Veterinary Parasitology 344 (2026) 110730

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Weight development of intermediate hosts infected by a *Cystoisospora ohioensis*-like coccidian (Apicomplexa: Cystoisosporinae): Experimental model in mice

Francisco Carlos Rodrigues de Oliveira [a], Samira Salim Mello Gallo [a,*], Carlos Wilson Gomes Lopes [b]

[a] *Animal Health Laboratory, Universidade Estadual de Norte Fluminense Darcy Ribeiro (UENF), Campos dos Goytacazes, Rio de Janeiro 28013-602, Brazil*
[b] *Department of Animal Parasitology, Veterinary Institute, Universidade Federal Rural do Rio de Janeiro (UFRRJ), Seropédica, Rio de Janeiro 23890-000, Brazil*

ARTICLE INFO

Keywords:
Cystoisosporosis
Host infectivity
Hypnozoites
Oocysts

ABSTRACT

This study aimed to evaluate clinical signs, visceral changes, and weight development in intermediate hosts infected with oocysts of a *Cystoisospora ohioensis*-like coccidian from naturally infected dogs. Using 135 albino mice divided into three groups—INFECTED (inoculated with oocysts), PAIR-FED (given the same food as infected mice), and CONTROL (fed *ad libitum*)—researchers monitored weight and pathological changes over 35 days. Mice were euthanized at various intervals post-inoculation (1–35 days), with organs from some INFECTED mice fed to dogs on day 60 for a biological assay. The INFECTED group showed higher body weight and weight gain than the CONTROL group, though organ weights were greater, leading to lower carcass yield. Tissue cysts with hypnozoites were found in the intestines (1st day), Peyer's patches (up to 5th day), and lymph nodes/spleen (up to 35th day). Dogs inoculated with hypnozoites (from infected mice) had a shorter prepatent period, longer patent period, and higher oocyst shedding compared to those given sporulated oocysts. The findings indicate that the *C. ohioensis*-like coccidian affect mouse weight, persist in visceral tissues for up to 60 days, and exhibit biological differences in infectivity between sporozoites and hypnozoites in dogs.

1. Introduction

The genus *Cystoisospora* was established by Frenkel (1977) to accommodate certain species formerly classified under genus *Isospora*, characterized by sporulated oocysts containing two sporocysts with four sporozoites each, the absence of Stieda bodies in the sporocysts, and the ability to form monozoic tissue cysts (hypnozoites) in intermediate hosts. Smith (1981), who recognized the involvement of an intermediate host, proposed placing *Cystoisospora* within the subfamily Cystoisosporinae under the family Sarcocystidae. Molecular studies subsequently confirmed the classification of mammalian *Cystoisospora* spp. within the Sarcocystidae family (Carreno et al., 1998; Franzen et al., 2000; Barta et al., 2001, 2005).

Within the subfamily Cystoisosporinae, the genus *Cystoisospora* comprises four species for which the dog is the definitive host: *C. canis*, *C. ohioensis*, *C. burrowsi*, and *C. rivolta*. Among these, *C. canis* is distinct because of its large, ovoid oocysts (28–34 μm), whereas the last three, with intermediate-sized oocysts (17–23 μm), are considered *C. ohioensis*-like coccidians or organisms owing to their overlapping size ranges (Lindsay et al., 1997a; Dubey and Lindsay, 2019). Other group, consisting of small oocysts (10–13 μm) shed in the feces of infected dogs, includes two members of the subfamily Toxoplasmatinae: *Neospora caninum*, which is clinically important, and *Heydornia heydorni* (syn. *Hammondia heydornii*), which is apparently nonpathogenic (Dubey, 1993). The developmental stages of these parasites are primarily found in the digestive tracts of young animals, which become infected by ingesting sporulated oocysts from the environment or monozoic cysts present in the viscera of previously infected intermediate hosts (Dubey and Mehlhorn, 1978). *Cystoisospora ohioensis* has been characterized as pathogenic when experimentally inocu-lated into puppies, which subsequently develop diarrhea as a clinical manifestation (Dubey, 1978; Buehl et al., 2006). Additionally, diarrhea associated with *C. ohioensis* has been reported in a naturally infected three-month-old puppy in South Korea, with the diagnosis confirmed by PCR (Lee et al., 2018).

The importance of *C. ohioensis*-like infection in dogs is significant due to its role as an enteric pathogen. Clinical coccidiosis caused by

* Corresponding author.
E-mail addresses: foliveira@uenf.br (F.C.R. Oliveira), samira.gallo@pq.uenf.br (S.S.M. Gallo), lopescwg@ufrrj.br (C.W.G. Lopes).

https://doi.org/10.1016/j.vetpar.2026.110730
Received 15 January 2026; Received in revised form 27 February 2026; Accepted 1 March 2026
Available online 2 March 2026

Cystoisospora spp. is a major cause of diarrhea in puppies and kittens, with adults often acting as subclinical carriers. Clinical signs can include diarrhea (sometimes with weight loss and dehydration), vomiting, anorexia, and depression, with death being a potential outcome in severe cases. The disease is often exacerbated by stress, overcrowding, or intercurrent infections. While *Cystoisospora canis* is often considered more pathogenic, *C. ohioensis*-like is frequently detected, with recent research confirming its role in severe infections. The pathogen is globally distributed and commonly affects young animals in high-density environments like kennels (Grellet and Mila, 2024; Morelli et al., 2025).

Cystoisospora ohioensis is widely distributed and fairly common in epithelial cells throughout the small intestine as well as in the cecum and colon of dogs, coyotes, red foxes, raccoons, and possibly dingoes (Levine, 1985). Dogs fed tissues from camels (Hilali et al., 1992, 1995), sheep (Hilali et al., 1992), pigs, donkeys, and buffaloes (Zayed and El-Ghaysh, 1998) naturally shed *C. ohioensis* oocysts in their feces; however, there has been no microscopic confirmation of monozoic tissue cysts. Experimentally, intermediate hosts include mice (*Mus musculus*), brown rats (*Rattus norvegicus*), golden hamsters (*Mesocricetus auratus*), cats (*Felis catus*) and dogs (*Canis lupus familiaris*), in which monozoic cysts have been found in viscera, mainly mesenteric lymph nodes, liver, and spleen (Levine, 1985). Frenkel and Dubey (1972), in describing mice as intermediate hosts of *Cystoisospora* spp. of felines as definitive hosts, characterized the formation of merozoites through endodyogeny as an asexual reproductive process occurring in the mucosa of the small intestine of interme-diate hosts. Subsequent studies led Dubey and Lindsay (2019) to suggest that the two zoites likely enter the same host cell rather than resulting from the division of a single zoite. The monozoic tissue cysts of *C. felis*, *C. rivolta*, and *C. ohioensis* increase in size over time but do not undergo division like other members of the Sarcocystidae family (Frenkel and Dubey, 1972; Dubey and Mehlhorn, 1978). Thus, the formation of monozoic cysts is considered one of the distinguishing characteristics of *Cystoisospora* spp. (Frenkel, 1977), a feature also observed in the asexual stages of *Cystoisospora* spp. in mouse tissues (Frenkel and Smith, 2003).

There is limited information regarding the economic importance of species within this genus, although the presence of the parasite has been reported in the tissues of naturally infected livestock (Hilali et al., 1992; Zayed and El-Ghaysh, 1998) as well as in experimentally infected livestock (Costa and Lopes, 1998; Carvalho Filho et al., 2003; Massad et al., 2003; Melo et al., 2003). Pathology associated with monozoic cysts have been reported for the genus *Cystoisospora* in immunosuppressed humans, particularly in cases involving *Cystoisospora belli*, the causative agent of human cystoisosporosis (Lindsay et al., 1997b). This organism has been identified as an opportunistic etiological agent in patients with acquired immunodeficiency syndrome (AIDS) and has been associated with chronic diarrhea, acalculous cholecystitis, and cholangiopathy (Benator et al., 1994; Walther and Topazian, 2009). Velásquez et al. (2022) reported that in this patient group, infection by *C. belli* may lead to disseminated cystoisosporosis, with the presence of monozoic tissue cysts in the intestinal lamina propria, lymph nodes, liver, and spleen (Restrepo et al., 1987; Michiels et al., 1994; Comin and Santucci, 1994; Velásquez et al., 2001; Frenkel et al., 2003a).

To diagnose coccidiosis in dogs, it is necessary to detect oocysts through microscopic examination (Levine, 1985) or identify the developmental stages of the parasite in mucosal scrapings during the acute phase of infection (Loss and Lopes, 1992a;b). Lee et al.(2018) examined the feces of a 3-month-old female dog with persistent diarrhea using real-time multiplex poly-merase chain reaction (PCR), which targeted 23 pathogens known to cause diarrheal syndromes. Sequence analysis was performed using nested PCR amplification of 18S ribosomal RNA. Oocysts were identified in the fecal smear. Although real-time multiplex PCR was positive for *Cyclospora cayetanensis*, the final diagnosis was *C. ohioensis* infection, which was confirmed through phylogenetic analysis of the 18S rRNA gene. Another relevant aspect is the difficulty in isolating oocysts of *C. canis* (Nemeséri, 1959; Frenkel, 1977) and *C. ohioensis* (Dubey, 1975a; Frenkel, 1977), as these species are most often shed in the feces of dogs along with other coccidia, such as *C. burrowsi* (Trayser and Todd, 1978) Rommel and Zielasko, 1981, *C. rivolta* (Dubey and Mahrt, 1978) Frenkel, 1977, as well as small oocysts of *Heydornia heydorni* (syn. *H. heydorni*) (Tadros and Laarman, 1976), *N. caninum* (Dubey et al., 1988), and some species of the genus *Sarcocystis*.

This research is justified by the scarcity of studies on C. ohioensis in intermediate hosts and its sanitary/economic impact. Investigating its extra-intestinal behavior and spoliative potential is fundamental due to immunosuppression, the dog-human relationship, and losses in animal productivity, aiming to improve diagnosis and control. Thus, from the diagnosis of the shedding of subspherical, disporic, tetrazoic coccidian oocysts of medium size by six dogs from the same litter—morphologically and morphometrically characterized as belonging to the *C. ohioensis*-like group—the objective of this research was to experimentally infect mice, describe possible clinical signs of cystoisosporosis in these animals, and evaluate whether there are alterations in the viscera of the infected animals and whether cystoisosporosis influences the weight development of the intermediate host using an experimental mouse model.

2. Materials and methods

2.1. Origin of the oocysts

Cystoisospora ohioensis-like oocysts were obtained from the feces of six 8-week-old puppies from the same litter. The animals were naturally infected with *Cystoisospora* spp. and lived freely in peridomestic urban areas of the city of Seropédica, State of Rio de Janeiro, Brazil. They were maintained in accordance with Law No. 6638 of May 8, 1979 (Brazil, 1979) which establishes procedures for the scientific use of animals. The animals were fed a commercial diet formulated for puppies provided ad libitum. Pens were cleaned daily and sanitized weekly using a flame broom. For 15 days, the feces were examined daily for oocyst detection and collection, according to Oliveira et al. (2000).

2.2. Oocyst sporulation

The collected fecal material was diluted at a ratio of 1:2 in a 2.5% potassium dichromate solution and aerated using a conventional aquarium pump (Alpha II, Vigor Ar, São Paulo) at room temperature. After 72 h of aeration, a drop of the suspension was placed between a microscope slide and coverslip and examined under a light microscope. Once the sporulation index reached 80% or higher, the fecal suspensions were transferred to 50 mL Falcon-type centrifuge tubes and centrifuged at 200 × g for 10 min. The sporulated oocysts were recovered from the supernatant using flotation in a saturated sugar solution (Oliveira et al., 2001a).

