Research Article:

MORPHOMETRIC DESCRIPTION OF *Cyclospora*-LIKE COCCIDIAN IN A STRAY DOG (CARNIVORA: CANIDAE) FROM NEPAL

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ABSTRACT

Cyclospora spp. cause intestinal cyclosporiasis and exists as a globally emerging pathogen in humans. However, the infection has also been reported from various animal hosts, including domestic canids. Very few articles have highlighted the presence of this coccidian in dogs, but interestingly, in each case; the oocyst morphology resembles that of human strains (*C. cayetanensis*). Here within, we have addressed a new description of the oocyst morphology of *Cyclospora*-like coccidian reported in the feces of a young female free-ranging street dog in Lalitpur Metropolitan City in central Nepal. Sporulation of oocyst is exogenous and completed within a week at 28°C with a 1:4 ratio in a 2.5% potassium dichromate solution. A mature oocyst was spherical, measuring 22.5 µm × 21.5 µm (22-23 µm × 21-22 µm, n = 10), with a shape index of 1.03 (1.0–1.05), an oocyst thickness of 0.5 µm, and a smooth and bilayered oocyst wall. Similarly, the oocyst was dizoic and was broadly elliptical sporozoites (average size: 9.9 µm × 8.3 µm, shape index: 1.2) with an inconspicuous nipple-shaped stieda body and sporocyst residuum.

सारांश

जनावरहरूमा लाग्ने *Cyclospora* प्रजातिको प्रोटोजोआले मानवको आन्द्राजन्य साइकलोस्पोरायसिस उत्पन्न गर्ने गर्दछ। यो विश्वव्यापी रूपमा उदाउँदो परजीवीजन्य रोगको कारकका रूपमा चिनिएको छ। यद्यपि, यसको सङ्क्रमण घरेलु कुकुर लगायत विभिन्न जनावरमा समेत देखिएको छ। कुकुरमा यस कक्सिडियनको उपस्थिति सम्बन्धी प्रकाशित विवरणहरू अत्यन्तै सीमित छन्, तर हालसम्म उल्लेख गरिएका सबै घटनाहरूमा ओसिस्टको आकार-रूप मानव प्रजाति *(C. cayetanensis)* सँग मिल्दोजुल्दो रहेको देखिन्छ। यस अध्ययनमा, ललितपुर महानगरपालिका (मध्य नेपाल) मा स्वतन्त्र रूपमा विचरण गर्ने एक पोथी भुस्याहा कुकुरको मलमा भेटिएको *Cyclospora* सँग मिल्दो जुल्दो कक्सिडियनको ओसिस्ट संरचनाको नयाँ विवरण प्रस्तुत गरिएको छ। ओसिस्टको स्पोरुलेसन बाह्यरूपमा सम्पन्न भएको थियो र २८° से. तापक्रममा एक साताभित्र पुरा भएको थियो, जसमा २.५% पोटासियम डाइक्रोमेट घोलमा १:४ अनुपात प्रयोग गरिएको थियो। परिपक्व ओसिस्ट गोलाकार थियो, जसको औसत आयाम २२.५ *µm* × २१.५ *µm* (२२-२३ *µm* × २१-२२ *µm, n* = १०) थियो । यसले १.०३ (१.०-१.०५) को आकार सूचकाङ्क देखायो। ओसिस्टको भित्तो ०.५ *µm* मोटाइको, दुई तहको संरचना देखियो । स्पोरोसिस्ट भित्र २ वटा सङ्क्रामक कोषहरू (डाइजोइक) भएको पाइयो। यसमा रहेका स्पोरोजोइटहरू चौडा अण्डाकार आकारका (औषत ९.९ *µm* × ८.३ *µm*) थिए, जसको आकार सूचकाङ्क १.२ थियो। साथै, हल्का मात्र देखिने स्टिडा बडी र स्पोरोसिस्ट रिजिडुम पनि थिए। यस अध्ययनले नेपालमा भुस्याहा कुकुरबाट *Cyclospora* सँग मिल्दो जुल्दो कक्सिडियन ओसिस्टको नयाँ स्वरूपको विवरण प्रस्तुत गरेको छ। यसले कुकुरमा देखिएको ओसिस्ट र मानवमा पाइने प्रजाति (*C. cayetanensis*) बीचको आकारगत समानतालाई समेत पुष्टि गर्दछ, जसले भविष्यमा आणविक स्तरमा थप अध्ययनको आवश्यकता औंल्याउँछ ।

