Journal of Nepal Agricultural Research Council
Vol.10:105-111, December 2024
ISSN: 2392-4535 (Print), 2392-4543 (Online)
DOI: https://doi.org/10.3126/jnarc.v10i1.73328

Cross-sectional Study on Sero-Prevalence of Porcine Reproductive and Respiratory Syndrome in Bagmati Province of Nepal

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Received: Sept. 29, 2024, Revised: Nov. 05, 2024, Accepted: Dec. 15, 2024, Published: December 2024.

The authors declare that there is no conflict of interest.

ABSTRACT

A total of 178 samples were collected from four districts of Bagmati provinve from March 2022 to January 2023 and the serum samples were tested for antibodies against PRRS virus by ID Screen® PRRS Indirect Enzyme-linked immunosorbent assay (ELISA) kit. Out of 178 samples, 28 samples were found to be positive for PRRSV antibodies indicating overall prevalence of 15.73% with the highest prevalence observed in Kathmandu (11.79%) and lower prevalence in Lalitpur district (3.93%). Notably, no sero-positive cases were detected in Kavrepalanchowk and Chitwan districts suggesting a localized distribution of PRRSV within the province, potentially influenced by differences in farming practices, biosecurity measures, or animal movement between districts. Age-wise analysis revealed significantly higher sero-prevalence in younger pigs aged 0-6 months (8.42%) and 7-12 months (6.74%) compared to older pigs over 12 months (0.56%). Female pigs exhibited a higher prevalence (10.67%) compared to males (5.05%), although this difference was not statistically significant. Farm management practices significantly impacted on PRRSV prevalence. The incidence of PRRSV infection was found to be more in farms which had relatively poor biosecurity measures and unhygienic environment. Hence, the findings of this study revealed the presence of PRRSV antibodies among pig populations of Bagmati province. For an effective control and prevention, strict biosecurity and quarantine measures along with continuous monitoring of the pig populations is highly important. Farmers should be educated about biosecurity, pig disease transmission and management and quarantine because majority of them are unaware of these issues.

Keywords: ELISA, PRRS, Sero-prevalence

सारांश

बागमती प्रदेशका विभिन्न जिल्लाहरूबाट मार्च २०२२ देखि जनवरी २०२३ सम्म कुल १७८ नमूनाहरू सङ्कलन गरिएको थियो र PRRSV Indirect Enzyme-linked Immunosorbent Assay (ELISA) किटद्वारा PRRSV भाइरस विरुद्धको एन्टिबडीका लागि सीरम नमूनाहरू परीक्षण गरिएको थियो। १७८ नमूनाहरूमध्ये, २८ नमूनाहरूमा PRRSV एन्टिबडी पोजेटिभ पाइयो जसले १५.७३% को समग्र व्यापकता देखाउँछ जसमा सबैभन्दा बढी काठमाडौं ११.७९% र त्यसपछि ललितपुरमा ३.९३% को व्यापकत देखिएको छ भने काभ्रेपलाञ्चोक र चितवन जिल्लामा कुनै पनि सेरोपोजिटिभ केसहरू फेला परेनन्। त्यसैगरी उमेर अनुसार ०-६ महिनामा ८.४२%, ७-१२ महिनामा ६.७४% र १२ महिनामा ०.५६% व्यापकता देखिएको छ। पोथी सुंगुरमा १०.६७% र भालेमा ५.०५% को व्यापकता पाइएको छ। खोर व्यवस्थापन अभ्यासहरूले PRRSV संक्रमणलाई महत्वपूर्ण रूपमा प्रभाव पारेको हुन्छ। PRRSV संक्रमणका घटनाहरू तुलनात्मक रूपमा कमजोर जैविक सुरक्षा उपायहरू र अस्वच्छ वातावरण भएकाहरूमा बढी पाइएको थियो। तसर्थ, यस अध्ययनको नतिजाले बागमती प्रदेशको सुंगुरहरूमा PRRSV एन्टिबडीको व्यापकता देखिएको छ। प्रभावकारी नियन्त्रण र रोकथामको लागि, सुँगुरमा यो रोगको निरन्तर अनुगमनसँगै कडा जैविक सुरक्षा र क्वारेन्टाइन उपायहरू अत्यन्त महत्वपूर्ण छ। कृषकहरूलाई जैविक सुरक्षा, व्यवस्थापन र क्वारेन्टाइनबारे शिक्षित गराउनु पर्ने देखिएको छ किनभने अधिकांश कृषकहरू यी विषयहरूबारे अनभिज्ञ छन्।

INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) is as a serious swine disease characterized by either reproductive failure in pregnant sows or respiratory distress particularly in sucking pigs (Wensvoort et al 1992). This viral disease was first discovered in the United States in 1987, later in Europe and in Asia in the early 1990s and has now spread worldwide causing enormous economic losses each year (Albina, 1997).

