Veterinary Parasitology 266 (2019) 34–55

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Review article

Coccidiosis in dogs—100 years of progress

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ARTICLE INFO

Keywords:
Cystoisospora canis
Cystoisospora ohioensis-like
Cystoisospora burrowsi
Cystoisospora neorivolta
Life history
Diagnosis
Epidemiology
Control

ABSTRACT

Until 1970, coccidian parasites of dogs were considered to have a direct fecal-oral life cycle like *Eimeria* in poultry. They were thought to be non-host specific and infect both dogs and cats. Studies conducted in the 1970s revealed that dog coccidia were host-specific and had transport or paratenic hosts that were infected with an encysted stage containing a single organism, the monozoic tissue cyst. There are still considerable confusion and uncertainties concerning the life cycles and pathogenicity of coccidian parasites of dogs. The present paper reviews the history, taxonomy, life cycles, pathogenicity, epidemiology, diagnosis, and treatment of conventional coccidian parasites previously called *Isospora* spp., currently designated *Cystoisospora* spp. that infect canines.

1. Introduction and history

Although coccidian parasites have been known in cat and dog feces for more than a century, until 1970 little was known regarding their clinical or public health significance. Until the report of Wenyon (1923), only one species of coccidia was thought to be present in the feces of cats and dogs. Wenyon (1923, 1926) reviewed earlier literature and concluded that Grassi (1879) probably named *Coccidium rivolta* first from the cat, and Stiles (1891) named *Coccidium bigemina* first from the dog. Lühe (1906) transferred these parasites to the genus *Isospora*. Wenyon (1926) believed that there were two "races" of *I. bigemina*, the small race developed in intestinal villar enterocytes whereas the large race was in the lamina propria.

Wenyon (1923) made the first distinction that there were at least three species of coccidia in cats and dogs, based on the oocyst size: (a) large oocyst size, ~40 µm long; (b) medium oocyst size, ~25 µm long; and (c) small oocyst size, ~10–12 µm long. Wenyon (1923) named the coccidia with large oocysts as *Isospora felis*.

The coccidian species with large and medium sized oocysts of dogs and cats were found to be host-specific and re-described (Nemeséri, 1959, 1960; Shah, 1970; da Rocha and Lopes, 1971; Lage et al., 1974; Dubey, 1975a, b; reviewed in Dubey, 2009). The *I. bigemina* (*Coccidium bigemina* of Stiles) turned out to be a mixture of numerous species of *Sarcocystis*, *Toxoplasma, Besnoitia*, *Neospora*, and *Hammondia* (Dubey et al., 2002; Dubey, 2009). Retrospectively, *I. bigemina* is *nomen nudum*, and not discussed further.

Nemeséri (1959, 1960) first separated coccidia with large-sized oocysts from dogs from those found in cats and named the dog parasite *Isospora canis* and partially described its endogenous stages. Dubey (1975a, b) separated the coccidia with medium sized oocysts and retained the name *I. rivolta* for the parasite in cat feces and named the dog parasite *Isospora ohioensis*. Subsequent studies revealed that there were two other *I. ohioensis*-like species in dogs and they were named *Isospora burrowsi* and *Isospora neorivolta* (Trayser and Todd, 1978; Dubey and Mahrt, 1978).

Until the discovery of the life cycle of *Toxoplasma gondii*, coccidia were considered to have a direct fecal-oral cycle. Frenkel and Dubey (1972) and Dubey and Frenkel (1972a) found extra-intestinal stages of *I. felis* and *I. rivolta* in cats and mice. An encysted stage (tissue cyst) of *I. felis* and *I. rivolta* was found in rodents (Frenkel and Dubey, 1972). Based on these observations, Frenkel (1977) and Dubey (1977) proposed new genera, *Cystoisospora* and *Levineia* for *Isospora* species of cats and dogs but *Levineia* was considered synonym of *Cystoisospora* based on priority of publication dates. Barta et al. (2005) supported this conclusion based on molecular phylogeny.

During the last 50 years much has been learned of coccidiosis in dogs. However, there is still controversy concerning the species and pathogenicity of coccidiosis in dogs (Mitchell et al., 2007). The objective of the present review is to summarize biology of dog coccidia, including taxonomy, life cycle, prevalence, pathogenicity, diagnosis, and control.

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https://doi.org/10.1016/j.vetpar.2018.12.004
Received 4 October 2018; Received in revised form 3 December 2018; Accepted 4 December 2018
0304-4017/ Published by Elsevier B.V.

2. Canine *Cystoisospora* species

This review concerns only *Cystoisospora* species. Currently, there are four species, *C. canis, C. ohioensis, C. burrowsi,* and *C. neorivolta.* Of these, *C. canis* is distinctive because of its large-sized oocysts, whereas the latter three are grouped as *C. ohioensis*-like because their oocysts overlap in size. Oocysts of all four species lack micropyle, and oocyst residuum; all have two sporocysts with no Stieda bodies and have four sporozoites.

2.1. *Cystoisospora canis* Nemeséri, 1959, 1960

2.1.1. Morphology and life cycle

Oocysts are ovoid and vary in size (Table 1). In general, there is agreement among measurements provided in four papers in Table 1 except the report of Abdel-Magied et al. (1982). The number of oocysts measured, medium of suspension, amount of pressure applied between coverslip and slide could all affect the measurements. For diagnostic purposes, the minimum length was 32 µm and the upper limit was 44 µm. Based on measurements obtained from a cloned isolate, sporulated oocysts were 34–40 × 28–32 µm with length-width ratio of 1.12-1.4. Sporocysts were 18–21 × 15–18 µm (Lepp and Todd, 1974).

Lepp and Todd (1974) described the endogenous development of their cloned culture of *C. canis* in coccidia-free dogs inoculated orally with 150,000 sporulated oocysts. One infected dog was necropsied daily from 1-12 days post inoculation (p.i.). Developmental stages were located throughout the small intestine and rarely in the colon. The distal part of ileum was the most parasitized site. All developmental stages were in the lamina propria; no stages were seen in surface epithelium; the host cell parasitized is unknown but thought to be an enterocyte. They reported three generations of schizonts. Trophozoites (uninucleate, round zoite) were seen days 1–3 p.i. and the first nuclear division occurred day 4 p.i. First generation schizonts matured 5-7 days p.i.; were 16-38 × 11-23 µm and contained 4-24 merozoites measuring 8–11 × 3–5 µm. The second generation schizonts occurred 6–7 days p.i., were 12–18 × 8–13 µm, and contained 3–12 merozoites measuring 11–13 × 3–5 µm. The third generation schizonts occurred 6–8 days p.i., were 13–38 × 8–24 µm, contained 6–72 merozoites, measuring 8–13 × 1.5–3.0 µm. The second and third generations were seen in the same parasitophorous vacuole (pv). Gamonts were seen 7–10 days p.i. No mention was made of schizonts occurring at 9–12 days p.i. Macrogamonts were 22–29 × 14–23 µm. Microgamonts were 20–38 × 14–26 µm, and contained numerous microgametes and a large residual body (Lepp and Todd, 1974). The prepatent period was 9–11 days (Lepp and Todd, 1974) or 10–12 days (Buehl et al., 2006).

Hilali et al. (1979) partially described the ultrastructure of schizonts and merozoites of *C. canis* in the intestines of dogs examined on day 5 p.i. Merozoites divided by endodyogeny; no multinucleated stage was found. Thus, the mode of division of *C. canis* schizonts is not clear.

Dubey and Lindsay (2019) re-evaluated life cycle stages of *C. canis* in the small intestine of two dogs necropsied 10 days p.i. after oral inoculation with 100,000 oocysts in experiments reported by Mitchell et al. (2007). The dogs started excreting oocysts on the nineth day. Asexual and sexual development stages were seen throughout the small intestine. Developmental stages were seen only in villar lamina propria; deeper parts of mucosa and glands of Lieberkühn were not parasitized (Fig.1). The parasitized host cell (probably enterocyte) nucleus was indented but rarely hypertrophied. Merozoites of different sizes were present, often in the same vacuole (Fig. 2). They were arranged singly, in pairs (Fig. 2A), and many within a vacuole (Fig. 1D, E). The maximum number of nuclei within developing merozoites in a group was eight, but it could not be discerned if they were individual nuclei or parts of merozoites. The largest meront/group was 53 x 35 µm. Rarely, there were groups with many nuclei (Fig. 2F). At least 80 nuclei were present but it was not clear if the multinucleated stage was a schizont or immature microgamont. Individual merozoites were 12–15 µm long and 3–6 µm wide and varied in intensity of staining in the same histological section. Merozoites in some meronts were tightly packed without empty spaces whereas others were loosely arranged with empty spaces among merozoites. Occasionally, merozoites in meronts were as small as 5 µm long. Merozoites in all meronts contained periodic acid Schiff (PAS)-positive granules (Fig. 2H).

Gamonts were seen in the lamina propria, often just below the enterocytes (Fig.3). Immature microgamonts were PAS-negative (Fig.3A). The more advanced microgamont stage could be definitively distinguished from immature schizont by the presence of peripherally arranged nuclei (Fig. 3 C, D). and the presence of PAS-positive bodies (Fig. 3C). A large eosinophilic and PAS-positive residual body was present in mature microgamonts (Fig. 2E). The microgametes were PAS-negative.

The macrogamonts contained a centrally located nucleus with prominent nucleolus (Fig. 2C). The wall forming bodies (WFB) were probably too small to be identified definitely in hematoxylin and eosin (HE)-stained sections but eosinophilic granules (presumed WFB) were visible in some specimens (Fig. 3 D, G). The macrogamonts were intensely PAS-positive (Fig. 3A).

Findings of asexual stages a day after dogs had started excreting oocysts indicated continued asexual multiplication beyond the prepatent period. The stages found resemble the three generations reported by Lepp and Todd (1974). The mode of division of the asexual generations remains unclear. Lepp and Todd (1974) schematically depicted as many as 20 separate nuclei in schizonts and as many as 72 merozoites in a group, but the details are not clear in their text or illustrations.

The results of our re-evaluation indicated that there are many generations that are difficult to determine because of the multiplication of merozoites without leaving the host cell. It is evident that cat and dog coccidia (*Cystoisospora* spp.) divide by more than one type of division, including endodyogeny (Dubey, 1978b). Therefore, the term “types” instead of asexual generations was proposed by Dubey (1978b) because of numerous generations within a given host cell. This kind of division is different from a series of generations of schizonts in *Eimeria* species (Levine, 1973). Dubey and Frenkel (1972b) first proposed the term “type” for intestinal stages of *T. gondii* because there were many generations of the parasite in feline enterocytes in the intestine and we concur that this more descriptive than generations. The development of schizonts of *Eimeria* of poultry can be artificially manipulated to reduce

Table 1
Measurements (µm) of *Cystoisospora canis* oocysts.

Oocysts	Sporocysts	Country	Reference
36-44 × 29-36 (39 × 32, n = NS)	NS	Hungary	Nemeséri (1960)
35-42 × 27-33 (38 × 30, n = 50)	18-24 × 15-18 (n = 50)	USA	Levine and Ivens (1965)
32-40 × 23-35 (36 × 30, n = NS)	NS	Brazil	da Rocha and Lopes (1971)
34-40 × 28-32 (36 × 30, n = 50	18-21 × 15-18 (20 × 16), n = 50	USA	Lepp and Todd (1974)
36-41 × 27-36 (NS)	NS	Egypt	Hilali et al. (1979)
40-50 × 25-40 (NS)	20-25 × 18-20 n = NS	Egypt	Abdel-Magied et al. (1982)
35-39 × 27-32 (37 × 29, n = 25)	19-32 × 15-18 (21 × 16, n = 25	USA	Mitchell et al. (2007)

NS = not stated.

