



Longitudinal study on foot and mouth disease virus in *Arunachali* yak and yak – cattle hybrid

MOKHTAR HUSSAIN¹✉, SNEHA BHOWMICK¹, DINAMANI MEDHI¹, S S BEGUM³, S SUBRAMANIAM², R P SINGH² and MIHIR SARKAR¹

ICAR-National Research Centre on Yak, Dirang, Arunachal Pradesh 790 101 India

Received: 3 October 2024; Accepted: 2 December 2024

ABSTRACT

This is the first longitudinal report on the *Arunachali* yak and yak-cattle hybrids of India, exhibiting the prevalence of antibodies against the structural proteins (SPs) and non-structural proteins (NSPs) of foot and mouth disease (FMD) virus from 2020 to 2023 in the West Kameng district of Arunachal Pradesh. Of the 1,160 samples, 145 (12.5%) were tested positive for anti-NSP antibody detection. The results showed a declining trend in seroprevalence, with rates of 56.36% in 2020, 44.16% in 2021, 13.76% in 2022, and 3.24% in 2023. Additionally, anti-SP antibody test demonstrated a sharp increase in protective antibody titer in both pre- and post-vaccinated animals starting in 2022. The analysis revealed protection levels of 73.73% (serotype O), 68.95% (serotype A), and 68.65% (serotype Asia 1). Additionally, following multiple vaccination rounds, the percentage of antibodies that are considered protective increased significantly, rising from 31.48% in 2021 to 99.85% in 2023. These findings suggest that while immunity to all serotypes improved over time, NSP seroprevalence remained low with no evidence of an outbreak or clinical infection. Although yak species are not currently covered under the National FMD Control Programme, those kept in organized farms are regularly vaccinated. This vaccination effort needs to be extended to field populations to better protect these species.

Keywords: Arunachal Pradesh, *Arunachali* yak, DIVA, Foot and Mouth disease, SPC-ELISA

Rearing *Arunachali* yak and yak-cattle hybrid is a significant source of agricultural production in Arunachal Pradesh and plays a vital role in the socio-economic growth of the farming community. Foot and Mouth disease (FMD), is endemic in many parts of the world and is highly contagious viral illness caused by the *Aphthovirus* genus of the *Picornaviridae* family, posing a threat to cloven-hoofed livestock (Grubman and Baxt 2004). Recovered cattle may also experience long-lasting physical weakness, preventing them from re-entering the economic production cycle (Azimi *et al.* 2023). In India, the ‘National FMD Control Programme’ (FMD-CP) controls the disease through prophylactic bi-annual mass vaccination (Subramaniam *et al.* 2013). The short-lived humoral immunity induced by inactivated vaccines necessitates regular vaccination of susceptible animal populations (Knight-Jones *et al.* 2015). The success of the disease control program depends not only on vaccination with high-quality vaccines but also on effective sero-surveillance activities (Arjkumpa *et al.* 2020). FMD sero-surveillance using serological test, i.e. 3AB3 non-structural protein (3AB3-NSP) ELISA has been

carried out regularly since 2008 (Tewari *et al.* 2021). The presence of NSP-antibodies is considered a differential marker for infection in vaccinated populations (Hayer *et al.* 2018). Currently, India is home to approximately 58,000 yaks, with around 24,700 of them living in Arunachal Pradesh. Of the roughly 2,500 Brokpas nationwide, about 1,200 live in this state (Shraddha 2024). Herd immunity not only protects vaccinated animals but also indirectly benefits unvaccinated animals by reducing the virus transmission rate, making it more critical than individual protection in FMD affected regions (Fine *et al.* 2011). In India, sero-monitoring by estimating antibody titers against structural proteins of FMDV serotypes O, A and Asia 1, has been carried out using Solid Phase Competitive ELISA (SPCE) developed by ICAR-National Institute of FMD, Bhubaneswar.

Since, the only published data available on seroprevalence, is the test conducted by Rout *et al.* (2017) in 2015–2016. Hence, to address the FMDV infection and vaccination status, a longitudinal study was conducted from 2020 to 2023 in the livestock-wildlife interface areas of the West Kameng district of Arunachal Pradesh.

