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Spatiotemporal distribution of foot-and-mouth disease in Nepal between 2019 and 2021

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Abstract

Foot-and-mouth disease (FMD) is a highly contagious viral disease affecting cloven-hoofed livestock. It is caused by the FMD virus (FMDV), which has seven distinct serotypes (O, A, C, SAT I, SAT II, SAT III, and Asia 1). In Nepal, FMD is a prevalent and economically important livestock disease, with hundreds of outbreaks yearly across different regions. However, there is limited understanding of the recent epidemiological trends of FMD in the past few years in Nepal. This study aims to analyze the spatial and temporal distribution of FMD in Nepal from 2019 to 2021. The FMD and TADs Investigation Laboratory, under the Government of Nepal, conducts annual risk-based surveillance of FMD in the country. The nonstructural protein (NSP) serosurveillance and serotyping (for outbreak confirmation) data from this laboratory were used for the study. The samples were collected either by the laboratory staff or were sent to the laboratory. Data analysis and mapping were performed using Epi info version 7.2.5.0 and QGIS version 3.22.5, respectively. Our findings revealed that 37.65% of samples ($n=417$) tested positive for serotyping. The highest number of positive cases occurred in March–April, followed by December. Geographically, the Terai region had the most positive cases, followed by hills and mountains. The positivity rate for serotyping did not significantly vary by animal species ($p>0.05$). Serotype O was the dominant serotype in all years, accounting for 98% of cases, while serotype A was found in only 2% of serotype-positive samples. In NSP serosurveillance, out of 3216 samples tested, 15.07% (474/3146; 95% CI, 13.86–16.36) tested positive. NSP seropositivity varied significantly by year ($p<0.001$) but not by eco-zone ($p>0.05$). In conclusion, FMD remains endemic in Nepal, with a consistent epidemiological pattern except that the Asia 1 serotype was not detected in the past years. We recommend expanding FMD surveillance activities to high-risk areas and collecting data on potential risk factors driving FMD infection in the country. This will enable the implementation of suitable control measures.

Keywords FMD, NSP serosurveillance, Serotyping, Transboundary animal diseases, Economics, Nepal

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Introduction

Foot-and-mouth disease (FMD) is a highly contagious viral disease of cloven-hoofed livestock and wild animals (Samuel and Knowles 2001). It is caused by FMD virus (FMDV) of the Aphthovirus genus of the *Picornaviridae* family (Grubman and Baxt 2004). The virus has seven genetically distinct serotypes (O, A, C, South African Territories (SAT) I, SAT II, SAT III, and Asia 1). Outbreaks of FMD have occurred in most countries worldwide with variations in the serotype distribution, and it is included as a listed disease by the World Organization for Animal Health (WOAH). Viral serotypes O, A and C are widely distributed in Europe, America, Asia and Africa, while the SAT I, SAT II and SAT III serotypes are limited to sub-Saharan Africa, with some exceptions (Jamal and Belsham 2013). FMD causes enormous economic losses due to decreased milk production owing to the loss of productivity of the affected animals and losses associated with treatment costs and trade barriers; however, the mortality in adult animals is generally low (1–5%) (Foot and mouth disease, 2023).

In Nepal, the livestock sector is an important subsector of agriculture. It contributes to approximately 11.5% of the total national gross domestic product (GDP) and 25.7% of the agricultural GDP (AGDP) as per the livestock census (2018/19) (Poudel et al. 2020). Nepal's animal population comprises approximately 7.41 million cattle, 5.13 million buffalo, 0.773 million sheep, 13.9 million goats, 1.5 million pigs, and 62 thousand yak/Nak (Ministry of Agriculture and Livestock Development 2023). Unfortunately, the livestock sector in Nepal is hindered by different endemic and emerging animal diseases along with other management problems and the unavailability of fodder and feed. Each year, hundreds of FMD outbreaks are reported throughout the seasons in all geographic regions of Nepal (Veterinary Epidemiology Centre 2016). Nepal formulated the National Control Strategic Plan for Foot-and-mouth disease for the phasewise control of FMD in the country and designated FMD as a notifiable animal disease in 2019. The government imports FMD vaccines and distributes them to the provinces based on disease risk. The Foot-and-mouth Disease and Transboundary Animal Diseases (FMD and TADs) Investigation Laboratory is the national laboratory for FMD diagnosis in Nepal and is also responsible for the serological monitoring of vaccinated animals and active surveillance for FMD.

