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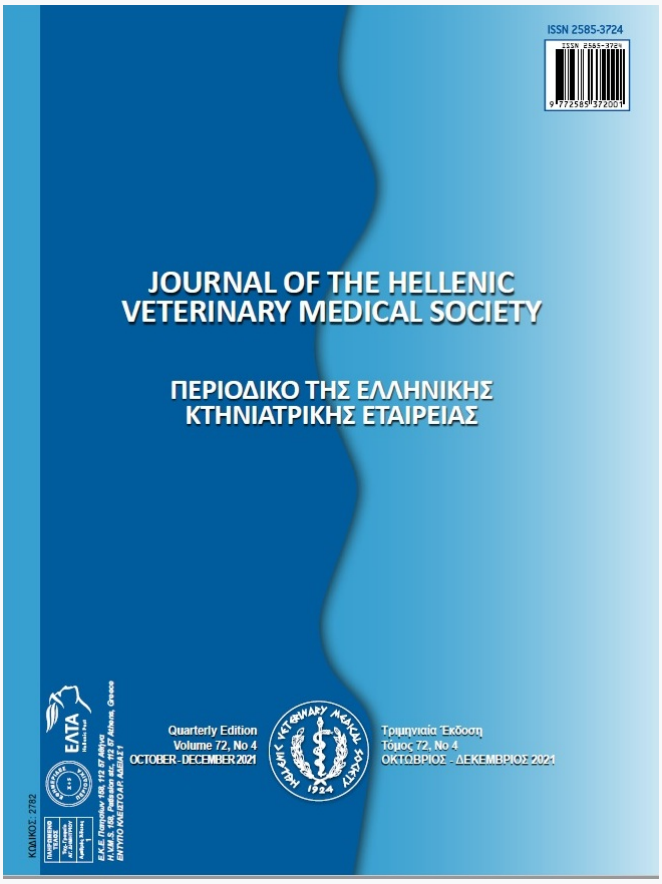

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Research article
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Diversity and prevalence of *Eimeria* species in goats of Nepal

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ABSTRACT: This study aimed to determine the prevalence of *Eimeria* and identify its different species in adult goats brought to the Kathmandu valley, Nepal, for commercial meat purposes. *Eimeria* spp. were present in 916 samples out of 991 examined samples (92.4%). A total of 15 different morphologic forms of *Eimeria* were detected, with the prevalence rates as follows: *E. ninakohlyakimovae* (83.0%), *E. alijevi* (75.2%), *E. capralis* (75.2%), *E. masseyensis* (67.2%), *E. hirci* larger form (63.2%), *E. tunisiensis* (47.3%), *E. charlestoni* (33.0%), *E. jolchejevi* larger form (32.4%), *E. arloingi* (32.4%), *E. caprina* (19.3%), *E. aspheronica* (16.5%), *E. jolchejevi* smaller form (13.5%), *E. christenseni* (9.4%), *E. hirci* smaller form (9.4%), and *E. caprovina* (8.1%). The current data support the fact that *Eimeria* species are predominant in adult populations. Therefore, coprologic diagnosis and anticoccidial treatments should be implemented in these populations before their transport to new sites to avoid the transmission.

Keywords: *Eimeria ninakohlyakimovae*; Coccidiosis; Goats; Morphometry

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INTRODUCTION

The genus *Eimeria* Schneider, 1875 (Apicomplexa: Eimeriidae) is a diverse and widespread coccidian parasite of the invertebrate and vertebrate hosts. As they are present in chicken, calves, sheep, goats, and rabbits, they are prioritized by the veterinary professionals and livestock scientists. This unicellular protozoan parasite is monoxenous because it completes its lifecycle in a single host. The host becomes infected with the consumption of food and water contaminated with sporulated *Eimeria* oocysts. Once oocysts enter the host, asexual and sexual reproductive cycles occur inside the host's intestinal epithelium. As a result, unsporulated oocysts are released in the feces. In the external environment, the oocysts undergo sporogony and become infectious. This coccidian parasite causes a disease called coccidiosis that is characterized by decreased growth, weight loss, decrease in the production of milk, wool, and hair, reduced fertility, enhanced mortality of goats, and severe economic loss (Khodakaram-Tafti and Hashemnia 2017). Therefore, the study of the role of *Eimeria* is important in livestock.