MATERIALS AND METHODS

Study area and sample handling: Over the course of four years (2020 to 2023), a total of 1,467 serum

Present address: ¹ICAR-National Research Centre on Yak, Dirang, Arunachal Pradesh; ²ICAR-National Institute on Foot and Mouth Disease, Arugul, Bhubaneswar; ³College of Veterinary and Animal Sciences, Arrabari, Kishanganj, Bihar. ✉Corresponding author email: hussainmokhtar61@gmail.com

samples for seromonitoring and 1,160 serum samples for serosurveillance were obtained from clinically healthy animals in different villages of West Kameng district. Blood samples were obtained from yak and yak-cattle hybrids (dzo and dzomo), with sample selection based on the availability of animals, geographic location and proximity. The samples were collected from the ICAR-NRC-maintained Nyukmadung Yak farm, (9,000 feet above mean sea level (msl)), and also from eight different yak-rearing tracts in West Kameng district: Chug (5,000 feet above msl), Thembang (9,000 feet above msl), Mandala (10,000 feet above msl), Lubrang (5,000 feet above msl), Khagoksa (5,000 feet above msl), Sela (13,7000 feet above msl), Dirang (4,910 feet above msl) and Nagagiji (10,500 feet above msl). Blood samples were taken from jugular veins of livestock using 18G needles. 2.0 ml aliquots of serum sample were prepared and stored at -20°C for future analysis.

Serological analysis: DIVA: An indirect ELISA was performed to detect antibodies against the FMDV 3AB-NSP protein, following the method described by Mohapatra *et al.* (2011). The indigenously developed rDIVA-FMD kit, designed according to OIE-approved guidelines, has been widely used for mass-scale comprehensive sero-surveillance in India. The reactivity of anti-3AB3 antibodies in the serum of an infected bovine animal is measured against purified recombinant 3AB3 NSP in an indirect ELISA format. A sample yielding an OD (optical density) value higher than the fixed cut-off ratio $\{(test\ serum\ sample\ mean\ OD/positive\ control\ serum\ mean\ OD) \times 100, or\ percent\ positivity\ (PP)\ value > 40\%\}$ is qualitatively diagnosed as positive for FMD virus infection.

Solid Phase Competitive ELISA (SPCE): The SPCE developed by ICAR-NIFMD, Bhubaneswar, is used for quantifying protective antibody titers in animals following FMD vaccination. If antibodies against the structural protein of FMDV are present in the test serum, they block the FMDV antigen, inhibiting the binding of homologous anti-FMDV guinea pig serum. This antibody inhibition is detected by adding a pre-titrated anti-guinea pig-HRPO conjugate and substrate, following the standard ELISA process. The protective antibody level is expressed as a \log_{10} titer, with a titer ≥ 1.65 (corresponding to the highest serum dilution resulting in 35% inhibition of OD as observed in the antigen control) is considered protective.

RESULTS AND DISCUSSION

Sero-prevalence of FMD: A total of 1,160 serum samples, collected over four years were analyzed using

the DIVA-ELISA to detect NSP antibodies. Of these 145 samples, 12.50%, tested positive for the 3AB3 NSP. Similar studies can be seen in Nepal (15.07%) (Dhakal *et al.* 2023) and Libya (19%) (Eldaghayes *et al.* 2017). Throughout the survey, the percentage of positive DIVA samples fluctuated from 3.24 % to 56.36% across the years (Table 1). The trend indicates a decline in positivity over the past four years, likely due to regular public awareness and vaccination programs. The higher sero-prevalence rate observed in 2020 and 2021 may be attributed to lack of awareness, lower vaccination coverage, or a smaller sample size that were included in the FMD-CP until 2022. However, as more villages were covered under the FMD-CP, seroprevalence rates gradually decreased in the year 2023. This decline can be linked to the expansion of the mass vaccination program under the NADCP, which covered all susceptible animals against FMD. In 2023, the NSP seroprevalence rate dropped to 3.24%, indicating fewer FMDV outbreak and suggesting an efficient control mechanism and robust serosurveillance system. However, the occurrence of DIVA positive cases in apparently healthy yak and its hybrids without clinical signs is concerning and warrants further investigation. Additionally, the close contact between migratory herds of domesticated cattle and these bovine species may facilitate virus transmission.