According to format of the REF (Gongal and Shrestha 2002; Adhikari et al. 2018; Upadhyaya et al. 2020), FMD is endemic in Nepal, with outbreaks caused by serotypes O, Asia 1 and A (Jha 2012; Adhikari et al. 2018). Since 1965, Nepal has reported the presence of four serotypes of FMD virus: O, A, C, and Asia 1 (Ferris et al. 1992). However, serotype C was not detected in Nepal after 1996 as per the Department of Livestock Service's technical report, 2015 (Adhikari et al. 2018). FMD has been reported throughout the year, with a higher incidence during the monsoon period (April–May) and postmonsoon period (October–November, Ferris et al. 1992; Gongal and Shrestha 2002; Veterinary Epidemiology Centre 2016). Giri and Parshin studied the different FMD virus serotypes prevalent in Nepal with seasonal variation in FMD outbreaks in different species of animals in different regions and ecozones between 2000 and 2007 (Giri et al. 2010). Similarly, Adhikari analyzed the distribution of FMD outbreaks between 2010 and 2015 in cattle, buffalo, goats, sheep, swine, and yak in different ecozones of Nepal (Adhikari et al. 2018) . However, the recent epidemiological patterns of FMD since 2015 have not been studied. There may be some changes in FMD outbreaks in the country after implementation of vaccination for control of the disease and strict requirements of animal health certificates for the import of live animals from neighboring countries. Thus, this study aimed to understand the spatial and temporal distribution of FMD in Nepal during recent years from 2019 to 2021.

Results

Disease confirmation (serotype data)

A total of 417 samples were tested during 2019–2021 for disease confirmation and serotyping. Out of the total samples tested, 37.65% ($n=157/417$) of samples were positive for FMDV. The percentage of positive samples was highest at 62.07% ($n=18/29$) in 2019, followed by 44.44% positive ($n=20/45$) in 2020 and 34.69% positive ($n=119/343$) in 2021.

The annual temporal pattern of FMD in Nepal was demonstrated by analyzing samples received by the laboratory in 2021, which covered most months of the year, with the highest number of samples and positive cases in the periods of January–February and November–December (Fig. 1). Figure 1 shows the highest number of outbreaks caused by serotype O and few outbreaks caused by serotype A, with no virus detected (NVD) in many samples. The results showed a higher percentage of positive samples during March (59%), April (64%) and December (43%).

The sample testing covered all three ecozones of the country with the highest number of samples from the Hill ecozone, followed by the Terai and Mountain ecozones (Table 1), which were roughly proportional to the number of susceptible livestock present in each ecozone. The percentage of samples that tested positive was highest in Terai, followed by Hill and Mountain, but was not significantly different between ecozones ($\chi^2=1.06$, df$=2$, $P=0.58$).

Suspected samples sent during the three years covered six out of seven provinces. The highest number of samples came from Bagmati Province, followed by Sudurpaschim, Gandaki, Lumbini, Madhesh, and Koshi Provinces (Table 2). The percentage positive among the provinces was not significantly different ($\chi^2 = 3.54$, df = 5, $P = 0.61$).

For the species-specific distribution, out of 399 samples available, the highest number of samples was collected from cattle (313), followed by buffalo (54) and pigs (25), and the lowest number of samples was from goats (7) (Table 3). The percentage of positive samples

