Review

Zoonotic implications of the swine-transmitted protozoal infections

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Abstract

Pig production is an important part of the economy in many countries. Domestic and wild pigs (Sus scrofa) are susceptible to a wide range of infectious and parasitic diseases. Some of these diseases are specifically limited to pigs while some of the other diseases are shared with other species of wildlife and domestic livestock. As the numbers and geographic distribution of wild and domestic swines continue to increase, it is certain that the number of contacts between these swines and domestic livestock will also increase, as will the probability of human exposure to the parasites of swine directly or indirectly. Here, we will discuss the protozoal infections of pigs, which have the potential to infect humans and provide reasonable risk assessment for zoonotic transmission.

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Keywords: Pig; Wild boar; Epidemiology; Parasite; Protozoa; Zoonosis

Contents

1. Introduction ................................................................. 190
2. Blastocystis sp. ............................................................ 190
  2.1. Infection and disease .............................................. 191
  2.2. Epidemiology ....................................................... 191
  2.3. Diagnostic techniques ............................................ 191
  2.4. Therapy ............................................................. 191
  2.5. Prevention and control .......................................... 192
3. Entamoeba polecki (Von Prowazek, 1912) ....................... 192
  3.1. Infection and disease .............................................. 192
  3.2. Epidemiology ....................................................... 192
  3.3. Diagnostic techniques ............................................ 192
  3.4. Therapy ............................................................. 193
4. Balantidium coli (Malmsten, 1857) ............................... 193
  4.1. Infection and disease .............................................. 193
  4.2. Epidemiology ....................................................... 194

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1. Introduction

For many years and in many countries zoonotic diseases, with their reservoirs in domestic and wild animals, have imposed a health burden, especially among the vast number of people living and working in rural areas. The health and socioeconomic impacts of zoonotic and parasitic diseases and related food-borne diseases are growing continuously and increasingly are being felt most particularly by developing countries. Apart from causing human morbidity and mortality, they hamper agricultural production, decrease availability of food, and create barriers to international trade. The problem of zoonoses has spread from predominantly restricted rural areas into regional and, in some cases, worldwide epidemics. This is due to the great changes of the previous decades, especially the increasing urbanization, most of which is inadequately planned. In addition, large movements of populations, opening up of badly needed new areas for food production, the increasing trade in meat, milk and other products of animal origin, the increasing number and speed of vehicles, and even tourism have contributed to expanding the impact of zoonotic diseases. Pig production is an important part of the economy in many countries. Domestic and wild pigs (*Sus scrofa*) are susceptible to a wide range of infectious and parasitic diseases. Some of these diseases are specifically limited to pigs while some of the other diseases are shared with other species of wildlife and domestic livestock. As the numbers and geographic distribution of wild and domestic swines continue to increase, it is certain that the number of contacts between these swine and domestic livestock will also increase, as will the probability of human exposure to the parasites of swine directly or indirectly. This article will review some of the protozoal infections of medical importance that wild and domestic swine may transmit to humans.

2. *Blastocystis* sp.

*Blastocystis* sp. has a wide variety of animal hosts worldwide. *B. hominis*-like organisms have been found in birds (including chickens and ostriches), non-human primates, domestic pigs, wild boars, horses, rodents, reptiles, amphibians, and insects (Boreham and Stenzel, 1993). To date, the taxonomic assessment of *Blastocystis* remains controversial. *B. hominis*, the species that infects humans, is more prevalent in under-developed and developing countries (Stenzel and Boreham, 1996). In recent years, there have been increasing reports on the association of the parasite with symptomatic gastrointestinal diseases, although its pathogenic potential still remains controversial (Tan, 2004).
2.1. Infection and disease

It is currently unclear whether *B. hominis* is a pathogen, commensal, or an opportunistic organism as there are numerous conflicting studies that either implicate or exonerate the parasite as a cause of intestinal disorders (Tan, 2004). In a comprehensive study, Clark (1997) showed extensive sequence variation between morphologically identical but genetically distinct *Blastocystis* organisms. This huge difference among clinical isolates may explain the protean nature of the infection. Clinical disease in infected individuals is not specific and includes diarrhea, abdominal cramps, and nausea. In more severe disease, profuse watery diarrhea and fever may be seen.

There are some limited reports dealing with the correlation between *Blastocystis* sp. and clinical disease in swine. In one study undertaken in Spain, no correlation was found between *Blastocystis* sp. and symptomatic disease when the intensity of infection was 5 cells per 40× field (Quilez et al., 1995). Additionally, Pakandl (1994) detected infections of up to eight *Blastocystis* sp. cells per 100× and did not find any correlation between intensity of *Blastocystis* sp. infection in pigs and the occurrence of diarrhea. In one more recent study undertaken in western Iran, all *Blastocystis*-infected wild boars from different age-groups were asymptomatic (Solaymani-Mohammadi et al., 2004).

