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Farm characteristics and sero-prevalence of porcine reproductive and respiratory syndrome virus (PRRSV) antibodies in pigs of Nepal

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Abstract

Background: Porcine reproductive and respiratory syndrome is a highly infectious disease of swine caused by PRRS virus (PRRSV).

Objectives: To evaluate the prevalence of PRRSV antibodies in the four districts of hilly and terai regions of Nepal. Toassess the farm characteristics through a questionnaire interview of farmersregarding management practices and PRRS.

Methods: A cross-sectional study was conducted from July 2020 to June 2021 to determine the sero-prevalence of PRRSV in pigs. A total of 180 porcine serum samples were collected from 23 pig farms and tested for PRRSV antibodies by ELISA. Alongside, farm characteristics were also assessed through questionnaire to determine the level of biosecurity measures in the farm, knowledge of the disease and possible control mechanisms.

Results: Out of 180 samples, 37 were tested positive resulting the overall sero-prevalence of 20.5%. There was significant association between different districts ($p < 0.05$) and PRRS prevalence. Prevalence of PRRSV antibody was found higher in Kaski district (10.5%) followed by Sunsari (8.8%) district. Based on age groups, highest prevalence was found in age groups of above 18 months (9.4%), followed by 13–18 months age groups (7.7%). Regarding the knowledge level of the disease, 43% of the farmers responded that they have heard about the disease. Biosecurity practices in the farm was found very poor where only 40% of the farms had disinfectant at the entrance of the farm and 25% pig farmers were found using separate boots while dealing with pigs.

Conclusions: The findings of this study reveal the presence of PRRSV antibodies in pigs of Nepal. In addition poor biosecurity measures, management practices and poor knowledge level about the disease among farmers highly affect in the control and prevention of disease thereby affecting the pig production and productivity. Therefore, government should develop and implement effective control measures and biosecurity programs.

KEYWORDS
ELISA, pigs, PRRS virus, sero-prevalence

1 | BACKGROUND INFORMATION

Porcine reproductive and respiratory syndrome is a highly infectious disease of swine caused by PRRS virus (PRRSV) which is an enveloped, positive sense single-stranded RNA virus within the family *Arteriviridae*. The disease was first recognised in North America in the mid to late 1980s and in Europe in 1990 and is now present in almost all pig production areas of the world (Keffaber, 1990). Based on genetic diversity, PRRSV has been categorised into two distinct species PRRSV 1 (European) and PRRSV 2 (North American) (Walker et al., 2021). In addition, due to distinct genetic diversity, PRRSV 2 is further divided into virus lineages (Kuhn et al., 2016). These two species have significant genetic and antigenic differences with approximately 60% nucleotide identity at the genome level (Nelsen et al., 1999; Wensvoort et al., 1992). Clinical outbreaks and long-term effects of PRRSV infection are highly variable. Clinical manifestation of disease depends upon the breed and age of the infected pig, pregnancy status and trimester of gestation of the infected sow/gilt. Generally, infection causes anorexia, fever, lethargy, pneumonia, agalactia, red/blue discoloration of the ears and vulva, subcutaneous and hindlimb oedema (Terpstra et al., 1991). In pregnant gilts/sows, it causes late - term abortions or premature farrowing with stillborn foetuses, partially autolysed foetuses, and mummified foetuses (Terpstra et al., 1991). PRRSV alone or in combination with other pathogens accelerates the morbidity and mortality in infected pigs of all ages resulting in the decrement of the productivity of animals. Studies have revealed the variability on the clinical signs showed by the virus between farms. In some sero-positive farm, respiratory signs are distinct (Heukelbach et al., 2003; Akao and Ohta, 2007) whilst some have periodic outbreaks of reproductive disease in breeding sows (Morrison, 2001).

The virus is present in nasal secretions, faeces and urine, and infected pigs can be long-term carriers. Virus can spread both through horizontal and vertical routes whereby the transplacental transmission of the virus induces late-term abortions most commonly in the last trimester. Further, it reduces farrowing rate, production of heterogeneous litters and results in a decrease in the number of weaned piglets.

