Chapter 10
Contributors to Faecal Water Contamination in Urban Environments

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Abstract Faecal contamination of water has both anthropogenic and zoogenic origins that can shade various point and nonpoint/diffuse sources of pollution. Due to the dual origin and number of sources of faecal contamination, there are immense challenges in the implementation of effective measures to protect water bodies from pollution that poses threats to human and environmental health. The main health threats refer to infections, illnesses and deaths caused by enteric pathogenic microbes, in particular those responsible for waterborne zoonoses. To detect and identify the origins and sources of faecal pollution simultaneously, various methods and indicators have been compiled into a comprehensive measuring toolbox. Molecular diagnostics using genetic markers derived from *Bacteroidales* 16S rRNA gene sequences are quite prevalent in the current methodological implementation for the identification of faecal contamination sources in water. For instance, a culture- and library-independent microbial source tracking toolbox combining micro- and molecular biology tests run as a three-step procedure has been implemented in Norway. Outcomes from the Norwegian studies have identified two general trends in dominance of contributors to faecal water contamination in urban environments. Firstly, there is a tendency of higher contributions from anthropogenic sources during the cold season. Secondly, the identification of the dominance of zoogenic sources in faecal water contamination during warm periods of the year.

Keywords *Bacteroidales* · Faecal indicator bacteria · Gene markers
Microbial source tracking · Water pollution

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Table 10.1 General concentrations of the most common microorganisms in human faecal matter of healthy or infected individuals (Edberg et al. 2000; WHO 2006)

Organisms	Numbers per gram of faecal matter
Bacteria:	
– *Escherichia coli (E. coli)*	10^9
– *Salmonella* spp.	10^4–10^8
– *Campylobacter jejuni*	10^6–10^9
– *Shigella* spp.	10^7
– *Vibrio cholerae*	10^7
Viruses:	
– Enteroviruses	10^4–10^9
– Rotaviruses	10^7–10^{11}
Protozoa:	
– *Cryptosporidium parvum*	10^7–10^8
– *Giardia intestinalis*	10^5–10^8
– *Entamoeba histolytica*	10^5–10^8
Helminths:	
– *Ascaris lumbricoides*	1–10^5
– *Schistosoma mansoni*	1–10^3
– *Clonorchis sinensis*	10^2

10.1 Concise Facts on Faecal Contamination

In general, faecal contamination refers to any kind of pollution caused solely or partially by faecal matter, or pollution that contains any portion of this matter. The faecal matter characterises wastes from metabolic processes occurring in a gastrointestinal/digestive tract (gut) of humans and other animals, and are defecated as faeces (solid or semisolid wastes) through anus (in most mammals) or as excreta (faeces and urine) through cloaca (in birds, reptiles and amphibians).

The gut is a habitat of trillions of various organisms among which bacteria dominate with approximately 500 different species (Marotz and Zarrinpar 2016; Quigley 2013). The gut bacteria comprise the major number of the microbes in the whole body and constitute about 10 times more than all body cells (Quigley 2013). Taking a vital part in the metabolic process, these microbes are continuously defecated; hence, faecal matter contains an abundance of live microorganisms (Marotz and Zarrinpar 2016). The number and variety of faecal microbes depend greatly on animal species, but even within the same sort, there are substantial variations. In humans, concentrations of faecal microbes vary according to, e.g., gender, age, health conditions, diet, physical activities, lifestyle and region of living (Table 10.1).

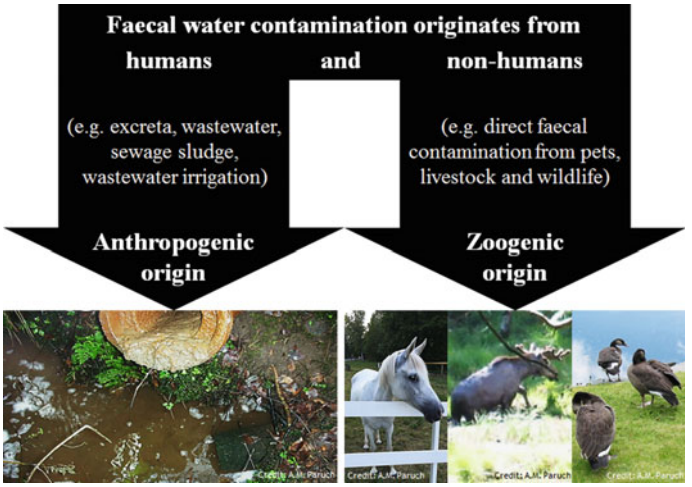

Fig. 10.1 Anthropogenic and zoogenic origins and multiple sources of faecal water contamination *Source* Authors

10.2 Origins and Sources of Faecal Water Contamination

Faecal contamination of water has both anthropogenic and zoogenic origins that can shade multiple sources of pollution (Fig. 10.1). The main human sources include direct disposal of excreta, leakages from sewers, overflows from sewage pumping stations, uncontrolled discharges from treatment plants, inadequate sewage sludge handling and insufficient performance of decentralised wastewater treatment systems. Non-human sources are characterised by animal faecal contamination directly from pets, livestock and wildlife, or indirectly from the improper utilisation of manure, slurry and other materials containing animal faeces. From all of these sources, a high number of faecal microbes (viruses, bacteria and parasites) can directly contaminate groundwater and surface water bodies (drinking-, irrigation- and recreation water) or indirectly from soil and vegetation through agricultural drainage, irrigation and organic fertilisation, particularly during and after heavy precipitation and subsequent run-offs (Paruch et al. 2015a).

