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Interesting differentiation of malicious poisoning in a wild leopard

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Abstract

Human–wildlife conflict in India has escalated due to habitat fragmentation and declining prey, often leading to retaliatory killing of large carnivores. This case report describes the forensic investigation of suspected malicious poisoning in leopards using an integrated diagnostic approach. Carcasses of a sub-adult male leopard, an adult female leopard, and a civet cat were recovered from the same locality in Maharashtra. Post-mortem examination revealed generalized visceral congestion, hepatic and renal necrosis, and haemorrhagic enteritis, warranting differential diagnosis for infectious diseases such as Feline Panleukopenia Virus (FPV).

Molecular analysis using PCR and qPCR assays tested negative for FPV, ruling out infectious etiology. Species identification of undigested stomach contents via cytochrome b gene sequencing confirmed the presence of goat (*Capra hircus*) DNA, indicating recent livestock predation. Field investigation subsequently revealed a decomposed goat carcass suspected to be deliberately poisoned. Toxicological analysis confirmed the presence of zinc phosphide.

The combined pathological, molecular, and toxicological findings established malicious poisoning as the cause of death, likely in retaliation for livestock depredation. This case highlights the importance of wildlife forensics in differentiating causes of mortality and emphasizes the ecological threat posed by indiscriminate poisoning. Strengthening forensic capacity and community-based conflict mitigation strategies is essential for effective conservation.

Keywords: Leopard, *Panthera pardus*, malicious poisoning, zinc phosphide, wildlife forensics, human–wildlife conflict, molecular diagnostics, toxicology

Introduction

Human wildlife conflict has reached its peak owing to the loss of habitat, habitat fragmentation and invasion by development infrastructure. The incidences of predation of livestock have increased due to the presence of the carnivores near human settlements. The predation of livestock leads to financial losses to the village community. Incidences of poisoning of the predated livestock as revenge have come to light in recent times. Malicious poisoning of the carcass often leads to massive deaths as scavengers are unintentionally poisoned (Radhakrishnan, 2018) [9]. From the conservation point of view, malicious poisoning of carcass is a threat to biodiversity as it can lead to large scale mortality. Under the Indian Wildlife Protection Act, 1972 all endangered animals are protected. The cases of poisoning in Indian wildlife are often under reported due to non-availability of laboratories for detection, decomposition of the carcass, improper packaging/ handling of the samples and long waiting period in the laboratories. There are reports of poisoning of a variety of wildlife with a range of chemicals ranging from insecticides, pesticides, chemical toxins, rodenticides etc.

Leopards are distributed throughout the Indian subcontinent and despite the stern competition for resources have adapted to survive at the wild-human interface. In recent times, leopard-human conflict has reached its pinnacle due to habitat loss, limited prey base, deforestation and encroachment of development infrastructure in protected areas. Leopards cause economic hardship by predation of the livestock and dogs. The cases of revenge poisoning have been

encountered in the country but poorly documented (Gupta *et al.*, 2021) [3]. This case report deals with the differential diagnosis of poisoning of leopards using molecular and forensic analysis.

Case Presentation

A carcass of a sub-adult male leopard was found during the forest patrolling at Semadoh under Sipna Division of Melghat Tiger Reserve, Amravati, Maharashtra, a systematic post mortem examination was carried out to probe the cause of death. The gross lesions were noted and samples were collected for further investigation. The samples were submitted to Wildlife research & Training Centre, Gorewada, Nagpur for diagnosis. The major findings included congestion of the lungs, heart, intestine, kidney and spleen along with necrotic foci on the liver and kidney. The serosal and mucosal surfaces of the intestine showed typical haemorrhagic bands. A day later a carcass of a female adult leopard and civet cat was recovered from the same area. The carcass was sent to Wildlife Research & Training Centre, Gorewada, Nagpur for the post mortem examination. The findings were similar to the findings reported in the male sub adult leopard. The stomach and intestinal content were sent for the forensic analysis. The description of lesions prompted a possibility of Feline Panleukopenia, hence intestinal content and visceral organs were utilised for the diagnosis of the disease. During the post mortem examination undigested meat was discovered in the stomach of the female leopard. The sample was collected for the species identification.

