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Unvaccinated Goats as Sentinel Animals for foot-and-mouth disease virus surveillance at the Wildlife– Livestock Interface: Serological and Molecular Evidence

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Abstract

Foot and mouth disease (FMD) is one of the most contagious viral diseases of cloven-footed animals. Detection of FMD virus (FMDV) circulation at the wildlife–livestock interfaces remains challenging as vaccinated animals may exhibit limited or no clinical signs. Unvaccinated goats can serve as sentinel-animals for field-level evidence of FMDV circulation. A three-year cross-sectional surveillance study (2021–2023) was conducted around Sanjay Dubri and Bandhavgarh Tiger Reserve, Madhya Pradesh, India. A total of 3278 animals (2457 bovines and 821 goats) were tested for detection of antibodies against FMDV non-structural protein (NSP). Bovine sera and a subset of goat sera were additionally tested for structural protein antibodies. Oropharyngeal fluid (OPF) samples from NSP-positive animals were tested using pan-serotype RT-qPCR for detection of FMDV. NSP-Ab was detected in both vaccinated bovines and unvaccinated goats, indicating exposure to natural FMDV infection. Among goats, NSP-seropositivity declined from 14.13% in 2021 to 8.63% in 2022 and to 3.0% in 2023. A similar decline was observed in RT-qPCR positivity among NSP-positive goats, from 45.0% in 2021 to 25.45% in 2022 and to 5.55% in 2023. In bovines, protective antibody response against FMD increased progressively during the period, while the proportion of NSP-reactive/RT-qPCR-positive animals declined concurrently. Two cattle OPF derived FMDV isolates collected in 2023 when characterized genetically were clustered within serotype A/ASIA/G-18/2019 lineage while the lone NSP-positive goat was shown as FMDV serotype A-positive in RT-PCR. It is suggested that goats can serve as marker of silent FMDV circulation at the WLIs.

Highlights

• Foot and mouth disease (FMD) viral RNA & NSP Ab was detected in unvaccinated goats.

• FMD virus (FMDV) circulation persisted despite rising bovine herd immunity.

• FMD-positive goat indicated circulation of FMDV in bovine vaccinated population.

• Goat surveillance provided an unbiased indicator of natural FMDV exposure.

• Integration of goat-based FMD surveillance strengthens FMD control programme.

Introduction

Foot-and-mouth disease (FMD) is one of the most economically important diseases of cloven-footed animals. The disease is caused by FMD virus (FMDV), a positive-sense single-stranded RNA virus belonging to the genus *Aphthovirus* within the family *Picornaviridae*. Seven immunologically distinct FMDV serotypes (O, A, C, Asia1, SAT1, SAT2 and SAT3) have been described, of which serotypes O, A and Asia1 are endemic in the Indian subcontinent (Grubman and Baxt 2004; Subramaniam et al. 2022). FMD causes fever, vesicular lesions, lameness, and severe production losses (Ranjan et al. 2016). India has implemented World Organisation for Animal Health (WOAH) endorsed FMD control program, under

which cattle and buffaloes receive periodic biannual trivalent inactivated FMD vaccine containing serotypes O, A and Asia1 (Ranjan et al. 2026). Sustained vaccination has markedly reduced the frequency and severity of clinical outbreaks along with reduction of FMD non-structural protein (NSP) antibody reactors in many regions of the country (ICAR-NIFMD 2025). Therefore, FMDV surveillance becomes more significant as the disease incidences have gradually declined. Laboratory-based surveillance using FMD NSP antibody detection, FMD viral RNA detection in oropharyngeal fluid (OPF), virus characterization and phylogenetic analysis are essential components of progressive FMD control programme (Brocchi et al. 2006).