2.2. Epidemiology

It now is clear that *Blastocystis* is a common parasite in pigs and wild boars and has a worldwide distribution (Quilez et al., 1995; Solaymani-Mohammadi et al., 2004). There are some reports in which the incidence approaches 70–95% in domestic pigs (Pakandl, 1991) and up to 25% in wild boars (Solaymani-Mohammadi et al., 2004). These high prevalence rates of the parasite in swine may represent a potential risk for human infection especially in animal handlers in developing countries. Zoonotic transmission of *B. hominis* has been postulated since epidemiological evidence suggested a relation between close contact with animals and human blastocystosis (Rajah Salim et al., 1999). In this study, it was shown that the occurrence of *B. hominis* in animal handlers was significantly higher compared with persons with no exposure to animals. Although the morphology of *Blastocystis* parasites isolated from some animal hosts was identical to human isolates by light and electron microscopy (Clark, 1997; Solaymani-Mohammadi et al., 2004), only a few animal isolates were proven to be identical to human isolates of the parasite. Using a restriction fragments length polymorphism (RFLP) analysis of small sub-unit ribosomal RNA gene (SSU-rRNA), Thathaisong et al. (2003) analyzed isolates from humans, a pig, and a horse, and concluded that *Blastocystis* isolates from a pig and a horse were monophyletic and closely related to *B. hominis* of humans. In one recent study, using analysis based on SSU-rRNA, it was shown that pig isolates were apparently *B. hominis* (Noel et al., 2003). In this study, human-to-animal transmission was proposed for pigs if humans are the primary hosts of *B. hominis*. In a more recent study, 19 isolates of the parasites from different animals were analyzed according to the SSU-rRNA sequences of these isolates (Abe, 2004), and it was shown that there was a high similarity between the isolates. The results of these studies reinforce the fact that *Blastocystis* organisms have low host specificity and there is a cross-contamination between animals and humans; many of the swine isolates of the parasite have zoonotic potential, or have cross-transmissibility among heterogeneous hosts (Clark, 1997).

2.3. Diagnostic techniques

Direct stool examinations with or without concentration is the method of choice for the diagnosis of the parasite. In recent years, an ELISA-based diagnostic test for detection of both antibodies and antigens has been used for the diagnosis of the infection in symptomatic and asymptomatic individuals (Zierdt et al., 1995). A more recent study (Kaneda et al., 2000) showed, by the indirect immunofluorescence test (IFA), that 70% of infected asymptomatic individuals were serum positive for *Blastocystis* antibodies. Inoculation of stool samples into culture media, including modified nutrient broth LES, has been used to diagnose swine infection (Pakandl, 1991).

2.4. Therapy

The need to treat *Blastocystis* infections is controversial and this stems from the uncertain pathogenicity of the organism. However, in cases where *Blastocystis* is implicated in gastrointestinal disease, the recommendation is usually antibiotic treatment. From the results of clinical reports, the drug of choice for chemotherapy of *B. hominis* appears to be metronidazole or other nitroimidazoles. However, metronidazole was ineffective in the eradication of the parasite in certain infected individuals, suggesting that some isolates are more drug resistant (Haresh et al.,
1999). Additionally, this study has shown variable levels of resistance to metronidazole in geographically diverse isolates of human B. hominis (Haresh et al., 1999). More recently, it has been shown that nitazoxanide is effective in the treatment of persistent enteritis associated with B. hominis infection in Egypt (Rossignol et al., 2005).

2.5. Prevention and control

Zoonotic transmission of B. hominis has been speculated since epidemiological studies suggested a linkage between close contact with animals and blastocystosis in humans (Rajah Salim et al., 1999). Control of zoonotic blastocystosis is challenging. Clearly, efforts directed at reducing the prevalence of the parasite in domestic animals, especially in pigs, would significantly reduce the risk of exposure. Prevention of blastocystosis at present requires interruption of the fecal–oral spread of the parasite by improved hygiene, sanitation, and water treatment.

3. Entamoeba polecki (Von Prowazek, 1912)

E. polecki is a cosmopolitan intestinal parasite of pigs, wild boars, and monkeys and may infect humans accidentally. This parasite is found commonly in wild and domestic pigs all around the world. It seems that the parasite is very prevalent in some parts of the world, i.e. in Papua New Guinea where domestic pigs are the main livestock. This protozoan parasite is essentially non-pathogenic to humans, but it is morphologically similar to and often confused with Entamoeba histolytica, a pathogenic species.

3.1. Infection and disease

Infection with E. polecki almost always is asymptomatic in humans, but debate remains about the possibility of nonspecific symptoms such as diarrhea, bloody stools, fever, nausea, vomiting, abdominal cramps, inspiratory restriction, and weight loss. There are case reports of E. polecki causing symptomatic infections in humans (Salaki et al., 1979). It seems that most intestinal colonizations by E. polecki in swine are asymptomatic.

3.2. Epidemiology

It is generally accepted that human may acquire infection as a result of close contact with animals that are reservoir hosts, mainly pigs and wild boars. This parasite has been reported on rare occasions from all parts of the world (Chacin-Bonilla, 1983), but the overwhelming majority of cases have been reported from Southeast Asian countries or from Southeast Asian refugees (Gay et al., 1985). In a recent study, Verweij et al. (2001) studied twelve human infections with Entamoeba spp. producing uninucleated cysts. Using phylogenetic analysis of partial ribosomal DNA sequences, the authors demonstrated that there were at least four distinct genetic types of uninucleated cyst-producing Entamoeba species that infect humans, two of which were related to E. polecki and Entamoeba chattoni and two of which had not been reported previously. The authors proposed “E. polecki-like” for the agents of all human infections with uninucleated cyst-producing Entamoeba species. Although human infections with uninucleated Entamoeba are regarded as rare zoonotic infections, in one study 2 of 20 samples from humans in rural villages in northern Ghana revealed the presence of E. polecki-like variant 3 (Verweij et al., 2003).

E. polecki is a frequent inhabitant of the intestine of wild and domestic pigs all around the world; the prevalence of infection may reach up to 25% in these animals (Pakandi, 1994; Solaymani-Mohammadi et al., 2004). Where man and pig live in close association and where sanitation is poor, pig-to-man transmission is considered to be the most likely source of human infection (Desowitz and Barnish, 1986). Most cases of human E. polecki infections were reported from rural and sub-rural areas where pigs are abundant and there is close contact between man and pigs (Barnish and Ashford, 1989; Dwyer, 2005). In Muslim countries, where domestic pigs are absent, it seems that wild boars are the main reservoir host of human infections (Solaymani-Mohammadi et al., 2004).