Due to the dual origin of various point and nonpoint/diffuse sources of faecal water pollution, there are immense challenges in the implementation of efficient measures protecting water bodies from contamination that poses threats to human and environmental health. Normally, it is possible to localise point sources of faecal water pollution (typically, direct discharge of wastewater), while it is more problematic to locate inflows from diffuse faecal pollution sources (usually, storm water-, urban water- and agricultural run-offs contaminated with faecal matter from humans, pets, livestock and wild animals). It is therefore crucial to identify the primary origins of faecal contamination as to act upon their elimination, if possible directly at the source or if this is not applicable then at least to minimise the exposure to various contam-

inating agents, and hence to reduce potential health risks of waterborne infections and diseases to humans and animals.

10.3 Health Risks Related to Faecal Water Contamination

The main health threats associated with faecal water contamination refer to infections, illnesses and, in plenty of cases, deaths caused by enteric pathogens (infectious agents causing diseases), in particular those causing waterborne zoonoses (zoonotic infections and diseases transmitted between animals and humans through water). It has been recently reported that 5 million people, including 1.5 million children, die every year as a result of water-related diseases (IWFA 2017). The numbers of infected and ill individuals are vastly higher, although not completely reported as some cases have been ignored due to minor abdominal and diarrhoeal symptoms. Mortality and burden of disease resulting from faecal contamination of water represent almost 10% of the total burden of human disease worldwide (WHO 2017).

Water-related infections and diseases are normally characterised by four main categories (Moe 2004), in which water is the major, but not the only transmission route of pathogens. These categories are defined as follows:

- water-borne infections representing classic examples in which pathogenic organisms enter water sources through faecal contamination,
- water-washed infections that occur due to lack of adequate water and sanitation facilities, hence poor hygienic manners,
- water-based infections that are caused generally by pathogens spending part of their life in the aquatic environments and
- water-related insect vectors which are associated with infections transmitted by insects breeding in or near water.

Water-related pathogenic organisms and toxins produced by these organisms (which are of particular health concern as they are significant virulence causes of microbial pathogenicity), as well as the infections and disease symptoms they cause, may be characterised by more than one category. In addition, the pathogenic microbes and toxins have various transmission pathways (Pond 2005; WHO 2011), with the most common routes through:

- ingestion (e.g. *E. coli*, *Salmonella* spp., *Vibrio cholerae*, *Shigella* spp., *Campylobacter* spp., *Helicobacter* spp., *Cryptosporidium parvum*, *Giardia intestinalis*, enteroviruses, noroviruses, hepatoviruses and rotaviruses),
- direct contact (e.g. *Pseudomonas aeruginosa*, *Aeromonas* spp., *Mycobacteria* spp., *Acanthamoeba* spp., *Naegleria* spp., *Leptospira* spp. and *Schistosoma* spp.) and
- inhalation and aspiration (e.g. *Legionella* spp. and *Mycobacteria* spp., adenoviruses and enteroviruses).

Table 10.2 General classification of water-related zoonoses (Moe 2004)

Classes	Examples of zoonotic diseases
1. Waterborne through drinking water	Balantidiasis, campylobacteriosis, cryptosporidiosis, cysticercosis, *E. coli* O157:H7, giardiasis, microsporidiosis, salmonellosis, toxoplasmosis, tularaemia and yersiniosis
2. Waterborne through recreational water	Cryptosporidiosis, giardiasis and leptospirosis
3. Water-based infections	Dracunculiasis and schistosomiasis
4. Water-washed infections	Cryptosporidiosis, giardiasis, hepatitis viruses
5. Water-related insect vectors	West Nile virus, Rift Valley fever virus, yellow fever virus, sleeping sickness
6. Water/wastewater aerosols inhalation	Legionellosis
7. Aquatic food consumption	Paragonimiasis

Some pathogenic microbes (e.g. *Aeromonas* spp., *Pseudomonas* spp., *Vibrio vulnificus* and *Vibrio parahaemolyticus*) can also colonise through wound infections (Pond 2005). Furthermore, other pathogens (e.g. *Campylobacter* spp., *E. coli*, *Salmonella* spp., *Vibrio* spp. and *Shigella* spp.) and toxic species (e.g. *Gambierdiscus, Gonyaulax*, *Gymnodinium* and *Paragonimus*) might be transmitted through a raw edible consumption of infected and faecally contaminated aquatic animals and plants (Moe 2004).

Based on the criteria of water-related infections and diseases, and transmission routes of pathogens and toxins, a general classification of water-related zoonotic diseases has been suggested by World Health Organization (Moe 2004). Seven main classes exemplifying common zoonoses have been distinguished, as presented in Table 10.2.

The criteria defining water-related infections and diseases are not only characterised by zoonoses as the pathogen pathways, but also have person-to-person and vector-borne transmissions (Moe 2004). Although the pathogenic organisms might come from both humans and animals, and/or occur naturally (e.g. *Legionella* spp. causing legionellosis), the zoonotic pathogens still comprise 75% of emerging infectious diseases (Bolin et al. 2004). Furthermore, human and animal faecal contaminations constitute the largest load of pathogens associated with waterborne disease transmission. The faecal pathogen groups with their associated organisms most common in human and animal excreta, as well as symptoms and diseases caused by these pathogens are presented in Table 10.3.

