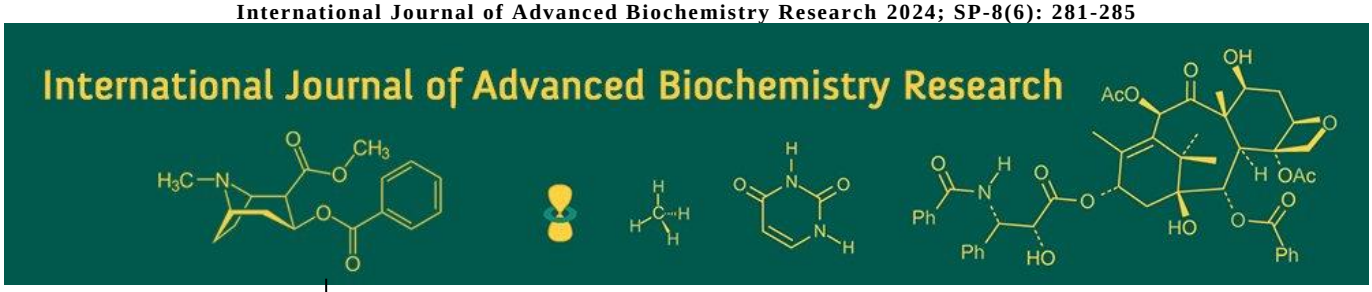

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First report of *Cysticercus tenuicollis* (*Taenia hydatigena cysticerci*) in an Indian Langur (*Semnopithecus entellus*)

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Abstract
Background: Taenia hydatigena is a widely reported tapeworm in domestic as well as wild animals. The larval form of the tapeworm causes *Cysticercus tenuicollis* in the intermediate hosts and attaches to the liver or peritoneum. The infection has been reported in non-human primates and its impact on the conservation of wild canids is unidentified.
Case Presentation: A five-year-old female Gray Langur was rehabilitated at Wildlife Research & Training Centre, Gorewada, Nagpur, India. The Langur was found dead and a post mortem examination was conducted. Two cysts were detected, one on the liver and the other on the large intestine. The cysts were dissected and processed for histopathology investigation. Molecular investigation confirmed *Cysticercus tenuicollis* through PCR and Sequencing. The sequence was submitted to public domain database. Phylogenetic analysis found similarity with previously reported sequences from Asia and Europe. This was the first evidence of Cysticercus tenuicollis from India in a non-human primate.
Conclusion: The case report provides evidence of *Cysticercus tenuicollis* in Gray Langur. There are definite research gaps in the knowledge about the spread and wild reservoirs of the infection. The need to access the impact of the disease in endangered canids is essential.

Keywords: *Cysticercus tenuicollis*, *Taenia hydatigena cysticerci*, Indian Langur

Introduction
Taenia hydatigena is a widely reported tapeworm in domestic as well as wild animals. The life cycle requires an intermediate host such as cattle, pig, sheep and horse, while, canids act as a definitive host [1]. The infection is non-zoonotic though isolated incidences of *Cysticercus tenuicollis* (*Taenia hydatigena cysticerci*) have been reported in humans. The source of infection in humans can be attributed to environmental contamination and the infection is transmitted by hand to mouth route. Water and vegetables contaminated with canid or carnivore faeces can act as a source of infection to humans and wild animals. There are a few reports of the infection in primates however the incidences are understudied in India and the subcontinent [2].
The pathogenesis involves intermediate host and definitive host without which the life cycle is incomplete. Herbivores and domestic animals pick up the eggs from contaminated feed, and harbour the worm for short period of time, the worm reaches maturity in its definitive host where the worm attaches to the intestine by means of the protoscolex and begins to form proglottids which mature to become gravid proglottids. The gravid proglottids are voided in the faeces by definitive host leading to release of eggs. The larval form (Cysticercus) persists in the liver for a brief period and settles down into the peritoneal cavity in the process it attaching to visceral organs like the intestine or liver and leads to cysticercosis in the intermediate host [3].
The infection in primates is majorly due to the contamination of the feed by carnivore faeces. An infective wild carnivore may continue to shed eggs in the faeces and be a source of infection to other intermediate hosts. The social organisation of Indian Langurs makes them more susceptible to the infection. The sylvatic cycle of transmission in wildlife is poorly understood. In the natural habitat of the langurs a range of carnivores can serve as definitive host including the canids (hyena, wild dogs, foxes, wolves etc.).

