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Cryptosporidium infections in birds - a review

Infecção por *Cryptosporidium* em aves - uma revisão

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Abstract

Cryptosporidiosis is one of the main protozoan infections in birds. It manifests as either a respiratory or a digestive illness, and it affects a very large number of avian species across several continents. The aim of this review is to report on the main results of studies on cryptosporidiosis among birds and the importance of these results to veterinary medicine and public health.

Keywords: *Cryptosporidium* spp., wild birds, poultry.

Resumo

A criptosporidiose constitui-se em uma das principais infecções por protozoários em aves, manifestando-se como enfermidade respiratória ou digestiva, em dezenas de espécies aviárias, em vários continentes. O objetivo desse trabalho foi relatar, por meio de revisão de literatura, os principais resultados de estudos sobre criptosporidiose em aves e sua importância para a medicina veterinária e saúde pública.

Palavras-chave: *Cryptosporidium* spp., aves selvagens, aves domésticas.

Introduction

Protozoa of the genus *Cryptosporidium* parasitize fish, amphibians, reptiles, birds and mammals. Protozoan biological cycles take place on the surface of the epithelial cells in the gastrointestinal and respiratory tracts, in the bursa of Fabricius, and, less frequently, in other organs (CURRENT et al., 1986; BARTA & THOMPSON, 2006; VALIGUROVÁ et al., 2008), causing clinical and subclinical infections (SANTÍN, 2013).

The first description of *Cryptosporidium* infection among birds was reported by Tyzzer (1929) and involved the cecal epithelium of chicken. Slavin (1955) described a new species of *Cryptosporidium* that was causing mortality among young turkeys and suggested the name *Cryptosporidium meleagridis*. Nearly two decades later, cryptosporidiosis was diagnosed among domestic geese (*Anser anser)* (PROCTOR & KEMP, 1974) and broiler chickens (FLETCHER et al., 1975). Current et al. (1986) described the biological cycle of *Cryptosporidium* in domestic chickens and named the species *Cryptosporidium baileyi.* The third valid species of this parasite, *Cryptosporidium galli*, was described by Pavlásek (1999) from the proventriculi of chickens and was later revised by Ryan et al. (2003a).

Cryptosporidiosis is one of the main protozoan infections among birds. It manifests as either a respiratory or a digestive disease, and it affects a very large number of avian species across all continents except Antarctica (Table 1). Various aspects of cryptosporidiosis among humans and animals have been addressed (RAMIREZ et al., 2004; XIAO et al., 2004; JEX et al., 2008; BOWMAN & LUCIO-FORSTER, 2010; RYAN, 2010), but the literature regarding the occurrence of *Cryptosporidium* infection among avian species is demonstrably sparse.

The objective of the present study was to report on the main results of studies on cryptosporidiosis among birds and the importance of these results to veterinarian medicine and public health by reviewing the literature.

Etiological Agent and Host Specificity

Cryptosporidium spp. are parasites classified as members of the phylum Apicomplexa, class Sporozoea, subclass Coccidia, order Eucoccidiida and family Cryptosporidiidae, which contains a single genus, *Cryptosporidium* (FAYER, 2008). However, there is evidence that the genus *Cryptosporidium* might be more closely related to the Gregarinia than to the Coccidia (BARTA & THOMPSON, 2006; CAVALIER-SMITH, 2014).

The classification of species within the genus *Cryptosporidium* is constantly being updated using molecular methods and data on morphology, biology and host specificity. There are descriptions of 27 to 30 different species of *Cryptosporidium*, although there is still

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Table 1. *Cryptosporidium* species and genotypes identified in birds using molecular diagnostic techniques.

Species/ genotype	Host Order	Site of infection	Gene target	Geographic origin	Reference
Cryptosporidium baileyi	Anseriformes, Cathartiformes, Charadriiformes, Columbiformes, Falconiformes, Galliformes, Gruiformes, Passeriformes, Piciformes, Psittaciformes, Strigiformes, Struthioniformes	Bursa of Fabricius, conjunctiva, kidneys, respiratory tract, cloaca, rectum	18S rRNA, Actin, HSP-70, COWP	Africa, Asia, Europe, North America, South America	Morgan et al. (2001), Ryan et al. (2003b), Chvala et al. (2006), Ng et al. (2006), Huber et al. (2007), Van Zeeland et al. (2008), Nakamura et al. (2009), Molina-López et al. (2010), Sevá et al. (2011), Qi et al. (2011), Wang et al. (2011), Coldwell et al. (2012), Schulze et al. (2012), Wang et al. (2012), Baroudi et al. (2013), Bougiouklis et al. (2013), Nakamura et al. (2014), Qi et al. (2014), Wang et al. (2014b), Li et al. (2015), Máca & Pavlásek (2015)
Cryptosporidium meleagridis	Columbiformes, Galliformes, Passeriformes, Psittaciformes	Small intestine, large intestine	18S rRNA, Actin, HSP-70, COWP, GP60	Africa, Asia, Europe, Oceania, North America, South America	Morgan et al. (2000), Morgan et al. (2001), Ryan et al. (2003b), Huber et al. (2007), Pagès-Manté et al. (2007), Nakamura et al. (2009), Qi et al. (2011), Silverlås et al. (2012), Wang et al. (2012), Baroudi et al. (2013), Wang et al. (2014b), Li et al. (2015), Máca & Pavlásek (2015), Reboredo-Fernández et al. (2015)
Cryptosporidium galli	Bucerotiformes, Galliformes, Passeriformes, Psittaciformes, Phoenicopteriformes	Proventriculus	18S rRNA, Actin, HSP-70	Asia, Europe, Oceania, South America	Ryan et al. (2003a), Ng et al. (2006), Antunes et al. (2008), Nakamura et al. (2009), Silva et al. (2010), Sevá et al. (2011), Qi et al. (2011), Nakamura et al. (2014)
Avian genotype I	Galliformes, Passeriformes	nd	18S rRNA, Actin	Oceania, South America	Ng et al. (2006), Nakamura et al. (2009)
Avian genotype II	Galliformes, Psittaciformes, Struthioniformes,	Cloaca, rectum, bursa of Fabricius	18S rRNA, Actin, HSP-70	Asia, Oceania, South America	Santos et al. (2005), Meireles et al. (2006), Ng et al. (2006), Nakamura et al. (2009), Sevá et al. (2011), Nguyen et al. (2013), Wang et al. (2014b)
Avian genotype III	Passeriformes, Psittaciformes,	Proventriculus	18S rRNA, Actin, HSP-70, COWP	Asia, Oceania, North America, South America	Abe & Makino (2010), Ng et al. (2006), Nakamura et al. (2009), Makino et al. (2010), Qi et al. (2011), Gomes et al. (2012), Nakamura et al. (2014), Ravich et al. (2014)
Avian genotype IV	Psittaciformes	nd	18S rRNA	Europe	Ng et al. (2006)
Avian genotype V	Psittaciformes	nd	18S rRNA, actin, HSP-70	Asia, South America	Abe & Makino (2010), Qi et al. (2011), Nakamura et al. (2014)
Goose genotypes I-V	Anserifornes	nd	18S rRNA	North America	Jellison et al. (2004), Zhou et al. (2004)
Black duck genotype	Anseriformes	nd	18S rRNA	Oceania	Morgan et al. (2001)
Eurasian Woodcock genotype	Charadriiformes	Proventriculus	18S rRNA, HSP-70	Europe	Ryan et al. (2003b)
Cryptosporidium andersoni	Galliformes	nd	18S rRNA, Actin	Europe	Ng et al. (2006)
Cryptosporidium muris	Caprimulgiformes, Struthioniformes	nd	18S rRNA, HSP-70, Actin	Asia, Europe	Ng et al. (2006), Qi et al. (2014)
Cryptosporidium parvum	Accipitriformes, Anseriformes, Charadriiformes, Galliformes, Passeriformes, Psittaciformes	Small intestine, caecum	TRAP-C2, beta-tubulin,18S rRNA, COWP	Asia, Europe, North America, South America	Graczyk et al. (1998), Zhou et al. (2004), Zylan et al. (2008), McEvoy & Giddings (2009), Nakamura et al. (2009), Gomes et al. (2012), Reboredo-Fernández et al. (2015)
Cryptosporidium hominis	Anseriformes	nd	18S rRNA	North America	Zhou et al. (2004)

