GLOBAL WATER PATHOGEN PROJECT

**PART THREE. SPECIFIC EXCRETED PATHOGENS: ENVIRONMENTAL AND
EPIDEMIOLOGY ASPECTS**

BALANTIDIUM COLI

Francisco Ponce-Gordo

Complutense University

Madrid, Spain

Kateřina Jirků-Pomajbíková

Institute of Parasitology Biology Centre, ASCR, v.v.i.

Budweis, Czech Republic

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Summary

To date, *Balantidium coli* is the only ciliate protozoan reported to infect the gastrointestinal track of humans. During its life cycle, two stages exist: the active trophozoite stage which inhabits the hindgut, and the resting/resistant cyst stage which is shed with the feces and is the transmissible stage to new hosts. The parasite can infect a wide range of mammals, pigs being the main reservoir. The organism has also been found in some birds.

In most cases, *B. coli* is not considered a public health problem because infections are usually asymptomatic; however, in some (still not clearly determined) circumstances the parasite could invade the intestinal mucosa causing a disease known as balantidial dysentery (balantidiasis) which could be fatal. Transmission from one infected individual to others is by a fecal-oral route in which contaminated drinking water or food are the main modes of transmission. Close contact with pigs and poor condition of the water sources are the main risk factors associated with the infection by the parasite.

Control measures includes the identification and treatment of infected people, the safe disposal of excreta (both of human and animal origin), avoiding the use of night soil as fertilizer for vegetables to be consumed raw, and the improvement in the quality of the water sources. There have not been any specific studies about *B. coli* cyst survival time in the environment or about specific inactivation processes during wastewater or drinking water treatment.

The protozoan ciliates of the genus *Balantidium* are transmitted by a fecal-oral route in which cysts are the infective stage. *Balantidium* cysts can be present in temperate and tropical surface waters worldwide. Among the species in this genus, there is only one infecting humans, *Balantidium coli*, and no subspecies, serotypes, or genetically diverse types have been named to date. The main reservoir of this species is swine. Human infections are related worldwide in relation to low sanitation or to contamination of drinking water sources with human and animal (especially swine) feces. Worldwide prevalence is low, estimated less than 1%, with most cases reported in a few endemic areas in which prevalence could rise up to 30%. Most infections are asymptomatic, but the organism could in some cases produce a severe infection and even the death of the host, usually associated with other concurrent illness. Waterborne transmission has been documented in some cases

1 Epidemiology of the disease and pathogen

1.1 Global burden of disease

1.1.1 Global distribution

Balantidium coli is reported worldwide although it is more common in temperate and tropical regions (Areán and Koppisch, 1956). The Philippines, Papua New Guinea, some Pacific Islands, some regions of Central and South America and central Asia are considered endemic areas (Fletcher et al., 2012; Kline et al., 2013; Owen, 2005; Schuster and Ramirez-Avila, 2008; Zaman, 1978).

In humans, the overall prevalence is estimated to be 0.02 to 1% (Areán and Koppisch, 1956; Boonjaraspinyo et al., 2013; Schuster and Ramirez-Avila, 2008; Walzer and Healy, 1982). It is possible that these numbers are an underestimation of the real situation as the parasite is considered usually asymptomatic and most reports and statistics are based on data from symptomatic or compromised patients (Cooper and Guderian, 1994; McCarey, 1952; Nuti et al., 1979; Walzer et al., 1973). In endemic areas of South America the prevalence has been reported to be 1 to 12% (Areán and Koppisch, 1956; Devera et al., 1999; Esteban et al., 1998), and even near 30% in Oceania (Kline et al., 2013; Walzer and Healy, 1982).

