



# Journal of the Hellenic Veterinary Medical Society

Vol 72, No 4 (2021)



# To cite this article:

GHIMIRE, T., ADHIKARI, R., & BHATTARAI, N. (2022). Diversity and prevalence of Eimeria species in goats of Nepal. *Journal of the Hellenic Veterinary Medical Society*, *72*(4), 3299–3306. https://doi.org/10.12681/jhvms.29363

# Diversity and prevalence of *Eimeria* species in goats of Nepal

# T.R. Ghimire<sup>1,\*</sup>, R.B. Adhikari<sup>2</sup>, N. Bhattarai<sup>3</sup>

<sup>1</sup>\*Department of Zoology, Tri-Chandra Multiple Campus, Tribhuvan University, Ghantaghar, Kathmandu, Nepal

<sup>2</sup>Third Pole Conservancy, Wildlife and Eco-health, Bhaktapur, Nepal

<sup>3</sup>National Biotechnology Research Center, Nepal Agricultural Research Council (NARC), Khumaltar, Lalitpur, Nepal

**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

Corresponding Author: Dr. Tirth Raj Ghimire, Department of Zoology, Tri-Chandra Multiple Campus, Tribhuvan University, Ghantaghar, Kathmandu, Nepal E-mail address: tirthprimate@gmail.com; tirth.ghimire@trc.tu.edu.np

Date of initial submission: 27-06-2020 Date of acceptance: 22-04-2021

#### T.R. GHIMIRE, R.B. ADHIKARI, N. BHATTARAI

#### **INTRODUCTION**

The genus Eimeria Schneider, 1875 (Apicomplexa: L 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, °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 °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).



#### **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).

ıean±standard		Sporocyst Residuum		Present	Present	Present	Present
n to maximum (n	Polar granules / Refractile globules			Present/ Present	Present/ Present	Present/ Present (Indistinct)	Present/ Present (Indistinct)
as minimur	t wall	Color		Greenish Brown	Green	Yellow, Green, and Red	Green and Purple
re expressed	Oocyst	Thickness		1-2 (1)	1-2 (2±0.1)	1-2 (1±0.4)	1 (1±0.0)
and Width aı all form.	dı	Shape		Mound- shaped	Mound- shaped	Dome- shaped	Nipple-like
l. Length d s.f.: sm	ropyle ca	Shape index (Vb)		1.8-5	7	1.8-2	2-3
e goats in Nepa : large form an	Mic	<i>Length</i> (μm) x Width (μm)	ylar cap	5-7 (6±0.6) x 1-4 (2±0.6)	6-8 (7±0.7) x 3-4 (3±0.3)	4-7 (6±0.8) x 2-4 (3±0.5)	3-4 (4±0.2) x 1-2 (2±0.1)
etected in the pylar cap. l.f.		Shape	Vith microp	Elongated and Ovoid	Ovoid	Ellipsoidal	Ellipsoidal
species d	porocyst	Shape index (Ub)		1.9-2.2	1.8-2	1.6-2	1.4-1.6
fferent <i>Eimeria</i> le species with	ø	Length (µm) x Width (µm)		11-15 (13±1.1) x5-8 (7±0.6)	14-16 (15±0.7) x 7-9 (8±0.8)	10-13 (12±0.9) x5-8 (7±0.7)	8-11 (9±0.9) x 5-8 (6±0.9)
oocysts of di neasured in th	ristics	Shape of oocyst		Ellipsoidal/ Ovoid	Ovoid	Ellipsoidal/ Ovoid	Ellipsoidal
acters of as been n	characte	Shape index (Vb)		1.4-1.5	1.4-1.6	1.3-1.5	1.1
orphologic chan of micropyle h	Oocyst	<i>Length</i> (μm) x Width (μm)		26-32 (30±1.6) x 17-23 (20±1.2)	34-38 (36±1.1) x 21-27 (24±1.8)	25-31 (28±1.6) x 17-23 (20±1.3)	18-22 (21±0.9) x 17-20 (18±1.1)
Supplementary 2: M deviation). The length		Eimeria species		E. arloingi	E. christenseni	E. hirci l.f.	E. hirci s.f.