The etiological agent of PRRS is porcine reproductive and respiratory syndrome virus (PRRSV), a small enveloped, single-strand positive-sense RNA virus of the family *Arteriviridae*. There are two genotypes, type 1 (European) and type 2 (North American) genotypes, which are now known as PRRSV-1 and PRRSV-2 respectively (Correia-Gomes et al 2022). PRRSV is one of the most rapidly evolving RNA viruses through the accumulation of mutations and recombinations (Fang et al 2022).

Pigs of any age are the only animals known to be naturally infected with PRRSV(Albina, 1997). Transmission occurs by either direct or indirect contacts and even vertically to fetuses (KC et al 2015). Virus is present in body secretions and excretions including saliva, nasal secretion, urine, semen, milk, and colostrum (Wills et al 1997). Pigs get infected through ingestion, inhalation, inoculation, insemination or by coitus (Pileri & Mateu, 2016). The virus can readily be spread by transport vehicles, fomites and personnel (Pitkin et al 2009).

Signs may include death, anorexia, pyrexia, agalactia, lethargy and sometimes skin discoloration (blue ears, blue vulvas, blue skin areas), laboured breathing, coughing and pneumonia. Reproductive signs include abortion, premature and late farrowing, stillborn, mummified or decomposing piglets as well as weak newborn piglets. Infertility, including delayed returns to oestrus, persistent rebreeding, and persistent anoestrus may also be seen (Wensvoort, 1993). Boars may show loss of libido and temporary reduction in semen quality (Hopper et al 1992).

PRRS was first confirmed in China in 1996 and has spread widely in China since then. In June 2006, there was an emergence of highly pathogenic PRRSV variants, which led to a pandemic that affected almost half of China. Over 2 million pigs were affected with over 400,000 fatal cases i.e. 20% mortality (Guo et al 2018; Li et al 2007). India reported its first outbreak of PRRS in the pig population of Mizoram state to OIE on the 26 June 2013 (Rajkhowa et al 2015).

In Nepal, this disease was serologically reported in pigs of Kathmandu valley in 2011 (Sharma et al 2016) while the outbreak of PRRS was reported in 2013 (Prajapati et al 2014). PRRS is an emerging disease and has been present in Nepal for about a decade now and is making a mark. PRRS outbreaks have occurred in the period when the pig industry in Nepal was booming and has caused great economic losses. The objective of this study was to determine the seroprevalence of PRRS in pigs of Kathmandu, Lalitpur, Chitwan and Kavrepalanchowk districts of Bagmati Province along with the farm features of pig farms such as housing, management, and biosecurity.

MATERIALS AND METHODS

Study area and sample size

A cross sectional study was conducted in 4 districts of Bagmati province, namely Kathmandu, Lalitpur, Kavrepalanchowk and Chitwan districts from March 2022 to January 2023. Pigs in these areas are mostly reared in clusters along the riverbanks. The sample size for the study was calculated by the following formula given by Open Epi. This is a free and open-source programme for epidemiological statistics and provides statistics for counts and measurement in descriptive and analytical studies. This programme uses the given equation to calculate the sample size:

Sample size (n) = [DEFF*Np (1-p)] / [(d2/Z21-α/2* (N-1)+p*(1-p)] where,
Population size (for finite population correction factor or fpc) (N) = 179363 (for Bagmati province)
Hypothesized % frequency of outcome factor in the population (p) = 20% +/- 5
Confidence limits as % of 100 (absolute +/- %) (d)= 5%
Design effect (for cluster surveys- DEFF) = 1

The calculated sample size for pig population in Bagmati Province is 246. However, due to the time limitation, lack of resources, farmer cooperation, closure of pig farms due to ASF outbreak, only 178 samples were collected.

Questionnaire survey

A questionnaire was made, and farmers were interviewed in order to gather basic information about pig raising, housing and management and health status. Farmers were questioned about their farming system, herd size, breeds, pen cleaning, feeding practices, waste disposal, biosecurity measures and quarantine practices.

Sample collection

A total of 178 samples were collected from 4 districts of Bagmati Province. Blood was drawn from ear and jugular veins using aseptic techniques, then placed in well labeled plain blood collection tubes and transported in cooler box to the laboratory of National Animal Health Research Center (NAHRC), Khumaltar. The blood samples were then centrifuged, and separated serum was transferred to eppendorf tubes. The tubes were well-labelled and stored in deep freezer at -79^{O}C until further testing.

