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Occurrence of lung flukes in wild felids across different forest ranges in The Nilgiris, Tamil Nadu, India

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Abstract

The paper presents the findings of a systematic study on occurrence of lung flukes in wild felids observed over a period of one year during postmortem examination across different forest ranges in the Nilgiris district of Tamil Nadu, India. A total of 22 wild felids comprising of 7 tigers and 15 leopards were screened during the Fourteen months period from December 2023 to January 2025. Detailed postmortem examination was conducted by the forest veterinary officer following standard procedures. The flukes were collected, labelled, processed as per standard procedures, identified and photographed. Out of these, 3 tigers (42.85%) and 3 leopards (20%) were found infected with lung flukes with an overall occurrence of 27.27%. Among the three infected tigers, two were males aged 3 and 1.5 years and other was a female aged 10 years. Among the three infected leopards, two were males aged 6 and 7 years and other was a female aged 12 years. Infected tigers were from Gudalur and Bitherkadu forest ranges whereas infected leopards were from Gudalur and Pandalur forest ranges. All lung flukes were found in lung tissue inside lung cysts. It was observed that 50% of cysts had single fluke and 50% of cysts had a pair of flukes. A total of 84 flukes were recovered from 56 cysts from the three infected tigers and a total of 33 flukes from 22 cysts were recovered from the three infected leopards. Overall total of lung flukes collected from all the six wild felines was 117 from 78 lung cysts. The findings revealed that lung fluke infection by Paragonimus sp. is common in wild felines in different forest ranges of The Nilgiris, Tamil Nadu, India in both male and female animals of all age groups. Pathological findings in the infected animals revealed several cysts or nodules in the lung tissue and each cyst containing the flukes surrounded by purulent fluid. The present observations on the occurrence of lung fluke infection with Paragonimus sp. and recovery of these flukes in large numbers will be important to map the status of fluke infection in felines in the wild and to frame strategies for control of lung flukes in these animals.

Keywords: *Occurrence, lung flukes, Paragonimus sp., wild felines, The Nilgiris*

Wild felines are among the important fauna in the forests of India. They play a vital role controlling the population of wild herbivores and ungulates, preventing overgrazing and protecting plant life in their habitats (Shrivatsav and Singh 2012). As apex predators, they indicate a healthy ecosystem with a diverse range of species and play a vital part in food chain. Several factors such as diseases, habitat fragmentation, poaching, territorial fights etc. threaten these animals and therefore it is crucial to preserve the population of these big cats (Varun, 2005). Parasitic helminths play a major role in

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affecting the health status of wild felines. It is important to study the parasitic dynamics in wild populations, especially predatory animals, as parasites can transfer to domestic animals and even humans (Marathe *et al*., 2002; Shirbhate, 2008). Several endoparasites (flukes, tapeworms, nematodes), ectoparasites (arthropods and acarines) and protozoa affect wild felines. Among helminths, one of the most significant diseases is caused by lung flukes (*Paragonimus* sp.), leading to high morbidity and mortality. Lung fluke infections have been observed based on faecal examinations from tigers (Arjun *et al*., 2017; Milind and Amrita 2019), leopards (Kinge *et al*., 2010), wolves (Shrikhande *et al*., 2008), wild cats (Chinchilla-Carmona *et al*., 2020). Based on necropsy findings, lung fluke infections have been reported in tigers (Rao and Acharjyo 1984; Arora and Das 1988; Rao and Acharjyo, 1991; Latha *et al*., 2000; Dharanesha *et al*., 2017, 2019), leopards (Pythal *et al*., 1993; Varadharajan and Pythal 1999; Dhoot *et al*., 2002), the golden cat (Rao and Acharjyo 1984, 1991), the striped hyena (Rao and Acharjyo 1995) and mongoose (Rao and Acharjyo 1984, 1991, 1995). Singh *et al*. (2016) studied the occurrence of *Paragonimus* sp. in free ranging tigers and leopards by examination of faecal samples and carcasses and recorded 39.5% prevalence. Paragonimiosis is considered one of the most pathogenic food borne parasitic diseases reported in wild carnivores (Parihar and Shrivastava 1988; Sano *et al*., 1994). Apart from India, lung flukes are geographically distributed across the globe (Siberia, Japan, Korea, China, Taiwan, Phillipines, Indonesia, Malaysia, Thailand, Sri Lanka, USA, Cost Rica etc.)