Case Presentation

A five-year-old female Indian langur (*Semnopithecus entellus*) was presented to Wildlife Rescue Centre, Gorewada, Nagpur for further care due to electrocution injuries. The langur was kept under quarantine for 30 days period after which it was shifted to the permanent enclosure for care and management. The animal received feed in the form of fresh fruits, pods and roasted gram. The animal was dewormed every quarterly and was vaccinated against rabies. The animal was found to be alert and active during the period of stay at Wildlife Rescue Centre. The animal was found dead after two years of captivity without any signs of illness or inappetence. The body weight and condition of the animal was found to be average. A necropsy was conducted at Wildlife Research & Training Centre, Gorewada, Nagpur to ascertain the cause of death. During the necropsy two pale yellow cysts were noticed in the peritoneal cavity (Fig. 1). One of the cysts was attached to the liver and contained 30 ml of yellowish fluid, while the other cyst was attached to the colon and contained 35 ml of fluid. On careful dissection two layers of the cysts were observed, the outer layer was transparent while the inner layer was white (Fig. 2). Histopathological examination was conducted on the liver to study the tissue changes, a cyst was found to compress the adjacent liver parenchyma (Fig. 3), the wall of the hydatid cyst was composed of acellular laminated membrane and inner nucleated germinal layer on H & E staining (Fig. 4). The other tissues were found to be normal.

To further investigate the infection 200 ul of the fluid from the cyst was utilised to isolate DNA using the DNeasy® Blood and Tissue Kit (Mfg. Qiagen Inc, MD, USA). Using the primers Thy-F (5'-TGAAGTTAGTAATTAAGTTTAA-3') and Thy-R (5'-AATCAAATGGAGTACGATTA-3') a Polymerase Chain Reaction targeting the NADH region was performed. The PCR conditions comprised of denaturation at 95°C followed by 35 cycles of denaturation at 94°C; annealing at 50°C and extension at 72°C, final extension at 72°C for 10 minutes. The PCR produced an amplicon of 450 bp which was purified using QIAquick PCR Purification Kit (Mfg. Qiagen Inc, MD, USA) and sequenced using forward and reverse primers (ABI 3130 automated DNA Sequencer, Mfg. Applied Biosystems, CA, USA) to ensure sequence consistency. The sequence so obtained was trimmed at ambiguous readings and consensus sequence was generated and submitted to nBLAST of NCBI. The sequence was found to be 99.74% identical to sequence reported in goats from China (Accession No. JN831270) and 99.47% identical to sequences reported from Nigeria in Goats (MN175586, MN175584) and Sheep (MN175580). Using the BankIT programme the sequence was submitted to public domain database NCBI and accession No. ON911502 was allotted to it. To understand the relatedness and evolution of the sequence similar sequences reported in wild and domesticated animals were retrieved from NCBI and utilised in the phylogenetic analysis using MEGA X software [4]. A neighbour joining tree was constructed using boot strap method with 1000 replications to maintain tree consistency. The sequences reported Indian subcontinents were given preference to understand the evolution (Table 1).

On phylogenetic analysis, the sequence was found to be 99.74% similar to sequence of *T. hydatigena* reported in a goat from China. The sequence also was similar to many reported sequences from the region MT776568 (99.21%), MW375360 and MT776619 (99.47%). The phylogenetic tree could be divided into three clades; clade I consisting of sequences Africa, Europe and Asia majorly reported in sheep and goats. Clade II can be divided into sub clade II A consisting of the query sequence (ON911502) along with sequences reported in dog, wild boar, sheep and goat from Asia and Europe. The clade III mainly consisted of Asian sequences reported in wild boar, goat and sheep (Fig. 5).

