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Cross-sectional serological study to estimate foot-and-mouth disease virus non-structural protein antibodies in randomly sampled small ruminants and pigs in Haryana during 2019 and 2020

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ABSTRACT

Foot-and-mouth disease (FMD) is highly contagious and affects various cloven-hoofed animals including cattle, buffalo, sheep, goat and pig. This study investigates the prevalence of foot-and-mouth disease virus (FMDV) non-structural protein antibodies (NSP-Abs) as an indicator of past or recent FMDV exposure in small ruminants and pigs sampled randomly in Haryana state of India, during 2019 and 2020. Serum samples were collected from 440 goats, 440 sheep and 216 pigs in 2019, and 436 goats, 436 sheep and 218 pigs in 2020, representing all 22 districts of Haryana. Samples were tested using an in-house indirect 3AB3 NSP ELISA. The results revealed positive NSP-Ab rates of 26.36% in goats, 13.18% in sheep, and 5.09% in pigs in 2019, and 24.54% in goats, 14.22% in sheep, and 13.30% in pigs in 2020, indicating their previous exposure to FMDV. In contrast, the NSP-Ab prevalence in the bovine population of Haryana was lower, reported at 7.3% (179/2450) in 2019 and 4.4% (173/3930) in 2020. This indicates a higher rate of FMDV circulation in goats, sheep and pigs compared to bovines in the state during the study period. Given this reversal in infection dynamics, there is an urgent need to strengthen intensive active surveillance of FMD in the bovine population by screening oropharyngeal fluid samples to follow-up NSP reactors. Additionally, implementing routine six-monthly vaccination of small ruminants and pigs along with active surveillance in Haryana is recommended to control FMD and reduce disease transmission.

Keywords: Foot-and-mouth disease virus, Goat, Haryana, Non-structural protein antibodies, Pig, Sheep, Surveillance

Foot-and-mouth disease (FMD), popularly known as the ‘fast-moving disease’, is one of the most important infectious diseases in veterinary medicine. Because of the high magnitude of economic consequences, FMD is graded as one of the most important diseases. The etiological agent, FMD virus (FMDV) belongs to the *Aphthovirus* genus in the *Picornaviridae* family (Grubman and Baxt 2004). Cattle and buffalo are the primary hosts for FMD, although the virus can affect other cloven-hoofed domestic ungulates such as sheep, goats and pigs, adversely impacting the livelihoods of farmers and national economies, causing a global economic challenge (Paton *et al.* 2010, Knight-Jones and Rushton 2013).

Haryana possesses vast agricultural land suitable for grazing livestock, with sheep and goats reared across various systems including intensive, semi-intensive and extensive setups. Sheep and goats play crucial roles in the smallholder livestock production system within the state. Sheep and goats are integral to smallholder livestock production systems in Haryana, where the total livestock population is approximately 7.04 million, including 0.288 million sheep, 0.335 million goats, and 0.108 million pigs. Since 2004, India has implemented an FMD control program that primarily focusses on immunizing bovine species. However, sheep, goats and pigs have not been routinely immunized under this program, despite their potential roles as carriers/contributors to FMD spread. Sheep and goats can harbor the FMD virus, while pigs can release contagious viral aerosols, contributing significantly to disease transmission. The present cross-sectional sero-surveillance study conducted in Haryana aimed to estimate the prevalence of FMDV infection-specific non-structural protein antibodies (NSP-Abs) in small ruminants and pigs. Serum samples were collected randomly from all 22 districts of Haryana during the consecutive years of 2019 and 2020 to assess the prevalence of past or recent FMDV exposure in these species.

MATERIALS AND METHODS

Study area and sample collection: The Planning Commission has divided India into 15 broad agro-climatic

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regions based on agro-climatic features, soil type, climate including temperature, rainfall and water resources availability, physiography, geological formations, cropping patterns, etc. Haryana falls in agroclimatic zone VI, called the Trans-Gangetic Plains Region. The state extends between 27°37' N and 30°35' N latitudes and 74°28' E and 77°36' E longitudes covering a geographic area of 44,212 km^2, i.e. 1.3% of the total area of the country. Five states including Rajasthan, Punjab, Himachal Pradesh, Uttarakhand, Uttar Pradesh and National Capital Territory of Delhi and union territory of Chandigarh surround the Haryana state. Agriculture and allied animal husbandry sectors greatly support the state economy prominently contributing to the state as well as national GDP. In 2019, serum samples from 440 goats, 440 sheep, and 216 pigs while in the year 2020, samples from 436 goats, 436 sheep, and 218 pigs were randomly collected from all the 22 districts of Haryana (Table 1). Regarding the species-wise number of serum samples collected during the two consecutive years, 20 sera from goats, 20 from sheep and 10 from pigs were collected from each of the 22 districts.