Wildlife–livestock interfaces (WLIs) are considered as one of the most intricate epidemiological settings for FMD surveillance. FMD has been reported in many wildlife species like deer, elephant and bison etc. (Biswal et al. 2015; Rout et al. 2016). Active surveillance is fundamental for assessing the effectiveness of national FMD control programme. The WLIs situated in Madhya Pradesh, India are protected forest ecosystems that harbour different FMDV susceptible wildlife species *viz.* deer, antelope, wild boar, Indian gazelle and bison. The neighbouring villages surrounding the protected forest consist of extensive livestock population *viz.* cattle, buffalo, sheep and goats. Villagers adopt open grazing system for their livestock. Therefore, livestock often enter buffer/core zones of the wildlife reserve leading to frequent interactions with wildlife species highlighting the necessity for FMD surveillance at the WLIs.

Serological investigation of antibodies (Ab) against FMDV structural proteins (SP) indicates vaccination and/or infection, whereas Abs against FMDV NSPs arises principally after FMDV replication in the host. NSP-Ab detection acts as an indicator of FMDV exposure in vaccinated population (Brocchi et al. 2006). Unlike cattle and buffalo, goats and sheep are usually not covered under regular vaccination program against FMD. Therefore, detection of NSP Abs or FMDV RNA in goats offers direct evidence of natural FMDV exposure. In addition, FMDV infection in goats is frequently asymptomatic/ subclinical. Hence they can be strategically monitored via specific laboratory methods to act as excellent markers of silent FMDV circulation (Pamia et al. 2026). Despite extensive goat population in India, relatively little attention has been given to their potential role as sentinel animals for FMD surveillance especially at the WLIs. Lack of FMD vaccination, close association with other livestock, extensive grazing behaviour and frequent movement through forest-edge habitats collectively suggest their considerable epidemiological value. The present investigation was undertaken to assess the utility of goats as sentinel animals for FMD surveillance at the WLI of Sanjay Dubri Tiger Reserve (SDTR) and Bandhavgarh Tiger Reserve (BTR) of Madhya Pradesh, India.

Materials and methods

Study area

The WLI of SDTR and BTR located in Madhya Pradesh, India was chosen as the study area (Fig. 1). These protected forest ecosystems comprise dense tropical deciduous forests supporting diverse populations of FMD susceptible wild ungulates, including chital (*Axis axis*), sambar (*Rusa unicolor*), barking deer

(*Muntiacus muntjak*), nilgai (*Boselaphustrago camelus*), wild pig (*Sus scrofa*), and gaur (*Bos gaurus*). Villages situated around the protected areas maintain mixed populations of cattle, buffalo, goat and sheep that frequently graze within forest fringes and buffer zones, resulting in repeated contact opportunities between domestic livestock and wildlife species (Ranjan et al. 2026). Such environmental conditions favour bidirectional transmission of infectious diseases, including FMDV.

Study design, animals and samples collection

A repetitive cross-sectional surveillance study was undertaken to investigate the circulation of FMDV at the WLI of SDTR and BTR from the year 2021 to 2023. Surveillance programme consisted of serological detection of FMD NSP antibodies, SP antibody titre determination against FMDV serotype O, A and Asia1 in vaccinated bovines and unvaccinated goats. Molecular detection of FMDV RNA in Oropharyngeal fluid (OPF) was done by real-time reverse transcription polymerase chain reaction (RT-qPCR). Phylogenetic analysis of FMDV isolated from OPF was carried out. A total of 3278 livestock consisting of 2457 bovines (cattle and buffaloes) and 821 goats were examined. Bovines were categorized into calves and adults, whereas all the goats included were adults. Under FMD control programme (FMDCP), bovines had received trivalent FMD vaccine against FMDV serotype O, A and Asia1, whereas goats remained unvaccinated throughout the study period. Approximately 5–10 ml of whole blood was collected aseptically from the jugular vein of each animal into sterile vacutainer tubes.

Serum was separated by centrifugation and stored at − 20°C until analysis. OPF samples were collected from FMD NSP-reactive animals using a sterile probang cup (Ranjan et al. 2018). Samples were transported under cold-chain and stored at − 80°C until RNA extraction.