Table 10.3 Examples of faecal pathogens causing acute disease outcomes (Kanarat 2004; Pond 2005; Suresh and Smith 2004; WHO 2006)

Pathogen groups and organisms	Symptoms and diseases
Bacteria:	
– *Escherichia coli (E. coli)*	Urinary tract infection, haemolytic-uraemic syndrome, colitis with diarrhoea
– *Salmonella* spp.	Fever, abdominal pain, diarrhoea or constipation
– *Campylobacter* spp.	Diarrhoea, abdominal pain, fever
– *Helicobacter pylori*	Gastritis, intestinal metaplasia and gastric cancer
– *Enterococcus faecalis*	Endocarditis and bacteraemia, urinary tract infection, meningitis
– *Clostridium perfringens*	Abdominal cramping and diarrhoea
– *Shigella* spp.	Shigellosis, abdominal pain, diarrhoea, fever
– *Vibrio cholerae*	Cholera, muscle cramps, vomiting, diarrhoea
Viruses:	
– Hepatitis A and E	Hepatitis, fever, abdominal pain, diarrhoea
– Adenoviruses	Respiratory disease, eye infection
– Rota-, noro-, enteroviruses	Gastroenteritis, fever, vomiting, abdominal pain, diarrhoea
Protozoa:	
– *Cryptosporidium parvum*	Cryptosporidiosis, fever, crampy abdominal pain, watery diarrhea
– *Giardia lamblia*	Giardiasis, fever, abdominal pain, diarrhoea
– *Entamoeba histolytica*	Amoebiasis, abdominal pain, bloody diarrhoea
Helminths:	
– *Ascaris lumbricoides*	Ascariasis, fever, abdominal swelling and pain, diarrhoea
– *Schistosoma mansoni*	Schistosomiasis, abdominal pain, diarrhoea, urinary tract infection
– *Clonorchis sinensis*	Clonorchiasis, abdominal pain, nausea, diarrhea
– *Diphyllobothrium latum*	Diphyllobothriasis, vomiting, abdominal discomfort, diarrhea
– *Fasciolopsis buski*	Fasciolopsiasis, abdominal pain, chronic diarrhoea, anemia

10.4 Detection of Faecal Water Contamination and Identification of Pollution Sources

To detect and identify the origins and sources of faecal pollution simultaneously, various methods and indicators have been combined into a comprehensive measuring toolbox. In general, there are two main methods that have been applied for tracking of faecal water contamination, chemical source tracking (CST) and microbial source tracking (MST). Various chemical substances and components as well as different microbial indicators and markers have been employed in these methods, which are therefore also known under other terms as, e.g., bacterial source tracking or faecal source identification (Field 2004).

When using the CST method, chemical detection may provide supplementary evidence on the faecal source (Staley et al. 2016; Harrault et al. 2014; Hartel et al. 2008). Caffeine, faecal sterols and stanols, bile acids, laundry brighteners, fragrances and pesticides can be used as chemical indicators and molecular tracers to aid in the identification of faecal inputs, but they do have limits to their use, as the chemical indicators respond differently to many environmental factors (Tran et al. 2015). Therefore, the CST methods should be applied in the combination with the MST methods.

Overall, there are two main categories within MST, i.e. culture-based and culture-independent methods. Both categories can further be subdivided into library-dependent and library-independent approaches (Hagedorn et al. 2011). Notably, under the first category, antibiotic resistance mapping (Olivas and Faulkner 2008) and other phenotypic methods, e.g. carbon-source utilisation profiling (Smith et al. 2010) and fatty acid methyl ester profiling (Duran et al. 2009) for source tracking, utilise the biological traits (phenotypes) to classify the sources. Genotypic library-dependent methods, like ribotyping, repetitive extragenic palindromic polymerase chain reaction, amplified fragment length polymorphism and pulsed-field gel electrophoresis, are DNA fingerprinting techniques based on the established amplicons' library (Field 2004). Sorting/clustering of microbe groups is accomplished by directly comparing the generated DNA polymorphisms (Carson et al. 2003). This is quite technically demanding, and the results are less reproducible. In comparison, the culture- and library-independent methods are remarkably more time efficient, are less labour intensive and are more accurate.

In the molecular culture- and library-independent methods, some faecal viruses have been selected as good candidates for detection purpose. For instance, human-specific adenoviruses and enteroviruses (Bambic et al. 2015), and bovine/ovine adenoviruses (Ahmed et al. 2013) are highly host specific. However, due to the small size of viruses and low viral load, a large amount of water is normally required for a concentrated sample. An enrichment step to facilitate the capture of viruses is also required. In terms of anaerobic bacterial genes, animal-specific *Bifidobacterium* spp. (e.g. *B. dentium* and *B. adolescentis*) became targets in markers development (Venegas et al. 2015). In addition, host-specific toxin genes in *E. coli* and Enterococci can be targeted for source determination, for example human-specific ST1b toxin (Moyo

et al. 2007), pig-specific ST1b toxin (Khatib et al. 2003) and enterococcal surface protein (Scott et al. 2005). As the toxin target genes are rare and thus need the enrichment procedure, the final detection may only be semi-quantitative and it also inherits instability due to the horizontal transfer of genes (Böhm et al. 2015). Host-specific *Bacteroidales* genetic markers are by far the most tested/optimised and exhibited in most cases geographical stability across USA, Canada, Europe, New Zealand and Japan (Kobayashi et al. 2013; Mieszkin et al. 2013; Sowah et al. 2017).

Regardless of the variety of markers that have been applied in MST surveys, many of them are still under comparable testing and verification processes, while others are less applied in practice. Yet, the molecular diagnostics using genetic markers derived from *Bacteroidales* 16S rRNA gene sequences are quite prevalent in the current methodological implementation for the identification of faecal contamination sources in water.