3.3. Diagnostic techniques

The mainstay method for diagnosing E. polecki in humans and swine is by the identification of trophozoites or uninucleated cysts in feces, utilizing preserved, stained, and microscopically examined stool specimens (Fig. 1). This method of diagnosis can be difficult, however, given the morphologic similarities between E. polecki and other intestinal amoebas such as E. histolytica and Entamoeba hartmanni. More recently, a reverse line blot hybridization assay was developed to detect a variety of Entamoeba species and genetic variants known to infect humans (Verweij et al., 2003). The assay is performed after amplification with general Entamoeba-specific primers, and could identify four genetic variants of E. polecki-like cysts as well as
E. histolytica, Entamoeba dispar, E. hartmanni, Entamoeba moshkovskii and Entamoeba coli and even mixed infections in a range of controls and fecal samples. This technique can be used as an additional standard for diagnosis of human and swine infections, epidemiological studies, and quality control for pathogenic and non-pathogenic amebic infections (Verweij et al., 2003).

3.4. Therapy

E. polecki has been treated successfully with three antiparasitic drugs, metronidazole, ornidazole, and furamide. Metronidazole in a regime similar to that used for E. histolytica infection (750 mg three times a day for 5 days) (Gay et al., 1985) or in combination with diloxanide furoate (Salaki et al., 1979) has been reported to be effective in the treatment of E. polecki infections.

4. Balantidium coli (Malmsten, 1857)

B. coli is the largest protozoan and the only ciliate parasite to infect humans. Some 50 species of the genus Balantidium have been described, often on size differences, but the validity of most of these species is still unresolved. Infection with this parasite is not common in humans, and only 1000 cases had been reported before 1980. Its presence has been reported in parts of the world as far north as Sweden, Finland and Northern Russia, with the highest prevalence rates in tropical and subtropical regions. Estimates of worldwide prevalence are usually <1%, although higher rates are reported in hyper-endemic areas and in some residential institutions (Solaymani-Mohammadi et al., 2004, 2005a,b). Although the ciliate parasite infects a wide range of mammals all around the world, it is generally accepted that domestic pigs and wild boars are the main reservoir hosts for human infections (Esteban et al., 1998; Solaymani-Mohammadi et al., 2005a; Walzer and Healy, 1982).

4.1. Infection and disease

In the overwhelming majority (or in some instances in 100%) of infected persons, the infection is asymptomatic, and cyst-passer carriers have no significant clinical symptoms (Esteban et al., 1998) or may only have mild diarrhea and abdominal discomfort. In invasive cases, trophozoites penetrate through the epithelium, mainly from the bottom of the crypts (Zaman, 1993). Proteolytic enzymes, e.g. hyaluronidases, are produced by the parasite that can break down the intestinal epithelium, resulting in invasion of the mucosa and colonic ulceration (Fig. 2). As a result, hemorrhagic lesions, perforation, secondary bacterial infections, and local generalized peritonitis can be seen.

The acute dysenteric form of infection may be mild, severe or fulminating with numerous trophozoites present in stool. Fulminating acute balantidiasis has been reported to have a case fatality rate of 30% (Esteban et al., 1998; Walzer and Healy, 1982). Patients may die of intestinal perforation, or fulminating dysentery with hemorrhage and shock resembling dysentery produced by pathogenic E. histolytica.

It seems that an extraintestinal involvement is secondary to intestinal infection. Spread via the lymphatic system may extend infection to the mesenteric lymph nodes, appendix, and terminal ileum. Other organ involvement may include the vagina, peritoneum, pleura and lungs, and urinary bladder (Ferry et al., 2004). In recent years, there have been some reports on
**B. coli** infection in immunocompromised patients, including HIV/AIDS patients, patients with malignancies and patients that have undergone organ transplantations (Cermeno et al., 2003).

Typically, swine do not show signs of infection (i.e. they are asymptomatic carriers); indeed, **B. coli** is believed to live commensally in the large intestine of swine (Solaymani-Mohammadi et al., 2004). However, infected pigs still can shed vast volumes of the parasite in their feces.

### 4.2. Epidemiology

The presence of **B. coli** is frequently reported in Central and South America (e.g. Brazil and Venezuela), The Philippines, Papua New Guinea, Iran, Central Asia and certain Pacific Islands. Although the parasite infects a wide range of mammals, including horses, sheep, bovines, rodents, wild boars and higher primates, domestic pigs are generally considered to be the most important natural reservoir hosts for human infection (Walzer and Healy, 1982). The incidence of human infection is higher where pigs share habitat with humans, and faecal contamination of food and water occurs. In an outbreak in Truk Islands in Micronesia, the extensive contamination of superficial and underground water supplies with pig feces following a typhoon was postulated as the source of infection (Walzer et al., 1973). **B. coli** is common in pigs (and in its relative, the wild boar), being found in 20–100% of pigs in various populations (Solaymani-Mohammadi et al., 2004). Although human balantidiasis is considered to be an uncommon infection, it has an increased prevalence in communities that live in close association with pigs. In Papua New Guinea, where pigs are the principal domestic animals, the infection rates among swine herders and slaughterhouse workers are as high as 28% (Barnish and Ashford, 1989). Human balantidiasis is moderately common in warm climates and is usually sporadic in cooler areas. In some parts of the world – Papua New Guinea, for example, where hog raising is common and hygiene standards are sub-optimal – human infections are frequently seen (Barnish and Ashford, 1989). In western countries, including the United States, human infections are relatively rare and in Islamic countries, where pigs are regarded as being untouchable, and their breeding and consumption is prohibited, balantidiasis is almost nonexistent (Solaymani-Mohammadi et al., 2004, 2005a,b). However, the presence of human balantidiasis in Iran, where the overwhelming majority of people are Muslim, is quite extraordinary (Solaymani-Mohammadi et al., 2005b). Some authors believe that human-to-human transmission is responsible for transmission of infection in this endemic focus, but it seems that the prevalence of human infection is too low to have a determining role in this regard (Solaymani-Mohammadi et al., 2004). More recently, it has been proposed that wild boars are likely involved in the transmission of human diseases, as a high rate of boars examined in different parts of Iran were infected by a ciliate resembling **B. coli** (Solaymani-Mohammadi et al., 2004, 2005a). Additionally, it has been hypothesized that camels may be responsible in part in the epidemiology of human disease in Iran (Cox, 2005).