The Nilgiris Biosphere Reserve is situated in the Nilgiris Mountains of the Western Ghats in the state of Tamil Nadu, South India and is home to several mammals including a very good population of wild felines such as tigers, leopards and panthers. Though there are several reports on lung fluke infection in wild felines from different parts of the country and abroad, a detailed systematic study on this infection in wild felines is very limited. Therefore, the present systematic study was undertaken to investigate the occurrence of lung fluke infection in wild felines over a one-year period through postmortem examination in different forest ranges of the Nilgiris district, Tamil Nadu, India.

Materials and methods

Study Area

The study was conducted in The Nilgiris which lies at the juncture of the Western Ghats and the Eastern Ghats. Its latitudinal and longitudinal coordinates are 11°12 N to 11°37 N and 76°30 E to 76°55 E, respectively (Fig. 1). Due to its high altitude, the Nilgiris had a significantly cooler climate than the surrounding plains. The region received substantial rainfall throughout the year, with monsoon seasons impacting the weather. It experiences summer, monsoon and winter seasons. The summer starts from March and extends up to June, followed by the monsoon season from July to September and the winter season is from October to February months. During summer the temperature reaches a maximum of 25 °C (77 °F) and a minimum of 10 °C (50 °F). During winter the temperature maximum is 20 °C (68 °F) and the minimum 0 °C (32 °F). The average annual rainfall of The Nilgiris is 1,920.80 mm. Thirteen different forest ranges were included in the study. The ecosystem of the study areas included tropical evergreen forests, Montane sholas and grasslands, semi-evergreen forests, moist deciduous forests, dry deciduous forests and thorn forests. Though it was a forest area, recreational activities, trekking activities and tourism had increased in the recent years.

Study animals

A total of 22 wild felines comprised of 7 tigers and 15 leopards were screened during the study period from December 2023 to January 2025 during post mortem examination. The animals screened were from different forest ranges of the Nilgiris, viz., Singara, Ovalley, Cherambadi, Kundha, Udagai North, Segur, Masinagudi,

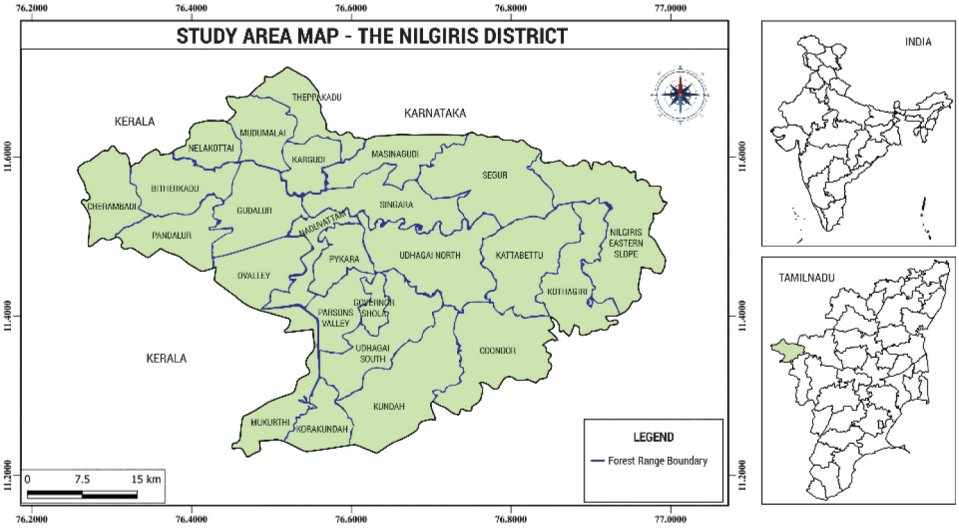

Fig. 1 Location map of the study area

Gudalur, Bitherkadu, Pandalur, Kothagiri and Theppakadu. Detailed postmortem examination was conducted by the forest veterinary officer following standard procedures.

Occurrence study across different forest ranges, age and sex of wild animals

The details of forest ranges, as well as age and sex of the infected wild felines were recorded to determine the distribution of parasites based on forest range, season, age and sex.