Keywords: Canids, *Cyclospora*-like coccidian, morphometry, sporulation

INTRODUCTION

Cyclospora (Schneider, 1881) is an intracellular apicomplexian parasite of the Eimeriidae family. Even though it has been evidenced to occur extra-intestinally (de Górgolas et al., 2001; Sifuentes-Osornio et al., 1995), it usually resides in the gastrointestinal tract and causes intestinal cyclosporiasis. Currently, it is a globally emerging human pathogen (Bartosova et al., 2021; BCCDC, 2023), with evidence of infection in more than 54 countries and disease outbreaks in more than 13 countries (Li et al., 2020). In Nepal, *Cyclospora* infections tend to show seasonality, with higher detection during summer and rainy season (Sherchand & Cross, 2001a). The earliest documented Cyclosporiasis outbreaks occurred in 1992 among expatriates (foreign visitors) and British soldiers (Sterling & Ortega, 1999). Thereafter, subsequent clinical and community studies in the Kathmandu Valley consistently detected *C. cayetanensis* in 3–9% of symptomatic and asymptomatic individuals, including children, slum residents, and hospitalized diarrheal patients (Bhandari et al., 2015; Bhattachan et al., 2019; Sherchand & Cross, 2001a). Additionally, a cross-sectional study reported a noticeable 17% prevalence among the ethnic Chepang community in central Nepal (Adhikari et al., 2021a). Similarly, *Cyclospora* oocysts have been detected in environmental samples such as sewage, irrigation canals, pond water, and leafy vegetables (Bhandari et al., 2015; Sherchan et al., 2010; Sherchand & Cross, 2001a). It is a food- and water-borne pathogen, which is why the infection usually occurs upon ingestion of environmentally sporulated oocysts via the oral route (Ortega & Sanchez, 2010). Besides humans, its oocysts have also been reported in the fecal samples of various animals like mussels (Aksoy et al., 2014), non-human primates (Chhetala et al., 2025; Li et al., 2015; Marangi et al., 2015), gorals (Adhikari et al., 2021), goats (Basnett et al., 2018), cattle (Li et al., 2007), and canids like dogs (Adhikari et al., 2023; Chu et al., 2004). Morphologically, a mature or fully sporulated oocyst of *Cyclospora* spp. contains two sporocysts, and each sporocyst contains two sporozoites; thus, its oocyst formula is 0.2.2 (Ghimire, 2010).

Considering domestic canids (dogs), cyclosporiasis was first confirmed morphometrically (8–10 µm in diameter, with a cluster of retractile globules) in two male young dogs from Brazil in 1996 and 1997 (Yai et al., 1997). The dogs exhibited diarrhea, vomiting, weight loss, and abdominal sensitivity, but they recovered following treatment (Yai et al., 1997). Following this report, Sherchand and Cross successfully reported the *Cyclospora*-like oocyst (8–10 µm, round and red-colored) in the fecal samples of 1–1 dog in two consecutive years (1999 and 2000) from endemic areas of Nepal (Sherchand & Cross, 2001b). Furthermore, using PCR, Chu and colleagues confirmed the presence of *C. cayetanensis* oocysts in both domestic and street dogs in Nepal (Chu et al., 2004). Four years later, similar morphotypes of this coccidian were reported (Ghimire et al., 2008). Subsequently, two urban street dogs were found to excrete *Cyclospora* oocysts (Adhikari et al., 2023). Despite attempts to record and explain cyclosporiasis in domestic canids, additional details on the pathology have not been clearly demonstrated.

Despite, several diagnostic methods, including molecular (PCR, qPCR, nested PCR, multiple PCR, sequencing, digital PCR), immunological (IFA, experimental ELISA) and food/water testing (Ultrafiltration+ PCR) have been developed, to confirm *Cyclospora* oocyst (Durigan et al., 2020; Hussein et al., 2022; Lee et al., 2010; Pieniazek et al., 1996), conventional microscopy, with accurate oocyst morphometry stills remains an essential first-line diagnostic approach, critical in routine diagnostic and field-based investigations (CDC, 2019). Additionally, microscopy provides clear visualization of oocyst morphology and enables differentiation from other coccidian oocysts, such as *Cryptosporidium* and *Cystoisospora,* which differ significantly in size and content. On the other hand, non-microscopic diagnostics can be limited by sample purity and resource availability (Maurer et al., 2011). Therefore, we prefer a combination of both microscopy and morphometry approaches to diagnose *Cyclospora* in the current study.