The samples were transported on ice to the laboratory and DNA was isolated using commercially available DNA stool kit (Mfg. Qiagen) and blood and tissue Kit (Mfg. Qiagen). The DNA was quantified using Qubit and a PCR test for Feline Panleukopenia was performed using protocol described by Sheikh *et al.*, 2017 [13]. Also, qPCR was performed as described by Singh *et al.*, 2013 [14]. The DNA recovered from undigested meat in the stomach was utilised for the species identification using cytochrome b based primers (Singh & Verma, 2003) [15]. The amplicon so obtained was purified using commercial kits and sequenced using Gene Analyser 3500 (Mfg. Applied Biosystems). The consensus sequence was built using the Mega X software and subjected to nBlast analysis using the NCBI database.

Result:

The PCR and qPCR test was negative for Feline Panleukopenia; the species identification PCR of the recovered meat provided an amplification of approximately 440 bp. The consensus sequence on blast analysis was found similar to goat (*Capra hircus*). The findings were communicated to the law enforcement agencies of the forest department. The team conducted search at the site where the carcasses were discovered. The search led to discovery of a decomposed goat carcass. The field investigation and enquiry revealed malicious poisoning of the kill by a farmer to seek revenge. The forensic lab confirmed the presence of zinc phosphide in the stomach contents of the male and female leopard.

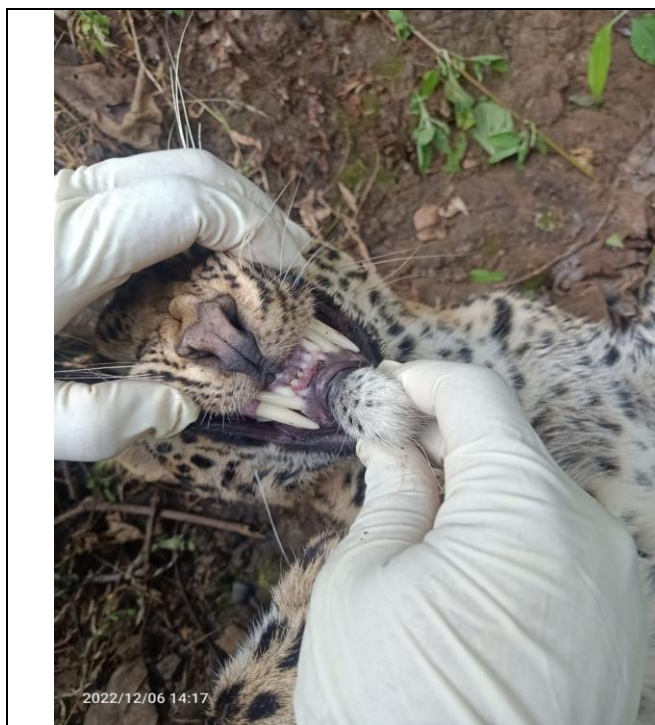


Fig 1: Leopard found dead in the protected area of Maharashtra.



Fig 2: Liver with foci of infarction and rounded borders



Fig 3: Ecchymotic haemorrhages on the serosal surface of the small intestine



Fig 4: Agarose Gel Electrophoresis of 1% gel for the detection of Feline Panleukopenia. Lane L: 100 bp ladder; Lanes 1-3 samples Liver, intestine and heart; Lane 4: Negative control; Lane 5: Positive control showing 680 bp amplification.

Capra hircus mitochondrial DNA, including all protein coding genes, isolate: G664
Sequence ID: [AB735752.1](#) Length: 12623 Number of Matches: 1

Range 1: 11820 to 12118 [GenBank](#) [Graphics](#) [▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
531 bits(287)	3e-146	296/300(99%)	1/300(0%)	Plus/Minus
Query 1	AATTAGTAGCATGGCGCCTAAGATATCTTTAATGGTGTAGTAAGGGTGAAATGGGATTTT	60		
Sbjct 12118	AATTAGTAGCATGGCGCCTAAGATATCTTTAATGGTGTAGTAAGGGTGAAATGGGATTTT	12059		
Query 61	ATCTGCGTCTGATGGAATTCCTGTGGGGTTGTTTCGATCCTGTTTCGTGGAGGAAAAGCAG	120		
Sbjct 12058	ATCTGCGTCTGATGGAATTCCTGTGGGGTTGTTTCGATCCTGTTTCGTGGAGGAAAAGCAG	11999		
Query 121	GTGGACTATGGCGAGGGCTGTGATGATGAATGGGAGGATAAAGTGAAGGCGAAAAATC	180		
Sbjct 11998	GTGGACTATGGCGAGGGCTGTGATGATGAATGGG-AGGATAAAGTGAAGGCGAAGAAATC	11940		
Query 181	GGGTGAGAGTGGCTTTGTCTACTGAGAATCCCCCTCAAATTCATTCGACTAGGTTTGTGC	240		
Sbjct 11939	GGGTGAGAGTGGCTTTGTCTACTGAGAATCCCCCTCAGATTCAATTCGACTAGGTTTGTGC	11880		
Query 241	CAATATATGGGATTGCTGAAAGAAAATTAGTGATGACTGTTGCCCTCAAATGATATT	300		
Sbjct 11879	CAATATATGGGATTGCTGAAAGAAATTAGTGATGACTGTTGCCCTCAAATGATATT	11820		

