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ORIGINAL RESEARCH ARTICLE



Prevalence of antibodies against classical swine fever in two districts of Nepal

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ABSTRACT

This study aimed to investigate the seroprevalence of Classical Swine Fever (CSF), caused by the Classical Swine Fever Virus (CSFV), in the Bhaktapur and Kavrepalanchok districts of Nepal. The primary objective was to determine the prevalence of antibodies against CSFV in the swine population, providing crucial insights for targeted interventions and control strategies. A cross-sectional study was conducted between January and March 2018. The research involved structured questionnaire surveys and blood sample collection from selected farms in the study area. A total of 184 serum samples were collected and subjected to testing using the IDEXX ELISA test kit. The study evaluated seroprevalence by considering variables such as district, sex, age groups, and housing systems. Out of the 184 serum samples collected, 34 were found to be seropositive for CSFV antibodies, resulting in an overall seroprevalence of 18.40%. The study identified significant variations in seroprevalence between different factors, including districts, sexes, age groups, and housing systems. These findings indicate a notable exposure of swine populations in the Bhaktapur and Kavrepalanchok districts to CSFV. This research highlights the considerable risk posed by CSF to the pig populations in the Bhaktapur and Kavrepalanchok districts of Nepal. The identified seroprevalence and variations among different factors emphasize the importance of targeted interventions and control strategies in these regions. The findings serve as a foundation for informed decision-making to mitigate the impact of CSF, safeguard swine health, and protect the livelihoods of those dependent on the swine industry. This study was made possible with the support of the Zoonosis Control Project under the National Animal Science Research Institute, Nepal Agriculture Research Council.

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INTRODUCTION

Classical swine fever (CSF), colloquially known as hog cholera, is a highly contagious viral disease that affects both domestic and wild pig populations on a global scale (Council, 2005). The

etiological agent responsible for this affliction is the classical swine fever virus (CSFV), a member of the Flaviviridae family. Owing to its rapid dissemination, heightened mortality rates, and significant economic ramifications, CSF constitutes a formidable menace to the worldwide pig industry (Yu *et al.*,

2019). The classical swine fever virus is characterized as a small, enveloped RNA virus harboring a single-stranded positive-sense genome (Gladue *et al.*, 2011). Through meticulous genetic scrutiny, it has been classified into three discernible genotypes (1, 2, and 3), with each genotype further partitioned into numerous sub-genotypes (Postel *et al.*, 2012). CSFV primarily affects pigs but can also infect wild boars, which act as a reservoir for the virus. The disease is characterized by a wide range of clinical manifestations, varying from subclinical infections to acute forms with high mortality rates (Postel *et al.*, 2019). Transmission of CSFV occurs primarily through direct contact between infected and susceptible animals. It can spread through respiratory secretions, feces, urine, and contaminated fomites. In addition, the virus can be transmitted via contaminated feed, water, and personnel involved in pig farming activities (Edwards *et al.*, 2000). International trade and movement of pigs also play a crucial role in the global dissemination of CSFV. Clinical signs of classical swine fever include high fever, anorexia, depression, lethargy, respiratory signs, hemorrhages, and reproductive disorders (Moennig *et al.*, 2003). Accurate and timely diagnosis of CSF is crucial for effective disease management. Laboratory techniques such as polymerase chain reaction (PCR), virus isolation, and serological tests are used for CSFV detection and differentiation from other diseases with similar clinical signs (Greiser-Wilke *et al.*, 2007). The World Organization for Animal Health (OIE) provides guidelines for the diagnosis and control of classical swine fever. Vaccination is an essential tool in CSF control strategies. Various vaccines have been developed, including live attenuated vaccines, inactivated vaccines, and marker vaccines (Greiser-Wilke and Moennig, 2004; Moennig, 2004). Moreover, this problem arises from the need to understand the presence of antibodies against CSF in the swine population in these regions, with the goal of informing and developing targeted control measures and strategies to safeguard pig populations. The research gap emerges from the limited focus on regional seroprevalence data for CSF, particularly in the context of these specific districts in Nepal. Despite the global recognition of CSF's impact, there is a dearth of localized information