1.1.2 Symptomatology

It remains undetermined whether *B. coli* alone is pathogenic. Trophozoites inhabit the intestine, feeding on bacteria and other intestinal contents. In most cases, infections are asymptomatic and the infected host shows no clinical signs, suggesting that this ciliate is an opportunistic parasite that could take advantage of the weakened status of the host caused by other infections, lesions, or diseases. In such cases, the parasite could invade the intestinal wall causing the disease known as balantidiasis or balantidial dysentery. In the chronic form of the disease, the symptoms varied from unspecific abdominal disorders (diarrhea, abdominal pain) to cramping rectal pain, nausea and vomiting, whereas in the acute form these symptoms can be accompanied by mucus and blood in feces, and in severe cases, hemorrhages and perforation could occur resulting in parasite dissemination to other tissues or even in the death of the host (Areán and Koppisch, 1956; Neafie et al., 2011; Zaman, 1978). In most of the human patients with extraintestinal balantidiasis, a concurrent intestinal infection was usually not diagnosed (Anargyrou et al., 2003; Dhawan et al., 2013; Sharma and Harding, 2003; Vasilakopoulou et al., 2003) but a common characteristic was that they suffered from another illness such as diabetes, hepatic disorders, pulmonary and renal disorders, HIV infection or cancer (e.g. Anargyrou et al., 2003; Clyti et al., 1998; Ferry et al., 2004; Karuna and Khadanga, 2014; Sharma and Harding, 2003; Vasilakopoulou et al., 2003). In the last few years, several cases have described the presence of *B. coli* in urine (Maino et al., 2010; Bandyopadhyay et al., 2013; Karuna and Khadanga, 2014; Khanduri et al., 2014), which stresses the importance of the examination of the urinary specimens.

The economic importance of balantidiasis is not well determined. In different studies it has been suggested that *B. coli* infections in animals could affect the development of

the offspring directly (by causing mortality or reducing the weight gain; (Bauri et al., 2012; Bilic and Bilkei, 2006) or indirectly (i.e., by affecting the milk composition in the mother; Hinde, 2007). There are no data regarding the economic status of balantidiasis in humans.

1.2 Taxonomic classification of the agent

1.2.1 Taxonomy

Balantidium coli, the first intestinal protozoan formally identified in humans, is the largest protozoan inhabiting the human intestine and the only ciliate known to infect humans. The first formal description of this organism was reported as *Paramecium coli* by Malmsten in 1857 (Malmsten, 1857) but soon afterwards it was renamed as *Balantidium coli* by Stein in 1863 (Stein, 1863). Some proposals to change this name were made during the 20th century but they were not accepted until recently. Based on genetic analysis, Pomajbíková et al. (2013) (Pomajbíková et al., 2013) proposed to reclassify the human species in a new genus, as *Neobalantidium coli* for the homeothermic clade. Chistyakova et al. (2014) (Chistyakova et al., 2014) considered this name as a junior synonym of *Balantioides coli*, which was proposed by Alexeief (1931) (Alexeief, 1931). Because the nomenclature is under revision and to avoid confusion for the readers, we use the name that remains accepted worldwide and is commonly used in the scientific and medical literature - *Balantidium coli*.

The parasite has been described in a large number of host species (see "Reservoirs"). Based on morphological differences of specimens gathered from different host species, or from results of cross-infection studies, some researchers proposed that isolates from pigs, guinea pigs, camels and several non-human primates were different species (e.g., (Hegner, 1934; McDonald, 1922). Such morphological differences could be related to differences in growth conditions (Levine 1961); this nutrient-related variability has been also observed in *in vitro* cultures (Barbosa et al., 2015; Levine, 1940). Failures by some authors in cross-infections studies (i.e., see Awakian, 1937; Schumaker, 1930) have also led to the implication that an isolate was host specific and therefore a separate species.