RESEARCH METHODS

Field survey

The field study was conducted among free-ranging street dogs in the Lalitpur Metropolitan Municipality in Central Nepal. From June to August 2020, a total of 63 fresh fecal samples were collected via opportunistic sampling at various locations within the study area. These samples were immediately preserved in 2.5% potassium dichromate ($K_2Cr_2O_7$) in sterile 20 ml vials. They were then transported to the Animal Research Laboratory and stored at 4°C in a refrigerator before microscopic examination.

Parasite examination and analysis

The samples were analyzed by formalin ethyl acetate sedimentation and saturated salt (45% weight/volume NaCl) flotation techniques as previously described in the literature (Adhikari et al., 2024; Zajac & Conboy, 2012). Furthermore, the *Cyclospora*-positive sample was incubated at 28°C in an incubator with 2.5% potassium dichromate at a 1:4 ratio (Adhikari et al., 2023; Ghimire et al., 2021). Potassium dichromate enhances oocyst sporulation by providing a strongly oxidizing and oxygen-rich environment while inhibiting bacterial and fungal overgrowth (Laverty et al., 2023; Li & Ooi, 2008), thereby creating optimal conditions for the division of sporoblasts and development of mature sporocysts containing sporozoites.

Thereafter, the sporulation state of the oocysts was examined at 24-hour intervals using a compound microscope (Optika Microscopes, Italy, B-383PLi) at total magnifications of 100x and 400x. Photomicrographs were taken using the camera (SXView 2.2.0.172 Beta, November 6, 2014, Copyright (C) 2013-2014), attached to the microscope, and the identification of the *Cyclospora* was confirmed based on the literature (Ghimire, 2010; Lainson, 2005; Smith et al., 1997) and the micrometry assessed by ImageJ 1.51k (National Institute of Health, USA).

Strategies of literature search

To review the literature, *Cyclospora* species recorded in canid and non-canid hosts were searched Online and those articles related to detection of these coccidia in street and domestic dogs were included. In addition, their reports in invertebrates and vertebrates were included in the discussion. However, the reports on human *Cyclospora* species were excluded.

RESULTS AND DISCUSSION

In the current study, only 1.6% (1/63) of fecal samples were positive for *Cyclospora* oocysts **(Fig. 1a, 1b)**. However, the positive sample contained a significant number of oocysts: <10 oocysts per microscopic slide with formal-ether sedimentation and >10 oocysts per microscopic slide with flotation.

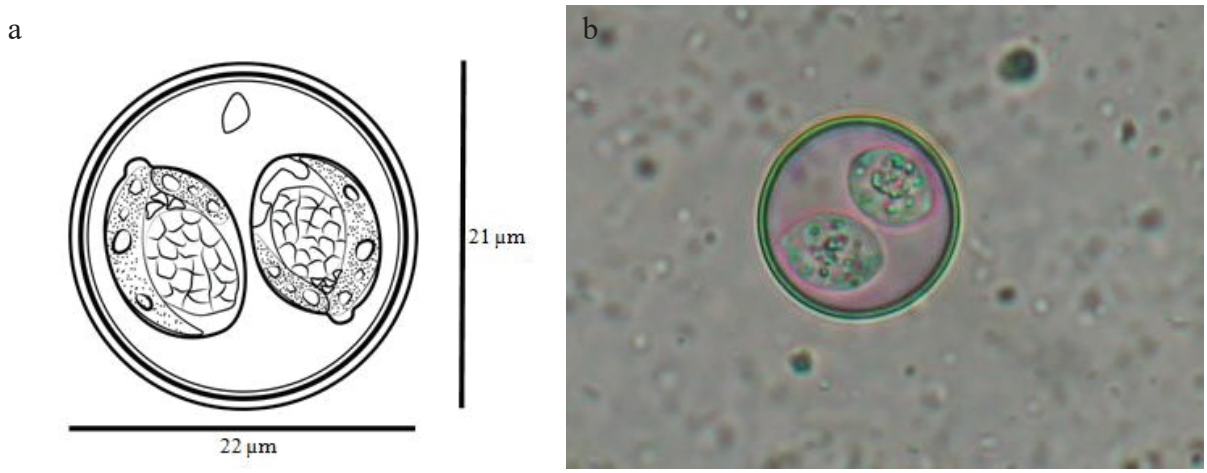

Fig. 1. Oocyst of *Cyclospora*-like coccidian a) Line drawing, b) In stool

Morphometric characters of *Cyclospora*-like coccidian

Description of the oocyst morphology

Oocyst characters: a) Shape of mature oocyst (Fig. 1): Oval, almost spherical b) Oocyst size: 22.5 µm × 21.5 µm (22-23 µm × 21-22 µm, n = 10); c) Shape index: 1.03 (1.0–1.0); d) Oocyst wall: bilayered, smooth-walled, and 0.5 µm thick; e) Oocyst residuum or polar granules: Single; f) Oocyst cap (micropyle): absent.