Serological analysis

The serum samples were tested for the presence of PRRS antibody by using ID Screen® PRRS Indirect Enzyme-linked immunosorbent assay (ELISA) kit manufactured by IDvet, France. The ID Screen® PRRS Indirect ELISA kit has high specificity and sensitivity and efficiently detects antibodies directed against PRRSV-1 and PRRSV-2 antibodies. The test was carried out as per the manufacturer's instructions and the microplate was read in an ELISA reader at 450 nm and optical density (OD) values were recorded.

Validation and interpretation of ELISA results

The test was validated when the mean value of the negative control OD (OD_{NC}) was less than or equal to 0.150 ($OD_{NC} \leq 0.150$) and when the difference of the mean values of the positive and negative controls (OD_{PC} and OD_{NC}) was greater than or equal to 0.150 ($OD_{PC} - OD_{NC} \geq 0.150$). For each sample, the S/P ratio was calculated as follows using the OD values of sample and control.

$S/P = (OD_{sample} - OD_{NC})/(OD_{PC} - OD_{NC})$

Samples presenting an S/P ratio

- Less than 0.4 were considered negative.
- Equal to or greater than 0.4 were considered positive.

Data analysis

The data entry was done in MS Excel 2016 and the analysis was performed in SPSS, version 27. The prevalence rate of PRRSV between different districts was analyzed using a Chi square test. Similarly, the effect of various factors in the prevalence rate was also analyzed through the Chi square test. If the expected frequency was less than five in more than 20% of cells, the Fisher exact probability test was used. Differences with $P<0.05$ were considered significant.

RESULTS

Out of 178 samples, 28 samples were found to be positive for PRRSV antibodies indicating overall prevalence of 15.73%. The overall prevalence of PRRSV is shown in **Table** 1. Prevalence was found higher in Kathmandu (11.79%), followed by Lalitpur (3.93%) and none of the samples from Kavrepalanchowk and Chitwan were found to be positive. For age-wise analysis, the animals were categorized into 3 age groups as 0-6 months, 7-12 months and >12 months. The highest prevalence was obtained in the age group of 0-6 months (8.42%), followed by age group of 7-12 months (6.74%) and least in age group >12 months (0.5%). The prevalence was found higher in females (10.67%) than in males (5.05%).

Table 1. Overall prevalence of PRRSV

Parameters	Sample number	Positive	Negative	P value
Districts				
Kathmandu	54	21 (38.9%)	33 (61.1%)	
Lalitpur	11	7 (63.6%)	4 (36.4%)	<0.001*
Kavrepalanchowk	21	0 (0.0%)	21 (100%)	
Chitwan	92	0 (0.0%)	92 (100%)	
Age				
0-6 months	66	15 (22.7%)	51 (77.3%)	
7-12 months	56	12 (21.4%)	44 (78.6%)	<0.001*
>12 months	56	1 (1.8%)	55 (98.2%)	
Sex				
Male	55	9 (16.4%)	46 (83.6%)	>0.1
Female	123	19 (15.4%)	104 (84.6%)	

*denotes significant

Questionnaire survey was conducted in 29 farms with their consent. Data of farm characteristics and biosecurity measures obtained from questionnaire survey are shown in **Table** 2. From the survey, it was found that food scraps and leftovers from hotels and restaurants were usual source of food for the pigs in majority of the farms. Other feedstuffs include kitchen waste, flour, bran and commercial feed. Of the total 29 farms visited, 22 farmers had non-integrated farming whereas the remaining 7 farmers were found to have integrated farming. 10 farms were found using food scraps and leftovers from hotels and restaurants as these are the only food for the pigs where 6 farms using commercial feed and the remaining farms using both source as a food for pig. There was poor implementation of biosecurity measures in the farms. Only 3 farms were found using separate clothing in the farm

Table 2. Farm characteristics and biosecurity measures

Parameters		Number	Percentage (%)
Farming	Integrated	7	24.13
	Nonintegrated	22	75.86
Herd size	<50	17	58.62
	50-100	7	24.13
	>100	5	17.24
Feeding	Commercial feed	6	20.68
	Food leftovers and swill feeding	10	34.48
	Both	13	44.82
Separate clothing	Yes	3	10.34
	No	22	75.86
	sometimes	4	13.79
Separate boots	Yes	15	55.17
	No	13	44.82
	sometimes	1	3.44
Foot dips	Yes	10	34.48
	No	19	65.51

And separate boots were used by only 15 farms. Out of 29, 10 farms had foot dips at the entrance. All farms were disposing wastes and dead bodies by burial within the farm premises or nearby. Dirty and unhygienic environment was a common observation in nearly half of the farms.

