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Seroprevalence of porcine reproductive and respiratory syndrome virus and classical swine fever virus in pigs of Mizoram, India

K. Lalhraipuii^{1*}, I. Shakuntala¹ and A. Sen²¹ICAR-RC for NEH Region, Mizoram Centre, Kolasib-796 081, India²Animal Health Division, ICAR-RC for NEH Region, Barapani-793 001, India*Corresponding Author Email : lhpuii@gmail.com

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Abstract

Aim: This study aimed to evaluate the prevalence of antibodies against two economically significant viral pathogens in domestic pigs like porcine reproductive and respiratory syndrome virus (PRRSV) and classical swine fever virus (CSFV) through a serological study in swine herds in Mizoram, India.

Methodology: Serum samples were collected from apparently healthy and suspected pigs of different age groups comprising weaners, growers and adult in four districts (Aizawl, Champhai, Kolasib and Serchhip) of Mizoram during February, 2018 to June, 2019. Seroprevalence of CSFV and PRRSV specific antibodies was detected by enzyme-linked immunosorbent assay (ELISA).

Results: Out of 420 samples, 117 samples were found positive for PRRSV, 171 samples for CSFV indicating a mean positivity of 27.86% and 40.48%. Presence of mixed infection was observed in 13.33% samples.

Interpretation: The present study demonstrated the extensive circulation of classical swine fever virus and porcine reproductive and respiratory syndrome virus among domestic pig populations of Mizoram, India.

Key words: Antibodies, CSF virus, ELISA, Pigs, PRRS virus, Serological study



420 serum samples were collected from apparently healthy and suspected pigs of different age groups from four districts (Aizawl, Champhai, Kolasib and Serchhip) of Mizoram

All serum samples were tested for PRRSV and CSFV-specific antibodies using a commercially available enzyme - linked immunosorbent assays (ELISA) kit according to the manufacturer's instruction

The overall prevalence of PRRSV and CSFV antibodies detected in the study period was 27.86% and 40.71% from four districts of Mizoram

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a highly infectious viral disease of swine. It was first recognized in 1987 in the United States of America and is characterized by reproductive failure in sows and respiratory tract illness in pigs (Keffaber, 1989; Albina, 1997; Pejsak *et al.*, 1997; Rossow, 1998). PRRSV belongs to *Arteriviridae* family in the order of *Nidovirales* within the genus *Arterivirus*, which is an enveloped virus with a linear positive-stranded RNA genome with an approximate diameter of 50–65 nm (Wensvoort *et al.*, 1991; Collins *et al.*, 1992). The virus causes abortions in late pregnancy, stillborn or weak piglets, reproductive failure, respiratory disease and high death rates in suckling, and weaned pigs (Zimmerman *et al.*, 2012). It became pandemic, covering North America, Europe and Asia in the subsequent years (Morin *et al.*, 1991; Wensvoort *et al.*, 1991; Baron *et al.*, 1992; Benfield *et al.*, 1992; Bøtner *et al.*, 1994; Kuwahara *et al.*, 1994). Presently, the disease occurred in most pig-producing countries and caused enormous economic loss to the swine industry (Thanapongtharm *et al.*, 2014; Scanlan *et al.*, 2019).

India has reported the first outbreak of PRRS in the pig population of Mizoram state to the Office International des Epizooties (OIE) on 26 June 2013. Since its first outbreak in 2013, PRRS had been established as an enzootic disease in pig population of Mizoram and caused huge loss to the pig farmers (Rajkhowa *et al.*, 2016). The disease was observed to affect all age groups including the pregnant sows with morbidity rate 44.01%-80.29% and mortality rate 14.51%-66.11% (Gogoi *et al.*, 2017). Classical swine fever virus (CSFV), also known as 'hog cholera', is a highly contagious and often fatal disease of domestic pigs and wild boar (Leifer *et al.*, 2010) economically affecting various parts of the world (Edwards *et al.*, 2000). Classical swine fever, classified under list-A diseases by Office International des Epizooties, is considered as a transboundary animal disease by Food and Agriculture Organization (Sarma *et al.*, 2008). It causes significant economic losses to the swine industry throughout the world. The disease is currently endemic to India especially in intensive pig rearing units of north-eastern, northern and southern states of India (Saini *et al.*, 2000; Sarma *et al.*, 2011; Malsawmkima *et al.*, 2015).