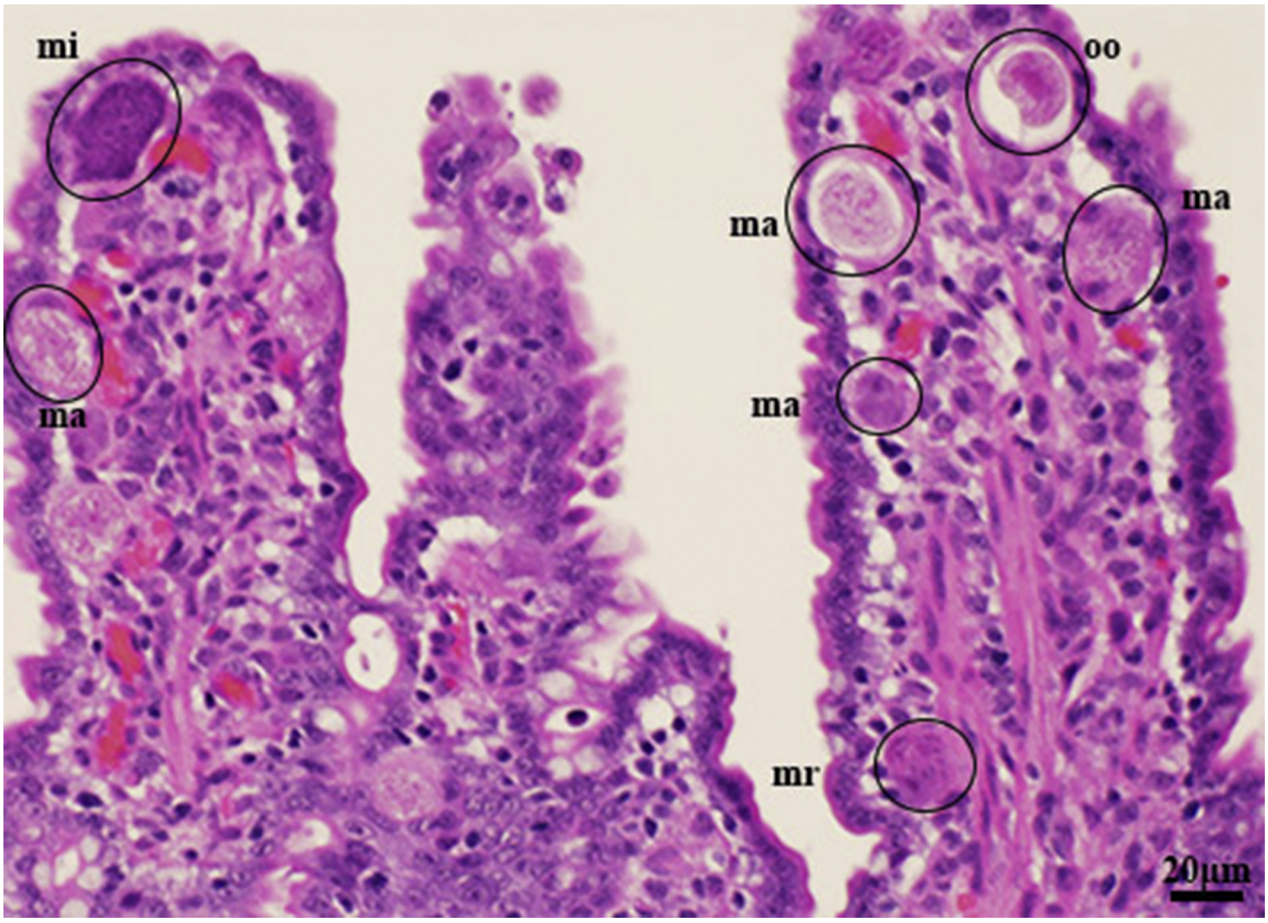

Fig. 1. Location of endogenous stages of *Cystoisospora canis* in jejunum of a dog, 10 days after oral inoculation with 100,000 oocysts. The stages are in lamina propria in the villous part. Note a meront (mr) at the base of the villous, and macrogamonts (ma), microgamont (mi), and oocyst (oo) just beneath the surface epithelium towards luminal part. Hematoxylin and eosin stain.

the size and location of schizonts and numbers of merozoites produced based on selection for precocious oocysts (i.e. earliest oocysts are selected) or oocysts that are serially passaged in chicken embryos (McDonald et al., 1986).

2.1.2. Paratenic hosts

Based on bioassays in dog, sheep, camel, donkey, pig, and water buffalo have been reported as paratenic hosts for *C. canis* (Hilali et al., 1992, 1995; Zayed and El-Ghaysh, 1998) Tissue cysts were identified and described ultrastructurally in the mesenteric lymph nodes of mice fed *C. canis* oocysts (Markus, 1983). Coyotes that ingested tissues of mice orally inoculated with *C. canis* oocysts excreted *C. canis* oocysts (Dubey, 1982).

2.1.3. In vitro cultivation

Cystoisospora canis and *C. ohioensis* sporozoites can be excysted in bile salts and trypsin like other coccidian parasites (Toyama et al., 1982; Fayer and Mahrt, 1972; Speer et al., 1973; Mitchell et al., 2009; Houk and Lindsay, 2013) and used for in vitro studies. Limited multiplication occurred by endodyogeny in several cell lines; schizonts were not seen (Fayer and Mahrt, 1972). Recent studies using *C. canis* sporozoites have demonstrated that monozoic tissue cysts would form in several cell lines of non-canine origin (Houk and Lindsay, 2013; Mitchell et al., 2009). (Fig. 4.). These monozoic cysts persisted up to 127 days, and zoites were motile after treatment with bile-trypsin excystation solution or acid-pepsin digestion solution (Fig. 4). The zoites entered new cells and became monozoic tissue cysts again. The tissue cyst wall was not dissolved by bile-trypsin but was by acid-pepsin solution (Houk and Lindsay, 2013). The ultrastructure of these in vitro monozoic tissue cysts (Houk and Lindsay, 2013; Mitchell et al., 2009) was virtually identical to those that formed in mouse mesenteric lymph nodes (Markus, 1983).

2.1.4. Experimentally-induced clinical disease

There is uncertainty concerning clinically induced coccidiosis in dogs (Table 2). Most experimentally infected dogs excreted large numbers of oocysts but were not ill. Dogs that excreted oocysts became immune and did not excrete oocysts after re-inoculation of oocysts (Lepp and Todd, 1974). Mitchell et al. (2007) reported weight loss, diarrhea, vomiting and lethargy in dogs fed 100,000 oocysts. Lesions included villous atrophy, and rare foci of inflammation. A focus of necrosis and denudation of the contents of lamina propria into the lumen are shown in Fig. 5. A microgamont, a merozoite and an oocyst are visible in luminal contents (Fig. 5).

Houk et al. (2013) demonstrated that dogs that previously excreted *C. ohioensis* were not immune to *C. canis*. Clinical signs were seen in some of the *C. ohioensis*-like infected dogs fed 100,000 *C. canis* oocysts. Some studies found association between diarrhea and *Cystoisospora* infections (Altreuther et al., 2011a; Buehl et al., 2006).

2.2. *Cystoisospora ohioensis (Dubey, 1975a, b) Frenkel, 1977*

2.2.1. Life cycle

Its life cycle has been studied in detail in newborn dogs fed oocysts or tissues of infected mice. Newborn dogs and littermates were used as controls to minimize extraneous coccidial infections. For the oocyst-

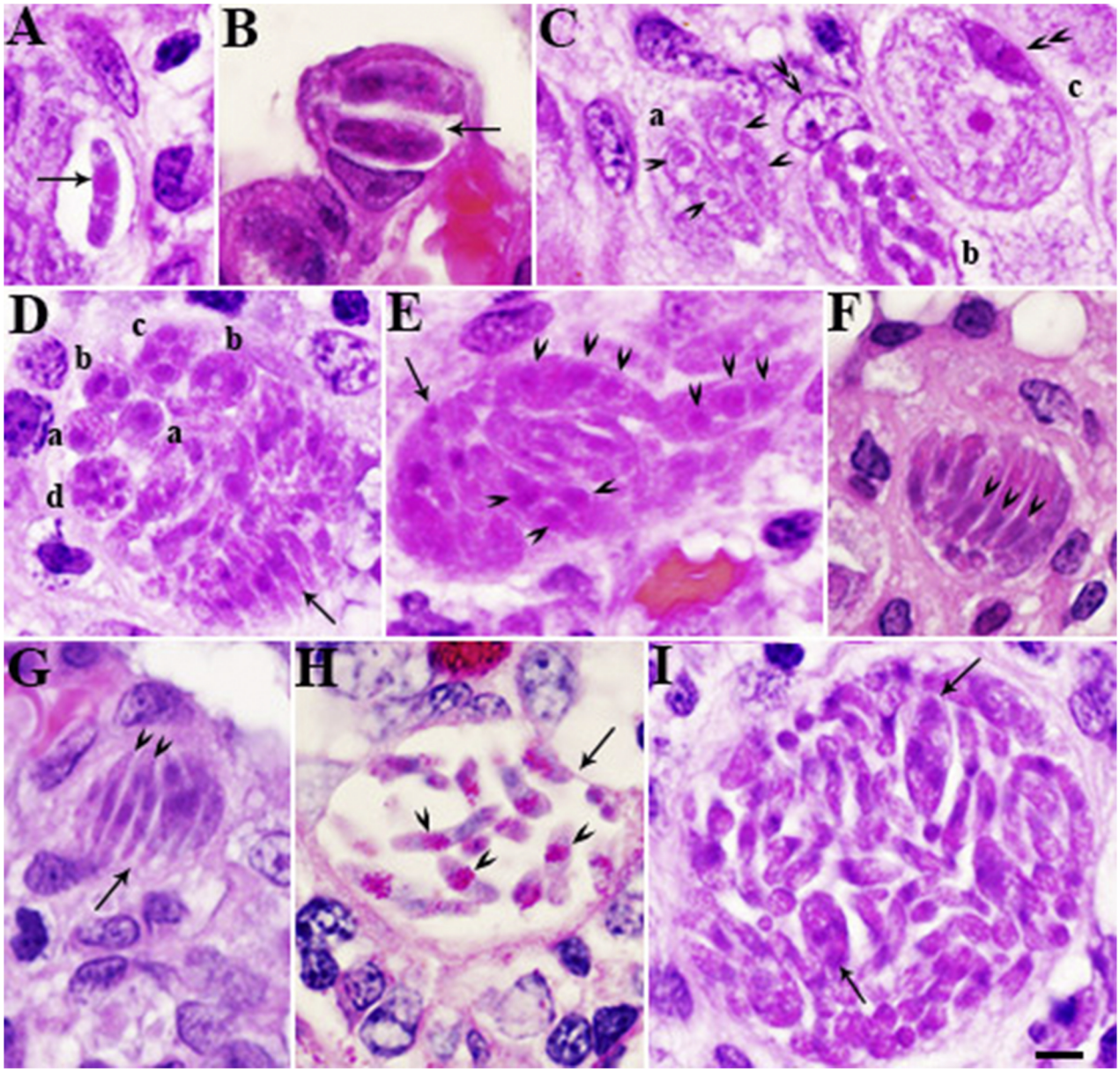

Fig. 2. Asexual stages of *Cystoisospora canis* in the lamina propria of small intestine of dogs 10 days after oral inoculation with 100,000 oocysts. A-G, I = Hematoxylin and eosin stain, H = periodic acid Schiff (PAS) with hematoxylin counter stain. Bar = 5 μm and applies to all parts. (**A**) Single uninucleated merozoite (arrow) in a parasitophorous vacuole (pv) (**B**) Two meronts in a pv (arrow). The nuclei are not clear. (**C**) Asexual and sexual stages. Double arrowheads point to host cell nucleus. (**a**) Merozoite-shaped meronts with large nuclei (arrowheads), (**b**) mature meront with merozoites, (**c**) a macrogamont with a prominent nucleolus. (**D**) Large pv containing uninucleated (**a**), binucleated (**b**), 4-nucleated (**c**), a meront with more than 5 nuclei (**d**) and several mature merozoites (arrow). (**E**) An elongated pv (arrow) containing merozoite-shaped meronts, each with 3 nuclei and other merozoites. (**F**) A mature meront with merozoites that have subterminal nuclei (arrowheads). (**G**) Meront (arrow) with slender merozoites (arrowheads). (**H**) Meront (arrow) with many loosely arranged merozoites. Note PAS-positive granules (arrowheads). (**I**) Large pv with many merozoites and at least 2 developing meronts (arrows).

induced infections, eight dogs were euthanized 6–120 h after feeding 1 million *C. ohioensis* sporocysts to each (Dubey, 1978a); to facilitate excystation of sporozoites in the gut of newborn pups, sporocysts had been liberated mechanically from sporulated oocysts. Irrespective of the inocula, stages were confined to surface epithelial enterocytes (Fig. 6). First divisional stages occurred in the jejunum at 48 h post-inoculation (hpi). At 48 hpi, zoites occurred in pairs in parasitophorous vacuoles (pv) of surface epithelial cells of the jejunum. The pv were 7–9 x 6 μm and the zoites were 7–9 × 2–5 μm in sections. At 72 hpi, uninucleated zoites, multinucleated zoites and meronts containing fully formed merozoites occurred in surface epithelial cells of the jejunum. At 96, 114 and 120 hpi, asexual multiplication occurred throughout the small and large intestine, mostly in the ileum. The number of asexual generations was not determined. At least two structurally distinct meronts were identified at 96–120 hpi. Type I meronts contained larger merozoites (11 x 3 μm) than those in Type II meronts (7-5 x 1-5 μm). Meronts were merozoite-shaped and contained up to 8 nuclei (Fig. 7 A–F); multinucleated round meronts were not seen. Uninucleated, binucleated and multinucleated zoites occurred within the same pv. Gamonts occurred in surface epithelial cells of the small intestine, cecum and colon, but predominantly in the ileum, 96–120 hpi. Macrogamonts were 13–17 × 11–13 (14.5 × 12.8) μm in sections and 21–26 × 17–25 (21.7 × 17.6) μm in smears. Microgamonts were 13–17 × 8–15 (15.3 × 11.4) μm in sections and 24–30 × 15–24 (27 × 19) μm in smears and contained up to 50 microgametes (Fig. 7 H).