10.5 Methodological Toolbox Discriminating Dominant Sources of Faecal Water Contamination

Numbers of host-specific *Bacteroidales* genetic markers have been developed to discriminate faecal pollution between human and different warm-blooded animal species (Dick et al. 2005; Layton et al. 2006; Reischer et al. 2007; Shanks et al. 2008; Tambalo et al. 2012). These can be further employed in various attempts focussing on providing detailed profiling of markers contributions in the faecal contamination and defining the dominant source(s) of this pollution. One of such attempts has been undertaken in Norway, where a culture- and library-independent MST toolbox has been utilised since 2013.

The Norwegian approach focusses on faecal contamination of aquatic ecosystems, mainly in urban and agricultural landscapes, as well as catchments of drinking water reservoirs, as these significantly influence human and environmental health. The developed methodological toolbox has been described in greater detail elsewhere (Paruch et al. 2015b). Briefly, it combines micro- and molecular biology and consists of three independent steps:

1. microbial analyses of faecal water contamination based on the detection of *E. coli*,
2. molecular DNA tests using real-time quantitative polymerase chain reaction (RT-qPCR) for the detection and quantification of host-specific *Bacteroidales* 16S rRNA genetic markers and
3. profiling of the genetic markers contribution in the detected faecal contamination.

In step one, *E. coli* bacteria have been used as the historical and most frequent faecal indicator employed. These bacteria have also often been applied in other MST studies on faecal water contamination (Åström et al. 2015; Shahryari et al. 2014; Tambalo et al. 2012). Although *E. coli* greatly satisfies most of the criteria of faecal indicator bacteria, i.e. has dominant faecal origin, is present in large numbers in

faeces of human and warm-blooded animals and is rapidly detectable by simple methods (Paruch and Mæhlum 2012), it does not satisfactorily fit into the criteria of a source identifier. This is due to low host specificity, possible replication in the environment, as well as geographic and temporal variability (Farnleitner et al. 2010; Field and Samadpour 2007; USEPA 2005). Therefore, bacteria belonging to the phylum *Bacteroidetes*, especially *Bacteroidales* spp., have widely been applied in various MST studies with molecular diagnostics based on RT-qPCR (Dick et al. 2005; Lamendella et al. 2009; Layton et al. 2006; Reischer et al. 2007; Shanks et al. 2008; Tambalo et al. 2012).

In step two, the performance of *Bacteroidales* genetic markers, in terms of sensitivity and specificity, needs to be evaluated prior to their adaptation. Furthermore, analyses of melting curves are strongly recommended as it has been proved that they are essential in discriminating strains of intestinal and non-intestinal *Bacteroidales* bacteria (Paruch and Paruch 2017). This is of high importance as *Bacteroidales* are environmental bacteria, but still species of the genus *Bacteroides* comprise the largest portion of the gut microbes and normally constitute about 30% of total faecal bacteria (Layton et al. 2006). They can even make up to 52% of human faecal flora (Dick et al. 2005) with concentrations up to 10^{11} organisms per gram of faeces (McQuaig et al. 2012). In addition, these bacteria are strictly anaerobic, having little potential for growth in the environment (Dick et al. 2005; USEPA 2005) and are highly host-specific, thus enabling distinguishable host's identification (Layton et al. 2006).

Since there are no significant correlations between *E. coli* bacteria and the host-specific *Bacteroidales* genetic markers (Harwood et al. 2014), only the percentage profile of markers contribution in the measured faecal contamination can be further assessed in step three.

10.6 Exposure of Urban Catchments to Various Faecal Pollution Sources

When faecal contamination of water occurs in urban areas, it is quite common to assume that it was caused primarily by leaks from sewer systems, uncontrolled discharges of wastewater, overflows from sewage pumping stations and floods after extreme precipitation. In addition, the practices of collecting urban run-off (mainly storm water, but also wash water after maintenance of roads, railways, bridges and tunnels) jointly with sewage into the sewer system contribute to overflows and overloading of wastewater treatment plants. Furthermore, climate change predictions on frequent episodes of extreme precipitation will also expect more overflows as the urban drainage systems get easily overloaded. These scenarios have already been observed, in particular when related to abrupt rainfall (usually short but intensive) followed by run-off predominating over water infiltration, especially in tight urban areas with dense surfaces (concrete, steel and asphalt). It is therefore often taken for granted that faecal water contamination in urban areas is mainly derived from

sewer systems; hence, it is entirely of anthropogenic origin. Such assumptions must, however, be revised since even the modern cities are also living areas for a variety of animal species, not just pets like dogs and cats, but also wildlife, and these can make a significant zoogenic contribution to faecal water contamination in urban catchments.

Not all that comes from the sewer system is anthropogenic. Sewerage pipelines offer suitable environments for common sewer rats, also known as Norwegian rats (*Rattus norvegicus*), which thrive with food residues and fat rests deposited in the sewer systems all year-round. This, in fact, is a historical problem of growing cities in which the rat concentration might exceed the city's population; e.g., there were reported more rats (up to one million individuals) than citizens of Oslo (Aftenposten 2013), three times more rats than people in Stockholm (DN 2016) and as many as four rats per 100 m of sewage pipeline in Copenhagen (Fettvett 2016). It has also been documented that excreta of rats represent a risk for public health as they contain both zoonotic and multiresistant bacteria (Guenther et al. 2013). In general, the large number and diversity of pathogens enter sewer systems through four main routes (Gerardi 2006), representing both anthropogenic and zoogenic origins:

1. domestic wastewater,
2. industrial wastewater, e.g. food production and processing,
3. inflow and infiltration of animal excrements and
4. excreta of inhabitants of sewer systems, mainly rats.

In addition, human and livestock wastes in urban areas can attract various animals not only to be fed occasionally, but also to live around (e.g. rats, pigeons and crows), and there is a high prevalence of human pathogens in such wildlife (Benskin et al. 2009; Scheffe 2007).