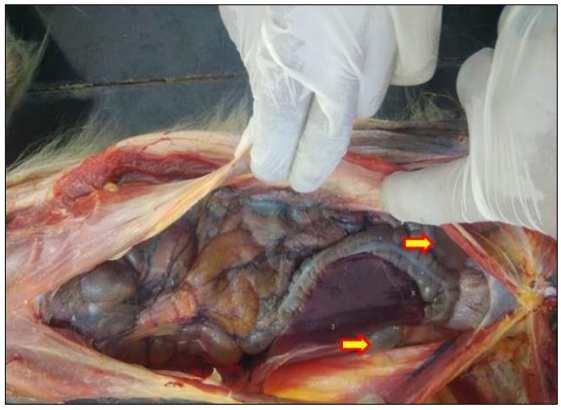

Fig 1: A location of cyst in the peritoneal cavity of the Indian langur (Arrows)

Fig 2: Dissected cyst recovered from the liver of the Indian langur

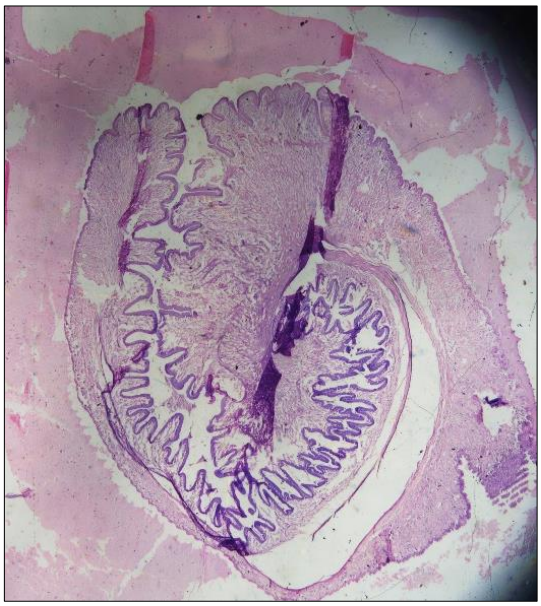

Fig 3: A hydatid cyst compressing adjacent liver parenchyma H&E stain 2x

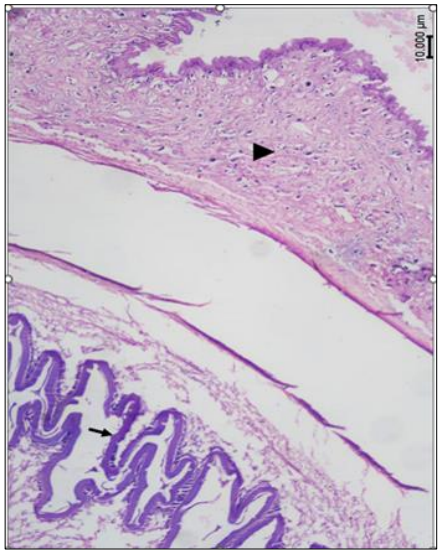

Fig 4: Higher magnification of Fig. 3 showing a hydatid cyst wall composed of acellular laminated membrane (arrow) and inner nucleated germinal layer (arrow head) H&E stain 20×