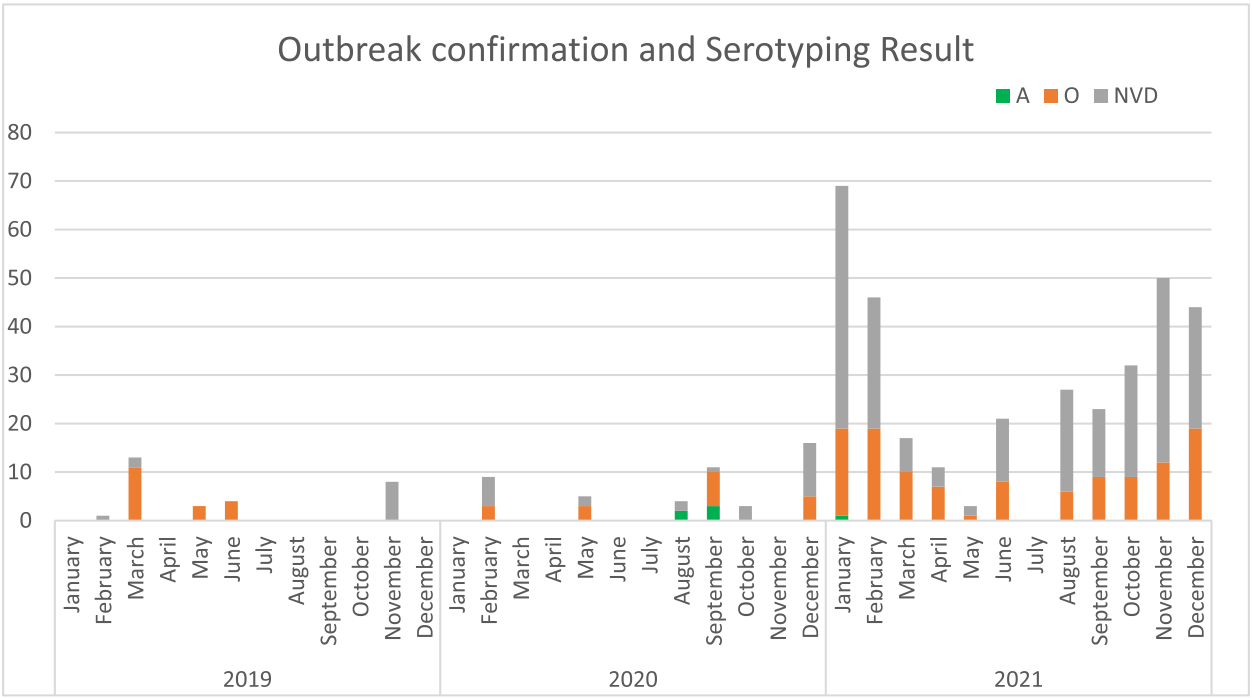

Fig. 1 Laboratory-confirmed outbreaks of FMD by month (2019–2021)

Table 1 Distribution of FMD in Nepal by Ecozone (2019–2021)

Ecozones	Number of samples	Number of positive samples	% of positive samples	95% CI (lower–upper)	χ^2	*P* value
Mountain	26	8	30.77	14.33–51.79	1.06	0.58
Hill	237	87	36.71	30.56–43.19		
Terai	154	62	40.26	32.45–48.46		

Table 2 Distribution of FMD in Nepal by Province (2019–2021)

Provinces	Number of samples	Number of positive samples	% of positive sample	95% CI (lower–upper)	χ^2	*P* value
Koshi	20	10	50.00	27.20–72.80	3.54	0.61
Madhesh	35	14	40.00	23.87–57.89		
Bagmati	180	68	37.78	30.67–45.29		
Gandaki	62	25	40.32	28.05–53.55		
Lumbini	55	21	38.18	25.41–52.27		
Karnali	0	0	0.00	-		
Sudurpaschim	65	19	29.23	18.60–41.83		

Table 3 Distribution of FMD in Nepal by susceptible species

Species	Number of samples	Number of positive samples	% of positive sample	95% CI (lower–upper)	χ^2	*P* value
Cattle	313	114	36.42	31.29–41.89	6.61	0.08
Buffalo	54	14	25.93	14.96–39.65		
Pig	25	13	52.00	31.31–72.20		
Goat	7	1	14.29	0.36–57.87		

did not vary statistically among large and small animal species ($\chi^2 = 6.61$, $P = 0.08$).

Overall, FMDV was detected in 37.65% (95% CI, 33.13–42.39) of samples during the 3-year period. Among the samples where FMDV was detected, serotype O was the most dominant serotype, accounting for 96% of samples (95% CI, 91.87–98.58), and was observed every year and in all ecozones. Serotype A accounted for a small proportion (1.91%, 95% CI, 0.4–5.48), was observed in samples from Bagmati and Sudurpaschim provinces in 2020 and 2021 but was absent in 2019 (Fig. 2). There were also incidences of coinfection with serotypes O and A (1.91%, 95% CI, 0.4–5.48) in the Kanchanpur district of Sudurpaschim Province in 2020. No virus was detected in 62.35% (95% CI, 57.61–66.87) of samples collected within the three years.

NSP serosurveillance

A total of 3146 samples were tested under active FMD NSP serosurveillance during 2019–2021. Overall, 15.07% ($n = 474/3146$, 95% CI, 13.86–16.36) of samples were positive for NSP over the three years. The annual pattern shows that the percentage of FMD seropositive samples was found to be highest in 2021, at 19.03% ($n = 235/1235$, 95% CI, 16.94–21.31), followed by 14.90% in 2020 ($n = 125/837$, 95% CI, 12.68–17.51) and 10.60% ($n = 114/1074$, 95% CI, 8.91–12.60). The percentage of seropositive samples for FMD NSP each year was found to be significantly different ($\chi^2 = 31.79$, $p < 0.001$).

