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Coprological survey of helminths in selected cervids of Southeast Asia

Olga A. Loginova*, Sergei E. Spiridonov, Alma B. Mohagan, Oleg O. Tolstenkov & Daria S. Alieva

ABSTRACT. Sambar (*Rusa unicolor*), Javan rusa (*R. timorensis*), and Philippine deer (*R. marianna*) were opportunistically fecal sampled in The Socialist Republic of Vietnam, in the Republic of Indonesia, and in the Republic of the Philippines, respectively, during 2022–2024. A total of 28 samples were analyzed. Eggs of trematodes of the superfamily Paramphistomoidea were found in *R. unicolor* faeces, larvae of nematodes of the family Protostrongylidae were found in *R. timorensis* faeces, egg of trematode of the family Fasciolidae was found in *R. marianna* faeces. Sample mean intensity of invasion was ≤ 12 trematode eggs per 1 g of faeces, < 1 nematode larva per 1 g of faeces, < 1trematode egg per 1 g of faeces, respectively. Sample prevalence was 75, 20, and 17 %, respectively. Protostrongylids are reported for Javan rusa (*R. timorensis*) for the first time. The discrepancy in the morphometric results of the detected trematode eggs with the literature data may suggest the discovery of new helminth species.

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Гельминтокопроскопическое исследование некоторых видов оленьих в Юго-Восточной Азии

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РЕЗЮМЕ. Образцы фекалий индийского замбара (*Rusa unicolor*), тиморского оленя (*R. timorensis*) и филиппинского замбара (*R. marianna*) были собраны в 2022–2024 годах в Социалистической Республике Вьетнам, Республике Индонезия и Республике Филиппины, соответственно. Всего было проанализировано 28 образцов. Яйца трематод надсемейства Paramphistomoidea были обнаружены в фекалиях *R. unicolor*, личинки нематод семейства Protostrongylidae были обнаружены в фекалиях *R. timorensis*, яйца трематод семейства Fasciolidae были обнаружены в фекалиях *R. marianna*. Средняя интенсивность инвазии по выборке составила ≤ 12 яиц трематод на 1 г фекалий, < 1 личинки нематод на 1 г фекалий, < 1 яйца трематод на 1 г фекалий, соответственно. Экстенсивность инвазии по выборке составила 75, 20 и 17 %, соответственно. Протостронгилиды обнаружены у тиморского оленя (*R. timorensis*) впервые. Расхождение в результатах морфометрии обнаруженных яиц трематод с данными литературы может позволить предполагать обнаружение новых видов гельминтов.

КЛЮЧЕВЫЕ СЛОВА: нематоды, трематоды, тиморский олень, филиппинский замбар, индийский замбар.

