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Epidemiological Assessment of Gastrointestinal Parasites in *Cuon alpinus* (Wild Dog) of Murlen National Park, Champai District, Mizoram, India

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Abstract: Amid growing human-wild life interaction and the close proximity at which human has settled around protected areas, the chances of zoonosis spillover and transmission of pathogenic diseases has increased by many folds. The present study was aimed to investigate the gastrointestinal parasites of *Cuon alpinus* (wild dog) at the Murlen National Park (MNP), located in Champai district, Mizoram, India and reviewing their possible transmission and pathogenicity. A total 19 scat samples were collected and analyzed using smear, flotation and Telemann's techniques. The overall parasite prevalence was 78.94% with species such as *Ascaris*, *Capillaria*, *Diphyllobothrium*, *Eimeria*, *Isospora*, *Paragonimus*, *Strongyle*, *Toxocara*, *Trichuris*, etc. Fresh samples yielded more parasites than older ones. The presence of zoonotic parasites highlights the public health risk to local communities, who rely on shared forest resources and untreated water, and have close contact with wildlife. The findings emphasize the need for long term parasitological monitoring and improved strategies to prevent disease spillover from wildlife to humans and domestic animals in protected areas like MNP.

Keywords: Coprological analysis, *Cuon alpinus*, Gastrointestinal parasites, Murlen National Park, Prevalence, Zoonosis

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Introduction

Natural systems are dynamic assemblages of many species that interact with one another as well as internal ecological processes. Microbes are an essential component of these systems, contributing to the management of the whole system and playing important roles in the biology of other creatures (Habtamu *et al*., 2017: Hauffe and Barelli, 2019). For many intestinal parasites, carnivores can serve as a permanent hosts and reservoirs, harmful diseases such as paragonimiasis, toxoplasmosis, sarcocystosis and cysticercosis (Beltrame *et al*., 2018). Numerous species of placental animals, sometimes known as carnivorans, make up the diverse scrotiferan order ‘Carnivora’. India is home to eight families, total 60 species, of the 250 wild carnivore species that exist throughout the world. These animals are housed in zoological gardens or biological parks for aesthetic, scientific and conservation reasons (Thawait *et al*., 2014).

In developing nations, parasitic illnesses, particularly those that can result in zoonosis, are the most important problems in terms of health, economy and society. More than 60 zoonotic parasites have definitive or reservoir hosts in carnivores (Sarvi *et al*., 2018). Children are especially at risk of infection when these animals are in close proximity to human due to their poor hygiene compared to adults and increased exposure to polluted soil harboring parasite eggs or cysts. Additionally, farmers and ranchers who frequently engage in agricultural and animal care are also in danger. Four categories can be used to classify zoonotic parasites that affect humans: (i) Direct zoonotic parasites are such as *Giardia*, *Cryptosporidium* and *Toxocara,* infect people from animals; (ii) *Ancylostoma* species and *Strongyloides stercoralis*, two saprozoonotic parasites that spread by soil or water; (iii) *Dipylidium caninum* and other *Fasciola* spp. are examples of invertebrate intermediary hosts that help spread meta-zoonotic parasites that harm humans; and (iv) Examples of vertebrate intermediate hosts for cyclo-zoonotic parasites that can infect humans include *Echinococcus granulosus* and *Taenia* spp. (Abu-Samra *et al*., 2011; Sarvi *et al*., 2018). Gastrointestinal parasites of dogs such *Toxocara* spp., *Ancylostoma* spp*.,* *Echinococcus* spp., *Dipylidium* spp., *Giardia* and *Cryptosporidium* spp. are important in terms of public health since they have the ability to spread diseases to humans. The symptoms and clinical manifestations of parasite infections in humans, such as those that result in hydatidosis, visceral larva migrans, coenurosis, creeping eruption, mesocestoidiasis and dipylidiasis are present (Ahmed *et al*., 2014; Berentsen *et al*., 2015; Sarvi *et al*., 2018).

In India, conservation areas are highly humanized landscapes where people, cattle and wildlife coexist closely. In these systems, parasites can harm multiple hosts. For wild carnivores, who serve as sentinels for determining their native prey and ecosystem conditions, these impacts remain uncertain. Disease transmission between wild and domestic animals, which contact humans, can act as infection sources (Pereira *et al*., 2023). Parasites can directly affect host survival through pathological effects (blood loss, tissue damage, abortion, deformities and death), and indirectly by reducing immunity and physical health (Thawait e*t al*., 2014). While many reports exist on zoonotic parasites in public areas, little research exists for specific locations like wildlife sanctuaries in North-Eastern India. Due to limited access, their epidemiological conditions may be unique. There is minimal information about intestinal parasitic infections and zoonotic parasite species in carnivorous animals in the Murlen National Park (MNP).