In Nepal, only few diagnostics surveys have been carried out to determine the prevalence of PRRSV. In 2011, the first serological survey of pig population in Kathmandu valley showed the prevalence of 32% for antibodies against PRRSV 2 species (formerly North American genotype) (Sharma, 2015). Subsequently, the first clinical cases of PRRS were confirmed in 2013 in Nepal (Prajapati et al., 2014). In 2014, a total of 200 pig sera samples from eight districts of four developmental regions of Nepal were screened for PRRSV antibody and showed a sero-prevalence of 18.5% (Mahesh et al., 2015). As vaccination against PRRS has not been started yet, these studies indicate that PRRSV has been present in Nepal since at least 2011. However, since the disease is not notifiable to government, there is no any exact data of PRRSV incidence though there is outbreak of disease among pig population. This study aims to evaluate the prevalence of PRRSV antibodies in the four districts of hilly and terai regions of Nepal. Alongside the farm characteristics through a questionnaire interview of farmers regarding management practices and PRRS were also studied.

2 | METHODOLOGY

2.1 | Study area and animals

A cross-sectional study was carried out with purposive sampling in pig farms of Kaski, Sunsari, Kavre and Lalitpur district from July 2020 to June 2021 (Figure 1). Purposive sampling was carried out as we included only interested pig farmers to participate in the study. The sampled pigs belonged to four exotic breeds, that is Landrace, Duroc, Hampshire, Yorkshire and the native breed Pakhribas Black pig. These samples were collected from multiple farms of study sites.

2.2 | Questionnaire survey

The farm owners of corresponding pig farm were interviewed and data were collected using a questionnaire form that included questions covering demographic information, biosecurity practices, knowledge about PRRS and other infectious diseases that can affect pigs. The demographic information included questions such as age, gender, educational status, number of children and number of pigs kept. A few questions were also asked relating to knowledge about PRRS disease, quarantine measures during introduction of new animals in the farm and managing sick animals. Initially the farmers were asked if they had heard about highly infectious diseases PRRS. There were further question relating to their attitudes and practices regarding PRRS, including how they dispose of animal waste and dead animals, and biosecurity measures in place to prevent the introduction and spread of disease.

2.3 | Sample collection

A total of 180 samples were collected from 23 pig farms of the terai (Sunsari district) and hilly regions (Kaski, Kavre, and Lalitpur districts) of Nepal. Blood samples were collected aseptically from the ear vein of pigs using a 3 ml sterile syringe. Blood was transferred to the plain vacutainer, that is, without containing EDTA and kept undisturbed until the clear serum was separated and then collected in a sterile