But most of these species have been considered synonyms of *B. coli* (Levine, 1961; Levine, 1985). However, *Balantidium suis* from pigs and *Balantidium caviae* from the guinea pig have infrequently been used in publications until recently (Schuster and Ramirez-Avila, 2008). Recent genetic analysis with isolates from pigs, gorillas, and humans (Ponce-Gordo et al., 2008; Ponce-Gordo et al., 2011) have shown some genetic variability within the species, but this is not related to the origin of the isolates from a particular host species; in fact, these results and others obtained with isolates from different mammalian hosts including elk, African buffalo, several suid species (i.e., domestic pig, red river hog and South American tapir) and non-human primates (Pomajbíková et al., 2013; Ponce-Gordo et al., unpublished) and from ostriches and rheas (Ponce-Gordo et al., 2008; Ponce-Gordo et al., 2011; Ponce-Gordo et al., unpublished) indicate that only one species infects warm-blooded animals (mammals and birds), *Balantidium coli*.

1.2.2 Physical description (morphology)

Balantidium coli has two stages in its life cycle: the active, feeding, replicating stage (the trophozoite) that is found most commonly in the lumen of the large intestine, and the nonreplicating encysted stage (the cyst) that develops in the lower colon and is excreted in the feces. Trophozoites (Figure 1) are large (usually 100-150 µm in length; the size range varies somewhat according to different authors) and ovoid, with the entire cell surface covered by cilia. A mouth-like opening, the cytostome, is located in the anterior part of the cell. There is an elongate or kidney-shaped macronucleus usually located in the posterior part of the cell and a smaller, round micronucleus which usually overlaps the macronucleus and is not easily observable. Some vacuoles can also be seen in the cytoplasm. The cysts (Figure 2) are also large (40-60 µm in diameter); they are round and contain a single cell surrounded by a thick cyst wall. In stained cysts the macronucleus and some vacuoles can be easily identified and, occasionally, cilia can be seen.

Figure 1 *Balantidium coli* trophozoite from a pig sample, unstained. Scale bar: 50 μ m.

Figure 2 *Balantidium coli* cysts from a pig sample. Scale bar: 50 μ m. Left: Unstained cyst. Right: Cyst stained with Lugol's iodine; an egg of *Trichuris suis* also shown for comparison purposes.

1.3 Transmission

1.3.1 Routes of transmission

Balantidium cysts are found in the feces of infected individuals. Balantidiasis is considered a waterborne and foodborne disease. The parasite is transmitted by the fecal-oral route, with the cysts, and possibly but less likely the trophozoites, being ingested with fecally-contaminated water and food. Transmission by coprophagia could occur in animals, and this possibility has been suspected for some human infections, especially in asylums, orphanages, prisons and mental institutions (Areán and Koppisch, 1956; Giacometti et al., 1997; Schuster and Ramirez-Avila, 2008).

It has been considered that the trophozoites of *B. coli* cannot survive passage through the stomach (Schuster and Visvesvara, 2004) and the cysts were the only infective stage (Schuster and Ramirez-Avila, 2008). However, recent studies on captive great apes infected with *B. coli* suggest that trophozoites might also be infective (Pomajbíková et al., 2010). This possibility should be further investigated; if confirmed, trophozoites would likely need to be ingested within a few hours after excretion of the stools as their survival in the environment is limited (Areán and Koppisch, 1956), whereas cysts can survive within the environment for ten days at room temperature (Rees, 1927) and for several weeks in pig feces, particularly if kept moist and away from direct sunlight (Schuster and Visvesvara, 2004). The cyst wall provides protection from stomach acid.

Outbreaks of balantidiasis are rare. Apart from two reports in mental institutions (Areán and Koppisch, 1956; Giacometti et al., 1997), only two other outbreaks were reported from southern Persia (McCarey, 1952) and from

the Pacific island of Truk (Walzer et al., 1973; Walzer and Healy, 1982). In Persia, 87 cases were thought to be caused by human-to-human transmission among Muslims in an oil refinery town, whereas the outbreak in Truk affected 110 persons after a devastating typhoon that caused widespread contamination of water supplies with pig feces.