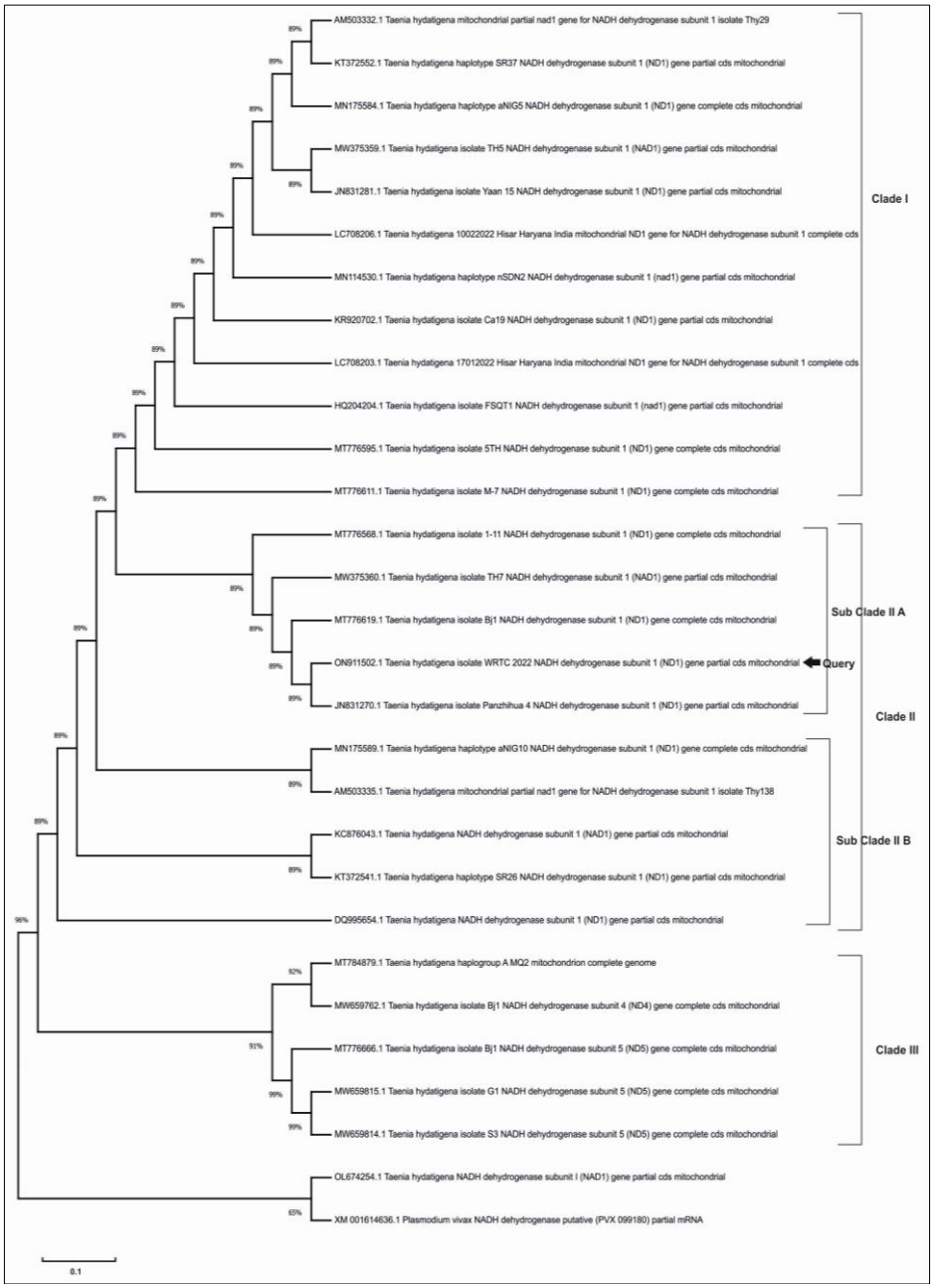

Fig 5: Phylogenetic Analysis of Sequence by Neighbour Joining Phylogenetic Tree using Bootstrap Method (1000 replications)

Table 1: List of sequences of *Taenia hydatigena* along with their attributes utilized for the neighbour-joining phylogenetic studies