nd: the site of infection was not reported.

some debate regarding which species are valid (ŠLAPETA, 2013; RYAN & HIJJAWI, 2015).

In birds, three species of *Cryptosporidium* have been reported, including *C. baileyi*, *C. galli* and *C. meleagridis*. Many genotypes have also been described, mainly based on molecular data (SMITH et al., 2007; XIAO & FAYER, 2008; XIAO & FENG, 2008; RYAN et al., 2014). The lack of biological, morphological or host specificity data has prevented the naming of new species related to *Cryptosporidium* avian genotypes (FAYER, 2010).

Cryptosporidium baileyi is the species most frequently diagnosed among birds, with reports of clinical or subclinical disease in 12 avian orders. Moreover, this is the most frequent species among the order Galliformes. *Cryptosporidium galli* has been found in several species of five different orders of birds, most frequently among Passeriformes and Psittaciformes, whereas *C. meleagridis* has been detected in four orders of birds, with infection occurring preferentially among the Galliformes (Table 1). *Cryptosporidium meleagridis* is the only avian species that infects mammals, and both natural and experimental infections have been reported (DARABUS, 1997; SRÉTER et al., 2000; AKIYOSHI et al., 2003; DARABUS & OLARIU, 2003).

Avian genotypes I, II, III, IV and V have been reported in birds (SANTOS et al., 2005; MEIRELES et al., 2006; NG et al., 2006; ABE & MAKINO, 2010), as have five goose genotypes (JELLISON et al., 2004; ZHOU et al., 2004), the black duck genotype and the Eurasian woodcock genotype (MORGAN et al., 2001) (Table 1).

There is still little information on the host specificity of the *Cryptosporidium* avian genotypes (Table 1). Avian genotype I has been found in canaries (*Serinus canaria*) and Indian peafowl (*Pavo cristatus*) (NG et al., 2006; NAKAMURA et al., 2009), whereas the presence of avian genotype III has been reported in several species of Psittaciformes and Passeriformes (NG et al., 2006; NAKAMURA et al., 2009; MAKINO et al., 2010; QI et al., 2011; GOMES et al., 2012; NAKAMURA et al., 2014).

The avian genotype II has been described in ostriches and in several Psittaciformes species (SANTOS et al., 2005; MEIRELES et al., 2006; NG et al., 2006; SEVÁ et al., 2011; NGUYEN et al., 2013). Although Wang et al. (2014b) reported the presence of avian genotype II in 0.78% (3/385) of fecal samples from chickens in China, Meireles et al. (2006) did not observe infection among chickens that were experimentally infected with avian genotype II and screened for *Cryptosporidium* infection using cytology, histology and oocyst screening in feces.

Infections by avian genotype IV and the Eurasian woodcock genotype have only been described once each: in the Japanese white-eye (*Zosterops japonicus*) and the Eurasian woodcock (*Scolopax rusticola*), respectively (NG et al., 2006). Regarding avian genotype V, which was first described by Abe & Makino (2010) among cockatiels (*Nymphicus hollandicus*), there have been two additional reports among birds of the order Psittaciformes (QI et al., 2011; NAKAMURA et al., 2014) and one report in reptiles (*Iguana iguana*) (KIK et al., 2011). The black duck genotype and the geese genotypes I to V have been described in the order Anseriformes and seem to have a narrower spectrum of hosts (JELLISON et al., 2004; ZHOU et al., 2004).

The infectivity of *C. parvum* to domestic chickens was assessed by Lindsay et al. (1987a) and Palkovič & Maroušek (1989), who observed clinical signs after intratracheal inoculation with oocysts. However, parasite colonization was found to be restricted to the respiratory tract, and low numbers of oocysts were produced. *Cryptosporidium* species that are more common among mammals are sporadically found in birds, either in association with clinical signs, such as *C. parvum* in the stone curlew (*Burhinus oedicnemus*) (ZYLAN et al., 2008), or asymptomatically in birds, as reported by Qi et al. (2014) for ostriches with *Cryptosporidium muris* present in their feces.

Epidemiological, Clinical and Pathological Aspects of *Cryptosporidium* spp. Infection in Birds

Although many recently reported *Cryptosporidium* infections in the intestinal and respiratory tracts and the bursa of Fabricius in birds are related, respectively, to the presence of *C. meleagridis* and *C. baileyi* (RYAN, 2010), the possible roles of other species or genotypes of *Cryptosporidium* in the etiology of infections that were not characterized molecularly cannot be disregarded. For this reason, in the present review, the denomination *Cryptosporidium* sp. was used for cases where molecular characterization was not performed, unless the authors defined the species of *Cryptosporidium*, as in several studies regarding *C. baileyi*.

There are numerous descriptions of infection by *Cryptosporidium* among several avian species, particularly dating from the 1980s and 1990s, in which the diagnoses were accomplished only through cytological or histopathological observations without molecular characterization of the species or the genotype (GOODWIN, 1989; SRÉTER & VARGA, 2000). The reported clinical signs were mostly related to the respiratory tract and the gastrointestinal tract and were sometimes associated with mortality. However, other tissues have been found to be colonized by *Cryptosporidium* sp. in clinical or subclinical infections: the bursa of Fabricius, ocular conjunctiva, middle ear, pancreas and kidneys (DHILLON et al., 1981; THAM et al., 1982; HOERR et al., 1986; MASON, 1986; RITTER et al., 1986; O'DONOGHUE et al., 1987; GOODWIN, 1988; NAKAMURA & ABE, 1988; GOODWIN, 1989; GOODWIN & BROWN, 1989; JARDINE & VERWOERD, 1997; MURAKAMI et al., 2002; SRÉTER & VARGA, 2000; RYAN, 2010).