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Introduction

The parasites of ruminants are very diverse (Poulin & Morand, 2000) and have different impact on their hosts up to death of the latter (Shanebeck *et al*., 2022). Helminths (parasitic worms) of ruminants are quite well studied for domesticated and (to the less extend) for wild hosts of temperate regions (Kutz *et al*., 2012; Kuznetsov *et al*., 2020; Kuznetsov, 2024), while the helminth fauna of the wild ruminants (especially cervids) of tropical and subtropical regions is much less investigated. The patterns of the geographical distribution of parasites are often even more complicated than those of their mammal hosts (Byers *et al*., 2019). The isolation events along with (re-)introductions, translocations, and other factors influenced diversity and distribution of ruminants and their parasites (Cromsigt *et al*., 2009). Within this framework Southeast Asia is an important terrain for the study of parasitic worms of the wild cervids, because periodic transgressions of ocean level caused repetitive events throughout the region, dividing and re-uniting islands and mammal populations (Hein & Kirwan, 2024). Some cervids inhabiting Southeast Asia are listed as Vulnerable in the IUCN. Those include three *Rusa* species: Javan rusa, *R. timorensis* de Blainville, 1822; Philippine deer, *R. marianna* Desmarest, 1822; and sambar, *R. unicolor* Kerr, 1792 (Hedges *et al*., 2015; MacKinnon *et al*., 2015; Timmins *et al*., 2015). Pryadko (1976) listed five species of helminths for *R. unicolor* (*Fascioloides magna*, *Taenia hydatigena larvae*, *T. lyncis larvae*, *Spiculopteragia houdemeri*, and *Rinadia andreevae*) in the Indochina (presently mainland Southeast Asia) without pointing the country. Rana *et al*. (2015) wrote about gastro-intestinal helminthes (mostly *Trichostrongylus* spp. and *Gaigeria pachyscelis*) in *R. unicolor* from Pakistan. Bernard *et al*. (2016) reported *Elaeophora* in *R. u. equina* from the zoo in North America (supposedly acquired there). Soundararajan *et al*. (2024) found *Cotylophoron cotylophorum*, *Fischoederius elongatus,* and *Setaria digitata* in *R. unicolor* in India. Portugaliza *et al*. (2015) reported *Fasciola gigantica* and *Paramphistomum* spp. in *R. marianna* from the Leyte Island (Philippines). Arif *et al*. (2024) found *Haemonchus contortus* in *R. timorensis* from the deer and sheep farm in Bogor Regency (Indonesia). Therefore, the helminth complex of *R. unicolor* has been studied relatively fully, while information on the helminths *R. timorensis* and *R. marianna* remains fragmentary. That is why every attempt to enrich our knowledge on the subject is worth trying.

The findings described below were generated as part of different expeditions set with other purposes. This is an opportunistic study, and all the extrapolations are hypotheses requiring broader sampling. The aim of this work was to study helminths of these three cervid species from Indonesia, Philippines, and Vietnam and, therefore, to contribute to conservation of vulnerable cervids of Southeast Asia.

Materials and methods

Fecal sampling

Fecal samples were collected for three host species of *Rusa*, inhabiting the area of Southeast Asia (Fig. 1, Table 1). Javan rusa individuals (*R. timorensis*) from the Komodo National Park in Rinca (Indonesia) were the only visible cervids there, whereas those from a private park in Bali (Indonesia) were kept with other cervids (supposedly, *Axis* spp.). Sambars (*R. unicolor*) from Cát Tiên National Park (Vietnam) were also kept with other cervids and bovids. Philippine deer (*R. marianna*) from Malaybalay Mimi Zoo in Mindanao (Philippines) were the only animals in their enclosure.

Faeces were collected from the ground shortly after host's defecation. Each sample was placed into individual container labeled with the host species, collection date, location, and collector's name. Fecal samples constituted of 10 to 15 pellets each. Sample sets 1 to 3 were stored dried (naturally), sample set 4 was stored at +4°C humid. Sample sets were delivered for further investigation to the Laboratory of Parasites' Systematic and Evolution of the Center for Parasitology of the A. N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences, Moscow, Russia. During the trip sample set 4 was in a thermal container with airline-approved refrigerants.

Taxonomical identification of cervid hosts

Host species were identified using the "Handbook of the Mammals of the World. Vol. 2. Hoofed Mam-