### 4.3. Diagnostic techniques

The diagnosis of balantidiasis in both humans and swine is based on microscopic detection of active trophozoites and/or cysts in fresh or formalin-fixed stool preparations (Fig. 3). **B. coli** is shed irregularly, and repeated examinations of stools are necessary for identification of the parasite (Solaymani-Mohammadi et al., 2004). Rectal biopsy can also provide specimens for diagnosis. In the case of pulmonary balantidiasis, examination of bronchoalveolar lavage may be useful (Anargyrou et al., 2003). The organism can be grown

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**Fig. 3.** **B. coli** trophozoites from pig feces. Haematoxylin and eosin stain. Magnification 1000×.
xenically, but has not been grown axenically. The media that have been used for *B. coli* xenic cultivation include many of the same ones used for *E. histolytica*, including LE, Robinson’s, and TYSGM-9, Balamuth’s, Jones’s, and Dobell’s HSre+ S. In contrast to other intestinal protozoa, *B. coli* grows over a broad temperature range (25–40 °C).

4.4. Therapy

The human disease (both intestinal and extra-intestinal) can be treated effectively with three antibiotics: tetracycline (500 mg/four times daily for 10 days), metronidazole (750 mg/three times daily for 3 days), and iodoquinol (640 mg three times daily for 20 days) (Yazar et al., 2004). However, in the treatment of acute balantidiasis, tetracycline is the drug of choice to eliminate *B. coli* trophozoites in humans.

Different therapeutic regimes, including chloroquine, niridazole (Ambilhar), and oxytetracycline (terramycin) have successfully been used in the treatment of swine balantidiasis (Verhulst and Shukla, 1976). It seems that terramycin is more effective in treatment of swine balantidiasis. For example, in a study in former Zaire, terramycin, at a dose rate of 15 mg/kg body weight administered twice daily, gave clinical recovery in all of the symptomatic pigs treated (Mwamba and Pandey, 1977).

4.5. Prevention and control

It is essential to note that people who have had the disease previously can become reinfected. Prevention requires effective personal and community hygiene-like measures used in preventing other oral–fecal transmitted protozoa: A clean water supply and hygienic living conditions can prevent the infection. Avoiding contact with pigs and fertilizer that is contaminated with pig excrement can also decrease the risk of infection among humans. In some instances where human-to-human transmission is possible, i.e. in institutions for mentally retarded individuals, monitoring the contacts of balantidiasis patients, and proper disposal of human feces is of the utmost importance. In rural areas, where wild boars are roaming freely, local farmers should take precaution to avoid infections (Solaymani-Mohammadi et al., 2004).

5. *Toxoplasma gondii* (Nicolle and Manceaux, 1908, 1909)

*T. gondii* is an intracellular protozoan parasite found in virtually all warm-blooded carnivores and omnivores including man. Transmission to man is commonly associated with exposure to either the oocyst stage in cat feces, or meat containing *T. gondii* tissue cysts. Also, it has been shown that pig’s meat is the most likely source of human exposure, since pigs are the only species shown to frequently harbor *T. gondii* and, therefore, pork may pose a risk to humans for exposure to *T. gondii* (Dubey and Beattie, 1988).

5.1. Infection and disease

Oocyst-transmitted infections may be more severe than tissue cyst-induced infections (Dubey et al., 1997). Enlarged lymph nodes, cervical or occipital lymphadenopathy, are the most frequently observed clinical form of postnatal toxoplasmosis in humans. Lymph nodes are not tender, do not suppurate, are usually discrete, and stay enlarged for less than 4–6 weeks. This situation may be associated with fever, nausea, fatigue, muscle pain, sore throat and headache.

Congenital toxoplasmosis is a potentially serious infection, which usually affects infants born to non-immune women. Most infants infected in utero are born with no obvious signs of toxoplasmosis on routine examination, but up to 80% develop learning and visual disabilities later in life if they are followed into adulthood (Carvalheiro et al., 2005). Neonatal clinical manifestations of congenital toxoplasmosis vary widely and include hydrocephalus, microcephaly, intracranial calcifications, chorioretinitis, strabismus, blindness, epilepsy, psychomotor or mental retardation, petechia due to thrombocytopenia, and anaemia. Toxoplasmic chorioretinitis can be seen in the setting of congenital or postnatally acquired disease as a result of acute infection or reactivation of the latent infections. Typical findings of toxoplasmic chorioretinitis include noticeably white focal lesions with an overlying and intense vitreal inflammatory reaction. By contrast with the favorable course of toxoplasmosis in almost all immunocompetent individuals, the disease can be life threatening in those who are immunocompromised. In these individuals, toxoplasmosis almost always happens as a result of reactivation of chronic infection (Bonnet et al., 2005). Encephalitis is the most important manifestation of toxoplasmosis in immunosuppressed patients as it causes the most severe damage to the patient.

