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Seroprevalence of Foot-and-Mouth Disease among Sheep and Goat Population across Ten Agro-climatic Zones of Odisha

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ABSTRACT

The aim of this study was to carry out sero-epidemiological study through detecting both nonstructural protein (NSP) and structural protein (SP)-antibodies (Abs) of FMD virus (FMDV) in small ruminant population across ten agro-climatic zones of Odisha. A total of 506 serum samples from 217 sheep and 289 goats were collected randomly covering all the ten agroclimatic zones of the state. The samples were subjected to 3AB NSP ELISA for detection of NSP-Abs and liquid phase blocking (LPB) ELISA to check the protective antibody status (log_{10} titre of ≥1.8) against all three serotypes in the trivalent vaccine. A total 49.31% sheep and 20.76 % goats were found to be positive for presence of NSP-Ab. The agro-climatic zone-wise prevalence of NSP-Ab with respect to sheep varied between 17.65% and 100%, while in goats it varied between 4.76% and 50%. In LPB ELISA, 2.08% goats and 4.14% sheep were found to have protective antibody against all three vaccine strains of serotypes O, A and Asia-1, which indicates poor herd immunity in these species. Hence these species should be regularly vaccinated along with large ruminants for effective control of the disease in the country.

Keywords: Foot-and-mouth disease, Goat, Odisha, Seroprevalence, Sheep

Foot-and-mouth disease (FMD) is caused by FMD virus (FMDV) belonging to genus *Aphthovirus* within the family *Picornaviridae*. It is mostly a disease of cloven-hoofed animals and considered as a serious threat to the livestock production sector across the globe. Hence OIE and FAO have declared it as "High Priority Disease" due to its severity and appealed to different countries to make effective groups for its strategic control (Saminathan *et al.,* 2016). Despite many eradication approaches, the disease is still prevalent in many parts of the world. In India, three serotypes of FMDV viz, O, A and Asia-1 are prevalent, out of which serotype O accounts for majority of the outbreaks across the country (Subramaniam *et al.,* 2013). Presence of antibodies (Abs) against FMDV nonstructural proteins (NSPs) confirms the previous viral replication in host irrespective of vaccination status. In the NSP-based ELISAs, 2B, 2C, 3A, 3AB, 3B, 3ABC and 3D have been used. The most reliable indicator of infection is indicated by antibody generated against 3ABC (Mahajan *et al*., 2013).

FMD is endemic in India (Subramaniam *et al.,* 2013) causing a huge economic loss (Bandyopadhyay, 2004; Pattnaik *et al.,* 2012). The country has 528 million FMD-susceptible animals (Pattnaik *et al.,* 2012), of which Odisha contributes 5% with 24.02 million FMD-susceptible livestock population including 14.28 million cattle, 1.439 million buffaloes, 1.758 million sheep, 5.974 million goats and 0.569 million pigs. The eastern region of India has been recorded with the highest prevalence (43%), which includes the state of Odisha, Bihar, West Bengal and Jharkhand (Subramaniam *et al.,* 2013). Still few prevalence reports of FMDV infection in small ruminants of Odisha is there. However, the present study reports the serological status of FMD covering all the ten agro-climatic zones of Odisha through detecting antibodies against both NSPs and SPs of FMDV. This preliminary

data on the seroprevalence of FMD in small ruminants of the state may be helpful for the control programme running in the country.

MATERIALS AND METHODS

Study area

Odisha is situated in the eastern part of India extending between 17.49N latitude to 22.34N latitude and from 81.27E longitude to 87.29E longitude. As per 19th Livestock census, 2012 (GOI, 2014), the state has 1591735 sheep and 6513087 goat population. The state possesses 10 agro-climatic zones spread across a total of 30 districts. Random sampling was carried out between October-2015 and April-2016 covering all the agro-climatic zones of the state. A total of 506 serum samples from 217 sheep (from 25 districts) and 289 goats (from 29 districts) were collected (Table 1 and 2). At the same time, the farmers/ veterinarians were interviewed about the history of previous incidences of FMD in their herd along with the vaccination status.

Detection of NSP-Abs

The 3AB NSP ELISA primarily developed for FMD serosurveillance in bovines in India by Mohapatra *et al.* (2011) was used with little modifications in the present study. Primarily the assay is based on the principle that antibodies against 3AB NSP are produced only in FMDV infected animals, not in uninfected and vaccinated animals with inactivated purified vaccine thereby acting as a serological marker for FMDV infection. The assay used in bovine sera has been reported to have an overall diagnostic sensitivity of 96% and diagnostic specificity of 99.1% and 96.4% for the naive and vaccinates, respectively. Rout *et al.* (2014) have earlier applied the assay for small ruminants. For sheep and goat, a serum dilution of 1:50 was followed and anti-species (anti-sheep and anti-goat) HRP conjugate was used with dilution @ 1:20,000. Serum samples producing corrected optical density (OD) values more ≥40% were considered as positive.

