<u>Original Research Article</u> First Records of Scent Marking with the Preorbital Gland in Banbakri Antelope (*Boselaphus tragocamelus*)

Comment [A1]: Really first record? I have heat that having preorbital gland near eye and using it scent marking is common for antelope.

ABSTRACT

Scent marking using the preorbital gland in the Banbakri antelope, also known as the white-footed antelope (Boselaphus tragocamelus), was studied at the Haryana Agriculture Farm, VKS College of Agriculture, Dumraon. This study investigated the behaviour of Banbakri antelopes concerning preorbital gland opening under various conditions, including stress, starvation, and maturity. The research aimed to assess this behaviour as part of a broader investigation into the phenotypic and behavioural characteristics of Banbakri/Nilgai to enhance their well-being and improve resource management. In mature Banbakri antelopes, the preorbital glands measured approximately 2.1 cm in length and 1.0 cm in width. These exocrine glands, appearing as trench-like slits of dark blue to black coloration, were located beneath the eyes. Under free-roaming conditions, both male and female B. tragocamelus rarely exhibited preorbital gland opening. However, the gland was observed to open under stress, particularly when an animal was restrained with a halter for the first time, either at night or occasionally during the day. Preorbital gland opening was also noted during maturity, hunger, and stressful conditions. The findings suggest that this behaviour may serve as a key indicator for differentiating between playful, courtship, and aggressive social behaviors, particularly in stressful situations or stages of maturity. Additionally, the gland may secrete pheromonal substances that facilitate olfactory communication.

To the best of our knowledge, no prior studies have reported the opening of the preorbital gland in Banbakri antelopes (*B. tragocamelus*). This study presents the first recorded observation of this behaviour in captive white-footed antelopes, contributing novel insights into their behavioural ecology.

Keywords: Antelope, habitat, stress, Banbakri calf, behaviour, pheromone.

1. INTRODUCTION

The importance of chemical communication in wild mammals is well recognized; however, our knowledge of this subject remains limited. The preorbital gland is a paired exocrine gland, appearing as a slit-shaped cavity located anterior to the eye. It is found in many ungulates, particularly in the families Bovidae, Cervidae, and various antelope species [1,2,3]. The preorbital gland serves different roles across artiodactyl species. Its opening functions in both visual communication and scent marking [4]. Most studies on preorbital glands have linked their use to various behaviors, including maternal care in red deer (Cervus elaphus) calves [5], alertness in captive pudu (*Pudu puda*) and red brocket deer (Mazama americana) [6], and sexual behaviors during aggressive interactions in rusa deer (*Rusa timorensis*) [7].

The Banbakri (Boselaphus tragocamelus), commonly known as Nilgai, exhibits unique ecological functions and mutualistic relationships with humans [8,9,10]. It is a large bovid species currently free-roaming in 18 Indian states, including almost all districts of Bihar [11]. Banbakri is protected under Schedule III of the Wildlife (Protection) Act of 1972 and is categorized as an animal of Least Concern by the IUCN [12]. In spite of many Indian states government have declared this white footed antelope, WFA, (Boselaphus tragocamelus) as vermin due to huge damage of crops and moreover some states have instructed to slay them in favour of agriculturists. However, it often receives negative attention in agrarian societies in India. Banbakri (B. tragocamelus) give positive sign and surviving an extreme ecological condition, it may be use as agriculture husbandry, animal transport, probably new source of food, medicine, and industrial product after domestication [11]. The Banbakri working as a soil doctor, their dung assist in agricultural farm and afforestation. This species primarily inhabits agricultural fields and undulating open habitats, avoiding forests, making it easier to observe. Several behavioural studies on Banbakri or Blue buck have been published [13,14,15,16,17]. Previous behavioural studies on Nilgai (WFA) Blue buck and Blackbuck have shown that urine and droppings play a significant role in territorial marking behaviour [2,18,19]. Compared to some cervids, the preorbital gland in Banbakri is pronounced [3]. However, there is no prior report describing the opening of the preorbital gland in the Banbakri, WFA, (Boselaphus tragocamelus) for any specific purpose.

