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A comprehensive serosurveillance of foot-and-mouth disease in pig population of Mizoram state in eastern Himalayan agro-climatic region in India

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Agriculture and livestock farming play an important role in livelihood earnings of majority of the inhabitants in India. The north-eastern region (NER) of the country comprising eight sister states occupies about 7% of the total land area and 4% of the total population of the country. The state of Mizoram, known as the 'land of blue mountains' falls within eastern Himalayan agroclimatic region in India. In smallholder farming systems in the villages of Mizoram, pig rearing occupies a vital position and is considered to be the most encouraging and appropriate livestock enterprise serving as an income source. Such small-scale pig sector has been reported to have greater potential to reduce poverty (Lanada *et al.* 2005).

Foot-and-mouth disease (FMD) is prevalent in NE region of India and significant cause of pig mortalities (Rout *et al.* 2024). FMD causes devastating losses during an outbreak situation. However, FMD-awareness among the farmers is low, and many have only fragmented knowledge about the spread and prevention of the disease. In India, three serotypes of FMD virus (FMDV) such as O, A, and Asia 1 are prevalent with more than 80% of the outbreaks attributed to serotype O (Subramaniam *et al.* 2022). As per the 20th Livestock Census-2019, All India Report (Ministry of Fisheries, Animal Husbandry and Dairying, New Delhi), India has a total pig population of 9.06 million, while Mizoram alone is home to 0.29 million pigs. This pig-dominant livestock population is under a constant threat of FMD due to several factors such as unrestricted movement of susceptible animals, animal trade, mixed animal husbandry practices, lack of vaccination practice, porous borders with FMD-endemic countries and poor zoo-sanitary measures during outbreaks. These conditions create a favourable and conducive environment for FMDV to flourish, mutate, and persist over time (Mishra *et al.* 1998). A major concern of the international community is combating infectious diseases among the poorest segment of the population (Fujii *et al.* 2014). Effective strategies to address these infectious diseases depend on the development of reliable epidemiological surveillance tools and methods. Serology has become an increasingly valuable resource and tool for the surveillance of a wide range of infectious diseases (Yman *et al.* 2016). Surveillance programmes based on laboratory screening tests are also used to document freedom from disease to facilitate trade (Stärk *et al.* 2000). Disease surveillance can help implement zoosanitary measures, including imposing restrictions on the movement of suspected animals. The NSP ELISA is pretty useful for the retrospective diagnosis of FMDV infection in pigs. The primary objective of this study was to estimate the seroprevalence of FMDV 3AB3 nonstructural protein antibodies (3AB3 NSP-Abs), an underlying indicator of infection/exposure of animals to FMDV, in randomly sampled pigs across all the eight districts of Mizoram state.

A simple random sampling method was followed to collect blood sample from pigs across Mizoram during 2014-2015. All eight districts targeting locally reared adult pigs were sampled without any sampling from organized farms. A questionnaire survey was also undertaken simultaneously, in which the farmers were interrogated about the vaccination status, management practices, incidence of outbreaks of FMD in their herds within the last 5 years preceding the study. Based on farmers' interviews and animal health records across the 8 districts, no history of FMD vaccination was apparent in the sampled pigs.

A total of 2996 blood samples of pigs were collected across 8 districts of Mizoram (Table 1). Pigs were bled through anterior venacava drawing 2 to 6 ml blood per animal and serum samples were separated by centrifugation at 3000 rpm for 15 minutes and transferred to 5 ml cryovials with proper identity. After completion of sampling at all identified locations, a single shipment of all samples was transported in cold chain to the Central FMD laboratory,

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Table 1. Table showing district and place-wise number of pigs sampled during the year 2014-2015 and serum samples found positive in 3AB3 NSP ELISA along with percentage positivity