Livestock production, especially goat farming, is becoming increasingly popular in Nepal. It plays an essential role in achieving the sustainable development goals (SDG) of the United Nations, mainly Goal 1: no poverty, Goal 2: zero hunger, and Goal 3: decent work and economic growth. Although the disease caused by *Eimeria* species has been recognized as a critical problem in the goat industry in this country for many years (Khakural 2003; Ghimire 2018; Ghimire and Bhattarai 2019). However, the study of occurrences of various *Eimeria* species in goats has not been investigated here yet. The understanding of various *Eimeria* species in goats is crucial because pathogenicity caused by different species is different, and even some *Eimeria* species lead to asymptomatic infection (Sayin et al. 1980; Soe 1989; Dai et al. 1991; Dai et al. 2006). Thus, species identification of *Eimeria* should be perfectly carried out to subsequently evaluate the parasite's lethality and follow the therapeutic options. This study aims to determine the prevalence of different *Eimeria* species present in the goats in Kathmandu valley, where thousands of the goats are imported from all over the country for commercial meat.

MATERIALS AND METHODS

Sample collection

The study was conducted on naturally-infected goats brought to the goat market and butcher shops from different parts of Nepal. No experimental infection was established during this research work. Goats were not directly involved in the study. From August 2019 to February 2020, 991 fresh fecal samples of male goats were collected from the local goat markets and butcher shops in Kathmandu, Nepal. The samples were immediately placed in sterile vials containing 2.5% potassium dichromate solution and stored in a refrigerator (4 Degree Celsius, ^{o}C) until further examination.

Laboratory processing, examination, and culture

The presence of *Eimeria* oocysts in the fecal samples was examined by the flotation method using 45% NaCl (1200 revolutions per minuteX5minutes) as standardized by laboratory and described in an earlier publication (Ghimire and Bhattarai 2019). Then, sporulation assays were performed in the *Eimeria*-positive samples. The fecal samples were placed in Petri dishes and incubated at 2.5% potassium dichromate (28 ^{o}C) up to 10 days (Adhikari et al. 2020). Sporulation time was calculated as the average time required for complete sporulation of 90% of the oocysts.

Sample identification

All oocysts were observed under a light microscope (Optika Microscopes Italy, B-383PLi) at a different magnification of X100, and X400. The images were taken using SXView 2.2.0.172 Beta (Nov 6, 2014) Copyright © 2013-2014. The identification of the oocysts was carried out using the parameters which include the oocyst characteristics (size, shape, shape index, color, the thickness of the wall, and presence of polar and refractile granules), sporocyst characteristics (size, shape, shape index, sporocyst residuum), presence or absence of micropyle and its cap, size, shape, and shape index (Soe 1989; Duszynski and Wilber 1997; Koudela and Bokova 1998; Wang et al. 2010). Different shapes of the oocysts were considered while identifying the species; for example, ellipsoid/ellipsoidal meant symmetrical elliptical in shape, ovoid meant egg-shaped with the broader end remote from the micropyle/polar cap, and urn-shaped meant egg-shaped with the broader end towards the micropyle/polar cap (Soe 1989). Species description was solely based on the literature (Soe 1989; Soe and Pomroy 1992).