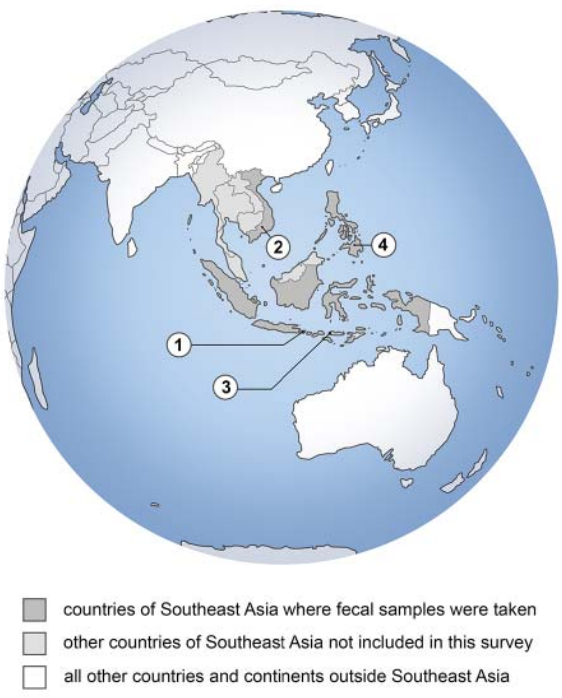

Fig. 1. Map of Southeast Asia indicating the sampling sites. Numbers correspond to identification numbers in Tab. 1. Drawing: O. Loginova.

Table 1. Collection data for fecal samples from cervids in Southeast Asia.

Sample set ID	Host species	Number of fecal samples	Location	Geographic coordinates	Date collected
1	Javan rusa (*Rusa timorensis*)	5	Bali, the Republic of Indonesia	8.3622 S, 115.1158 E	03 April 2022
2	Sambar (*Rusa unicolor*)	12	The Socialist Republic of Vietnam	11.4240 N, 107.4320 E	25 April 2022
3	Javan rusa (*Rusa timorensis*)	6	Rinca, the Republic of Indonesia	8.6538 S, 119.7158 E	05 March 2024
4	Philippine deer (*Rusa marianna*)	5	Mindanao, The Republic of the Philippines	8.1585 N, 125.1346 E	20 May 2024

Table 2. Helminths recovered from faeces of studied cervids in Southeast Asia.

Host species (Locality)	Trematodes				Nematodes	
	Paramphistomoidea (egg)		Fasciolidae (egg)		Protostrongylidae (L1)	
	Intensity	Prevalence	Intensity	Prevalence	Intensity	Prevalence
R. timorensis (Bali, Indonesia)	–	–	–	–	–	–
R. unicolor (Vietnam)	(1–36) 8 ± 4	9 (75%)	–	–	–	–
R. timorensis (Rinca, Indonesia)	–	–	–	–	2	1 (17%)
R. marianna (Philippines)	–	–	1	1 (20%)	–	–

Intensity: min-max numbers of helminth specimens per 3 g of host faeces (in brackets), mean ± SEM are given if $n > 1$; number of helminth specimens per 3 g of host faeces is given if $n = 1$ (n = number of positive fecal samples).
Prevalence: number of positive fecal samples and ratio (in brackets) are given.

mals" (Wilson & Mittermeier, 2011) based on the appearance (Fig. 2) and location of studied cervids.

Taxonomic nomenclature was also checked via online version of the "Mammal Species of the World. A Taxonomic and Geographic Reference" (Wilson & Reeder, 2005).

Fecal examination

A total of 28 fecal samples were surveyed of helminths in accordance with the National Standard of the Russian Federation GOST R 54627–2011 "Agricultural ruminant animals. Methods of Laboratory Helminthological Diagnostics" (National Standard…, 2013). Larvoscopy was performed via Vajda's method: a water drop was placed on a microscopic glass slide and 3 fecal pellets were placed into this water; after 40 min, the pellets were removed, and the liquid was studied under the microscope for the larvae of nematodes. Ovoscopy targeting eggs of nematodes and cestodes was conducted via double-centrifugation Darling's method. It requires first centrifugation performed in fecal slurry of feces and tap water, and second centrifugation where the sediment of the previous step is homogenized with the flotation solution (saturated solution of sodium chloride and glycerol 1:1). Ovoscopy targeting eggs of trematodes was a traditional fecal sedimentation (Verocai *et al*., 2020). Each ovoscopic fecal sample was 3 grams.