In the north-eastern region, the outbreaks of classical swine fever virus have been reported from Nagaland, Mizoram, Meghalaya and Assam (Rahman *et al.*, 2001; Sarma *et al.*, 2011; Rajkhowa *et al.*, 2013; Malsawmkima *et al.*, 2015; Mukherjee *et al.*, 2018; Barman *et al.*, 2020). The Department of Veterinary and Animal Husbandry, Government of Mizoram, has identified the CSF as disease of special economic importance for Mizoram in their Annual Report (2002-2003). The disease is characterized as a hemorrhagic fever in its acute form leading to chronic and clinically inapparent form (Edwards *et al.*, 2000; Moennig, 2000). The causative agent 'CSF virus' (CSFV) belongs to family *Flaviviridae*, a member of genus *Pestivirus*, is a small, enveloped virus with a non segmented, single-stranded positive RNA genome (Gong *et al.*, 2016).

In North-eastern India, especially Mizoram, the majority of livestock farmers are dependent on healthy pig population for their livelihood. The swine population plays a major role for the socio-economic status of the state of Mizoram. According to 19th Quinquennial Livestock Census of India (2012) data reproduced in the Mizoram Economic Survey 2017-18, total population of Livestock in Mizoram is 3,84,604 where pigs constitute the largest group (69.33 %), followed by cattle (9.95%), goat (5.7%). Porcine reproductive and respiratory syndrome virus and classical swine fever virus are economically important swine viruses causing reproductive failure/respiratory symptoms in pigs (Liu *et al.*, 2013; Chen *et al.*, 2019). It stands clear that the health of pig population should be given high priority, and diagnosis of such economically important diseases like reproductive problems becomes crucial for sustaining pig production. In view of the above, this study was conducted to determine the antibody prevalence of PRRSV and CSFV infection in pigs raised in different districts of Mizoram.

Materials and Methods

Ethical approval: As per Committee for the Purpose of Control and Supervision of Experiments on Animal Guidelines, a study involving clinical samples does not require the approval of Institute Animal Ethics Committee. However, the samples collected for the present study followed standard sample collection methods without causing any harm or stress to the animals (Parasuraman *et al.*, 2010).

Study area and data collection: In all 420 serum samples were collected from apparently healthy and suspected pigs of different age groups comprising weaners, growers and adult from organized and unorganized farms from four districts (Aizawl, Champhai, Kolasib and Serchhip) of Mizoram, India during February, 2018 to June, 2019. These districts were selected due to its close proximity to the border of Myanmar where Mizoram shares a porous international border of 510 km with Myanmar. There are chances of growing risk for animal populations to become exposed to transboundary animal disease pathogens.

Sample collection: Approximately, 4-5 ml of blood was collected from intra-cardiac in piglets and ear vein in older pigs. Blood samples were collected in sterile vacutainer tubes without ethylenediaminetetraacetic acid (EDTA) and centrifuged at 1000 rpm for 3 min. Serum was separated and stored at -20 °C before examination.

Serology: All serum samples collected from apparently healthy and suspected pigs of different age groups were tested for PRRSV-specific antibodies using a commercially available enzyme-linked immunosorbent assays (ELISA) kit.

Serum samples were considered positive for PRRSV antibody if the optical density value of the sample to that of positive control was ≥ 0.4 according to the manufacturer's instruction. Similarly, for detection of CSFV-specific antibodies commercially available ELISA kit was used following

manufacturer's instructions. The results obtained were expressed as positive and negative based on the manufacturer's recommended cutoff value (Table 1).

Results and Discussion

Serum samples were collected randomly from organized and unorganized farms of four districts of Mizoram, India bordering Myanmar since majority of the pigs were imported through the international border illegally, not only for slaughtering but also for breeding purpose. Antibodies to PRRSV and CSFV were detected in pig population in these four districts (Aizawl, Champhai, Serchhip and Kolasib) from February, 2018 to June, 2019. The overall prevalence of PRRSV antibodies detected in the study period was 27.86% in Table 2 which was comparatively less compared to earlier reports where 61.96 % PRRSV seroprevalence was reported in Aizawl district, Mizoram during the outbreak period from March to June, 2013 (Rajkhowa et al., 2016). Previous studies have reported a prevalence of 18.5 %, 17.2%, 3.62% and 0.84% PRRSV antibody sero-positive samples in pigs from Nepal, Vietnam and North East, India (Mahesh et al., 2015; Pegu et al., 2017; Mukherjee et al., 2018; Lee et al., 2020) which is relatively lesser than the present study.