Serological assay: The FMD NSP test was conducted at ICAR-NIFMD, Bhubaneswar using the in-house r3AB3 NSP ELISA kit as described earlier (Mohapatra *et al.* 2011). The r3AB3 NSP ELISA is an indirect ELISA detecting antibodies against the non-structural 3AB3 protein and was originally developed for bovine species. The kit has been used for FMD serosurveillance in India since 2008. In order to assess antibodies against FMDV 3AB3 NSP in caprine, ovine and porcine species, the protocol was modified further (Rout *et al.* 2014). Test serum samples of sheep, goat, and pig were diluted at the rate of 1:50, 1:50 and 1:20, respectively and respective anti-species horse radish peroxidase (HRP) conjugated antibodies were diluted at the rate of 1:12000. The final optical density (OD) values were measured at 492 nm. Serum samples from three distinct species that had adjusted OD values ≥55% of that of the corresponding positive controls were considered positive for FMDV 3AB3 NSP-Abs.

Statistical analysis: Serological data were entered into MS Excel (Microsoft®Excel, Washington, Microsoft Office 365). A descriptive analysis of FMD seroprevalence was performed with 95% confidence intervals (CI).

RESULTS AND DISCUSSION

Overall percentage of NSP-seropositive goat, sheep and pig: A total of 1096 and 1090 serum samples were collected during 2019 and 2020, respectively, from the small ruminant and pig populations of Haryana and analyzed using 3AB3 NSP ELISA. The serum samples collected from goats showed a higher NSP antibody prevalence than those from sheep and pigs. The per cent NSP antibody positivity in goat samples was 26.36% (116/440) and 24.54% (107/436) in 2019 and 2020, respectively, while in sheep, it was determined to be 13.18% (58/440) and 14.22% (62/436). Serum samples collected from pigs in 2019 and 2020 revealed positivity in 5.09 (11/216) and 13.30 (29/218) per cent of the animals, respectively.

Table 1. 3AB NSP seropositive percentage of sheep, goat and pig in different districts of Haryana during 2019 and 2020

District	% seropositive goat		% seropositive sheep		% seropositive pig	
	2019	2020	2019	2020	2019	2020
Ambala	30	30	5	15	0	40
Bhiwani	80	15	0	15	0	20
Charkhi Dadri	5	5	0	15	40	0
Faridabad	5	45	30	10	0	10
Fatehabad	10	10	15	25	0	10
Gurugram	35	20	35	30	0	0
Hisar	65	20	40	30	0	0
Jhajjar	30	35	10	10	0	40
Jind	35	45	30	5	10	40
Kaithal	0	45	0	0	0	10
Karnal	30	45	5	15	0	10
Kurukshetra	15	30	20	0	0	0
Mahendragarh	5	15	5	0	0	0
Nuh	5	0	20	5	0	10
Palwal	15	56.3	0	50	0	50
Panchkula	20	10	10	10	0	10
Panipat	5	0	15	0	20	0
Rewari	55	30	15	30	0	0
Rohtak	70	15	35	30	0	50
Sirsa	35	40	0	15	30	0
Sonipat	15	35	0	10	10	0
Yamunanagar	15	0	0	0	0	0
Total	26.36	24.54	13.18	14.22	5.09	13.30

The district-wise seropositivity status of goat, sheep, and pig during the two consecutive years (2019 and 2020) is depicted in Table 1, that were scattered across most of the districts of the state of Haryana. By and large, there was not much overall difference in the prevalence estimates of NSP-Ab during 2019 and 2020 in small ruminants, whereas for pig the percentage prevalence in 2019 was 5.09% and hiked to 13.30% in 2020.

The seroprevalence for sheep (n=120/876) was estimated to be 13.698% (95% CI: 1.59, 3.854); for goat (n=223/876) was 25.456% (95% CI: 3.3, 6.8) and for pig (n=40/234) samples was 17.094% (95% CI: 0.2, 1.5) as shown in Table 2. The seropositive status of sheep, goats and pigs sampled during 2019 and 2020 indicates their recent or past exposure to the FMD virus.

Haryana has historically demonstrated effective implementation of FMD control program, with consistently low recorded incidences of FMD compared to other Indian states. The NSP seropositivity rates in the bovine population of the state over the past four years (2018-2021) have ranged from 4.4% to 8.1% (ICAR-DFMD, Annual Reports 2018-2021), indicating relatively stable disease control status. In 2021, only a single FMDV serotype O incidence was reported in a migratory goat herd from a neighboring state, while three outbreaks occurred in 2019 affecting cattle and pigs (Dahiya *et al.* 2022). The current study indicates a moderately higher NSP seropositivity in sampled goats, sheep and pigs compared to the bovine population, likely attributed to intensive biannual vaccination of cattle and buffalo populations. This reversal of infection dynamics highlights the urgent need for strengthened active surveillance in the bovine population, utilizing oropharyngeal fluid sampling to identify the carrier animals. The highly dynamic nature of small ruminant populations with annual replacement rate of 30-40% (Singh and Bandyopadhyay 2015), complicates disease monitoring. The movement of small ruminants from surrounding regions into Haryana may introduce occult FMDV infections not reflected in the resident population's disease status at the sampling time. To address this, strict legal enforcement of small ruminant movement control is essential. If allowed, incoming animals should undergo quarantine and FMD vaccination before integration with the resident animal population in order to reduce the disease transmission risks. These measures are critical for maintaining disease control and preventing outbreaks in Haryana's livestock populations.