The importance of cryptosporidiosis in commercial poultry production has not yet been determined because few studies on the influence of natural infection by *Cryptosporidium* spp. on the production parameters of these birds have been conducted. Snyder et al. (1988) investigated antibodies against *Cryptosporidium* spp. by means of the indirect ELISA technique among broiler chickens in the United States and observed that the flocks that presented the best performance were negative for *Cryptosporidium*. However, positive *Cryptosporidium* serology was not clearly correlated with poor performance. Other authors have reported positive correlations between the presence of *C. baileyi* infection in broiler chickens and decreased weight gain, greater

incidence of airsacculitis, increased mortality and greater carcass condemnation rates in slaughterhouses (GORHAM et al., 1987; GOODWIN et al., 1996).

Infection by *Cryptosporidium* spp. in several species of wild and domestic birds has been demonstrated by many studies, with the reported prevalence values ranging from 0.8 to 44.4% (Table 2).

Intestinal Infection by *Cryptosporidium* sp., *C. meleagridis* and *C. parvum*

In turkeys, infection by *C. meleagridis* either presents subclinical characteristics (BERMUDEZ et al., 1988; WOODMANSEE et al., 1988) or has a clinical manifestation in the form of enteritis (SLAVIN, 1955; GOODWIN et al., 1988). In some cases, the infection is associated with other infectious agents (WAGES & FICKEN, 1989). Clinical infection is characterized by decreased weight gain, diarrhea, small intestine distention by gas and mucus, and the presence of evolutionary stages of *Cryptosporidium* in the proximal and distal portions of the small intestine (GOODWIN et al., 1988; GHARAGOZLOU et al., 2006).

Although *C. meleagridis* infects domestic chickens (LINDSAY et al., 1989), clinical cryptosporidiosis related to intestinal infection occurs infrequently. Additionally, there are only occasional reports of intestinal cryptosporidiosis in domestic chickens, which is usually subclinical or associated with clinical signs in co-infections with other etiological agents (TYZZER, 1929; ITAKURA et al., 1984; GOODWIN, 1988; GOODWIN & BROWN, 1989).

Infection by *Cryptosporidium* sp. has been correlated with the occurrence of enteritis and high mortality among quail, with the presence of diarrhea, small intestine containing clear aqueous fluid, cecum containing brown and foamy fluid, atrophy of the intestinal villi and presence of detached enterocytes in the intestinal lumen, as well as epithelial colonization by *Cryptosporidium* (HOERR et al., 1986). *Cryptosporidium* sp. in either natural infections (RITTER et al., 1986) or experimental infections (GUY et al., 1987), with or without associations with reoviruses, causes severe intestinal infection and high mortality among quail, in addition to presenting synergism with reoviruses.

One report described *C. meleagridis* infection in a breeding farm of red-legged partridges (*Alectoris rufa*) with clinical signs characterized by diarrhea and coughing, morbidity of 60-70% and mortality of 50% (100/200). On the same farm, during a later outbreak, mortality reached 89% (400/450). Evolutionary stages of *Cryptosporidium* were present in the respiratory tracts and intestines of these birds. Because molecular characterization was only

Table 2. Worldwide prevalence of *Cryptosporidium* spp. in wild and domestic birds.

Host species	Geographic origin	Species/genotype	% positive for *Cryptosporidium* spp. (No. positive/No. sampled)	References
Alectoris rufa	Czech Republic	*C. baileyi, C. meleagridis*	22 (145/663)	Máca & Pavlásek (2015)
Anas platyrhynchos	China	*C. baileyi*	16.6 (92/564)	Wang et al. (2010)
Columba livia domestica	Iran	*Cryptosporidium* spp.	2.94 (3/102)	Radfar et al. (2012)
	China	*C. baileyi, C. meleagridis*	0.8 (2/244)	Li et al. (2015)
Coturnix coturnix japonica		*C. baileyi, C. meleagridis*	13.1 (239/1818)	Wang et al. (2012)
Gallus gallus domesticus	China	*C. baileyi, C. meleagridis*	8.9 (179/2015)	Wang et al. (2010)
	Algeria	*C. baileyi, C. meleagridis*	34.4 (31/90)	Baroudi et al. (2013)
	China	Avian genotype II, *C. baileyi, C. meleagridis*	9.87 (38/385)	Wang et al. (2014b)
Meleagris gallopavo	Iran	*Cryptosporidium* spp.	35.3 (17/60)	Gharagozlou et al. (2006)
	USA	*C. parvum*	6.3 (5/79)	McEvoy & Giddings (2009)
	Algeria	*C. meleagridis*	43.9 (25/57)	Baroudi et al. (2013)
Several species	Australia	Avian genotypes I, II, III, *C. andersoni, C. baileyi, C. galli, C. muris*	6.25 (27/430)	Ng et al. (2006)
	Brazil	Avian genotypes I, II, III, *C. baileyi, C. galli, C. meleagridis*, *C. parvum*	4.86 (47/966)	Nakamura et al. (2009)
	China	Avian genotypes I, II, III, *V, C. baileyi, C. galli, C. meleagridis, C. parvum*	8.1 (35/434)	Qi et al. (2011)
	Brazil	Avian genotype II, *C. baileyi, C. galli*	6.6 (16/242)	Sevá et al. (2011)
	Nigeria	*Cryptosporidium* spp.	7.4 (66/890)	Bamaiyi et al. (2013)
	Spain	*C. meleagridis*, *C. parvum*	8.3 (36/433)	Reboredo-Fernández et al. (2015)
Struthio camelus	Brazil	*Cryptosporidium* spp.	44.4 (50/77)	Oliveira et al. (2008)
	China	*C. baileyi*	11.7 (53/452)	Wang et al. (2011)
	Vietnam	Avian genotype II	23.7 (110/464)	Nguyen et al. (2013)
	China	*C. baileyi, C. muris*	10.2 (31/303)	Qi et al. (2014)

performed on the intestinal content, from which *C. meleagridis* was identified, the authors of the study suggested that the respiratory infection could have been caused by *C. meleagridis*, although it was an unusual location, or could have been due to co-infection with *C. baileyi* (PAGÈS-MANTÉ et al., 2007).

Enteritis due to *Cryptosporidium* sp. has been reported among pigeons, with clinical signs such as diarrhea, hyperemia and intestinal distension and the presence of evolutionary stages of the parasite in the epithelium of the small intestine (ÖZKUL & AYDIN, 1994).

The importance of the prevalence of intestinal infection among Psittaciformes has not been determined. However, intestinal cryptosporidiosis associated with clinical signs has been observed among budgerigars (*Melopsittacus undulatus*) (GOODWIN & KRABILL, 1989), cockatiels (*Nymphicus hollandicus*) (GOODWIN & KRABILL, 1989; LINDSAY et al., 1990), ring-necked parrots (*Psittacula krameri*) (MORGAN et al., 2000) and lovebirds, which exhibited a high mortality rate (*Agapornis* sp.) (BELTON & POWELL, 1987).

