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Cover: The nine vultures of India, digital art made on Krita by Dupati Poojitha.

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First documented case of flunixin residue in a Himalayan Vulture *Gyps himalayensis* Hume, 1869 (Aves: Accipitriformes: Accipitridae) in India: conservation and veterinary implications

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Abstract: Non-steroidal anti-inflammatory drugs (NSAID), particularly diclofenac, have been widely identified as a major cause of vulture deaths across Asia, leading to significant population declines. The impact of other veterinary NSAIDs, including flunixin, remains poorly documented. This study reports the first confirmed case of flunixin residue in a wild Himalayan Vulture *Gyps himalayensis* (Hume, 1869) in India. A juvenile vulture was rescued from Jaldapara National Park, West Bengal, and transferred to the Buxa Vulture Conservation Breeding Centre & Aviary at Rajabhatkhawa (West Bengal) for treatment and rehabilitation. Despite medical intervention, the bird died. Necropsy revealed extensive visceral gout, indicative of renal failure. Toxicological analysis confirmed the presence of flunixin residues in the tissues (stomach contents showed the highest level of flunixin with 903.9 ng/g, followed by the kidney with 214.3 ng/g, and the liver with 67.6 ng/g). This report highlights the requirement for careful monitoring of veterinary NSAID usage in India by trained professionals for the conservation of endangered vulture populations.

Keywords: Buxa Vulture Conservation Breeding Centre and Aviary, non-steroidal anti-inflammatory drugs (NSAIDs), renal failure, veterinary pharmaceuticals, visceral gout, vulture conservation, West Bengal, wildlife toxicology.

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INTRODUCTION

Vultures play a crucial ecological role as obligate scavengers, preventing the spread of diseases by efficiently disposing of animal carcasses. The Himalayan Vulture *Gyps himalayensis* (Hume, 1869) is a resident of the mountains of central Asia, the Himalaya, southern & eastern Tibet, and China (Ali & Ripley 1978). While breeding adults remain in their nesting territories for most of the year, juveniles, and sub-adults migrate to the plains of southern and southeastern Asia during the winter (Naoroji 2006; Rasmussen & Anderton 2012). This seasonal movement is primarily driven by reduced food availability at the high-altitude regions due to whiteout, leading to lesser chance of success in securing food in competition with dominant adults (BirdLife International 2024), and the need to conserve energy in harsh winter conditions. Additionally, young vultures, not yet engaged in breeding, exhibit dispersal behaviour as part of their survival strategy and future range expansion (Yong & Kasorndorkbua 2008). With the return of favourable conditions, they migrate back to their breeding grounds in summer.

As juveniles and sub-adults migrate to the plains of southern and southeastern Asia, they become exposed to anthropogenic threats, including veterinary drug residue in livestock carcasses, the primary food source. In contrast, breeding adults that remain in the high-altitude regions of the Himalaya are relatively shielded from this threat, as NSAID-laced carcasses are less common in these remote and sparsely populated regions. The indiscriminate use of NSAIDs in veterinary medicine has had catastrophic consequences for vulture populations worldwide. Diclofenac, in particular, has been linked to the catastrophic decline of several *Gyps* species in southern Asia (Oaks et al. 2004; Pain et al. 2008). Although diclofenac, along with three other NSAIDs (aceclofenac, ketoprofen, and nimesulide), are banned for veterinary use in India (Ministry of Health and Family Welfare, Government of India 2008, 2023, 2024), there are reports (Cuthbert et al. 2011; Down To Earth 2022) of continued illegal use of these NSAIDs meant for human use for veterinary purposes.

Flunixin, a potent NSAID, is commonly administered to livestock for pain management, and inflammation control. Flunixin, similar to diclofenac, aceclofenac, ketoprofen, and nimesulide, is suspected to induce renal failure in *Gyps* vultures, leading to fatal visceral gout (Zorrilla et al. 2014). Although flunixin is legally approved for veterinary use in India, its toxicity to vultures is suspected, highlighting the need for experimental testing of the drug's toxicity in vultures (Galligan et al. 2020). Until now, there has been no documented case of flunixin poisoning in Himalayan Vultures in India. The present case reports the necropsy and toxicological findings of the first documented instance of flunixin-associated mortality in a wild Himalayan Vulture in India, highlighting a significant conservation concern for this species, and an urgent need for comprehensive monitoring of the use of this NSAID in veterinary practice.