As a waterborne parasite, *B. coli* could be transmitted by drinking water but also by recreational water. In countries with high sanitation standards, transmission is mainly due to an occasional contamination (i.e., in swimming pools) or a process failure within water utilities (Bellanger et al., 2013), while in the developing world it could be the consequence of inadequate or insufficient sewage treatment and water supply systems that can be affected by adverse environmental conditions (e.g., the typhoon in Truk) (Plutzer and Karanis, 2016; Thompson and Smith, 2011).

1.3.2 Reservoirs

Balantidiasis is a zoonotic disease. The parasite has been described in a wide range of mammalian hosts other than humans, including non-human primates, pigs and wild boars, cattle, sheep, goats, camels, equids and rodents (Hegner, 1934; Levine, 1961; Levine 1985); *Balantidium* infecting ostriches and rheas might also be *B. coli* (Ponce-Gordo et al., 2008; Ponce-Gordo et al., 2011). On the other hand, *Balantidium* species from poikilothermic hosts have been erroneously reported as *B. coli* and their hosts (i.e., turtle, cockroaches) have been incorrectly listed as suitable hosts of *B. coli* (i.e., Areán and Koppisch, 1956; Neafie et al., 2011).

The most important reservoirs are the domestic pig and

wild boars. Other hosts potentially important in the transmission of *B. coli* to humans are rodents, wild boars, sheep, goats, camels and horses. Domestic pigs are infected worldwide with *B. coli*, with prevalence ranging from 50 to 100% of the animals examined (Hindsbo et al., 2000; Ismail et al., 2010; Schuster and Ramirez-Avila, 2008; Weng et al., 2005; Yatswako et al., 2007; Yin et al., 2015). In pigs, these ciliates are non-invasive and non-pathogenic (Schuster and Visvesvara, 2004). Among the studies, prevalence and the intensity of infection varied between gender and age groups and in some cases opposite results were found between the studies, but probably the main factors affecting infection rates were the management practices at each farm and the veterinary care (Schuster and Ramirez-Avila, 2008). In general, facilities with cement floors, adequate cleaning protocols, and sewage disposal reduced the prevalence among pigs (Ismail et al., 2010; Giarratana et al., 2012; Weng et al., 2005; Yatswako et al., 2007;).

There are very few studies on the prevalence of *B. coli* in wild boars and feral pigs. The data available indicate that prevalences are lower than in domestic pigs, ranging from 4 to 70% depending on the study (Navarro-Gonzalez et al., 2013; Solaymani-Mohammadi et al., 2004). Other suids such as the Central American tapir has been found infected with *B. coli* (Cruz Aldan et al., 2006), but its epidemiological importance in relation to the transmission of *B. coli* to humans is unknown.

The epidemiology of *B. coli* in some Muslim countries has not been clearly determined, but reservoirs other than pigs have been proposed to be of local importance. Although pig farming is forbidden in Muslim countries, human balantidiasis exists in some regions, such as Iran (Solaymani-Mohammadi et al., 2005a; WHO 1979). It has been proposed that wild boars might act as the main reservoirs (Solaymani-Mohammadi et al., 2005a; Solaymani-Mohammadi et al., 2005b); other authors have suggested camels (Cox 2005), donkeys (Khan and Fallon 2013), and sheep and goats (Jamil et al., 2015). In Iran and neighboring countries, the parasite has been found in cattle and buffaloes (Mirzaei and Khovand 2015; Samad, 2011; Tarrar et al. 2008) but most of the reports have been based on the cyst stage, which is indistinguishable from the cattle ciliate *Buxtonella sulcata* (Sultan et al., 2013) so the importance of ruminants (cattle and buffaloes) as reservoirs of *B. coli* should be considered with caution.

Balantidium coli has been found in several rodent species. Rats have been considered as a possible source of infection of *B. coli* for humans and as a source of spreading the cysts in the environment (Awakian, 1937; Bogdanovich, 1955; Schumaker, 1930). Other rodents, such as the lowland paca (*Cuniculus paca*) which is raised as a source of proteins in some Central American countries, can harbor this ciliate Matamoros et al. (Matamoros et al., 1991) gave a prevalence of 5.7% in farmed pacas in Costa Rica and may play a role in the transmission of the parasite to humans in these regions.