2.3. Oocyst concentration

The sporulated oocysts were concentrated by resuspension in a saturated sugar solution followed by additional centrifugation (200 × g for 5 min). The resulting supernatant was poured into a 14 cm diameter Petri dish, over which the bottom of a 15 cm Petri dish was placed, creating an interface between the lid (the base of the 15 cm Petri dish) and the convergent meniscus of the sugar solution surface. At 15-minute intervals over 1 h, the upper dish was removed so that its bottom could be rinsed with phosphate-buffered saline (PBS), pH 7.2, using a 10 mL syringe fitted with a hypodermic needle. The suspension of sporulated oocysts obtained from washing with PBS was collected using a funnel placed in a glass beaker. These oocysts were subjected to another round of centrifugation, followed by two consecutive resuspensions in PBS to eliminate potassium dichromate residue and excess sucrose. The final sediment, containing a high concentration of oocysts, was resuspended

in a small volume of PBS and transferred to a new sterile 50 mL Falcon tube. The total volume of feces excreted by the dogs was con-centrated to a final volume of 50 mL and stored at 4 °C until use (Oliveira et al., 2000).

2.4. Oocyst counting and morphometry

An aliquot of purified oocyst suspension was placed in a Neubauer chamber for counting. The average number from five counts of oocysts quantified in the four outer reticulated areas—the area used for leukocyte quantification—was multiplied by 2.5, which is the chamber height correction factor, corresponding to the number present in 1 mm^3 of the material. To convert the number of oocysts per mL of the concentrated sus-pension, the number of oocysts found in 1 mm^3 was multiplied by 1000. Thus, the total number of oocysts recovered from all the samples collected from all the animals was equal to the number of oocysts quantified in one mL multiplied by the total volume of the concentrated suspension. The major diameter (MD) and minor diameter (md) of sporulated oocysts were measured one hundred times via a K-15X micrometer eyepiece (PZO, Poland) coupled with a Leitz Westler microscope (H.M. Lux, Germany). The morphometric indices (MIs) were obtained by the ratio of MD to md and were calculated for both sporulated oocysts and the sporocysts within each oocyst (Oliveira et al., 2001b).

2.5. Standardization of the inoculum and infection of the mice

For the concentrated sample, the total number of oocysts in the sample was previously evaluated, and the inoculum was standardized for each mouse to 10^5 sporulated oocysts of the *C. ohioensis*-like group in 0.5 mL of PBS.

A total of 135 female Swiss Webster mice, 6 weeks old and weighing an average of 25 g, were obtained from the Central Animal Facility at FIOCRUZ. They were housed in 27 polypropylene cages (five mice per cage) and divided into three groups (n = 45 per group). The INFECTED group received oral inoculum via gavage and *ad libitum* feed. The other two groups received PBS as control inoculum: the CONTROL group had *ad libitum* feed, while the PAIR-FED group was given the same amount of feed as the INFECTED group. All mice had water *ad libitum* and were maintained in compliance with Law No. 6638/1979 (Brazil, 1979).

2.6. Evaluation of body weight development

The relative weights of the mice, whether infected or not, were proportionally assessed through daily weight measurements of each animal over 35 days. The influence of food intake on weight was evaluated by comparing the mean body weight of the infected group with that of the control groups.

Five mice from each group were euthanized in a CO_2 chamber at 1, 3, 5, 9, 14, 21, 28, 35, and 60 days after infection (DAI), in accordance with Law No. 6638 of May 8, 1979 (Brazil, 1979). Animals euthanized between 1 and 35 DAI underwent necropsy and evisceration. Their organs—stomach, small intestine, large intestine, Peyer's patches, mesenteric lymph nodes, spleen, liver, lungs, and heart—were collected, identified, and weighed using a precision balance (Mettler PE 360, USA). For each subject, the proportional weight of the viscera relative to the live body weight was determined. Similarly, the carcass weight post-evisceration was comparatively assessed.

2.7. Recovery of hypnozoites from organs

For each of the five mice euthanized from 1 to35 DAI, their organs were collected, weighed, and individually minced into small fragments using a mortar and pestle. A 0.9% saline solution was then added to achieve a final homogenate volume of 5 mL, which was transferred to a 50 mL plastic centrifuge tube. *Cystoisospora ohioensis*-like hypnozoites were recovered according to the methodology described by Dubey (1977), with slight modifications as proposed by Oliveira et al. (2001a).

2.8. Infectivity of sporozoites and hypnozoites

To determine the viability of inocula containing 10^5 sporulated oocysts of the *C. ohioensis*-like group and hypnozoites present in the viscera of mice from the INFECTED group, a pregnant bitch (in her final trimester) from Rio de Janeiro was selected and maintained at the UFRRJ experimental station. The bitch received daily oral treatment with trimethoprim (90 mg) and sulfadiazine (410 mg) (Triglobe®, Astra Química e Farmacêutica Ltda., Tamboré, São Paulo, Brazil) for 10 days and served as a puppy donor. All six puppies of both sexes born to this bitch were dewormed at 25 days of age (Basken® Plus, König do Brasil Ltda; single dose of 120 mg/kg body weight), weaned at 35 days, and housed in individual cages. Throughout the experiment, the animals received commercial puppy feed and water *ad libitum*. Two puppies were inoculated with 0.5 mL of a concentrated suspension containing 10^5 sporulated oocysts. Another two puppies were fed organs from INFECTED group mice (euthanized at 60 DAI). The remaining two puppies served as controls: one received 0.5 mL of PBS orally, while the other was fed homogenized organs from CONTROL and PAIR-FED group mice.

2.9. Recovery of oocysts from the biological assay

Oocysts recovered from the feces of two dogs inoculated with sporulated oocysts and two dogs fed viscera from mice in the INFECTED group were quantified daily using a modified Sheather's sucrose flotation technique, with slight adaptations as follows: the total fecal matter excreted daily by the dogs was collected, weighed, and homogenized. A 1-gram aliquot was placed in a 125 mL Erlenmeyer flask containing 100 mL of distilled water. The material was mixed to obtain a homogeneous suspension, from which 10 mL was withdrawn and transferred to a 15 mL Falcon centrifuge tube. The sample was subsequently centrifuged at 200 × g for five minutes. The supernatant was discarded, and the sediment was resuspended in saturated sugar solution before being centrifuged again under the same conditions (200 × g for 5 min). A disposable Pasteur pipette was used to add saturated sugar solution until a meniscus formed. A coverslip was placed on top for five minutes and then carefully transferred to a standard glass slide. The total number of oocysts present between the slide and coverslip was counted and multiplied by 10 to obtain the number of oocysts per gram of feces (OPG). The total daily oocyst output was calculated by multiplying the OPG value by the total weight (in grams) of feces collected each day. The remaining fecal material containing oocysts was further processed for concentration, sporulation, and morphological/morphometric analysis of the oocysts, following the same protocol used for oocysts collected from naturally infected dogs and initial donor animals in this study.

2.10. Statistical analysis

The daily mean OPG excreted and total daily output, along with the mean meas-urements and morphometric indices of oocysts and sporocysts from both naturally infected dogs and those inoculated were compared using Student's t test. Weight parameters and organ percentages in mice from INFECTED, PAIR-FED, and CONTROL groups were compared via Tukey's test. Additionally, the mean values of MD, md and MI of oocysts and sporocysts excreted by naturally infected dogs, dogs experimentally infected with sporulated oocysts, and dogs fed viscera (hypnozoites) from mice infected with sporulated oocysts were calculated and compared by Tukey's test. The number and percentage of hypnozoites in the viscera of the mice that were fed sporulated oocysts were also calculated. For statistical analysis of the data, GraphPad InStat software was used.

3. Results

A total of 26.73 × 10^9 oocysts were recovered from the six naturally infected dogs over 15 days of shedding. None of the puppies presented clinical manifestations, and the texture of the collected feces was consistent in terms of their characteristic color and odor. The daily shedding behavior of the six puppies during the collection period can be observed in Fig. 1a.

The sporulated *C. ohioensis*-like oocysts had a subspherical to spherical shape, with an oocyst wall consisting of two membranes averaging 0.29 μm in thickness and lacking a micropyle. The sporocysts were ellipsoidal in shape, with a smooth and thin wall enclosing four sporozoites inside, and no Stieda, Substieda, or Parastieda bodies were observed, nor were polar granules or oocyst residues. Sporocyst residues appeared as fine granular material that formed small clumps that aggregated into larger, denser granules (Fig. 2). The mean dimensions and standard deviations of the MD, md and MI, as well as the highest observed values for oocysts and sporocysts, are shown in Table 1.

The average live weights (ALWs) of the mice in the INFECTED group were consistently lower than those in the CONTROL group, whereas compared with those in the PAIR-FED group, their average live weights followed a similar trend throughout the experiment (Fig. 3a). When these parameters were assessed on the days when five mice from each group were euthanized, this observation was less evident. The ALW values of the statistically compared groups are shown in Table 2. The mean weight of the CONTROL group at 15 DAI was significantly greater ($p \leq 0.05$) than that of the INFECTED and PAIR-FED groups, whereas at the same time point, the INFECTED and PAIR-FED groups did not significantly differ ($p \geq 0.05$). The ALW of the CONTROL group was greater than that of the PAIR-FED group at 35 DAI, with statistically significant differences ($p \leq 0.05$) at 15, 16, 17, 18, 26, 27, and 28 DAI. The ALW of the PAIR-FED group did not differ significantly ($p \geq 0.05$) from that of the INFECTED group (Table 2).

The average weight gain (AWG) of the mice in the CONTROL group was lower than that of the INFECTED group until the 9th DAI, after which it increased and remained high until the end of the experiment, except at the 16th DAI (Table 3 and Fig. 3c). There was no significant difference ($p \geq 0.05$) throughout the experiment, except at the 1st DAI, when the AWG of the CONTROL group was significantly lower ($p \leq 0.05$) than that of the INFECTED group (Table 3). As observed for ALW, when the AWG parameters were assessed on the days when five mice from each group were euthanized, this trend was less evident (Fig. 3d). The PAIR-FED group had AWG values similar to those of the INFECTED group until the 5th DAI, after which its performance became inferior, with statistically significant differences ($p \leq 0.05$) on the 8th, 9th, 15th, 16th, 17th, and 27th DAI (Fig. 3c and d). Similarly, the performance of the CONTROL group was inferior to that of the PAIR-FED group, with statistically significant differences ($p \leq 0.05$) on the 15th, 16th, 17th, 19th, and 27th DAI (Table 3).

Compared with that of the PAIR-FED group, the average carcass weight (ACW) of the mice in the INFECTED group was significantly lower ($p \leq 0.05$) on the 21st DAI, whereas on the 28th DAI, the ACW of the PAIR-FED group was significantly lower ($p \leq 0.05$) than that of the CONTROL group. At both time points (21st and 28th DAI), the ACW of the INFECTED group was lower than that of the CONTROL group, although the difference was not statistically significant ($p \geq 0.05$) (Table 4).