The intensive growth of the human population results in extending urban areas to huge metropolises, great agglomerations, megacities and supercities (megalopolises). These expanding areas reduce the natural habitat of wildlife, which in many cases adopt their lifestyle to the new situations and move close to, or into the cities, where food and settlements can be easily found. In addition, an increased trend in the development of "green cities" and an extensive evolution of "blue-green" solutions for urban run-off also open various options for habitats of different animals in the cities. For instance, the re-opening of watercourses that previously run in pipes is not only an important strategy for meeting challenges of changing climate, but it offers an attractive landscape element for the city population. These areas also attract wildlife and create new inner-city ecosystems. Consequently, a large variation in urban wildlife can be found and contribute to faecal water contamination. This wildlife is represented mostly by different species of birds (e.g. gulls, pigeons, crows, rooks, ravens), in particular waterfowl (swans, geese and ducks), and wild mammals (e.g. raccoons, foxes, rats, beavers and bats).

Apart from the habitats of wildlife in cities, there are also an increasing number of pets, particularly dogs and cats, in proportion to the city's population. Dogs, in particular, are the significant hosts for pathogenic microbes, e.g. *Giardia* spp. and *Salmonella* spp. (Schueler 2000). Furthermore, it becomes quite popular to

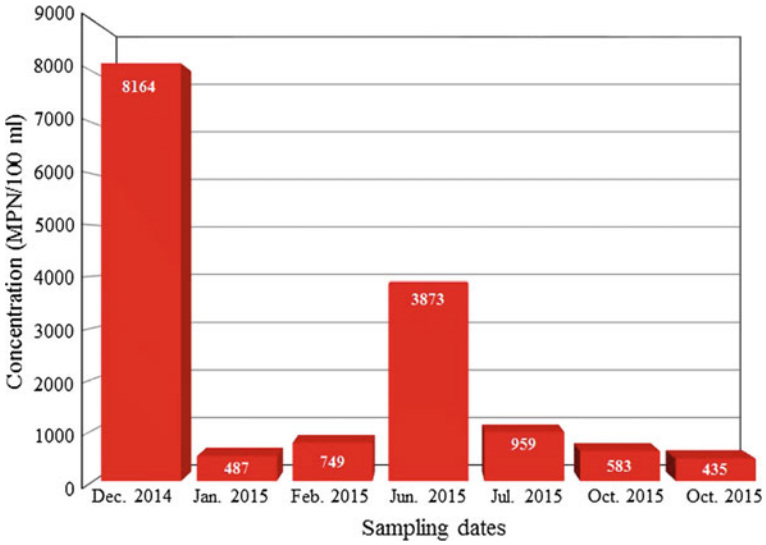

Fig. 10.2 Concentrations of *E. coli* bacteria expressed as the most probable number (MPN)/100 ml of water samples collected from Aker River, Oslo, Norway *Source* Authors

establish "educational" or "hobby" farms with livestock to be presented year-round for city people. Another trendy wave that comes to the cities is to organise riding clubs/centres. It is therefore quite common that cattle and horses grazing pastures become natural elements and landscapes of the urban catchments.

The zoogenic contribution to faecal water contamination in urban areas can be dominant in some periods, as reported by recent Norwegian MST studies (Paruch et al. 2017). One of the studies was conducted on water samples from Akerselva (Aker River) that flows through Oslo, the capital and the most populated city in Norway. Although the samples were collected at irregular intervals (from December 2014 to October 2015), all of them were faecally polluted (Fig. 10.2). The highest *E. coli* concentration was observed at the same occasion as the human marker revealed its dominance (96%) in faecal water contamination (Fig. 10.3). Anthropogenic origin of this contamination was detected in all water samples, but most samples had dominant zoogenic origin, with highest genetic marker contribution of 97% to faecal water contamination. Another study was performed on water samples from Blåveisbekken, a stream which in large part flows in a culvert in Ski town located approximately 20 km south-east of Oslo. All the samples collected during the course of nearly two years (from November 2014 to September 2016) revealed faecal contamination (Fig. 10.4). Almost all the highest *E. coli* concentrations represented anthropogenic origin, while zoogenic origin was dominant in only one-third of the samples (Fig. 10.5).

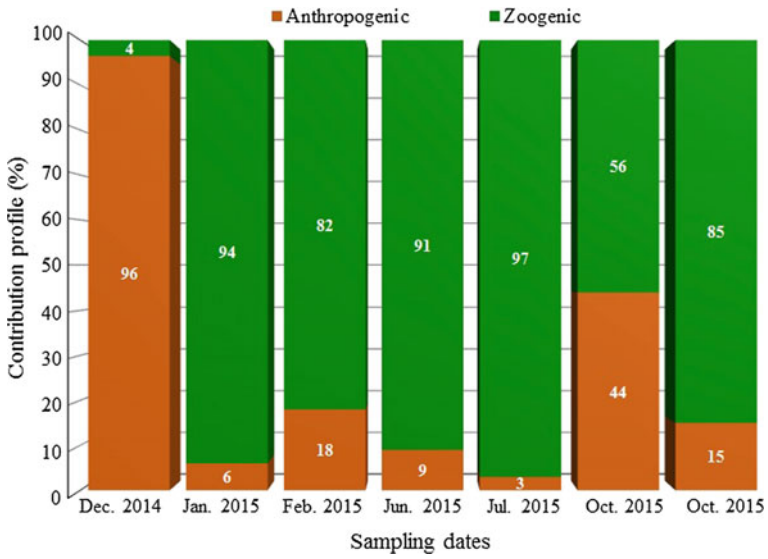

Fig. 10.3 Contribution profile of genetic markers in faecal contamination of water samples collected from Aker River, Oslo, Norway *Source* Authors