1.3.3 Incubation period

There are no data on prepatent and incubation periods

of *B. coli* in humans. According to experimental infections in piglets and non-human primates, the incubation period ranges from 3 to 6 days (Yang et al., 1995).

1.3.4 Period of communicability

The infectious dose or dose-response for trophozoites or cysts is not known. The disease is communicable (infectious) for as long as organisms are excreted, but it should be noted that in chronic balantidiasis the parasite is identified in the stools only sporadically (Areán and Koppisch, 1956). Cysts can survive much longer (weeks) outside the host body whereas trophozoites die at a faster rate (hours) (Areán and Koppisch 1956; Schuster and Visvesvara, 2004). This information is important for understanding human-to-human transmission, because cysts are rarely detected in human feces and trophozoites are often found in cases of dysentery (Areán and Koppisch, 1956; Kennedy and Stewart, 1957).

1.3.5 Population susceptibility

Persons working with animals appear at greatest risk of acquiring balantidiasis, especially those in contact with pigs (the main reservoir host of *B. coli*). Pig-to-human transmission was recorded in areas where humans live in close proximity with domestic pigs and even share habitation (Ferry et al., 2004; Sharma and Harding, 2003). In most cases, people at greatest risk live in poor hygienic conditions (Barnish and Ashford, 1989; Esteban et al., 1998; Owen, 2005). Others at risk work in abattoirs where pig intestines are handled; farmers working with pig feces; zookeepers working with apes carrying *B. coli* infections, or are veterinarians or veterinary students (Anargyrou et al., 2003; Schuster and Visvesvara, 2004). In regions where pigs are not reared such as Muslim countries, the contact with other reservoirs such as wild boars, camels, small ungulates or equids could be the source of infection. In all cases, a poor hygienic environment or poor personal hygiene (see below - hygiene measures) would facilitate both the animal-to-human and the human-to-human transmission.

Another important factor is the health status of the person, which is of paramount importance for the onset of the infection and of the disease. This includes the physical condition, concurrent pathologies, immunological status and immunodepression (Anargyrou et al., 2003; Maino et al., 2010; Vasilakopoulou et al., 2003; Yazar et al., 2004;) and diet (nutritional status) of each person (Schovancová et al., 2013). *Balantidium coli* is usually detected in persons with other infectious agents (other protozoa, helminths, bacteria, viruses), with systemic diseases, or suffering malnourishment. All of these conditions complicate the medical management and the prognosis (Walzer and Healy, 1982). Although there is no conclusive evidence that these other infections render the host more susceptible to *B. coli* (Schuster and Ramirez-Avila 2008), the extraintestinal locations of the parasite are usually associated with other concurrent illness (e.g., Anargyrou et al., 2003; Clyti et al., 1998; Ferry et al., 2004; Karuna and Khadanga, 2014; Khanduri et al., 2014; Sharma and Harding, 2003;

Vasilakopoulou et al., 2003). Other factors that might facilitate balantidiasis include the virulence of *B. coli* subpopulations (still not confirmed) and the number of infecting forms ingested (Schuster and Ramirez-Avila, 2008).

1.4 Population and individual control measures

The populations at greatest risk are people living in close contact with reservoir hosts (as in many rural areas), people living in poor sanitation environments with limited, unconditioned, or absent adequate infrastructures for water supply and sewage disposal; people with poor hygienic habits, those at low socio-economical levels, and those residents and workers in institutions (as asylums, orphanages, prisons and mental institutions) where hygienic supervision is inadequate (Schuster and Ramirez-Avila, 2008; Yatswako et al., 2007). Human infection is rare in temperate areas and in industrialised countries (Ferry et al., 2004). In France a clinical case of balantidiasis in a not immunocompromised patient, who lived alone, with no history of travel or chronic inflammatory bowel disease was recently reported (Bellanger et al., 2013). The patient was not working in contact with animals. He reported having been on a hike the previous weekend during which he had drunk water through a polyurethane hydration pouch bladder. The patient insisted that he did not refill the hydration system during the hike. In the authors' opinion, the persistence of residual water in the patient's hydration pouch bladder was the source of the contamination (Bellanger et al., 2013).