Sporocyst characters: a) Number: 2 (Dizoic); b) Shape: Broadly elliptical; c) Size: Average 9.9 µm × 8.3 µm (9-11 µm × 7-10 µm); d) Shape index: 1.2 (1.1–1.4); e) Stieda body: Present (nipple-like but inconspicuous); f) Sporocyst residium: Present; g) Refractile granules: Present

Taxonomic summary

Host: Free-ranging street dog (canid), young female <1 year
Type locality: Urban city within Lalitpur Metropolitan Municipality, Nepal
Geo-climatic conditions: Sub-tropical (25°C average annual temperature) and monsoon season
Type material: Fresh stool sample (Semi-solid consistency)
Prevalence: 1.6% (1 out of 63 fecal samples)
Examination technique: Copro microscopic and sporulation assays
Sporulation time: A week (7 days)
Pattern of infection: Single infection
Pathology: No apparent

Till date, extensive studies regarding human cyclosporiasis have been performed, and the infection has usually been confirmed by microscopy methods based on oocyst morphology and its staining properties. *Cyclospora cayetanensis* is a well-known human strain with an oocyst diameter of 8–10 µm (Soave, 1996). Interestingly, a similar oocyst morphotype has been detected only in canids to date (Table 1). In this circumstance, owing to the coprophagous feeding behavior of dogs (Hart et al., 2018) and the absence of tissue invasion, some researchers have claimed its presence in dogs' feces reflects the passage of the ingested oocysts through the GI tract (Ortega & Sherchand, 2015). On the other hand, a few authors suggested that cyclosporiasis in canines may have resulted from the ingestion of free-living nematodes, insects, and rotifers (Ortega & Sanchez, 2010). In this regard, we compared the morphometric findings of the oocyst of *Cyclospora* spp. recorded from canids and other vertebrates (Table 1), and we found that the oocyst morphology recorded in our study is quite different from the oocyst recorded in similar hosts previously. Also, oocyst residuum or polar body and oocyst ranges *C. angimurinensis* recorded from *Chaetodipus hispidus* are similar (Ford et al., 1990).

Thus, the current finding implies the possibility of two hypotheses: first, free-ranging canids can be the natural host of this coccidian, and second, the infection may have occurred via cross-transmission with rodents or through ingestion of rodents, indicating dogs as accidental or paratenic hosts. But the scenario for the second hypothesis might not be completely explained due to the age and gender factor of current host (< 6 months of age; female) because hunting success increases with age, and probably males are good hunters (Wierzbowska et al., 2016).
The detection of *Cyclospora* oocysts in only a single female dog (<6 months of age) in our study may reflect a combination of ecological, age-related behavioral, and environmental factors. The young positive dog is an urban street dog found roaming in Gwarko, Lalitpur Municipality. This is why it has easy access to human–waste–contaminated environments; such conditions increase opportunities for exposure to sporulated oocysts shed by infected humans or animals, including rodents. Moreover, younger puppies tend to explore their surroundings more extensively, ingest soil, and consume discarded food or fecal material more frequently than adult dogs, increasing their chance of encountering contaminated human waste, which has been previously associated with *Cyclospora* infection in Nepal (Adhikari et al., 2023; Bhandari et al., 2015; Sherchand et al., 2007). The extreme morphological variations of these parasites indicate that either parasites from dogs excreted as wandering hosts or new species of parasites may be found inside the canids. However, limited contact between most other sampled dogs may be the reason behind the absence of infection in the remaining population. But the presence of oocysts in even one animal highlights their potential role as mechanical carriers**,** suggesting they may facilitate environmental dissemination and increase the likelihood of indirect human transmission in settings with poor sanitation.