Collection of flukes

During post mortem examination, the entire lung tissue was carefully screened for pathological lesions and presence of flukes by the Forest Veterinary Officer. The pulmonary parenchyma surrounding the cystic lesions was incised to recover lung flukes. All recovered flukes were stored in separate sterile plastic containers with 10% formalin, labelled and despatched to the Department of Veterinary Parasitology, Veterinary College and Research Institute, Theni for processing and species identification. Some flukes were flattened and stored in 10% formalin and despatched. The label included details such as the date of post mortem and sample collection, forest range where the post mortem was conducted, information about the animal (species, age, and sex), details of flukes collected, location of flukes and the sample identification number.

Once the samples were received, the details were recorded. The flukes from different wild felids were counted separately and documented. The flukes were processed, stained with borax carmine and mounted in DPX as per Tongbram *et al.* (2020) and identified based on the descriptions of Soulsby 1982 and Tongbram *et al*. (2020). Photographs of gross flukes were taken using NIKON Digital camera-mirrorless-Z5 model with NIKKOR Z-24-70 MM F/4S lens. Photographs of mounted flukes on microscopic slides were taken using Trinocular microscope (Lawrence and Mayo-Lynx, CAT. No: LM-52-3000, Sl. No: 500700) under 4x magnification with a photomicrograph and computer attachment.

Results and discussion

Morphological features of lung flukes and ova

The lung flukes were identified as *Paragonimus* sp. Fresh worms were coffee-bean like in appearance and had a reddish-brown hue. They were ovoid, plump, abruptly rounded anteriorly and somewhat tapering posteriorly, measured 7.5-12 mm in length and 4-6 mm in breadth (Fig. 2).

Microscopically, the integument had scale-like spines, either entire or toothed covering all surfaces of the fluke. The oral sucker was ventro-terminal in position. The ventral sucker was located in the middle of the body. Genital pore opened immediately behind the ventral sucker. Irregularly, deeply lobed testes were placed side by side midway between ventral sucker and posterior end. Ovary was large, lobed and pre-testicular. Cirrus sac was absent. A highly dendritic vitellarial network was observed (Fig. 3). Eggs were yellowish brown, oval-shaped, and had a thick shell with an operculum at one end (Fig. 4).

Morphological features of the adult lung flukes including shape and size, spiny cuticle, suckers, ovary and testes, intestinal caeca, vitellaria, as well as the shape, size, and shell characteristics of the ova matched with the observations of Singh *et al*. (2012). All the morphological features of adult lung flukes and ova in the present study corroborated with the findings of Tongbram *et al*. (2020) and Soulsby (1982).

Recovery of lung flukes from lung tissue cysts

All lung flukes were found in lung tissue inside lung cysts. It was observed that 50% of cysts had single fluke and 50% of cysts had a pair of flukes. A total of

Fig. 2. *Paragonimus* sp. flukes - Gross appearance without magnification (Left) and magnified view under stereoscope (Right)

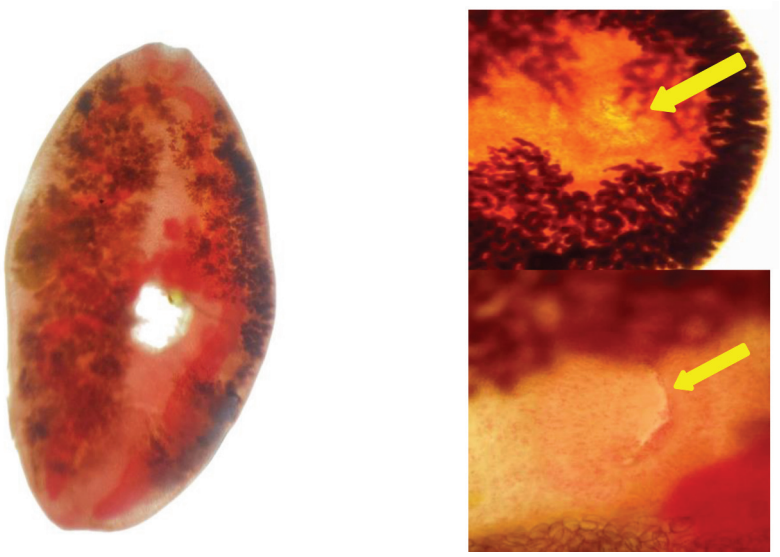

Fig. 3. *Paragonimus* sp. fluke stained with Borax Carmine and mounted in DPX, oral sucker (Top-Right) and ventral sucker (Bottom-Right)