1.4.1 Hygiene measures - hand washing, disinfection

The best means of protection against *B. coli* infection is by providing adequate clean water for drinking and other uses (Schuster and Ramirez-Avila, 2008). Because it is unrealistic to attempt to render reservoir animals (mainly pigs) *B. coli*-free, the best option is to keep animals and their feces away from contaminating water sources used for human consumption.

Chlorine, at concentrations normally used for ensuring water safety, is not effective against cysts of *B. coli* (Schuster and Ramirez-Avila, 2008). Water boiling is acceptable. Individual hygiene measures include washing hands with soap and clean water after being in contact with reservoir hosts or before handling food, and washing fruits and vegetables with clean water.

1.4.2 Drug therapy

Until the 1950s, about one third of persons with severe, acute balantidiasis would die (Areán and Koppisch, 1956). Since then, the use of antibiotics has clearly improved the prognosis and now most fatal cases are associated with another concurrent pathology. *Balantidium coli* infections are easily treated with antibiotic therapy, provided that the correct diagnosis is made in time (Knight, 1978). For humans, treatment consists of tetracycline (500 mg four times daily for 10 days; not recommended for pregnant women or for children under 8 years old), metronidazole

(750 mg three times daily for 5 days) or iodoquinol (640 mg three times daily for 20 days). It is advisable to give the patient a starch-free diet (Areán and Koppisch, 1956; Schovancová et al., 2013). Pigs (the main reservoir of *B. coli*) can be treated with oxytetracycline.

2 Environmental Occurrence and Persistence

2.1 Detection methods

Trophozoites of *B. coli* rapidly disappear outside the host (Areán and Koppisch, 1956) and only the cysts can be found in environmental samples. There are no specific or standardized methods for detection and those applied for other enteric protozoa and helminths can be used with *B. coli*.

In fecal samples, trophozoites can be easily detected by microscopy in saline smears by their size and their slow motion; in fixed samples, the morphology of the macronucleus can be easily recognized in both trophozoites and cysts in temporary smears stained with iodine. Other staining methods such as hematoxylin-eosin or trichrome are also useful. Cysts can be recovered using common coprological techniques (i.e., centrifugation methods for concentration). In samples from cattle and buffaloes, the diagnosis based on the identification of cysts should not be considered confirmatory because other ciliates (namely *B. sulcata*) with morphologically identical cysts could also be present. Complementary genetic analysis should be made in this case. For this purpose, the sequences corresponding to the ribosomal genes (the small subunit rDNA and the 5.8s rDNA, and the internal transcribed spacers -ITS- 1 and 2), although showing some genetic heterogeneity, can be used (Pomajbíková et al., 2013; Ponce-Gordo et al., 2008; Ponce-Gordo et al., 2011).

In environmental samples, the *B. coli* trophozoites would not be detectable (they disappear soon after the passage of the stools; Areán and Koppisch, 1956) and only the cysts would be found. However, it is not possible to differentiate on morphological grounds the cysts of *B. coli* from those of other *Balantidium* species (i.e., amphibian or fish balantidia), or even from those of other ciliates (as *B. sulcata* from cattle), and genetic analysis should be made to correctly identify the species.