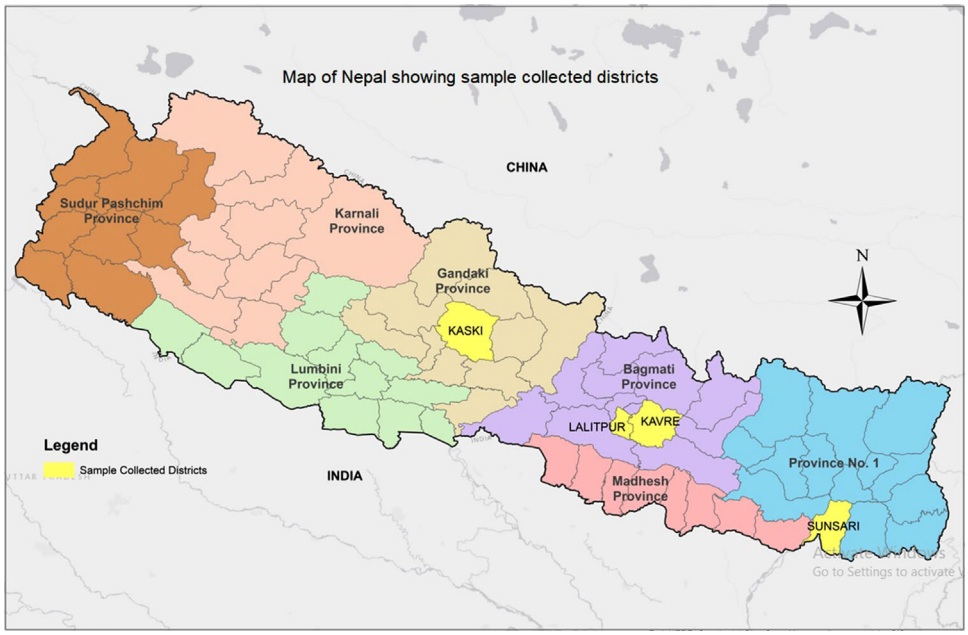

FIGURE 1 Map of Nepal showing sample collected districts

Eppendorff tube. The serum samples were labelled and transported on ice to the National Animal Health Research Centre, Khumaltar for examination by ELISA. The serum samples were kept there at −40°C until examination.

2.4 | Serological analysis

Serum samples were analysed by an indirect enzyme linked immunosorbent assay (I-ELISA) kit, which was manufactured by ID. Vet innovative diagnostics, France. This diagnostic kit detects antibodies directed against PRRSV-1 and PRRSV-2 strains in serum and plasma. The assay was performed as directed by the manufacturer's protocol and the optical density (OD) values were obtained using an ELISA reader at a wavelength of 450nm.

2.5 | Validation of ELISA results

The test is validated if: the mean value of the Negative Control OD (OD_{nc}) is less than or equal to 0.150 ($OD_{nc} \leq 0.150$). The difference of the mean values of the Positive and Negative Controls (OD_{pc} and OD_{nc}) is greater than or equal to 0.150 ($OD_{pc} - OD_{nc} \geq 0.150$). Results were expressed as sample positive ratio (*S*/*P*) as follows using the corrected sample and control values:

$S/P = \text{OD sample} - OD_{nc}/OD_{pc} - OD_{nc}$

Samples presenting an *S*/*P* ratio (*S*/*P*): less than 0.4 are considered negative and equal to or greater than 0.4 are considered positive.

2.6 | Statistical analysis

The data analysis was done using Excel and association of different variables were analysed using the chi-square test with significance level defined.

3 | RESULTS

3.1 | Results from serological study

Out of 180 sera samples, 37 were found to be positive against PRRSV indicating the overall sero-prevalence to be 20.6%. Table 3 shows the overall prevalence of PRRSV in pigs. There was significant association between different districts ($p < 0.05$) and PRRS prevalence. Prevalence of PRRSV antibody was found higher in Kaski district (10.5%) followed by Sunsari (8.8%) district and lower in Kavre and Lalitpur district (Figure 2). The sexwise distribution of PRRSV prevalence in pig showed 3.3% sero-positivity in male and 17.2% in female. There was significant association between different districts ($p < 0.005$) and PRRS prevalence.

Based on age groups, highest prevalence was found in age groups of above 18 months (9.4%), followed by 13–18 months age groups (7.7%). In addition, herd history obtained from farmers revealed that piglet death shortly after birth, abortions and stillbirths were common occurrences on all the farms. Higher prevalence of PRRSV antibody (10.55%) was observed in animals with history of abortions, stillbirths, piglet mortality shortly after birth and infertile pigs.