District	Place of Collection	Total number of samples tested	Found positive
Lawngtlai	Lawngtlai-III	3	0
	Lawngtlai-IV	23	2
	College Veng	12	2
	Bazar Veng	16	0
	Council Veng	15	0
	Chanmari-II	14	1
	Lawngtlai I	28	1
	Total	111	6 (5.40%)
Aizawl	Bethlehem Veng	8	4
	East Phaileng	6	0
	Tuithiang Veng	3	0
	Bawngkawn Veng	19	0
	Bethlehem Vengthlang	18	0
	Venghlui	27	1
	Chanmari West	37	0
	Aibawk	9	0
	Tachhip	4	0
	Thingsulthliah	49	0
	Tlungvel	16	0
	Sailam	5	0
	Pehlawn	10	1
	Saitual	38	3
	Durtlang	64	5
	Sihphir	19	0
	Thingsul Tlangnuam	22	0
	Maubuang	5	0
	Sateek	4	0
	Ramhlun North	8	0
	Muallungthu	11	0
	Republic	23	0
	Electric Veng	5	0
	Republic Veng	10	0
	Khatla South	7	5
	Aizawl Venglai	15	1
	Total	442	20 (4.52%)
Lunglei	Sazaikawn	37	1
	Chanmari	96	7
	Thingfal	13	1
	Chanmari-II	50	3

contd...

Table 1 continue

District	Place of Collection	Total number of samples tested	Found positive
	Farm Veng	47	0
	Lungsen	29	0
	Lungpuizawl	24	0
	Lunglawn	42	10
	Haulawng	27	7
	Mualthuam	22	3
	Serkawn	33	12
	Rangte	24	4
	Lungsen 'N'	10	1
	Lungsen 'S'	9	2
	Leite	12	0
	Rotlang 'E'	7	1
	Thlengang	3	2
	Ramlaitui	13	0
	Total	498	54 (10.84%)
Champhai	Ngur	10	0
	Vengsang	24	0
	Kahrawt	10	0
	Hmunhmeltha	71	2
	Zokhawthar	67	3
	Khuangleng	9	3
	Ngopa	10	0
	Khawbung	3	0
	Hnahlan	1	0
	Zotlang	30	0
	Ruantlang	30	0
	Zote	29	0
	Tlangsam	35	0
	Vapar	20	1
	Melbuk	35	0
	Keifang Tlang	35	1
	New Champhai	25	1
	Mualkawi	30	0
	Total	474	11 (2.32%)
Saiha	Tuipang	28	3
	Zawngling	22	7
	Bualpui Ng	36	8
	New Colony	1	1
	Vengpui-II	1	0
	Meisavaih East	24	4
	Meisatla I	37	16
	New Saiha East	18	9

contd...

Table 1 continue

District	Place of Collection	Total number of samples tested	Found positive
	Tlangkawn	9	3
	New Saiha	23	2
	Thingsen	12	9
	Rawmibawk	7	3
	Siata	18	5
	Sangau	15	4
	Vengthar	10	2
	Zero Point	24	1
	Kawlchaw	17	0
	Theiva	19	3
	Phura	21	4
	Total	342	84 (24.56%)
Kolasib	Venglai	30	14
	Tumpui	18	4
	Hmar Veng	6	6
	Vairengte	44	12
	Kawnpui	57	16
	Khuangpuilam	17	6
	Project Veng	31	16
	Lungdai	20	2
	Bairabi	20	0
	Total	243	76 (31.27%)
Mamit	W. Phaileng	126	6
	Dinthar	47	2
	Zawlnuam	94	2
	Lallen	17	0
	Chhippui	15	2

contd...

Table 1 concluded

District	Place of Collection	Total number of samples tested	Found positive
	Luangpawl	23	3
	Charkawn	33	1
	Lungsir	26	0
	Damparengpui	20	0
	Khawhnai	5	0
	Teirei Forest	7	0
	Tuirum	7	0
	Total	420	16 (3.80%)
Serchhip	Hmunzawl	10	0
	Piler	20	1
	Sialsir	22	0
	Baktawng	91	5
	Hmawngkawn	6	1
	Chhingchhip	43	8
	Serchhip III & IV	55	23
	Dinthar	72	37
	New Serchhip	42	0
	East Lungdar	20	3
	Sailulak	10	8
	Lungchhuan	10	1
	Leng	4	1
	Chhiahtlang II	23	4
	Chhiahtlang I	28	10
	Sialhau	10	4
	Total	466	106 (22.74%)
	Grand Total	2996	373 (12.44%)

Mukteswar, Uttarakhand, and stored at -80°C until used.