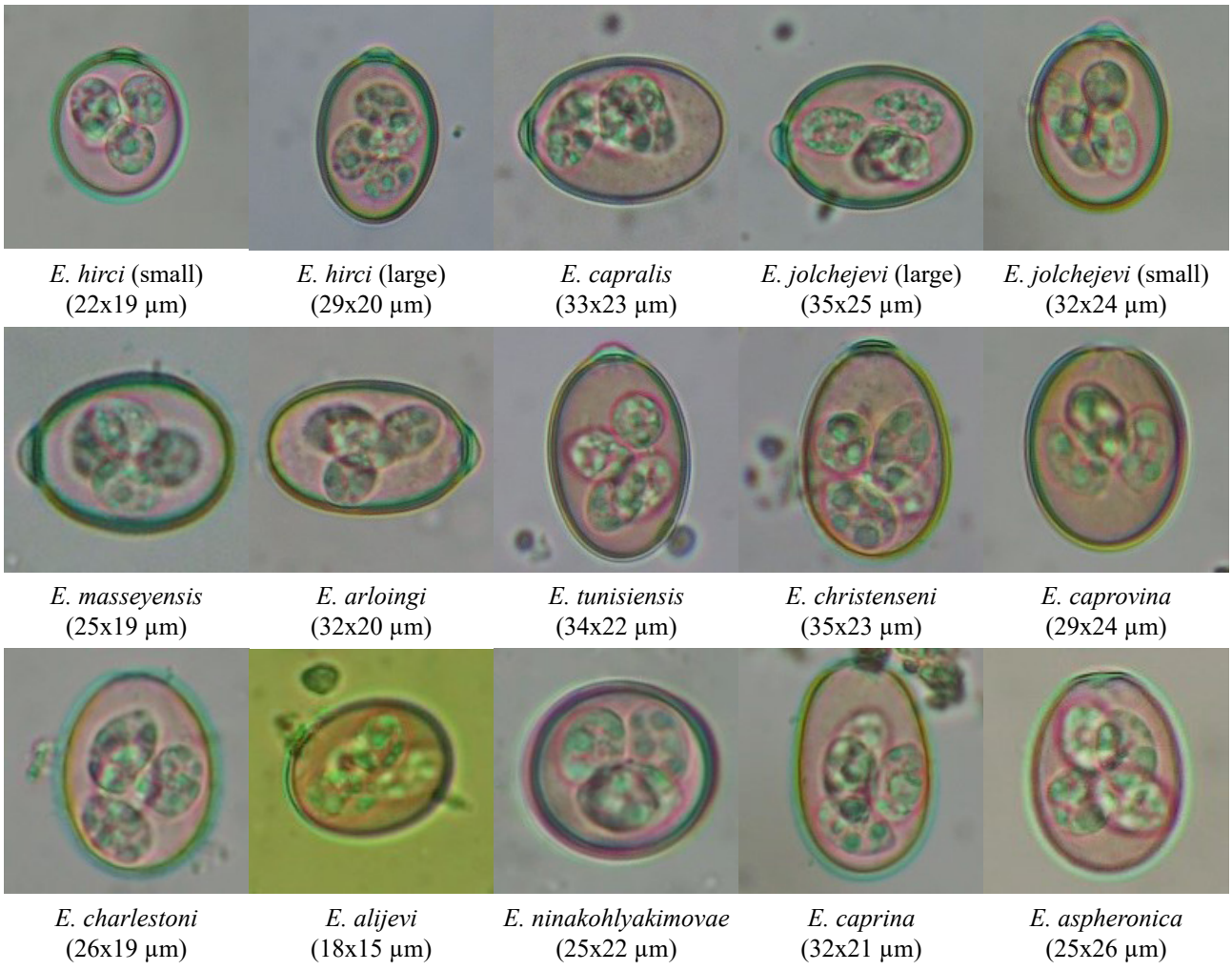

Fig 1. Different *Eimeria* species (X400) detected in goats in Kathmandu, Nepal

Data analysis

At least 100 sporulated oocysts from each species of *Eimeria* were used in the investigation of the morphometric identification of various species. The GraphPad Software, Inc (Prism 5.00, 2007) was used to calculate mean, range (minimum to maximum), and standard deviations of length and width of oocysts, sporocysts, micropylar cap and thickness of oocyst wall. Prevalence of the *Eimeria* was calculated by dividing the total numbers of *Eimeria* positive samples by whole numbers of collected samples.