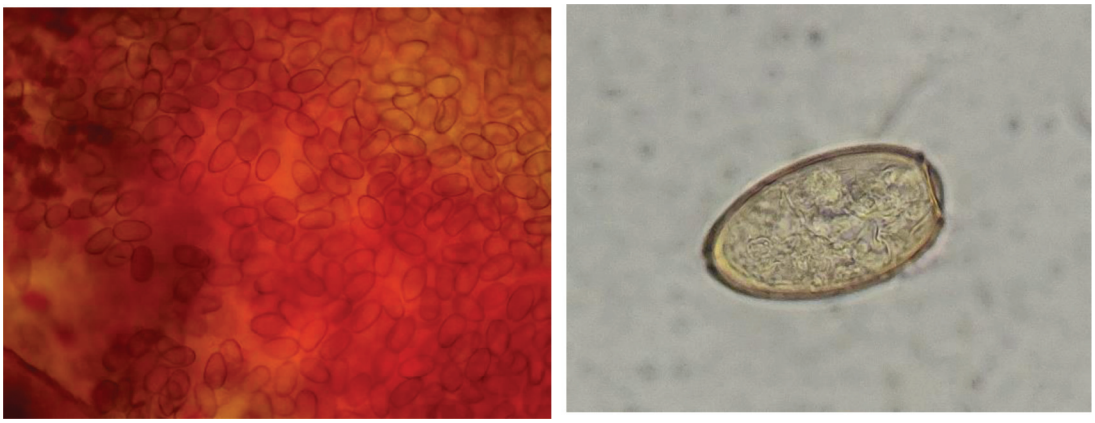

Fig. 4. Eggs of *Paragonimus* sp. fluke (Left-10X and Right-40X)

Fig. 5. Lung cysts harbouring lung flukes in an infected Tiger

84 flukes were recovered from 56 cysts from the three infected tigers and a total of 33 flukes from 22 cysts were recovered from the three infected leopards. A total of 117 lung flukes were collected from 78 lung cysts across all six wild felines examined. Among tigers, the highest infection was observed in a 10-year-old female harbouring 54 flukes from 36 cysts and the lowest infection was observed in a 3-year-old male tiger harbouring 12 flukes from 8 cysts (Fig. 5, 6). Among leopards, the highest infection was observed in a male animal aged 6 years harbouring 15

Fig. 6. Lung flukes embedded in lung tissue of tiger (Left) and flukes removed from lungs (Right)