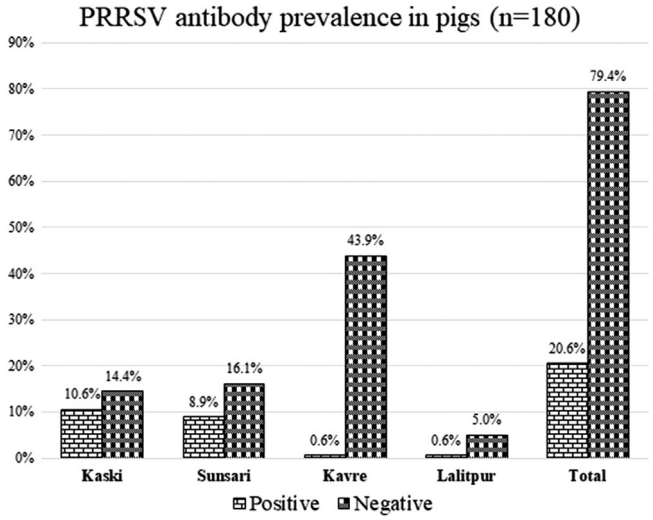

FIGURE 2 PRRSV antibody prevalence in pigs

3.2 | Results from the questionnaire interview

The study was conducted in 23 farms of hilly and terai regions of Nepal. Among them, 65.21% of the farmers were male participants and the remaining 34.7% were female. Regarding the education level of pig farmers, 47.82% had primary level education whereas 26.08% had higher education level. Demographic and farm characteristics were shown in Table 1 whereas management and biosecurity practices applied by the farmers were shown in Table 2. From the survey, it was found that the common feedstuffs given to pigs include feed waste from hotels and restaurants, kitchen leftover rice bran, wheat bran, flour, maize bran and few commercial farm feed commercial pellet feed. The survey result showed that the farmers' seem very interested and serious in seeking help when pigs show serious health problems indicating their positive attitudes towards veterinarians. Regarding knowledge level of PRRS, 43% of the participants responded that they have heard of the disease PRRS and the remaining 56.2% responded they have not heard about the disease.

It was observed that most of the farms have very poor biosecurity measures. About 25% pig farmers were found using boots while dealing with animals whereas none of them were found using gloves. Whilst few were found (1%) to use separate clothing during animal management, 40% farms have placed disinfectant at the entrance of the farm (Figure 3).

4 | DISCUSSION

PRRS is an economically important disease that is known to cost $663 million/ per year to the US swine industry (Holtkamp et al., 2013) showing an increase from $560 million/year in 2005 (Neumann et al., 2005). An effective control of disease depends upon the surveillance and early detection of disease. This study presents the sero-prevalence of PRRSV antibodies to be 20.3% in pig populations of the study sites. In the hilly

TABLE 1 Socio-demographic characteristics of the pig farmers

Parameters	*n*	%
Sex		
Male	15	65.21
Female	8	34.7
Age		
20–30	6	26.08
31–40	5	21.73
41–50	8	34.78
>50	4	17.39
Educational level		
Primary level	11	47.82
Secondary level	6	26.08
High School	6	26.08
Knowledge about PRRS		
Yes	10	43.47
No	13	56.52
Types of farming		
Integrated farming	8	34.78
Nonintegrated farming	15	65.21
Pig herd size		
10–50	9	39.13
51–100	7	30.43
101–150	4	17.39
>150	3	13.04
Farming type		
Integrated	8	34.79
Non Integrated	15	65.21
Pig farming duration		
Up to 5 years	15	65.21
Above 5 years	8	34.79