The tissue cyst-induced cycle was also studied in six newborn dogs;

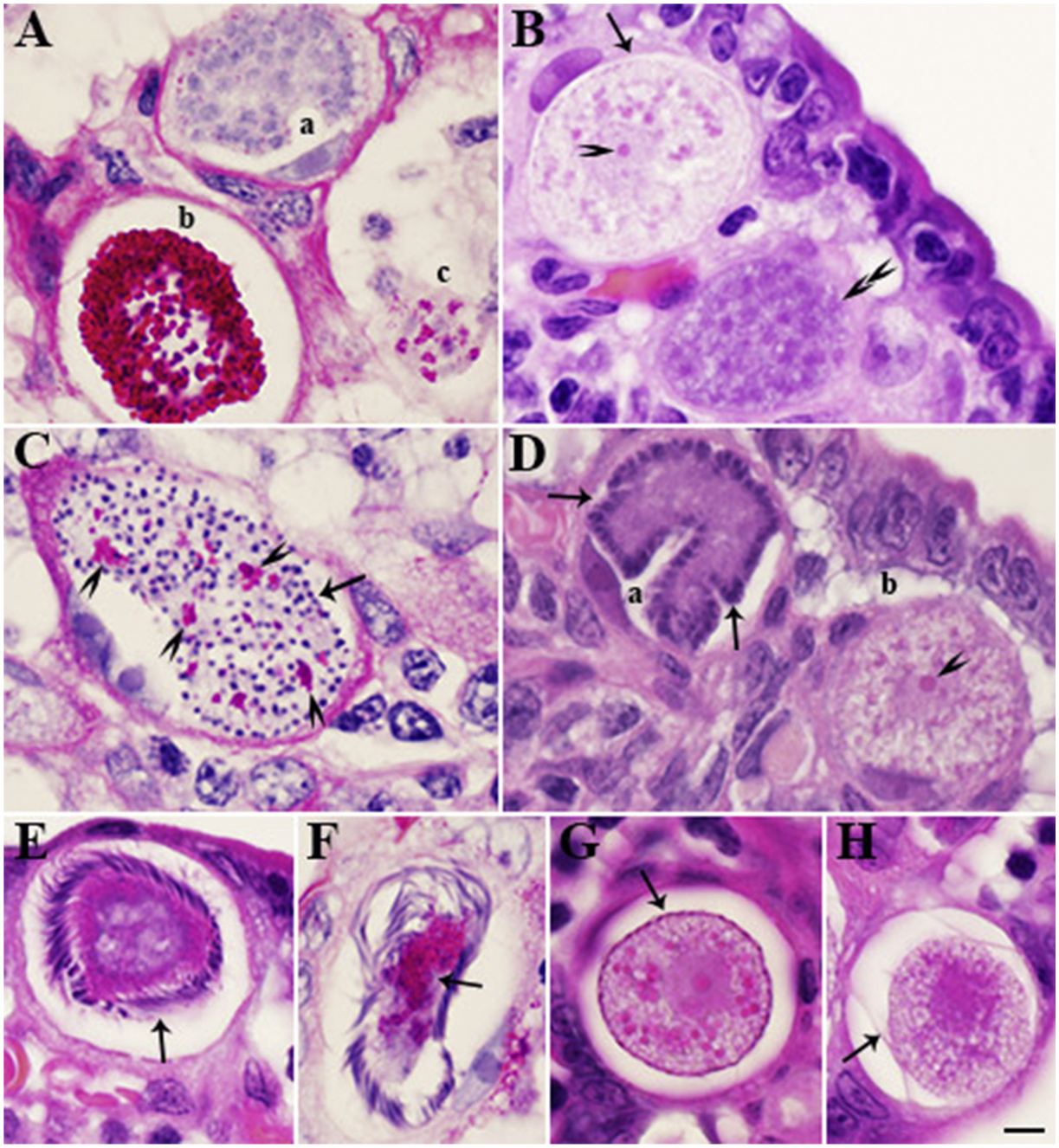

Fig. 3. Sexual stages of *Cystoisospora canis* in histological sections of small intestine of dogs 10 days after oral inoculation with 100,000 oocysts. A, C, F = periodic acid Schiff (PAS) reaction counterstained with hematoxylin, B, D-E, G, H = hematoxylin and eosin stain. Bar = 5 µm and applies to all parts. All stages are in the lamina propria. (**A**) Multinucleated structure (a) considered microgamont is PAS-negative, oocyst with intensely PAS-positive sporont (b), and a mature meront (c) with 5 merozoites with PAS-positive granules. (**B**) Macrogamont (arrow) with a prominent nucleolus (arrowhead) and few granules in the cytoplasm. Also, note a microgamont-like structure (double arrowheads). (**C**) Microgamont with numerous condensed nuclei (arrow) and few residual body-like PAS-positive structures (arrowheads). (**D**) Microgamont (a) with peripherally located nuclei (arrows) and a macrogamont (b) with a large nucleolus (arrowhead). (**E**) Microgamont (arrow) with a large residual body. (**F**) Microgamont (arrow) with prominent PAS positive bodies in the cytoplasm. (**G**) Macrogamont (arrow) with prominent nucleus and eosinophilic bodies in the cytoplasm. (**H**) Oocyst with crumpled oocyst wall (arrow).

the dogs were euthanized 6–120 h after feeding tissues of laboratory mice that had been infected with C. *ohioensis* oocysts 17 days earlier. Stages were not seen in the dogs killed at 6 and 12 h p.i. Asexual and sexual stages were structurally similar in size and location to the oocyst-induced cycle but their development was faster by 24 hpi in the mouse-induced cycle. Oocysts were excreted unsporulated (Fig. 7J).

Extra-intestinal stages were not found in sections of mesenteric lymph nodes, spleen, lung, liver, heart, skeletal muscle or brain of dogs. However, biological evidence indicated that *C. ohioensis* invaded the spleens and mesenteric lymph nodes of dogs fed oocysts; dogs fed individual extraintestinal tissues excreted oocysts (Dubey, 1978a). Oocysts sporulated within 96 h (Baek et al., 1993).

2.2.2. *Paratenic hosts*

Mice, sheep, camel, donkey, pigs, and water buffalo can be paratenic hosts for *C. ohioensis*. Tissue cysts were identified in mesenteric lymph nodes of mice fed *C. ohioensis* oocysts (Dubey and Mehlhorn, 1978) (Fig. 8). Dogs fed tissues of experimentally infected mice

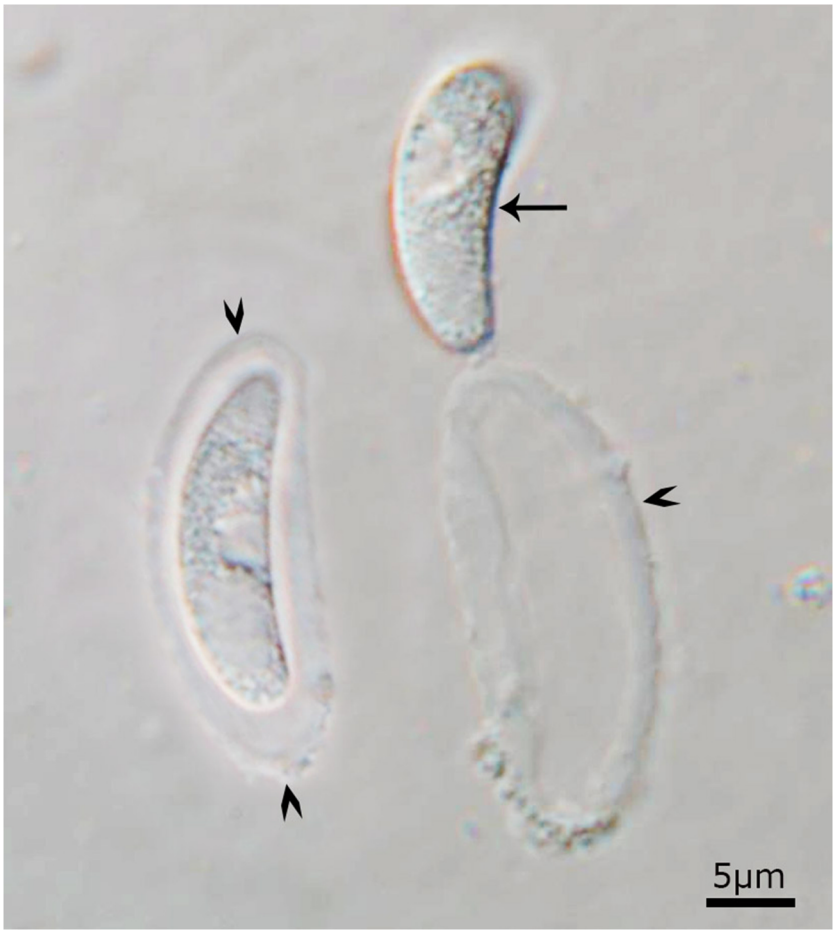

Fig. 4. Tissue cysts of *Cystoisospora canis* in cell culture. A tissue cyst (opposing arrowheads), excysted sporozoite (arrow), and an empty cyst (arrowhead).

excreted *C. ohioensis* oocysts (Dubey, 1978a). Dogs fed tissues of naturally exposed tissues of camel (Hilali et al., 1992, 1995), sheep (Hilali et al., 1992) swine, donkey, and water buffalo (Zayed and El-Ghaysh, 1998) excreted *C. ohioensis* oocysts; however, there was no microscopic confirmation of tissue cysts. Tissue cysts can be excysted by pepsin digestion (Oliveira et al., 2001).

2.2.3. Pathogenicity and immunity

In experimentally infected young pups, *C. ohioensis* was pathogenic (Dubey, 1978b; Buehl et al., 2006). The inoculated pups developed diarrhea. Histologically, stunted villi, necrosis and desquamation of villar tips were found (Dubey, 1978b). Weaned pups inoculated with *C. ohioensis* became immune as judged by lack of excretion of oocysts after challenge (Dubey, 1978b).

C. ohioensis-associated diarrhea was reported in a three-month-old dog from Korea; the diagnosis was confirmed by PCR (Lee et al., 2018).

2.3. Cystoisospora neorivolta (Dubey and Mahrt, 1978) Frenkel, 1977

2.3.1. History and background

Mahrt (1967; Mahrt, 1968) microisolated an oocyst and developed a cloned culture of *C. rivolta*-like parasite from the feces of a dog in Illinois, USA as part of his Ph.D. dissertation. Transmission of this isolate of *C. rivolta* from a dog to cats was inconclusive because cats were not raised coccidia-free (Mahrt, 1966). He studied endogenous stages in coccidia-free dogs killed 24–144 hpi. The prepatent period was six days. Endogenous stages were seen in mainly in the small intestine and rarely in cecum and colon. Stages were in the lamina propria (Fig. 9). Initially, only one generation of schizonts was recognized. The stages described differed from the stages of *C. ohioensis* described by Dubey (1978b). Unfortunately, Mahrt did not record the measurements of oocysts and the culture had been destroyed by the time *C. ohioensis* oocysts were described (Dubey, 1975b). Fortunately, histological sections from the study by Mahrt were available and were reevaluated by Dubey. Information is summarized in Table 3. Based on these observations, a new

Table 2
Experimentally induced *Cystoisospora canis* coccidiosis in dogs.

Dose	*C. canis* isolate	Breed of dog	Number of dogs inoculated	Age of dogs	Clinical signs	Reference
5×10^3	Hungary	Not stated	Not stated	Not stated	No	Nemeséri (1960)
$5\text{-}8 \times 10^4$	Hungary	Not stated	Not stated	Not stated	Yes	Nemeséri (1960)
$1\text{-}1.5 \times 10^5$	Illinois	Not stated	25	6-8 weeks	No	Lepp and Todd (1974)
$6 \times 10^2\text{-}2 \times 10^4$	Austria	Beagle	26	3 weeks	Yes	Buehl et al. (2006)
1×10^5	Virginia	Beagle	22	6-8 weeks	Yes	Mitchell et al. (2007)
$2.5\text{-}5 \times 10^4$	Virginia	Beagle	81	8-9 weeks	No	Reinemeyer et al. (2007)