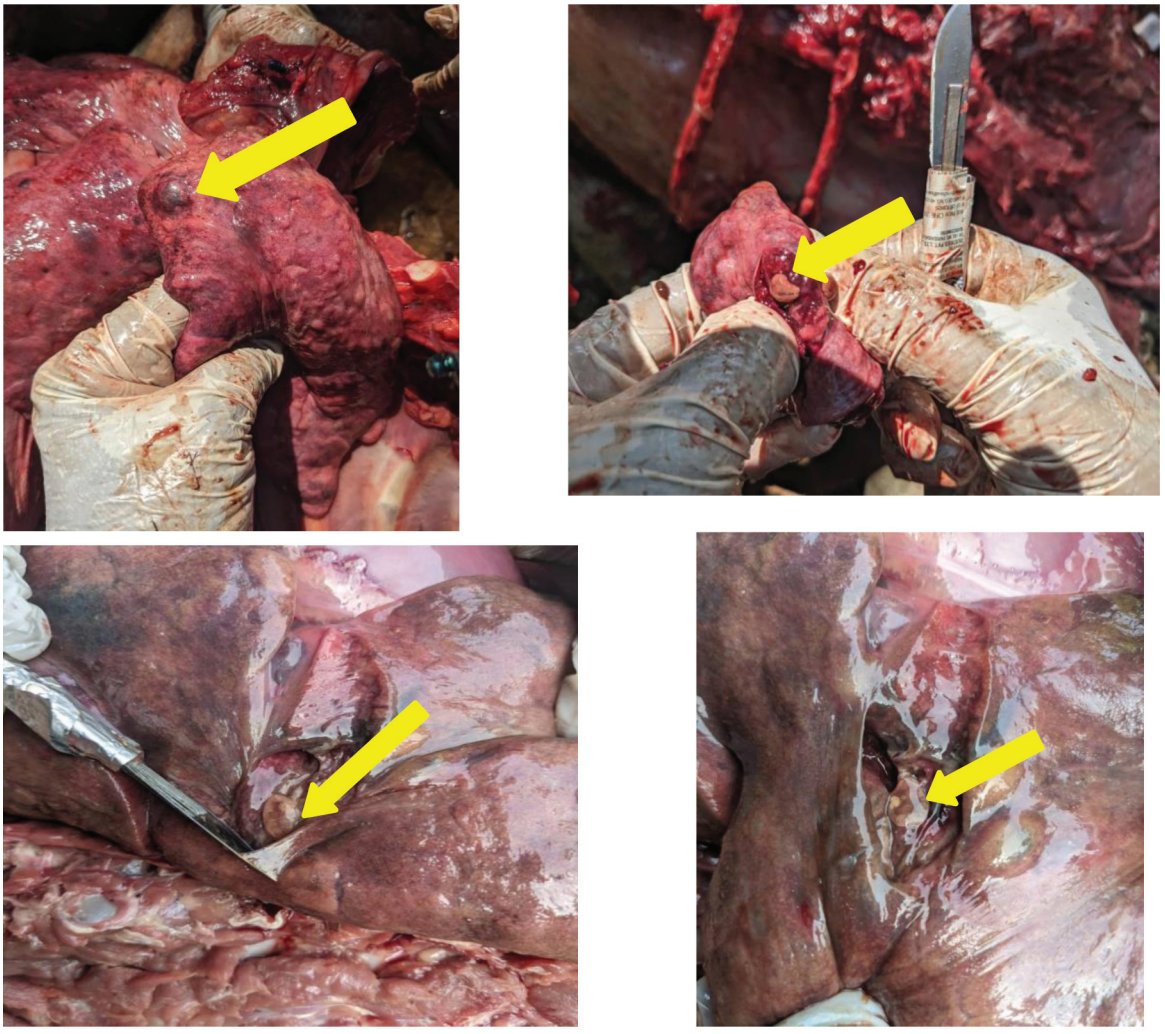

Fig. 7. Lung cysts caused by lung flukes (Top) and flukes embedded in lung tissue of leopard *in situ* (Bottom)

flukes from 10 cysts and the lowest infection was observed in a 12-year-old female leopard harbouring 6 flukes from 4 cysts (Fig. 7). Rao and Acharjyo (1991) observed cysts containing one or more lung flukes from a Golden cat and Royal Bengal tiger during necropsy at Nandakanan Zoo and cysts with flukes were observed in one or both lungs.

Dharnesha *et al*. (2019) observed lung fluke cysts in both lobes of lungs in a tiger from Mysore and observed 2-3 number of lung flukes in each cyst. Dharnesha *et al*. (2017) also observed paired lung flukes in cysts from both lungs of a Royal Bengal tiger during post mortem at Rajiv Gandhi National Park, Nagarahole, Mysore, Karnataka. The present observations were in line with the above findings wherein cysts were found in both the lobes of the lungs and lung flukes were found inside the cysts. However, the present observation revealed that 50% of the lung cysts contained a single lung fluke, while the remaining 50% harboured paired flukes, with infection in both the lung lobes.

Occurrence of lung fluke infection

Out of 22 wild felids comprising 7 tigers and 15 leopards, 6 (27.27%) were found to be infected with lung flukes. Out of 7 tigers, 3 (42.85%) and out of 15 leopards, 3 (20%) were found infected with lung flukes. Lung flukes affect a wide range of feline wild animals such as lions, tigers, leopards etc. and has been reported worldwide. Arjun *et al*. (2017) reported a prevalence of 41.6% (25 out of 60 animals) of paragonimosis in tigers from Wayanad wild life sanctuary based on analysis of scat samples which is almost similar to the present study where the occurrence in tigers was 42.85%. Observations on lung fluke infection during necropsy of tiger have been recorded at Nandakanan zoo (Rao and Acharjyo 1984, 1991), Corbett National Park in Uttar Pradesh (Arora and Das 1988), Kanha National Park in Madhya Pradesh (Parihar and Shrivatsava 1988), Vandalur Zoological Park in Tamil Nadu (Latha *et al*., 2000) and Assam State Zoo in Guwahati (Nashiruddullah and Chakraborty 2001) indicating a wide prevalence of this parasite across different Indian states. Additionally, Sano *et al*. (1994) recovered eggs of *Paragonimus* sp. from four tigers at Kanha National Park. Dharnesha *et al*. (2019) reported *Paragonimus* sp. in an 8-year-old female Royal Bengal tiger from Rajiv Gandhi National Park, Nagarahole, Mysore district, Karnataka. Dharnesha *et al*. (2017) also reported paragonimosis in an adult male tiger.