The purpose of this study was to determine how frequently scat samples taken from the environment of the MNP, Mizoram, India, had parasites that are important to public health. The findings of this study are essential for expanding our understanding of the gastrointestinal parasite ecology of wild dog and suggest prevention of the transmission of infectious zoonotic diseases to nearby humans or other animals in the National Park.

Materials and Methods

Study area

Murlen National Park (MNP)is in the Indian state of Mizoram's Champhai district (Fig. 1). The park area is 200 km^2 (77 sq. m) in size. The park is near to the Chin Hills and is located around 245 km east of Aizawl, lies latitude: 23°37'0.59"N and longitude: 93°17'60.00"E. In the same district, it is located to the north of Lengteng Wildlife Sanctuary. It has an area of about 100 km^2. A wide diversity of vegetation and wildlife can be found in the Murlen forest's tropical, semi-evergreen and

Fig. 1: Map of Murlen National Park and fecal Collection coordinates

sub-montane habitats. In this park, there have been records of 15 species of animals, 150 species of birds, 35 species of medicinal plants, different species of bamboos and several species of orchids. There are many different animals that are found in this area, including sambar, barking deer, Malayan giant squirrel, Himalayan black bear, serow, Hume's pheasant, kalij pheasant, grey partridge and dark-rumped swift (EFCCD, 2022).

Sample collection

Between April and May 2023, fresh fecal samples were gathered from 10 am to 3 pm with the assistance of research aides. Fresh fecal samples were identified as wet fecal deposits of wild dogs (*Cuon alpinus*) containing mucus. Approximately 10 g of deposited fecal matter was collected using sterilized disposable gloves (Table 1). Scat samples were diligently searched for along forest paths and streams in MNP using a random sampling method (Figs. 2a, c). A portion of the scat, primarily the middle section, was placed in airtight zip bags, and each sample was examined macroscopically for consistency and the presence of adult worms or proglottids (Fig. 3). The collected samples were transported on ice to the laboratory of Department of Zoology, Mizoram University, Aizawl and stored at 4°C until they were analyzed.

Laboratory analysis

Processing of fecal samples

Three different methods were used for examination and identification of gastrointestinal parasites in scat samples of wild dogs. It includes Smear Technique, Flotation Technique (mostly useful for the examination of eggs of nematodes) and the Telemann's method as described by Vandir *et al*. (2021), Takano *et al.* (2024) and Regidor-Cerrillo *et al.* (2020), respectively, with slight modifications.

(i) *Smear technique*

Fecal material was prepared as a thin smear with normal saline. Debris were removed and again few drops of saline solution (0.85% of NaCl) was added and mixed well; examined under a 10X microscope.

(ii) *Flotation technique*

Following the floatation procedure, nematode eggs were screened. A small amount of faeces (2 g) was thoroughly mixed with 10 ml of 10% formalin; the mixture was then filtered through cheesecloth over a funnel to eliminate the coarse excrement, 0.85% saline was poured through the debris on the cheesecloth. The filtrate was poured into a 15 ml centrifuge tube and centrifuged at 500 rpm for

Table 1: Coordinates of scat collection sites during the field survey

S. No.	Latitude	Longitude
1	23°38'54.01°N	93°17'25.04°E
2	23°38'52.27°N	93°17'26.07°E
3	23°38'50.53°N	93°17'29.88°E
4	23°38'43.00°N	93°17'43.84°E
5	23°38'41.80°N	93°17'46.64°E
6	23°38'41.52°N	93°17'51.45°E
7	23°38'37.44°N	93°18'7.95°E
8	23°38'37.54°N	93°18'10.12°E
9	23°38'32.15°N	93°18'16.43°E
10	23°38'11.42°N	93°18'36.67°E
11	23°38'24.91°N	93°18'28.38°E
12	23°38'48.18°N	93°17'30.15°E
13	23°38'43.43°N	93°17'46.43°E
14	23°38'31.20°N	93°17'36.01°E
15	23°38'15.98°N	93°17'30.24°E
16	23°38'12.61°N	93°17'30.40°E
17	23°37'58.54°N	93°17'23.63°E
18	23°38'52.15°N	93°17'25.85°E
19	23°38'38.48°N	93°17'49.13°E

