in a greater balance of goods over harms for the human being in question. (ibid., 117)

From this perspective, human beings are "turned" into patients if they *can* be treated in a particular way in the context of medicine: If the being is brought to a physician and *can* be medically treated so that health-related interests are promoted and protected, it is considered a patient and *should* be treated accordingly. This is independent of capacities that are eventually considered as necessary or sufficient for personhood.

These criteria can easily be brought to veterinary medicine. However, are they sufficient to turn animals into patients? Although the criteria are in principle plausible, we have one major concern: Whereas it is clear that in human medicine, patients that can benefit from medical treatment should be treated accordingly for their own sake, this is not the case in animals. As Chervenak et al. rightly state, clinical practice in human medicine is evidently under the obligation to protect and promote the interests of the patient (ibid., 115). What else should be the

end and the legitimization of the intervention other than their health-related interests as sketched under the definition of medicine as a practice? Whereas this seems clear in the human sphere in most cases, it is not when it comes to animals.

When animals are treated with regard to their health-related interests, it remains open whether a medical intervention that results in a greater balance of goods over harms also *aims* at the animal's benefit and is carried out for the animal's sake. To illustrate this point, we take the example of a veterinary clinician using animals in a clinical trial. If the animal is used to gather data and gain knowledge that can be published and used to treat other animals, we can of course *not* rightly speak of this animal as a patient. Even if an ill (not ill made) animal in a clinical trial recovers from illness through medical intervention, this does not justify referring to that animal as a patient, if the sole intention is to gather data. The reason for this conclusion is that the end of treating the animal in the trial is not to promote and protect the health-related interests[1] of the animal, but instead other animals or also humans by gaining knowledge – therefore the animal is considered as a proband instead. In other words, if an animal is treated with the *intention* that others benefit from knowledge gained by its treatment, this animal is used as an instrument to serve the interests of others even though – as a matter of fact – health-related interests are protected and promoted. In such cases, health-related interests are not the end of the clinical treatment but means to other ends. However, if the clinical trial is carried out in order to protect and promote the health-related interests of the animals in question as an end, the animal is rightly referred to as a patient. Therefore, we believe that also the *right intention* – namely, the end to protect and promote the animal's health-related interests – is a necessary condition to consider animals as animal patients.

In the following we are going to elaborate on this in more depth. For this purpose we use the irritating fact that very strong critics of animal use in research like Tom Regan indicate that some animal experiments can be morally justified (Regan 2004, 387). How is this possible, if – as Regan holds – using animals as means to other ends is morally wrong? According to Regan, experiments can be justified if the ends of the experiment are the health-related interests of the animal in question. If the gained additional knowledge is only a "side product" of the medical treatment, the health-related obligations toward the animal are respected.[2] The experiment is in line with moral respect for the animal just like in the case of managing an injury. The reason for this conclusion is that the animal itself and its health-related interests – and not the benefit of others – are *the end of the actor's action/intention*.

This point is of great importance when we look at different actions carried out by veterinarians. Take for instance veterinary treatments in the farming sector. Not all actions of veterinarians are directed toward the health-related interests of the animal

[1] Whenever we speak of health-related interests in animals *presumed* health-related interests are meant. Whether the presumed interests are the interests of an animal in question is of course a difficult question to be answered and not in scope of this article.

[2] We are claiming that to serve presumed health-related interests is a necessary condition for regarding animals as patients. Whether other or plural ends are in accordance with treating animals with moral respect remains open but seems possible.

as the end. For example, if a pig's health is restored in order to regain productivity, the ultimate purpose of the action is not to promote health-related interests of the animal, and consequently, we should not refer to it as patient. In such cases, other ends like economic efficiency or productivity are served. Veterinary skills are used but obviously not directed toward the health-related interests of the animal as the major end of veterinary medicine.[3] Dehorning of cattle, castrating pigs, artificial insemination, etc. are good examples of actions that serve ends other than the health-related interests of animals. Another example would be a cow with mastitis. If the health of the cow is cared for to sustain its productivity, the end of veterinary action is not the cow's presumed health-related interest but the farmer's (in productivity). The cow's health is only secondary and a means to economic ends. In a nutshell, the argument goes that we can only refer to animals as patients as long as they are treated with regard to medicine's end, which is to protect and promote health-related interests.

We can draw on a debate in human medical ethics to clarify this position. The ethicist Pellegrino (Pellegrino 1999) argues for a sharp linguistic distinction between the goals and the ends of medicine. The ends are "[…] tied to the nature of medicine, to its essence. Ends serve to define medicine. Without certain ends, the activity in question does not qualify as medicine. The ends of medicine distinguish it from other arts and sciences which have different ends. To convert the ends of medicine to the purposes of economics, politics, or professional prerogative, transforms medicine into economics, politics, or professional preference" (Pellegrino 1999). In brief, medical knowledge and skills are used for medicine only when they are used to pursue medicine's ends, which Pellegrino – according to Veatch (2000) – restates as activity that meets the needs of a particular patient, to cure, care, help, or heal. As we have seen, medical knowledge and skills can also be used for other goals or purposes that are not tied to the essence of medicine. And, if actions are not directed to medicine's end, there is no reason to talk about a patient.