*Toxoplasma* generally does not make pigs ill. Transplacental infection appears to be less common than post-natal infection. Most pigs acquire subclinical infection, and clinical toxoplasmosis occurs mostly in young pigs. The parasite can persist in the edible tissues.
of pigs and other food animals for years, perhaps for life. The parasite has been found in virtually all body muscles of pigs. Most *T. gondii* infections in swine are subclinical, but toxoplasmosis can cause clinical signs in pigs of all ages. Clinical toxoplasmosis has been reported most often in nursing pigs. Infected pigs are born dead, sick, or become sick within 3 weeks after birth; some remain clinically normal. Labored respiration is the most common clinical sign of toxoplasmosis in newborn piglets. Other clinical signs include fever, general weakness, diarrhea, nervous signs and rarely, loss of vision. *Toxoplasma* can cause mummified fetuses and stillborn piglets. A blood test for *T. gondii* antibodies in body fluids of the fetus can detect toxoplasmosis (Dubey and Beattie, 1988).

### 5.2. Epidemiology

The prevalence of *T. gondii* infection was found to range from as low as 4% to as high as 69% in pigs from the USA (Dubey et al., 2005), with similar values for other countries (de Sousa et al., 2006). Rural rearing, exposure to farm animals, and rearing of pigs have been identified as increasing the risk of human *Toxoplasma* seropositivity (Weigel et al., 1999). Domestic pigs are considered an important source of *T. gondii* infection in humans (Dubey and Beattie, 1988), and acute toxoplasmosis has been reported in humans that have consumed uncooked infected meat from pigs (Choi et al., 1997). Several studies have attempted to elucidate the sources of pig infections with *T. gondii* based on serological information and parasite isolation from feed, soil, and animals living in and around pig farms. These studies demonstrate that there are at least three possible sources of pig infections: ingestion of oocysts in soil, eating infected rodents, and cannibalism (Dubey et al., 1986). Of the meat sources, pork has always been considered to be a major source of *Toxoplasma* infection, whereas beef has not been shown to contain infectious *Toxoplasma* parasites (Dubey and Beattie, 1988). Recent studies have shown a significant difference between the seroprevalence of *T. gondii* in fattening pigs from large and small-scale commercial farms that practise good hygiene and backyard-reared scavenging pigs. These surveys show the importance of modern intensive husbandry systems in reducing the prevalences of *T. gondii* infection in domestic pigs (Hove et al., 2005). Assadi-Rad et al. (1995) showed risk factors associated with transmission of *T. gondii* to pigs kept in different management systems in Tennessee, and concluded that sows associated with cats were 2.6 times more likely to be seropositive for *T. gondii* than sows that had not contacted with cats. Additionally, it was shown that pigs of different ages from an outdoor farm had anti-toxoplasmal antibodies much more than pigs from an intense management indoor farm (Venturini et al., 2004). In this study, it was concluded that the prevalence of *T. gondii* antibodies among sows seemed to be related to the facilities and management of the farms. It has been estimated that the meat of one pig is eaten by approximately 300–400 individuals, and it has been shown that all edible parts of an infected pig may contain *Toxoplasma* cysts and should be considered infectious (Dubey, 1986). Hill et al. (2004) has warned that several methods of curing meat may not result in the killing of infectious cysts of the parasite. Additionally, it has been demonstrated that wild boars are infected by *T. gondii* in different parts of the world (Hejlicek et al., 1997; Gauss et al., 2005). In one study undertaken in Spain, using MAT, anti-toxoplasmal antibodies were found in 34.8% of wild boars examined (Gauss et al., 2005). In some parts of the world, wild boar meat is eaten raw by local people, and this may increase the risk of human infection with this parasite. The results of serological studies indicated the widespread exposure to *T. gondii* among wild boars, suggesting that this animal could represent a public health risk for persons that handle or consume raw or undercooked infected wild pig meat (Gauss et al., 2005).

### 5.3. Diagnostic techniques

Diagnosis of toxoplasmosis in humans is made by biological, serological, histological, and molecular methods, or by some combination of the above. Clinical signs of toxoplasmosis are non-specific and are not sufficiently characteristic for a definite diagnosis.

Diagnosis can be made by finding *T. gondii* in host tissue removed by biopsy or at necropsy (Fig. 4). A rapid diagnosis may be made by microscopic examination of impression smears of lesions with one of the Romanowsky’s stains (the Giemsa stain being very satisfactory). Electron microscopy can aid diagnosis. *T. gondii* tachyzoites are always located in vacuoles. Tissue cysts are usually spherical, lack septa, and the cyst wall can be stained with a silver stain. The bradyzoites are strongly positive on periodic acid Schiff (PAS) staining. Immunohistochemical staining of parasites with fluorescent or other types of labeled *T. gondii* antisera can aid in diagnosis (Dubey and Beattie, 1988; Hill and Dubey, 2002).

There are several serological methods that may be used in laboratory diagnosis of human and animal
toxoplasmosis. These tests include the Sabin–Feldman dye test, the indirect hemagglutination assay, the indirect fluorescent antibody assay (IFA), the direct agglutination test, the latex agglutination test (LAT), the enzyme-linked immunosorbent assay (ELISA), and the immunosorbent agglutination assay test (IAAT) (Chandramukhi, 2004). The IFA, IAAT and ELISA have been modified to detect immunoglobulin M (IgM) antibodies. The IgM antibodies appear sooner after infection than the IgG antibodies and disappear faster than IgG antibodies after recovery (Buffolano et al., 2005). In addition, it is possible to maintain the tachyzoites of the parasite in vitro in cell culture media, i.e. VERO and HEla, in order to produce antigenic materials.