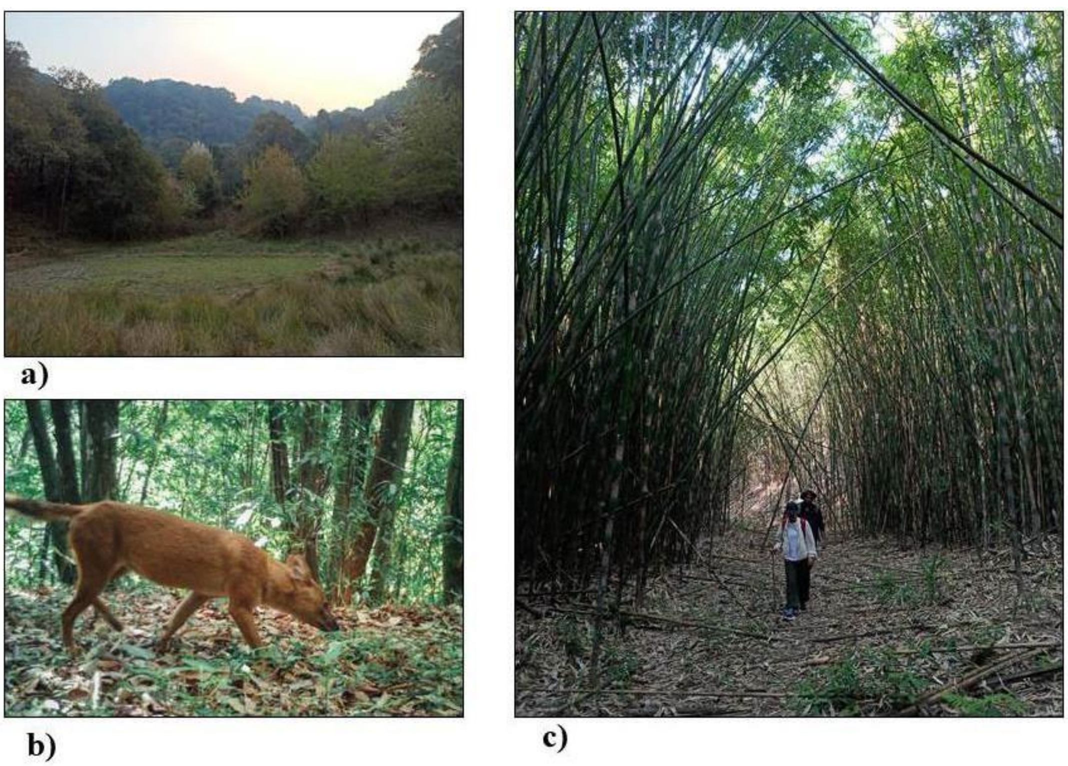

Fig. 2: (a) and (c) Habitat that served through; and (b) *Cuon alpines.*

Fig. 3: (a) Fresh Scat, (b) Dry Scat, (c) Pugmark of wild dog, (d)Photography and examination of morphometric features of fecal sample.

Table 2: Prevalence percentage of parasites in 19 scat samples of *Cuon alpinus*

Total No. of Sample	**No. of positive sample**	**No. of negative Sample**
19	15	4
Prevalence Percentage of parasite	78.94%	21.05%

10 min. The supernatant was discarded. The sediment was then mixed with 2 ml of zinc sulphate solution and again centrifuged at 500 rpm for 2 min. Few drops of surface layer of the floatation fluid were placed on a clean-dry glass slide and covered with a cover slip; examined under a 40X microscope.

(iii) *Telemann's method*

In Telemann's method, 1 g of scat sample mixed with 5 ml of 5% acetic acid. The mixture was then filtered using cheesecloth, poured few drops of ether through cheesecloth and centrifuged at 1500 rpm for 1 min. The sediment was collected after centrifugation and diluted with distilled water. Few drops of solution were placed on a glass slide and covered with cover slip; examined under 40X microscope.