From this perspective it is no surprise that the use of the term "animal patient" is generally attributed to companion animals where (supposedly) everything is done for the sake of, and in the presumed interest of, the animal. Even if an animal's clinical treatment harms the animal significantly, like chemotherapy, and the benefits are doubtable (e.g., a few additional weeks to live), the intention to serve the presumed health-related interest of the animal prevails and gives reason to call the animal a patient. This argument can also explain why it is often but not always problematic when the term "patient" is attributed to farmed animals or animal models in research. The health of these animals is mainly a means to other ends – namely, productivity or knowledge – and not for their own sake. If, however, their health-related interests were the aim of clinical intervention, probands could turn into patients.

[3] At this point we leave it open whether there is only one end to veterinary medicine or more, and whether this leads to many medicines (cf. Grimm 2016b). With regard to human medicine, this question was addressed by Veatch (cf. Veatch 2000).

Against this background we complement the two criteria of Chervenak et al. (1996) with a third one and bring their idea to the animal field: An animal is a patient if (a) it is presented to a veterinarian; (b) when there exist veterinary measures that are reliably expected to be efficacious, in that they are reliably expected to result in a greater balance of goods over harms for the animal in question; and (c) the ends of the veterinary intervention are the animal's presumed health-related interests and not the interests of others.

15.4 Recognizing Animals as Patients: Are All Patients Equal?

We have argued that animals are rightly called patients under specific circumstances. In the following, we aim at a clearer understanding of what behavior is in line with treating an animal as a patient. As indicated, this is also to say that animals are treated with moral respect. Therefore, an analysis of duties toward patients in human medicine will be used. In this analysis Tom L. Beauchamp's and James F. Childress' book *Principles of Biomedical Ethics* (Beauchamp et al. 2009) serves as a valuable source. They describe four moral principles that are relevant in medical ethics and applied to patients and to medical contexts in general: *autonomy*, *non-maleficence*, *beneficence*, and *justice*. According to Beauchamp and Childress, these four principles should guide clinical practice. Their principles represent major normative dimensions leading to fundamental obligations toward patients in the field of human medicine. In the following, we will sketch the possibilities and limits of transferring these principles from human to animal patients and illustrate some further ethical dimensions of the term "animal patient."

1. *The principle of autonomy* holds that one should not ignore, insult, demean, or be inattentive to other's rights to self-governed action (cf. Beauchamp et al. 2009, 103).
2. *The principle of non-maleficence* "imposes an obligation not to inflict harm on others" (ibid., 149).
3. *The principle of beneficence* embraces "all forms of action intended to benefit other persons" (ibid., 197). The difference to the principle of non-maleficence lies in the positive duty to support well-being instead of the negative duty *not* to harm others or prevent them from harm.
4. *The principle of justice* is concerned with the distribution of resources, e.g., that everyone gets an appropriate share according to its needs (ibid., 241f). In the field of human medicine, this principle is applied to reflect upon the distribution of organ donations in a morally justifiable way.

Being acknowledged as a patient is identified with being a receiving end of these moral principles and the correlated moral duties of actors in the medical context. There are differences between the four principles regarding their applicability to animals. Initially, we start with the largely uncontroversial claim that we should not

inflict unjustified harm to patients (non-maleficence) and should contribute to their health-related interests (beneficence).

As we have already seen, aiming at the health-related interests of animals and acting in accordance with the *principle of beneficence* reflects respect for the animals' moral status and make them animal patients. The principle is rather uncontroversial when it comes to good nutrition, enrichment of the housing environment, clinical treatment of a broken leg, etc. The principle becomes questionable when, for example, it is not entirely clear whether a clinical intervention has a therapeutic or esthetic aim. Consider a dog with dental braces. Can this treatment indeed be considered therapeutic or just enhancement without therapeutic character? According to the three criteria outlined above, it could be argued that the dog's health-related interests are not the end of veterinary action in this case. However, if the dog suffers from adverse effects due to its tooth position, the aim to end this suffering by means of the positive effects of the dental braces makes the dog a patient. Whether or not all available technical possibilities during medical treatment shall be used to benefit animals is likewise an issue of intense debate. Whereas we usually think that all possibilities should be used to restore human health, this is not so clear in the case of animals (Yeates 2013, 114), where limiting factors are included for instance, the coverage of financial costs. Since clinical treatments for animals presently have to be paid on a private basis, the bandwidth of clinical treatments varies from one extreme to the other according to the owner's financial situation and willingness to pay. We will have a closer look at this issue when we focus on the principle of justice.