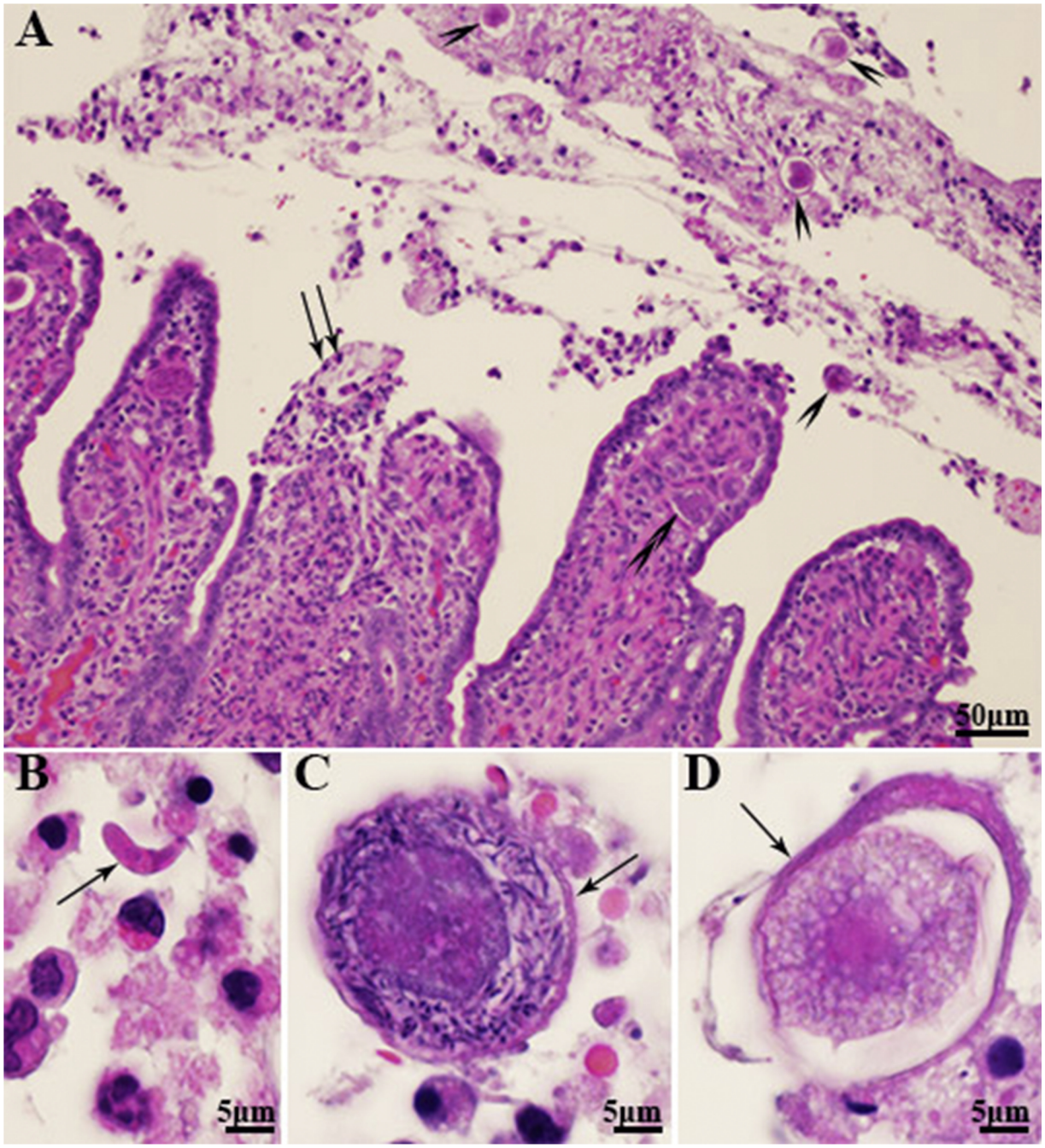

Fig. 5. Enteritis in a dog 10 days after oral inoculation with 100,000 *Cystoisospora canis* oocysts. Hematoxylin and eosin stain. (**A**) Focal desquamation of epithelium (double arrow) and exudation of lamina propria contents including parasite stages (arrowheads) into lumen. Double arrowheads point to parasite stages in the lamina propria. (**B**) Merozoite (arrow) with inflammatory cells in lumen. (**C**) Degenerating microgamont (arrow). (**D**) Oocyst (arrow) in lumen.

name, *C. neorivolta* was proposed for the Mahrt coccidian. There is no other information available regarding this parasite. The tissue stage of *C. neorivolta* is unknown.

Dogs experimentally infected with *C. neorivolta* remained asymptomatic (Mahrt, 1967).

2.4. *Cystoisospora burrowsi* (Trayser and Todd, 1978) Frenkel, 1977

Trayser and Todd (1978) described life cycle of a coccidian using a cloned culture of oocysts in Illinois, USA. The oocysts were smaller than those of *C. ohioensis* (Table 3). Rommel and Zielasko (1981) found oocysts of *C. burrowsi* in Germany and contributed to its life cycle. They found that mice and rats can act as its paratenic hosts (Rommel and Zielasko, 1981). The prepatent period was 7–11 days after ingestion of infected mouse tissues (Rommel and Zielasko, 1981) like to that after ingestion of oocysts (Trayser and Todd, 1978). Oocysts were 17–22 × 16–19 (20.3 × 17.3) µm as per Trayser and Todd (1978) and 16–23 × 14–22 (mean 20.6 × 18) µm as per Rommel and Zielasko (1981); the size did not change after three passages in dogs (Rommel and Zielasko, 1981). Dogs experimentally infected with *C. burrowsi* remained asymptomatic.

2.5. *Cystoisospora ohioensis-complex*

It is not certain whether *C. burrowsi* is different from *C. neorivolta*. There are no archived specimens of *C. burrowsi.* Attempts by one of us

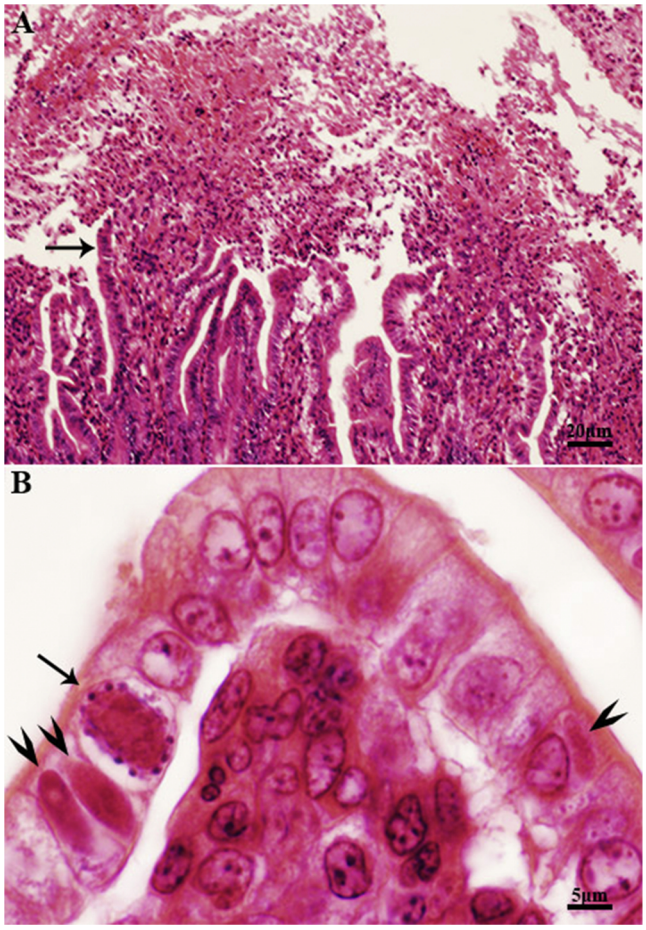

Fig. 6. Severe enteritis and endogenous stages of *Cystoisospora ohioensis* in surface epithelium of the small intestine of an experimentally infected dog. Hematoxylin and eosin stain. (**A**) Outpouring of the contents of lamina propria into the gut lumen. Arrow points to a meront; stages are not clearly visible at this magnification. (**B**) Higher magnification of an intact villus. Note, microgamont (arrow) and meronts (arrowheads).

(Dubey) in 1978 to obtain histologic material from Dr. K.S. Todd were unsuccessful (Dubey, 1978 unpublished). Initial life cycles of both *C. neorivolta* and *C. burrowsi* were studied in the same laboratory. As said earlier, Mahrt did not measure the oocysts he used to describe life cycle and the histological description of schizonts in both studies are incomplete. The comparison of schizonts that Trayser and Todd (1978) made between Mahrt's coccidian and *C. burrowsi* is not valid because of incomplete description of schizonts in both studies. Therefore, the real identity of these species will remain unknown. Therefore, for diagnostic purposes, this group of parasites is called *C. ohioensis*-like.

2.5.1. Endogenous development of a C. ohioensis-like organism in a naturally infected dog

Dubey et al. (1978) reported clinical coccidiosis in a dog in association with a *C. ohioensis*-like organism. It differed structurally and biologically from *C. ohioensis* (Table 3). Asexual and sexual stages were in the lamina propria and surface epithelium of small intestine, cecum and colon, and glands (Fig. 10). The merozoites and meronts stained poorly by hematoxylin and eosin and details were often not visible even at 1000x magnification. More details could be seen in smears made from infected intestine (Fig. 11). Ultrastructurally, meronts contained merozoites and multinucleated structures within the same pv (Fig.12) (Dubey, 2018). The case is of interest because the development of the parasite is described in a naturally-infected dog, and lesions were associated with the *C. ohioensis*-like parasite.

3. Transmission of *Cystoisospora* spp.-like oocysts from wild canids to domestic dogs

Wild canids are thought to share *C. canis* and *C. ohioensis*-like oocysts but little is known of their cross transmission to the domestic dog. *C. canis* of domestic dog was transmitted successfully to coyote (*Canis latrans*) (Dubey, 1982). For this, eight Swiss Webster mice were each inoculated with 100,000 *C. canis* oocysts from dog feces. Fourteen days later, infected mice were fed to 4 laboratory-reared coccidia-free 3.5 months old coyote pups; two littermate coyotes served as uninoculated controls. The inoculated coyotes excreted *C. canis* oocysts with a prepatent period of 8 or 9 days; the control pups did not excrete oocysts. Transmission via infected mice was done to avoid spread of infection via oocysts.

Bledsoe (1976) transmitted *Cystoisospora vulpina* (Syn. *Isospora vulpina*) from silver foxes (*Vulpes vulpes*) to the domestic dog; *C. vulpina* oocysts are morphologically *C. canis*-like. Five coccidia-free dogs fed oocysts from the fox excreted *C. vulpina* oocysts with prepatent period of 6 or 7 days; 1 control uninoculated dog did not excrete oocysts. These studies indicate that these parasites might not be strictly host-specific.

4. Clinical coccidiosis

Before the recognition of enteric viral pathogens of dogs and effective immunization against distemper virus infection in the 1960's, coccidiosis was considered a common disease affecting as many as 50% of dogs (Hall and Wigdor, 1918; Lee, 1934; Emmerson and Dashoff, 1943; Catcott, 1946; Huang et al., 1954; Smith and Edmonds, 1959; Levine, 1964). An excellent account was given by Perry (1952) who listed individual case histories of 56 dogs presented in the past five years at the Small Animal Clinic at the Tuskegee Institute School of Veterinary Medicine that opened in 1947. In the last 50 years there are only a few reports of clinical coccidiosis in dogs (Dubey et al., 1978; Oduye and Bobade, 1979; Correa et al., 1983; Olson, 1985; Wu et al., 1993; Penzhorn et al., 1992; Randhawa et al., 1997; Conboy, 1998; Junker and Houwers, 2000; Gal et al., 2007). Depression, weakness, loss of appetite, diarrhea and dehydration were the common clinical signs and a positive response to anticoccidial therapy was considered to support diagnosis (Randhawa et al., 1997). Concurrent infections with other pathogens complicated the diagnosis (Gal et al., 2007). In a clinic in Brazil, 66 cases of coccidiosis were diagnosed between 1972 and 1978 (Correa et al., 1983). These cases occurred throughout the year and clinical signs persisted 1–15 days. Abdominal pain (100%), vomiting (25%), diarrhea (90%), and anorexia (80%) were present in 97% of cases. Neurological, respiratory and circulatory disturbances were seen in more than 50% of cases. Therefore, it is uncertain if all clinical signs were due to coccidiosis. Coccidiosis was considered the sole disease in 51% of cases, and in the remaining 49% concurrent infections or other maladies were noted including canine distemper virus infection in 7%, hookworms in 32%, *Dipylidium caninum* infections in 4%, and organic insecticide poisoning in 6% (Correa et al., 1983). Olson (1985) provided detailed clinical history of 3 dogs with coccidiosis; the dogs were 3, 5, and 22 months old and repeated fecal examinations were needed for diagnosis of *C. ohioensis* infection. All three dogs responded to anti-coccidial therapy. Retrospectively, 21 other cases of clinical coccidiosis were diagnosed in years from 1969 to 1980 at his institution and *C. ohioensis* was the most common cause (18 of 21 cases) and *C. canis* was diagnosed in 5 cases (Olson, 1985).

An outbreak of coccidiosis associated with *C. ohioensis* in puppies housed in a kennel was reported by Oduye and Bobade (1979). The dogs were reported to have hemorrhagic diarrhea with abdominal pain and dehydration. However, the pups were mongrels and positive response to anticoccidial therapy was the main diagnostic criterion.