regarding the extent of CSF in these areas. Furthermore, vaccination programs should be designed based on epidemiological factors and the disease situation in a particular region (Stroh *et al.*, 2019). However, vaccination alone is not sufficient, and it should be combined with strict biosecurity measures, surveillance, and prompt response to outbreaks. Preventing the introduction and spread of CSFV is crucial to maintaining the health and productivity of pig populations. Classical swine fever had significant impacts on the pig industry globally, with outbreaks reported in various countries, including China (Gao *et al.*, 2020). This research primarily aimed to assess the seroprevalence of Classical Swine Fever (CSF) in Kavrepalanchok and Bhaktapur districts of Nepal. This involves determining the presence of antibodies against CSF in the swine population, providing crucial insights into the disease's prevalence and aiding in the development of targeted control measures in these specific regions.

MATERIALS AND METHODS

Ethical approval

The blood collection was conducted by taking Ethical Approval from Ethical committee of Institute of agriculture and Animal Science, Date: Dec 2017 to Dec 2018.

Study design and study area

This cross-sectional study was conducted from January to March, 2018 in the Two districts viz. Bhaktapur and Kavrepalanchok District. These districts were selected because pig industry is growing rapidly in these areas and there is huge movement of pigs from these areas to rest of Nepal.

Questionnaire survey

A structured questionnaire survey was conducted on the selected farms to gather relevant information. The questionnaire covered i) Owner's name, ii) Number of pigs, iii) Age, iv) Feeding system, v) Vaccination status, vi) Purpose of rearing, vii) Waste management, viii) Disposal of carcass, ix) History of abortion, and x) Housing system.



Figure 1. Map of research area.

Sampling population, sample size, and sampling procedure

According to data from the Ministry of Livestock Development (MOLD), the pig population in Bhaktapur and Kavrepalanchok districts was 1780 and 6789 respectively. The sample size was calculated using EpiTool epidemiological calculators (www.epitool.ausvet.com.au) (Villarta Jr and Asaad, 2014). A total of 184 samples were collected, with 92 samples each from Bhaktapur and Kavrepalanchok districts. Purposive sampling was employed for selection.

Collection of samples

A total of 184 blood samples were collected from both districts. Approximately 6-8 ml of blood was drawn from the jugular vein of each pig (Schwartz and Smallwood, 1977). The blood was collected into plain blood collection tubes coated with clot activators, and the tubes were gently inverted 3-5 times. Blood clotting was allowed to occur within a time frame of 15-30 minutes.

Serum preparation

Clear serum samples were transferred from the tubes to serum vials using a micropipette. In cases where serum clarity was not achieved, samples were centrifuged at 10,000 rpm for 5 minutes. The obtained serum samples were stored at -20 degrees Celsius for further analysis.

Testing of the samples

The stored serum samples underwent testing using the IDEXX ELISA test kit for Classical Swine Fever, following the manufacturer's instructions and established protocols. This comprehensive methodology was implemented to determine the seroprevalence of Classical Swine Fever in Bhaktapur and Kavrepalanchok districts, enabling accurate data collection and analysis for the research study. The laboratory testing of the samples followed the IDEXX ELISA Test kit protocol: all reagents were allowed to reach 18-26°C before use, and then gently mixed by vortexing using separate pipettes. Next, 50 µl of Sample Diluent was added to all wells designated for testing and to the control wells. Subsequently, 50 µl each of the Positive and Negative Controls were added to their respective wells. Following this, 50 µl of the serum or plasma sample was added to the remaining plate, ensuring a separate pipette tip for each sample. The contents of the microwells were then mixed by gently tapping the plate or using a shaker for microtiter plates. The samples were then incubated for 2 hours (+5 min) at 18-26°C. In both cases, the plates were tightly sealed or incubated in a humid chamber using plate covers to prevent evaporation. Each well was subsequently washed three times with approximately 300 µl of Wash Solution. Liquid contents were aspirated from all wells after each wash. After the final aspiration, any residual wash fluid was tapped from each plate onto absorbent material. Plate drying was to be avoided between washes and before the addition of the next reagent. Following this, 100 µl of conjugate was added to each well. The samples were incubated for 30 minutes (±2 min.) at 18-26°C, sealed or in a humid chamber using plate covers. The previous steps of washing were repeated,