Considering the live weight of each individual, the average percentage carcass weight (ACW%) was established. In this case, the ACW% of the CONTROL group was consistently greater than that of the INFECTED group, except on the 14th and 28th DAI. However, these differences were not statistically significant ($p \geq 0.05$). On the 21st DAI, the ACW% of the INFECTED group [n = 5 ($\bar{X}$=59.73 ± 2.04) 95% CI] was significantly lower ($p \leq 0.05$) than that of the PAIR-FED group [n = 5 ($\bar{X}$=63.39 ± 1.76) 95% CI]. On the same day, the ACW% of the CONTROL group [n = 5 ($\bar{X}$=61.13 ± 1.93) 95% CI] was greater than that of the INFECTED group and less than that of the PAIR-FED group, but the difference was not statistically significant ($p \geq 0.05$) (Fig. 3e).

To determine whether organ (viscera) weight influences the carcass yield of infected animals, the live weight of each animal was subtracted from its carcass weight (LW-CW). As shown in Fig. 3e and comparing it with Fig. 3f, an inverse relationship was observed between the average percentage carcass yield (CY%) and viscera weight (LW-CW) in the INFECTED group. This pattern was not observed in the PAIR-FED and CONTROL groups.

No differences were found among animals in the INFECTED, PAIR-FED, and CONTROL groups ($p \geq 0.05$) regarding the average percentage weights of the stomach (SW%) and lungs (LW%) at any of the DAIs when the mice were euthanized throughout the experiment. With respect to the average heart weight (HW%), animals in the CONTROL group [n = 5 ($\bar{X}$=0.85 ± 0.11) 95% CI] presented significantly greater HW% ($p \leq 0.05$) than did those in the INFECTED [n = 5 ($\bar{X}$=0.65 ± 0.11) 95% CI] and PAIR-FED [n = 5 ($\bar{X}$=0.65 ± 0.09) 95% CI] groups, but only on the 9th DAI. On the same day, these latter two groups did not significantly differ ($p \geq 0.05$).

On the 21st DAI, the average percentage weight of the small intestine (AWSI%) relative to the carcass weight (Fig. 4a) in the CONTROL group [n = 5 ($\bar{X}$=4.43 ± 1.40) 95% CI] was significantly lower ($p \leq 0.05$) than that in the PAIR-FED group [n = 4 ($\bar{X}$=9.39 ± 1.21) 95% CI]. During the same period, the AWSI% of the INFECTED group [n = 5 ($\bar{X}$=6.35 ± 2.8) 95% CI] was greater than that of both the CONTROL and PAIR-FED groups; however, these differences were not statistically significant ($p \geq 0.05$). When the average percentage weight of the large intestine (AWLI%) relative to the carcass weight was analyzed (Fig. 4b), a significant difference was observed on the 1st DAI. The AWLI% of the CONTROL group [n = 5 ($\bar{X}$=10.47 ± 1.88) 95% CI] was significantly greater ($p \leq 0.05$) than that of the PAIR-FED group [n = 5 ($\bar{X}$=7.57 ± 1.35) 95% CI] but was not significantly different ($p \geq 0.05$) from that of the INFECTED group [n = 5 ($\bar{X}$=9.11 ± 1.28) 95% CI].

Owing to the small size of Peyer's patches in the mice from all three

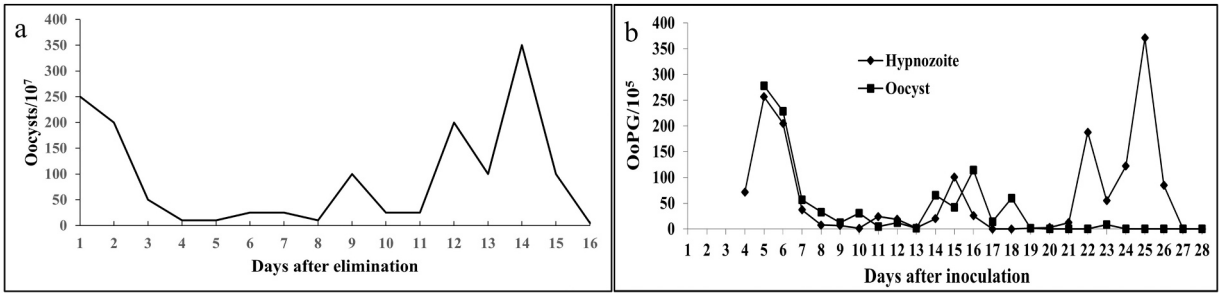

Fig. 1. *Cystoisospora ohioensis*-like oocysts shed daily by dogs. a, Total oocysts excreted by six naturally infected 8-week-old puppies. b, Mean daily excretion of oocysts per gram of feces (OPG) divided by 1000 (OPG/1000) from two puppies inoculated with viscera from five mice previously infected with 10^5 sporulated oocysts of *C. ohioensis*-like species.

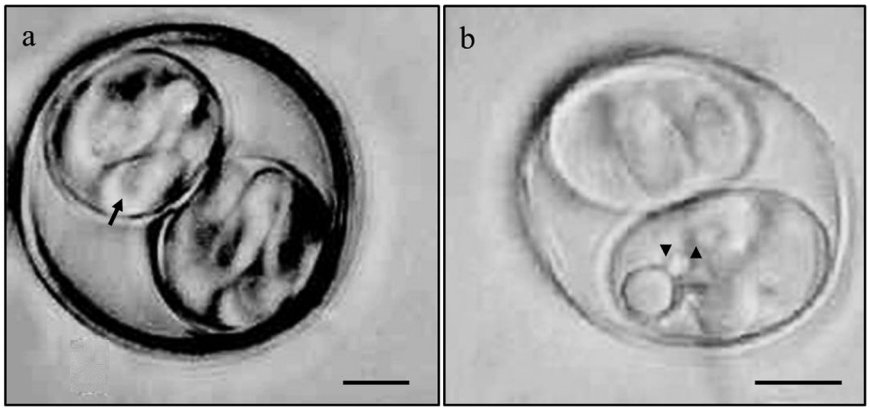

Fig. 2. Photomicrograph of sporulated oocysts of *Cystoisospora ohioensis*-like species excreted in feces by (a) naturally infected 8-week-old dogs and (b) dogs experimentally infected with 10^5 sporulated oocysts at 35 days of age. Highlighted in (a) sporozoites (arrow) and (b) fine granular residual bodies (arrowhead). Scale bar: 5 μm.

Table 1
Dimensions in micrometers of oocysts and sporocysts and morphometric indices of *Cystoisospora ohioensis*-like organisms, excreted by naturally infected dogs, experi-mentally infected with sporulated oocysts (sporozoites), and fed viscera (hypnozoites) from five mice infected with sporulated oocysts.

Route of Infection	n[a]	Diameters		Morphometric
Measured forms		Major	Minus	Index
Natural				
Oocyst	100	21.39 ± 1.71 (18.40–26.80)	19.23 ± 1.91 (14.40–25.00)	1.11 ± 0.08 (0.98–1.42)
Sporocyst	100	15.48 ± 1.42 (12.20–19.00)	10.24 ± 0.96 (8.40–13.00)	1.51 ± 0.13 (1.08–1.83)
Experimental using sporozoites				
Oocyst	100	25.28 ± 2.12 (19.84–29.76)	23.32 ± 2.18 (17.67–27.59)	1.08 ± 0.06 (1.00–1.34)
Sporocyst	100	18.02 ± 1.91 (13.02–21.70)	11.59 ± 1.57 (8.68–19.84)	1.57 ± 0.12 (1.00–1.97)
Experimental using hypnozoites				
Oocyst	100	26.42 ± 1.69 (22.32–30.07)	24.47 ± 1.36 (21.39–27.28)	1.08 ± 0.04 (1.00–1.20)
Sporocyst	100	18.97 ± 1.77 (11.47–22.63)	12.00 (16.43–7.13)	1.59 ± 0.17 (1.13–2.07)

[a] Number of oocysts measured.

groups, the average percentage weight of Peyer's patches (PPW%) could not be determined on the 1st DAI (Fig. 4c). A significantly greater weight ($p \leq 0.05$) of PPW% was observed in the INFECTED group [$n = 5$ ($\bar{X}$=0.36 ± 0.08) 95% CI] than in the CONTROL group [$n = 5$ ($\bar{X}$=0.23 ± 0.09) 95% CI] on the 5th DAI (Fig. 4c). On the 3rd and 5th DAI, the PPW% of the PAIR-FED group was higher than that of the INFECTED group, although not significantly ($p \geq 0.05$), and subsequently became lower (also not significantly, $p \geq 0.05$) on the 9th, 14th, and 21st DAI. By the 35th DAI, the PPW% values of all three groups were practically identical (Fig. 4c).

Changes in the average percentage weight of mesenteric lymph nodes (MLNW%) were more clearly observed from the 9th DAI onward. A significantly greater MLNW% ($p \leq 0.05$) was found between the PAIR-FED [$n = 5$ ($\bar{X}$=9.39 ± 1.21) 95% CI] and CONTROL [$n = 5$ ($\bar{X}$=4.42 ± 1.40) 95% CI] groups on the 21st DAI, with values returning to similar levels by the 28th DAI (Fig. 4d). On the 28th DAI, mice in the PAIR-FED group presented significantly greater MLNW% ($p \leq 0.05$) values than those in the INFECTED group did [$n = 5$ ($\bar{X}$=3.75 ± 0.35) 95% CI], whereas no significant difference was observed compared with the CONTROL group [$n = 5$ ($\bar{X}$=4.32 ± 0.82) 95% CI].

The average percentage spleen weight (SW%) of the animals in the INFECTED group was greater throughout most of the experiment than that of the PAIR-FED and CONTROL groups. Specifically, the SW% of the INFECTED group [$n = 5$ ($\bar{X}$=1.33 ± 0.29) 95% CI] was significantly greater ($p \leq 0.05$) than that of the CONTROL group [$n = 5$ ($\bar{X}$=0.94 ± 0.09) 95% CI] but not significantly different ($p \geq 0.05$) from that of the PAIR-FED group [$n = 5$ ($\bar{X}$=1.23 ± 0.21) 95% CI] at the end of the experiment on the 35th DAI (Fig. 4e).

The average percentage liver weight (LW%) of the mice in the INFECTED group increased from the 3rd DAI onward. A significantly greater LW% ($p \leq 0.05$) was observed in the INFECTED group [$n = 5$ ($\bar{X}$=12.16 ± 2.10) 95% CI] than in the PAIR-FED group [$n = 5$ ($\bar{X}$=9.26 ± 0.77) 95% CI] on the 9th DAI, whereas no significant difference ($p \geq 0.05$) was found versus the CONTROL group [$n = 5$ ($\bar{X}$=9.60 ± 1.40) 95% CI] on that day. Compared with those of the other groups, the LW% of the INFECTED group subsequently decreased but remained nonsignificant ($p \geq 0.05$), ultimately reaching similar values among all three groups by the 35th DAI (Fig. 4f).

In all the mice from the CONTROL and PAIR-FED groups, no *C. ohioensis*-like hypnozoites were observed in the organs after peptic digestion, nor were the hypnozoites recovered from the stomach, heart, lungs, or liver of the INFECTED group. From this group, a total of 2.4×10^3 hypnozoites were recovered exclusively from the small and large intestines on the 1st DAI, from Peyer's patches between the 1st and 9th DAI, and from mesenteric lymph nodes and the spleen across all 35 evaluated DAIs (Table 5). However, by the 35th DAI, a significant reduction in the absolute number of isolates was observed in mesenteric lymph nodes and the spleen, which was accompanied by a relative decrease in isolation from mesenteric lymph nodes (4.42%) and a relative increase in isolation from the spleen (95.58%). The number of hypnozoites and their percentage relative to the total number of hypnozoites recovered daily from necropsies of various viscera are detailed in Table 5.