Results

A total 19 scat samples of wild dogs (*Cuon alpinus*) were collected from the field surveys at Murlen National Park. 10 scat samples were relatively old (few days old) while 9 samples were fresh in nature. The prevalence of gastrointestinal parasites in the examined samples of *Cuon alpinus* was found to be 78.94% with 15 samples testing positive for parasite (Table 2).

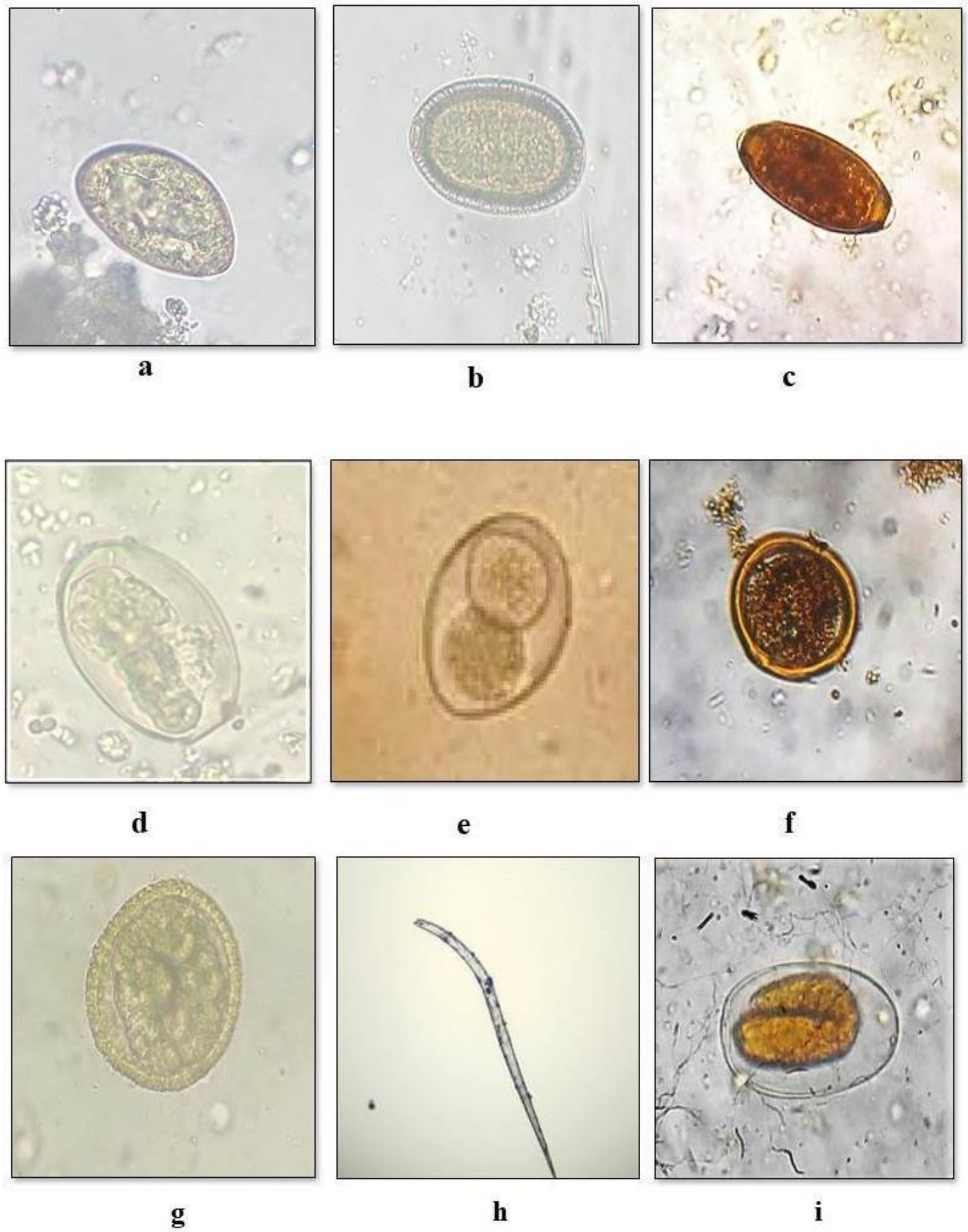

Fig. 4: (*a*) *Diphyllobothrium* sp., (b) *Capillaria* sp., (c) *Trichuris* sp., (d) *Paragonimus* sp., (e) *Isospora* sp., (f) *Ascaris* sp., (g) *Toxocara* sp., (h) First stage larva of *Strongyle*, and (i) Adult *Strongyle.*

In this study, different types of gastrointestinal parasites were observed and recorded such as *Ascaris* sp., *Diphylobotrium* sp., *Isospora* sp., *Paragonimum* sp., *Strongyle* sp., *Toxocara* sp., *Trichuris* sp. etc. (Fig. 4). Large number of debris such as plants hail-follicles, insects wings, pollen grains *etc*. were also observed during the examination process (Fig. 5). Maximum number of parasites found in *Cuon alpines* in MNP were that of *Isopora* species and *Stongyle* species (Fig. 6).