Taxonomical identification of helminths

Preliminary identification of obtained eggs and larvae of helminths was based on their morphology and morphometry. For eggs, morphologic criteria included structure of an egg membrane, presence or absence of operculum, color, type of content. Morphometric criteria included length and width. For larvae, morphologic criteria included structure of intestine (homogenous appearance or distinct cells), type of tail (spikes, spines). Mobility and position at rest were also taken into consideration. Morphometric criteria included total length of larva, buccal cavity depth, distance from anterior end to nerve ring, to excretory pore, to esophageal-intestinal junction, to genital primordium, to anus; maximum body width; distance from esophageal-intestinal junction to genital primordium; tail length; tail extensions (if present). Bright field light microscopy was used to study eggs and larvae. Morphometry was performed using micrographs of helminths. For this purpose an optical microscope Micmed-6 (LOMO-MA, Russia) was equipped with a digital photo camera 5D Mark II (Canon, Japan) via the C-mount adapter (LOMO-MA, Russia). Micrographs were taken at 400× magnification. Measurements were taken using Figi/ImageJ Version 1.2.4 RRID:SCR_003070 software (National Institutes of Health, USA). The program was set using the microscope calibration slide OMP (LOMO-MA, Russia) and operated in the Straight Line mode (for eggs)

and Segmented Line mode (for larvae). The reference literature was used to identify obtained eggs and larvae of helminths (Skrjabin, 1948, 1949; Boev, 1975; Kontrimavichus *et al*., 1976).

Statistical analysis

The statistical analysis was performed using the Analysis ToolPak of Microsoft Excel 2007 RRID:SCR_016137 software (Microsoft Corporation, USA). The following parameters were calculated: 1) Egg's width to length ratio; 2) Sample mean intensity of invasion (mean number of helminths in a stage of an egg or a larva per 1 g of host's faeces); 3) Sample prevalence (proportion of number of positive fecal samples to number of studied fecal samples). Intensity and Prevalence were calculated for sample sets (implying that one sample characterizes one animal), not populations.

The arithmetic mean value and the standard error of the mean (SEM) were determined for: 1) Linear dimensions of helminths; 2) Egg's width to length ratio; 3) Sample intensity of invasion (where possible); 4) Sample prevalence (Blaker, 2000).