Detection of SP-Abs

The Liquid phase blocking (LPB) ELISA was used for measuring serotype-specific SP-Ab titre against all three serotypes of FMDV as per the method described earlier by Rout *et al.* (2014). The results were expressed as percentage reactivity for each serum dilution as follows:

$$\text{Percentage reactivity} = (OD_{\text{mean of each test serum dilution}} / OD_{\text{mean of antigen control}}) \times 100.$$

Antibody titres were expressed as the $\log_{10}$ of reciprocal of serum dilutions giving 50% of the absorbance recorded in the antigen control wells. The samples with $\log_{10}$ titre of ≥1.8 were declared to have sufficient protective antibody.

Statistical analysis

Analysis of data was done using software downloaded from the website http://www.real-statistics.com. The chi-square value was calculated on data at 5% significance level and then P values were calculated at respective df. P values more than 0.05 were taken as non-significant for the respective category and *vice-versa*.

RESULTS AND DISCUSSION

A total of 49.31% sheep (46.80% males and 51.22% females) and 20.76% goats (22.22% males and 19.48% females) were found to be positive for NSP-Ab. The varying degree of prevalence rate among sheep and goats were at statistically different level ($p<0.05$), but not significant among male and female ($p\geq0.05$) (Table 1). During estimation of SP-Abs, only 2.08% (6/289) goats and 4.14% (9/217) sheep were found to have protective $\log_{10}$ titre of ≥1.8 against all three vaccine strains of serotypes O, A and Asia-1 indicating extremely poor herd immunity. Mohanty *et al.* (2015) reported a higher percentage of NSP-Ab prevalence in goats (38.33%) and a lower in sheep (3.93%) in coastal belts of Odisha. An overall prevalence of 38% NSP-Abs and 20.7% SP-Abs was recorded among goats of Odisha by Ranabijuli *et al.* (2010). Rout *et al.* (2014) reported the prevalence of 3AB NSP-Abs in 20.35% sheep and 13.60% goats sampled across many states in India. Similarly, Bora *et al.* (2014) reported 2.85% cattle and 0.76% buffaloes to have 3AB NSP-Abs in samples collected from different districts of Haryana and found higher virus activity in Bhiwani, Jhajjar and Sirsa (all FMD-CP districts). Mohan *et al.* (2014) reported 9.62% prevalence of NSP-Abs among female buffaloes.

Table 1: Overall seroprevalence of FMD with respect to species and sex

	Sheep			Goat		
	Male	**Female**	**Total**	**Male**	**Female**	**Total**
Total number of serum samples Taken	94	123	217	135	154	289
Positive (%)	44(46.80)	63(51.22)	107(49.31)	30(22.22)	30(19.48)	60(20.76)
$\chi 2$	0.415			1.786		
P- Value	0.5194			0.1814		
$\chi 2$	45.68					
P- Value	<0.0001					

In our study, both the agro-climatic zone-wise and district-wise prevalence rates were recorded. The zone-wise prevalence of NSP-Ab among sheep was found to be the highest in North Central Plateau and South Eastern Ghat (100%), following the lowest in Western Undulating Zone (17.65%). The same in goat population was found to be the highest in North Central Plateau (50%), while the lowest in Eastern Ghat Highland (4.76%). The variations recorded in different zones with respect to sheep and goats are at statistically significant level ($p<0.05$) (Table 2). Out of the total samples collected from sheep across 25 districts, Mayurbhanj and Malkangiri were found to have the highest (100%) prevalence, followed by the lowest in Jajpur, Ganjam, Kalahandi and Sonepur (0%). Again, out of the total sera collected from goats across 29 districts, the highest prevalence was recorded in Mayurbhanj (100%) and the lowest in Puri, Ganjam, Kandhamal, Koraput, Sambalpur and Bolangir (0%) (Table 2).