The viability of sporocysts in *C. ohioensis* oocysts inoculated into two puppies (designated the SPOROZOITE group) was confirmed when these animals began shedding a total of 9×10^5 oocysts over 24 days starting from the 5th DAI (Fig. 1b). Similarly, the viability of hypnozoites present in monozoic tissue cysts from the viscera of mice infected with 1×10^5 sporulated oocysts was demonstrated when the other two puppies (designated the HYPNOZOITE group) shed a total of 16.2×10^5 oocysts over 27 days starting from the 4th DAI (Fig. 1b). Neither of the two puppies inoculated orally with the same volume of inoculum (0.5 mL of PBS) containing organs from the CONTROL or PAIR-FED group mice shed oocysts in their feces. All six puppies maintained a healthy

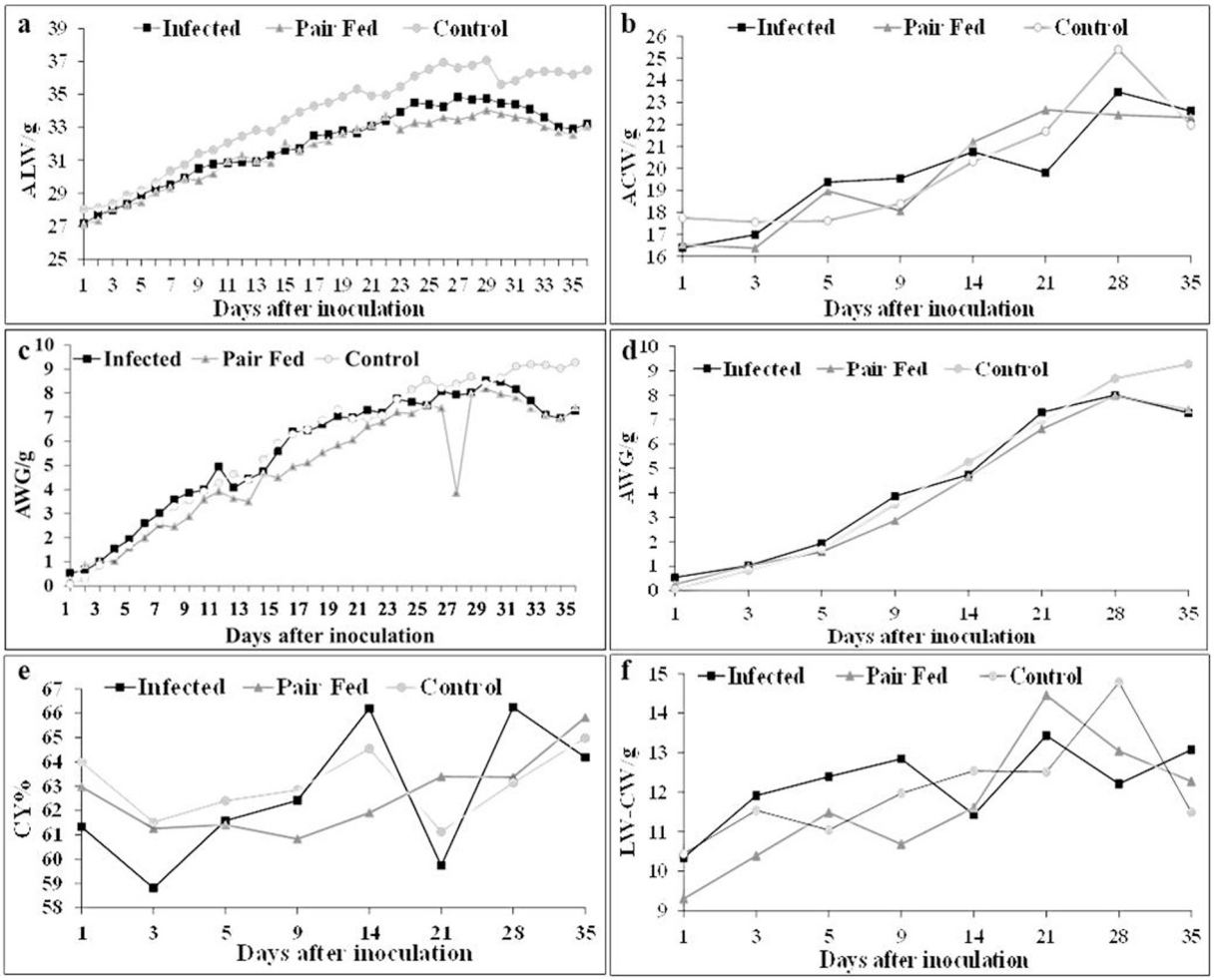

Fig. 3. Mean body weight development of mice inoculated with sporulated oocysts of *Cystoisospora ohioensis*-like species (Infected), pair-fed according to the mean daily con-sumption of their infected group counterparts (Pair-Fed), and fed *ad libitum* (Control): (a) Daily average live weight in grams over 35 experimental days (ALW/g); At 15 DAI, the Control group was higher ($p \leq 0.05$) than both the Infected group and Pair-Fed group. From 15–18 and 26–28 DAI, the Control group remained higher ($p \leq 0.05$) than the Pair-Fed group; (b) average carcass weight on days when five animals from each group were euthanized (ACW/g); (c) daily average weight gain in grams over 35 experimental days (AWG/g); (d) average live weight gain on days when five animals from each group were euthanized; The Pair-Fed group showed lower values ($p \leq 0.05$) than the Infected group at 8, 9, 15–17, and 27 DAI; and the Control group at 15–17, 19, and 27 DAI. (e) av-erage percentage carcass yield (CY%) on days when five animals from each group were euthanized; At 21 DAI, the Infected group was lower ($p \leq 0.05$) than the Pair-Fed group and (f) average live weight minus average carcass weight (Viscera weight) on days when five animals from each group were euthanized.

appearance without signs of infection or notable gastrointestinal symptoms. The mean dimensions and standard deviations of MD, md and MI, along with the maximum and minimum measurements of oocysts and sporocysts recovered from the HYPNOZOITE and SPOROZOITE groups, are shown in Table 1. The MD measurements of oocysts from the HYPNOZOITE group were significantly greater ($p \leq 0.05$) than those from the SPOROZOITE group, whereas the md and MI measurements were not significantly different ($p \geq 0.05$) between the groups. The daily oocyst shedding patterns were similar between the HYPNOZOITE and SPOROZOITE groups until the 21st DAI, when the HYPNOZOITE group presented a renewed increase in oocyst excretion (Fig. 1b).

4. Discussion

Cystoisosporosis is a common condition in dogs, and the most frequent clinical aspects are depression, weakness, loss of appetite, diarrhea, and dehydration (Oduye and Bobade, 1979; Correa et al., 1983; Olson, 1985; Penzhorn et al., 1992; Wu et al., 1993; Randhawa et al., 1997; Buehl et al., 2006; Altreuther et al., 2011). However, contrasting reports exist regarding the pathogenicity of these coccidia. While some studies associate *Cystoisospora* with enteritis (Dubey et al., 1978; Mitchell et al., 2007), others have observed no clinical signs in infected animals. He et al. (2012) did not observe any signs or symptoms of coccidiosis in 20 dogs shedding *C. ohioensis*-like oocysts. Similarly, Barrera et al. (2024), in a one-year study of 524 fecal samples from dogs in a kennel, diagnosed 46 (8.8%) animals as positive for *Cystoisospora* spp. but noted that none exhibited signs of coccidiosis. In the present study, none of the dogs—whether naturally infected, infected with 1×10^5 sporulated oocysts, or fed viscera containing hypnozoites from mice previously infected with 1×10^5 sporulated oocysts—exhibited any signs related to coccidiosis. These results may be related to the distinction of endogenous developmental stages, with *C. ohioensis*, according to Dubey (2019), being confined to the superficial intestinal epithelium, which differs from *C. rivolta* and *C. burrowsi*, whose endogenous stages occur predominantly in the lamina propria. Thus, we cannot infer that the naturally infected dogs and oocyst donors in this study were or had been infected by *C. ohioensis* but rather by the group of medium-sized coccidia referred to as the *C. ohioensis*-like complex.

Oocysts were shed in significant numbers by all six naturally infected puppies, as well as by the two puppies fed hypnozoites contained in the viscera of previously infected mice and by the two puppies orally

Table 2
Average live weight in grams (ALW) of mice inoculated with sporulated oocysts of *Cystoisospora ohioensis*-like organisms (INFECTED), fed the average daily intake of their counterparts in the infected group (PAIR-FED), and fed *ad libitum* (CONTROL).

DAI[a]	n[b]	GROUPS[c]					
		INFECTED		PAIR-FED		CONTROL	
0	45	27.22 ± 2.74		27.11 ± 2.22		28.04 ± 2.80	
1	45	27.69 ± 2.88		27.28 ± 2.39		28.10 ± 2.60	
3	40	28.33 ± 3.18		28.30 ± 2.01		28.88 ± 2.77	
5	35	29.30 ± 3.44		29.06 ± 2.30		29.61 ± 2.82	
9	30	30.77 ± 2.86		30.19 ± 2.56		31.28 ± 3.22	
14	25	31.60 ± 3.04		32.02 ± 3.07		33.44 ± 3.18	
15	20	31.70 ± 2.78	(K)	31.57 ± 2.44	(K, Y)	33.94 ± 3.33	(Z)
16	20	32.66 ± 2.70	(KY)	32.02 ± 2.36	(Y)	34.30 ± 3.35	(K, Z)
17	20	32.56 ± 2.44	(KY)	32.18 ± 2.36	(Y)	34.50 ± 3.54	(K, Z)
18	20	32.80 ± 2.25	(KY)	32.62 ± 2.58	(Y)	34.86 ± 3.60	(K, Z)
19	20	32.14 ± 3.20	(K)	32.90 ± 2.66	(K, Y)	35.32 ± 3.64	(Y, Z)
21	20	33.42 ± 2.21		33.75 ± 3.05		34.95 ± 3.93	
25	15	34.25 ± 1.83	(K)	33.60 ± 2.45	(K, Y)	36.93 ± 4.50	(Y, Z)
26	15	34.83 ± 2.02	(KY)	33.45 ± 2.44	(Y)	36.61 ± 4.31	(K, Z)
27	15	34.68 ± 2.01	(KY)	33.66 ± 2.59	(Y)	36.76 ± 4.46	(K, Z)
28	15	34.75 ± 2.03	(K, Y)	34.06 ± 2.70	(Y)	37.07 ± 4.34	(K, Z)
35	10	33.22 ± 4.73		33.08 ± 3.42		36.47 ± 4.09	

[a] Days after inoculation.
[b] Number of samples in each group.
[c] Letters, in parentheses, equal in the lines, ALW does not differ according to the Tukey Test ($p \geq 0.05$) with a 95% confidence interval.

infected with sporozoites contained in sporulated oocysts (Fig. 1a and b). The number of oocysts shed daily by the dogs in the groups inoculated with hypnozoites and sporozoites was similar until the 21st DAI, when the HYPNOZOITES group presented a new increase in the number of oocysts shed (Fig. 1b). The animals in this group reached peak daily shedding on the 25th DAI and continued shedding oocysts until the 27th DAI, differing from the animals in the SPOROZOITE group, which peaked on the 5th DAI and shed oocysts until the 24th DAI (Fig. 1b). The prepatent period of *C. ohioensis* can range from 13 to 23 days (Mahrt, 1967; Dubey, 1975a), and the prepatent period observed by Rocha and Lopes (1971) in dogs inoculated with sporulated oocysts was 3–7 days. In induced infections, the observation of gametocytes in the intestinal cells of dogs at 96 and 114 h postinoculation with hypnozoites and sporozoites, respectively, suggests a biological distinction between the two parasitic forms (Dubey, 1977). Dubey and Lindsay (2019), in their review of canine coccidiosis, reported that the asexual and sexual stages in cycles induced by sporozoites or hypnozoites are structurally similar in size and location, but the hypnozoite-driven cycle develops 24 h faster. This was evident in our research results, where dogs inoculated with hypnozoites had a shorter prepatent period, shed a greater number of oocysts, and exhibited a longer patent period, reinforcing the hypothesis that the two parasitic stages are biologically distinct, as suggested by Dubey (1977) and Dubey and Lindsay (2019). The oocyst shedding pattern in naturally infected dogs was similar to that in dogs fed viscera from previously infected mice (hypnozoites), suggesting that

Table 3
Average weight gain in grams (AWG) of mice inoculated with sporulated oocysts of *Cystoisospora ohioensis*-like organisms (INFECTED), fed the average daily intake of their counterparts in the infected group (PAIR-FED), and fed *ad libitum* (CONTROL).