Molecular diagnosis does not depend on an immune response, and allows direct detection of the parasite in biological samples. Thus, they can be used to establish a diagnosis when serological tests are not definitive. Real-time PCR is very sensitive and is a promising technique that is capable of providing a quantitative result (de Sousa et al., 2006). Since the early 1990s, prenatal diagnosis of congenital disease is based on detection of *Toxoplasma* DNA in the amniotic fluid by polymerase chain reaction (Thalib et al., 2005).

In the recent years, numerous diagnostic tests including modified agglutination test (MAT) (Dubey, 1997), microplate-ELISA (Arko-Mensah et al., 2000), latex agglutination test and Sabin–Feldman dye test (Hejlicek et al., 1997) have been used in diagnosis of the disease in domestic and wild pigs. However, the MAT has been shown to be superior to other serological tests currently available and compares well to bioassay for detection of *T. gondii* infection (Dubey, 1997). Bioassaying in laboratory mice and cats is also a sensitive method to obtain tachyzoites and tissue cysts of the parasite from infected organs of pigs. In a study carried out on Iowa sows, the success of isolation in cats (65.6%) was approximately twice that in mice (31.7%) (Dubey et al., 1995). In the recent years, the detection of *T. gondii* DNA in pork samples by polymerase chain reaction (PCR) has been reported, but there are no data on the specificity and sensitivity of this method to detect *T. gondii* in meat samples (Hill and Dubey, 2002). A highly sensitive method using a real-time PCR and fluorogenic probe was found to detect *T. gondii* DNA from as few as four bradyzoites (Jauregui et al., 2001).

### 5.4. Therapy

The combination of pyrimethamine and sulphadiazine remains the mainstay for treatment of *T. gondii*; spiramycin, diaminodiphenylsulphone, atovaquone, and clindamycin, are also used to treat toxoplasmosis especially in patients with sulfa allergies (Derouin et al., 1991). In swine, the disease is usually asymptomatic or sub-clinical, and treatment seldom warranted.

### 5.5. Prevention and control

To prevent exposure to *Toxoplasma*, the hands of people handling meat should be washed thoroughly with soap and water before they begin other tasks (Dubey and Beattie, 1988). All cutting boards, sink tops, knives and other materials coming in contact with uncooked meat should also be washed with soap and water. Washing is effective because the stages of *T. gondii* in meat are killed by contact with soap and water (Dubey and Thayer, 1994). Tissue cysts in pork meat are killed by heating the meat throughout to 67–8°C (Dubey et al., 1990) or by cooling to −13°C. *Toxoplasma* tissue cysts are also killed by exposure to 0.5 krad of gamma irradiation (Dubey and Thayer, 1994). In a prospective study carried out in Norway, the following were predictors for *T. gondii* seroconversion during pregnancy: eating raw or undercooked mutton; washing kitchen knives infrequently after preparation of raw meat prior to handling another food item; cleaning the cat litter box; eating raw or undercooked minced meat products; eating raw or undercooked pork; and eating unwashed raw vegetables or fruits (Kapperud et al., 1996). Pregnant women should avoid contact with cats, soil and raw meat. Pet cats should be fed only dry, canned, or cooked food. The cat litter box should be emptied every day, a task to be avoided by pregnant women. Gloves should be worn while gardening. Vegetables should be washed thoroughly before eating...
because they may have been contaminated with cat feces. Expectant mothers should be aware of the dangers of toxoplasmosis. At present there is no vaccine to prevent toxoplasmosis in humans.

It has been suggested that most pigs become infected after birth either from the ingestion of oocysts in feed and water contaminated with infected cat feces or by eating tissue cysts from other infected animals. The following measures could be used to prevent pigs from being infected by the parasite: keeping cats out of the swine barns, feed, and water, removal of dead pigs immediately to prevent cannibalism, rodent control by rodenticides, not by cats, and prohibition of feeding of uncooked garbage to pigs (Dubey and Beattie, 1988).

6. Sarcocystis suihominis (Heydorn, 1977)

Sarcocystis species are intracellular protozoan parasites with an intermediate-definitive host life cycle based on a prey-predator relationship. Humans are the definitive host (i.e. host to the intestinal stage) of at least two species: Sarcocystis hominis and S. suihominis. Most Sarcocystis species infect specific hosts or closely related host species. For example, humans and some primates are definitive hosts for S. hominis and S. suihominis after eating raw meat from cattle and pigs, respectively (Dubey and Powell, 1994). Three species of Sarcocystis have been recognized from pigs: Sarcocystis miescheriana, Sarcocystis porcifelis, and S. suihominis; the final hosts of these species are dogs, cats, and humans, respectively; from these three species infecting suids, only S. suihominis uses humans as the definitive host.

6.1. Infection and disease

There are limited surveys on human intestinal sarcocystosis, predominantly from European countries. Volunteers in Germany who ate raw pork containing S. suihominis tissue cysts became infected, shed oocysts, and had dramatic symptoms 6–48 h later, including bloat, nausea, loss of appetite, stomach ache, vomiting, diarrhea, difficulty in breathing, and rapid pulse (Heydorn, 1977).

Three species of Sarcocystis have been recognized from pigs: S. miescheriana, S. porcifelis, and S. suihominis of which only S. suihominis is of public health importance. In swine hosts, mild infections do not usually cause clinical signs in naturally infected animals, but weight gain and meat quality of infected pigs may be reduced over the whole fattening period. In heavy infections, intermediate hosts, i.e. swines, are the most affected by Sarcocystis in terms of disease. After ingestion of sporocysts and subsequent migration of sporozoites through the body vessels, acute lesions (edema, hemorrhages and necrosis) develop. Lesions are associated with maturation of the second generation of meronts within the endothelial and subendothelials cells. The most common pathological alterations observed are myositis, petechial hemorrhages of heart and serosae, edema, necrosis and hemorrhage of lymph nodes. After the acute phase, cysts of Sarcocystis may be found in various muscular tissues of swine, generally without any significant pathological changes (Avapal et al., 2004).