Detection of antibodies against FMDV structural protein (SP) and non-structural protein (NSP)

Bovine serum samples and a subset of goat sera were analysed for detection of antibodies against FMDV O, A and Asia1 serotypes using solid-phase competitive ELISA (SPCE) (Ranjan et al. 2026). For the years 2021 and 2022, 30 FMD NSP-positive goat sera were randomly selected for SPCE analysis. In 2023, only three NSP-positive sera were available which were analysed together with 15 NSP-negative sera (total = 18) to evaluate serotype-specific antibody responses. Serum samples collected from bovine and goats were tested for antibodies against FMDV NSP using r3AB3 NSP indirect ELISA (Mohapatra et al. 2011; Rout et al. 2014). Only the NSP-positive/SPCE-positive results were considered as evidence of previous natural FMDV infection, whereas NSP-negative sera served as negative controls for retrospective serotyping.

Detection of FMD viral genome

All NSP-positive cattle and goats were screened for the presence of FMD viral genome in OPF using multiplex RT-PCR (Giridharan et al. 2005) and RT-qPCR (Biswal et al. 2023). FMD viral RNA was extracted from OPF using commercial viral RNA extraction kit (QIAamp Viral RNA Kits, Qiagen). A sample was interpreted as positive when the amplification curve exhibited a characteristic sigmoid profile with a cycle threshold (Ct) value of ≤ 40. Appropriate positive and negative controls were included in each amplification run to ensure assay reliability.

Virus isolation, VP1 sequencing and phylogenetic analysis

Rescue of infectious FMDV was carried by chemical transfection method (Ranjan et al. 2016b). Two field isolates designated as A/IND_IP2MP/08/2023 and A/IND_IP3MP/09/2023, obtained from cattle OPF collected during 2023, were selected for VP1 gene sequencing. PCR amplification of VP1 coding region of FMDV isolates were done as described by Hemadri et al (2002) using the primer combination ARS4 and NK61 (Knowles et al. 2016), with Pfu DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA). The PCR amplified products were purified using commercially available Gel Extraction Kit (QIAquick Gel Extraction Kit, QIAGEN, Hilden, Germany). Subsequently, the PCR purified product was sequenced using an ABI 3500xL 24 capillary Genetic Analyzer (Applied Biosystems, ThermoFisher) with the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, phylogenetic and genetic analyses of VP1 nucleotide sequences aligned with reference sequence of FMDV serotype A were carried out using ClustalW, and MEGA 12 software (Kumar et al. 2024). Maximum Likelihood method with 1,000 bootstrap replications was used to reconstruct the phylogenetic relationships to determine the genetic lineage of FMDV serotype A circulating in the ecosystem.

Statistical analysis

Prevalence of FMD NSP Ab, FMDV serotype-specific SP antibody, and RT-qPCR positivity were demonstrated as percentages with corresponding 95% confidence intervals (CI). Chi-square test was used to determine the differences in FMD prevalence among the years and animal categories. Cochran–Armitage trend test was used to estimate the temporal trends in prevalence. A $p\text{-value} < 0.05$ was considered statistically significant.

Results

A total of 3,278 domestic livestock (bovine and goats) were investigated during the three-year surveillance study at the WLIs. The study population comprised 2,457 bovines (1,026 calves and 1,431 adults) and 821 adult goats. Annual surveillance included 954 animals in 2021, 1,768 animals in 2022 and 556 animals in 2023. Details of the study population and laboratory findings are summarized in Table 1.

Table 1
Summary of serological and molecular surveillance of FMD virus at the wildlife–livestock interface of Sanjay Dubri Tiger Reserve and Bandhavgarh Tiger Reserve, Madhya Pradesh, India (2021–2023).