RESULTS

The current study revealed a total of 916 *Eimeria* positive fecal samples (92.4%) out of 991 examined samples (Supplementary 1). We detected a total of 13 species of *Eimeria* with a total of 15 different morphometric forms (Fig 1) (Supplementary 2). The prevalence of all of these species was as follows: *E. arloingi* (32.4%), *E. christenseni* (9.4%), *E. hirci l.f.* (63.2%), *E. hirci s.f.* (9.4%), *E. jolchejevi l.f.* (32.4%), *E. jolchejevi s.s.* (13.5%), *E. masseyensis* (67.2%), *E. capralis* (75.2%), *E. tunisiensis* (47.3%), *E. alijevi* (75.2%), *E. aspheronica* (16.5%), *E. caprina* (19.3%), *E. caprovina* (8.1%), *E. charlestoni* (33.0%), and *E. ninakohlyakimovae* (83.0%) (Supplementary 1).

Interestingly, *E. ninakohlyakimovae* was the common parasite in different fecal samples; for example, it was found to be along with 11 different species in different fecal samples. There was no sample with a single infection. All positive fecal samples were concomitantly positive for different numbers, for example, with three (1.3%), four (27.7%), five (8.1%), seven (18.9%), eight (30.9%), nine (4.8%), and ten (0.7%) species of *Eimeria* (Supplementary 1).

Supplementary 1: Prevalence of each *Eimeria* sp. in the fecal samples of goats. N represents total numbers of fecal samples examined (N=991) and n represents total numbers of particular *Eimeria* sp. positive fecal samples. Prevalence rate was calculated by using 100n/N formula for particular species or concomitant infection. l.f.: large form and s.f.: small form.

Species of *Eimeria*	n	Prevalence (%)
E. ninakohlyakimovae	823	83.0
E. alijevi	745	75.2
E. capralis	745	75.2
E. masseyensis	666	67.2
E. hirci l.f.	626	63.2
E. tunisiensis	469	47.3
E. charlestoni	327	33.0
E. arloingi	321	32.4
E. jolchejevi l.f.	321	32.4
E. caprina	191	19.3
E. aspheronica	164	16.5
E. jolchejevi s.f.	134	13.5
E. christenseni	93	9.4
E. hirci s.f.	93	9.4
E. caprovina	80	8.1
Concomitant infections		
Triplet	13	1.3
Quadruplet	275	27.7
Pentuplet	80	8.1
Heptuplet	187	18.9
Octuplet	306	30.9
Nonaplet	48	4.8
Decaplet	7	0.7
Total infected	**916**	**92.4**

DISCUSSION

As per our knowledge, this is the first published report on the diversity and prevalence of *Eimeria* species in the goats of Nepal. The high prevalence rate accompanied by mixed infections up to ten species and the presence of 13 different species indicates that *Eimeria* is a dominant coccidian parasite in Nepal's goats. Although there are 17 accepted species of *Eimeria* from goats (Levine 1988; Soe and Pomroy 1992), we have detected 15 different morphologic forms. Among the 15 morphologic forms identified in this study, only four are significant for coccidiosis. These species include *E. ninakohlyakimovae, E. arloingi, E. christenseni,* and *E. caprina* that have been proved as etiologic agents of hemorrhagic diarrhea and lesions in the GI tracts in goats (Sayin et al. 1980; Aumont et al. 1984; Norton 1986; Koudela and Bokova 1998; Dai et al. 2006; Hashemnia et al. 2012). However, the former is regarded as the most pathogenic species resulting in extra-intestinal complications (Dai et al. 1991). Although the latter three species are considered to be pathogenic, *in vivo* experiments with *E. ninakohlyakimovae*, *E. christenseni,* and *E. arloingi* have resulted in sub-clinical coccidiosis which is characterized by a decrease in the intake of dry matter, momentary diarrhea or slight constipation, and a very large oocyst excretion (Aumont et al. 1984; Aumont et al. 1986). The various results indicate that these *Eimeria* species lead to both clinical and subclinical manifestations, and the effect depends on the different species involved.