The outcomes of sero-surveillance are largely reliant on prior natural infections, which lead to the creation and persistence of NSP-Abs in susceptible population. Regarding wildlife, it has been documented that FMDV can persist in African buffalo for upto 5 years (Alexandersen *et al.* 2002). Therefore, high positive rate in 2020, may suggest that FMDV can persist for a very long time in yak and its hybrids. Additionally, high positive rate also reflects inadequate vaccination intensity, topological inaccessibility, unreliable sample collection and narrow surveillance in 2020. After that, sero-prevalence rates steadily decreased (by as much as 15%) as more villages were included in the FMDCP. Factors such as herd size, pasture, and water availability likely contribute to the observed variation in FMDV infection status. This high positivity (56.36%) in 2020 align with research conducted in India (Krishnamoorthy *et al.* 2022), Nigeria (Lazarus *et al.* 2012) and in Ethiopia (Bandaw *et al.* 2024) showing 45.00%, 72.60% and 46.88% positivity respectively. However, lower seroprevalence of FMD in 2023 (3.24%) were consistent with prior reports: 2.74% in Indian Elephants (Rout *et al.* 2023), 8.9% in South Omo Zone cattle (Molla *et al.* 2010) and 14.90% in Ethiopian cattle (Tesfaye *et al.* 2016). The NSP-Ab prevalence for the

Table 1. Trend of DIVA-positive sample

Year	No. of samples tested for DIVA	No. of positive samples	% of positive samples
2020	55	31	56.36%
2021	120	53	44.16%
2022	276	38	13.76 %
2023	709	23	3.24 %
Total	1,160	145	12.5%

randomly sampled yak and mithun population in Arunachal Pradesh was 25.12% (in yaks) and 2.27% (Mithun) during 2015-16 (Rout *et al.* 2017). It is also noteworthy that regions with DIVA-positive results did not report any clinical cases of FMD in yak.

Sero-monitoring of FMD: To effectively control FMD, several key components are essential, including systematic vaccinations, post-vaccination sero-monitoring, and active as well as passive disease surveillance. Post-vaccination sero-monitoring is vital for assessing the level of protection at both the individual animals and herds/farms (Leon *et al.* 2009 and Caporale *et al.* 2012). In West Kameng district of Arunachal Pradesh, the fourth round of vaccinations has been successfully completed, with proper documentation. The SPCE results showed that, out of 797 pre-vaccination and 670 post-vaccinated sera samples, 55.33%, 47.42% and 48.68% demonstrated antibody titers greater than log₁₀ 1.65 in pre-vaccination samples while 73.73%, 68.95%, and 68.65% did so in post-vaccinated sera samples of serotype O, A and Asia 1 respectively from 2021 to 2023 (Fig. 1).

vaccination. Additionally, most of the samples collected after 2022 were from organized farms, wherein regular vaccination and awareness programmes are being practised. Thus, the samples from these farm showed good protective titre against FMDV serotypes O, A and Asia 1, indicating almost 100% vaccination in 2023 (Table 2).

The number of yak and yak-cattle hybrids vaccinated against FMD has significantly increased since the launch of the “FMD Control Program” by the Government of India, which has had a significant impact due to well-structured vaccination strategy. The trend of DIVA-positive cases has also declined over the past four years, mirroring the serological status of FMD in cattle and buffalo in the Andaman and Nicobar Islands (Sunder *et al.* 2015).