Year	Species (No. examined)	Animal category	NSP-positive n/N (%)	SPCE / Protective antibody (Serotype O) n/N (%)	SPCE / Protective antibody (Serotype A) n/N (%)	SPCE / Protective antibody (Serotype Asia1) n/N (%)	RT-qPCR-positive n/N (%)†
2021	Bovines (685)	Calves	33/369 (8.94)	16/120 (13.33)	16/120 (13.33)	20/120 (16.66)	18/40 (45.00)
		Adults	73/316 (23.10)	22/120 (18.33)	18/120 (15.00)	19/120 (15.83)	13/40 (32.50)
	Goats (269)	Adults	38/269 (14.13)	28/30 (93.33)*	0/30 (0.00)	0/30 (0.00)	18/40 (45.00)
2022	Bovines (1316)	Calves	24/505 (4.75)	71/145 (48.96)	59/145 (40.69)	61/145 (42.07)	12/66 (18.18)
		Adults	139/811 (17.14)	186/319 (58.31)	140/319 (43.89)	130/319 (40.75)	24/80 (30.00)
	Goats (452)	Adults	39/452 (8.63)	16/30 (53.33)*	0/30 (0.00)	0/30 (0.00)	14/55 (25.45)
2023	Bovines (456)	Calves	0/152 (0.00)	22/37 (59.46)	18/37 (48.65)	21/37 (56.76)	0/37 (0.00)
		Adults	64/304 (21.05)	50/77 (64.93)	40/77 (51.95)	45/77 (58.44)	2/77 (2.59)
	Goats (100)	Adults	3/100 (3.00)	0/18 (0.00)**	1/18 (5.56)**	0/18 (0.00)	1/18 (5.56)

† RT-qPCR was performed on the designated goat and bovine samples included for molecular investigation.

* For goats in 2021 and 2022, SPCE was performed on 30 randomly selected NSP-positive sera to retrospectively identify the infecting FMDV serotype

** For goats in 2023, SPCE was performed on 18 sera comprising 3 NSP-positive and 15 NSP-negative sera. The single serotype A-positive result originated from an NSP-positive goat, while all 15 NSP-negative sera were negative against serotypes O, A, and Asia1

Serological surveillance of FMD in bovines and goats

Antibodies against FMDV NSPs were detected in both vaccinated bovines and unvaccinated goats throughout the surveillance period, indicating natural exposure to FMDV. Among bovine calves, NSP

seropositivity declined significantly from 8.94% (95% CI: 6.3–12.2) in 2021 to 4.75% (95% CI: 3.1–7.0) in 2022, and no NSP-positive calves were detected in 2023 (0/152). Adult bovines consistently exhibited higher NSP seropositivity than calves, with prevalence of (23.10%), (17.14%), and (21.05%) during 2021, 2022 and 2023, respectively. Among adult goats, NSP seropositivity gradually declined from (14.13%) in 2021 to (8.63%) in 2022 and (3.00%) in 2023.

FMDV serotype-specific protective antibody responses

Protective antibody prevalence against FMDV serotype O, A and Asia 1 increased markedly both in bovine calves and adults throughout the study period. Among bovine calves, the proportion of animals with protective antibody prevalence against serotype O increased gradually from 13.33% in 2021 to 48.96% in 2022 and 59.46% in 2023. A similar upward trend was observed for serotype A, with protective antibody prevalence increasing from 13.33% in 2021 to 40.69% in 2022 and 48.65% in 2023, and for serotype Asia1, increasing from 16.66% to 42.07% and 56.76%, respectively. Adult bovines showed comparable trends with protective antibody prevalence against FMDV serotype O increased from 18.33% in 2021 to 58.31% in 2022 and 64.93% in 2023. For serotype A, the corresponding prevalence increased from 15.00% to 43.89% and 51.95%, while for serotype Asia1, it increased from 15.83% to 40.75% and 58.44%, respectively. These findings indicate progressive increase in vaccine-induced herd immunity among bovines following repeated FMD vaccination campaigns implemented under the national programme.