MATERIALS AND METHODS

Case details and clinical presentation

The Buxa Vulture Conservation Breeding Centre & Aviary, situated at Rajabhatkhawa of Alipurduar District of West Bengal in India, serves as a conservation breeding centre for three Critically Endangered *Gyps* species of vultures, including White-rumped Vulture *Gyps bengalensis*, Long-billed Vulture *Gyps indicus*, and Slender-billed Vulture *Gyps tenuirostris*. Additionally, the centre functions as a rescue, and rehabilitation facility for vultures in the region. Since its establishment in 2006, the centre has received 95 rescued Himalayan vultures, and successfully released 80 individuals back in their natural habitat after treatment (Chakraborty et al. 2024). On 19 December 2024, a juvenile Himalayan Vulture *Gyps himalayensis* was rescued in a weakened state in Jaldapara National Park, West Bengal. The bird was promptly transported to the centre for treatment and rehabilitation. The vulture exhibited symptoms of lethargy, dehydration, and anorexia, and was unable to fly. It was identified as a juvenile Himalayan Vulture based on its overall dark plumage (except for a whitish head), distinctly darker than juvenile Eurasian Griffons *Gyps fulvus*, and lacking their rufous tinge. The bird had a long, pointed buffy-brown ruff with pale shaft streaks, dark brown upperparts, and conspicuously streaked buff-white scapulars, and upper wing coverts. Its flight feathers and tail were blackish-brown, with a dark brown crop patch, and the underparts were heavily streaked buffy-white especially on the body. These plumage features are consistent with juvenile *Gyps himalayensis* as described by Naoroji (2006).

Symptomatic treatment was initiated to stabilise the bird's condition. The treatment regime included:

- 40 ml of Dextrose Normal Saline (DNS) intravenously (IV) for rehydration and electrolyte replenishment.

- 0.5 ml of Atropine Sulphate intravenously (IV) to alleviate respiratory distress and stabilise cardiac function.

- 100 mg of Intacef Tazo intravenously (IV) a combination antibiotic (Ceftriaxone and Tazobactam) to treat suspected bacterial infections.

- 1 ml of Tribivet intravenously (IV) is used as a supportive multivitamin injection to treat vitamin B-complex deficiencies and boost recovery of the vulture.

Condition of the bird progressively deteriorated despite administration of supportive care. It succumbed to its illness on 22 December 2024. A necropsy was subsequently conducted to determine the underlying cause of death.

Necropsy examination

A comprehensive necropsy examination was performed. The major findings of the necropsy examination included:

- Extensive deposition of uric acid crystals on visceral organs (visceral gout), indicating renal failure (Image 1).
- No evidence of external trauma or underlying diseases.
- An empty gastrointestinal tract, suggesting prolonged anorexia.

Tissue samples were collected for further toxicological analysis to determine the underlying cause of death.

Toxicological analysis

The tissue samples (liver, kidney, and stomach contents) from the carcass were collected, labelled, and frozen immediately for further analysis. The samples were then transported to the Salim Ali Centre for Ornithology and Natural History (SACON) at Coimbatore, India, for ecotoxicological screening. Liquid chromatography-mass spectrometry (LC-MS/MS) was used to detect any residue of NSAIDs. The samples were screened for residues of 14 NSAIDs, including - diclofenac, aceclofenac, ketoprofen, ibuprofen, naproxen, paracetamol, mefenamic acid, meloxicam, nimesulide, piroxycam, tolfenamic acid, indomethiocin, flunixin, and carprofen. This comprehensive screening aimed to detect any potential NSAID contamination that may have contributed to the vulture's death.

RESULTS

Toxicological analysis revealed presence of notable levels of flunixin in the samples. Residues of other targeted NSAIDs were below detection limit in all the samples. The findings are summarised in Table 1.

Among the tissues analyzed, stomach contents showed the highest level of flunixin (903.9 ng/g), followed

Image 1. Presence of visceral gout characterised by extensive deposition of uric acid crystals on the liver of the rescued Himalayan Vulture's carcass.

by the kidney (214.3 ng/g), and liver (67.6 ng/g), which had the lowest concentration. The presence of uric acid crystal deposition in the viscera (Image 1), indicative of visceral gout, was also noted during the necropsy.

Although pharmacokinetics of flunixin in *Gyps* vultures are poorly documented, Ramzan et al. (2012) demonstrated flunixin meglumine toxicity in broiler chickens, with dose-dependent mortality (20–60%) and associated increases in serum uric acid, and creatinine. The study indicated that flunixin meglumine caused similar toxicity in birds as diclofenac. Previous studies have linked diclofenac residues (0.051–0.643^{-1} µg/g in kidneys) in *Gyps* vultures to renal failure and visceral gout (Oaks et al. 2004). During post-mortem examination, clear visceral gout, as extensive deposition of uric acid, was observed in the vulture. Further, toxicological analysis of tissue samples for 14 NSAIDs (table 1), only flunixin was detected at significant concentrations in the liver, and kidney. Therefore, it can be inferred that flunixin was one of the reasons for the death of the vulture in the present case.