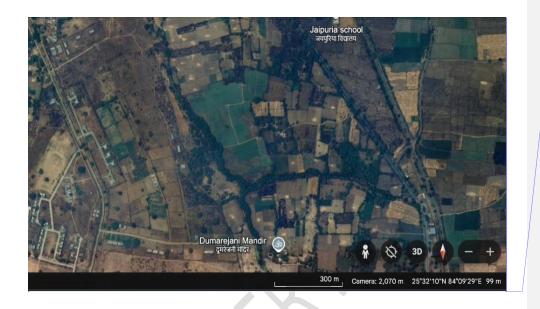
This study documents, for the first time, the opening of the preorbital gland in Banbakri under various contexts, expanding our understanding of this elegant species' behaviour and the functional role of its preorbital gland.

2. MATERIALS AND METHODS

Comment [A2]: Should the name be written i capital letters?

Comment [A3]: ??

The study was conducted on a Haryana Agricultural Farm (Banbakri Research Farm) at VKS College of Agriculture, Dumraon, Buxar, located at 25.53° N latitude and 84.15° E longitude, at an elevation of 76±4 meters (250±6 feet). Showing in google map-1.



Comment [A4]: Ensure all figures and table are included and referenced correctly.

Figure 1: Google map showing the study area Haryana Agriculture farm Dumraon

Dumraon is a subdivision of District Buxar and harbours many fascinating wild mammalian species, including the WFA (*Boselaphus tragocamelus*), Prasad et al 2020 [20]. The behaviour of male and female Banbakri was observed in captivity (Fig. 2a & b). During winter, the Banbakri was kept indoors in a 10x20 feet room. Occasionally, it was released into a large outdoor enclosure during summer, winter, and rainy seasons. The enclosure was surrounded by wire fencing approximately 200 by 150 feet (LxW) and 6 feet in height. The enclosure had a savanna-like appearance during the rainy and winter seasons, with most of the area covered by short grass interspersed with large trees. The boundaries of the enclosure were densely vegetated with herbs, shrubs, and trees, including cucumbers, cabbage, Moong, Green gram (*Vigna radiata*) Pigeon pea, (*Cajanus cajan*) Jharbar shrubs, (*Ziziphus mauritiana*) and various grasses. Trees such as sesame, (*Dalbergia sisso*) Neem, (*Azadirachta indica*). Babool, (*Acacia nilotica*) and other wild species were also present. The WFA was fed a diet consisting of fruits, vegetables, grasses, and the fruits, seeds, and leaves of Ber, arhar, and wild trees.

Comment [A5]: ??

Comment [A6]: And did you forget to write the scientific name for these three plant?

Comment [A7]: I think you should mention universally understandable name (common/ Engi name or scientific name so that everyone can understand



Fig.2. a, Banbakri calf siting in the field and b. its close view of preorbital gland

During rearing, the appearance of the preorbital glands in calves, juveniles, and maturing was noted, and photographs were taken whenever possible, using ad libitum sampling [21] (Altmann, 1974). Observations were conducted daily for over a year (16 months), from October 2023 to January 2025. All observations of marking and gland opening were made between 07:00 and 10:30 am, as the Banbakri was most active during this period.

3. RESULTS AND DISCUSSION

Banbakri of the sub family Bovinae mark with the small preorbital gland beneath the each eye. The structure of this gland differs from of other ruminants. A pair of preorbital glands in Banbakri (*Boselaphus tragocamelus*) were small, shiny black, oily in appearance, and covered with densely distributed hair (Fig. 2). In mature females, the glands measured approximately 2.1 cm in length and 1.0 cm in width. These exocrine glands were located beneath the eyes (Fig. 2b). The rate of gland opening was high in newborn calves and gradually decreased with habituation. Preorbital gland opening was uncommon under free-roaming conditions for both males and females. However, the gland was observed to open under stress, such as when the animal was tied with a halter in controlled conditions during the night or occasionally during the day. The colour of the gland secretion ranges from colourless to blackish-bluish. The details of preorbital gland opening are summarized in Table 1 and Fig. 3.