Retrospective testing of selected goat sera using SPCE showed that FMDV serotype O was the most prevalent serotype responsible for natural infection. In 2021, Abs against serotype O was detected in 28/30 (93.3%) NSP-positive goat sera, declining to 16/30 (53.3%) in 2022, while no Abs against serotypes A or Asia1 were detected in either year. In 2023, SPCE analysis of 18 goat sera, comprising 3 NSP-positive and 15 NSP-negative, identified Abs against serotype A in one NSP-positive goat with, while all samples were negative for serotypes O and Asia1. Overall, 44 of 60 (73.3%) NSP-positive goat sera were positive for serotype O and 1 of 3 (33.3%) NSP-positive sera collected in 2023 showed evidence of serotype A, with no evidence of serotype Asia1 infection throughout the study. RT-qPCR results supported the serological findings, with viral RNA detected in 18/40 (45.0%), 14/55 (25.5%), and 1/18 (5.6%) goats during 2021, 2022, and 2023, respectively.

Detection of FMDV RNA among NSP-reactive animals

All RT-qPCR positive samples were subjected to chemical transfection and genome detection. Only two FMDV field isolates were rescued from the OPF collected from the bovine and no virus was rescued from the goat. RT-qPCR detected FMD viral RNA among NSP-positive animals in both vaccinated bovines and unvaccinated goats, confirming active or recent viral replication within the study population. Among bovine calves, RT-qPCR positivity declined gradually from 18/40 (45.00%) in 2021 to 12/66 (18.18%) in 2022, and no positive calf was detected in 2023 (0/37). Adult bovines demonstrated RT-qPCR positivity of 13/40 (32.50%), 24/80 (30.00%), and 2/77 (2.59%) during 2021, 2022 and 2023, respectively. Among

adult goats, RT-qPCR positivity declined from 18/40 (45.00%) in 2021 to 14/55 (25.45%) in 2022 and 1/18 (5.55%) in 2023.

Phylogenetic characterization of circulating FMDV

VP1 nucleotide sequence was determined for two bovine OPF FMDV isolates designated as A/IND_IP2MP/08/2023 and A/IND_IP3MP/09/2023 (Fig. 2). Maximum-likelihood phylogenetic analysis based on VP1 region demonstrated that both isolates clustered within the A/ASIA/Genotype G-18/2019 lineage of FMDV serotype A together with recently circulating Indian field isolates (Fig. 2). Bootstrap analysis provided strong statistical support for clustering of the isolates with contemporary field strains, indicating that viruses circulating in the SDTR–BTR landscape belong to the currently predominant lineage responsible for recent outbreaks in India. Although goat-derived samples were not sequenced, the concurrent detection of FMDV RNA in unvaccinated goats confirmed that natural FMDV infection occurred within the same WLI during the study period. The prevalence of FMDV NSP antibodies differed significantly among surveillance years for bovine calves and goats (Chi-square trend test, $p < 0.05$) whereas no consistent temporal trend was observed among adult bovines. Similarly, RT-qPCR positivity among NSP-reactive goats gradually declined significantly between 2021 and 2023 **(**$p < 0.05$**)**. Vaccinated bovines demonstrated significantly higher serotype-specific protective antibody prevalence in 2022 and 2023 than in 2021 $p < 0.001$**).**

Discussion

The present study provides serological and molecular evidence of FMDV circulation in livestock present in the buffer/ core area of SDTR and BTR, Madhya Pradesh, and highlights the potential role of unvaccinated goats as sentinel animals in the WLIs. The strategic surveillance revealed distinct epidemiological information from two livestock populations. Vaccinated bovines primarily provided information on FMD vaccine-induced herd immunity through SPCE, while NSP ELISA and RT-qPCR identified animals that had experienced natural FMDV infection despite vaccination. In contrast, goats were not vaccinated and therefore all NSP-positive or RT-qPCR-positive goats provided evidence of natural FMDV exposure despite the absence of clinical signs of FMD. Antibodies against FMDV NSPs were detected throughout the surveillance period in both vaccinated bovines and unvaccinated goats, confirming that natural virus exposure occurred at the WLIs. The presence of FMDV NSP antibodies in vaccinated bovines especially in calves indicated FMD infection despite vaccination, whereas NSP antibodies detected in goats represented natural FMDV exposure/infection since goats were unvaccinated at the WLI ecosystem. Since repeated vaccination of bovines can reduce the utility of clinical disease alone as an indicator of FMDV circulation (Anusha et al. 2026) especially when NSP contaminated vaccine is used (Hayer et al. 2018) unvaccinated goats are potentially valuable for surveillance under this scenario.