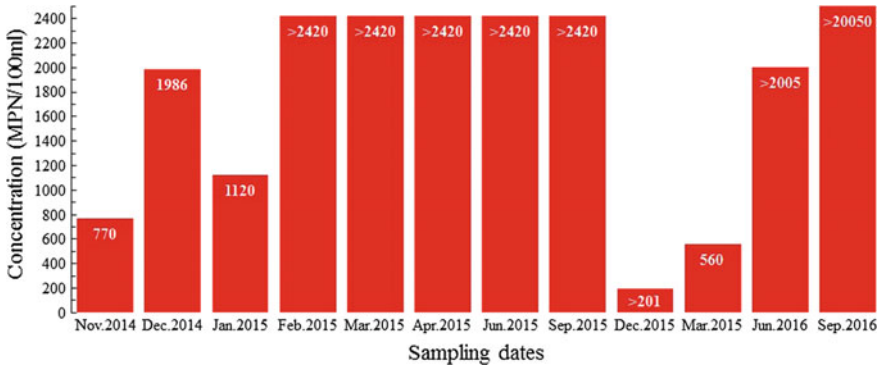

Fig. 10.4 Concentrations of E. coli bacteria expressed as the most probable number (MPN)/100 ml of water samples collected from Blåveisbekken stream, Ski, Norway *Source* Authors

Results from the Norwegian MST studies exhibited two general trends in dominance of contributors to faecal water contamination in urban environments. In the cold season, particularly in autumn, winter and spring, the observed tendency shows higher faecal contributions from anthropogenic sources. While during warm periods of the year, the tendency shifts to dominance of zoogenic sources in faecal water contamination.

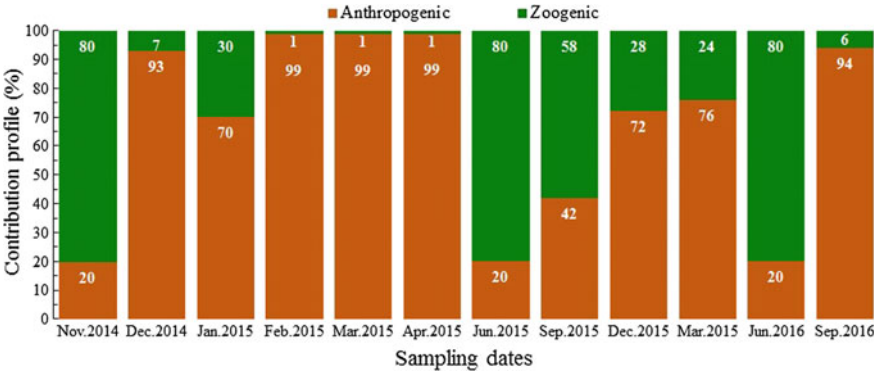

Fig. 10.5 Contribution profile of genetic markers in faecal contamination of water samples collected from Blåveisbekken stream, Ski, Norway *Source* Authors

References

Aftenposten (2013) Opp mot en million rotter i Oslo. https://www.aftenposten.no/osloby/i/212oy/Opp-mot-en-million-rotter-i-Oslo. Accessed 6 Aug 2017

Ahmed W, Sritharan T, Palmer A, Sidhu JP, Toze S (2013) Evaluation of bovine feces-associated microbial source tracking markers and their correlations with fecal indicators and zoonotic pathogens in a Brisbane, Australia, reservoir. Appl Environ Microbiol 79(8):2682–2691

Åström J, Pettersson TJ, Reischer GH, Norberg T, Hermansson M (2015) Incorporating expert judgments in utility evaluation of *Bacteroidales* qPCR assays for microbial source tracking in a drinking water source. Environ Sci Technol 49(3):1311–1318

Bambic DG, Kildare-Hann BJ, Rajal VB, Sturm BS, Minton CB, Schriewer A, Wuertz S (2015) Spatial and hydrologic variation of *Bacteroidales*, adenovirus and enterovirus in a semi-arid wastewater effluent-impacted watershed. Water Res 15(75):83–94

Benskin CMcWH, Wilson K, Jones K, Hartley IR (2009) Bacterial pathogens in wild birds: a review of the frequency and effects of infection. Biol Rev 84:349–373. https://doi.org/10.1111/j.1469-185X.2008.00076.x

Böhm ME, Huptas C, Krey VM, Scherer S (2015) Massive horizontal gene transfer, strictly vertical inheritance and ancient duplications differentially shape the evolution of *Bacillus cereus* enterotoxin operons *hbl*, *cytK* and *nhe*. BMC Evol Biol 10(15):246. https://doi.org/10.1186/s12862-015-0529-4

Bolin C, Brown C, Rose J (2004) Emerging zoonotic diseases and water. In: Cotruvo J, Dufour A, Rees G, Bartram J, Carr R, Cliver DO, Craun GF, Fayer R, Gannonp VPJ (eds) Waterborne zoonoses: identification, causes, and control. WHO, London, UK, pp 21–26

Carson CA, Shear BL, Ellersieck MR, Schnell JD (2003) Comparison of ribotyping and repetitive extragenic palindromic-PCR for identification of fecal *Escherichia coli* from humans and animals. Appl Environ Microbiol 69:1836–1839

Dick LK, Bernhard AE, Brodeur TJ, Santo Domingo JW, Simpson JM, Walters SP, Field KG (2005) Host distributions of uncultivated fecal *Bacteroidales* bacteria reveal genetic markers for fecal source identification. Appl Environ Microbiol 71(6):3184–3191