This difference may be due to the geographical location, time of sample collection, test procedures, sample size etc. The present finding corroborates the reports of Gogoi et al. (2017) who revealed 28.26% seropositivity. The report on systematic studies on PRRSV has not been reported from India, although genomic and phylogenetic studies are available (Rajkhowa et al., 2015, 2016). Interestingly, animals with positive PRRSV antibody have been also found positive for CSFV antibody, i.e., 13.33% serum samples showed the presence of PRRSV and CSFV. The presence of PRRSV can also have profound effect on the vaccination efficiency because PRRSV is immunosuppressive and it seriously inhibits the immune response to CSF vaccine (Tao et al., 2013; Mao et al., 2015; Zhou, 2019). During the study period, Kolasib districts of Mizoram showed the highest prevalence (34.69%) of PRRSV antibodies as compared to other three districts of Mizoram, i.e., Aizawl (25.00%), Champhai (17.9%) and Serchhip (34.5%) as shown in Fig. 1. In the first outbreak of PRRSV in Mizoram on June, 2013 (Rajkhowa et al., 2015) no PRRSV positive samples were detected from Kolasib districts of Mizoram. This study showed an extensive circulation

of PRRSV as vaccination against this disease is not practiced in Mizoram and, hence, possibility of antibody production through vaccination is completely ruled out. There was high prevalence of PRRSV in more than 6 month-old pigs (38.1%) as compared to pigs less than 6 months (13.6%) (Fig. 2). Similarly, 29.7% female serum samples showed higher prevalence to PRRSV as compared to males (25.9%).

This finding is in corroboration with the results of Mahesh et al. (2015) who reported high prevalence of PRRSV in more than 6 month-old pigs than pigs less than 6 month of age and also higher prevalence of PRRSV in female serum as compared to males. In this study, the overall prevalence of CSFV antibodies detected was 40.71% (Table 2) which was comparatively less when compared to previous pan India surveillance finding of 594 serum samples from 12 states and 287 tissue samples tested from 13 states of India using commercial ELISA kits in which mean prevalence of CSFV antibodies in suspected sera was 63.3% and CSFV antigen in the suspected samples was 76.7% (Nandi et al., 2011). The present study is in corroboration with the earlier study where 45.15% seroprevalence was detected (Mukherjee et al., 2018) but higher than reported by earlier worker, i.e., 37% (Patil et al., 2018). The study revealed the endemicity of the diseases in Mizoram since majority of the samples were collected from apparently healthy and suspected animals. Unrestricted movement of pigs from one region to another within the country and across the porous international border surrounding the North Eastern States, unavailability of adequate doses of vaccine and poor public awareness are some of the important factors that might have helped in perpetuating the disease in the state (Malsawmkima et al., 2015).

Detection of virus-specific antibody in animals indicates an indirect evidence of virus persistence in the farms. This implies that swine population acts as a major carrier of viruses and thus plays an important role in its dissemination. Harboring these viral pathogens without showing any distinguishable symptoms, these animals may be considered as reservoir host as viremia is prolonged and most infections are subclinical (Mukherjee et al., 2018). In the present study, Kolasib districts of Mizoram has shown the highest prevalence (54.1%) of CSFV antibodies compared to Aizawl (45%), Champhai (23.2%) and Serchhip (42.7%) districts as shown in Fig. 1. High seroprevalence of CSFV could be due to lack of awareness about vaccination

Table 1: ELISA kit used for the detection of specific antibody with cutoff value

Agent ^a	ELISA test ^b	Positive threshold ^c
CSFV	HERDCHEK CSFV Ab (IDEXX)	Blocking % \geq 40%
PRRSV	HERDCHEK PRRS 3X Ab (IDEXX)	OD \geq 0.4

^aCSFV=Classical swine fever virus2, PRRSV=Porcine reproductive and respiratory syndrome virus. ^bELISA=Enzyme linked immunosorbent assay. ^cOD=Optical Density

Table 2: District-wise details of positive serum samples by ELISA

District	No. of samples	Viral diseases	
		PRRSV	CSFV
Aizawl	100	25 (25.00)	45 (45.00)
Champhai	112	20 (17.85)	26 (23.21)
Serchhip	110	38 (34.54)	47 (42.73)
Kolasib	98	34 (34.69)	53 (54.08)
Total:	420	117 (27.86)	171 (40.71)