By far the most striking case with a definitive diagnosis of clinical coccidiosis due to *C. ohioensis*-like organism was in a 10 weeks-old Chihuahua pup with a history of weight loss. The pup had yellow pasty

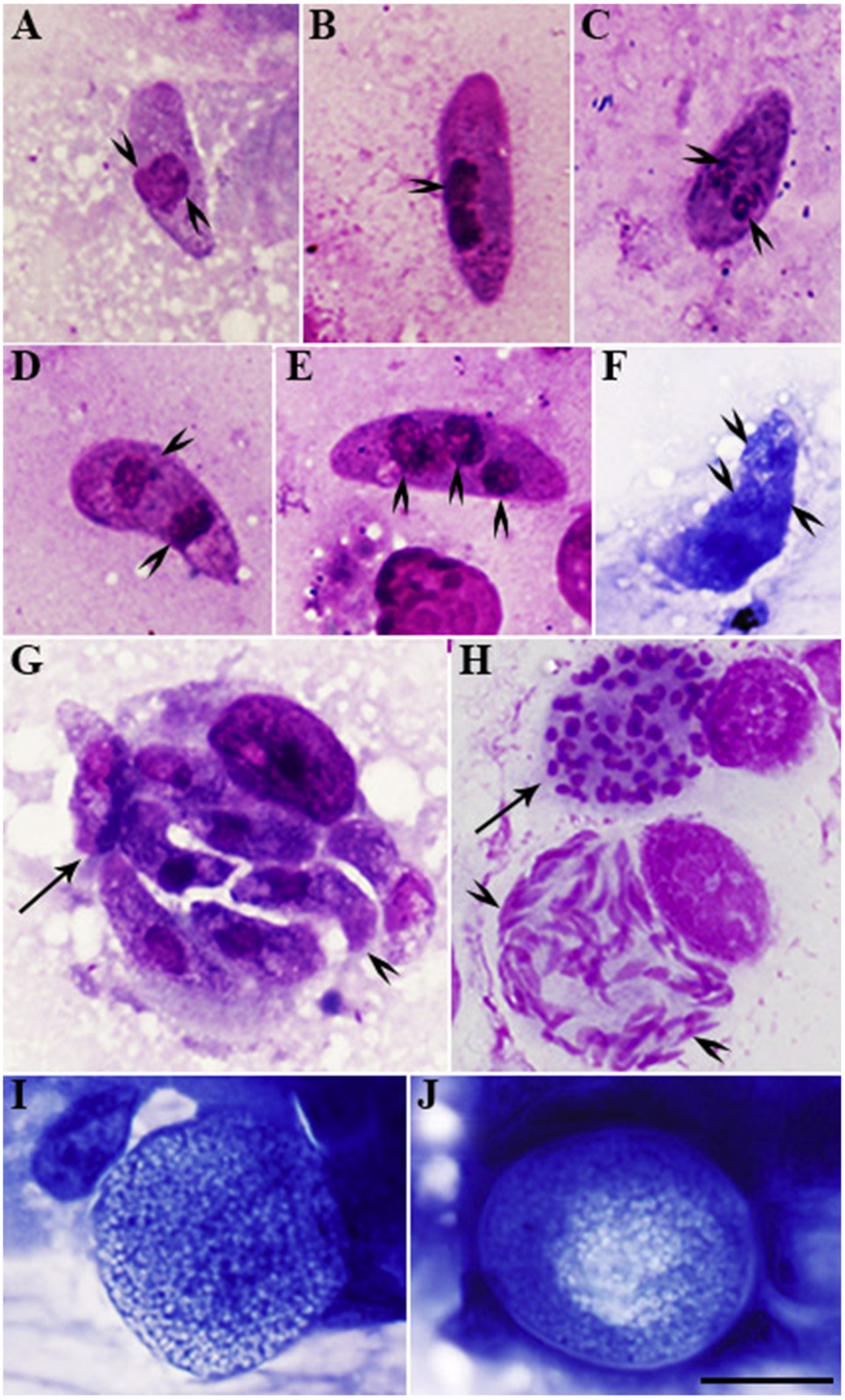

Fig. 7. Asexual and sexual stages of *Cystoisospora ohioensis* in smears of intestine of experimentally infected dogs (Dubey, 1978a). Giemsa stain. Bar = 10 μm and applies to all parts. (**A-D**) Division of nuclei into 2 nuclei (arrowheads). Two daughter merozoites are visible within 1 merozoite in Fig. 7 C. (**E**) Merozoite-shaped meront with 3 nuclei (arrowheads). (**F**) Meront with more than 4 nuclei (arrowheads). (**G**) Meront (arrow) with at least 8 mature merozoites (arrowhead). (**H**) An immature microgamont (arrow) and a mature microgamont with microgametes (arrowheads). (**I**) Macrogamont. (**J**) Oocyst.

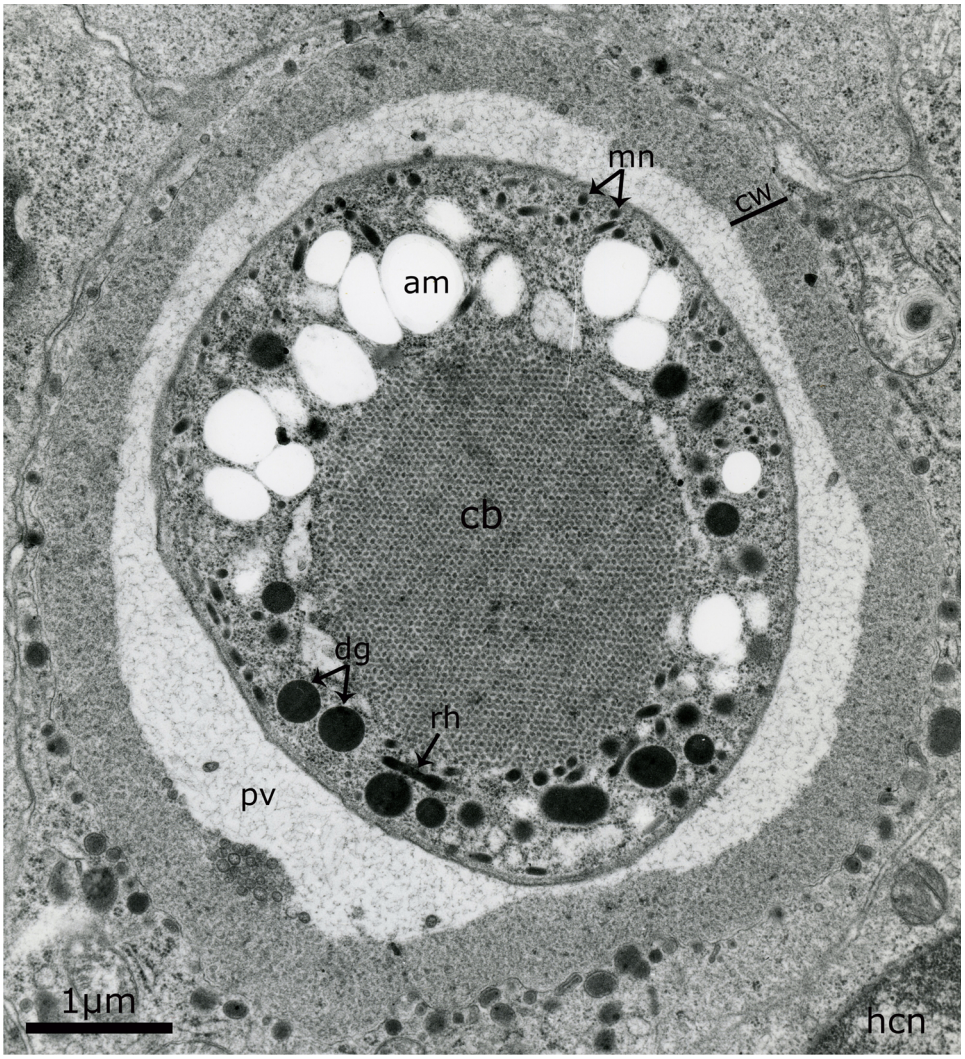

Fig. 8. Transmission electron micrograph of tissue cyst of *Cystoisospora ohioensis* in mesenteric lymph node of an experimentally infected mouse. Note cross/oblique section of sporozoite containing a large crystalloid body (cb), numerous amylopectin granules (am), a rhoptry (rh), and dense granules (dg). The sporozoite is enclosed in a parasitophorous vacuole (pv) surrounded by a cyst wall (cw). (Modified from Dubey and Mehlhorn, 1978).

feces and died despite sulfaguanidine therapy for two days (Dubey et al., 1978). Severe enteritis was associated with asexual and sexual stages of the *C. ohioensis*-like organism.

5. Epidemiology

Coccidia are prevalent worldwide (Table 4). The ingestion of food and water contaminated with oocysts is the major mode of transmission of *Cystoisospora* infections in dogs. Although *Cystoisospora* stages occur in extra-intestinal organs, there is no evidence for lactogenic or congenital transmission. The role of paratenic hosts in the natural epidemiology of coccidial transmission is unknown. Experimentally, newborn puppies are susceptible to infection. Coccidiosis is a disease of young dogs and most become infected by 4 months of age (Buehl et al., 2006).

A detailed epidemiological investigation was conducted by Penzhorn et al. (1992) in a commercial kennel in South Africa. The kennel was well managed and the bitches were housed individually on cement floors with an outside. Feces were collected from all 34 brood bitches; their feces were collected 2 weeks before the expected partus and until 6 weeks post-partum. Feces from pups were collected 2–3 weeks after birth and were tested as pooled samples; feces of pups were licked/eaten by their dams. Oocysts were counted in 1 g samples using McMaster chambers. The following observations were made: (a) Coccidial oocysts were detected in 26% of 484 samples from bitches and 51.7% of 387 pooled samples from pups —the species identified were *C. canis* 26.4%, and *C. ohioensis*-like in the remaining samples. (b) During the first 2 weeks post-partum oocysts were recovered from only 4 bitches and in low numbers. (c) During the 2nd and 3rd week oocysts were recovered from all but 1 bitch. (d) Oocysts were detected in pups starting at 3 weeks and by week 5 oocysts were seen in 15 of 16 litters. The oocyst excretion peaked at 5 weeks reaching 139,600 per gram of feces. (e) There was no pattern of association between oocyst excretion in pups and bitches. (f) Diarrhea was recorded in 17 of 34 litters. (g). The authors concluded that the most likely source of infection in pups was the oocysts excreted by their dams.

Similar results were reported by Daugschies et al. (2000) in a follow up of coccidial infections in bitches and their litters in Germany. In the first trial, oocysts were detected in feces of 3% of bitches and 80–100% of 104 litters; pooled feces were tested weekly until 7 weeks of age. Feces of bitches were tested before and after parturition. Similar findings were noted dogs from a second trial of 110 puppies from 21 litters from 1 breeding facility; most (93%) had *C. ohioensis*-like oocysts.

In an Austrian study, of the 980 dogs whose ages were known, most infected dogs were positive by 4 months of age and dogs older than one year rarely (< 1%) excreted oocysts (Buehl et al., 2006). In another

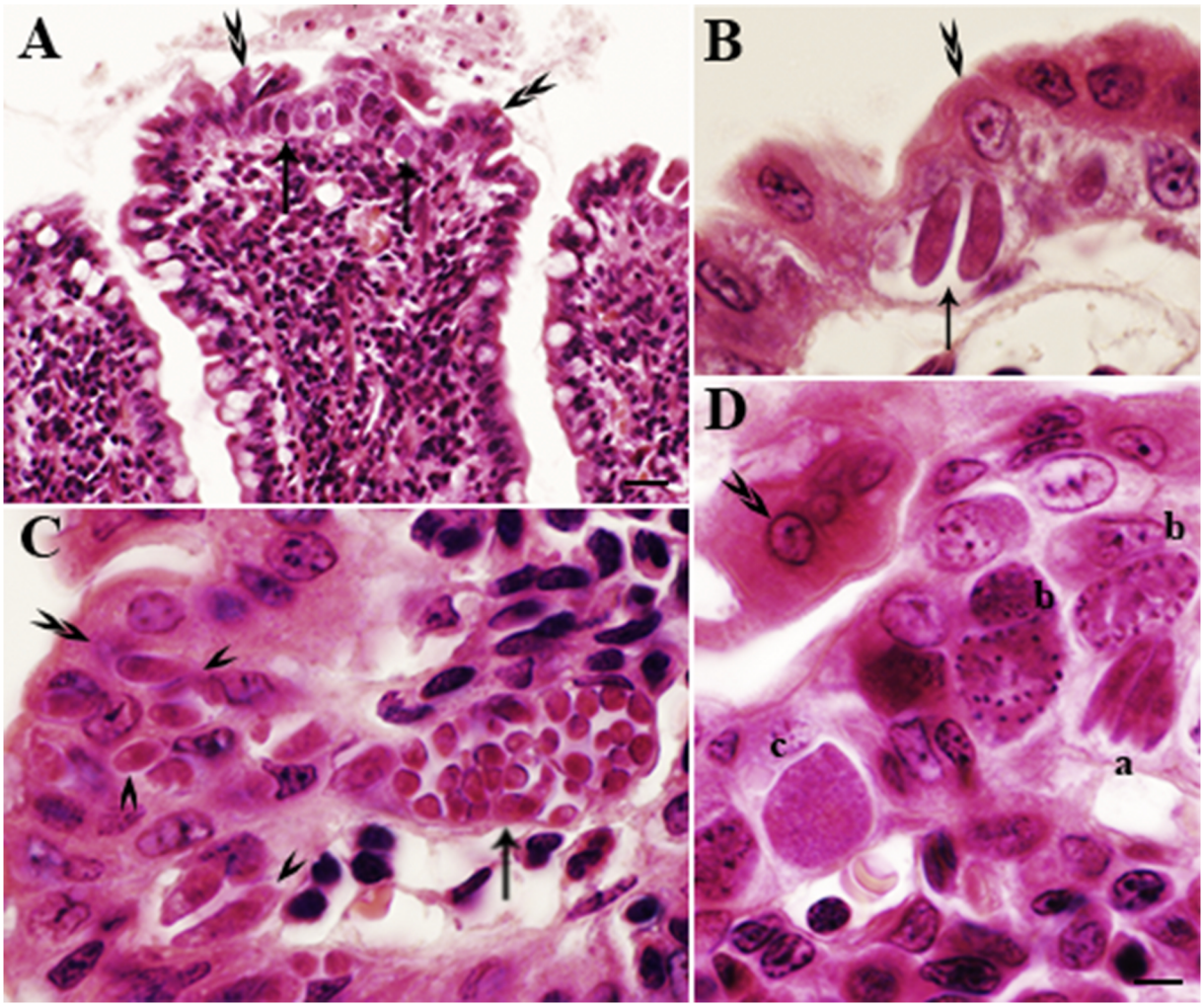

Fig. 9. Asexual and sexual stages of *Cystoisospora neorivolta* in the ileum of an experimentally infected dog (from Dubey and Mahrt, 1978). Hematoxylin and eosin stain. The surface epithelium is indicated by double arrowheads. (**A**) Endogenous stages in the lamina propria (arrows) just beneath the epithelium. (**B**) Two merozoite-shaped meronts in a parasitophorous vacuole (arrow). (**C**) An elongated meront (arrow) with many mature merozoites. Arrowheads point to several individual merozoites and meronts. (**D**) Mature meront (a), microgamonts (b), and a macrogamont (c) in the lamina propria. Double arrowheads point to hypertrophic /enlarged host cell nucleus of an enterocyte. Bar in A = 20 µm, Bar in B, C, D = 5 µm.