For NSP serosurveillance, samples were collected from the Hill and Terai ecozones only. In the Hill ecozone, the percentage of NSP seropositive individuals was 14.61% ($n = 359/2457$, 95% CI, 13.27–16.06), while the percentage of NSP seropositive individuals in Terai was 16.69% ($n = 115/689$, 95% CI, 14.09–19.66). There was no significant difference ($\chi^2 = 1.81$, $p < 0.177$, OR = 1.17, 0.93–1.47) in the percentages of seropositivity between ecozones.

Figure 3 shows that out of 19 districts that were sampled for NSP serosurveillance, 15 districts were found to be positive for FMD NSP in 2019. However, the sampling was limited to six districts in 2020, with four districts positive for FMD NSP. All the districts that were sampled for NSP in 2021 were positive.

The percentage of samples seropositive for FMD NSP was found to be highest in cattle, followed by goats and buffalo (Table 4). The seropositive value varied statistically between species ($\chi^2 = 45.56$, $P < 0.000001$).

Discussion

This study was conducted using FMD and TADs investigation laboratory data from 2019 to 2021. The data of samples collected from quarantine offices were excluded in this study, as these samples were collected from the animals imported via the quarantine stations during major festivals. These animals are typically slaughtered within a week or immediately after import, and as such, the samples may not be reflective of the national animal population. Furthermore, including those samples could lead to the misleading prevalence of the disease within the country. The NSP surveillance results show that FMD is endemic to Nepal, as the disease occurred throughout the study period of 2019–2021, with an overall seropositivity of 15%. Prior studies conducted by researchers (Adhikari et al. 2018; Gongal and Shrestha 2002) have underscored the endemic status of FMD in Nepal.

Out of the three ecozones, the Hill ecozone had reported a higher frequency of outbreaks; however, all ecozones had roughly similar proportions of positive cases, similar to results from Giri and Parshin (Giri et al. 2010; Adhikari et al. 2018).

The spatial analysis shows the consistent presence of FMD in Bagmati, Gandaki, and Sudurpaschim provinces each year. These are the provinces with frequent movements of a large number of animals, with Sudurpaschim province sharing a border with India. The percentage of positive samples is higher during December-January and April-March, which is similar to findings by Giri and Parshin (Giri et al. 2010; Adhikari et al. 2018). The high rate of positives during December-January is attributed to residual effects of legal or illegal animal trade at border areas, as well as the frequent movement of animals within the country during major festivals in August-October. Although the imported animals are held for a brief duration or are immediately slaughtered, there exists a risk of transmitting infections to other animals in close proximity. The