Milind and Amrita (2019) reported 80.7% prevalence of *Paragonimus* sp. infection in leopards at Katepurna Wild life sanctuary, Akola, India which was significantly higher compared to the present observation of only 20% in leopards. Kinge *et al*. (2010) observed eggs of *Paragonimus* sp. in a trapped leopard at Maharajbagh zoo, Nagpur. Lung fluke infection was reported from a clouded leopard by Hiregowdar and Pethkar (1970) and was also found in a leopard during post mortem and coprological examination in Thiruvananthapuram Zoo (Pythal *et al*., 1993; Varadharajan and Pythal 1999). Dhoot *et al*. (2002) observed lung fluke infection in a leopard at Maharajabagh zoo, Nagpur coprologically.

Singh *et al*. (2016) observed 39.5% prevalence of paragonimosis in free ranging tigers and leopords from different tiger reserves of Central India. Out of 16 carcasses necropsied, 6 (37.5%) harboured lung flukes and out of 27 faecal samples examined, 11 (40.7%) was positive for eggs of *Paragonimus* sp. These observations indicated a higher prevalence when compared to the present study where the occurrence in tigers and leopards combined was only 27.27%.

Chinchilla-Carmona *et al*. (2020) conducted a parasitological study of 623 faecal samples of 616 wild felines (Jaguar, Ocelot and Cougar) collected from five different regions in Costa Rica and observed 41.9% prevalence of paragonimosis in these wild felines which was higher than the present observation in tigers and leopards put together. Patton and Rabinowitz (1994) reported *Paragonimus* sp. in leopard and tiger from Thailand. Dubey (2023) did a detailed review on endemic *P. kellicotti* infections in animals and humans in USA and Canada with emphasis on life cycle stages and pathogenesis. Doanh *et al*. (2015) detected *Paragonimus* sp. ova in 7 out of 120 faecal samples collected from wild animals in Da Krong Nature Reserve, Da Krong district, Quang Tri Province, Vietnam which was comparatively a low level of infection.

Although several reports exist on prevalence of lung flukes in tigers, leopards and other wild felines, the present study reports the occurrence of lung flukes in tigers and leopards systematically observed over a period of one year across different forest ranges of the Nilgiris. The findings indicate that the infection is substantially present among wild felines in this region. Lung fluke infections in wild felines is acquired by ingestion of second intermediate hosts such as crabs and cray fishes which contain the metacercaria stages. The variations in the prevalence of lung flukes in tigers and leopards could be attributed to the availability of appropriate intermediate hosts in the forest area. Predation by wild felines on other animals like jungle cat, mongooses, leopard cats etc. can result in lung fluke infections as the lower carnivore diet includes fishes and crabs (Arjun *et al*., 2017) and these animals are commonly found in the present study area. Several hosts such as wild boar, deer and rodents act as paratenic host for lung flukes where the flukes fail to develop to adulthood after ingestion of metacercaria infected crustaceans, but remains viable as juveniles and wild carnivores can become infected by ingesting the meat of these paratenic hosts (Singh *et al*., 2012; Singh *et al*., 2016). Potential paratenic hosts such as wild boars, deer etc. are in plenty in the present study area and could have been additional sources of infection to tigers and leopards apart from crabs and crustaceans. Choubisa (2024) opined that fresh water sources in India may serve as sources for dangerous trematode infections such as lung flukes in wild animals. This is because the faeces contaminated with trematode eggs is carried by rain water to available fresh water sources where snail intermediate hosts are available in plenty. In the present study area as well, numerous such water bodies are present, containing snails, crabs, cray fishes etc. that could serve as potential sources of lung fluke infection to these wild felines.