Table 3
Comparison of 4 *Cystoisospora ohioensis*-like organisms (modified from Dubey et al., 1978).

	C. ohioensis	*C. neorivolta*	*C. burrowsi*	*C. ohioensis*-like
Location of endogenous stages	Entire length	Distal one-half	Distal three-fifths	Distal one-half
Small intestine	Epi	LP + Epi	LP + Epi	LP + Epi
Vilus				
Number of asexual generations	4 or more	4 or more	2	2 or more
Meronts	Present	Present	Not reported	Present
Multinucleated zoites				
Microgamonts	13-17[a] x 8-15	10-22 × 7-18	13-27 × 10-21	7-12 × 5-10
Sections	(15.3 × 11.4)	(14.2 × 10.1)	(19.8 × 14.1)	(9.6 × 7)
Smears	24-30 × 15-24	No data	No data	15-25 × 9-19
	(21.7 × 17.6)			(19.5 × 15.6)
Macrogamonts	13-17 × 11-12	11-15 × 9-13	11-25 × 8-18	10-16 × 9-13
Sections	(14.5 × 12.8)	(12.6 × 10.8)	(17.1 × 11.5)	(12.5 × 10.8)
Smears	21-26 × 17-25	No data	No data	12-24 × 12-21
	(21.7 × 17.6)			(18-1 × 15.7)
Oocysts	15-19 × 13-16	13-15 × 11-13	No data	12-17 × 10-13
Sections	(16.5 × 14.6)	(12.6 × 11.4)	No data	(13 × 11.5)
Smears	18-27 × 16-25	No data		16-23 × 14-20
	(22.3 × 20.6)			(19 × 16.4)

(Abbreviations: Epithelium (Epi), Lamina propria (LP) + Epi).
[a] range (average), measurements in µm).

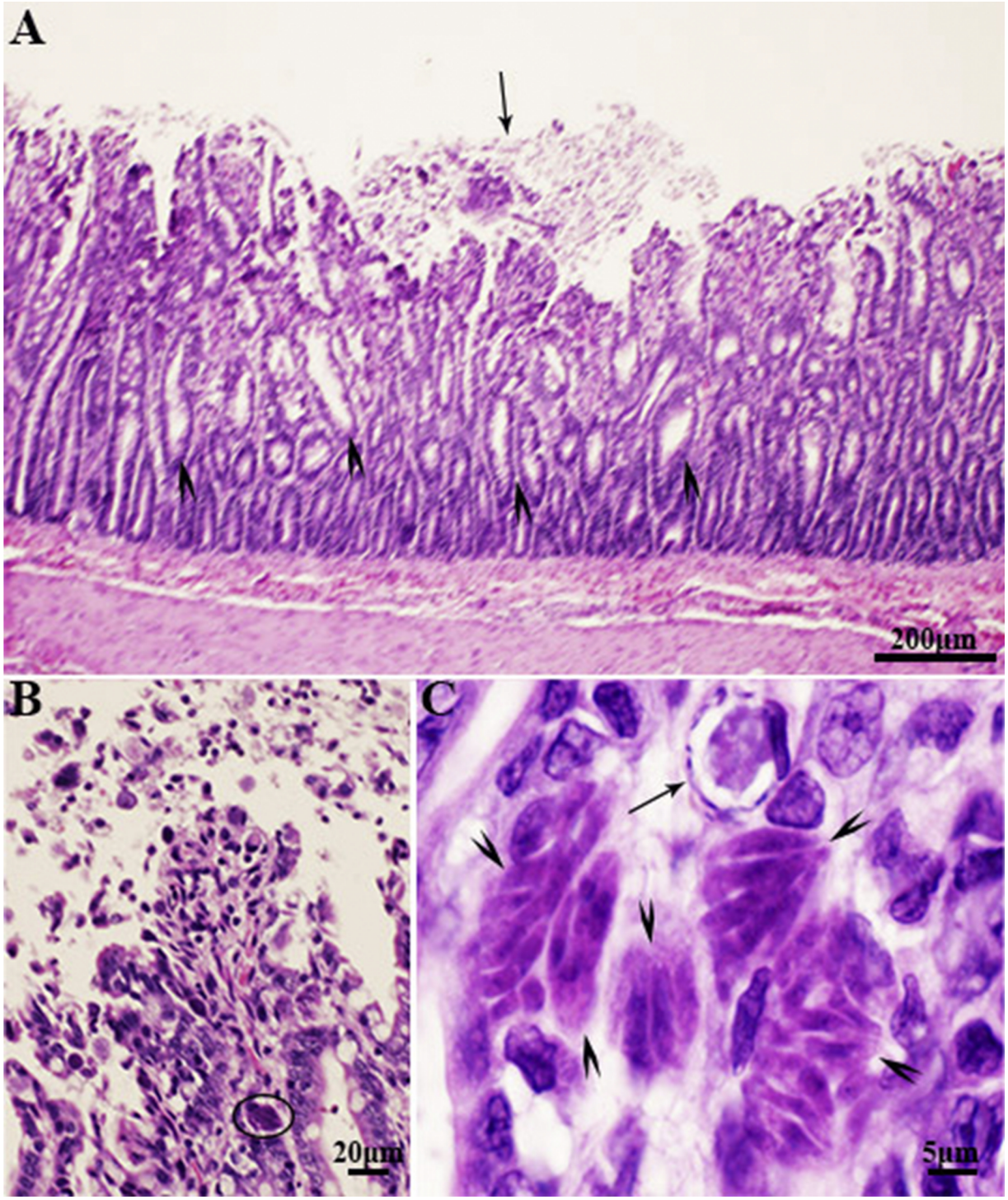

Fig. 10. Colitis in a naturally infected dog in association with a *C. ohioensis*-like organism from the case reported by Dubey et al. (1978). Hematoxylin and eosin stain. (**A**) Low magnification to show focal desquamation (arrow) and dilated gland (arrowheads). (**B**) Higher magnification to show desquamated host cells and a meront (circled). (**C**) Higher magnification of meronts (arrowheads) and a microgamont (arrow) in the lamina propria.

study from Austria, three-week-old puppies were already excreting oocysts; as many as 95,250 oocysts per gram were detected in some pups (Rauscher et al., 2013)

These findings obtained on dogs from breeding establishments were corroborated by Barutzki and Schaper (2013) in a very large sample of pet dogs. Among the 24,677 feces of dogs submitted to a commercial diagnostic laboratory (Barutzki and Schaper, 2011), data from 2319 dogs up to 1 year of age were analyzed with respect to enteropathogens. *Cystoisospora* spp. oocysts were first seen at the beginning of 4 weeks of age. Peaks of infection were at week 6 for *C. ohioensis*-like organisms, and week 15th for *C. canis*. Co-infections of *Toxocara canis*, *Giardia* sp. were seen starting at 6 weeks of age.

In a study of families of dogs in Germany, *Cystoisospora* spp. oocysts were detected in feces of 41 of 50 litters and in 25 of 37 bitches (Gothe and Reichler, 1990a, b); the prevalence of *C. canis* (16 litters) was much lower than that of *C. ohioensis*-like (41 litters) parasites.

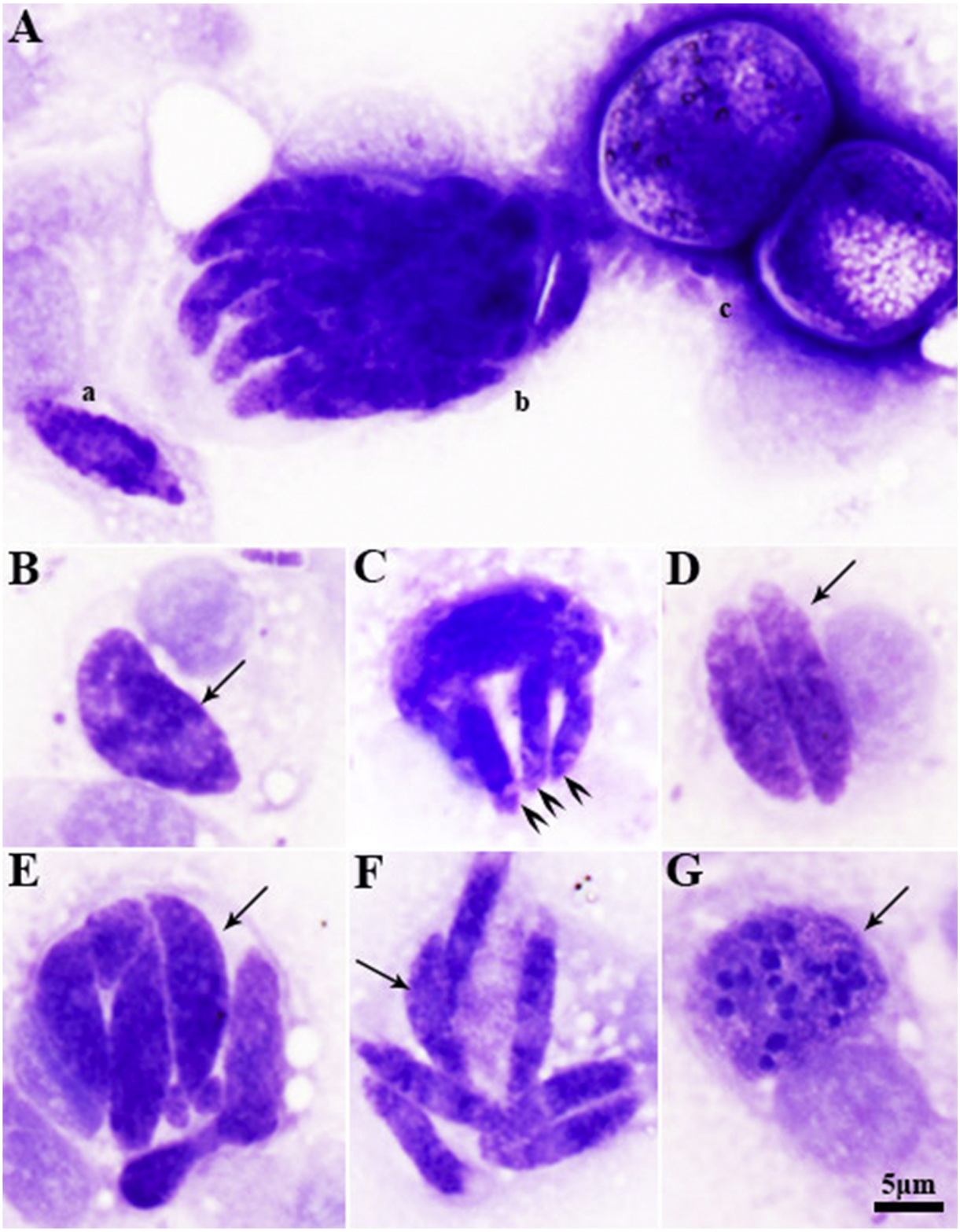

Fig. 11. Endogenous stages of a *C. ohioensis*-like organism in intestinal smears of a naturally infected dog. Giemsa stain. (**A**) Meront/gamont (a), meront with budding merozoites (b) and 2 oocysts (c). (**B**) A pear shaped meront (arrow) with more than 6 nuclei. (**C**) Merozoites (arrowheads) budding from a multinucleated mass. (**D**) Pair of intracellular merozoites (arrow). (**E**) mature meront with merozoites. (**F**) Slender merozoites (arrow). These merozoites are thinner than merozoites in Fig. 2 E. (**G**) Immature microgamont with many nuclei (arrow).