Among the three different methods that were used for examination of parasites in the scat samples, smear technique and floatation technique resulted in 15 yield each while Telemann's method resulted in eight parasites (Table 3). As obvious, fresh samples were found to contain higher

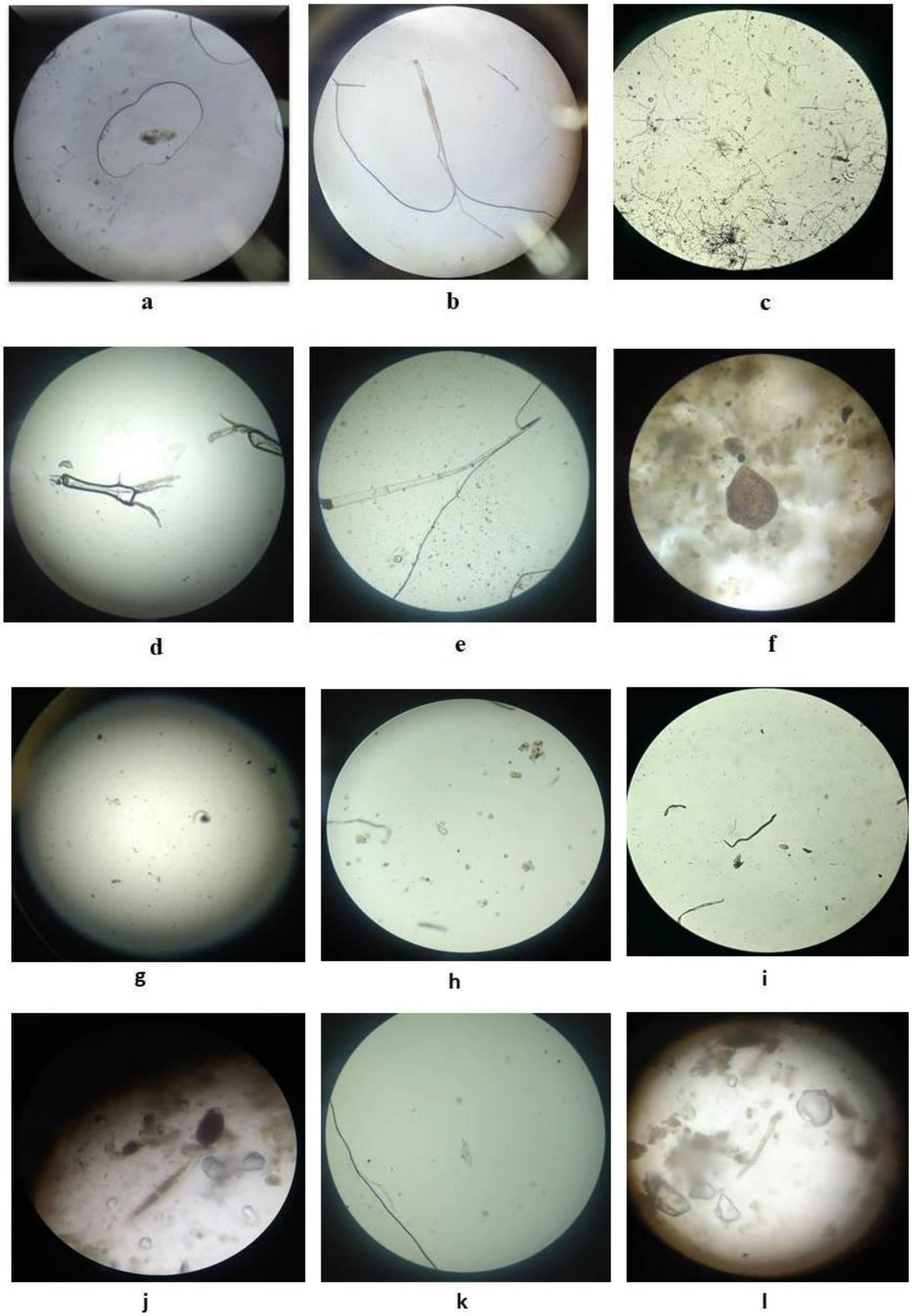

Fig. 5: Derbis, oocyste, plant matters and unidentified parasites in the examined scat samples.