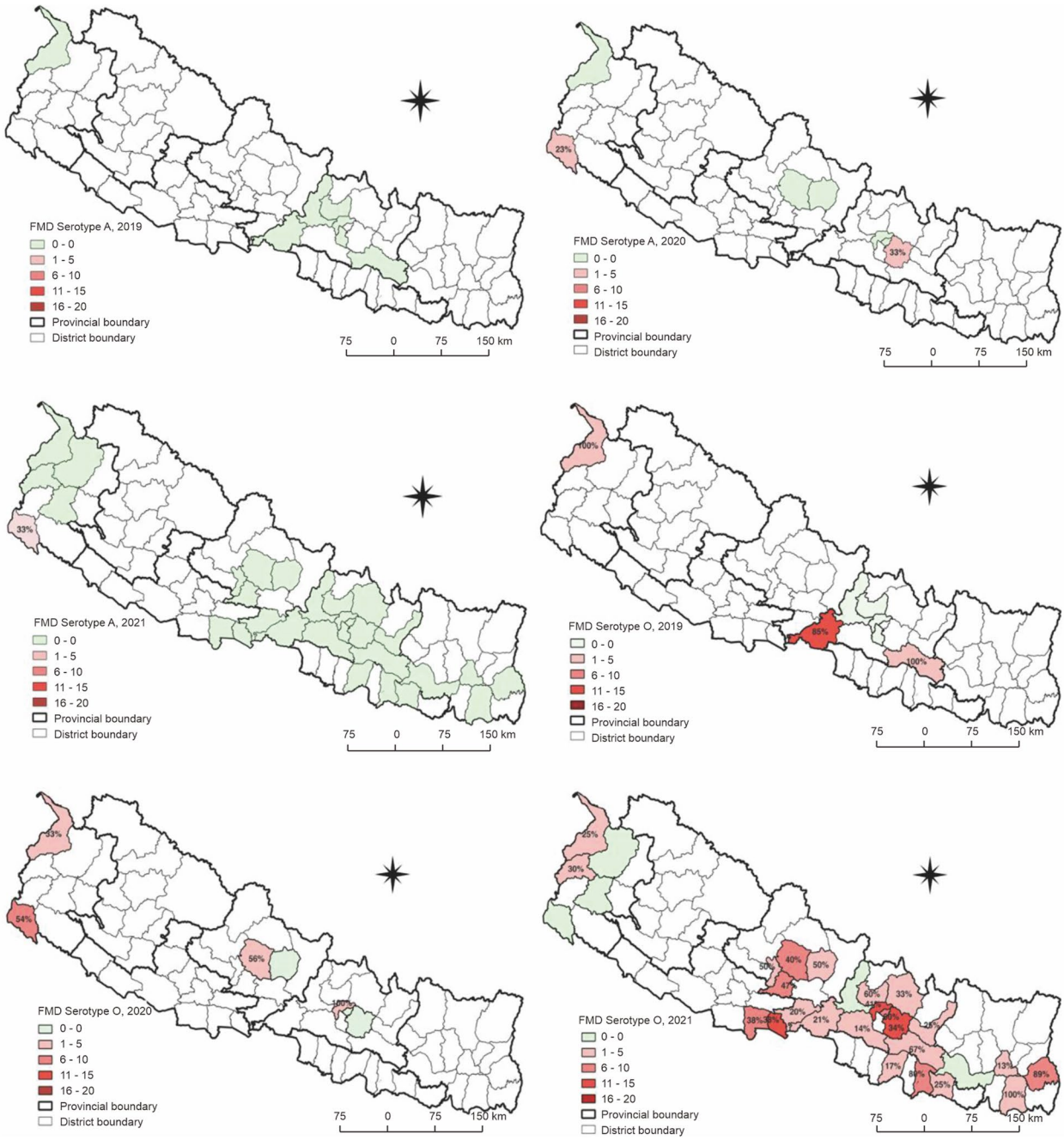

Fig. 2 Distribution of FMD serotypes with percent positivity for the stated serotype, 2019–2021

movement of animals increases the possibility of contact between infected and susceptible animals and elevates susceptibility due to physiological stress endured during transportation, which is in line with the result from Chanchaidechachai et al. 2021 and Osmani et al. 2019, about the distance-dependent and transmission of FMD by animal movement (Chanchaidechachai et al. 2021; Osmani et al. 2019).

Throughout the 3 years under study, serotype O was the dominant serotype in Nepal, with sporadic instances of serotype A (2%). These findings align with research conducted by Adhikari (Adhikari et al. 2018). However, in contrast to earlier studies (Gongal and Shrestha 2002; Giri and Parshin 2010; Adhikari et al. 2018), serotype Asia 1 was not observed during 2019–2021. This absence may be attributed to the use of the FMD trivalent vaccine