Despite extensive knowledge about the virus, disease and FMD vaccines, FMD remains a significant threat to the global livestock industry. Various factors, such as local customs, religious practices, and trade of live animals in local markets, pose major barriers to controlling this disease (Oyewusi *et al.* 2015). And certain limitations like cold chain maintenance, challenges in handling wild yak

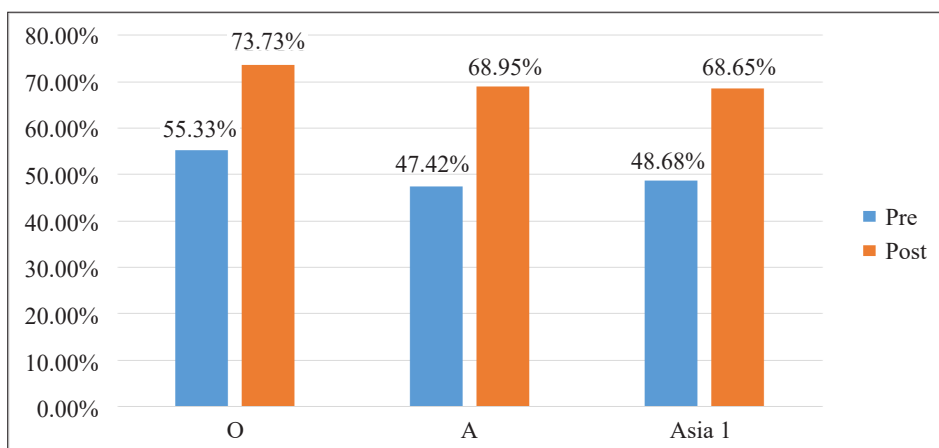


Fig. 1. Percentage of protective antibodies of each serotype, pre- and post-vaccination

The increase in overall protection across all three serotypes (Table 2) can be attributed to repeated vaccination, as indicated by field data and SPCE test results. Furthermore, the proportion of antibodies deemed protective rises dramatically after several vaccine rounds, from 31.48% in 2021 to 99.85% in 2023 (Fig. 2).

These findings suggest that the biannual vaccination practice has been effective in enhancing vaccine-induced immunity in yak and yak-cattle hybrids. The high level of herd immunity observed in these species is particularly significant, as most are routinely subjected to FMD

during vaccination, semi-wild behaviour, harsh weather conditions in high altitude areas, antigenic match between heterologous circulating field strain and vaccine, the regime (timing, frequency, and herd-level coverage) used to deliver the vaccine etc. are possible demur in Arunachal Pradesh. As the socio-economic landscape evolves, it is becoming more important to focus on preserving yak and yak cattle hybrids, which play a crucial role as farm animals in high-altitude areas.

In conclusion, yaks and yak-cattle hybrids face significant challenges due to infectious diseases like FMD,

Table 2. Pre-vaccination & post-vaccination sero-monitoring result

Year	Pre-vaccination			Post vaccination		
	O	A	Asia 1	O	A	Asia 1
2021	21.97%	8.96%	11.21%	39.89%	28.28%	26.26%
2022	32.50%	22.47%	22.09%	75.94%	72.15%	73.41%
2023	98.36%	97.06%	99.02%	99.69%	99.82%	99.57%

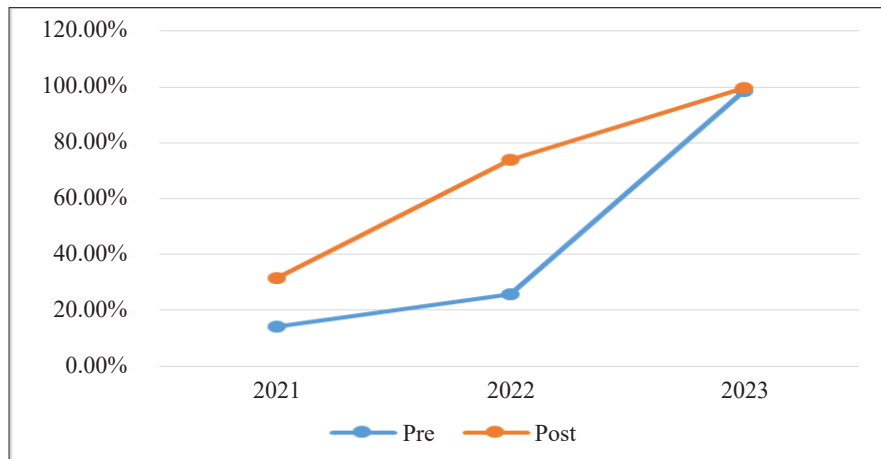


Fig. 2. Overall trend of FMD vaccinal antibody response in yak and yak-cattle hybrids.

which is often transmitted through close contact. To mitigate this risk, it is crucial to maintain a safe distance between yak and other species. Routine prophylactic immunization against FMD is essential for safeguarding these animals, thereby improving the livelihoods of tribal communities in hilly regions through enhanced livestock protection.