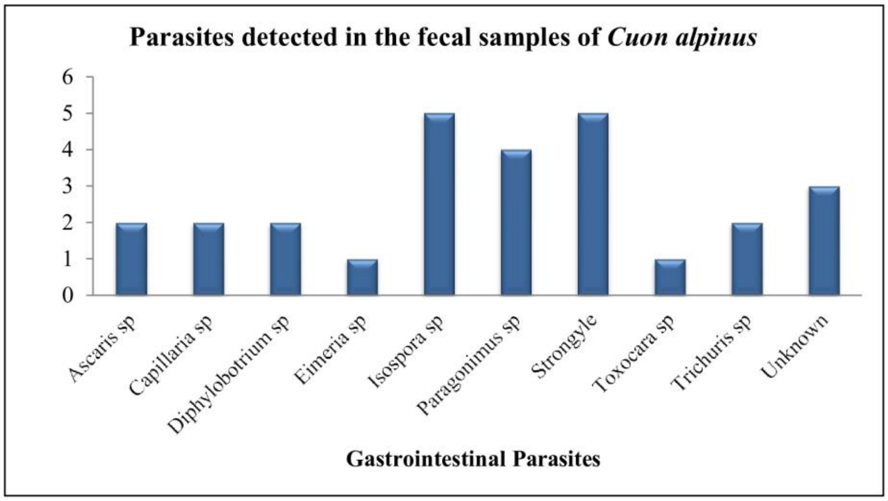

Fig. 6: Prevalence of Parasites in *Cuon alpinus*

Table 3: Number of parasites from sample collection

Smear Technique		No. of Parasites	Floatation Technique		No. of Parasites	Telemann's Method		No. of Parasites
Dry sample	S 1	1	**Dry sample**	S 1	0	**Dry sample**		None
	S 2	2		S 2	0			
	S 3	2		S 3	0			
	S 4	1		S 4	2			
	S 5	1		S 5	0			
	S 6	0		S 6	0			
	S 7	0		S 7	0			
	S 8	1		S 8	0			
	S 9	0		S 9	0			
	S 10	0		S 10	0			
Fresh Sample	S 1	1	**Fresh sample**	S 1	0	**Fresh Sample**	S 1	0
	S 2	0		S 2	1		S 2	1
	S 3	0					S 3	1
	S 4	0		S 3	2		S 4	0
	S 5	3		S 4	2		S 5	0
	S 6	0		S 5	4		S 6	0
	S 7	0		S 7	**2**		S 7	0
	S 8	3		S 8	**1**		S 8	4
	S 9	0		S 9	**1**		S 9	2
	Total	**15**			**15**			**8**

number of parasites than the dried once. However, dried or old samples were found to have more numbers of free-living nematodes, mites, first stage larva of hookworms and common tapeworms.

Discussion

There has been few research on the diversity of parasites among carnivores in the wild, whereas zoonotic investigations have primarily focused on zoos due to the risk of exposure to the general public (Figueiredo *et al*., 2021). While earlier studies centred on comprehending the relationship between the host and the microorganism and its implications (Meyer *et al*., 2022); recent research has switched the emphasis to zoonotic illnesses and the creation of evidence-based risk assessments for both people and animals as a whole (Keatts *et al*., 2021; Nath *et al*., 2021).

The present study provides the evidence of gastrointestinal parasites in animal species of Murlen National Park, located in Champai district of Mizoram, India. From the scat sample of *Cuon alpinus*, it was found that total 15 scat samples were tested positive for parasites, after their faeces have been examined by direct smear, flotation method and Telemann's method. The overall infection rate with gastrointestinal parasites from the sample collected is 70.98 % which is exceptionally high. Parasite species found in this study is known to be harmful to both humans and animals (including visitors and animal handlers).