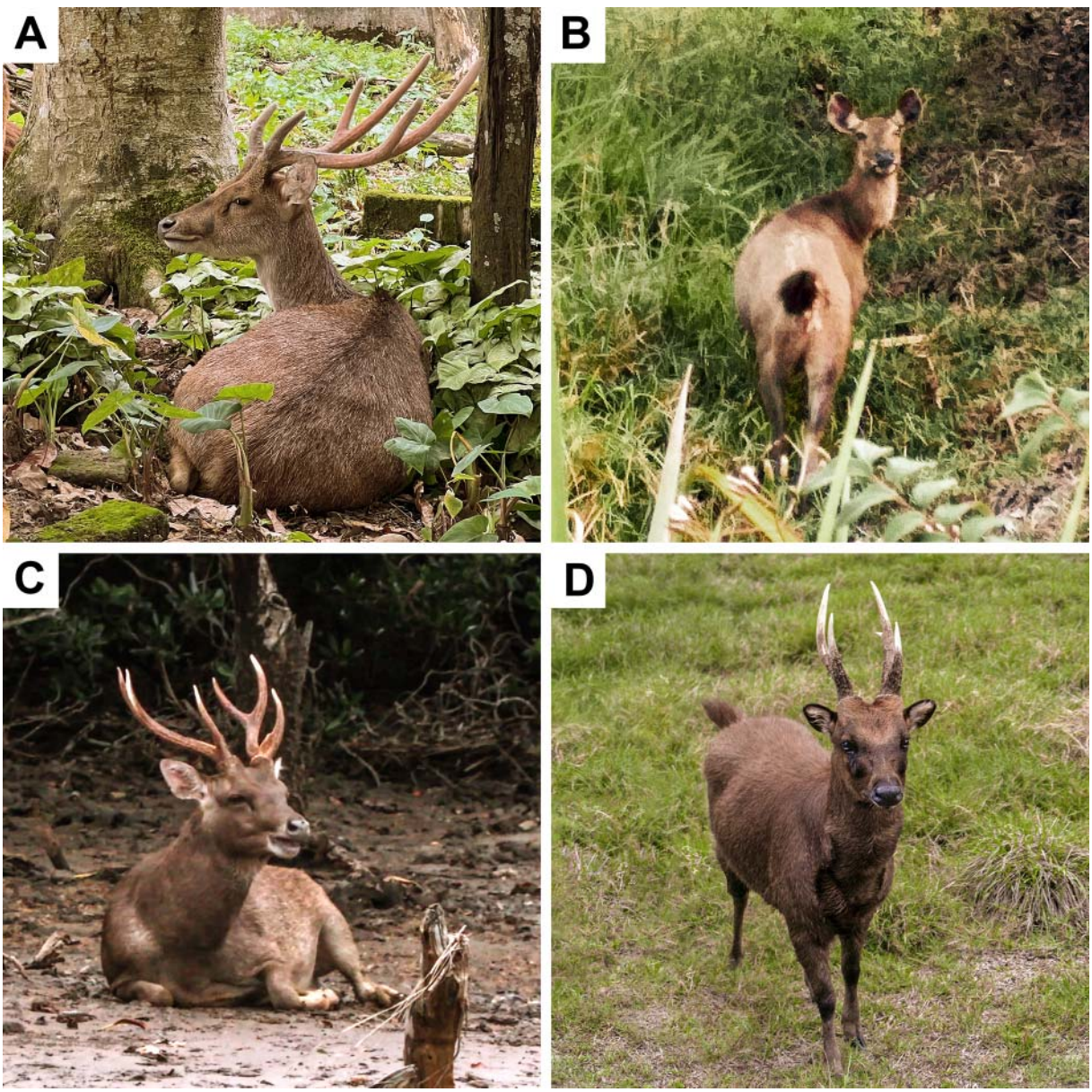

Fig. 2. Appearance of studied cervid host species in Southeast Asia: A — Javan rusa (*R. timorensis*) in Bali, the Republic of Indonesia, photo A. Stetyukha; B — sambar (*R. unicolor*) in the Socialist Republic of Vietnam, photo O. Tolstenkov; C — Javan rusa (*R. timorensis*) in Rinca, the Republic of Indonesia, photo D. Alieva; D — Philippine deer (*R. marianna*) in Mindanao, the Republic of the Philippines, photo S. Spiridonov.