Although infection by *C. parvum* is not common among birds, Zylan et al. (2008) described a case of catarrhal enteritis and mortality among stone curlews (*Burhinus oedicnemus*) in Saudi Arabia.

Infection by *Cryptosporidium* sp., *C. baileyi* and Avian Genotype II in the Ocular Conjunctiva, Respiratory Tract, Bursa of Fabricius, Rectum and Cloaca

Cryptosporidium sp. and *C. baileyi* are frequently regarded as etiological agents for infections in the upper respiratory system, middle ear and ocular conjunctiva of wild birds, such as owls (*Otus scops*), swallows (*Petrochelidon pyrrhonota*), falcons (*Falco cherrug* and *Falco rusticolus X Falco cherrug*) and red grouse (*Lagopus lagopus scoticus)* (VAN ZEELAND et al., 2008; MOLINA-LÓPEZ et al., 2010; COLDWELL et al., 2012; LEY et al., 2012; BOUGIOUKLIS et al., 2013; BAINES et al., 2014), and of domesticated birds, such as domestic chickens (BLAGBURN et al., 1991), geese (*Anser anser* f. *domestica*) (CHVALA et al., 2006), turkeys (GLISSON et al., 1984), ducks (MASON, 1986; O'DONOGHUE et al., 1987), peacocks (MASON & HARTLEY, 1980) and pheasants (RANDALL, 1986).

Respiratory infection may be restricted to the upper respiratory tract, or it may disseminate to the lower respiratory tract, including the bronchia, lungs and air sacs (Figure 1a - c). This may occur with *C. baileyi* alone or in association with other etiological agents of respiratory infections in chickens, such as *Escherichia coli* (Figure 1d) and the infectious bronchitis virus, and can result in high mortality (GOODWIN, 1989; BLAGBURN et al., 1987; BLAGBURN et al., 1991; MEIRELES et al., 1999). Several reports have been published on infections by *Cryptosporidium* sp. in turkeys and quails, with or without associations with other etiological agents (THAM et al., 1982; TARWID et al., 1985; MURAKAMI et al., 2002).

Oral infection by *C. baileyi* is generally subclinical, although there may be decreased weight gain, which may only be transitory (BLAGBURN et al., 1987; LEVY et al., 1988; MEIRELES et al., 1998a). After oral or intratracheal infection, *C. baileyi* colonizes the bursa of Fabricius, which presents slight hyperemia and mucus on the mucosal surface. The exudation of products generated from the inflammation, especially heterophils, plasma and cell residues, results in the deposition of a caseous exudate in the lumen of the bursa of Fabricius (Figure 2a) (GUY et al., 1988; MEIRELES et al., 1998b).

Divergent reports exist on the effects of cryptosporidiosis on the immune system. *Cryptosporidium baileyi* causes a severe infection in the bursa of Fabricius (Figure 2b), which is the organ responsible for the humoral immune response in birds (SCOTT, 2004). Although experimental infections with *C. baileyi* have been found to present diffuse chronic superficial purulent bursitis with epithelial hyperplasia and hypertrophy (Figure 2c) and slight lymphoid atrophy (GUY et al., 1988; LEVY et al., 1988; GOODWIN & BROWN, 1989; MEIRELES et al., 1998b), no influence on the humoral immune response of chickens has been observed (BLAGBURN et al., 1987; MEIRELES et al., 1998b; ABBASSI et al., 2000). Nevertheless, other reports have shown that *C. baileyi* infection had a suppressive effect on the humoral immune response of birds to the pathogenic virus or vaccine virus for Marek's disease (NACIRI et al., 1989), the Gumboro disease vaccine virus (LEVY et al., 1988), reoviruses (GUY et al., 1988), the Newcastle disease vaccine virus (RHEE et al., 1998a; ELADL et al., 2014), the infectious bronchitis vaccine virus (RHEE et al., 1998b), *Brucella abortus* (RHEE et al., 1998c) and the avian influenza vaccine virus (HAO et al., 2008; ELADL et al., 2014).

In addition to the possible immunosuppression caused by *C. baileyi*, even if only transitory, the association of *C. baileyi* with other infectious agents may result in high mortality and decreased weight gain among chickens. Among the agents that may present synergistically with *C. baileyi* are the virus vaccine (Rispens) for Marek's disease (ABBASSI et al., 2000), the avian infectious anemia virus (HORNOK et al., 1998), the Gumboro disease virus (LEVY et al., 1988) and reoviruses (GUY et al., 1988). In contrast, Meireles et al. (1995) did not observe any synergism among broiler chickens that were experimentally infected with *Toxoplasma gondii* and *C. baileyi.*

There have been reports that infection by *Cryptosporidium* sp. in ostriches resulted in prolapse of the phallus and cloaca (ALLWRIGHT & WESSELS, 1993; BEZUIDENHOUT et al., 1993; PENRITH & BURGER, 1993; PENRITH et al., 1994) and in pancreatic necrosis (JARDINE & VERWOERD, 1997). The avian genotype II colonizes the epithelium of the cloaca (Figure 2d) and, less frequently, the rectum and bursa of Fabricius of ostriches. The nfection results in prolapse of the cloaca (Figure 3a), particularly if stressful conditions lead to immunosuppression or if there are poor husbandry practices relating to feed, water or hygiene (SANTOS et al., 2005).

Infection in the Proventriculus by *Cryptosporidium* sp., *Cryptosporidium galli, C. muris*, Avian Genotype III and the Eurasian Woodcock Genotype

Cryptosporidium galli infects several species of birds of the orders Bucerotiformes, Galliformes, Passeriformes, Phoenicopteriformes and Psittaciformes (Table 1). The pathogenicity of the gastric