Nearly all (99%) dogs (wild and domestics) harbour one or more zoonotic species of gastrointestinal parasites (Traub *et al*., 2002). With such rapidly changing landscape and human encroachment into wildlife areas, spillovers of diseases from wild animals to humans are inevitable. In the rural areas like MNP conditions are more detoured with poor water and sanitization facilities thus increasing the changes of zoonotic infections. The present study reports the presence of parasites such as *Ascaris* sp., *Paragonimus* sp., *Trichuris* sp., and other free-living nematodes that have the potential to infect humans and their domestic animals when come into contact. Grazing of domesticated mithun/gaur (*Bos gaurus*) and other cattles into MNP also can act as carriers of zoonotic parasites that can ultimately infects swine, poultry etc. causing an economic burden for the locals.

Since more than 75% of human diseases are of zoonotic origin, it is important to understand the dynamics between wildlife, domestic animals and humans inhabiting the vicinity of protected forest, and to conduct more such research on zoonotic parasites for timely prevention of diseases and save lives.

Furthermore, despite receiving appropriate care for feeding, watering and maintaining hygiene in captivity, it has been found that keeping wild animals in zoos increases their susceptibility to many parasites illness (Kashid *et al*., 2002). The nematodes and some coccidian parasites are transmitted by faecal-oral route through contaminated feed, water and soil and have the ability to accumulate in captivity environments since they do not require an intermediary host (Thawait *et al*., 2014). According to the study, gastrointestinal helminth parasites like trematodes, cestodes, and nematodes are more common in the MNP carnivores than protozoa. Water features and agricultural fields close to MNP are also attractive to a variety of animals, including civets, leopard cats, and crab-eating mongooses (*Herpestes urva*), which may act as possible sources of contaminants (faeces matters) (Gouda *et al*., 2020). For drinking and other purposes, the local people rely directly on the natural water streams that flow through the reserve, which may be another key role in the spread of zoonoses in humans and livestock. The likelihood of cross-transmission is additionally enhanced by the local population's (21%) reliance on wild animals as bushmeat and for ethno-medical purposes (Solanki *et al*., 2016). A number of species of small carnivores had used agricultural fields in the vicinity of protected areas as a peri-habitat, and there is a significant likelihood that they are susceptible to zoonotic illnesses. According to Gibb *et al*. (2020); Plowright *et al*. (2020) and Keatts *et al*. (2021), it is only a matter of time before zoonotic parasites break the nexus, find a new host in the form of livestock, and ultimately become dominant among humans due to an increase in landscape alteration, migration of people into wilderness areas, shrinking of wildlife habitats, free-ranging animals, and seasonal trends.

Conclusion

Mammal parasite diversity has long been a valuable source of knowledge regarding the relationship between diet and the micro biome, anthropogenic food, the effects of changing land use patterns, *etc*. However, because of the emergence of zoonoses in wildlife, the emphasis has switched to a meta-organism conservation strategy. According to this study, a wild canine sample taken from MNP in the Champai area had an unusually high total infection rate with gastrointestinal parasites of 70.98%. From the perspective of public health, this is also crucial. Due to the intimate contact that animal carers have with wild mammals, there is a potential of infection transfer. These animals may also serve as reservoir hosts for a number of these zoonotic parasites, which could eventually cause active transmission to the human population. The study identified a number of infectious diseases, including *Paragonimus* spp., *Capillaria* spp., *Toxocara* spp. And *Strongyle* spp., which can cause major health issues or disorders if left untreated. Additionally, these parasites pose a risk to domestic animals, which are crucial resources for the inhabitants living close to MNP. The risk of parasite spill over is at an all-time high due to the rising interaction of human wildlife with wild bushmeat, untreated water, and frequent excursions to protected areas. Due to the fact that our study only relied on morphological identification, a long-term epidemiological study of parasites is also required in order to fully understand the parasitism, prevent potential recurrence of the infection in wild animals, and stop cross-transmission to humans.

Ethical Statement

This study was conducted after obtaining the permission from Murlen National Park authorities, and none of the animal was harmed.

Author Contributions

Boro Hathorkhi Hala: Conceptualization; *Boro Hathorkhi Hala* and *Sarmah Manash Pratim*: Data curation and formal analysis; *Sarmah Manash Pratim*, *Kalita Dikshita* and *Nath Ankur*: Resources; *Sarmah Manash Pratim* and *Boro Hathorkhi Hala*: Visualization; *Sarmah Manash Pratim*, *Kalita Dikshita* and *Nath Ankur*: Original draft writing, review and editing. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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