DAI[a]	n[b]	GROUPS[c]					
		INFECTED		PAIR-FED		CONTROL	
1	45	0.53 ± 0.61	(K)	0.25 ± 0.75	(K, Y)	0.05 ± 0.66	(Y, Z)
2	40	0.66 ± 0.76	(KY)	0.87 ± 0.98	(Y)	0.28 ± 0.76	(K, Z)
3	40	1.01 ± 0.98		1.01 ± 0.98		0.82 ± 1.11	
5	35	1.93 ± 1.34		1.58 ± 1.33		1.68 ± 1.12	
8	30	3.58 ± 1.45	(K)	2.45 ± 1.13	(Y, Z)	3.30 ± 1.66	(K, Z)
9	30	3.86 ± 1.47	(K)	2.86 ± 1.28	(Y, Z)	3.53 ± 1.70	(K, Z)
14	25	4.74 ± 2.03		4.65 ± 1.30		5.26 ± 1.54	
15	20	5.59 ± 1.61	(K, Y)	4.48 ± 1.61	(Y)	5.93 ± 1.58	(K, Z)
16	20	6.39 ± 1.53	(K)	4.94 ± 1.69	(Y)	6.29 ± 1.55	(K, Z)
17	20	6.44 ± 1.85	(K)	5.10 ± 1.73	(Y)	6.49 ± 1.56	(K, Z)
19	20	7.03 ± 1.91	(K, Z)	5.82 ± 1.85	(K, Y)	7.32 ± 1.71	(Z)
21	20	7.30 ± 2.05		6.61 ± 1.92		6.95 ± 2.02	
27	15	7.93 ± 1.93	(K)	3.84 ± 1.33	(Y)	8.37 ± 2.32	(K, Z)
28	15	8.00 ± 2.24		7.97 ± 2.22		8.68 ± 2.26	
35	10	7.27 ± 4.71		7.41 ± 3.29		9.27 ± 2.47	

[a] Days after inoculation.
[b] Number of samples in each group.
[c] Letters, in parentheses, equal in the lines, AWG does not differ according to the Tukey Test ($p \geq 0.05$) with a 95% confidence interval.

Table 4
Average carcass weights in grams (ACW) relative to live weights of mice inoculated with sporulated oocysts of *Cystoisospora ohioensis*-like organisms (INFECTED), fed the average daily intake of their counterparts in the infected group (PAIR-FED), and fed *ad libitum* (CONTROL).

DAI[a]	n[b]	GROUPS[c]					
		INFECTED		PAIR-FED		CONTROL	
1	5	16.40 ± 0.65		16.54 ± 1.12		17.75 ± 2.75	
3	5	16.99 ± 1.43		16.38 ± 0.74		17.56 ± 1.65	
5	5	19.38 ± 1.35		18.98 ± 0.36		17.62 ± 1.91	
9	5	19.55 ± 1.05		18.07 ± 1.55		18.40 ± 1.31	
14	5	20.75 ± 3.59		21.19 ± 3.11		20.30 ± 1.65	
21	5	19.81 ± 2.13	(K, Z)	22.66 ± 0.41	(Y)	21.68 ± 0.93	(Y, Z)
28	5	23.47 ± 0.92	(K, Y)	22.43 ± 1.30	(Y)	25.40 ± 1.54	(K, Z)
35	5	22.61 ± 1.00		22.31 ± 2.41		21.96 ± 1.30	

[a] Days after inoculation.
[b] Number of samples in each group.
[c] Letters, in parentheses, equal in the lines, ACW does not differ according to the Tukey Test ($p \geq 0.05$) with a 95% confidence interval.

the six naturally infected dogs had ingested hypnozoites through contaminated food. In 1995, Dubey reported that dogs infected with

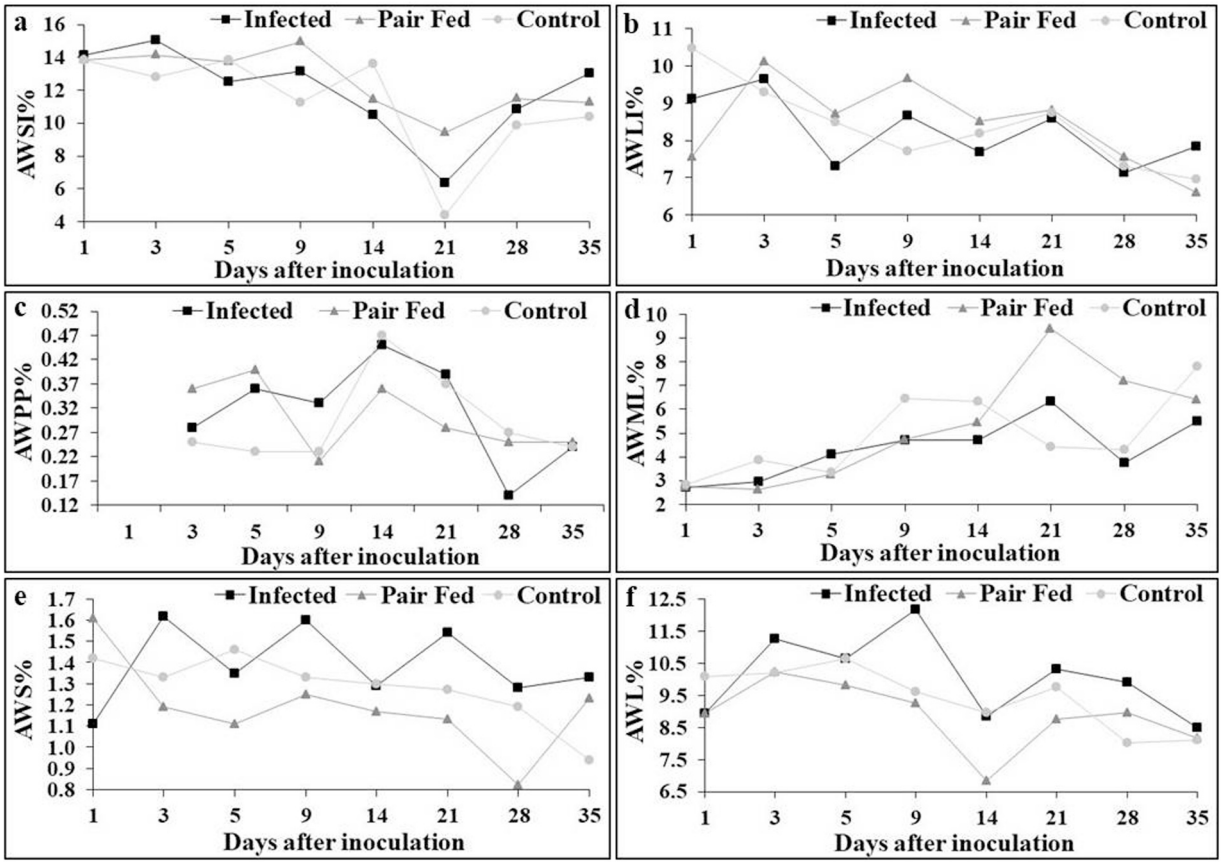

Fig. 4. Average percentage weights of the small intestine (AWSI%) (a), in the 21 DAI, the Control group was significantly higher ($p \leq 0.05$) than the Pair-Fed group; Large intestine (AWLI%) (b), Peyer's patches (AWPP%) (c), at 5 DAI, the Infected group was higher ($p \leq 0.05$) than the Control group; Mesenteric lymph nodes (AWML%) (d), at 21 DAI, the Pair-Fed group was higher ($p \leq 0.05$) than the Control group; Spleen (AWS%) (e), at 35 DAI, the Infected group was higher ($p \leq 0.05$) than the Control group; Liver (AWL%), at 9 DAI, the Infected group was higher ($p \leq 0.05$) than the Pair-Fed group; (f) relative to the carcasses of five mice inoculated orally with 105 sporulated oocysts of *Cystoisospora ohioensis*-like species (Infected), five fed the average daily intake of their counterparts in the infected group (Pair-Fed), and five fed *ad libitum* (Control) per evaluation day.

Table 5
Hypnozoites quantified by recovery from the viscera of five mice inoculated with sporulated oocysts of Cystoisospora ohioensis-like organisms.

DAI[a]	HYPOZOITES/VISCERA										TOTAL	
	Small intestine		Large intestine		Peyer's patch		Mesenteric lymph node		Spleen			
	n[b]	(%)[c]	n	%	n	%	n	%	n	%	n	%
1	102	17.62	147	25.39	135	23.31	86	14.85	109	18.83	579	100
3	-	-	-	-	50	7.17	506	72.60	141	20.23	697	100
5	-	-	-	-	28	25.00	44	39.29	40	35.71	112	100
9	-	-	-	-	9	4.89	60	32.61	115	62.50	184	100
14	-	-	-	-	-	-	38	18.45	168	81.55	206	100
21	-	-	-	-	-	-	60	23.90	191	76.10	251	100
28	-	-	-	-	-	-	35	12.96	235	87.04	270	100
35	-	-	-	-	-	-	5	4.42	108	95.58	113	100
GRAND TOTAL	102	4.23	147	6.09	222	9.20	834	34.58	1.107	45.90	2.412	100

[a] Days after inoculation.
[b] Number of hypnozoites per viscera.
[c] Percentage of hypnozoites recovered per organ relative to the total visceral load.

1×10^5 oocysts of *C. canis* or fed tissues from mice previously infected with 1×10^5 oocysts produced similar numbers of oocysts and had comparable prepatent periods (Dubey, 1975b). However, in our experiment, dogs inoculated with 1×10^5 sporulated oocysts had a longer prepatent period than those fed viscera from mice previously inoculated with 1×10^5 sporulated oocysts (Fig. 1b).

The subspherical appearance of the oocysts and ellipsoid shape of the sporocysts shed by the six naturally infected dogs, as well as the measurements of the major and minor diameters of the oocysts and sporocysts, were compatible with those observed by Rocha and Lopes (1971) and Dubey (1992) for *C. rivolta* and with those characterized by Lindsay et al. (1997a) as intermediate-sized oocysts from dogs with dimensions ranging from 17 to 23 μm, grouped as *C. ohioensis*-like. In the present study, the measurements of MD, md and MI of oocysts and sporocysts from the six naturally infected dogs were smaller than those of oocysts and sporocysts shed by the two puppies inoculated with sporulated oocysts and the two puppies fed mouse viscera containing hypnozoites. These differences in measurements may be related to

varying proportions of medium-sized oocysts being shed, corresponding to the species *C. ohioensis*, *C. burrowsi* and *C. rivolta*, which cannot be distinguished morphologically (Lindsay et al., 1997a).