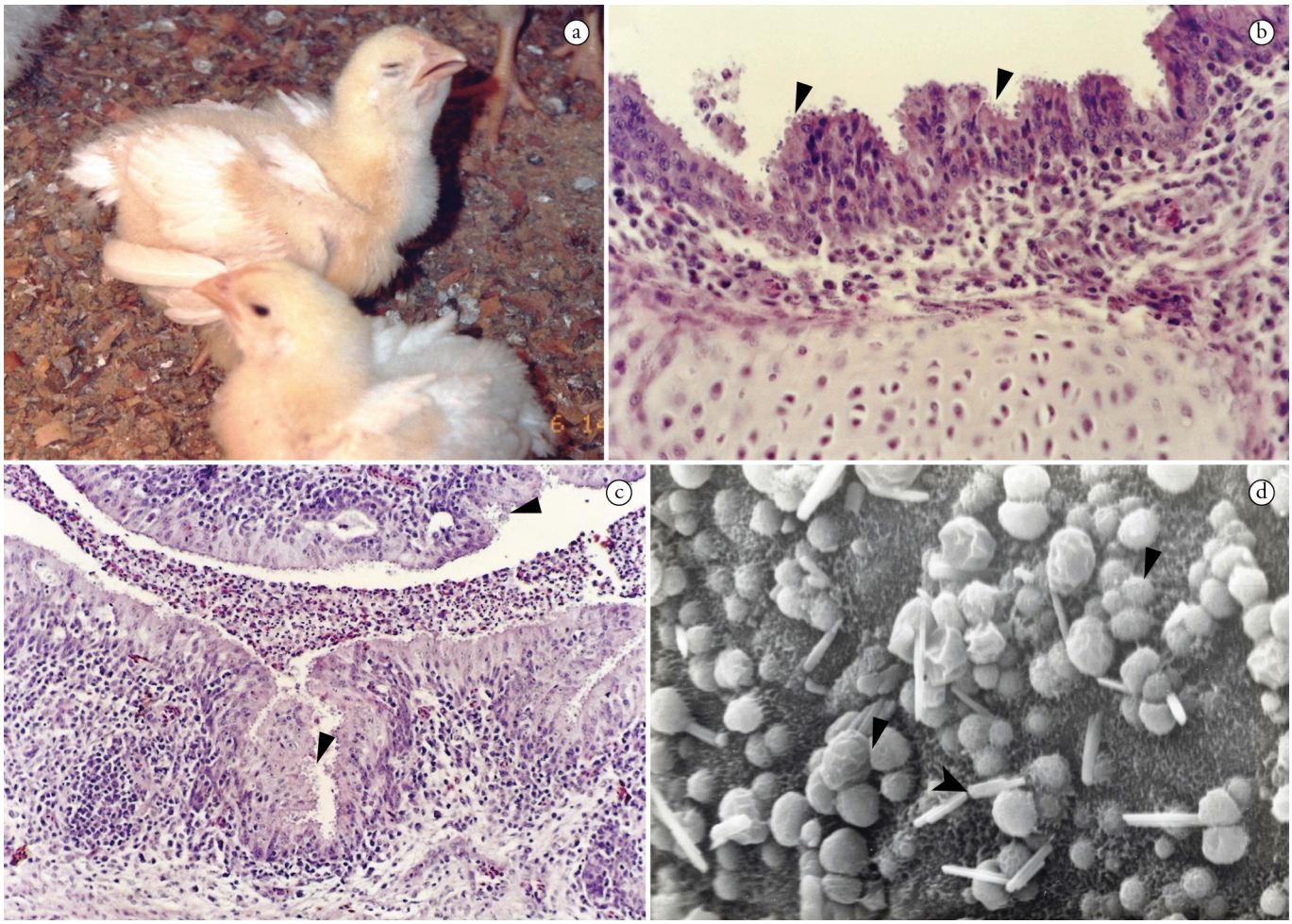

Figure 1. a: Chicken showing dyspnea after intratracheal inoculation of *C. baileyi* oocysts. **b:** Light micrograph of chicken trachea. Developmental stages of *C. baileyi* adhered to the epithelial surface (arrows), epithelial hyperplasia and infiltration of inflammatory cells in the submucosa (H&E stain, 400x). **c:** Light micrograph of chicken lung tissue. Developmental stages of *C. baileyi* adhered to the bronchial epithelial surface (arrows), inflammatory cells infiltrating both submucosa and epithelium, and inflammatory exudate filling the bronchial lumen (H&E stain, 200x). **d:** Scanning electron micrograph of chicken tracheal epithelium showing severe loss of cilia and concomitant infection with C. baileyi (arrow) and *Escherichia coli* (arrowhead) (2,000x).

species of *Cryptosporidium* has not yet been determined. Gastric infections by *C. galli* or *Cryptosporidium* sp. may be subclinical or associated with clinical signs characterized by apathy, diarrhea, weight loss, and sporadic mortality (BLAGBURN et al., 1990; CLUBB, 1997; MORGAN et al., 2001; ANTUNES et al., 2008; SILVA et al., 2010).

Infection with *C. galli* is characterized by intermittent and chronic shedding of oocysts in the feces. *C. galli* is able to infect young and adult birds and cause chronic gastric infection similar to *C. serpentis* in snakes (SILVA et al., 2010). Thus, Antunes et al. (2008) and Silva et al. (2010) suggested that infections by *C. galli* could be responsible for chronic proventriculitis in birds, which would predispose them to secondary infections by other pathogens.

The avian genotype III has also been found among several species of Passeriformes and Psittaciformes (Table 2). As with *C. galli*, avian genotype III causes chronic gastric disease, with clinical signs that include vomiting, weight loss and macroscopic and microscopic lesions in the proventriculus (MAKINO et al., 2010; RAVICH et al., 2014).

Cryptosporidium muris and *C. andersoni* infect several species of mammals and are occasionally related to clinical signs (SANTÍN, 2013). In birds, *C. muris* and *C. andersoni* oocysts may be present in fecal samples, possibly due to an actual infection or to being mechanically transported (NG et al., 2006). Subclinical infection by *C. muris*, which has apparently adapted to a new host, has been described among adult ostriches in China (QI et al., 2014).

There is only one report of infection in the proventriculus that was caused by the Eurasian woodcock genotype; this infection was described in the Czech Republic in a Eurasian woodcock (*Scolopax rusticola*) that died during the quarantine period (RYAN et al., 2003b).