The parameters and reagents, in particular the dilution of test serum and the porcine species-specific horse radish peroxidase (HRP) conjugated antibodies of the validated r3AB3 NSP ELISA kit used for screening FMDV NSP-antibodies (NSP-Abs) in bovine serum (Mohapatra *et al.* 2011) were modified and optimized to assess antibodies against 3AB3 NSP in pigs. For this, porcine test serum samples along with the positive and negative controls were diluted at 1:20 ratio in the diluent buffer. Subsequently, anti-porcine HRP conjugated antibodies (Komabiotech, South Korea) were dispensed at 1:12000 dilutions. Serum samples producing corrected optical density (OD) values ≥55% of that of the positive control were considered positive. Corrected OD was obtained after subtracting the background OD or OD of blank media (here it is the diluent buffer) from the test sample OD. It typically refered to the OD measurement from a spectrophotometer adjusted for background interference.

In order to corroborate the 'no vaccination' situation as obtained from history, representative serum samples were tested for protective anti-structural antibody against FMDV serotypes O, A and Asia 1 using in-house LPB ELISA kit as per the procedure described earlier (Ranabijuli *et al.* 2010). For this, two-fold dilution (from 1:16 to 1:128) of serum samples was tested. The results were expressed as percentage reactivity for each serum dilution as follows:

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

The antibody titres were expressed as logarithm of reciprocal of serum dilutions giving 50% of the absorbance recorded in the antigen control wells.

FMD is highly contagious and is arguably one of the

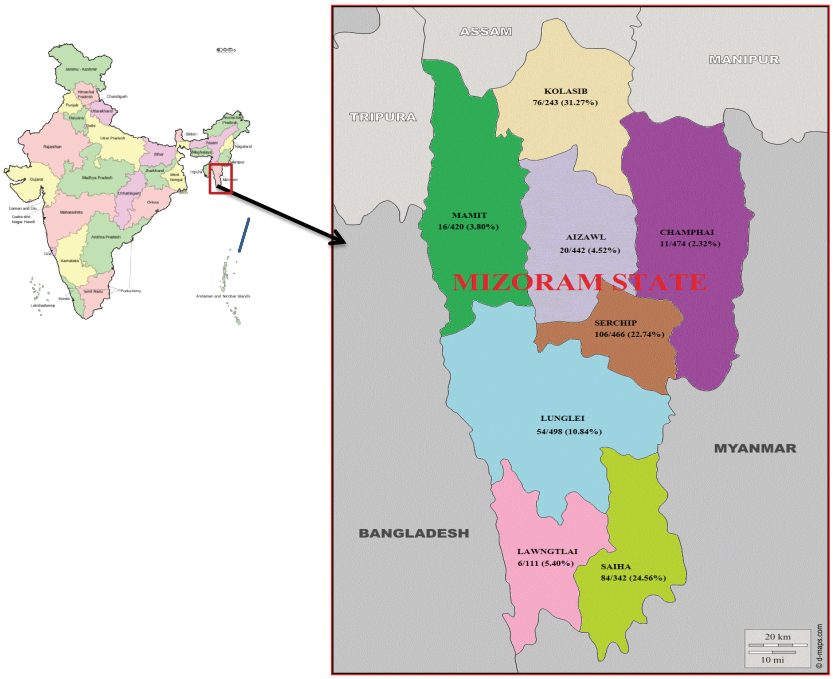

Fig.1. Map of Mizoram state showing district-wise NSP-Ab seropositive pig population during the year 2014-2015. Eight different districts are depicted with different colours. The number of pig serum samples collected and tested from each district is shown in the denominator, whereas those found positive is denoted in the numerator part along with the percentage positivity in each district of the state. The figures in parentheses denote percent positivity in 3AB3 NSP ELISA for the respective district.

most important livestock diseases globally in terms of economic impact significantly constraining the livestock productivity, and profoundly impacting the livelihoods of the rural communities. Infected pigs are important from an epidemiological perspective and are potent emitters or amplifiers of aerosolized FMDV (Donaldson *et al.* 1987). According to Alexandersen *et al.* (2003), one infected pig can produce up to 60-times more airborne virus per day compared to sheep and cattle. Despite efforts, the disease is still endemic in Asia, Africa, and a few countries in South America (World Organization for Animal Health, 2024). The economic devastation caused by FMD outbreaks in pigs is well exemplified from the Taiwanese pig industry in March 1997, where the total cost was estimated at US$4,050 million (Donaldson 1999).