When a female Banbakri (WFA) was released into the outdoor enclosure after being kept indoors for several days, the first thing she usually did was mark. She primarily marked the trunks, branches, and leaves of saplings growing around the edges and middle of the enclosure. She moved from sapling to sapling, often covering the entire perimeter one or

Comment [A8]: I could not find the sample si Small sample size and limited observation period could reduce the generalizability of the results. Clarifying this details in methodology section wor enhance the research credibility.

Comment [A9]: Beneath or in front of each ev

Comment [A10]: It can be written in thisway The frequency of gland opening was highest in newborn calves and decreased with habituation over time

Comment [A11]: ??

more times before beginning to feed or rest. It is likely that the preorbital gland secretes a scent that calves or other Banbakri can detect, possibly serving as a form of communication. **Table 1: Preorbital Gland Opening Observations in** *Boselaphus tragocamelus*

S. No.	Condition of Preorbital gland	Month/Year
1	Opened after birth, secreting scent	2-5 October 2023
2	Closed during normal conditions	6-16 October 2023
3	Opened during 2–4 hours of hunger	17 October 2023
4	Opened during stress (e.g., tied with a halter)	15–20 May 2024
5	Opened as females approached sexual maturity	25 January 2025





We observed the glands opening and closing in captive Banbakri while nursing their calves, suggesting a role in scent communication between mother and calf. This is the first report documenting preorbital gland opening in Banbakri (*Boselaphus tragocamelus*). The preorbital gland serves different roles in many ungulates such as sheep, horse, antelope, cow and few marsupials apocrine glands are widely distributed act as scent glands to produce pheromones. Pheromone-containing secretions from the gland may help establish dominance, particularly in preparation for breeding, marking territory, or providing pleasurable sensations to the animal [22]. It has previously been reported and being used for marking or display during the precopulatory stage in Eld's deer [23] and in Indian sambar [2,14] also been established the diurnal variation and distribution of scent marking behaviour of captive male (*Antelope cervicapra*) also influence of the marker. Whereas [24] recorded for sexually receptive red deer hinds during soliciting behaviour to the stag, although preorbital gland

opening by females in a sexual context appears to be much rarer. Therefore, we suggest that preorbital gland opening may be an important behavioural indicator of real maturity of the individual in the sexual context and also in stress condition. Our findings are also in line with the suggestion of [1.2,7] reported, that olfactory signals from the preorbital gland may have some role in the sexual behaviour of cervids and cervicapra.



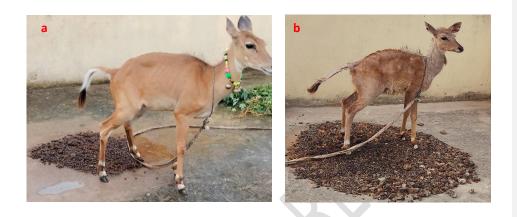
Figure 4. a & b: Opening & close view of preorbital gland during maturation



Figure 5. a&b Banbakri scent marks by rubbing its face against leaves and branches of trees

In the present observation, it was noted that the Banbakri or Blue Bull (*Boselaphus tragocamelus*) exhibited the ability to discriminate scents deposited near food substances, branches, trunks, leaves of trees, bushes and grasses. Banbakri may be rely heavily on the scent glands to communicate with other members of their species, and possibly even with member of other species. This behaviour may be used to indicate presence, attract males for mating, and signal social behaviour (Fig. 4 and 5a&b). Both males and females often selected specific locations for urination, defecation, and preorbital gland scent marking, (Figure 6.

a&b). We often observe Banbakri sniffing faecal pellets or urine on the ground, which may have further behavioral consequences. However, during the rut, urine left by females is particularly attractive to males. The male invariably stops, sniffs, and licks the urine, repeatedly displaying the flehmen response to each urine patch he encounters, similar to behaviors reported in other ungulates [24,25].



Figure,6a&b Both males and females often selected specific same locations for defecation and urination.