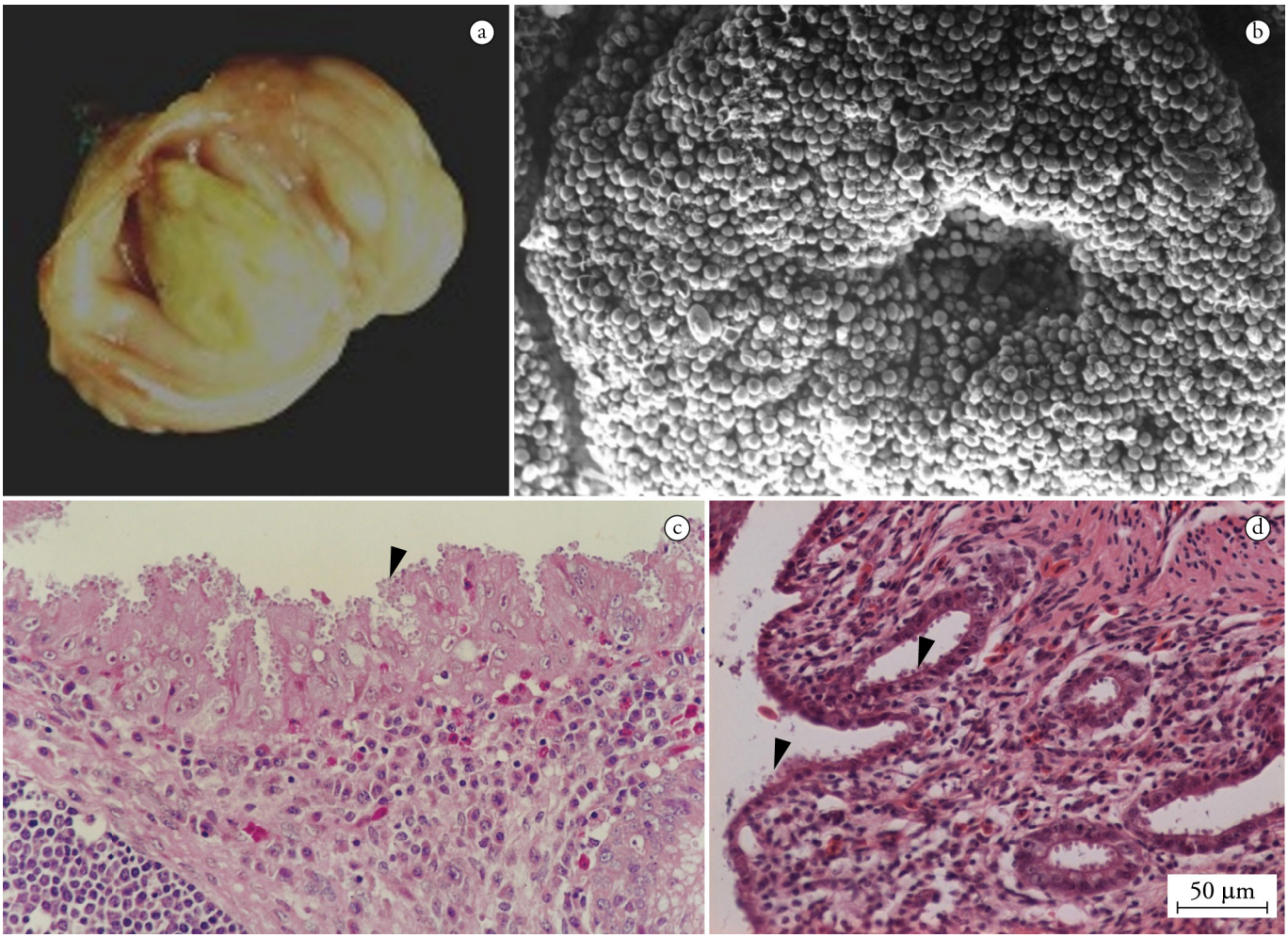

Figure 2. a: Caseous exudate filling the lumen of bursa of Fabricius of a chicken infected with *C. baileyi*. **b:** Scanning electron micrograph of bursa of Fabricius of a chicken infected with *C. baileyi*. Massive infection with parasite developmental stages covering the epithelial surface (700x). **c:** Light micrograph of chicken bursa of Fabricius. Developmental stages of *C. baileyi* adhered to the epithelial surface, epithelial hyperplasia and inflammatory cells infiltrating both submucosa and epithelium (H&E stain, 400x). **d:** Light micrograph of ostrich urodeum. Developmental stages of *Cryptosporidium* avian genotype II adhered to the epithelial surface and inflammatory cell infiltration in the submucosa (H&E stain, 400x).

Infection by Other Avian Genotypes of *Cryptosporidium*

Tissue tropism or the clinical importance of other genotypes of *Cryptosporidium* among birds has not been determined. Avian genotype I and avian genotype V show genetic similarity to *C. baileyi* and avian genotype II (ABE & MAKINO, 2010; MEIRELES et al., 2006; NG et al., 2006). Because species with greater genetic similarity present similar tissue tropism, as observed with *C. parvum* and *C. meleagridis*, with *C. baileyi* and avian genotype II and with *C. galli* and avian genotype III (XIAO et al., 2004; NG et al., 2006), it is likely that avian genotype I and avian genotype V colonize the final portion of the intestine, cloaca, bursa of Fabricius or respiratory system, and avian genotype IV, the proventriculus.

The hosts of the other avian genotypes described to date are as follows: avian genotype I: the canary (*Serinus canaria*) (NG et al., 2006; NAKAMURA et al., 2009) and the Indian peafowl (*Pavo cristatus*) (NAKAMURA et al., 2009); avian genotype IV: the Japanese white-eye (*Zosterops japonicus*) (NG et al., 2006); and avian genotype V: the cockatiel (*Nymphicus hollandicus*) (ABE & MAKINO, 2010; QI et al., 2011) and the blue-fronted parrot (*Amazona aestiva*) (NAKAMURA et al., 2014).

Diagnosis of Cryptosporidiosis Among Birds

Experience is a fundamental factor in diagnosing cryptosporidiosis because *Cryptosporidium* oocysts are small in comparison with other coccidians, do not present sporocysts, are difficult to