DN (2016) Dagens Nyheter, Råttinvasion i Stockholm—och de föredrar Östermalm. http://www.dn.se/sthlm/rattinvasion-i-stockholm-och-de-foredrar-ostermalm/. Accessed 21 Sept 2016

Duran M, Yurtsever D, Dunaev T (2009) Choice of indicator organism and library size considerations for phenotypic microbial source tracking by FAME profiling. Water Sci Technol 60(10):2659–2668

Edberg SC, Rice EW, Karlin RJ, Allen MJ (2000) Escherichia coli: the best biological drinking water indicator for public health protection. Symp Ser Soc Appl Microbiol 29:106S–116S

Farnleitner AH, Ryzinska-Paier G, Reischer GH, Burtscher MM, Knetsch S, Kirschner AKT, Dirnböck T, Kuschnig G, Mach LR, Sommer R (2010) *Escherichia coli* and enterococci are sensitive and reliable indicators for human, livestock and wildlife faecal pollution in alpine mountainous water resources. J Appl Microbiol 109:1599–1608

Fettvett (2016) Rotterace i avløpsnettet. http://fettvett.no/rotterace.html. Accessed 6 Aug 2017

Field KG (2004) Faecal source identification. In: Cotruvo J, Dufour A, Rees G, Bartram J, Carr R, Cliver DO, Craun GF, Fayer R, Gannonp VPJ (eds) Waterborne zoonoses: identification, causes, and control. WHO, London, UK, pp 349–366

Field KG, Samadpour M (2007) Fecal source tracking, the indicator paradigm, and managing water quality. Water Res 41:3517–3538

Gerardi MH (2006) Wastewater bacteria. John Wiley and Sons Inc, Hoboken, NJ, USA

Guenther S, Wuttke J, Bethe A, Vojtěch J, Schaufler K, Semmler T, Ulrich RG, Wieler LH, Ewers C (2013) Is fecal carriage of extended-spectrum-β-lactamase-producing *Escherichia coli* in urban rats a risk for public health? Antimicrob Agents Chemother 57(5):2424–2425. https://doi.org/10.1128/AAC.02321-12

Hagedorn C, Harwood VJ, Blanch A (2011) Microbial source tracking: methods, applications and case studies. Springer, New York

Harrault L, Jarde E, Jeanneau L, Petitjean P (2014) Development of the analysis of fecal stanols in the oyster *Crassostrea gigas* and identification of fecal contamination in shellfish harvesting areas. Lipids 49(6):597–607

Hartel PG, Rodgers K, Moody GL, Hemmings SN, Fisher JA, McDonald JL (2008) Combining targeted sampling and fluorometry to identify human fecal contamination in a freshwater creek. J Water Health 6(1):105–116

Harwood VJ, Staley C, Badgley BD, Borges K, Korajkic A (2014) Microbial source tracking markers for detection of fecal contamination in environmental waters: relationships between pathogens and human health outcomes. FEMS Microbiol Rev 38:1–40

IWFA (2017) Institute Water for Africa, Water and health. https://www.water-for-africa.org/en/health.html. Accessed 26 June 2017

Kanarat S (2004) What are the criteria for determining whether a disease is zoonotic and water related? In: Cotruvo J, Dufour A, Rees G, Bartram J, Carr R, Cliver DO, Craun GF, Fayer R, Gannonp VPJ (eds) Waterborne zoonoses: identification, causes, and control. WHO, London, UK, pp 136–150

Khatib LA, Tsai YL, Olson BH (2003) A biomarker for the identification of swine fecal pollution in water using the STII toxin gene from enterotoxigenic *Escherichia coli*. Appl Microbiol Biotechnol 63(2):231–238

Kobayashi A, Sano D, Okabe S (2013) Effects of temperature and predator on the persistence of host-specific Bacteroides-Prevotella genetic markers in water. Water Sci Technol 67(4):838–845

Lamendella R, Santo Domingo JW, Yannarell AC, Ghosh S, Di Giovanni G, Mackie RI, Oerther DB (2009) Evaluation of swine-specific PCR assays used for fecal source tracking and analysis of molecular diversity of swine-specific "*Bacteroidales*" populations. Appl Environ Microbiol 75:5787–5796

Layton A, McKay L, Williams D, Garrett V, Gentry R, Sayler G (2006) Development of Bacteroides 16S rRNA gene TaqMan-based real-time PCR assays for estimation of total, human, and bovine fecal pollution in water. Appl Environ Microbiol 72(6):4214–4224

Marotz CA, Zarrinpar A (2016) Treating obesity and metabolic syndrome with fecal microbiota transplantation. Yale J Biol Med 89(3):383–388

McQuaig S, Griffith J, Harwood VJ (2012) Association of fecal indicator bacteria with human viruses and microbial source tracking markers at coastal beaches impacted by nonpoint source pollution. Appl Environ Microbiol 78(18):6423–6432

Mieszkin S, Caprais MP, Le Mennec C, Le Goff M, Edge TA, Gourmelon M (2013) Identification of the origin of faecal contamination in estuarine oysters using *Bacteroidales* and F-specific RNA bacteriophage markers. J Appl Microbiol 115(3):897–907

Moe CL (2004) What are the criteria for determining whether a disease is zoonotic and water related? In: Cotruvo J, Dufour A, Rees G, Bartram J, Carr R, Cliver DO, Craun GF, Fayer R, Gannonp VPJ (eds) Waterborne zoonoses: identification, causes, and control. WHO, London, UK, pp 27–45

Moyo SJ, Maselle SY, Matee MI, Langeland N, Mylvaganam H (2007) Identification of diarrheagenic *Escherichia coli* isolated from infants and children in Dar es Salaam, Tanzania. BMC Infect Dis 9(7):92. https://doi.org/10.1186/1471-2334-7-92