There was a marked increase in the protective Ab responses against FMDV serotypes O, A and Asia1 in the vaccinated bovines from 2021 to 2023 while the retrospective SPCE analysis showed a clear temporal shift in the serotypes associated with natural FMDV infection in unvaccinated goats. During

2021 and 2022, antibodies were detected exclusively against FMDV serotype O, indicating that this serotype dominated among naturally infected goats at the WLI. In contrast, SP-Ab against FMDV serotype A was only detected during 2023 in one of the three NSP-reactor goats which suggest sporadic circulation of this serotype within the area. The gradual decline in RT-qPCR positivity for FMDV and the parallel increase in protective immunity suggest an overall reduction in FMDV circulation at the WLI. Detection of FMDV RNA among NSP-positive goats provides an additional layer of epidemiological information that may not be noticed through routine clinical reporting and signifies that goats can provide independent evidence of virus circulation even when FMD vaccination has substantially increased herd immunity among bovine population.

Phylogenetic analyses of two cattle-derived isolates demonstrated that FMDV circulating at the WLI belonged to the contemporary serotype A lineage A/ASIA/Genotype G-18/2019. This lineage was first detected in India in 2019 and remained the predominant serotype A lineage since its emergence. The phylogenetic finding confirms that the field viruses isolated in the present study are genetically related to currently circulating FMDV serotype A. Although sequencing was not performed on goat-derived samples, detection of FMDV RNA in goats, together with the genetic characterization of cattle derived isolates, provides evidence of natural FMDV transmission within the same epidemiological landscape. However, these findings should be interpreted cautiously: phylogenetic analysis establishes the lineage of the virus detected in cattle but does not demonstrate that the goat-derived viruses belonged to the same lineage as sequence data from goat samples were unavailable. Future investigations incorporating systematic wildlife sampling together with whole-genome sequencing would be needed to elucidate FMDV transmission pathways among wildlife, goats and bovines.

Conclusions

The study demonstrated that goat-based surveillance can identify silent FMDV circulation even when FMD prevalence is significantly low. Integration of NSP serology, molecular diagnosis and epidemiology could augment FMD surveillance and contribute to effective FMD control in India.

Declarations

Conflict of interest

The authors declare no competing interests.

Ethical approval

The study was conducted as per the Institutional Animal Ethics Committee (IAEC) protocol (P13-03/2023)

Author Contribution

Rajeev Ranjan: Conceptualization, methodology, investigation, formal analysis, data curation, funding, visualization, writing-original draft; Himanshu R Joshi: Resource, Methodology, Investigation, writing-review & editing; Vinay Pandey: Investigation, writing- review & editing; Abhay Sengar: Resources, Investigation, writing- review & editing; Nitin Gupta: Resources, Investigation, writing- review &editing; Samarendra Das: Statistical analysis, Software, Writing- review &editing; Jitendra Kumar Biswal: Methodology, analysis, writing- review & editing; Smrutirekha Mallick: Methodology, analysis, writing-review & editing; Saravanan Subramaiam: analysis, supervision, writing- review & editing.

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Data Availability

Data are available from the corresponding author upon reasonable request.