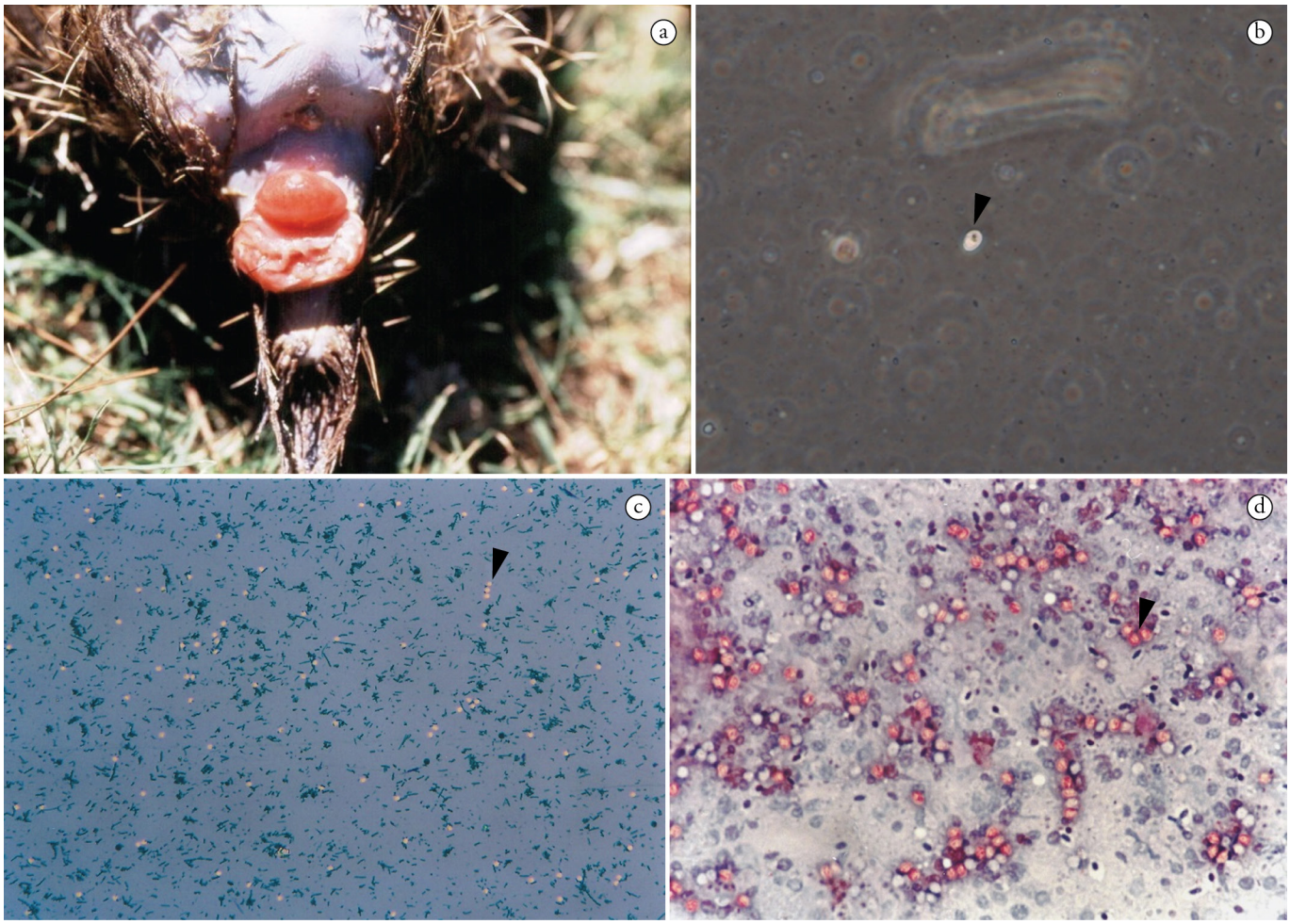

Figure 3. a: Cloacal prolapse in ostrich chick infected with *Cryptosporidium* avian genotype II. **b:** Single oocyst of *C. galli* (arrow). Fecal sample from a chronically infected adult canary processed using the Sheather's centrifugal flotation procedure (Phase contrast microcopy, 400x). **c:** Numerous oocysts of *Cryptosporidium* avian genotype II (arrow). Fecal sample from an ostrich chick processed using the ether centrifugal sedimentation procedure (Malachite green negative stain, 100x). **d:** Light micrograph of chicken bursa of Fabricius mucosal smear. Developmental stages of *C. baileyi* (arrow) (Safranin methylene blue stain, 200x).

observe, and are morphologically similar to fungi and yeast spores (CASEMORE, 1991). In samples with few oocysts in particular, care is needed to avoid false-positive results in fecal samples examined using the most common diagnostic methods, such as acid-fast staining or viewing oocysts under an optical microscope after concentration with saturated solutions of sugar, zinc sulfate or sodium chloride.

False-negative results are also common in samples with a low number of oocysts because of the low sensitivity of the staining techniques (JEX et al., 2008). In infections with *C. galli*, chronic shedding occurs, and few oocysts (Figure 3b) are observed per slide (ANTUNES et al., 2008). The amount of oocyst shedding and the patent period of infection with *C. baileyi* and *C. meleagridis* vary according to the age and species of the host (SRÉTER & VARGA, 2000).

The diagnostic methods using microscopy that are most used and least expensive involve screening for oocysts after centrifugal flotation in Sheather's solution, followed by phase contrast microscopy (Figure 3b) or bright-field microscopy (CARDOZO et al., 2008; TEIXEIRA et al., 2011a) and any of the many staining techniques for fecal samples, including negative malachite green staining (Figure 3c) (ELLIOT et al., 1999) and acid-fast staining (HENRIKSEN & POHLENZ, 1981; ORTOLANI, 2000; CARDOZO et al., 2008). In morphometric studies, morphological and morphometric alterations in oocysts should be considered when fecal smears are subjected to staining techniques (MEIRELES & FIGUEIREDO, 1992; CARDOZO et al., 2005).

Several staining techniques are useful for screening of the evolutionary stages of *Cryptosporidium* in histological sections and in mucosal smears, including hematoxylin and eosin, safranin-methylene blue (Figure 3d) and acid-fast stains (Figure 4). Furthermore, *Cryptosporidium* DNA can be detected in tissue sections using fluorescent *in situ* hybridization (LATIMER et al., 1988; CHVALA et al., 2006; JEX et al., 2008).

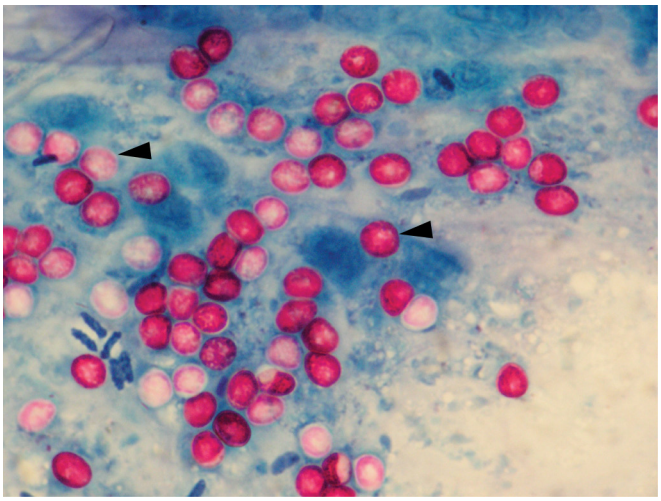

Figure 4. Light micrograph of lesser seed-finch proventriculus mucosal smear. Developmental stages of *C. galli* (arrows) (Kinyoun acid-fast stain, 1,000x).

Immunological methods for *Cryptosporidium* spp. diagnosis have been extensively reviewed (JEX et al., 2008; CHALMERS & KATZER, 2013). The detection of *Cryptosporidium* by capture enzyme-linked immunoassays (ELISA) or direct fluorescent antibody (DFA) assays using commercially available antibodies have been extensively adopted in fecal and environmental samples; as a rule, they present higher sensitivity and higher specificity than oocyst-staining techniques.

The antigens targeted by capture ELISA and DFA present cross-reactivity among the different species of *Cryptosporidium*, and therefore, does not allow species-specific diagnosis (GRACZYK et al., 1996; JEX et al., 2008; CHALMERS et al., 2011; TEIXEIRA et al., 2011b). Although both methods are commonly applied to detect *C. parvum* antigens (JEX et al., 2008), they may be useful for the diagnosis of avian cryptosporidiosis (RICHTER et al., 1994; GRACZYK et al., 1996; ROHELA et al., 2005; PAGÈS-MANTÉ et al., 2007).