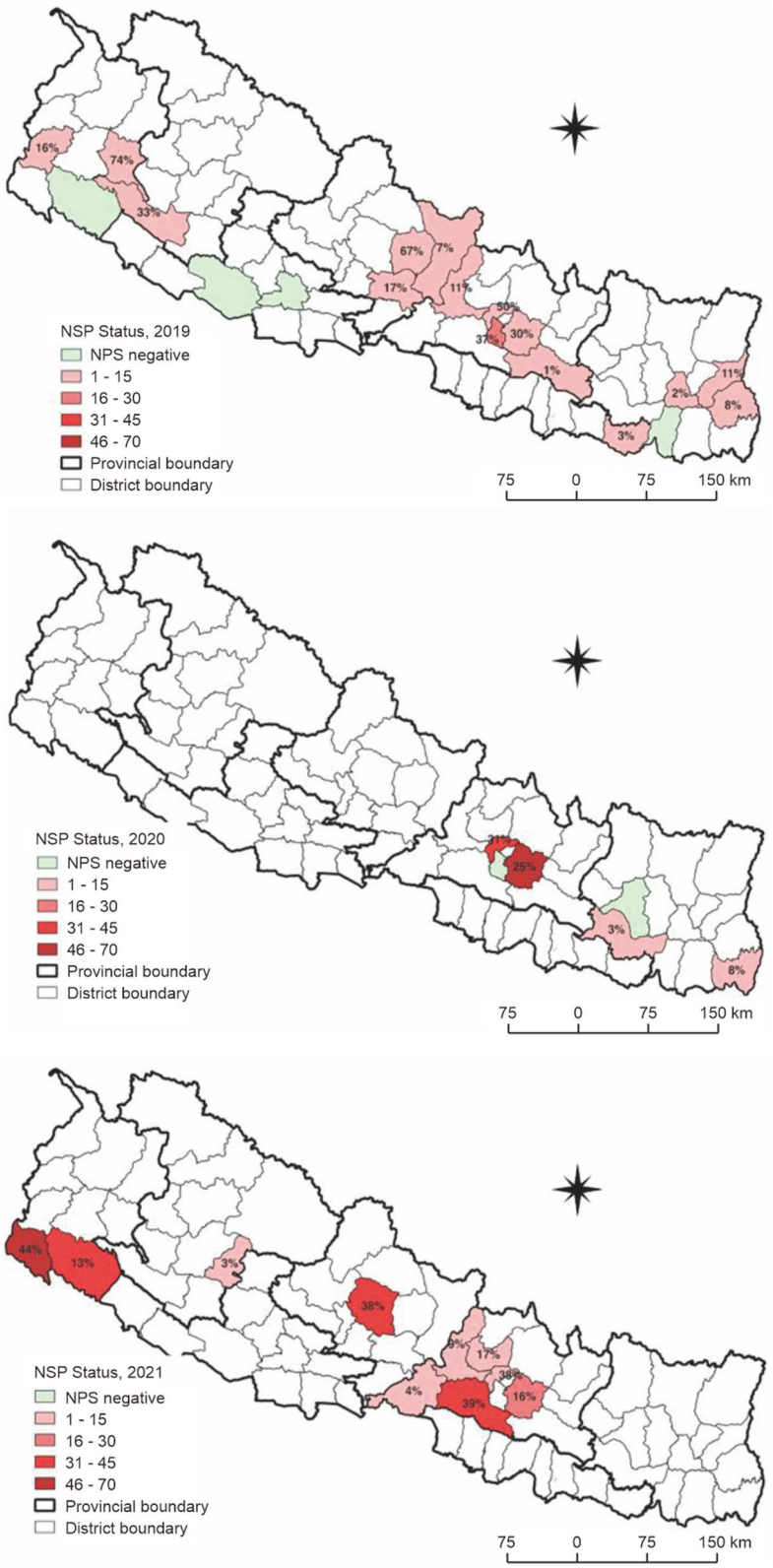

Fig. 3 FMD distribution according to NSP serosurveillance with percent positive for NSP, 2019–2021

Table 4 Distribution of FMD by species according to NSP serosurveillance, 2019–2021

Species	Number of samples	Number of positive samples	Prevalence %	95% CI (lower–upper)	χ^2	*P* value
Buffalo	102	9	8.82 (9/102)	4.11–16.09	45.56	< 0.000001
Cattle	2126	364	17.12 (364/2126)	15.58–18.78		
Goat	311	51	16.40 (51/311)	12.7–20.92		
Sheep	294	9	3.06 (9/294)	1.41–5.73		
Pig	13	0	0 (0/13)	0–24.71		

(O, A and Asia 1). Lee also stated that the serotype Asia 1 has not been reported since 2007 in Vietnam (Lee et al. 2020). The vaccines are procured by the farmers themselves and are also supplied by the government to the outbreak areas and priority regions, as indicated in the National FMD Control Plan. There were also occurrences of coinfection in 2% of the samples for serotypes O and An in Sudurpaschim Province. A similar type of coinfection was documented by Mahajan (Mahajan et al. 2021).

The available data, although limited, point to cattle as the most affected species, followed by buffalo, similar to a study by Lee (Lee et al. 2020). A considerable percentage of goats tested positive (38%) in NSP serosurveillance but were negative during serotyping. Similarly, a small percentage of sheep tested positive in NSP serosurveillance but were negative during serotyping. Conversely, pigs tested negative for NSP serosurveillance but yielded positive results for serotyping. The higher percentage of samples that tested negative (62.35%) during serotyping may be due to the extended period of time between sample collection and testing and the quality of the samples.

The prevalence of FMD is likely to be underestimated. There is a wide variation in the number of samples received and collected by the laboratory between the years of this study period, most likely due to the restrictions imposed by the coronavirus disease 2019 pandemic. Additionally, the serotyping data did not include samples from the Karnali province, despite its significant animal population. The mountain ecozone is not covered by NSP serosurveillance, despite registering a 30.77% positivity rate in serotyping. This bias may be introduced due to the limited access to animal health professionals, transportation means, and other topographical inaccessibility, which hinder the transportation of samples to the laboratory. Likewise, for NSP surveillance, there is an absence of continuity in surveillance activities in districts that exhibited a high percentage of positive samples in previous years. However, there is a possibility that these districts may have been analyzed through alternate laboratory activities, such as vaccine seromonitoring and NSP monitoring in samples from quarantine and high-risk areas, but this information was not accessible.