The lower ALW of the animals in the INFECTED group throughout the experiment, which became statistically significant from the 15th DAI compared with that of the CONTROL group (Table 2), and the AWG of the PAIR-FED group, which was consistently lower than that of the INFECTED group (Table 3), support the hypothesis that the reduced performance of intermediate hosts in terms of daily live weight and weight gain is related to the development of infective forms for the subsequent establishment of extraintestinal hypnozoites, where an increase in visceral weight (Fig. 3f) when parasitized masks the cachectic effect of *C. ohioensis*-like species. These observations were also detected in mice inoculated with sporulated oocysts of *C. rivolta* (=*C. ohioensis*-like) by Brösike et al. (1982) and with *C. felis* by Loss and Lopes (1992c), Freire and Lopes (1996), and Costa and Lopes (1994). The differences in ALW and AWG between the INFECTED and CONTROL groups are likely related to the anorexia that follows infection, as described by Loss and Lopes (1992c).

Studies with cattle (Fayer and Frenkel, 1979; Wolters et al., 1980); buffalo, sheep, and swine (Zayed and El-Ghaysh, 1998; Melo et al., 2003; Carvalho Filho et al., 2003); rabbits (Costa and Lopes, 1998); and broiler chickens (Massad et al., 2003) have indicated that production animals can harbor varying numbers of *Cystoisospora* genus hypnozoites in their viscera. However, whether parasitic action in these organs influences weight gain in these animals or causes significant lesions remains unknown. Recurrent clinical disease is common in both immunocompetent and immunosuppressed humans infected with *C. belli*, which is believed to be due to reactivation of the hypnozoites present in tissues and their migration to the human intestinal tract (Lindsay et al., 1997b; Velásquez et al., 2001). Immunosuppressed patients frequently experience clinical disease relapse after discontinuation of *C. belli* treatment (Boyles et al., 2012). Ultimately, the interference of this parasitism on weight development in humans and animals remains unknown, whereas economic production of animal protein requires increasingly greater financial investments to meet demand. Our research suggests that animals may have impaired zootechnical performance, as occurred with mice in the INFECTED group during the establishment of hypnozoites in target organs or due to anorexia induced during this process. This hypothesis is reinforced by the fact that mice in the PAIR-FED group showed lower ALW and AWG performance than the INFECTED group, resulting from feed control that increased stress levels and the lack of nutrients necessary for the development of healthy animals.

Biological assays in cats have indicated that infectious stages of *C. felis* and *C. rivolta* can develop in gerbils, guinea pigs, rabbits, and chickens fed sporulated oocysts (Oliveira et al., 2007). In addition to the results of this study, it can be inferred that infection of mice with *C. ohioensis*-like and potentially other intermediate hosts negatively affects carcass weight. The increased organ weight in the parasitized animals (Fig. 3f) contributed to a lower carcass yield (Tables 3 and 4), since parasitism also reduced the ALW (Fig. 3a, b and Table 2) and AWG (Fig. 3c, d and Table 3) of the infected mice.

Freire and Lopes (1996) reported no differences in the mean weight percentages of the heart, lungs, kidneys, and brain in mice inoculated with sporulated oocysts of *C. felis*. Dubey (1978) previously failed to demonstrate hypnozoites or extraintestinal stages of *C. ohioensis* in histopathological sections of the heart, lung, and brain, leading Freire and Lopes (1996) to suggest that these organs, including the kidneys, are rarely parasitized by *Cystoisospora* spp. In our research, no differences in average stomach weight percentage (ASW%) or average heart weight percentage (AWH%) were detected between the groups throughout the experiment. The average weight percentages of the small intestine (AWSI%) and large intestine (AWLI%) of mice in the INFECTED group were greater, although not significantly so, likely because of the presence of transient parasites in these organs (Table 5). Considering the viscera where *Cystoisospora* spp. has been previously reported and the organ weight percentages observed in this experiment, these organs are not frequently parasitized by C. ohioensis-like organisms, as previously reported with other *Cystoisospora* spp. All aforementioned authors reported alterations in the average weight percentages of Peyer's patches (AWPP%), mesenteric lymph nodes (AWML%), spleen (AWS%), and liver (AWL%) of animals inoculated with *Cystoisospora* spp., which is consistent with our observations for *C. ohioensis*-like organisms. Dubey (1979) demonstrated that sporozoites released after excystation of *C. rivolta* sporulated oocysts most frequently invade mouse mesenteric lymph nodes, remaining infected for at least 23 months, potentially explaining the increased AWML% in our study (Fig. 4d). Freire and Lopes (1996) reported increased AWL% in infected mice from the 3rd to 18th DAI, whereas in our experiment, the AWL% increase occurred from the 3rd to 9th DAI (Fig. 4f), followed by a decrease, with no differences among groups until the 35th DAI. These cyclic alterations, also observed in AWPP% and AWML%, may be due to spontaneous flow of parasite hypnozoite forms during their biological cycle development in the paratenic host. Our results revealed that during parasite passage through organs, their weight percentages increased (Fig. 4a,f), possibly due to elimination of some forms while others became encysted. Since parasite multiplication does not occur in the intermediate host, the organ weight percentages returned to normal during the chronic infection process, as observed with AWPP% (Fig. 4c), AWML% (Fig. 4d), and AWL% (Fig. 4f). However, the spleen did not follow this pattern, with AWS% (Fig. 4e) increasing throughout the entire process, including the chronic phase, suggesting that this organ best harbors and maintains *C. ohioensis* parasitic forms for the longest duration.

Previous research on *Cystoisospora* spp. showed minimal parasite presence in organs like the heart, lungs, and kidneys, with no significant weight changes (Freire and Lopes, 1996; Dubey, 1978). In the present study, infected mice exhibited temporary, non-significant weight increases in the small intestine and liver, likely due to transient parasites. Consistent with prior studies (Dubey, 1979; Brösike, 1981; Brösike et al., 1982; Loss and Lopes, 1992c; Costa and Lopes, 1994; Freire and Lopes, 1996), an increase in the weight were observed in the Peyer's patches, mesenteric lymph nodes, liver and spleen. Since parasite multiplication does not occur in the intermediate host, the organ weight percentages returned to normal during the chronic infection process. However, the spleen did not follow this pattern, with AWS% (Fig. 4e) increasing throughout the entire process, including the chronic phase, suggesting that this organ best harbors and maintains *C. ohioensis* parasitic forms for the longest duration. This suggests these organs, particularly the spleen, are primary sites for harboring *C. ohioensis*-like hypnozoites during chronic infectionas described by Freire and Lopes (1996), with weight fluctuations reflecting the migration and encystment of these non-multiplying parasite forms.

The combination of lower carcass yield and higher visceral weight in mice reflects the imbalance between catabolism and energy demand imposed by *C. ohioensis*-like infection. This occurs because the host prioritizes resources to fight the infection, diverting glucose and lipids to immune cells rather than muscle or adipose storage, leading to reduced carcass yield, as described by Lochmiller and Deerenberg (2000). Additionally, intestinal inflammation likely activates pro-inflammatory cytokines that stimulate muscle protein degradation to provide amino acids for the immune system and tissue repair, as described by Argilés and López-Soriano (1999) in cachexia. According to Chapman et al. (2013), the increased weight of visceral organs reflects the intense immune and inflammatory response in the tissue, which increases vascular permeability, causing edema and fluid accumulation that contributes to weight gain without actual metabolic mass gain. These mechanisms reflect the host's prioritization of fighting infection at the expense of normal growth and metabolism.

Parasite dissemination in rodent organs occurs similarly to other coccidia through phagocytes (Mehlhorn and Markus, 1976; Dubey and Mehlhorn, 1978; Boch et al., 1981; Markus, 1983). Freire and Lopes (1996) suggested both hematogenous and lymphatic routes for

extraintestinal dissemination of *Cystoisospora* spp. in intermediate hosts. Liver and spleen enlargement in the initial days postinoculation results from rapid parasite spread via the bloodstream, whereas slower dissemination likely occurs through the lymphatic system, sequentially increasing lymphoid organ size. Our research revealed temporary production losses in immunocompetent intermediate hosts, which are likely exacerbated in immunocompromised animals—a common scenario in meat and dairy production systems due to the confinement stress required for increased productivity.

Freire and Lopes (1996) observed *C. felis* hypnozoites in the kidneys, lungs, and heart, attributing this to accidental distribution rather than a normal parasitic access route. The absence of hypnozoites in the stomach, heart, and lungs in our study suggests these organs are not preferential for *C. ohioensis*-like organisms in mice, a hypothesis supported by visceral weight evaluation. No hypnozoites were observed in the livers of INFECTED group mice in our research. Considering that the liver is a known site for hypnozoites in intermediate hosts infected with other *Cystoisospora* species (Brösigke, 1981; Brösigke et al., 1982; Costa and Lopes, 1994; Freire and Lopes, 1996), we cannot conclusively state that the liver was free from parasitism, as a significant cellular mass remained after the peptic digestion of liver tissue, requiring substantial sample dilution to improve hypnozoite visualization.

In the spleen, a progressive increase in recovered hypnozoites was observed, indicating this organ is the primary target of *C. ohioensis*-like organisms in mice. Brösike (1981) noted a strong predilection of *C. rivolta* for mesenteric lymph nodes, whereas Freire and Lopes (1996) reported that *C. felis* prefers the spleen and liver. The dynamics of *C. ohioensis*-like organisms in our study suggest a faster hematogenous route to the spleen between the 1st and 3rd DAI, and a slower lymphatic migration route from the 9th DAI onward, indicating a more lymphotropic distribution. Decreased parasite recovery at the 35th DAI may reflect either parasite elimination or survival bias of digestion-resistant stages (Lindsay et al., 2014), supporting the hypothesis of immunological regulation where *C. rivolta*-like species are more easily controlled than *C. felis* (Fayer and Frenkel, 1979; Long, 1982).

Studies analyzing the deleterious effects of *Cystoisospora* spp. are scarce, and no research has addressed the pathogenic and nutrient-depleting aspects of *C. ohioensis*-like organisms in intermediate hosts. In livestock, confinement stress aimed at high productivity is a key factor in opportunistic infections. Our findings demonstrate that *C. ohioensis*-like species invade extraintestinal organs, increasing organ size and masking body weight, ultimately impairing carcass yield. These results challenge the traditional view of non-definitive hosts as merely "carriers" with a harmonious parasite-host relationship. Based on reduced weight gain and carcass yield alongside increased visceral weight, non-definitive hosts should be reclassified as true intermediate hosts, as infection significantly impacts their physiology. This nutrient-wasting effect stems from immune prioritization over growth, with the spleen identified as the primary target organ. These findings reveal important production losses in livestock and potential implications for immunocompromised individuals, where hypnozoite reactivation may exacerbate disease, highlighting this coccidiosis as an economically relevant condition requiring attention in animal production systems.

CRediT authorship contribution statement

Carlos Wilson Gomes Lopes: Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Formal analysis, Conceptualization. **Samira Salim Mello Gallo:** Writing – review & editing, Validation, Resources, Methodology, Formal analysis, Data curation, Conceptualization. **Francisco Carlos Rodrigues de Oliveira:** Writing – original draft, Validation, Software, Project administration, Methodology, Investigation, Data curation, Conceptualization.