6.2. Epidemiology

Man is an obligatory final host for two species of Sarcocystis (i.e. S. hominis, S. suihominis) and a possible intermediate host for other still unspecified species. It is difficult to specify the frequency of Sarcocystis species infecting humans, which are not examined always in routine laboratory examinations. In one study in Germany, Bussieras (1994) found a rate of 2% in humans in Germany, while 3–30% of the pigs in Germany were found to carry tissue cysts of S. suihominis.

Sarcocysts resist refrigeration at −2 °C, but are killed at −5 °C (48 h) and −20 °C (24 h). The sporocysts are evacuated by the final hosts at the end of the pre-patent period (from 11 to 18 days for the parasitic species of the man). The sporocysts have a longevity of about 1 year in humid environment, but reduced to 2 or 3 months in dry medium; they resist temperatures as low as −20 °C for 48 h. Their resistance is also substantial to disinfectants; only 10% ammonia is known to exert a lethal effect on the sporocysts (Fayer, 2004).

6.3. Diagnostic techniques

Sporocysts of S. suihominis are excreted 11–13 days after ingesting pork. Sporocysts can be seen by bright-field microscopy in a fecal flotation wet mount just beneath the coverslip as oblong or cylindrical in shape. Within the sporocysts, long and teardrop-shaped sporozoites may be observed. Flotation based on high-density solutions incorporating sodium chloride, cesium chloride, zinc sulfate, sucrose, Percoll, Ficoll-Hypaque, and other such density gradient media is preferred to formalin–ethyl acetate and other sedimentation methods. Because sporocysts of different species overlap in size and shape, species cannot be distinguished from one another solely by microscopy (Saito et al., 1998).
The overwhelming majority of swine infections are asymptomatic. In heavy infections, infected animals are usually emaciated, showing purpura of the skin of the ear, legs, and buttocks. Grossly, petechial to ecchymotic hemorrhages are seen on the serosal surface of the stomach, heart, liver, kidneys, with moderate to severe hemorrhages on the serosal surface of the small intestine and large intestine in the majority of cases (Avapal et al., 2004). *Sarcocystis* in swine can be detected in meat by direct observation of macroscopic sarcocysts or microscopic examination of histologic sections. Grinding meat, artificially digesting it in a solution of pepsin and hydrochloric acid, centrifuging the digest, and microscopically examining the pellet for the presence of bradyzoites can be used to inspect larger quantities of meat (Fayer, 2004). An ELISA test, using *S. miescheriana* as antigen, an indirect haemagglutination test (IHA), using antigens from *Sarcocystis gigantea* and indirect immunofluorescence test (IFA) have also been used to detect swine sarcocystosis (Damriyasa et al., 2004).

6.4. Therapy

There is no known prophylaxis or therapeutic treatment for intestinal sarcocystosis in humans. Infections are self-limiting, of short duration, and often asymptomatic. The efficacy of co-trimoxazole or furazolidone remains to be demonstrated. Because of the paucity of reported treatment cases and the lack of any controlled studies, there is no basis for evaluation, and therefore no course of treatment can be recommended as superior to any other at this time (Fayer, 2004). There is not known treatment in swine.

6.5. Prevention and control

Fresh chuck roast and round steak, as well as rare roast beef and hamburger, may contain infectious bradyzoites. Cooked products such as beef bologna and beef frankfurters, as well as frozen hamburger and frozen flaked sandwich steaks, were not infectious (Fayer, 2004). Chemoprophylaxis using the anticoccidial drugs amprolium and salinomycin was effective in preventing severe illness and death in experimentally infected calves and lambs (Fayer, 2004), but there is no report of attempted prophylaxis in humans.

To prevent infection of food animals, they must be prevented from ingesting the sporocyst stage from human feces in contaminated water, feed, and bedding. When such preventative measures cannot be assured and meat might be harboring cysts, it should be thoroughly frozen for 2 days or more or thoroughly cooked to kill infectious bradyzoites. These measures will prevent the development of intestinal stages where humans might serve as definitive hosts (Fayer, 2004).

7. Trypanosoma (*Schizotrypanum*) cruzi (Chagas, 1909)

The protozoan *T. cruzi* is the etiological agent of Chagas’ disease, a chronic, and incapacitating condition afflicting millions of people in Central and South America (WHO, 2002). The disease is a zoonosis in which the parasite is transmitted to humans through the feces of blood sucking triatomine bugs. Because of a higher risk of exposure to infected triatomines, infection is primarily present among people who live under poor conditions in rural settlements. American trypanosomiasis (Chagas’ disease) is a parasitic zoonosis endemic to the Americas. Over 200 species/subspecies of mammals and 120 triatomine species are known to be susceptible to *T. cruzi* infection. Dogs, opossums, domestic and wild pigs, rodents, and armadillos act as major reservoirs in human-related environments (Fujita et al., 1994).