The *principle of non-maleficence* is a second fundamental principle that we find in human and veterinary medicine. As Beauchamp and Childress state, physical harm is much easier to detect than mental harm (cf. Beauchamp et al. 2009, 152f). They also dive into the question of euthanasia and frame it within the principle of non-maleficence. Contrary to standard practice in human medicine, there is virtually no hesitation, Beauchamp and Childress claim, to consider killing part of medical care in veterinary medicine (cf. Beauchamp et al. 2009, 184). Although in specific cases, such as convenience euthanasia, this lacks empirical proof (cf. Hartnack et al. 2016), euthanasia is often framed as an important moral responsibility of the veterinary profession when it can be considered mercy killing (cf. Grimm et al. 2016b, 96–98; Hartnack et al. 2016). Basically, the principle of non-maleficence can be understood similarly in the human and the animal field: "Do no harm without justifying reason!" If no harm were allowed at all for whatever reason, most clinical interventions would be prohibited since most of them start by harming in order to promote health-related interests. Interventions in the bodily integrity of a living being are considered as necessary and/or justified because of the presumed interests of the animal patient.

Regarding the *principles of autonomy and of justice*, we can detect significant differences between the application to humans and to animals. Concerning autonomy one could ask: Can an animal be seen as an autonomous being at all? How can we know about its autonomy? How can we respect it? In the majority of human cases, autonomy refers to *informed consent* in clinical contexts. Its fundamental

requirement is that a competent patient must give her consent to clinical treatment voluntarily and on the basis of relevant knowledge. Information about the treatment, a recommended course of action, and the understanding of both are fundamental preconditions. These elements are the necessary conditions to be able to proceed to the "consent elements" in the strict sense, which are the decision in favor of the procedure and the authorization of a physician (cf. Beauchamp et al. 2004, 111–113; Beauchamp et al. 2009, 120f). They constitute a threshold that is not only high for animals but also for many human beings (cf. Rogers 2014) and may even be too high for either in some cases. It requires a clear utterance of preferences against the background of a traceable understanding of the relevant information given by the clinic staff. Since we cannot deliberate with a companion dog about its favored treatment or if it would chose euthanasia, the *informed owner consent* mirrors the idea that the owner has to decide with regard to the health-related interests of her animal.

However, the ethical difficulty with autonomy should not be seen in the lack of verbal expression of volition in animals only. Major difficulties lie in the obligation to take decisions for the animal as a patient. Although some theorists suggest using common-sense intuitions is enough in human medicine (cf. Hoerster 1998, 122f), one can also assume that the patient's individual biography, interests, and preferences play a role in such vital decisions (cf. Huth 2011). This is also true for nonhuman patients. Animals never utter their preferences verbally, but we think to know at least to a certain extent about their state and what is presumably in their interest. If the animal is treated as a patient, the moral duty to take honest effort to find out what is best for the animal according to the animal patient's interest is prior (with all the given limitations).

What we see here is that treating animals as patients might at times present medical staff with even more difficult questions than in human medicine. Since medical treatment has to aim for the benefit of an animal that cannot verbalize its own interests, unlike most humans, people have to take the responsibility to decide for the animal patient without knowing with certainty whether they act in its interest. Questions like "Can it be in the interest of the animal to be euthanized?" and "Is a painful life for the animal worse than no life at all?" emerge. In this field, tough decisions have to be faced.

The principle of justice illustrates a problematic sphere of clinical treatment of animals. Although animal owners are legally bound to take care for their animals and pay for clinical treatment, respectively, in many countries, no public health system for animals guarantees a minimal and fair standard for animal medical care as in human medical care. For this reason, the limits of transferability are quickly reached here. If we frame differences according to wealth or willingness to pay as manifesting injustice, the consequence would be a moral claim on a public health system for (certain) animals. If we frame this question differently and argue that this diverges from the situation in human health, we implicitly admit that justice is another matter when it comes to animals. Therefore, a bundle of questions arise immediately: Are animals our equals insofar we can owe them justice like we owe it to humans? If this were the case, the differences between the access to medical

treatments for animals that depends on the owner's economic situation and willingness to pay would be pure injustice.

Within human medical ethics, the debate about how to understand and apply Beauchamp's and Childress' principles to patients started in 1979 with the first edition of their book. In veterinary medicine we are only at the beginning of elaborating on what it means to apply principles of medical ethics to animal patients. For that reason, only minor conclusions can be made so far. However, taking decisions carefully and deciding after extended deliberation with regard to the animal's health-related interests are probably the best sign that one has considered moral obligations toward animal patients.

15.5 Synopsis: The Complexities of Health

We have tried to show how the concept of "patient" can be transferred to animals. Being an animal patient was introduced as a concept that mirrors moral status in clinical practice. Three criteria were outlined and elaborated in order to give a transparent account of what is to be understood under "animal patient": An animal is a patient if (a) it is presented to a veterinarian; (b) when there exist veterinary measures that are reliably expected to be efficacious, in that they are reliably expected to result in a greater balance of goods over harms for the animal in question; and (c) the end of the veterinary intervention are the animal's presumed health-related interests and not the interests of others. When it comes to moral principles and their transfer from human medical ethics to veterinary medical ethics, we are at the beginning of a debate that may continue in the future. The four principles of biomedical ethics, described by Beauchamp and Childress, are not in every respect easily transferred to animals. In particular, obvious limits of transferability are reached when we deal with autonomy and justice. Finally, we can conclude that although the transfer of knowledge is possible and plausible, we are only at the beginning when it comes to a clear understanding of what it means to respect animal patients morally with regard to the principles of beneficence, non-maleficence, autonomy, and justice.

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