Preorbital glands in other ungulates secrete complex mixtures of chemical compounds, and variations in gland size and positioning may reflect differences in social structures and marking behaviours [26, 27]. Red deer calves reportedly open their preorbital glands during stressful procedures, while relaxed calves do not [27]. In contrast, no such behaviour was observed in wild female Barasingha (*Rucervus duvaucelii*)³. Our study aligns with previous findings that olfactory signals from preorbital glands may play a role in sexual behaviour in cervids and other ungulates [1,2,7]. This study may prove to be a potential exist and drug development for producing new antimicrobial agents against pathogenic skin microorganism [28].

4. CONCLUSION

In conclusion, this is the first report of preorbital gland opening in Banbakri (*B. tragocamelus*). We observed this gland open in Banbakri antelope under stress, starvation, or sexual maturity contexts may serve as an essential behavioural indicator. This could help distinguish between playful, courtship, aggressive, social status, and other behaviours to communicate with other member of Banbakri (*Boselaphus tragocamelus*). These findings may be pave the way for further research on communication in this species and its potential applications in antimicrobial drug development.

Comment [A12]: •The jump from behavioural communication to antimicrobidrug development feels abrupt. A brief explanation of how the gland's function might relate to antimicrobial properties would help bridge this gap.

Comment [A13]: Can you rephrased it for a better readability

Comment [A14]: How and why?

Comment [A15]: Sometimes I felt like I didn't get it right when I read it. You should make a language simpler and easily understandable and errorless.

REFERENCES:

- Ceacero, F., Landete-Castillejos, T., Barto^{*}sová, J., García, A.J., Barto^{*}s, L., Komárková, M., Gallego, L., 2014. Habituating to handling: factors affecting preorbital gland opening in red deer calves. J. Anim. Sci. 92, 4130–4136.
- Rajagopal, T. and Archunan, G., 2011. Histomorphology of preorbital gland in territorial and non-territorial male blackbuck *Antelope cervicapra*, a critically endangered species, Biologia, 66(2):370-378. DOI: 10.2478/s11756-011-0015-4
- Pluhácek, Francisco Ceacero, Peter Lupták 2015, First records of preorbital gland opening in rare wild Barasingha (*Rucervus duvaucelii*) in social contexts may help to explain this phenomenon in cervids. Behavioural Processes. 119;28–31. http://dx.doi.org/10.1016/j.beproc.2015.07.009
- 4. Lawson, R.E., Putman, R.J., Fielding, A.H., 2000. Individual signatures in scent gland secretions of Eurasian deer. J. Zool. 251, 399–410.
- Barto^{*}sová, J., Ceacero, F., Barto^{*}s, L., 2012. Pre-orbital gland opening: part of sucking behaviour in red deer (*Cervus elaphus*) calves. J. Anim. Sci. 90, 3207–3212.
- MacNamara, M., Eldridge, W.D., 1987. Behaviour and reproduction in captive Pudu (Pudu puda) and red brocket (Mazama americana), a descriptive and comparative analysis. In: Wemmer, C.M. (Ed.), Biology and Management of Cervidae. Smithsonian Institution Press, Washington, D.C, pp. 371–386.
- Ceacero, F., Pluháčcek, J., Komárková, M., Zábransk'y, M., 2015. Pre-orbital gland opening during aggressive interactions in rusa deer (*Rusa timorensis*). Behav. Proc. 111, 51–54.
- 8. Kyle R (1990) An antelope for All Seasonings: New Scientist: 126(1711):54-57.
- Prasad, S. Ahmed, R., 2016. The Indian Antelope Nilgai (*Boselaphus tragocamelus*) for all interests; A Now hope in Indian Farming. Progressive research. An International Journal 1(sp-x): 6638-6641.
- Ortega-Santos JA, Hewitt DG, Campbell TA 2016 Nilgai in south Texas: Nuisance or Asset? Society of Range Management. 1-2. https://rangelandgedeway.org.
- Prasad, S., 2022. High Time of Pacing Nilgai Antelope (*Boselaphus tragocamelus*) into Mainstream as Community Conservation for Influencing Macroeconomics. Asian Journal of Research in Zoology. 5(1): 21-30. DOI: <u>10.9734/ajriz/2022/v5i130128</u>.