7.1. Infection and disease

At the site of parasite entry, an inflammatory lesion, know as chagoma, may develop. Most persons with acute Chagas’ disease have only mild symptoms. However, children and, less frequently adults, may develop severe symptoms after an incubation period of 7–14 days (Tanowitz et al., 1992). During the acute stage, patients may experience a mild illness with fever, malaise, unilateral painless edema of the eyelids (“Romana’s sign”), conjunctivitis, hepatosplenomegaly and lymphadenopathy. These symptoms resolve spontaneously in 1–3 months (Tanowitz et al., 1992). Acute symptoms occur only in a few people (i.e., 1%), leading most people to be unaware of their infection. Years after the initial infection, approximately 10–30% of patients will develop chronic Chagas’ disease; this may occur 10–20 years after the primary infection. Cardiac effects are most common, with enlargement, apical aneurysms, mural thrombi, and disturbances in the conduction system (Fig. 5). Chronic Chagas’ disease is associated with a decrease in Auerbach’s and Meissner’s plexuses. It seems that most *T. cruzi* infections in pigs are asymptomatic with no apparent clinical symptoms (Salazar-Schettino et al., 1997).
7.2. Epidemiology

Domestic mammals play an important role in maintaining the infection as they are in close contact with both triatomines and human settings. Natural and experimental infections in domestic pigs have been documented (Salazar-Schettino et al., 1997). In the Brazilian Amazon Basin, it was shown that the domestic pig was heavily infested with the vector Panstrongylus geniculatus and was one of the animals that the vector fed on (Valente et al., 1998). Also, the domestic pig was one of the feeding resources of Triatoma braziliensis, one the most important vectors of Chagas’ disease in Brazil (Costa et al., 1998). Additionally, in a study that was done in one of Paraguay’s most highly endemic areas, 2/20 pigs showed anti-T. cruzi antibodies using a direct agglutination test (DAT) (Fujita et al., 1994). These authors considered pigs as one of the possible animal reservoir hosts for human T. cruzi infections in the endemic regions of Paraguay. Recently, natural infection of pigs has been reported from an endemic region of Mexico, showing that this animal host may play an important role in maintaining domestic cycle of the human disease (Salazar-Schettino et al., 1997).

7.3. Diagnostic techniques

Xenodiagnosis is one of the methods traditionally used to diagnose the parasite in latent or chronic phases of the disease. In this method, laboratory-reared triatomine bugs are allowed to feed on the blood of an infected patient. The feces and intestine of the nymphs are examined after 1 and 2 months for the presence of the parasite.

Immunodiagnosis is widely used because nearly all T. cruzi-infected individuals in the chronic phase develop antibodies against the parasite. In the chronic phase of the disease, antibodies are predominantly of the IgG class, whereas IgM antibodies are more frequently in the acute phase of the disease. ELISA, IFA, and IHA are the ones that are widely used (Leiby et al., 2000). Recently, the use of synthetic peptides and recombinant antigens has improved the sensitivity and specificity of the serodiagnostic tests (Saez-Alquezar et al., 2000). More recently, the performance of the Chagas Stat-Pak rapid immunochromatographic test with a standard ELISA in the serodiagnosis of Chagas’ disease in Central America was assessed, and it was found that this rapid test is a sensitive and specific alternative to the ELISA (Ponce et al., 2005).

The sensitivity of PCR techniques is higher compared with xenodiagnosis; however, this sensitivity depends on the level of parasitemia of the individuals. Detection of T. cruzi kinetoplast DNA by PCR is a potentially powerful tool for the parasitological diagnosis of Chagas’ disease; this assay is able to detect as low as one parasite/20 ml blood and has the sensitivity of 96–100% (Vallejo et al., 1999). PCR amplification also has been used in archeoparasitic studies and in chronically infected persons as well as in congenital infections (Guhl et al., 1997).

Different parasitological (Romanowsky’s dyes stained thick and thin smears), serological (DAT, IHA), culture methods (Novy, Mac Neal and Nicolle medium), xenodiagnosis, and bioassay in mice have been used to diagnose infection in reservoir hosts including domestic pigs (Fujita et al., 1994; Salazar-Schettino et al., 1997).

7.4. Therapy

Nifurtimox (8–10 mg/kg/day in four divided doses for 120 days in adults) is active against trypomastigotes and amastigotes. This drug interferes with the parasite’s carbohydrate metabolism by inhibiting the pyruvic acid synthesis. Benznidazole (a nitroimidazole) at 5–7 mg/kg/day in two divided doses for 30–90 days in adults has more trypanocidal activity than nifurtimox. Both compounds show adequate efficacy against the acute
7.5. Prevention and control

In the absence of vaccines or adequate drugs for large-scale treatment, the reduction of disease burden critically depends on the control of transmission by triatomine vectors and infected blood transfusion. Several multinational initiatives have been launched with that purpose. Ten years of concerted action in the Southern Cone have resulted in the elimination of transmission by *Triatoma infestans* (the most widespread domestic vector) from vast areas of the region. Incidence of human infection has dropped by an average of 94% in the area, and by 65% in Latin America (WHO, 2002). In endemic areas, transmission by transfusion is another important source of infection. To prevent transfusion-transmitted cases, serosurveys of blood donors as well as testing of blood should be performed in endemic areas (Galel and Kirchhoff, 1996). In addition, seropositivity of an organ donor should be considered a contradiction to transplantation.

8. Conclusion

Parasitic infections in swines are of considerable economic importance causing chronic production losses as a result of reduced weight gain, weight loss and reduced milk production. Several surveys have indicated that outdoor production of pigs results in heavier and more prevalent parasitic infections compared to conventional intensive production under indoor conditions. Pigs are naturally omnivorous, and indoor systems allow the easy collection of fecal waste, preventing pigs from being infected and transmitting infection to humans. In addition, education of farmers and stockmen is crucial to successful on-farm control of food-borne swine-transmitted zoonoses, and an understanding of why control measures are necessary, and how they can be applied, will improve compliance with protocols and procedures. To conclude, the occurrence of these infections underscores the need for better collaboration between those involved in human health, and those whose job it is to deal with the health of animals.

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