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Figures

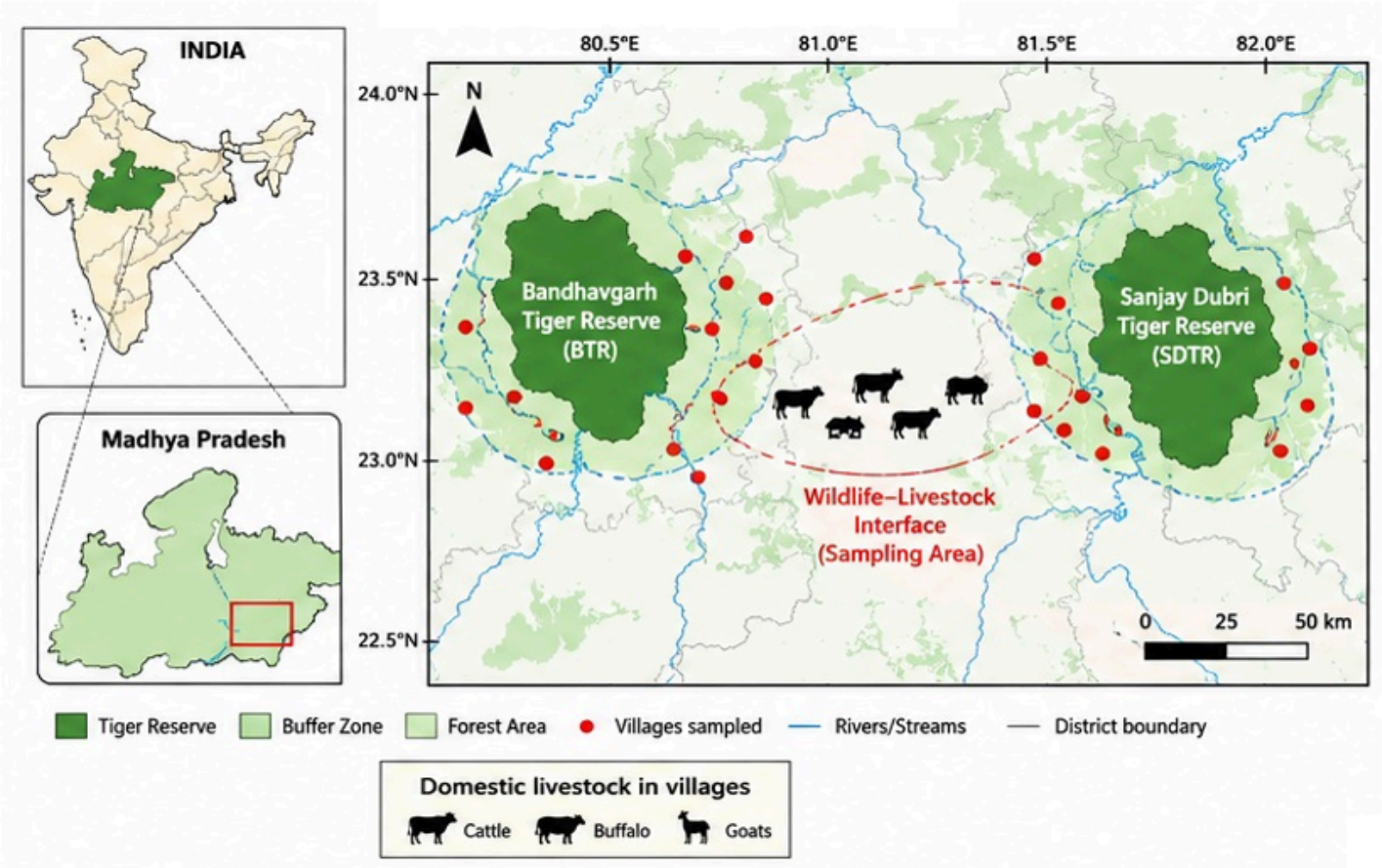


Figure 1

Wildlife-livestock interface area of Sanjay Dubri Tiger Reserve and Bandhavgarh Tiger Reserve, Madhya Pradesh, India selected for foot-and-mouth disease virus surveillance