In the present study, the 3AB3 indirect NSP ELISA was successfully applied to screen random pig sera for FMDV infection-specific NSP-Abs, which, in turn, is an indicator of previous exposure of the animals to the virus. In the 3AB3 NSP ELISA, 373 out of 2996 (12.44%) samples were found positive for NSP-Abs and its percentage varied by districts as evident from the table 1. The district-wise number of serum samples tested and number of samples found positive in 3AB3 NSP ELISA, along with percentage positivity are presented in Figure 1. On the contrary, in LPB ELISA, none of the pigs were found to have protective $\log_{10}$ antibody titre of ≥1.8 against FMDV serotypes, confirming 'no vaccination' scenario in pigs in the region. Moreover, in India, systematic vaccination campaigns under Government-endorsed FMD control programme, named as Livestock Health and Disease Control Programme (LHDCP) that have been in place, cover only domestic large ruminants. In India, due to lack of control over animal movement across the states, FMD spreads easily from one area to another. It is important to note that the apparent prevalence of FMDV 3AB3 NSP-Abs in the bovine population of Mizoram state during the study period was 9.76% (Annual Report DFMD 2014-2015). While confirming the seropositivity in pigs sampled in this study, the possibility of virus exchange between cattle and pigs in the region cannot be ruled out. Mizoram shares international borders with Bangladesh and Myanmar, making it critical in terms of the transmission and spread of transboundary animal diseases. It is well established that FMDV infected pigs being amplifier hosts may pose severe threat to other susceptible domestic livestock also. Therefore, pigs should be routinely vaccinated against FMD and included in the ongoing surveillance and control programmes of the country, along with strict adherence to biosecurity measures.

Serological test demonstrating NSP-Ab helps in distinguishing FMDV infected from vaccinated and uninfected animals (Clavijo *et al.* 2004), for which NSP ELISAs have become an integral companion to vaccination-centric control and surveillance programmes worldwide. Vaccination-based control and elimination of FMD has been proven effective in many developed countries, such as mainland Europe (Lombard *et al.* 2007) and South

America (Naranjo and Cosivi 2013). Therefore, preventive vaccination combined with systematic serosurveillance for NSP-Ab and monitoring of protective SP-Ab status can significantly contribute to controlling the disease in the country. The use of this indigenously developed 3AB3 NSP ELISA validated as a simple, cost-effective diagnostic assay for large scale serosurveillance of pigs offers a clear advantage over commercial test kits due to cost implications for countrywide large-scale serosurveillance. Several researchers have applied different NSP ELISAs for testing pig sera (Chung *et al.* 2002; Chung *et al.* 2003; Lee *et al.* 2004; Bruderer *et al.* 2004) for FMD. Following the 1997 Taiwanese epidemic, 3AB NSP antigen was used in serological surveillance programme to facilitate the FMD eradication campaign and evaluation of the mass vaccination programme (Chung *et al.* 2002).

In conclusion, 3AB3 NSP ELISA was optimized and applied for a state-wide surveillance to assess the FMD status in pigs in Mizoram. The apparent seroprevalence of 3AB3-Abs in the randomly sampled pigs suggested virus exposure in these species. Additionally, the LPB ELISA indicated the non-existence of protective anti-structural antibodies, which are crucial to preventing disease and virus transmission. The data generated here on apparent seroprevalence of FMD, although restricted to a single state, may pave the way for an elaborate countrywide sero-epidemiological study in the pig population, which is critical to effective implementation of disease control strategies. The findings of this study can further be considered in strengthening the FMD control programme in India by including pigs in different states including the NE region.

SUMMARY

The primary objective of this study was to estimate the seroprevalence of foot-and-mouth disease virus (FMDV) 3AB3 nonstructural protein (NSP) antibodies in pig population of Mizoram. The 3AB3 NSP indirect ELISA, used for bovines, was modified using anti-porcine IgG-horse radish peroxidase (HRP) conjugate and successfully applied for serosurveillance of FMD in pig population of Mizoram. In total, 2996 randomly collected pig serum samples were tested using 3AB3 NSP ELISA, wherein 373 samples (12.44%) were found positive for NSP antibodies indicating past exposure to FMDV. Further, a set of representative serum samples was subjected to liquid phase blocking (LPB) ELISA to assess the level of protective structural antibody against FMDV serotypes O, A and Asia 1, but none of the pigs were found to have a protective $\log_{10}$ antibody titre of ≥1.8 against any of the three FMDV serotypes, indicating a 'no vaccination' situation in pigs in the region. FMDV-infected pigs being the amplifier hosts, excrete enormous quantity of virus and may pose a severe threat to other domestic livestock. Hence, pigs need to be included in ongoing surveillance and control measures, including prophylactic vaccination coupled with zoosanitary measures.

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