7

3303

# J HELLENIC VET MED SOC 2021, 72(4) ПЕКЕ 2021, 72(4)

Present (Mostly aggregated)	Present (Mostly aggregated)	Present	Present	Present (Large)		Absent
Present/Present (Prominent)	Present/ Present	Present (Fragmented)/ Present	Present/ Present	Present (Fragmented)/ Present		Present/ Present
Yellowish Green	Blue, Yellow, and Green	Greenish Yellow	Dark Green	Greenish Yellow		Greenish Blue
1-2 (2±0.4)	1-2 (2±0.4)	1 (1±0)	1-2 (1±0.2)	1-2 (2±0.3)		1 (1±0.0)
Truncated- cone shaped	Half-moon -shaped	Dome- shaped	Dome- shaped	Dome- shaped		ı
2-2.5	2-2.5	2-4	1.4-4	2-2.5		ı
5-8 (7±0.8) x 2-4 (3±0.5)	5-8 (6±0.7) x 2-4 (3±0.5)	4-6 (5±0.7) x 1-3 (2±0.2)	4-7 (6±0.7) x 1-5 (3±0.6)	5-8 (6±0.6) x 2-4 (3±0.2)		ŗ
Elongated and Ovoid	Elongated and Ovoid	Elongated and Ovoid	Ovoid	Elongated and Ovoid	vlar cap	Elongated and Ovoid
1.7	1.6-1.7	0.8-1.4	1.6-1.7	1.6-2	microp	1.8
$\begin{array}{c} 10\text{-}17\\ (14\pm1.8) \text{ x}\\ 6\text{-}10 \ (7\pm0.9) \end{array}$	10-16 (13±1.3) x 6-10 (7±0.9)	4-13 (10±1.4) x 5-9 (6±0.7)	10-14 (12±0.9) x 6-9 (7±1.0)	12-16 (14±0.9) x 6-10 (8±1.2)	Without	7-11 (9±0.7) x 4-6 (5±0.7)
Urn-shaped/ Ellipsoidal	Ellipsoidal / Urn-shaped	Ellipsoidal or Broadly ovoid	Ellipsoidal	Ellipsoidal (Large)		Ovoid/ Sub- spherical
1.4-1.5	1.3-1.5	1.3	1.4	1.5		1.1-1.3
32-41 (35±1.8) x 23-28 (25±1.7)	28-33 (31±1.3) x 19-25 (22±1.4)	20-27 (25±1.3) x 16-21 (19±1.2)	26-33 (29±1.6) x 18-23 (20±1.3)	30-38 (34±1.8) x 20-26 (22±1.3)		$15-22 (17\pm1.5) x 13-17 (15\pm1.0)$
E. jolchejevi l.f.	E. jolchejevi s.f.	E. masseyensis	E. capralis	E. tunisiensis		E. alijevi

J HELLENIC VET MED SOC 2021, 72(4) ПЕКЕ 2021, 72(4)