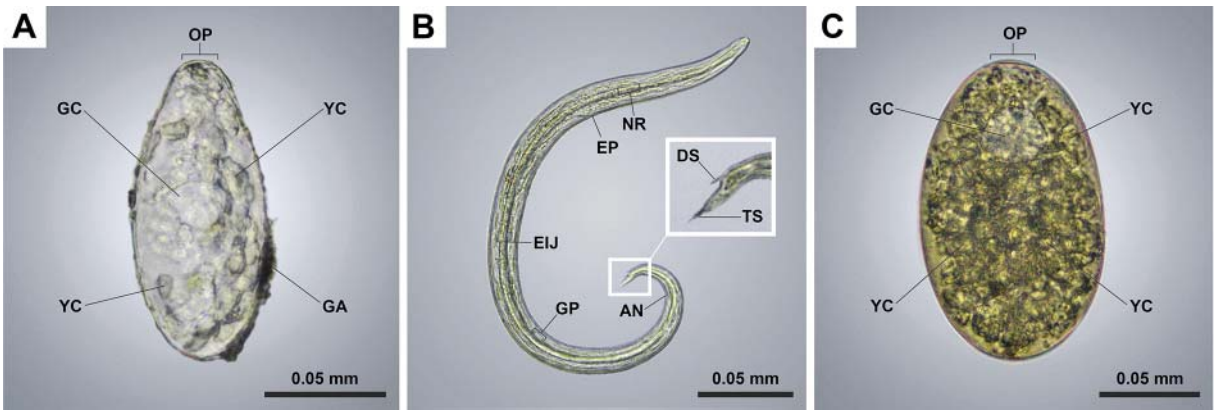

Fig. 3. Diagnostic stages of helminths obtained from faeces of studied cervids in Southeast Asia: A — Paramphistomoidea egg (dead) from faeces of sambar (*R. unicolor*); B — Protostrongylidae L1 (dead) from faeces of Javan rusa (*R. timorensis*); C — Fasciolidae egg (live) from faeces of Philippine deer (*R. marianna*). Abbreviations: AN — anus; DS — dorsal spine; GA — garbage; GC — germ cell; GP — genital primordium; EIJ — esophagus-intestinal junction; EP — excretory pore; NR — nerve ring; OP — operculum; TS — tail spike; YC — yolk cell., Bright field light microscopy, 400× magnification, helminths are in tap water under cover slips. Photos O. Loginova.

Results

Our initial coprological survey of helminths in three *Rusa* species in Southeast Asia revealed: 1) rumen fluke (superfam. Paramphistomoidea) in sambar (*R. unicolor*) in The Socialist Republic of Vietnam; 2) nematodes of the fam. Protostrongylidae in Javan rusa (*R. timorensis*) in the Republic of Indonesia; 3) liver flukes (fam. Fasciolidae) in Philippine deer (*R. marianna*) in the Republic of the Philippines. Sample mean intensity of invasion was ≤ 12 trematode eggs per 1 g of faeces, < 1 nematode larva per 1 g of faeces, < 1trematode egg per 1 g of faeces, respectively. Sample prevalence was 75, 20, and 17%, respectively.

Diagnostic stages of obtained helminths are presented in Fig. 3. Additional light micrographs of the

Table 3. Measurements (in micrometers, μm) of trematode eggs from faeces of studied cervids in Southeast Asia.

Trematode egg	Length (min–max)	Mean ± SEM	Width (min–max)	Mean ± SEM	Egg's index (min–max)	Mean ± SEM
Paramphistomoidea (n = 10)	102–125	115 ± 2	57–66	59 ± 0.9	1.71–2.13	1.94 ± 0.05
Fasciolidae (n = 1)	160	–	90	–	1.78	–

Table 4. Measurements of nematode larvae from faeces of studied cervids in Southeast Asia.

Parameter	L1 #1	L1 #2	Mean ± SEM
Total length, μm	376	397	386.5 ± 10.5
Buccal cavity depth, μm	4	3	3.5 ± 0.5
Distance from anterior end to beginning of nerve ring, μm	63	68	65.5 ± 2.5
Distance from anterior end to excretory pore, μm	91	97	94 ± 3.0
Distance from anterior end to esophagus-intestinal junction, μm	184	186	185 ± 1.0
Distance from anterior end to beginning of genital primordium, μm	236	241	238.5 ± 2.5
Distance from anterior end to anus, μm	335	356	345.5 ± 10.5
Esophagus length to total length ratio, %	49	47	48 ± 1.0
Maximum body width (at the esophagus-intestinal junction), μm	16	21	18.5 ± 2.5
Distance from esophagus-intestinal junction to middle of genital primordium, μm	57	62	59.5 ± 2.5
Tail length (distance from anus to the tip of tail), μm	33	30	31.5 ± 1.5
Dorsal spine length, μm	2	3	2.5 ± 0.5
Tail spike length, μm	6	7	6.5 ± 0.5

first stage larvae (L1) at 400× and 1000× magnification are available as three sets of raw files at:

https://www.researchgate.net/publication/396465862_DSL_raw_micrographs_x400_P1;

https://www.researchgate.net/publication/396466420_DSL_raw_micrographs_x400_P2;

https://www.researchgate.net/publication/396466364_DSL_raw_micrographs_x1000.

Intensity of invasion and prevalence rates (in relation to sample sets) are presented in Table 2.

Morphometric characteristics of trematode eggs found in faeces of studied cervids are presented in Table 3. Morphometric characteristics of nematode larvae found in faeces of studied cervids are presented in Table 4.