Table 1. *Cyclospora* spp. reported from different hosts with oocyst micrometry

***Cyclospora* spp.**	**Associated hosts**	**Oocyst micrometry (µm)**	**Literature**
Canid hosts			
Cyclospora-like coccidian	Street dog	22-23×21-22	Current study
Cyclospora sp.	Domestic dogs	8-10	(Yai et al., 1997)
Cyclospora sp.	Street dog	8-10	(Sherchand & Cross, 2001b)
Cyclospora cayetanensis	Domestic and Street dogs	8-10	(Chu et al., 2004)
Cyclospora sp.	Street dogs	8-10	(Ghimire et al., 2008)
Cyclospora sp.	Street dogs	9×9	(Adhikari et al., 2023)
Cyclospora spp.	Domestic dogs	-	(Rai & Ghimire, 2026)
A previous study in the non-canid hosts			
C. cayetanensis	*Homo sapiens* (Primates)	8.8×8.6	(Ortega et al., 1994)
Cyclospora schneideri	*Anilius scytale scytale* (Aniliidae)	19.8×16.6 (15.1×13.8 - 25.7×20.1	(Lainson, 2005)
Cyclospora sp.	Baboon	8-10	(Smith et al., 1997)
Cyclospora macacae	*Macaca mulatta*	8.49±0.55×8.49±0.49	(Li et al., 2015)
C. glomericola	*Glomeris* sp.	25-36×9-10	(Schneider, 1881)
C. caryolytica	*Talpa europaea, Mogera wogura*	18×12.5	(Schaudinn, 1902)

C. viperae	*Coluber scalaris, Coronella austriaca, Natrix viperinus, Vipera aspis*	16.8×10.5	(Phisalix, 1923)
C. babaulti	*Vipera berus*	17×10	(Phisalix, 1924a)
C. scinci	*Scincus officinalis*	10×7	(Phisalix, 1924b)
C. tropidonoti	*Natrix natrix, Natrix stolata*	17×10	(Phisalix, 1924c)
Cyclospora sp.	*Hemidactylus frenatus*	16.9-30×16.7-26	(Yamamoto, 1933)
C. naniae	*Ninia sebae sebae*	14.6×13.3	(Lainson, 1965)
C. cerrcopitheci	*Cercopithecus aethiops*	8-10×8-10	(Eberhard et al., 1999)
C. talpae	*Talpa europaea*	15-18×10-12	(Pellérdy & Tanyi, 1968)
C. megacephali	*Scalopus aquaticus*	18.5×15.7	(Ford & Duszynski, 1989)
C. ashtabulensis	*Parascalops breweri*	18×14.3	(Ford & Duszynski, 1989)
C. parascalopi	*Parascalops breweri*	16.5-13.6	(Ford & Duszynski, 1989)
C. *angimurinensis*	*Chaetodipus hispidus*	19-24×16-22	(Ford et al., 1990)
C. colobi	*Colobus guereza*	8-9×8-9	(Eberhard et al., 1999)
C. papionis	*Papio anubis*	8-10×8-10	(Eberhard et al., 1999)
Cyclospora sp.	*Macaca mulatta*	10×10	(Chhetala et al., 2025)
Cyclospora sp.	Goat	9×9	(Ghimire & Bhattarai, 2019)
Cyclospora sp.	Goral	9-12×8-10	(Adhikari et al., 2021b)
Cyclospora sp.	Cattle	10×10	(Li et al., 2007)
Cyclospora spp.	Cattle/Pigs	-	(Rai & Ghimire, 2026)
Cyclospora spp.	Buffalo male calves	-	(Thapa Magar & Ghimire, 2023)

CONCLUSION

We have described a new specimen of *Cyclospora*-like coccidian from a free-ranging street dog from central Nepal. Since free-ranging dogs are easily available in almost every urban area of Nepal, further study should be conducted with a large population size from different regions, and a molecular study must be conducted. In this context, the current findings serve as baseline data for further study, and this coccidian must be considered by parasitologists and veterinarians during routine fecal examinations, suggesting the immediate utilization of this research as a novel finding.

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AUTHOR CONTRIBUTIONS

Author A: Conceptualization; field and laboratory survey; methodology; data analysis; and original draft preparation; reviewing and editing

Author B: Conceptualization; laboratory analysis; data analysis; writing; reviewing; editing; and supervision

Both authors approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interest regarding this article's research, authorship, and /or publication. The second author (TRG) previously worked as Scientific Officer at Nepal Academy of Science and Technology (NAST), Lalitpur, Nepal and the first author worked as Research Assistant there.

ETHICS APPROVAL

The authors declare that the study was conducted on naturally infected street dogs. No experimental infection was established during this research work. None of the animals were touched or harmed during this study. The permissions to collect fecal samples of the free-ranging dogs were obtained from Lalitpur Metropolitan City and Lalitpur Metropolitan Veterinary Service (Lalitpur, Nepal).

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