*Figures in parenthesis indicates percentage

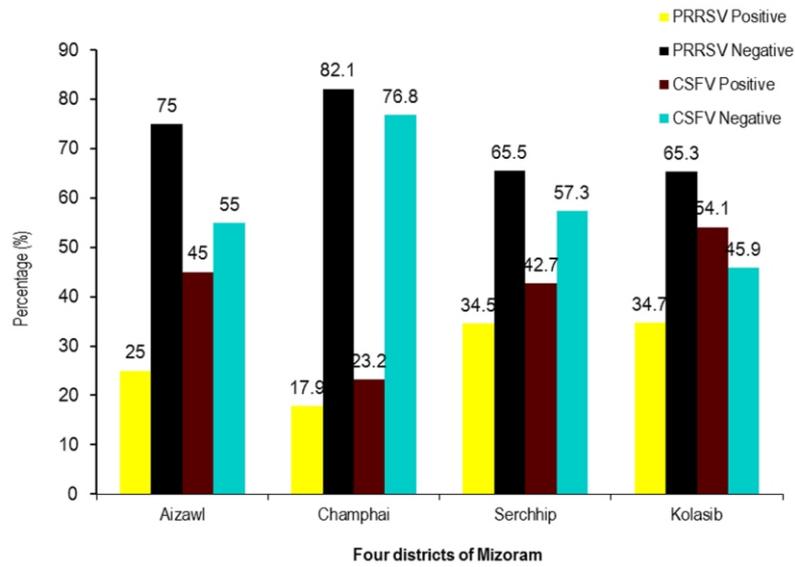


Fig. 1: Seroprevalence of PRRSV and CSFV in four districts of Mizoram.

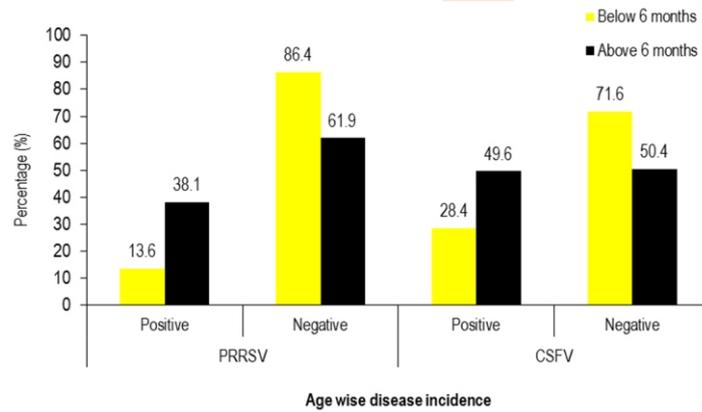


Fig. 2: Age wise seroprevalence of PRRSV and CSFV in Mizoram.

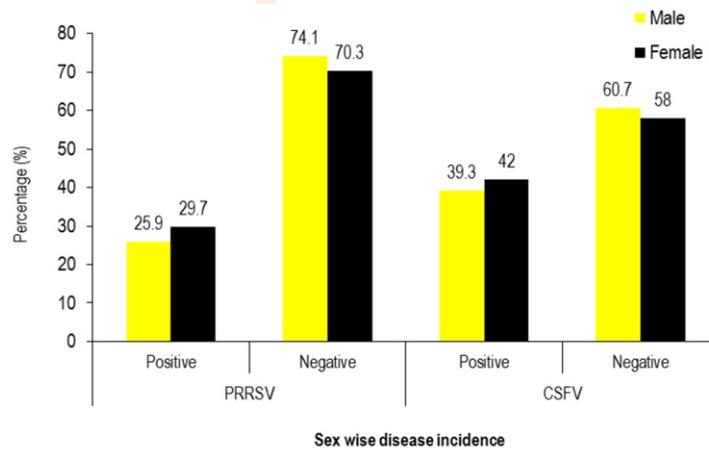


Fig. 3: Sex wise seroprevalence of PRRSV and CSFV in Mizoram.

programme of CSFV in unorganized farms or due to inappropriate titer of vaccine, cold chain abuse, and inappropriate vaccine dose leading to vaccination failure (Nandi *et al.*, 2011; Mukherjee *et al.*, 2018). There was high prevalence of CSFV in more than 6 months old pigs (38.1%) as compared to pigs less than 6 months old (28.4%). Similarly, 60.7% male serum samples showed higher prevalence to CSFV as compared to females (58%) in Fig. 3. It could be due to higher sample size in more than 6 months compared to less than 6 months old pigs. The study revealed an alarmingly high prevalence of PRRSV and CSFV antibody among different age group of pigs from organized and unorganized farms of different districts of Mizoram which is retarding the growth of swine industry in the state. To the author's best knowledge, this is the first observation on the seroprevalence of CSFV and PRRSV from different districts of pig population in Mizoram, India. This baseline data will be useful for formulation of control and eradication programmes.

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