Cattle and buffalo in rural India are often raised alongside goats. Antibodies to FMDV NSPs have been detected in small ruminants up to 3 years post-infection, but carriers of the virus cannot be detected for that long (Paton *et al.* 2009). The duration of persistent infection with FMDV varies significantly among species, with cattle showing persistence for about 3.5 years, compared to 9 months in sheep and 4 months in goats (Moonen and Schrijver 2000). However, the epidemiological impact of this persistence on disease transmission remains debatable. In Haryana state, vaccination is practiced among the bovine population, but animal movement between districts and neighboring states remains uncontrolled. Live animal trade has been implicated in introducing and spreading diseases like FMD, potentially contributing to increased seroprevalence rates in cattle, buffalo and goats in recent years.

Small ruminants, like sheep and goats, play a crucial role in the transmission of FMD. The disease has been introduced into disease-free countries through various means, including the movement of infected small ruminants. For instance, the illegal sheep trade from Turkey to Lesbos in 1994 led to an FMD outbreak in Greece. Similarly, the 1999 outbreak of FMD in south-east Asia was linked to the importation of goats from Myanmar. The 2001 FMD outbreak in the United Kingdom highlighted the role of sheep in disease spread, with additional sheep movements extending the spread of virus to Scotland, Wales, Northern Ireland and France. Due to the ease of transporting small ruminants between farms and across state lines, illegal and unregulated movements are common, often escaping notice by animal husbandry authorities. In earlier instances, illegal trade as well as the illegal nature of movements were found as major risks for the introduction of FMD (Hartnett *et al.* 2007). Therefore, authorities in Haryana and similar regions may consider implementing strict laws to control the movement of small ruminants as a measure to prevent the spread of FMD.

Infected sheep and goats often do not exhibit typical clinical symptoms of FMD, and their signs can mimic those of other diseases. Due to this less obvious presentation, the epidemiological role of sheep and goats in FMD has historically been overlooked. In adult small ruminants, clinical signs of FMD are usually mild, mainly manifesting as vesicles on the feet leading to lameness, with less common occurrences of vesicles and erosive lesions in the mouth and tongue (Barnett and Cox 1999, Kitching and Hughes 2002). The subdued nature of FMD symptoms in

Table 2. Statistical analysis of samples tested in 3AB3 NSP ELISA

Species	Year of sampling				Antibody prevalence	p-value	95% Confidence Interval
	2019		2020				
	Total Samples	Positive Samples	Total Samples	Positive Samples			
Goat	440	116	436	107	25.45662	$p>0.05$	$3.324 \leq \mu \leq 6.811$
Sheep	440	58	436	62	13.69863014	$p>0.05$	$1.599 \leq \mu \leq 3.854$
Pig	220	11	218	29	9.21	$p>0.05$	$0.287 \leq \mu \leq 1.53$

sheep and goats, combined with their frequent movement, makes disease detection challenging. Herds that practice transhumance or nomadic grazing can unknowingly spread the infection to other herds long before disease diagnosis is established, as observed with other viral diseases (Singh *et al.* 2004, Singh 2011). Shipping and trade involving live sheep and goats are more common globally compared to other susceptible species to FMD. Despite these challenges, in mixed farming settings like those in India, the role of small ruminants in FMD transmission is significant and warrants attention for disease elimination efforts. Understanding and addressing these factors are crucial for effective disease control and prevention.

Given the prevalence of FMD in small ruminants and pigs, it is recommended to implement routine FMD vaccination and promote zoo-sanitary practices and good husbandry to minimize virus introduction and spread. The relatively higher prevalence rates observed in small ruminants and pigs underscore the need for intensified and comprehensive FMD surveillance in the bovine population. To achieve disease-free status, Haryana should consider expanding vaccination efforts to include goats and sheep, alongside bovines and implement enhanced biosecurity measures in pig farms. These measures collectively aim to prevent and control FMD within the state.

In conclusion, the investigation revealed evidence of higher FMD virus circulation rates in goats, sheep and pigs compared to cattle and buffalo populations. This underscores the urgent need for intensive active surveillance in bovine populations by screening oropharyngeal fluid samples to follow up on NSP reactors. Furthermore, the state of Haryana should enforce movement controls on small ruminants and other FMD-susceptible animals entering its territory. Animals allowed entry should undergo quarantine and FMD vaccination before mingling with resident populations. Intensive vaccination efforts coupled with active surveillance strategies in cattle and buffalo populations aiming to build sustained herd immunity and eliminate virus circulation among susceptible animals is essential. To accelerate disease elimination, vaccination of goats and sheep, albeit a smaller proportion, should be implemented alongside stringent biosecurity measures in pig farms and movement restrictions on livestock from neighboring states. These comprehensive measures collectively may pave the way towards achieving disease freedom in Haryana.

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