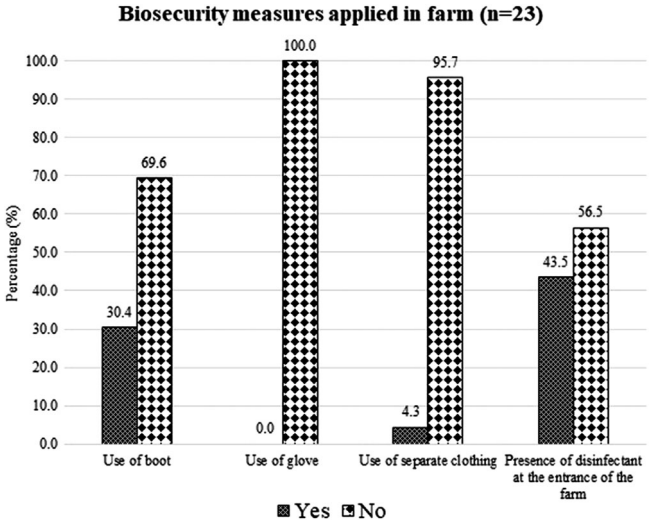

FIGURE 3 Biosecurity measures applied in farm

TABLE 2 Management and biosecurity practices in the farm

Features	*n*	%
Use of apron/separate clothing while dealing with pig		
Yes	1	4
No	22	96
Wear boots		0
Yes	7	30
No	16	70
Use of gloves		
Yes	0	0
No	23	100
Presence of disinfectant at the entrance of the farm		
Yes	10	43
No	13	57
Management of pig excreta		0
Sale	1	4
Use in crop farming	22	96
Have you encounter problems of abortion in pig?		0
Yes	22	96
No	1	4
Problem of death piglets?		0
Yes	22	96
No	1	4
Isolation of sick pigs		0
Yes	6	26
No	17	74
Quarantine of new pig before you introduced into the farm?		0
Yes	6	26
No	17	74
Distance of quarantine place		0
Near to adjacent pig pen (within 25 m)	23	100
Far from pig pen (>25 m)	0	0
Do you know that diseases transfer from pig to pig and farm to farm?		0
Yes	23	100
No	0	0
Heard about PRRS?		0
Yes	15	65
No	8	35
Washing of hands after touching pig or after dealing with pig?		0
Yes	23	100
No	0	0
Vaccinations		
Classical Swine fever	7	30
FMD	3	13
Both CSF and FMD	10	43
No vaccination	3	13

and terai region, the prevalence was observed to be 11.66% and 8.88%, respectively, which was found statistically significant. However, due to sample limitations, it could not represent the correct figure of seroprevalence in the hilly and terai regions of Nepal but it showed that the PRRSV is circulating in pig populations. Furthermore, this study limits in identifying the changes of disease situation over time as it is a cross-sectional study.

Antibodies against PRRS which can be detected by ELISA are produced after 9–13 days of infection (Yoon et al., 1995). The antibodies decline over time but are reported to persist for up to 28 months (Dee et al., 2000; Desrosiers and Boutin, 2002). Within 3–4 months of exposure, the virus will be cleared from the body and therefore the presence of antibodies mostly indicates the absence of virus but past infection (Wills et al., 2003). The sero-positivity of young piglets born on a farm indicated the presence of virus in the farm but this study limits the samples from young piglets. A screening study of PRRSV (PRRSV 1 and PRRSV 2) antibodies in Nigeria showed the prevalence of 53.8%, which is higher than the findings of our study (Aiki-Raji et al., 2018). Likewise, in Great Britain, the herd prevalence of PRRSV was reported to be 39.8% in 2004 (Evans et al., 2008). In Myanmar, serological survey of 331 samples showed the PRRSV prevalence of 41.1% (WIN and OO, 2017), which is higher than the findings of this study. In India, a serological survey study conducted in 2019 revealed the seropositivity to be 27.86% (Lalhruaipuii et al., 2020). In Nepal, serological survey conducted in 2011 and 2014 showed the prevalence of 32% and 18.5%, respectively (Sharma et al., 2016; Mahesh et al., 2015). This study found that abortion, stillbirths, death of piglets shortly after birth, infertility and poor growth were common in all the seropositive farms. Since the economic analysis of the impact of disease has not been carried out in the country, the exact loss to the farmers and country is unknown. However, it is believed that it is causing a huge loss to the farmers, thereby discouraging the farmers to continue pig farming since there is no animal insurance and loss coverage.

In this study, high sero-positivity (17.78%) was found in female and less in male (3.8%) pigs, which was statistically insignificant. Similarly Mahesh et al. (2015) reported the higher prevalence in female compared to male pigs. In most of the farms, the number of boars is less as they are kept mostly for breeding. Out of 35 boars, 7 were found positive for PRRS antibodies indicating that they may act as a potential source of infection as the viruses are shed in semen. Boars used for natural mating make contact with infected sows and during the process of mating they may get infected (Benfield, 2004).