Discussion

Our study revealed rumen flukes (Paramphistomoidea), liver flukes (Fasciolidae) and protostrongylids in the faeces of studied cervid hosts inhabiting Southeast Asia.

Since fecal sample set 2 was dry, eggs of Paramphistomoidea were found dead as expected, because they are very sensitive to drying (Fig. 3A). It can be seen that the germ cell has lost its characteristic round shape, and numerous yolk cells stuck from the inside to the walls of the egg. Garbage (stuck on the outside walls of the egg) is typical for Paramphistomoidea eggs that have spent some time in humidity (in contrast to Fasciolidae eggs which surface usually remains clean regardless its development stage). Differential diagnoses for these rumen flukes (based on egg appearance and reports for cervid hosts) include at least: *Paramphistomum* species (*P. cervi* Zeder, 1790, *P. leydeni* Nasmark, 1937) (Eduardo, 1982); *Calicophoron daubneyi* Dinnik, 1962 (Rehbein *et al*., 2024); *Cotylophoron cotyphorum* (Fischoeder, 1901) (Soundararajan *et al*., 2024), and *Fischoederius elongatus* (Poirier, 1883). The latter was once reported exactly for *R. unicolor* (Rehbein *et al*., 2024). Egg measurements of these rumen flukes are presented in Table 5.

All the above mentioned rumen flukes seem to produce bigger eggs than those that we found in cervids in Southeast Asia (Table 3). However, a correlation between the size of trematode eggs and the size of their hosts, as well as with location of the host was discovered (Valero *et al*., 2001). Therefore, the same Paramphistomoidea may produce eggs with different size ranges once parasitizing cervids, not bovids. Alternatively, some other trematode was found, or a new species might have been encountered.

In the sample set 3 two larvae were found (Fig. 3B). According to G-shape position at rest, presence of a dorsal spine along with a tail spike, and other characteristics they belong to fam. Protostrongylidae. However, it is hard to say if they are lung worms, brain worms or muscle worms. Differential diagnoses for these protostrongylids (based on dorsal-spined larvae appearance and reports for cervid hosts) include at least: *Elaphostrongylus*, *Parelaphostrongylus*, *Varestrongylus*, and *Pneumostrongylus* species (Boev, 1975; Kontrimavichus *et al*., 1976). Found larvae (Tab. 4) are most similar to *E. panticola* including measurements (Kontrimavichus *et al*., 1976). This species was reported not only for different cervid hosts (*Cervus elaphus* Linnaeus, 1758, *C. nippon* Temminck, 1838, and *Alces alces* Linnaeus, 1758), but also for Asia. How-

Table 5. Measurements (in micrometers, μm) of Paramphistomoidea eggs according to literature data and our study.

Family	Species	Length (min–max)	Width (min–max)	Reference	Host species[1]
Gastrothylacidae	*F. elongatus*	125–135	65–70	Fischoeder, 1903	*Bubalus bubalis kerabau*
Paramphistomidae	*C. daubneyi*	127–140	59–75	Dinnik, 1962	*Bos taurus*
	C. cotyphorum	125–135	65–68	Fischoeder, 1903	*Bos taurus*
	P. cervi	145–156	75–82	Skrjabin, 1949	no data
	P. leydeni	156	78	Skrjabin, 1949	*Bos taurus*
Unknown	Unknown	102–125	57–66	This study	*Rusa unicolor*

[1] Host species from which trematodes were obtained and described.

Table 6. Measurements (in micrometers, μm) of Fasciolidae eggs according to literature data and our study.

Species	Length (min–max)	Width (min–max)	Reference	Host species[1]
F. hepatica	130–145	70–90	Skrjabin, 1948	no data
F. gigantica	125–157	60–100	Cobbold, 1855	*Giraffa camelopardalis*
F. magna	109–168	75–100	Skrjabin, 1948	no data
P. fasciolaemorpha	126–135	81–90	Skrjabin, 1948	*Alces alces*
Unknown	160	90	This study	*Rusa mariana*

[1] Host species from which trematodes were obtained and described.

ever, some new species-specific protostrongylid might have been encountered in our research. Despite positive background of extracting DNA from single larvae (Loginova *et al*., 2023) this time our attempts were not successful. Therefore, morphology and morphometry allows us only solid family level of taxonomic identification, or a cautious guess. In case it is *Elaphostrongylus* species indeed, its adults inhabit brain and connective tissue, whereas larvae at some point occupy respiratory system.