Fig 5: Sequence alignment with subject sequence showing 99% identity with *Capra hircus* (goat) sequence

Discussion

Leopards (*Panthera pardus*) are one of the most adaptable large carnivores and are widely distributed across India. They have been increasingly coming into conflict with humans due to habitat fragmentation, depletion of natural prey, and encroachment of developmental infrastructure (Athreya *et al.*, 2013) [1]. Livestock depredation by leopards is a significant issue in many parts of India, particularly in states such as Maharashtra, Uttarakhand, Himachal Pradesh, and Madhya Pradesh (Odden *et al.*, 2014) [7]. Studies indicate that leopards exhibit a high degree of behavioural plasticity, enabling them to thrive even in human-dominated landscapes, often preying upon domestic livestock, including goats, cattle, and dogs (Kolipaka *et al.*, 2017) [8]. The economic burden of livestock loss often leads to retaliatory actions by local communities, including poisoning, electrocution, and snaring. Leopards, being highly elusive, are difficult to track, and indirect evidence such as pugmarks, scat, and camera trap data are often used to monitor their movements (Dutta *et al.*, 2021) [2]. While government compensation schemes exist, delays in processing claims and inadequate compensation frequently drive local communities to take matters into their own hands. Revenge killing of large carnivores is a persistent issue in India, primarily executed through poisoning, electrocution, and snaring. Poisoning remains one of the most common and detrimental methods, as it not only targets the predator but also results in collateral damage to scavengers such as

vultures, hyenas, and other carnivores (Radhakrishnan, 2018b) [10]. Poisoning involves the use of various chemicals such as organophosphates, carbamates, zinc phosphide, and rodenticides to lace carcasses. Zinc phosphide, identified in the current case, is a potent rodenticide known to cause severe pulmonary and gastrointestinal damage (Gupta *et al.*, 2021b) [4]. The indiscriminate use of poison disrupts the ecological balance and poses a significant threat to non-target species. Electrocution occurs when farmers and poachers install high-voltage electric fences or tamper with power lines to electrocute wild animals. Electrocution incidents have been frequently reported in states like Karnataka and Madhya Pradesh, where power lines run through forest fringes (Sivakumar *et al.*, 2019) [11]. Snaring involves the use of metal wire snares, commonly set for small game but inadvertently trapping leopards and other large carnivores. Injuries caused by snares often lead to prolonged suffering and eventual death due to infections and starvation (Gubbi *et al.*, 2019) [6]. In wildlife forensics, differentiating between infectious diseases and poisoning is crucial for accurate diagnosis and legal investigation. In the present case, differential diagnosis was necessary to rule out infectious causes, particularly Feline Panleukopenia Virus (FPV), which shares overlapping clinical signs such as haemorrhagic enteritis and multi-organ congestion (Steinel *et al.*, 2001) [12]. However, molecular diagnostics, including PCR and qPCR assays, confirmed the absence of FPV, strengthening the hypothesis of toxicological

causation. Species identification through molecular forensic methods plays a pivotal role in wildlife crime investigations. In this case, DNA extracted from the undigested meat in the female leopard's stomach was amplified using cytochrome b-based primers, confirming the presence of goat (*Capra hircus*) DNA (Singh & Verma, 2003) [15]. This finding was critical in linking the poisoned goat carcass to the leopard's mortality, strengthening the forensic evidence against the perpetrators. The use of molecular techniques such as DNA barcoding and species-specific PCR assays enables precise identification of prey species, aiding in forensic investigations and legal proceedings (Iyengar, 2014) [5]. The integration of molecular forensic tools with traditional necropsy findings provides a robust framework for investigating wildlife crimes and enforcing conservation laws.

Conclusion

This case highlights the urgent need for improved forensic capabilities in India to detect and document poisoning cases in wildlife. The findings underscore the importance of rapid and systematic necropsy procedures, molecular diagnostics, and toxicological analysis in differentiating between natural and anthropogenic causes of wildlife mortality. Strengthening law enforcement, enhancing awareness among local communities, and promoting coexistence strategies are critical steps toward mitigating human-wildlife conflicts and preventing retaliatory killings.

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