Our results were obtained from the fecal samples of male goats transported from different parts of the country for commercial meat purposes. These goats appeared clinically healthy and robust, although the detection of various *Eimeria* species indicated that they might have a mild to moderate or asymptomatic infection. In this context, the goats can act as asymptomatic carriers for different *Eimeria* species. It is believed that healthy goats can resist coccidiosis without developing clinical signs; however, stress situations during transportation or underfeeding and the associated factors may disrupt the immune system, which leads to a huge effect of *Eimeria* (Chartier and Paraud 2012; Mohamaden et al. 2018). These explanations and our exploration indicate that constant monitoring of *Eimeria* should be carried out routinely in the future, especially for the adult goats that might act as transmitting means of these coccidia.

CONCLUSIONS

In conclusion, based on our laboratory findings, the current study revealed that *Eimeria* species are dominant in Nepal's goats. It has already been suggested that goat kids are severely affected by *Eimeria* with high morbidity and mortality. However, the current study detected *Eimeria* in more than 90% of the goat adult populations. Therefore, to avoid transmission, coprologic diagnosis and anticoccidial treatments should be carried out in the goat populations before transporting them to other sites.

ACKNOWLEDGEMENTS

The authors would like to acknowledge those people who permitted the collection of fecal samples from goats.

CONFLICT OF INTEREST

We declare that we have no conflict of interest. The work is a part of the research proposal of the Red Book (075/076, 076/077, Planning Division, Nepal Academy of Science and Technology, NAST).

Supplementary 2: Morphologic characters of oocysts of different *Eimeria* species detected in the goats in Nepal. Length and Width are expressed as minimum to maximum (mean±standard deviation). The length of micropyle has been measured in the species without micropylar cap. l.f.: large form and s.f.: small form.

Eimeria species	Oocyst characteristics			Sporocyst			Micropyle cap			Oocyst wall		Polar granules / Refractile globules	Sporocyst Residuum
	Length (µm) x Width (µm)	*Shape index (l/b)*	*Shape of oocyst*	*Length* (µm) x Width (µm)	*Shape index (l/b)*	*Shape*	*Length* (µm) x Width (µm)	*Shape index (l/b)*	*Shape*	*Thickness*	*Color*		
With micropylar cap													
E. arloingi	26-32 (30±1.6) x 17-23 (20±1.2)	1.4-1.5	Ellipsoidal/ Ovoid	11-15 (13±1.1) x5-8 (7±0.6)	1.9-2.2	Elongated and Ovoid	5-7 (6±0.6) x 1-4 (2±0.6)	1.8-5	Mound-shaped	1-2 (1)	Greenish Brown	Present/ Present	Present
E. christenseni	34-38 (36±1.1) x 21-27 (24±1.8)	1.4-1.6	Ovoid	14-16 (15±0.7) x 7-9 (8±0.8)	1.8-2	Ovoid	6-8 (7±0.7) x 3-4 (3±0.3)	2	Mound-shaped	1-2 (2±0.1)	Green	Present/ Present	Present
E. hirci l.f.	25-31 (28±1.6) x 17-23 (20±1.3)	1.3-1.5	Ellipsoidal/ Ovoid	10-13 (12±0.9) x5-8 (7±0.7)	1.6-2	Ellipsoidal	4-7 (6±0.8) x 2-4 (3±0.5)	1.8-2	Dome-shaped	1-2 (1±0.4)	Yellow, Green, and Red	Present/ Present (Indistinct)	Present
E. hirci s.f.	18-22 (21±0.9) x 17-20 (18±1.1)	1.1	Ellipsoidal	8-11 (9±0.9) x 5-8 (6±0.9)	1.4-1.6	Ellipsoidal	3-4 (4±0.2) x 1-2 (2±0.1)	2-3	Nipple-like	1 (1±0.0)	Green and Purple	Present/ Present (Indistinct)	Present