Diclofenac, a non-steroidal anti-inflammatory drug inhibits cyclooxygenase (COX) enzymes. In vultures, COX inhibition impairs renal prostaglandin synthesis, reducing glomerular filtration, and uric acid excretion.

Table 1. Concentration of flunixin found in the tissues of the Himalayan Vulture.

NSAIDSs concentration in tissue samples				
Unit = ng/g				
	NSAIDs screened	Tissue samples		
		Liver	Kidney	Stomach content
1	Diclofenac	BDL	BDL	BDL
2	Aceclofenac	BDL	BDL	BDL
3	Ketoprofen	BDL	BDL	BDL
4	Ibuprofen	BDL	BDL	BDL
5	Naproxen	BDL	BDL	BDL
6	Paracetamol	BDL	BDL	BDL
7	Mefenamic acid	BDL	BDL	BDL
8	Meloxicam	BDL	BDL	BDL
9	Nimesulide	BDL	BDL	BDL
10	Piroxycam	BDL	BDL	BDL
11	Tolfenamic acid	BDL	BDL	BDL
12	Indomethiocin	BDL	BDL	BDL
13	**Flunixin**	**67.6**	**214.3**	**903.9**
14	Carprofen	BDL	BDL	BDL
BDL—Below detection limit | Detection limit—20 ng/g				

Image 2. Presence of uric acid crystals on the interior wall of the trachea of the Himalayan Vulture's carcass.

This leads to hyperuricemia, urate crystal deposition, and visceral gout (Oaks et al. 2004; Naidoo & Swan 2009). Oaks et al. (2004) reported the range of diclofenac residues in the kidneys of vultures that died of visceral gout from 0.051–0.643 µg/g. Flunixin is also a non-steroidal anti-inflammatory drug, and in the present case the concentration of flunixin has been detected 214.3 ng/g (equivalent to 0.2143 µg/g) in the kidney of the affected Himalayan Vulture. However, there is a lack of information on whether flunixin, like diclofenac, inhibits COX enzymes in vultures.

The detection of flunixin residues in the tissue samples, confirmation by the testing agency about the probable cause of death, and the observed symptoms of gout, lead us to conclude that flunixin poisoning was the most probable cause of death in this Himalayan Vulture.

DISCUSSION

Visceral gout is characterised by the extensive deposition of uric acid crystals on visceral organs, leading to inflammation, tissue damage, and organ dysfunction. In this present case, visceral gout was found on the liver surface (Image 1). Notably, uric acid crystals were also present on the inner wall of the trachea (Image 2), indicating a severe case that compromised the respiratory system. Uric acid crystals in the trachea can cause inflammation, blockage of the airways, and respiratory distress. The presence of visceral gout, coupled with uric acid crystals in the trachea, suggests that the vulture's death was likely caused by complications arising from kidney disease, and visceral gout developed on the liver as a consequence of flunixin poisoning. Cuthbert et al. (2007), also reported that flunixin has the potential to cause renal damage in birds. Therefore, in this case also, flunixin could be a precipitating factor. However, high flunixin residue in the stomach indicates a recent exposure. The Himalayan Vulture is currently listed as Near Threatened on the International Union for Conservation of Nature (IUCN) Red List. Their global population is estimated to be between 66,000–334,000 mature individuals (BirdLife International 2021), and is protected in India under Schedule-I of the Wild Life (Protection) Amendment Act, 2022 (Government of India 2022). This case underscores the pressing need to limit the veterinary use of flunixin along with other NSAIDs.

This study provides the first confirmed evidence of flunixin residue in a Himalayan Vulture *Gyps himalayensis* in India. The study could not identify the source of exposure to flunixin, which could have been anywhere within its former range.

CONCLUSION

The Himalayan Vulture's ecological importance cannot be overstated, and the drastic decline in its population is alarming. As a scavenger, it plays a crucial role in maintaining the health and balance of ecosystems by disposing of dead animals, and preventing the spread of diseases. This first reported case of flunixin residue in a Himalayan Vulture in India highlights the urgent need for monitoring of flunixin usage in veterinary use. Further research on flunixin toxicity in scavenging raptors is required to establish safe veterinary drug policies and to ensure a steady supply of safe food sources, such as carcasses, to ensure their survival.

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