3304

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

#### REFERENCES

- Adhikari RB, Maharjan M, Ghimire TR (2020) Prevalence of gastrointestinal parasites in the frugivorous and the insectivorous bats in Southcentral Nepal. Journal of Parasitology Research 2020:8880033. doi: 10.1155/2020/8880033
- Aumont G, Yvore P, Esnault A (1986) Experimental coccidiosis in goats.
  2. Effect of parasitism on nutritional balances and some blood parameters. Annales de Recherches Vétérinaires 17 (2):191-196
- Aumont G, Yvoré P, Esnault A (1984) Experimental coccidiosis in goats. 1. Experimental model. Effects of parasitism on the feeding behaviour and the growth of animals; intestinal lesions. Annales de Recherches Vétérinaires 15 (4):467-473
- Chartier C, Paraud C (2012) Coccidiosis due to *Eimeria* in sheep and goats, a review. Small Ruminant Research 103 (1):84-92. doi:10.1016/j. smallrumres.2011.10.022
- Dai YB, Lin MC, Zhang SX, Fu AQ (1991) Hepatic coccidiosis in the goat. International Journal of Parasitology 21 (3):381-382. doi:10.1016/0020-7519(91)90045-9
- Dai YB, Liu XY, Liu M, Tao JP (2006) Pathogenic effects of the coccidium *Eimeria ninakohlyakimovae* in goats. Veterinary Research Communications 30 (2):149-160. doi:10.1007/s11259-006-3228-1
- Duszynski DW, Wilber PG (1997) A guideline for the preparation of species descriptions in the Eimeriidae. The Journal of Parasitology 83 (2):333-336
- Ghimire T (2018) Investigating the contribution of parasites in the domestic animals researched at the Nepal Academy of Science and Technology (NAST). International Journal of Infectious Diseases 73:316. doi:10.1016/j.ijid.2018.04.4132
- Ghimire TR, Bhattarai N (2019) A survey of gastrointestinal parasites of goats in a goat market in Kathmandu, Nepal. Journal of Parasitic Diseases 43 (4):686-695. doi:10.1007/s12639-019-01148-w
- Hashemnia M, Khodakaram-Tafti A, Razavi SM, Nazifi S (2012) Experimental caprine coccidiosis caused by *Eimeria arloingi*: morphopathologic and electron microscopic studies. Veterinary Research

Communications 36 (1):47-55. doi:10.1007/s11259-011-9511-9 Khakural G (2003) Surveillance of goat diseases in the Western Hills of

- Nepal. Nepal Journal of Science and Technology 5 (1):37-40 Khodakaram-Tafti A, Hashemnia M (2017) An overview of intestinal coccidiosis in sheep and goats. Revue de Médecine Vétérinaire 167:9-20
- Koudela B, Bokova A (1998) Coccidiosis in goats in the Czech Republic. Veterinary Parasitology 76 (4):261-267. doi:10.1016/S0304-4017(97)00147-7
- Levine ND (1988) The Protozoan Phylum Apicomplexa, vol II. CRC Press Inc, Boca Raton
- Mohamaden WI, Sallam NH, Abouelhassan EM (2018) Prevalence of *Eimeria* species among sheep and goats in Suez Governorate, Egypt. International Journal of Veterinary Science and Medicine 6 (1):65-72. doi:10.1016/j.ijvsm.2018.02.004
- Norton C (1986) Coccidia of the domestic goat *Capra hircus*, with notes on *Eimeria ovinoidalis* and *E. bakuensis* (syn. *E. ovina*) from the sheep *Ovis aries*. Parasitology 92 (2):279-289. doi:10.1017/S0031182000064052
- Sayin F, Dincer S, Milli U (1980) The life cycle and pathogenicity of *Eimeria arloingi* (Marotel, 1905) Martin, 1909, in Angora kids and an attempt at its transmission to lambs\*. Zentralblatt für Veterinärmedizin Reihe B 27 (5):382-397. doi:10.1111/j.1439-0450.1980.tb01706.x
- Soe AK (1989) Coccidia (Protozoa: Apicomplexa) of domesticated goat *Capra hircus* in New Zealand. Massey University, New Zealand, New Zealand
- Soe AK, Pomroy WE (1992) New species of *Eimeria* (Apicomplexa: Eimeriidae) from the domesticated goat *Capra hircus* in New Zealand. Systematic Parasitology 23:195-202. doi:10.1007/BF00010872
- Wang CR, Xiao JY, Chen AH, Chen J, Wang Y, Gao JF, Zhu XQ (2010) Prevalence of coccidial infection in sheep and goats in northeastern China. Veterinary Parasitology 174 (3-4):213-217. doi:10.1016/j.vetpar.2010.08.026