Funding

This research received no external funding.

Declaration of Competing Interest

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.

Acknowledgement

We sincerely thank everyone at the Department of Animal Parasitology of the Universidade Federal Rural of Rio de Janeiro who in some way helped in carrying out this research.

The Article Processing Charge for the publication of this research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (ROR identifier: 00x0ma614).

References

Altreuther, G., Gasda, N., Schroeder, I., Joachim, A., Settje, T., Schimmel, A., Hutchens, D., Krieger, K.J., 2011. Efficacy of emodepside plus toltrazuril suspension (Procox R® oral suspension for dogs) against prepatent and patent infection with *Isospora canis* and *Isospora ohioensis*-complex in dogs. Parasitol. Res. 109 (1), S9–S20. https://doi.org/10.1007/s00436-011-2398-0.

Argilés, J.M., López-Soriano, F.J., 1999. The role of cytokines in cancer cachexia. Med. Res. Rev. 19, 223–248. https://doi.org/10.1002/(sici)1098-1128(199905)19:3<223::aid-med3>3.0.co;2-n.

Barrera, J.P., Montoya, A., Marino, V., Sarquis, J., Checa, R., Miró, G., 2024. *Cystoisospora* spp. infection at a dog breeding facility in the Madrid region: Infection rate and clinical management based on toltrazuril metaphylaxis. Vet. Parasitol. Reg. Stud. Rep. 48, 100971. https://doi.org/10.1016/j.vprsr.2023.100971.

Barta, J.R., Martin, D.S., Carreno, R.A., Siddall, M.E., Profousj-Uchelka, H., Hozza, H., Powles, M.A., Sundermann, C., 2001. Molecular phylogeny of the other tissue coccidia: *Lankesterella* and *Caryospora*. J. Parasitol. 87, 121–127. https://doi.org/10.1645/0022-3395(2001)087[0121:MPOTOT]2.0.CO;2.

Barta, J.R., Schrenzel, M.D., Carreno, R., Rideout, B.A., 2005. The genus Atoxoplasma (Garnham 1950) as a junior objective synonym of the genus Isospora (Schneider 1881) species infecting birds and resurrection of Cystoisospora (Frenkel 1977) as the correct genus for Isospora species infecting mammals. J. Parasitol. 91, 726–727. https://doi.org/10.1645/GE-3341.1.

Benator, D.A., French, A.L., Beaudet, L.M., Levy, C.S., Orenstein, J.M., 1994. *Isospora belli* infection associated with acalculous cholecystitis in a patient with AIDS. Ann. Intern. Med. 121, 663–664. https://doi.org/10.7326/0003-4819-121-9-199411010-00006.

Boch, J., Göbel, E., Heine, J., Erber, M., 1981. *Isospora*-Infektionen bei Hund und Katze. *Berl. Munch.* Tierarztl. Woche 94, 84–391.

Boyles, T.H., Black, J., Meintjes, G., Mendelson, M., 2012. Failure to eradicate Isospora belli diarrhea despite immune reconstitution in adults with HIV—a case series. PLoS One 7, e42844. https://doi.org/10.1371/journal.pone.0042844.

Brazil. Law No. 6,638 of May 8, 1979. It establishes procedures for the scientific use of animals. Available at: ⟨https://www.planalto.gov.br/ccivil_03/leis/1970-1979/l6638.htm.⟩.

Brösike, S., Heine, J., Boch, J., 1982. Der nachweis extraintestinallen entwicklingstadien (Dormozoiten) in extraintestinall mit *Cystoisospora rivolta* oozysten infizierten Mausen. Kleintierprax 27, 25–34.

Brösike, S., 1981. Untersuchungen an extraintestinalen entwicklungsstadien (Dormozoiten) von Cystoisospora rivolta der Katze in der Maus. Diss. Zur. Erlangung der Tiermed. Dr. der Tierarztl. Muchen 37.

Buehl, I.E., Prosl, H., Mundt, H.C., Tichy, A.G., Joachim, A., 2006. Canine isosporosis - epidemiology of field and experimental infections. J. Vet. Med. 53, 482–487. https://doi.org/10.1111/j.1439-0450.2006.00973.x.

Carreno, R.A., Schnitzler, B.E., Jeffries, A.C., Tenter, A.M., Johnson, A.M., Barta, J.R., 1998. Phylogenetic analysis of coccidia based on 18S rDNA sequence comparison indicates that *Isospora* is most closely related to *Toxoplasma* and *Neospora*. J. Eukaryot. Microbiol. 45, 184–188. https://doi.org/10.1111/j.1550-7408.1998.tb04523.x.

Carvalho Filho, P.R., Melo, P.S., Massad, F.V., Lopes, C.W.G., 2003. Determinação da infecção de suínos por *Cystoisospora felis* (Wenyon, 1923) Frenkel, 1977 (Apicomplexa: Cystoisosporinae) através de prova biológica em felinos livres de coccídios. Rev. Bras. Parasitol. Vet. 12, 37–42.

Chapman, D.H., Barta, J.R., Blake, D., Gruber, A., Jenkins, M., Smith, N.C., Suo, X., Tomley, F.M., 2013. A selective review of advances in coccidiosis research. Adv. Parasitol 83, 93–171.

Comin, C.E., Santucci, M., 1994. Submicroscopic profile of Isospora belli enteritis in a patient with acquired immune deficiency syndrome. Ultrastruct. Pathol. 18, 473–482. https://doi.org/10.3109/01913129409023222.

Correa, W.M., Correa, C.N.M., Langoni, H., Volpato, A.O., Tsunoda, K., 1983. Canine isosporosis. Canine Pract. 10, 44–46.