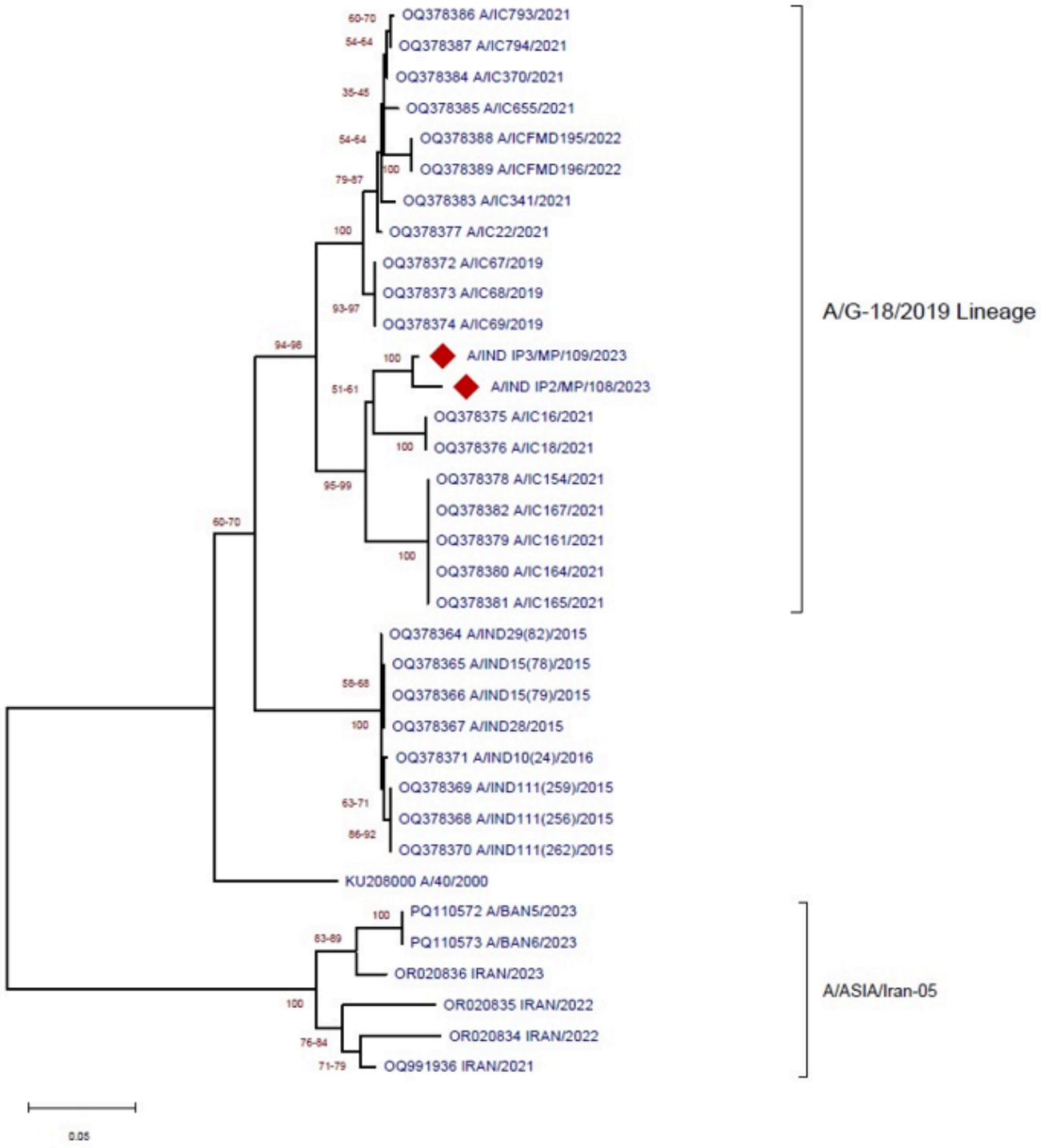


Figure 2

Fig. 3 Maximum-likelihood phylogenetic tree based on VP1 nucleotide sequences showing the relationship of cattle-derived isolates A/IND_IP2/MP/108/2023 and A/IND_IP3/MP/109/2023 with representative FMDV serotype A reference strains. Both isolates clustered within the Genotype G-18 (non-deletion 2019 lineage)