Conclusions

FMD has caused an enormous hindrance to the development of the livestock sector of Nepal. During 2019–2021, the disease was present throughout the year, with higher incidences during March–April and December. The majority of cases occurred in the Terai ecozone, followed by the Hill ecozone. The study revealed an average seropositivity of 15% for FMD NSP. Serotype O is the predominant serotype causing disease in Nepal, with sporadic instances of serotype A. Serotype Asia1 was not detected throughout the study period. Among all susceptible livestock, cattle are the most affected species. The Government of Nepal will soon implement the National Control Strategic Plan to control FMD in Nepal, and this analysis on the spatiotemporal distribution of the disease using laboratory data from 2019–2021 has provided a visualization of the current distribution pattern of the disease as a valuable resource for authorities in implementing risk-based surveillance. This study has also emphasized the importance of continuous surveillance, the establishment of a robust veterinary network, and the implementation of movement control for the management of this disease.

Methods

Study area

Nepal is a landlocked country in South Asia, sharing its eastern, western, and southern border with India and its northern border with China. Nepal has three distinct ecological regions: the Mountains, Hills, and Terai. The mountain region is in the northern part of the country, which ranges from approximately 4,800 to 8,848 meters above sea level (masl) and has a temperate to alpine climate. The Hill region ranges from 610 to 4,800 masl and has a subtropical to subtemperate climate. The Terai region is the southern part, which lies between 67 and 610 masl and has a tropical climate (Ministry of Health of Nepal 1996; Bhatta et al. 2014). For administrative purposes, Nepal is divided into 7 provinces, 77 districts and 753 local levels. Among the

77 districts, 16 districts are in the Mountain region, 40 districts are in the Hilly region and 21 districts lie in the Terai region (Administrative Divisions of Nepal 2023). The districts exhibit a range of areas, spanning from a minimum of 119 square kilometers to a maximum of 7,889 square kilometers. The average area among the districts is 1,911.44 square kilometers, with a standard deviation of 1,139.70 square kilometers, indicating the variability in their sizes.

The number of cattle and buffalo is highest in the Terai region, the number of goats and pigs is highest in the Hills region, and the number of sheep is highest in the Mountain region (Statistical Information on Nepalese Agriculture, 2020/21). This study includes data from the districts covered during sampling for both routine surveillance and outbreak investigation.

Sampling coverage

The FMD and TADs Investigation Laboratory in Nepal is responsible for serosurveillance and testing for foot-and-mouth disease (FMD) and other transboundary animal diseases. The laboratory has its own annual work plan and target for the surveillance of FMD in susceptible livestock (cattle, buffalo, sheep, goat, pig, and yak) covering different districts of Nepal, as currently, there is no national surveillance protocol for FMD. The serum samples are randomly collected from animals in major live animal markets, quarantine check posts (at the border as well as internal quarantine stations), haat bazzars (local markets) during major festivals, buffer areas of national parks and high-risk areas, as defined by the National FMD Control Program for carrier status surveillance (i.e., NSP surveillance) in different months of a year. The NSP samples are also sent to the laboratory from outbreak areas by the regional laboratory or district hospital, the Veterinary Hospital and Livestock Service Expert Centre (VHLSEC), for disease confirmation.

Additionally, samples (epithelium, lesion tissue, discharge, vesicular fluid, and buccal/pharyngeal swabs) were collected from the disease-suspected animals for serotyping. The samples are collected by laboratory staff or by veterinary professionals at the respective offices. They are tested in the FMD and TADs laboratory using a sandwich enzyme-linked immunosorbent assay (ELISA) or real-time polymerase chain reaction (RT–PCR). Usually, more than one sample is collected per outbreak, and each sample represents an individual animal.

For this study, the following two types of data were analyzed:

1. Routine NSP surveillance data
2. Data from serotyping (outbreak confirmation)

The districts that were sampled for serotype and NSP serosurveillance are shown in Fig. 4.

Data cleaning and processing

The NSP surveillance and serotype data collected over 3 years (2019–2021) were obtained from the annual bulletin and database of the FMD and TADs Investigation Laboratory of the Government of Nepal. Data cleaning and processing were conducted using MS Excel version 2013.