Costa, P.S., Lopes, C.W.G., 1998. Avaliação do parasitismo por Cystoisospora felis (Wenyon, 1923) Frenkel, 1977 (Apicomplexa: Cystoisosporinae) em coelhos tipo carne. Braz. J. Vet. Parasitol. 7, 15–19. https://doi.org/10.1590/S1984-29612011000300012.
Costa, P.S., Lopes, C.W.G., 1994. Hipnozoítas de *Cystoisospora felis* (Apicomplexa: Cystoisosporinae). Rev. Bras. Ciênc. Vet. 1, 35–36. https://doi.org/10.4322/rbcv.2015009.
Dubey, J.P., Capenter, J.L., Speer, C.A., Topper, M.J., Uggla, A., 1988. Newly recognized fatal protozoan disease of dogs. J. Am. Vet. Med. Assoc. 192, 1269–1285. PMID: 3391851.
Dubey, J.P., Lindsay, D.S., 2019. Coccidiosis in dogs—100 years of progress. Vet. Parasitol. 266, 34–55. https://doi.org/10.1016/j.vetpar.2018.12.004.
Dubey, J.P., Mahrt, J.L., 1978. *Isospora neorivolta* sp. n. from the domestic dog. J. Parasitol. 64, 1067–1073. PMID: 739301.
Dubey, J.P., Mehlhorn, H., 1978. Extraintestinal stages of *Isospora ohioensis* from dogs in mice. J. Parasitol. 64, 689. PMID: 682070.
Dubey, J.P., Weisbrode, S.E., Rogers, W.A., 1978. Canine coccidiosis attributed to an *Isospora ohioensis*-like organism: a case re-port. J. Am. Vet. Med. Assoc. 173, 185–191.
Dubey, J.P., 1975a. *Isospora ohioensis* sp. n. proposed for *I. rivolta* of the dog. J. Parasitol. 61, 462–465.
Dubey, J.P., 1975b. Experimental *Isospora canis* and *Isospora felis* infection in mice, cats, and dogs. J. Protozool. 22, 416–417. https://doi.org/10.1111/j.1550-7408.1975.tb05195.x.
Dubey, J.P., 1978. Life cycle of *Isospora ohioensis* in dogs. Parasitol 77, 1–11. https://doi.org/10.1017/s0031182000048654.
Dubey, J.P., 1979. Life cycle of *Isospora rivolta* (Grassi 1879) in cats and mice. J. Protozool. 26, 433–443. https://doi.org/10.1111/j.1550-7408.1979.tb04650.x.
Dubey, J.P., 2019. Re-evaluation of merogony of a *Cystoisospora ohioensis*-like coccidian and its distinction from gametogony in the intestine of a naturally infected dog. Parasitol 146, 740–745. https://doi.org/10.1017/S0031182018002202.
Dubey, J.P., 1977. *Toxoplasma, Hammondia, Besnoitia, Sarcocystis* and other tissue cyst-forming coccida of man and animals. In: Kreier, J.P. (Ed.), Protozoa. Academic Press, New York, pp. 102–219.
Dubey, J.P., 1992. *Toxoplasma, Hammondia, Besnoitia, Sarcocystis* and other tissue cyst-forming coccidia of man and animals. In: Kreier, J.P. (Ed.), Protozoa. Academic Press, New York, pp. 120–128.
Dubey, J.P., 1993. *Toxoplasma, Neospora, Sarcocystis* and other tissue cyst forming coccidia of humans and animals. In: Kreier, J.P. (Ed.), Parasitic Protozoa. Academic Press, New York, pp. 5–57.
Fayer, R., Frenkel, J.K., 1979. Comparative infectivity for calves of oocysts of feline coccidia: *Besnotia, Hammondia, Cystoisospora, Sarcocystis* and *Toxoplasma*. J. Protozool. 65, 756–762. PMID: 117090.
Franzen, C., Müller, A., Bialek, R., Diehl, V., Salzberger, B., Fätkenheuer, G., 2000. Taxonomic position of the human intestinal protozoan parasite *Isospora belli* as based on ribosomal RNA sequences. Parasitol. Res. 86, 669–676. https://doi.org/10.1007/pl00008550.
Freire, R.B., Lopes, C.W.G., 1996. Distribuição de hipnozoítas de *Cystoisospora felis* (Weyon, 1923) Frenkel, 1977 (Apicomplexa: Sarcocystidae) em camundongos albinos experimentalmente infectados. Rev. Bras. Parasitol. Vet. 5, 23–28.
Frenkel, J.K., Dubey, J.P., 1972. Rodents as vectors for feline coccidia *Isospora felis* and *Isospora rivolta*. J. Infect. Dis. 125, 69–72. https://doi.org/10.1093/infdis/125.1.69.
Frenkel, J.K., Silva, M.B., Saldanha, J.C., De Silva-Vergara, M.L., Correia, D., Barata, C. H., Silva, E.L., Ramirez, L.E., Prata, A., 2003a. Presença extra-intestinal de cistos unizóicos de *Isospora belli* em paciente com SIDA: relato de caso. Rev. Soc. Bras. Med. Trop. 36, 409–412. https://doi.org/10.1590/S0037-86822003000300014.
Frenkel, J.K., Smith, D.D., 2003. Determination of the genera of cystforming coccidia. Parasitol. Res. 91, 384–389. https://doi.org/10.1007/s00436-003-0969-4.
Frenkel, J.K., 1977. *Besnoitia wallace* in cats and rodents: with a reclassification of other cyst-forming isosporoid coccidia. J. Parasitol. 63, 611–628. PMID: 407344.
Grellet, A., Mila, H., 2024. Endoparasitic diseases in breeding kennels: a frequent and complex problem requiring a holistic approach. Animals 14, 1–10.
He, P., Li, J., Gong, P., Huang, J., Zhang, X., 2012. Cystoisospora spp. from dogs in China and phylogenetic analysis of its 18S and ITS1 gene. Vet. Parasitol. 23, 254–258. https://doi.org/10.1016/j.vetpar.2012.05.025.
Hilali, M., Fatani, A., Al-Atiya, S., 1995. Isolation of tissue cysts of *Toxoplasma, Isospora, Hammondia* and *Sarcocystis* from camel (*Camelus dromedarius*) meat in Saudi Arabia. Vet. Parasitol. 58, 353–356. https://doi.org/10.1016/0304-4017(94)00727-t.
Hilali, M., Nassar, A.M., El-Ghaysh, A., 1992. Camel (*Camelus dromedarius*) and sheep (*Ovis aries*) meat as a source of dog infection with some coccidian parasites. Vet. Parasitol. 43, 37–43. https://doi.org/10.1016/0304-4017(92)90046-c.
Lee, S., Kim, J., Cheon, D.S., Moon, E.A., Seo, D.J., Jung, S., Shin, H., Choi, C., 2018. Identification of *Cystoisospora ohioensis* in a diarrheal dog in Korea. Korean J. Parasitol. 56, 371–374. https://doi.org/10.3347/kjp.2018.56.4.371.
Levine, N.D., 1985. Veterinary Protozoology. Iowa State University Press, Ames, p. 414.
Lindsay, D.S., Dubey, J.P., Blackburn, B.L., 1997a. Biology of *Isospora* spp. from humans, nonhuman primates and domestic animals. Clin. Microbiol. Rev. 10, 19–34. https://doi.org/10.1128/CMR.10.1.19.
Lindsay, D.S., Dubey, J.P., Toivio-Kinnuncan, M.A., Michiels, J.F., Blagburn, B.L., 1997b. Examination of extraintestinal tissue cysts of *Isospora belli*. J. Parasitol. 83, 620–625.
Lindsay, D.S., Houk, A.E., Mitchell, S.M., Dubey, J.P., 2014. Developmental Biology of *Cystoisospora* (Apicomplexa: Sarcocystidae) Monozoic Tissue Cysts. J. Parasitol. 100, 392–398. https://doi.org/10.1645/13-494.1.
Lochmiller, R.L., Deerenberg, C., 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? Oikos 88, 87–98. https://doi.org/10.1034/j.1600-0706.2000.880110.x.
Long, P.L., 1982. The Biology of the Coccidia. Univ Park Press, Baltimore, p. 502.
Loss, Z.G., Lopes, C.W.G., 1992a. Alguns aspectos clínicos na infecção experimental por *Cystoisospora felis* (Wenyon, 1926) Frenkel, 1976 (Apicomplexa: Cystoisosporinae) em gatos. Arq. UFRRJ 15, 79–84.
Loss, Z.G., Lopes, C.W.G., 1992b. Aspecto patológicos da infecção experimental por *Cystoisospora felis* (Wenyon, 1926) Frenkel, 1976 (Apicomplexa: Cystoisosporinae) em gatos. Arq. UFRRJ 15, 113–119.
Loss, Z.G., Lopes, C.W.G., 1992c. Efeito da infecção experimental por *Cystoisospora felis* (Apicomplexa: Cystoisosporinae) no ganho de peso de camundongos. Arq. UFRRJ 15, 109–111.
Mahrt, J.L., 1967. Endogenous stages of the life cycle of *Isospora rivolta* in the dog. J. Parasitol. 14, 754–759. https://doi.org/10.1111/j.1550-7408.1967.tb02073.x.
Markus, M.B., 1983. The hypnozoite of *Isospora canis*. S. Afr. J. Sci. 79, 117.
Massad, F.V., Oliveira, F.C.R., Albuquerque, G.R., Lopes, C.W.G., 2003. Hipnozoítas de *Cystoisospora ohioensis* (Dubey, 1975) Frenkel, 1977 (Apicomplexa: Cystoisosporinae) em frangos. Rev. Bras. Ciênc. Vet. 10, 57–58. https://doi.org/10.4322/rbcv.2015.269.
Mehlhorn, H., Markus, M.B., 1976. Electron microscopy of stages of *Isospora felis* of the cat in the mesenteric lymph nodes of the mouse. Z. Parasitenkd. 51, 25–29. https://doi.org/10.1007/BF00380524.
Melo, P.S., Carvalho Filho, P.R., Lopes, C.W.G., Flausino, W., Oliveira, F.C.R., 2003. Hypnozoites of *Cystoisospora felis* (Wenyon, 1923) Frenkel, 1977 (Apicomplexa: Cystoisosporinae) isolated from piglets experimentally infected. Rev. Bras. Parasitol. Vet. 12, 57–59.
Michiels, J.F., Hofman, P., Bernard, E., Saint Paul, M.C., Boissy, C., Mondain, V., LeFichoux, Y., Loubiere, R., 1994. Intestinal and extraintestinal *Isospora belli* infection in an AIDS patient. A second case report. Pathol. Res. Pract. 190, 1089–1094. https://doi.org/10.1016/S0344-0338(11)80908-8.
Mitchell, S.M., Zajac, A.M., Charles, S., Duncan, R.B., Lindsay, D.S., 2007. *Cystoisospora canis* Nemeséri, 1959 (syn. *Isospora canis*), infections in dogs: clinical signs, pathogenesis, and reproducible clinical disease in beagle dogs fed oocysts. J. Parasitol. 93, 345–352. https://doi.org/10.1645/GE-1024R.1.
Morelli, S., Di Cesare, A., Traversa, D., Astuti, C., Lallone, I., Tsokana, C.N., Damiani, D., Beall, M., Buch, J., do Amaral Grossi, D., Peterson, S., Grimaldi, G., Damiani, C., Paoletti, B., Diakou, A., 2025. Occurrence of *Cystoisospora* spp. and other intestinal parasites in dogs and cats with diarrhea. Vet. Parasitol. 338, 1–7.
Nemeséri, L., 1959. Beiträge Zur Ätiologie der Coccidiose der hunde. 1. *Isospora canis* sp. n. Acta Vet. Hung. 10, 95–99.
Oduye, O.O., Bobade, P.A., 1979. Studies on an outbreak of intestinal coccidiosis in the dog. J. Small Anim. Pract. 20, 181–184. https://doi.org/10.1111/j.1748-5827.1979.tb07028.x.
Oliveira, F.C.R., Albuquerque, G.R., Munhoz, A.D., Lopes, C.W.G., Massad, F.V., 2001a. Hipnozoítas de *Cystoisospora ohioensis* (Dubey, 1975) Frenkel, 1977 (Apicomplexa: Cystoisosporinae) recuperados de órgãos de camundongos através da digestão péptica. Rev. Bras. Parasitol. Vet. 10, 29–35.
Oliveira, F.C.R., Massad, F.V., Albuquerque, G.R., Lopes, C.W.G., 2001b. *Cystoisospora ohioensis* (Dubey, 1975) Frenkel, 1977 (Apicomplexa: Cystoisosporinae) do cão: diferenças biológicas associadas com as vias de infecção. Rev. Bras. Ciênc. Vet. v. 8, 147–150.
Oliveira, F.C.R., Albuquerque, G.R., Munhoz, A.D., Lopes, C.W.G., 2000. Oocysts of *Cystoisospora ohioensis* (Dubey, 1975) Frenkel, 1977 (Apicomplexa: Cystoisosporinae) in naturally infected puppies from a litter. Rev. Univ. Rural SéR. Ciênc. Vida 22, 107–111.
Oliveira, F.C.R., Stabenow, C.S., Massad, F.V., Lopes, C.W.G., 2007. Hypnozoites of *Cystoisospora* Frankel, 1977 (Apicomplexa: Cystoisosporinae) in Mongolian gerbil lymph nodes and their transmission to cats free of coccidia. Rev. Bras. Parasitol. Vet. 16, 72–76. PMID: 17706007.
Olson, M.E., 1985. Coccidiosis caused by *Isospora ohioensis*-like organisms in three dogs. Can. Vet. J. 26, 112–114. PMID: 17422514.
Penzhorn, B.L., De Cramer, K.G.M., Booth, L.M., 1992. Coccidial infection in German shepherd dog pups in a breeding unit. J. S. Afr. Vet. Assoc. 63, 27–29. PMID: 1569537.
Randhawa, S.S., Juyal, P.D., Kalra, I.S., 1997. Clinical Isosporiasis in a racer greyhound dog. Indian Vet. J. 14, 413–414.
Restrepo, C., Macher, A.M., Radany, E.H., 1987. Disseminated extraintestinal isosporiasis in a patient with acquired immune deficiency syndrome. Am. J. Clin. Pathol. 87, 536–542. https://doi.org/10.1093/ajcp/87.4.536.
Rocha, E.M., Lopes, C.W.G., 1971. Comportamento da *Isospora canis*. Isospora felis e Isospora rivolta em infecções Exp. em C. ães e Gatos. Arq. UFRRJ 1, 81–96.
Rommel, M., Zielasko, B., 1981. Untersuchungen uber den Lebenszyklus von *Isospora burrowsi* (Trayser and Todd, 1978) aus dem Hund. Berl. Tierarztl. Wschr. 94, 87–90. PMID: 7225057.
Smith, D.D., 1981. The Sarcocystidae: *Sarcocystis, Frenkelia, Toxoplasma, Hammondia* and *Cystoisospora*. J. Parasitol 28, 262–266. https://doi.org/10.1007/BF00928042b.
Tadros, W., Laarman, J.J., 1976. *Sarcocystis* and related coccidium parasites: a brief general review, together with a discussion on some biological aspects of their life cycles and a new proposal for their classification. Act. Leid. 44, 1–107. PMID: 829199.
Trayser, C.V., Todd, K.S., 1978. Life cycle of *Isospora burrowsi* n sp (Protozoa: Eimeriidae) from the dog *Canis familiaris*. Am. J. Vet. Res. 39, 95–98. PMID: 629454.
Velásquez, J.N., Carnevale, S., Mariano, M., Kuo, L.H., Caballero, A., Chertcoff, A., Ibáñez, C., Bozzini, J.P., 2001. Isosporosis and unizoite tissue cysts in patients with acquired immunodefciency syndrome. Hum. Pathol. 32, 500–505. https://doi.org/10.1053/hupa.2001.24326.

Velásquez, J.N., Etchart, C.B., Astudillo, O.G., Chertcoff, A.V., Pantano, M.L., Carnevale, S., 2022. *Cystoisospora belli*, liver disease and hypothesis on the life cycle. Parasitol. Res. 121, 403–411. https://doi.org/10.1007/s00436-021-07406-2.

Walther, Z., Topazian, M.D., 2009. *Isospora* cholangiopathy: case study with histologic characterization and molecular confirmation. Hum. Pathol. 40, 1342–1346. https://doi.org/10.1016/j.humpath.2009.01.020.

Wolters, E., Heydorn, A.O., Laudahn, C., 1980. Cattle as intermediate host of *Cystoisospora felis*. [Das Rind als Zwischenwirt von *Cystoisospora felis*]. Berl. Tierarztl. Wschr. 93, 207–210.

Wu, D.H., Jin, H., Zhang, H.D., Xu, H.K., 1993. A case of mixed infection of canine distemper and intestinal protozoa. Chin. J. Prev. Vet. Med. 19, 32–33. https://doi.org/10.1177/10406387211011949.

Zayed, A.A., El-Ghaysh, A., 1998. Pig, donkey and buffalo meat as a source of some coccidian parasites infecting dogs. Vet. Parasitol. 78, 161–168. https://doi.org/10.1016/s0304-4017(98)00130-7.