6. Diagnosis

Cystoisospora infection in dogs can be diagnosed by identification of the unsporulated oocysts with any of the fecal flotation methods commonly used to diagnose parasitic infections. In dogs, only *C. canis* can be identified with certainty by oocyst size and shape (Fig.13). The oocysts of the other three other species of *Cystoisospora* namely *C ohioensis, C. burrowsi,* and *C. neorivota*, may overlap in size, and their

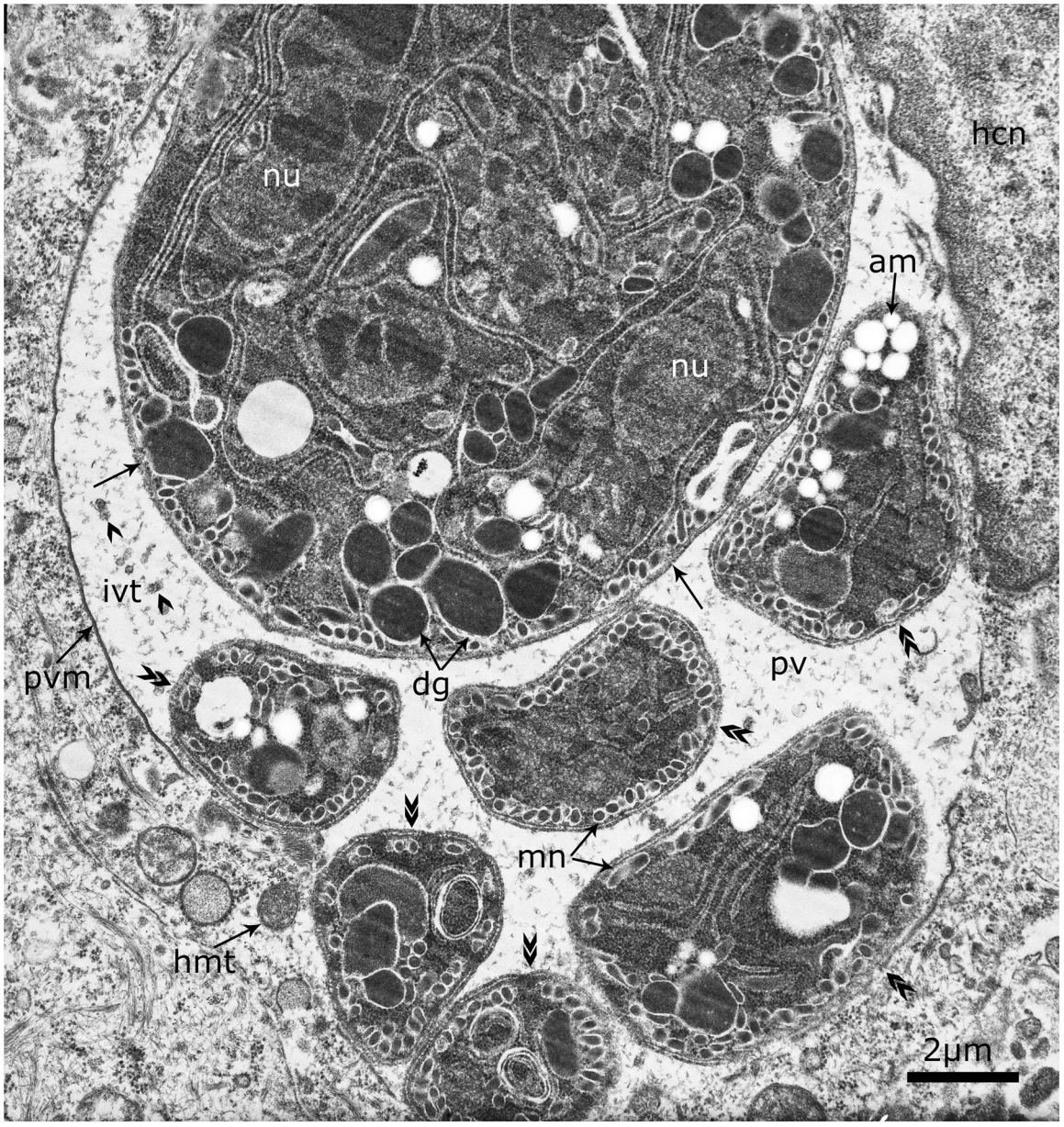

Fig. 12. Transmission electron micrograph of a *Cystoisospora ohioensis*-like organism in ileum of a naturally infected dog. Two groups of merozoites are enclosed in parasitophorous vacuolar membrane (pvm) in an enterocyte. Note 1 large meront (arrows) and 6 free merozoites (double arrowheads) outside the main group. The merozoites in the main group are twisted and individual boundaries are not clearly demarcated. Note numerous intravacuolar tubules (ivt) in the parasitophorous vacuole (pv). Also, note nucleus (nu), micronemes (mn), amylopectin granules (am) and numerous dense granules (dg). Host cell nucleus (hcn) and host mitochondria (hmt) are closely applied to the parasite.

distinction is not clinically important. Rarely, epithelial casts may be found in feces; schizonts, merozoites, and partially formed oocysts can be found in smears made in normal saline (not water). Unsporulated oocysts measuring 10–14 μm should be considered *Hammondia/Toxoplasma/Neospora* and specific PCR assays are needed for definitive identification (Schares et al., 2005). Unlike other coccidia, *Sarcocystis* species oocysts/sporocysts are excreted sporulated. They would not be seen in dogs or puppies who were not fed meat.

Molecular data using the 18S rRNA and ITS 1 genes indicates close phylogenetic similarity between dog and cat *Cystoisospora* species (Carreno et al., 1998; Barta et al., 2005; Samarasinghe et al., 2008; Matsubayashi et al., 2011; He et al., 2012), and this information may be useful in differential diagnosis of *C. ohioensis*-like parasites (Lee et al., 2018).

7. Treatment and prevention

Several medicines including sulfonamides, sulfonamides with antifolates, amprolium, spiramycin, diclazuril, toltrazuril, totrazuril sulfone (ponazuril) and combinations of these been used to treat coccidiosis in dogs (Duberman, 1960; Rao, 1968; Dürr, 1976; Brunnthaler, 1977; Kirkpatrick and Dubey, 1987; Aning and Ologun, 1992; Daugschies et al., 2000; Lloyd and Smith, 2001; Reinemeyer et al., 2007; Dubey et al., 2009; Lappin, 2010); dosages were summarized by Dubey and Greene (2012). Currently, toltrazuril and ponazuril are most commonly used (Table 5); medication with these compounds reduced or stopped oocyst excretion both in naturally exposed and experimentally infected dogs. In the trial performed by Rommel et al. (1986), 10 beagles were inoculated orally with 100,000 oocysts of *C. burrowsi*; 5 dogs were given

Table 4
Prevalence of *Cystoisospora* in feces of dogs.

Country	Region	No. of dogs	Number of positives (%)			Remarks	Reference
			C. canis	*C. ohioensis*-like	*C.* sp.	Source, others	
Albania	Tirana	111	19 (17.1)	35 (31.5)		Intestinal tracts of stray dogs	Xhaxhiu et al. (2011)
Argentina	Buenos Aires	2,193	66 (3.0)	264 (12.0)		Pets	Fontanarrosa et al. (2006)
Australia	Victoria	690	20 (2.9)	110 (15.9)		Shelter	Blake and Overend (1982)
Australia	Sydney	110			6 (5.5)	Clinics	Collins et al. (1983)
Australia	Southern Victoria	493			39 (7.9)	Pets, veterinary, kennel, stray dogs and fecal samples from public areas	Johnston and Gasser (1993)
Australia	Kimberley	182			4 (2.2)	Aboriginal communities	Meloni et al. (1993)
Australia	Perth	421	29 (6.9)	19 (4.5)		Refuges, pet shops, vet clinics, exercise areas, breeding kennels	Bugg et al. (1999)
Australia	Not specified	1400	15 (1.1)	49 (3.5)		Pets	Palmer et al. (2008)
Austria	Carinthia	220			11 (5.0)	Clinics. Stated only coccidia, no genus	Krebitz (1982)
Austria	Vienna	1,246	83 (6.7)	122 (9.8)		Shelter, pets	Supperer and Hinaidy (1986)
Austria		3,106			155 (5.0)	Includes all coccidia	Arnold et al. (2004)
Austria	Vienna	3,590	89 (2.5)	168 (4.7)	314 (8.7);17.8% had both *C canis* and *C. ohioensis* - like	Diagnostic center	Buehl et al. (2006)
Austria	Vienna and surrounding	1486	13 (0.9)			Species not distinguished	Hinney et al. (2017)
Belgium		2,432	32 (1.3)	61 (2.5)	5 (0.2)	Brussels dogs	Cotteleer and Famerée (1980)
Belgium	Brussels	52			2 (3.8)	Necropsied dogs	Gerin et al. (1980)
Belgium	Several regions	2,324	85 (3.7)			Stray	Vanparijs et al. (1991)
Brazil	Guanabara	251	13 (5.2)	19 (7.5)		Not stated	Lage et al. (1974)
Brazil	Rio de Janeiro	197	6 (3.0)	43 (21.8)		Stray	Franken et al. (1975)
Brazil	São Paulo	167	5 (3.0)	2 (1.2)	2 (1.2)	Not stated	Ogassawara et al. (1978)
Brazil	São Paulo	271			23 (8.5)	Pets and stray	Oliveira-Sequeira et al. (2002)
Brazil	Santa Catarina	158			10 (6.3)	Stray	Blazius et al. (2005)
Brazil	Paraná	280			21 (7.5)	Clinics	Tesserolli et al. (2005)
Brazil	Ribeirão Preto	331			11 (3.3)	Feces pools from recreational areas	Capuano and de Melo Rocha (2006)
Brazil	Santa Maria	109			20 (18.3)	Pet shops and breeding kennels	da Silva et al. (2008)
Brazil	Botucatu	254			9 (3.5)	Pets and stray	Katagiri and Oliveira-Sequeira (2008), 2010
Brazil	Botucatu	872 feces			75 (8.6)	Clinic	Torrico et al. (2008)
Brazil	Minas Gerais	141			2 (1.4)	Feces from 42 squares in the city of Belo Horizonte	Ribeiro et al. (2013)
Brazil	São Paulo	278			33 (11.9)	Urban and rural pets	Oliveira-Arbex et al. (2017)
Bulgaria	Stara Zagora	20			2 (10.0)	Stray	Georgieva et al. (1999)
Cambodia	Preah Vihear	94	13 (13.8)			Pets	Schär et al., (2014)
Canada	Several regions, northwest	959		85 (8.9)		Pets	Unruh et al. (1973)
Canada	Montréal	239	4 (1.7)	83 (34.7)		Stray	Seah et al. (1975)
Canada	Prince Edward Island	209			Pet store 49% of 69, 13% of 78 from clinic, 6% of 62 from shelter	Various sources. Only dogs < 1 year age were included	Uehlinger et al. (2013)
Canada	Several regions	1,086			113 (10.4)	Shelter	Villeneuve et al. (2015)
Chile	San Miguel	480	9 (1.9)	18 (3.8)		Urban areas	Gorman et al. (1989)
Chile	Santiago	972			89 (9.2)	Pets	López et al. (2006)
Czech Republic	Prague	500	16 (3.2)	6 (1.2)		Feces from 10 Prague housing developments	Valkounová (1982)
Czech Republic	Brno	663	2 (0.3)	14 (2.1)		Clinic	Svobodová et al. (1984)
Czech Republic	South Moravia	699	51 (7.3)	26 (3.7)		Farm dogs	Borkovcová (2003)
Czech Republic	Prague	4,320 feces			449 (10.4)	Fecal samples from public areas, animal shelter, rural areas	Dubná et al. (2007)
Denmark	Zealand	31			7 (22.6)	Coccidia spp. in hunted dogs	Tønsberg et al. (2004)
Ecuador	Galapagos Islands	97	4 (4.0)			Pets	Gingrich et al. (2010)
Egypt	Dakahlia Governorate	125	122 (97.6)	49 (39.2)		Stray. Oocysts measured	Abdel-Magied et al., (1982)

(*continued on next page*)

Table 4 (*continued*)