Results showed that a higher prevalence of PRRSV was recorded in the age groups of above 18 months (about 7.7%) followed by pigs of age group 13–18 months. A statistically significant difference ($p < 0.005$) was observed between pigs of different age groups (Table 3). The study

TABLE 3 Overall prevalence of PRRSV in pigs

Variable	No sampled	PRRSV	*p* Value
Topography			
Hilly region	135	21 (11.66)	0.004*
Terai region	45	16 (8.8)	
Location			
Kaski	45	19 (10.5%)	<0.0001*
Sunsari	45	16 (8.8%)	
Kavre	80	1 (0.5%)	
Lalitpur	10	1 (0.5%)	
Sex			
Female	145	31 (17.2%)	0.6491
Male	35	6 (3.3%)	
Age (months)			
0–6	26	1 (0.5%)	<0.0001*
7–12	75	5 (2.7%)	
13–18	29	14 (7.7%)	
>18	50	17 (9.4%)	

*Denotes significance.

showed that out of 37 positive samples, 19 had history of abortion and the remaining 18 had other history such as lack of feeding, or a previous PRRS outbreak a long time ago.

The presence of antibodies against PRRSV was found to be higher in the farms where practices such as wearing boots, gloves and using separate clothing while dealing with the animals were poor. Around 40% pig farms had disinfectant at the entrance of the farm while pig farmers using boots were found to be 25% indicating poor biosecurity in the farm, which can increase the risk of introduction of disease. Inadequate biosecurity measures, lack of knowledge about PRRSV, and wrong feeding habits increase the risk of introduction and spread of disease. In a country like Nepal, where there are no national strategies to control PRRSV, farmers' behaviours can impact on the occurrence of disease epidemics. In order to know about the knowledge level of farmers about PRRS, they were asked some questions relating to PRRS and its effect on pigs. Around 43% farmers have heard about PRRSV; however, it was noticed that they lacked detailed knowledge of the disease suggesting the necessity of disease awareness program. Most of the farmers were also found keeping other animals along with pig. Around 34% farmers had integrated farming along with poultry, goat and fish farming.

The introduction of PRRS can be minimised through the practice of geographical isolation, buying animals only from known negative stock, and quarantine of animals before introduction into the farm. However, these practices were not found to be applied by farmers in the pig farm during the study, which might lead to constant circulation and reintroduction of virus in the farm thereby increasing the risk of spread of the disease (personal observation). The unhygienic condition of farms, ignorance in biosecurity measures, and dirty environment of farms observed in this study are of epidemiological importance. Studies have indicated that mechanical transport and transmission via contaminated farm materials and environment as well as contaminated vehicles, which are capable of conveying the virus over significant distances contribute to spread of diseases (Mengeling et al., 2000; Pitkin et al., 2009). Therefore, for prevention of the introduction of disease, production systems and biosecurity should be highly considered (Corzo et al., 2010; Rathkjen and Dall, 2017). Most of the pig sheds in Nepal are very poor with one production site and a layout comprising gestation, farrowing, nursery and growing are also not reasonable. Many farms are still continuous flow sites and lack good biosecurity systems. So, these observations suggests that disease awareness programs of PRRS covering its aetiology, disease transmission, clinical signs and control and quarantine measures should be conducted for pig farmers to minimise the disease occurrence. Detailed study on PRRSV transmission dynamics and patterns of clinical disease is highly essential which will help in controlling the disease.

5 | CONCLUSION

The findings of this study revealed that PRRSV antibodies are present in pig populations of the study sites. Poor biosecurity measures of the farm and the lack of awareness among pig farmers all contribute to continued transmission of the disease thereby affecting the pig production and productivity in the country. Therefore, government should develop and implement effective control measures and biosecurity programs to minimise the losses caused by the disease.

AUTHOR CONTRIBUTIONS

Meera Prajapati: conceptualisation, investigation, methodology. Madhav Prasad Acharya: data curation. Prakash Yadav: resources. Frossard Jean-Pierre: data curation, writing – review & editing.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as no animal was treated in this study.

DATA AVAILABILITY STATEMENT

Data will be available upon request.

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PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.1011.

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