E. jolchejevi l.f.	32-41 (35±1.8) x 23-28 (25±1.7)	1.4-1.5	Urn-shaped/ Ellipsoidal	10-17 (14±1.8) x 6-10 (7±0.9)	1.7	Elongated and Ovoid	5-8 (7±0.8) x 2-4 (3±0.5)	2-2.5	Truncated-cone shaped	1-2 (2±0.4)	Yellowish Green	Present/ Present (Prominent)	Present (Mostly aggregated)
E. jolchejevi s.f.	28-33 (31±1.3) x 19-25 (22±1.4)	1.3-1.5	Ellipsoidal / Urn-shaped	10-16 (13±1.3) x 6-10 (7±0.9)	1.6-1.7	Elongated and Ovoid	5-8 (6±0.7) x 2-4 (3±0.5)	2-2.5	Half-moon -shaped	1-2 (2±0.4)	Blue, Yellow, and Green	Present/ Present	Present (Mostly aggregated)
E. masseyensis	20-27 (25±1.3) x 16-21 (19±1.2)	1.3	Ellipsoidal or Broadly ovoid	4-13 (10±1.4) x 5-9 (6±0.7)	0.8-1.4	Elongated and Ovoid	4-6 (5±0.7) x 1-3 (2±0.2)	2-4	Dome-shaped	1 (1±0)	Greenish Yellow	Present (Fragmented)/ Present	Present
E. capralis	26-33 (29±1.6) x 18-23 (20±1.3)	1.4	Ellipsoidal	10-14 (12±0.9) x 6-9 (7±1.0)	1.6-1.7	Ovoid	4-7 (6±0.7) x 1-5 (3±0.6)	1.4-4	Dome-shaped	1-2 (1±0.2)	Dark Green	Present/ Present	Present
E. tunisiensis	30-38 (34±1.8) x 20-26 (22±1.3)	1.5	Ellipsoidal (Large)	12-16 (14±0.9) x 6-10 (8±1.2)	1.6-2	Elongated and Ovoid	5-8 (6±0.6) x 2-4 (3±0.2)	2-2.5	Dome-shaped	1-2 (2±0.3)	Greenish Yellow	Present (Fragmented)/ Present	Present (Large)
Without micropylar cap													
E. alijevi	15-22 (17±1.5) x 13-17 (15±1.0)	1.1-1.3	Ovoid/ Sub-spherical	7-11 (9±0.7) x 4-6 (5±0.7)	1.8	Elongated and Ovoid	-	-	-	1 (1±0.0)	Greenish Blue	Present/ Present	Absent

E. aspheronica	26-33 (29±2.1) x 18-25 (21±1.7)	1.3-1.4	Ovoid and flat at micropylar end	11-14 (12±1.3) x 5-8 (7±0.9)	1.8-2.2	Elongated and Ellipsoidal	4-7 (6,0.8)	-	-	1-2 (1±0.1)	Pinkish Green	Present/ Unknown	Present
E. caprina	29-37 (32±2.6) x 21-25 (23±1.1)	1.4-1.5	Ellipsoidal/ Ovoid, and flat at micropylar end	12-14 (13±0.8) x 6-8 (7±0.6)	1.8-2	Elongated and Ovoid	4-7 (5,0.6)	-	-	1-2 (1±0.4)	Green	Present (Fragmented)/ Unknown	Present
E. caprovina	23-31 (28±2.5) x 19-24 (22±1.3)	1.2-1.3	Ovoid/ Sub-spherical and flat at micropylar end	11-14 (13±1.0) x 6-8 (7±0.7)	1.8	Elongated and Ovoid	4-6 (6,0.5)	-	-	1-2 (1±0.3)	Green, Yellow & Purple	Present/ Unknown	Present
E. charlestoni	20-26 (23±1.7) x 15-19 (17±1.0)	1.3-1.4	Ellipsoidal and flat at micropylar end	10-14 (11±0.9) x 5-7 (6±0.7)	2	Ellipsoidal/ Ovoid	3-5 (4,0.5)	-	-	1 (1±0)	Light to Dark Green	Present/ Present (Prominent)	Absent/ Invisible
E. ninakohlyakimovae	20-29 (23±1.6) x 16-22 (19±1.4)	1.3	Ellipsoidal/ Ovoid	10-14 (11±0.8) x 4-9 (6±0.7)	1.6-2.5	Elongated and Ovoid	-	-	-	1-2 (1±0.4)	Purple and Green	Present (Fragmented)/ Present (Non prominent)	Present (Aggregated)

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