Country	Region	No. of dogs	Number of positives (%)			Remarks	Reference
			C. canis	*C. ohioensis*-like	*C.* sp.	Source, others	
Egypt	Ismailia	500	23 (4,6)	65 (13,0)	88 (17,6)	Feces from the road sides and public places	Abou-Eisha and Abdel-Aal, (1995)
France		500			25 (5.0)		Petithory and Ardoin (1990)
France	Paris	93			8 (8.6)	Clinic	Beugnet et al. (2000)
France	St. Pierre	57	5 (8.8)			Pets, shelter	Bridger and Whitney (2009)
Germany	Not stated	512			37 (7.2)	Data includes all coccidia	Brahm (1974)
Germany	Hamburg, Hanover	565	13 (2.3)	13 (2.3)		Shelter, clinics	Pötters (1978); Boch et al. (1981)
Germany	South-Germany	500	4 (0.8)	20 (4.0)		Pets; *C. burrowsi*: 9 (1.8%)	Böhm (1979); Boch et al. (1979), 1981
Germany	Rhineland-Palatinate	725			51 (7.1)	Stated only coccidia, no genus	Jonas (1981)
Germany	Northwest Germany	3,029		130 (4.3)		Rural and urban	Bauer and Stoye (1984)
Germany	Munich	554			32 (5.8)	Stated only coccidia, no genus	Deumer (1984)
Germany	Hannover	92	1 (1.0)	4 (4.3)		Shelter	Schwennicke (1985)
Germany	Berlin	141			10 (7.0)		Jungmann et al. (1986)
Germany	Wuppertal	1,246			140 (11.2)	Mentioned coccidia, no genus	Emde (1988)
Germany	Southern Germany	50 litters and 37 bitches	8 (16.0) litters and 4 (10.8) bitches	18 (36.0) litters and 9 (24.3) bitches		Kennel (see text)	Gothe and Reichler (1990a,b)
Germany	Northern Germany	3,329			140 (4.2)	Pets, clinics from 1984-1991	Epe et al. (1993)
Germany	Rostock	1,555			31 (2.0)		Dibbert and Methling (1995)
Germany	Northern Germany	2,289			49 (2.1)	Pets, clinics from 1993-1997	Epe et al. (1998)
Germany	Not specified	8,438	219 (2.6)	464 (5.5)	499 (5.9)	Clinic	Barutzki and Schaper (2003)
Germany	Central Germany	270	5 (2.0)	24 (9.0)		Shelter	Cirak and Bauer (2004)
Germany	Northern Germany	1,281			30 (2.3)	Pets, clinics from 1998-2002	Epe et al. (2004)
Germany	Halle	340	2 (0.6)	4 (1.1)		Clinics, shelter	Gottschalk and Prange (2004)
Germany		141			5 (3.5)		Beck (2006)
Germany	Several regions	24,677	588 (2.4)	973 (3.9)	1,391 (5.6)	Pets (see text)	Barutzki and Schaper (2011)
Germany	Lower Saxony	445			11 (2.5)	Stray and foster	Becker et al. (2012)
Germany	Several regions	2,319	See text	See text		Pets, < 1 year-old (see text)	Barutzki and Schaper (2013)
Germany	Several regions	2,731			154 (5.6)	Clinics	Raue et al. (2017)
Ghana	Greater Accra region	380			33 (8.7)	Pets and hunting	Johnson et al. (2015)
Greece	Thessaloniki	232		9 (3.9)		Pets and hunting	Haralabidis et al. (1988)
Greece	Serres	281			11 (3.9)	Shepherd and hunting	Papazahariadou et al. (2007)
Greece	Several regions	1,036			129 (12.4)	Clinic, shelter	Symeonidou et al. (2017)
Hungary		220	18 (8.2)				Nemeséri (1960)
Hungary	Eastern and Northern Hungary	490			17 (3.5)	Pets, animal ambulance service and shelter	Fok et al. (2001)
India	Maharashtra	385	18 (4.6)	6 (1.5)	9 (2.3)	Stray	Shastri (1989)
India	Assam	101			2 (2.0)	Pets	Traub et al. (2002)
Iran	Teheran	255		10 (4.0)		Pets	Mirzayans et al. (1972)
Iran	Kerman	100	1 (1.0)			Pets	Mirzaei and Fooladi (2013)
Iran	Hamadan	1,500			41 (2.7)	Pets and stray	Sardarian et al. (2015)
Iraq	Basra Province	93	6 (6.5)			Clinics	Al-Jassim et al. (2017)
Italy	Rome	100		14.0		Not stated	Pellegrino et al. (1953)
Italy	North	52			0		Petithory and Ardoin (1990)
Italy	Naples	6,288				395 (6.2%). Only coccidia mentioned	Sanna et al. (1993)
Italy	Naples	415	17 (4.1)			Feces collected from public sites	Rinaldi et al. (2006)
Italy	Veneto and Abruzzi	406			85 (21.0)	Pets, kennel and stray	Capelli et al. (2006)
Italy	Tuscany	239			18 (7.5)	Pets	Riggio et al. (2013)
Japan	Tochigi City	1979 y (262); 1991 y (260)			6 (5.5); 10 (11.6)	Pets	Asano et al. (1992)
Japan	Hachinohe	1,105			81 (7.3)	Pets	Itoh et al. (2009)
Japan	Saitama	906	5 (0.6)	19 (2.1)		Shelter	Yamamoto et al. (2009)
Korea	Chonbuck	412		66 (16)			Baek et al. (1993)
Morocco	Rabat	57	2 (3.5)			Stray	Pandey et al. (1987)

(*continued on next page*)

Table 4 (*continued*)

Country	Region	No. of dogs	Number of positives (%)			Remarks	Reference
			C. canis	*C. ohioensis*-like	*C.* sp.	Source, others	
Netherlands		224	3 (1.3)	3 (1.3)		Shelter	le Nobel et al. (2004)
Nigeria	Ibadan	203			29 (14.3)	Feces from streets	Ayinmode et al. (2016)
New Zealand	North Island	481	19 (4.0)	44 (9,2)			McKenna and Charleston (1980)
Poland							Borecka et al. (1999)
Poland	Several regions	831			4 (0.5)	Asylums and clinics	Balicka-Ramisz et al. (2004)
Poland		135			10 (7.4)	*Cystoisospora* spp.	Michalczyk and Sokól (2008)
Poland	Chelmno	339			37 (10.9)	Feces collected from 7 sites from accessible public areas	Felsmann et al. (2017)
Portugal	Évora	126			7 (5.5)	Shelter, clinics	Ferreira et al. (2011)
Portugal	Cantanhede	301			12 (4.0)	Rural	Cardoso et al. (2014)
Portugal	Ponte de Lima	592			56 (9.5)	Hunting, farm dogs and feces from environment	Mateus et al. (2014)
Romania	Several regions	52	4 (7.7)	12 (23.1)		Shelter, kennels, shepherd and household	Mircean et al. (2012)
Russia	Perm	505			32 (6.3)	Clinics	Sivkova and Sogrina (2015)
Slovakia	Bratislava	457			19 (4.1)	Feces from public sites	Totková et al. (2006)
Slovakia	Several regions	752			78 (10.4)	Pets, guard-dogs, hunting dogs, shelter and center for import/export of animals	Szabová et al. (2007)
South Africa	Durban and Coast	240			3 (1.3)	Stray	Mukaratirwa and Singh (2010)
Spain	Zaragoza	81			8 (9.9)	Pets and stray	Causapé et al. (1996)
Spain	Barcelona	505			3 (0.6)	Pets, stray. Stated only coccidia, no genus	Gracenea et al. (2009)
Spain	Murcia	265	27 (10.2)			Pets, shelter, stray	Martínez-Carrasco et al. (2007)
Spain	Córdoba	1,800	396 (22.0)		184 (10.2)	Homeless or housed	Martínez-Moreno et al. (2007)
Spain	Madrid	1,562			47 (3.0)	Shelter	Miró et al. (2007)
Switzerland		662			30 (4.5)		Seiler et al. (1983)
Switzerland	Lugano	371			11 (3.0)	Shelter	Deplazes et al. (1995)
Switzerland	Bern	505			19 (3.7)	Clinics, pets and farm dogs	Sager et al. (2006)
Switzerland	Zurich	402			8 (2.0)	Samples from 14 grassland areas, disposal units for dog waste bags	Hauser et al. (2015)
Taiwan		376	28 (7.5)			Feces from imported dogs	Ho et al. (2006)
Thailand	Bangkok	229			23 (10.0)	Pets	Inpankaew et al. (2007)
Thailand	Nakhon Nayok	500			0	Shelter	Rojekittikhun et al. (2014)
United Kingdom	Not specified	608			11 (1.8)	Hearing dogs. Stated only coccidia, no genus	Guest et al. (2007)
USA	Ohio	113	4 (3.5)	5 (4.4)		Clinics	Catcott (1946)
USA	Illinois	139	22 (15.9)	25 (18.0)		Pets, stray and breeding kennels dogs; morphology described	Levine and Ivens (1965)
USA	New Jersey	660	90 (13.6)	70 (11.0)		Stray	Burrows and Lillis (1967)
USA	Chicago	846			14 (1.6)	Urban	Jaskoski (1971)
USA	Ohio	500	9 (1.8)	18 (3.6)		Stray	Streitel and Dubey (1976)
USA	Missouri	2,093			398 (19.0)	Pets	Becker et al. (1977)
USA	Atlanta	143			13 (9.1)	Pets and kennels	Stehr-Green et al. (1987)
USA	Pennsylvania	6,555	80 (1.2)	129 (2.0)		Clinic	Gates and Nolan (2009)
Venezuela	Maracaibo	614			50 (8.1)	Pets	Ramírez-Barrios et al. (2004)
Zambia	Several regions	540			31 (5.7)	Pets	Nonaka et al. (2011)

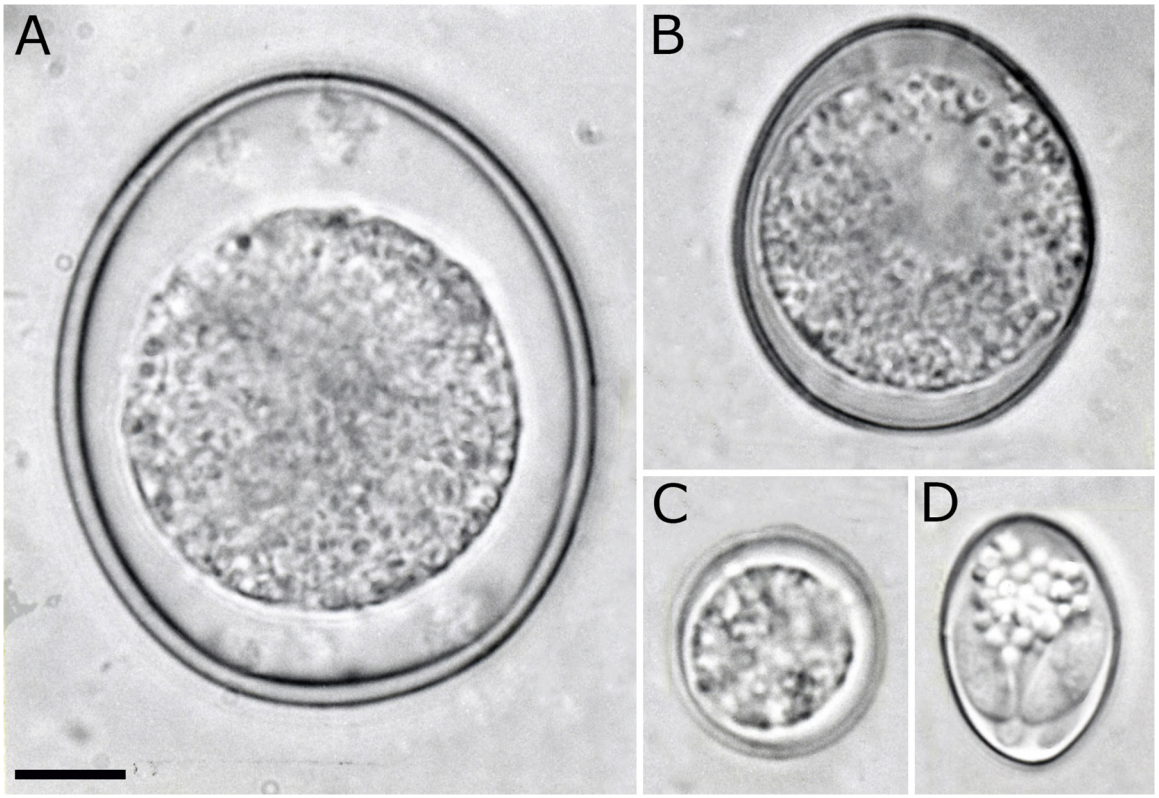

Fig. 13. Unsporulated oocysts of canine coccidia. (**A**) *Cystoisospora canis,* **(B)** *C. ohioensis,* **(C)** *Hammondia heydorni*, and (**D**) *Sarcocystis* sp. All canid coccidia, except *Sarcocystis*, are excreted in feces unsporulated. Bar = 5 µm. (Modified from Dubey, 1976).

medicated food and 5 were untreated. The unmedicated dogs excreted 7.5–37 million oocysts. No oocysts were excreted by medicated dogs and they became immune as evidenced by no oocyst excretion after challenge (Rommel et al., 1986). Dosages of 10 mg/kg are effective in reducing oocysts and clinical signs (Daugschies et al., 2000). Toltrazuril is relatively non-toxic even at the dose of 250 mg/kg (Charles et al., 2007). Supportive therapy is needed to prevent dehydration.

Dogs should be kept indoors and not fed raw meat. General hygienic measures should be practiced in kennels to minimize dissemination of oocysts. Although general disinfectants that do not harm humans are ineffective for killing coccidian oocysts, they remove many other contaminants. Treatment of equipment with hot water (> 70 °C) is most effective killing coccidian unsporulated oocysts. Attempts should be made to minimize stress during transport of puppies.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

Table 5
Efficacy of Toltrazuril and ponazuril for coccidiosis in dogs.

Preparation	Dose mg/kg/body weight	Formulation	*Cystoisospora* infection	No. of dogs	Reference
Toltrazuril	10	Mixed in food, 2-6 days p.i.	Experimental *C. burrowsi*	5	Rommel et al. (1986)
Toltrazuril	10,20,30	5% oral suspension	Experimental	18	Daugschies et al. (2000)
Toltrazuril	10,20,30	5% oral suspension	Natural *C. ohioensis*	104 litters	Daugschies et al. (2000)
Ponazuril[b]	20-250	5% oral suspension	Natural *C. ohioensis*	55	Charles et al. (2007)
Ponazuril[b]	20-50	5% oral suspension	Experimental *C. canis*	75	Reinemeyer et al. (2007)
Procox[−a]	9	Oral suspension	Natural	234	Altreuther et al. (2011b)
Procox[−a]	9	Oral suspension	Experimental *C. canis, C. ohioensis*-like	75	Altreuther et al. (2011a)
Procox[−a]	9	Oral suspension	Natural, *Cystoisospora* spp.	28	Rauscher et al. (2013)
Ponazuril	20-250	Paste, oral	Natural *C. canis*, *C. ohioensis*	43	Litster et al. (2014)

[a] Emodepside plus toltrazuril, 0.45 mg emodepside plus 9 mg toltrazuril/kg body weight oral (Bayer).
[b] Toltrazuril sulfone, 5% ponazuril oral suspension (Bayer).

Conflict of interest

None

Ethical statement

No experiments performed. Data reviewed.

Acknowledgments

We would like to thank, Camila Cezar, Fernando Murata, Andressa Ferreira da Silva, Meghan Sadler, and Oliver Kwok for their help in preparation of this paper. We are indebted to Prof. Christian Bauer for his help with this paper.

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