Olivas Y, Faulkner BR (2008) Fecal source tracking by antibiotic resistance analysis on a watershed exhibiting low resistance. Environ Monit Assess 139:15–25

Paruch AM, Mæhlum T (2012) Specific features of *Escherichia coli* that distinguish it from coliform and thermotolerant coliform bacteria and define it as the most accurate indicator of faecal contamination in the environment. Ecol Indic 23:140–142

Paruch L, Paruch AM (2017) The importance of melting curve analysis in discriminating faecal and environmental *Bacteroidales* bacteria. Microbiol 86(4):536–538. https://doi.org/10.1134/S0026261717040117

Paruch AM, Mæhlum T, Robertson L (2015a) Changes in microbial quality of irrigation water under different weather conditions in Southeast Norway. Environ Process 2:115–124. https://doi.org/10.1007/s40710-014-0054-2

Paruch L, Paruch AM, Blankenberg A-GB, Bechmann M, Mæhlum T (2015b) Application of host-specific genetic markers for microbial source tracking of faecal water contamination in an agricultural catchment. Acta Agric Scand 65(S2):164–172

Paruch AM, Paruch L, Mæhlum T (2017) Kildesporing av fekal vannforurensing med molekylærbiologiske metoder—Eksempler på undersøkelser i Norge (Source tracking of fecal water contamination by molecular biology methods—Examples of surveys in Norway). NIBIO Rapport 3/66, Aas, Norway

Pond K (2005) Water recreation and disease: plausibility of associated infections, acute effects, sequelae and mortality. WHO/IWA, London

Quigley EM (2013) Gut bacteria in health and disease. Gastroenterol Hepatol (NY) 9(9):560–569

Reischer GH, Kasper DC, Steinborn R, Farnleitner AH, Mach RL (2007) A quantitative real-time PCR assay for the highly sensitive and specific detection of human faecal influence in spring water from a large alpine catchment area. Lett Appl Microbiol 44(4):351–356

Scheffe L (2007) Reducing risk of *E. coli* O157: H7 contamination. Nutrient Management Technical Note No 7. USDA, NRCS, Washington, DC, USA

Schueler TR (2000) Microbes and urban watersheds: concentrations, sources, and pathways. In: Schueler TR, Holland HK (eds) The practice of watershed protection. Center for Watershed Protection, Ellicott City, Md, pp 68–78

Scott TM, Jenkins TM, Lukasik J, Rose JB (2005) Potential use of a host associated molecular marker in *Enterococcus faecium* as an index of human pollution. Environ Sci Technol 39(1):283–287

Shahryari A, Nikaeen M, Khiadani Hajian M, Nabavi F, Hatamzadeh M, Hassanzadeh A (2014) Applicability of universal *Bacteroidales* genetic marker for microbial monitoring of drinking water sources in comparison to conventional indicators. Environ Monit Assess 186(11):7055–7062. https://doi.org/10.1007/s10661-014-3910-7

Shanks OC, Atikovic E, Blackwood AD, Lu J, Noble RT, Santo Domingo J, Seifring S, Sivaganesan M, Huagland RA (2008) Quantitative PCR for detection and enumeration of genetic markers of bovine fecal pollution. Appl Environ Microbiol 74(3):745–752

Smith A, Sterba-Boatwright B, Mott J (2010) Novel application of a statistical technique, random forests in a bacterial source tracking study. Water Res 44(14):4067–4076

Sowah RA, Habteselassie MY, Radcliffe DE, Bauske E, Risse M (2017) Isolating the impact of septic systems on fecal pollution in streams of suburban watersheds in Georgia, United States. Water Res 108:330–338

Staley ZR, Grabuski J, Sverko E, Edge TA (2016) Comparison of microbial and chemical source tracking markers to identify fecal contamination sources in Humber River (Toronto, Ontario, Canada) and associated storm water outfalls. Appl Environ Microbiol 82(21):6357–6366

Suresh K, Smith HV (2004) Tropical organisms in Asia/Africa/South America. In: Cotruvo J, Dufour A, Rees G, Bartram J, Carr R, Cliver DO, Craun GF, Fayer R, Gannonp VPJ (eds) Waterborne zoonoses: identification, causes, and control. WHO, London, UK, pp 93–112

Tambalo DD, Fremaux B, Boa T, Yost CK (2012) Persistence of host-associated *Bacteroidales* gene markers and their quantitative detection in an urban and agricultural mixed prairie watershed. Water Res 46(9):2891–2904

Tran NH, Gin KY, Ngo HH (2015) Fecal pollution source tracking toolbox for identification, evaluation and characterization of fecal contamination in receiving urban surface waters and groundwater. Sci Total Environ 15(538):38–57

USEPA (2005) United States Environmental Protection Agency, Microbial source tracking guide document. Office of Research and Development, EPA-600/R-05/064, Washington, DC

Venegas C, Diez H, Blanch AR, Jofre J, Campos C (2015) Microbial source markers assessment in the Bogota River basin (Colombia). J Water Health 13(3):801–810

WHO (2006) World Health Organization, Guidelines for the safe use of wastewater, excreta and greywater. In: Wastewater and excreta use in aquaculture, vol 3. WHO Press, Geneva, Switzerland

WHO (2011) World Health Organization, Guidelines for drinking-water quality, 4th edn. WHO Press, Geneva, Switzerland

WHO (2017) World Health Organization, Mortality and burden of disease from water and sanitation. http://www.who.int/gho/phe/water_sanitation/burden/en/index2.html. Accessed 26 June 2017