2.2 Data on occurrence in the environment

2.2.1 Feces

Only very little data are known about intensity of infection of *B. coli* in pig or human samples, because generally it is difficult to choose an appropriate quantification method for ciliates. The only study by Růžicová et al. (2014) reported a few data on the intensity of infection of *B. coli* during calibration of the diagnostic and quantitative method FLOTAC® on both stages (Růžicová et al., 2014). The cysts were quantified in pig feces, whereas trophozoites in chimpanzees, which similarly as human feces contain trophozoite stages of *B. coli*. Despite it is optimized on chimpanzee feces, it can be

used also for the detection and quantification of human infections.

2.2.2 Sewage and sludge

Few data are available on the presence of *B. coli* cysts in environmental samples, and care should be taken when interpreting the data available to date because they are based on unreliable cyst morphology (see "Detection methods").

The presence of the parasite has been reported in large numbers in sludge from a wastewater processing facility in Bahrain but the origin was uncertain, because prevalence among the residents served by the processing facility was found to be negligible (two positive patients out of over 18,000 samples examined) and pigs were absent (Amin, 1988). In another report, *Balantidium* cysts were found in the sewage water in Bangladesh; the samples were collected from different places and *B. coli* cysts were found in three places although in low intensity ranging between 5 to 7.5×10^4 stages cysts per liter (Khanum et al., 2012).

2.2.3 Drinking waters

The balantidiasis outbreak of Truk followed a typhoon affecting an island with primitive catchment water supplies, low hygiene standards, and high numbers of pigs in close contact with humans. The combination of these factors resulted in the contamination of the water supplies (Walzer et al. 1973). Apart from this outbreak derived from a catastrophic situation, there are no published outbreaks of balantidiasis in relation to water supplies. However, several authors have raised concerns about the risk of epidemics. In Australia, *B. coli* was detected in feral pig populations that were foraging and wallowing in major public drinking water catchments (Hampton et al., 2006). The authors speculated that chances of pathogens reaching the main water supply might be high.

In Hyderabad, India, *B. coli* cysts were detected in 61 of 232 water samples (26.3% positive) collected in hostels, small restaurants, households, and various public places. The source of the contaminated samples was the water stored in overhead tanks and various other containers (Jonnalagadda and Bhat, 1995). In three districts in

Pakistan, *B. coli* cysts were detected in all three sources of drinking water used for examination (tap water, ponds, and drain water) with total prevalence 5.78% (26/450) (Ayaz et al., 2011). A recent case report of a patient suffering from dysentery caused by *B. coli* related to water was reported in France by Bellanger et al. (Bellanger et al., 2013).

2.2.4 Soil

The only information about the occurrence of *B. coli* in the soil is from Nigeria where *B. coli* cysts were found in water and soil samples in two out of the seven pig farming areas (Yatswako et al., 2007). The prevalence of *B. coli* in pig population in the studied area ranged between 35.3 and 75.7 % (207 samples positive for *B. coli* out of 402 collected samples in total).

In southwestern Nigeria, intestinal parasites, including *B. coli*, were detected on selected vegetables from open markets; the contamination was attributed to contaminated soil (Ogbolu et al., 2009). In Brazil several protozoan and helminth species including *B. coli*, were detected on field-grown strawberries although the prevalence was quite low (da Silva et al., 2014).

2.3 Persistence

There are few published data on the survival and viability of *B. coli* cysts in the environment. As mentioned above, the cysts could survive for several weeks in moist feces away from direct sunlight (Schuster and Visvesvara, 2004). Early studies in parasitology suggest that the cysts survive in the environment for at least ten days at room temperature (Rees, 1927), whereas trophic ciliates can survive for as long as ten days in the environment (Zaman, 1978).

3 Reduction by sanitation management

There are no data available about the inactivation or removal of *B. coli* cysts by sanitation treatment processes. Because of their larger size (40-60 μ m in diameter) it is possible that cysts are removed by sedimentation or filtration more so when compared to the removal of smaller protozoan cysts and helminth eggs. This suggests that the cysts will end up in the sludge and would remain an indicator of sanitation effectiveness for solid fecal waste treatment technologies.

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