Only one egg of liver fluke was found in the sample set 4 (Fig. 3C). Due to humidity of the faeces this egg was live. However, it was too little to try DNA extraction. Our previous positive experience of extracting DNA from trematode eggs was related to processing more that 60 live eggs for each attempt (Loginova *et al*., 2024). Egg's appearance and cervid hosts reported offer at least the following differential diagnoses: *Fasciola hepatica* Linnaeus, 1758 (Loginova *et al*., 2024), *F. gigantica* Cobbold, 1855 (Soundararajan *et al*., 2024), *Fascioloides magna* Bassi, 1875 (Skrjabin, 1948), and *Parafasciolopsis fasciolaemorpha* Ejsmont 1932 (Skrjabin, 1948). Egg measurements of these liver flukes are presented in Tab. 6.

Found egg (Table 3) tends to two Fasciolidae species reported for cervids according to its measurements: *F. gigantica* and *F. magna*. Egg length range of *F. gigantica* is somewhat smaller than the length of the found egg (125–157 μm vs. 160 μm), but this difference can be also due to different host species (*Giraffa camelopardalis* (Linnaeus, 1758) vs. *R. marianna*). It is important that *F. gigantica* was reported not only for the same cervid host that we studied (*R. marianna*), but also for the same area, the Philippines (Portugaliza *et al*., 2015). Despite the absence of molecular test in that publication, typical appearance of *F. gigantica* (presented in Fig. 3 of that article) leaves no doubt in its correct species identification. Alternatively, egg size range of *F. magna* matches the measurements for the found egg completely. Moreover, in contrast to *Fasciola* species inhabiting bile ducts, *Fascioloides* inhabits liver parenchyma. The way *Fascioloides* eggs enter intestine is still discussed, but it was noted that for some hosts only very few eggs are found during fecal tests like in our case (Králová-Hromadová *et al*., 2016). On the other hand, Valero rightly notes that "the absence of a direct relationship between number of flukes present and number of eggs shed is well known. Egg production oscillations in trematodes in general and *Fasciola* in particular throughout their lifespan is also well known" (Valero *et al*., 2002). Thus, found egg might be either *F. gigantica* or *F. magna*.

Values of intensity of invasion in this study are interpreted by the National Standard of the Russian Federation GOST R 54627–2011 "Agricultural ruminant animals. Methods of Laboratory Helminthological Diagnostics" (National Standard…, 2013) as low intensity. However, in contrast to high number of eggs or larvae in faeces, small number of found helminths may or may not correlate with the real intensity of invasion (Valero *et al*., 2002). All three kinds of discovered helminths have complex life cycles involving gastropods (fresh-water snail for trematodes and fresh-water or terrestrial gastropods for nematodes) (Skrjabin, 1948, 1949; Boev, 1975; Kontrimavichus *et al*., 1976). All three kind of discovered helminths pose a mortal threat to studied cervids, because lethal outbreaks or individual lethal cases were reported for cervids infected with Paramphistomoidea, Protostrongylidae, and Fasciolidae (Davidson *et al*., 2020; Benchohra *et al*., 2024).

Within the framework of conservation of vulnerable cervids in Southeast Asia this study should be continued with broader sampling and taking into account seasonal dynamics.

Conclusion

Given the insufficient study of the helminth fauna of artiodactyls of the family Cervidae in Southeast Asia, the helminth species identified in this study should be considered the first documented record for the surveyed areas of this region. Protostrongylids are reported for the *R. timorensis* for the first time. However, infestation rates remain low.

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