To better understand the epidemiology of FMD and protect these rare and threatened species in the remote, high-altitude region of north-eastern regions India, particularly in Arunachal Pradesh, more field studies, genomic research, and serological investigations are needed. Identifying the factors influencing disease spread and gaining a deeper understanding of FMD subtype circulation are key to control the disease. Effective control of the disease requires regular vaccination with higher coverage under endemic settings. Additionally, improving and expanding veterinary infrastructure and services by adopting and applying the advanced technology and scientific management practices, are crucial for conserving these economically significant species, which hold cultural importance for tribal herders in the high-altitude yak tract of India.

ACKNOWLEDGEMENTS

The authors thank DAHD-LHDCP, Government of India for providing the necessary funding support in carrying out this study under LHDCP on FMD project at ICAR-NRC on Yak, Dirang.

REFERENCES

- Alexandersen S, Zhang Z and Donaldson A I. 2002. Aspects of the persistence of foot-and-mouth disease virus in animals-the carrier problem. *Microbes and Infection* **4**(10): 1099-1110.
- Arjkumpa O, Yano T, Prakotcheo R, Sansamur C and Punyapornwithaya V. 2020. Epidemiology and national surveillance system for foot and mouth disease in cattle in Thailand during 2008–2019. *Veterinary Sciences* **7**: 99.
- Azimi S M, Mohammadian B and Khezri M. 2023. Seroprevalence of antibodies to non-structural protein of foot and mouth disease virus in vaccinated dairy cattle. *Veterinary Research Forum* **14**(12): 681-84.
- Bandaw T, Gebremeskel H F, Muluneh A, Mengistu T S and Kebede I A. 2024. Seroprevalence and molecular detection of foot and mouth disease virus in cattle in selected districts of Wolaita Zone, Southern Ethiopia. *Scientific Reports* **14**(1): 7929.
- Caporale V, Giovannini A and Zepeda C. 2012. Surveillance strategies for foot and mouth disease to prove absence of disease and absence of viral circulation. *Revue Scientifique et Technique* **31**: 747–59.
- Dhakal S P, Pandey K, Upadhyaya M, Karki S, Ramdam N, VanDyk S and Premashtira S. 2023. Spatiotemporal distribution of foot-and-mouth disease in Nepal between 2019 and 2021. *Animal Diseases* **3**: 39.
- Eldaghayes I, Dayhum A, Kammon A, Sharif M, Ferrari G, Bartels C, Sumption K, King D P, Grazioli S and Brocchi E. 2017. Exploiting serological data to understand the epidemiology of foot-and-mouth disease virus serotypes circulating in Libya. *Open Veterinary Journal* **7**(1): 1-11.
- Fine P, Eames K and Heymann D L. 2011. Herd immunity: a rough guide. *Clinical Infectious Diseases*. **52**(7): 911-16.
- Grubman M J and Baxt B. 2004. Foot-and-mouth disease. *Clinical Microbiology Reviews* **17**: 465–93.
- Hayer S S, VanderWaal K, Ranjan R, Biswal J K, Subramaniam S, Mohapatra J K, Sharma G K, Rout M, Dash B B, Das B, Prusty B R, Sharma A K, Stenfeldt C, Perez A, Delgado A H, Sharma M K, Rodriguez L L, Pattnaik B and Arzt J. 2018. Foot-and-mouth disease virus transmission dynamics and persistence in a herd of vaccinated dairy cattle in India. *Transboundary and Emerging Diseases* **65**: e404–15.
- Knight-Jones T J D, Bulut A N, Gubbins S, Stark K D, Pfeiffer D U, Sumption K J and Paton D J. 2015. Randomised field trial to evaluate serological response after foot-and-mouth disease vaccination in Turkey. *Vaccine* **33**(6): 805–11.
- Krishnamoorthy P, Karthika N, Sangeetha T R, Suresh K P, Sridevi R and Shome B R. 2022. Foot and mouth disease prevalence in cattle and buffaloes from India determined by systematic review and meta-analysis. *Indian Journal of Animal Sciences* **92**(6): 682-92.
- Lazarus D D, Schielen W J G, Wungak Y, Kwange D and Fasina F O. 2012. Sero-epidemiology of foot-and mouth disease in some Border States of Nigeria. *African Journal of Microbiology Research* **6**(8): 1756-61.
- Leon E A, Stevenson M A, Fernandez D, Robiolo B, Aznar M N, Duffy S J, Spath E J A and La Torre J. 2009. Serological evaluation of a foot-and-mouth vaccination campaign in young cattle In Buenos Aires Province, Argentina. Proceedings of the