DISCUSSIONS

Pig farming is a critical component of livestock production for indigenous communities across Nepal. This is one of the fastest-growing sectors, offering substantial potential for poverty alleviation due to pigs' high reproductive rates and efficient feed conversion, even when fed low-quality feed (McLean & Graham, 2022). Outbreak of PRRSV was first reported in Nepal in 2013 (Prajapati et al 2014), since then, the disease has been spreading widely causing significant losses. As PRRSV is not classified as a

notifiable disease, its outbreaks are often underreported. Farmers frequently retain seropositive pigs or those that have recovered from the disease and may sell piglets to other farms, facilitating disease transmission and persistence. This has contributed to an increase in cases involving reproductive failures and thereby creating difficulties in the control and prevention of disease.

The findings of this study highlight significant insights into the sero-prevalence of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and explore associated farm management practices in Bagmati Province. The overall sero-prevalence was observed to be 15.73%, which is lower than the findings of Sharma et al 2016, who reported a prevalence of 31.63% in Kathmandu Valley and (Prajapati et al 2023) documented a prevalence of 20.5%. Variations in prevalence could be attributed to differences in sample size, collection timing, geographic factors, and testing methodologies. The detection of PRRSV antibodies in Nepalese pig populations highlights exposure to the virus, particularly since vaccination against PRRSV is not practiced in the country.

The results demonstrate a district-wise variation, with the highest prevalence observed in Kathmandu (11.79%) and lower prevalence in Lalitpur (3.93%). Notably, no sero-positive cases were detected in Kavrepalanchowk and Chitwan districts. This suggests a localized distribution of PRRSV within the province, potentially influenced by differences in farming practices, biosecurity measures, or animal movement between districts.

Age-wise analysis revealed significantly high sero-prevalence in younger pigs aged 0-6 months (8.42%) and 7-12 months (6.74%) compared to older pigs over 12 months (0.56%). This pattern indicates that younger pigs are more susceptible to PRRSV infection, likely due to naïve immune systems or higher exposure risks during early growth phases. This age-related disparity underscores the need for targeted vaccination or management strategies for younger pig populations.

The sex of pigs showed no statistically significant difference in sero-prevalence ($p=1.0$), with females (10.67%) exhibiting slightly higher rates than males (5.05%). This minor variation could reflect differences in exposure, immune responses, or sample size discrepancies, though further investigation is required to establish a definitive correlation.

Studies have shown varying PRRSV prevalence rates. In India, Punjab and Mizoram reported prevalences of 22.2% and 27.8% respectively (Amninder et al 2019; Lalhruaipuii et al 2020), which are higher than this study's findings. However, prevalence rates in China reached 62.56% (Zhao et al 2022), significantly surpassing the levels observed in Nepal. Similar trends of higher prevalence have been reported in Thailand, Scotland, and Nigeria (Aiki-Raji et al 2018; Correia-Gomes et al 2022; Tummaruk et al 2013).

Biosecurity measures were inconsistently practiced among farms. Only 13.79% of farmers used separate clothing, and just 10.34% used aprons consistently, while 55.17% used separate boots, only 34.48% employed foot dips at farm entrances. These lapses in biosecurity practices highlight a lack of awareness and resources among farmers, increasing the risk of PRRSV introduction and spread.

Environmental conditions also played a role. The presence of insects, particularly houseflies, and poor hygiene in farms are epidemiologically significant, which was demonstrated by Pitkin et al 2009 indicating PRRSV RNA could be transmitted via fomites, personnel, and environmental factors.

Farms with better hygiene and biosecurity measures reported fewer cases, underscoring the critical need for improved farm management practices. These findings emphasize the importance of education and training to farmers on biosecurity protocols for preventing and controlling PRRSV effectively. Tailored intervention strategies, including improving feeding practices, enhancing biosecurity, and promoting vaccination programs, are essential for mitigating PRRSV's impact on pig farming in Nepal.

CONCLUSION

This study confirms the prevalence of PRRSV among pigs in Bagmati. The detection of antibodies in serum samples, combined with the absence of vaccination practices in Nepal, indicates that pigs were naturally exposed to the virus at some point in their lives. Key factors contributing to the spread and outbreaks of PRRSV include limited farmer awareness, traditional pig farming systems, inadequate biosecurity measures, and poor quarantine practices. Given the significant economic losses caused by PRRSV across all stages of pig production, this study underscores the critical need for continuous monitoring to accurately assess the disease's status. Developing and implementing effective control measures, including enhanced biosecurity practices and farmer education is essential to mitigate the impact of PRRSV in Nepal's pig farming industry.

ACKNOWLEDGEMENTS

Authors thank Nepal Agricultural Research Council for the funding. Technical and Administrative staffs of National Animal Health Research Center, Ms Gita Shrestha and Mr Rabin Chaudhary as well as all the farmers of the study sites are highly acknowledged for their cooperation and time.

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