Various factors like nomadic behaviour, unofficial trades, stress, nutrition and co-housing with other animals may play role in exposure of FMDV in small ruminants. Most often high temperature, low relative humidity (RH) and low rainfall increase the severity of most infectious diseases through induction of stress (Subramaniam *et al.,* 2013), which might have been the predisposing factors behind virus circulation in different agro-climatic zones. Due to quasispecies nature of the virus, animals infected with one serotype of FMDV remain vulnerable to infection with other serotypes (Alexandersen *et al.,* 2003; Jamal and Belsham, 2013). Samples were collected from unvaccinated flocks and the results of LPB ELISA also correlated with the same. FMD vaccination is not followed among small ruminants of the state. Moreover, frequent exposure to FMDV occurs in small ruminants having access for mixing with large ruminants by the farmers. These unrecognized/sub-clinically infected small ruminants may act as a source of infection for other susceptible animals through their secretions and excretions (Ranabijuli *et al*., 2010; Hegde *et al.,* 2016). Again the duration of persistence of FMDV infection in sheep is 9 months, while in goat it is for 4 months after infection (Farooq *et al*., 2017). Thus, the observed difference in the apparent prevalence of NSP-Ab between sheep and goats might be due to the probable variability in the degree of virus replication and its persistence in the said species. One of the major constraints in FMD control remains on these carrier animals after the outbreak due to the risk they pose in transmitting the virus (Tiwari, 2017). It has been cited that sheep and goats frequently affected during the last decades across small ruminant farms of Israel constitutes 27% of the total FMD outbreaks in the country despite vaccination (Elnekave *et al.,* 2016). The importance of sheep has already been highlighted in the outbreak of FMD in the United Kingdom during the year 2001 (Kitching and Hughes 2002). The difficulties in detecting FMD among sheep and goats for a considerable period of time are the most important obstacles for FMD control, which need an extensive study on course of the disease in these animals. Taking into consideration of the susceptibility and prevalence status of FMDV infection among sheep and goats, they should be included under FMD vaccination programme. India is a country possessing world's largest livestock sector supporting the rural economy. So, extensive studies along with vaccination are required in these animals along with large ruminant population so as to effectively control the disease in the state and in the country.

Table 2: Seroprevalence of FMD among different districts and zones of Odisha

Sl. No.	District	Agro-climatic zones of Odisha	Total positive/Total sample tested (%)			
			Sheep	Goat	Sheep	Goat
1	Sundergarh	North Western Plateau	4/7(57.14)	3/10(30)	11/16	4/19
2	Deogarh		7/9(77.78)	1/9(11.11)	(68.75)	(21.05)
3	Keonjhar	North Central Plateau	—	3/21(14.29)	15/15(100)	18/36(50)
4	Mayurbhanj		15/15 (100)	15/15 (100)		
5	Bhadrak	North Eastern Coastal Plain	2/10 (20)	2/11 (18.18)	8/39 (20.51)	4/27 (14.81)
6	Baleswar		6/14 (42.86)	2/16 (12.5)		
7	Jajpur		0/15(0)	—		
8	Puri	East & South Eastern Coastal Plain	4/11 (36.36)	0/10 (0)	24/54 (44.44)	10/50 (20)
9	Cuttack		4/9(44.44)	2/10(20)		
10	Khordha		4/7(57.14)	3/7(42.86)		
11	Nayagarh		4/8(50)	3/9(33.33)		
12	Jagatsinghpur		6/10(60)	1/7(14.29)		
13	Kendrapada		2/9(22.22)	1/7(14.29)		
14	Rayagarh	North Eastern Ghat	3/8(37.5)	6/11(54.54)	3/11 (27.27)	7/38 (18.42)
15	Kandhamal		—	0/10(0)		
16	Ganjam		0/3(0)	0/7(0)		
17	Gajapati		—	1/10(10)		
18	Koraput	Eastern Ghat High Land	6/9(66.67)	0/11(0)	14/19 (73.68)	1/21 (4.76)
19	Nabarangapur		8/10(80)	1/10(10)		
20	Malkangiri	South Eastern Ghat	9/9(100)	2/8(25)	9/9(100)	2/8(25)
21	Kalahandi	Western Undulating Zone	0/9(0)	2/11(18.18)	3/17 (17.65)	3/23 (13.04)
22	Nuapara		3/8(37.5)	1/12(8.33)		
23	Bargarh	Western Central Table Land	—	2/11(18.18)	8/21 (38.09)	5/48 (10.42)
24	Sambalpur		2/5(40)	0/5(0)		
25	Bolangir		—	0/11(0)		
26	Sonepur		0/5(0)	1/5(20)		
27	Boudh		1/3(33.33)	1/8(12.5)		
28	Jharsuguda		5/8(62.5)	1/8(12.5)		
29	Anugul	Mid Central Table Land	7/8(87.5)	2/9(22.22)	12/16 (75)	6/19 (31.58)
30	Dhenkanal		5/8(62.5)	4/10(40)		
	χ^2				59.295	28.095
	P- Value				<0.0001	0.0009

Based on the findings, it may be concluded that FMD is prevalent among sheep and goat population of Odisha. So further study can be carried out in future to study the course of infection and effective vaccination programme is necessary for control of the disease.

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