Table 1. Details of lung fluke infection in tigers and leopards from different forest ranges of the Nilgiris

Sl. N.	Wild animal species	Forest Range	Details of animal (Age and Sex)	Total number of lung cysts (from both lungs together)	Total number of lung flukes recovered
1.	Tiger	Gudalur	3 years old, male	8	12
2.	Tiger	Bitherkadu	10 years old, female	36	54
3.	Tiger	Bitherkadu	1.5 years old, male	12	18
Total (Tiger)				**56**	**84**
4.	Leopard	Gudalur	7 years old, male	8	12
5.	Leopard	Gudalur	12 years old, female	4	6
6.	Leopard	Pandalur	6 years old, male	10	15
Total (Leopard)				**22**	**33**
Grand Total				**78**	**117**

Occurrence of lung fluke infection with respect to age, sex of animals and forest ranges

Out of the three infected tigers, two were males and one was female and among the three infected leopards, two were males and one was female. The youngest animal with lung fluke infection was a 1.5-year-old male tiger while the oldest was a 12-year-old female leopard. The age range of infected animals was from 1.5 years to 10 years (Tiger) and 6 years to 12 years (Leopard). It has been reported that lung flukes can survive in the final host for 1-20 years even in the absence of specific therapy (Singh *et al*., 2012) and in the present study the heavily infected tiger with 36 cysts and 54 lung flukes was a 10-year-old female. The lung fluke infection was also observed in two younger male tigers aged 1.5 years and 3 years indicating the occurrence of infection in all age group of tigers. On the contrary, the leopard which harboured 10 cysts with 15 lung flukes was only a six-year-old male. Another 7-year-old male leopard had 8 cysts and 12 flukes, but a 12-year-old female leopard harboured 4 cysts and 6 flukes. Hence lung flukes' infection in tigers and leopards occur irrespective of age and sex of the animal. The variations in the prevalence of lung flukes in animals could be attributed to their immune status, feeding behaviours, accessibility to potential intermediate hosts, territorial ranges and other physiological factors. Infection by lung flukes in carnivores could occur from young age when the animals initially feed upon aquatic fauna, particularly infected crabs and cray fishes which act as second intermediate hosts (Soulsby 1982). The forest ranges in the study area, the Nilgiris receive rainfall during the monsoon season from June to September months every year and several lentic and lotic water bodies are available which are conducive for the growth, survival and multiplication of the intermediate hosts for lung flukes.

Distribution of lung fluke infected animals among different forest ranges revealed that two out of three infected tigers were from Bitherkadu forest range and the third tiger was from Gudalur forest range. With respect to leopards, two infected leopards were from Gudalur forest range and one was from Pandalur forest range. Overall, the highest lung fluke infection was observed in wild felines from Gudalur forest range (50%), followed by Bitherkadu forest range (33.33%) and Pandalur forest range (16.66%) (Table 1). These variations could be attributed to the availability of water bodies in the forest range areas containing potential first intermediate hosts which are *Semisulcospira* and *Melania* sp. snails, second intermediate hosts such as crabs (*Maydelliathelphusa lugubris*, *Potamiscus* sp.), cray fish (*Camberoides* sp.) and shrimps such as *Acrohrachium* and *Caridina* (Tongbram *et al*., 2020).