- Mallon, D. P. 2008. *Boselaphus tragocamelus*, In: IUCN 2012 Red list of Threatened species. Version 2012.2. <u>www.iucnredlist.org</u>.
- Prasad, S., Prabhakar, C.S., Sah, R.K, Kumar, A., 2019. Is domestication of Nilgai possible? Current science 116(7): 1045-1046. doi.org/10.18520/cs2Fv1162Fi72F1045-1046.
- 14. Leslie, D.M., 2008. *Boselaphus tragocamelus* (Artiodactyls: Bovidae) Mammalian species American society of Mammologist. 813: 1-16. 1–16, <u>doi.org/10.1644/813.1</u>.
- 15. Chopra G, Rai D. A 2009. Study on the ecology of Nilgai (*Boselaphus tragocamelus*, Pallas) and its status as an unconventional pest of agricultural in and around Beer-Sonty reserve forest. Haryana, India. Journal of Applied and Natural Science. 1(2):245-249.
- 16. Oguya BRO, Eltringham SK. 2009. Behavioural of nilgai (*Boselaphus tragocamelus*) antelope in captivity. Journal of Zoology. 223(1):91-102.
- 17. Bohra HC, Goyal SP, Ghosh PK, Prakash I 1992 Studies on the ethology and ecophysiology of the antelopes of the Indian Desert. Ann. Arid Zone, 31:83–96.
- 18. Kusum, 2018. Studies on, the ranging pattern and dung piles habit of Nilgai (*Boselaphus tragocamelus*) around Jodhpur, Rajasthan, India. IJRAR. 5; 555-559.
- Rajagopal T., Manimozhi A. & Archunan G. 2010. Diurnal variation in scent preorbital gland marking behaviour of captive male Indian Blackbuck (*Antelope cervicapra*) and its territorial significance. Biol. Rhythm Res. DOI:10.1080/092910110036931.
- Prasad S., Singh D. K., Kumar U., and Kumar S., 2020. Biodiversity and Ecology of Wild mammals in Dumraon, Buxar Bihar, J. Exp. Zool. India Vol. 23, No. 1, pp. 643-648, www.connectjournals.com/jez
- Altman J., 1974. Observational study of behaviour sampling methods. Behaviour. 40:227-265.
- 22. Kaufmann, B. 2024. Understanding Deer gland, Huntley llinols: Hunting Network LLC, 2024. Retrieved, 20.11.2024.
- Blakeslee, C.K., Rice, C.G., Ralls, K., 1979. Behaviour and reproduction of captive brow-antlered deer Cervus eldi thamin (Thomas1918). SäugetierkundlicheMitteilungen 27,114–127.
- 24. Azeve do Cvm, Menezes, AAL, Queiroz, J. M., Moreing, L.F.S 1996. Circadian and ultradian periodicity of grooming behaviour in family groups of common marmosets (*Callithrix facchus*) in captivity. Biol. Rhythm Res. 24(3):374-385.

- 25. Gosling LM, Roberts SC. 2001. Scent marking by male mammals: cheat proof signals to competitors and mates. Adv Study Behav. 30:169–217.
- 26. Burger, B.V. 2005. Mammalian semi chemicals" In Schutz, s(ed.). the chemistry of pheromones and other semi chemicals II (PDF). Topics in current chemistry. vol. 240. Berlin springer-Verlag. PP-231-278. doi: 10.1007/b98318.
- Bartusova-Vichova, J., Bartos, L., and Svecora. L. 2007. Preorbital gland opening in red deer (*Cervus elaphus*) calves as an indicator of stress, J. Anim. sci. 8: 494-996. doi: 10.2527/jas. 2006. 446.
- 28. Wood, W.F., 2010. "Synthesis and antimicrobial of activity of long chain 3, 4 epoxy,
 -2- alkenones" Scientia Pharmaceutica, 78(4):745-751