- 12th International Symposium on Veterinary Epidemiology and Economics (ISVEE). Available at www.sciquest.org.nz (accessed August 1, 2024).
- Mohapatra J K, Pandey L K, Sanyal A and Pattnaik B. 2011. Recombinant non-structural polyprotein 3AB-based serodiagnostic strategy for FMD surveillance in bovines irrespective of vaccination. *Journal of Virological Methods* **177**: 184–92.
- Molla B, Ayelet G, Asfaw Y, Jibril Y, Ganga G and Gelaye E. 2010. Epidemiological study on foot-and-mouth disease in cattle: Seroprevalence and risk factor assessment in South Omo Zone, South-western Ethiopia. *Transboundary and Emerging Diseases* **57**(5): 340–47.
- Oyewusi I K and Talabi A O. 2015. Control strategies for foot and mouth disease with particular reference to Nigeria. *African Journal of Livestock Extension* **15**(1): 9-17.
- Rout M, Bhattacharya D, Prusty B R, Chatterjee N, Bera A K, Doley J, Deori S, Medhi D, Bam J, Hanah S S, Deb S M and Jamal. 2017. Foot and mouth disease virus infection-specific non-structural protein antibodies detected in population of Mithun (*Bos frontalis*), yak (*Bos grunniens*) and their hybrids maintained in farms and villages of Arunachal Pradesh, India. *Indian Journal of Animal Sciences* **87**(9): 1090–91.
- Rout M, Deka P, Nair N S, Karikalan M, Manjunatha V, Sahoo N, Sharma A K, Mohapatra J K and Singh R P. 2023. Serological evidence of foot-and-mouth disease virus non-structural protein antibodies in Indian Elephants (*Elephas maximus indicus*). *Journal of Animal Research* **13**(5): 833-37.
- Shraddha Warde. 2024. Arunachal Pradesh to promote yak milk products. Nuffoodspectrum. Available at: <https://nuffoodspectrum.in/2024/02/23/arunachal-pradesh-to-promote-yak-milk-products.html>. (Accessed at: November, 19 2024).
- Subramaniam S, Pattnaik B, Sanyal A, Mohapatra J K, Pawar S S, Sharma G K, Das B and Dash B B. 2013. Status of foot-and-mouth disease in India. *Transboundary and Emerging Diseases* **60**:197e203.
- Sunder J, Balasundaram S K, Sharma G and Pattnaik B. 2015. Serological status of foot and mouth disease in cattle and buffalo of Andaman and Nicobar Islands of India. *Advances in Animal and Veterinary Sciences* **3**(8): 461-65.
- Tesfaye A, Mengistu A and Rufael T. 2016. Sero-prevalence status of foot and mouth disease in the North Western Amhara Regional State, Ethiopia. *Ethiopian Veterinary Journal* **20**(2): 43-53.
- Tewari A, Ambrose H, Parekh K, Inoue T, Guitian J, Nardo A D, Paton D J and Parida S. 2021. Development and validation of confirmatory foot-and-mouth disease virus antibody ELISAs to identify infected animals in vaccinated populations. *Viruses* **13**: 914.