Sr. No.	Accession No.	Species	Country	Reference
1.	MT776568	Dog	China	[18]
2.	JN831270	Goat	China	Direct Submission
3.	MN175584	Goat	Nigeria	Direct Submission
4.	KR920702	NA	Iran	Direct Submission
5.	LC708206	Goat	India	Direct Submission
6.	MW375360	Sheep	Slovakia	Direct Submission
7.	KC876043	Dog	Turkey	Direct Submission
8.	MT784879	Sheep	China	Direct Submission
9.	MT776611	Sheep	China	Direct Submission
10.	MT776595	Dog	China	Direct Submission
11.	HQ204204	Dog	China	Direct Submission
12.	AM503332	Dog	Kenya	Direct Submission
13.	MN175589	Goat	Nigeria	Direct Submission
14.	MN114530	Sheep	Sudan	Direct Submission
15.	KT372552	Sheep	Italy	Direct Submission
16.	LC708203	Sheep	India	Direct Submission
17.	MW375359	Sheep	Slovakia	Direct Submission
18.	OL674254	Chamois	Slovakia	Direct Submission
19.	ON911502	Indian Langur	India	Direct Submission
20.	JN831281	Goat	China	Direct Submission
21.	AM503335	Dog	Kenya	Direct Submission
22.	DQ995654	Sheep	India	Direct Submission
23.	MW659762	Wild Boar	China	Direct Submission
24.	MT776666	Wild Boar	China	Direct Submission
25.	MT776619	Wild Boar	China	[19]
26.	KT372541	Wild Boar	Italy	Direct Submission
27.	MW659815	Goat	Pakistan	Direct Submission
28.	MW659814	Sheep	Pakistan	Direct Submission

Discussion and Conclusion

The case report is the first evidence of *Cysticercus tenuicollis* from India in non-human primates. India is home to many species of wildlife including the primates. Due to loss of habitat, lack of feed, water scarcity and competition for resources in the natural habitat, the primates are distributed uniformly in the sylvatic, urban and rural areas of the country. In recent years monkey menace has been recorded in urban and rural areas of the country [5, 6]. The availability of food and water sources near human settlements has attracted monkey troops to human habitations. Some of the troops have permanently settled near human occupancies and interact with humans on a regular basis. In the past *Taenia hydatigena cysticerci* has been documented in a wide variety of species including the wolves, red foxes, corsac foxes, and snow leopards from Mangolia [7]; European wolves from Poland [8]. Wild boar has been found to be an important species in dissemination of the infection to carnivores in sylvatic life cycle in Italy [9]. India has also reported the infection in species like sheep [10], [11], pig [12] and goat [11], [13]; however, no evidence of the infection of primates has been reported from the country. Feral dogs are distributed throughout the country and considered a major threat to human communities due to the potential to contaminate water and food sources [14]. Majority of the cases of *Cysticercus tenuicollis* have been reported in captive wildlife [15], very few reports have emerged from wild or wild captured individuals. Human infection have been reported both from rural and urban India [16]. The finding marks the lack of investigation in wildlife especially canids and felids of the Indian subcontinent. Considering the conservation value of wild canids, a systematic surveillance system needs to be developed to access the impact of the infection on domestic, human and wild population at risk [17]. Though this is an isolated incidence of *Cysticercus tenuicollis*, an effort to probe the incidence at molecular level has been made. Extensive efforts to understand the prevalence in the wild-human-domestic interface is essential. Under Indian conditions, special emphasis must be given to screen wild animals that are present at the human-wild interface to understand the spread of infection and to safeguard public health.

Abbreviations

PCR: Polymerase Chain Reaction
NCBI: National Center for Biotechnology Information
DNA: Deoxyribonucleic Acid

Declarations

Ethics approval and consent to participate: The study is a post-mortem examination, and the sample was drawn for diagnosis of the animal presented to Wildlife Research & Training Centre, Gorewada, Nagpur; hence does not draw the ethical committee approval. However, as per the existing Wildlife Protection Act, 1972 permission from Principal Chief Conservator of Forest (Wildlife), Maharashtra State was sought to vide No. Desk-22(8)/Res/CR-59(19-20)/2370/20-21, Nagpur, date 7 January 2021 for the study and publication of scientific findings. All the sample collection during the study has been executed as per ARRIVE guidelines.

Consent for publication: Not Applicable.

Availability of Data & Materials: The sequence identified in the study is available in the public domain database of NCBI under Accession No. ON911502.
https://www.ncbi.nlm.nih.gov/nuccore/on911502

Competing interests: The authors declare that they have no competing interests.

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Authors' Contributions

KSM major contributor and involved in all phases of research, including writing the manuscript, performing the PCR and sequencing. USV, GAP, BBK, PMD, PM and KMP collected samples and performed the histopathology. KRM performed phylogenetic analysis and assisted in manuscript preparation. All authors have read and approved the final manuscript.

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