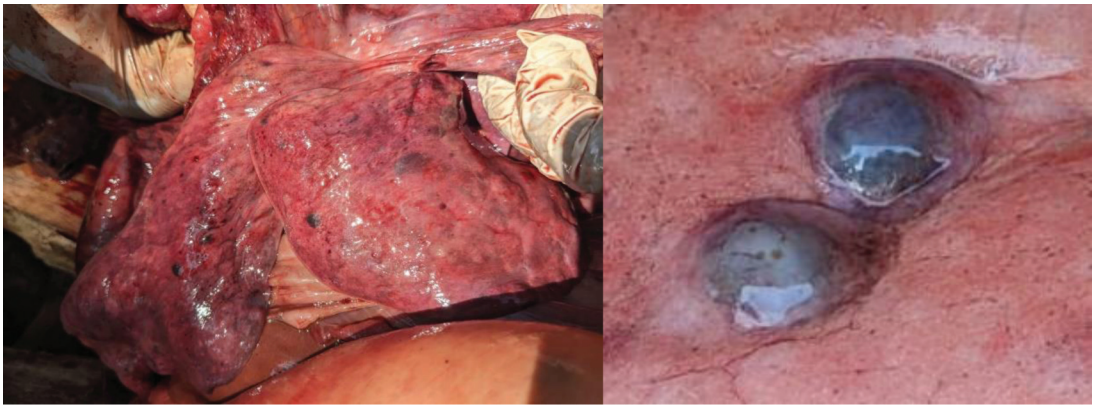

Fig. 8. Pseudo tubercle formation in the lungs of lung fluke infected leopard and lung cysts with flukes surrounded by purulent fluid

Pathological observations in lung fluke infected wild felines

Pseudotubercle formation, atelectasis and emphysema were observed in the lungs of affected animals. Each cyst contained a single fluke or pair of flukes surrounded by purulent fluid mixed with blood and eggs (Fig. 8).

Although lung flukes invade lung parenchyma and reside within cysts, they typically do not cause severe damage and mortality due to this parasite is rare (Kinge *et al*., 2010). Rao and Acharjyo (1991) and Parihar and Shrivastava (1988) observed lung cysts containing one or more reddish oval shaped lung flukes along with patches of atelectasis and emphysema as observed in the present study. Lung damage caused by lung flukes results from migration of fluke, toxic metabolites released by flukes, presence of fluke eggs and the host's immune response which induces severe inflammation (Blair *et al*., 1999). The excretory and secretory products of adult *P. westermani* contain cystein protease enzymes responsible for invoking immunological process during infection (Lee *et al*., 2006; Na *et al*., 2006). In the present study, the gross pathological findings in lungs like numerous cystic lesions with congestion, haemorrhage, oedema, atelectasis, emphysema in the lungs along with single and paired flukes inside the cysts, brownish, necrotic exudates within the cysts were similar to the findings of Singh *et al*. (2012).

The lungs are vital organs for respiration, and their impairment may reduce hunting ability of wild tigers and leopards. As a result, affected animals may move into buffer zones of forests to prey on weaker domestic animals and also avoid terrestrial fights. This behavior could lead to increased man-animal conflict. Pulmonary paragonimiasis may thus be an important contributing factor to mortality in these animals (Dharnesha *et al*., 2017).

High levels of lung fluke infection can lead to high mortality and morbidity across all age groups in wild felines, resulting in the loss of physical fitness and reproductive capacities. As apex predators with low predation risk, animals such as tigers tend to carry higher parasitic loads compared to other predators (Arjun *et al*., 2017). In the present study also occurrence of lung flukes was high in tigers (42.85%) compared to leopards (20%). This variation could be attributed to their territorial ranges, exposure to potential intermediate and paratenic hosts and feeding preferences. Lung fluke infection could be one of the attributing factors to the mortality of the wild felines. The forest ranges in The Nilgiris are home to several species of snails (Soundararajan *et al*., 2018) including those involved in the life cycle of lung flukes and chances of wild felines and other carnivores acquiring lung fluke infections is high. Since animals such as tigers and leopards are part of food chain in the forest ecosystem, their healthy survival is very vital (Milind and Amrita 2019). Routine monitoring of the presence of parasites in wild felids is imperative for the formulation and implementation of measures to prevent and control these important zoonotic parasitic infections. Though there are reports about lung flukes in different wild animal species the present study reports these infections in tigers and leopards from different forest ranges of the Nilgiris, Tamil Nadu observed over a period of Fourteen months. The present data will assist wildlife health officials in mapping the occurrence of lung fluke infections in wild felines and in formulating necessary control and preventive measures to protect these valuable animals from the potentially fatal effects of lung fluke infection.

Conclusion

The present data will help to map the occurrence of parasites affecting wild felines in the Nilgiris, Tamil Nadu. The influence of climatic factors on the abundance and diversity of the first and second intermediate hosts for lung flukes also needs to be explored. These data gathered in the present study will help zoologists, veterinarians, parasitologists and allied professionals for designing lung fluke control strategies and this will be highly essential for the development of future disease control campaigns in wild felines.

Conflict of interest

The authors declare that they have no conflict of interest.

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