Although the oocysts of some species present distinct morphology and morphometry, microscopic analysis does not allow species characterization because small variations exist in these parameters, and in many cases, the oocysts may be identical between different species or genotypes (RYAN, 2010). However, a presumptive diagnosis of gastric, intestinal or respiratory/bursal/cloacal cryptosporidiosis in birds can be accomplished by the presence of ellipsoidal oocysts measuring 7.5-8.5 × 6.0-6.4 µm; spherical, irregularly spherical or slightly elongated oocysts measuring 4.5-6.0 × 4.2-5.3 µm; or ovoid oocysts measuring 6.0-7.5 × 4.8-5.7 µm, respectively (CURRENT et al., 1986; LINDSAY et al., 1989; RYAN, 2010).

Molecular characterization of *Cryptosporidium* is performed by means of PCR, followed by either restriction fragment length polymorphism (RFLP) or sequencing of the amplified fragments. The gene most used for determining the species or genotype is 18S rRNA (RYAN et al., 2014). In comparison with the species of *Cryptosporidium* found in mammals, few sequences for the *Cryptosporidium* of avian species have been published in GenBank. When better resolution is needed to identify genetically similar species or genotypes, the relevant sequences of avian *Cryptosporidium* that are available relate to actin gene, heat shock protein gene (HSP-70) and *Cryptosporidium* oocyst wall protein gene (COWP) (Table 1). The 60-kDa glycoprotein (GP60) gene is used for subtyping *C. meleagridis* in molecular epidemiology studies (STENSVOLD et al., 2014; WANG et al., 2014a).

The genetic similarity between avian genotype II and avian genotype V in the 18S rRNA gene is 99.9%. Only a substitution of G by A in positions 329 and 378 differentiates the sequences of avian genotype II (DQ290031) and avian genotype V (AB471646), respectively. Because of this genetic similarity and the possibility of intraspecies variations in the 18S rRNA gene, classification of these two avian genotypes is recommended only after at least one gene that presents greater interspecies polymorphism, such as the HSP-70 gene or the actin gene (ABE & MAKINO, 2010; NG et al., 2006; MEIRELES et al., 2006), has been analyzed.

Species-specific diagnosis using molecular biology techniques is also possible. Recently, Nakamura et al. (2014) developed a real-time PCR specifically for diagnosing *C. galli* and avian genotype III.

Treatment and Prophylaxis

Many drugs have been tested for the treatment of cryptosporidiosis, but the US Food and Drug Administration (FDA) has approved only nitazoxanide for use in humans (SMITH & CORCORAN, 2004; STRIEPEN, 2013). Although halofuginone has shown variable efficacy, an effective drug for the prophylaxis and treatment of animal cryptosporidiosis is still lacking (LINDSAY et al., 1987b; SRÉTER et al., 2000; SHAHIDUZZAMAN & DAUGSCHIES, 2012).

Cryptosporidium oocysts are resistant to environmental stress and to the disinfectants commonly used in avian facilities. The prevention and control of avian cryptosporidiosis must rely on rigorous measures related to nutritional and sanitary management to prevent exposure to oocysts and the prophylaxis of concomitant diseases that are commonly associated with avian cryptosporidiosis (SANTOS et al., 2005; SRÉTER & VARGA, 2000; SILVA et al., 2010; SHAHIDUZZAMAN & DAUGSCHIES, 2012).

Importance for Public Health

More than 90% of human *Cryptosporidium* infections are related to *C. hominis* or *C. parvum*, although there are sporadic reports of infections with other *Cryptosporidium* species or genotypes. Among the *Cryptosporidium* species and genotypes of avian hosts, only *C. meleagridis* has a wider host spectrum and is able to infect humans; in fact, it is the third most common cause of human cryptosporidiosis. In some countries, such as Peru and Thailand, *C. meleagridis* is responsible for 10-20% of human *Cryptosporidium* infections, with a frequency similar to that of infection by *C. parvum* (XIAO & FENG, 2008; CHALMERS & GILES, 2010; ELWIN et al., 2012; INSULANDER et al., 2013).

Silverlås et al. (2012) reported the possible zoonotic transmission of *C. meleagridis* in Sweden when samples with identical nucleotide

sequences for the 18S rRNA and HSP-70 genes were found in hens, broiler chickens and an infected person. Further studies using phylogenetic analysis of multiple *loci* have suggested that the *C. meleagridis* found in birds may be related to isolates from humans and that birds may constitute an infectious source of human infections by *C. meleagridis* (STENSVOLD et al., 2014; WANG et al., 2014a).

Epidemiological studies have reported the presence of *C. meleagridis* in domestic birds and environmental samples. In Algeria, a report showed a high prevalence of *C. meleagridis*: 34% (26/90) in chickens and 44% (25/57) in turkeys (BAROUDI et al., 2013). However, in China, a low prevalence was described for *C. meleagridis* among broiler chickens (0.52%; 2/385) (WANG et al., 2014b), laying hens (0.19%; 3/1542) (WANG et al., 2010) and quails (0.22%; 4/1818) (WANG et al., 2012). Li et al. (2012) found *C. meleagridis* in 24.4% (22/90) of the wastewater samples collected from four cities in China.

The oocysts of *C. parvum* are sporadically present in fecal samples of asymptomatic birds that are kept either as pets or in zoos. In most situations, the birds represent only a mechanical transporter of oocysts (NAKAMURA et al., 2009; QUAH et al., 2011). However, even if birds are rarely infected by the *Cryptosporidium* species that are associated with mammals, aquatic birds mechanically transport the oocysts of zoonotic species, such as *C. parvum* and *C. hominis*, and may participate in the epidemiological chain of human cryptosporidiosis by means of environmental contamination (GRACZYK et al., 1998; ZHOU et al., 2004; GRACZYK et al., 2008; PLUTZER & TOMOR, 2009).

Concluding Remarks

Among the three species and various genotypes of *Cryptosporidium* identified in birds, only partial information is available regarding their economic, clinical, pathological and epidemiological characteristics and their importance for public health. Most of this information concerns *C. baileyi*, *C. galli*, *C. meleagridis*, avian genotype II and avian genotype III. Birds are kept as pets, for ornamental purposes, in zoos, in wildlife conservation centers and for commercial poultry production. The importance of determining the various aspects of cryptosporidiosis as a zoonosis or as a disease with significance regarding the health of birds is undeniable.

Reports of clinical disease associated with the presence of *Cryptosporidium* spp. among birds are increasingly frequent. Cryptosporidiosis on commercial farms remains understudied, perhaps because it is a subclinical disease or because it presents clinical signs that are not pathognomonic. Moreover, cryptosporidiosis is not among the diseases that are routinely diagnosed in avian pathology laboratories.

Several aspects of avian cryptosporidiosis, particularly its pathogeny among domestic birds, were more frequently studied during the 1980s and 1990s. After this period, the focus of research on avian cryptosporidiosis was directed toward the detection and classification of species and genotypes of *Cryptosporidium* and their roles as zoonotic agents. New research related to natural or experimental infection by *Cryptosporidium* spp. among domestic and wild birds could elucidate factors that are still undefined, such as the importance of this parasite as a primary infection agent and its interaction with other etiological agents of infections in the gastrointestinal tract, respiratory tract and bursa of Fabricius.

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