Data at the National FMD and TADs Investigation Laboratory were recorded on Nepali dates, which were converted to Gregorian calendar dates. For NSP surveillance, data from samples collected at quarantine check posts and suspected outbreak areas were excluded from the analysis. This is because the samples collected from animals at quarantine check posts may not be representative of the national population, as these animals are

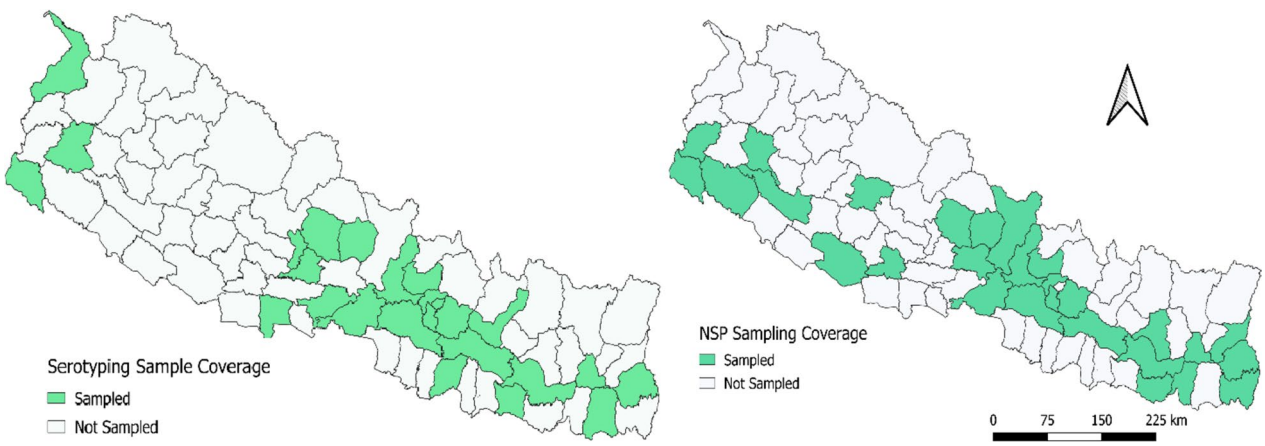

Fig. 4 Districts sampled for serotyping and NSP in 2019–2021

imported from neighboring countries, which may have a disease prevalence different from that in Nepal. Likewise, the NSP results from suspected outbreak areas could also mislead the disease's prevalence in the national population.

Data analysis

For this study, the individual sample received by the laboratory was designated the epidemiological unit. For serotyping, samples that were positive by ELISA and/or PCR were considered FMD-positive cases. The mean percentage of positive samples for the 3 years was calculated. The monthly distribution of the cases in different years was visualized using a bar graph, and serotype-specific distribution was visualized in maps using QGIS version 3.22.5. Additionally, the specieswise variation in the percentage of positive samples was calculated. The spatial pattern of FMD in Nepal was determined by analyzing samples collected from different ecozones, provinces, and serotype distributions.

From the NSP surveillance data, the mean percentage of seropositivity over 3 years as well as the percentage seropositive for individual years were calculated. The distribution of subclinical FMD cases was visualized with NSP results using QGIS maps. Statistical analyses in this study were conducted using the chi-square test for independence to assess whether there was a significant relationship between each variable (ecozones, provinces and species) and disease occurrence at a confidence level of 95%.

Abbreviations

%	Percentage
AGDP	Agricultural Gross Domestic Product
CI	Confidence interval
df	Degree of freedom
ELISA	Enzyme-linked Immunosorbent Assay
FMD and TADs	Foot and Mouth Disease and Transboundary Animal Diseases
FMD	Foot and Mouth Disease
FMDV	Foot and Mouth Disease Virus
GDP	Gross Domestic Product
GF-TADs	Global Framework for the Progressive Control of Transboundary Animal Diseases
masl	Meters above sea level
MOALD	Ministry of Agriculture and Livestock Development
MOHP	Ministry of Health and Population
NSP	Nonstructural protein
QGIS	Quantum Geographic Information System
RT–PCR	Real-time Polymerase Chain Reaction
SAT	South African Territories
VHLSEC	Veterinary Hospital and Livestock Service Expert Center
WOAH	World Organization for Animal Health
WTO	World Trade Organization

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Authors' contributions

SPD, SK, SP and SV conceived the idea. KRP and NR provided the laboratory data. SPD and SK performed the data analysis. SP, SV, SK, MU, and KRP provided the overall supervision and guidance. SPD wrote the first draft of the manuscript. SPD, SK, SP and SV revised the manuscript. All the authors have read and endorsed the final version.

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Availability of data and materials

The datasets used and/or analyzed during the current study are not publicly available due to their origin from government sources. However, they are available from the corresponding author upon reasonable request with the permission of the FMD and TADs Investigation Laboratory, Nepal.

Declarations

Ethics approval and consent to participate

NA

Consent for publication

NA

Competing interests

The authors declare that they have no competing interests.

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