and then 100 µl of TMB Substrate N.12 was added to each well. The samples were incubated for 10 minutes (±1 min.) at 18-26°C, away from direct light. The reaction was halted at 10 minutes by adding 100 µl of Stop Solution N.3 to each well, following the same order as the substrate solution addition. Finally, measurements were taken on the absorbance of the samples and controls at 450 nm, or using a dual wavelength of 450 nm and 650 nm on a microplate reader (using air as a blank). Mean absorbance values were then calculated for each test sample and the controls.

Statistical analysis

Data entry and analysis were conducted using RStudio (Verzani, 2011). The association between various variables was examined using the chi-square test, with significance set at $P < 0.05$. Graphical representation and tabulation were performed using MS Excel-2016.

RESULTS AND DISCUSSION

The study aimed to assess the seroprevalence of Classical Swine Fever (CSF) in two districts, Bhaktapur and Kavrepalanchok of Nepal. The serological analysis was conducted on a total of 184 swine samples collected from different farms in the study area. Overall, out of the 184 samples, 34 tested positive for CSF, resulting in an overall prevalence rate of 18.40%. The remaining 150 samples tested negative, accounting for 81.50% of the total (Table 1). In Bhaktapur district, a total of 92 serum samples were collected, out of which 28 tested positive for CSF, resulting in a seropositivity rate of 30.50%. Conversely, 64 samples tested negative, accounting for 69.50% of the total. In Kavrepalanchok district, 92 samples were collected, with 6 showing positive results (6.52%), while 86 samples tested negative (93.40%). The difference in seroprevalence between the two districts was statistically significant ($P < 0.0001$) (Table 1 and Figure 3). This research indicates the presence of antibodies against Classical Swine Fever in the serum samples obtained from pigs across various regions of Nepal. This finding suggests that these animals likely encountered CSFV at some point in their lifespan, as vaccination against this disease is not a standard practice in Nepal. Therefore, the likelihood of antibody production through vaccination can be entirely ruled out. The sero-prevalence found in this study i.e. 18.4%, is less than the findings of (Deka et al., 2021) where prevalence rate was found to be 6.30% in India. The results indicate a notable variation in the seroprevalence of classical swine fever (CSF) between the two districts, Bhaktapur and Kavrepalanchok. Bhaktapur exhibited a significantly higher prevalence rate compared to Kavrepalanchok (30.50% vs. 6.52%). This discrepancy may be attributed to various factors such as husbandry practices, biosecurity measures, and proximity to potential sources of infection. Further investigation is needed to elucidate the specific reasons for this disparity (Dione et al., 2018). Among the sampled population, 88 were male and 96 were female. Among males, 20 samples

Table 1. Total distribution of serum and seropositivity.

Parameters	Sample number	Result			P- Value	
		Positive serum	Positive	Negative Serum		
District	Bhaktapur	92	28	30.50%	P- Value <0.0001	
	Kavrepalanchok	92	6	6.52%		
Sex	Male	88	20	22.72%	P- Value <0.0001	
	Female	96	14	14.58%		
Age	Bellow six month	64	10	15.63%	P- Value <0.0001	
	Above six months	120	24	20%		
Housing system	Intensive	104	18	17.30%	P- Value <0.0001	
	semi-intensive	66	10	15.15%		
	Free range	14	6	42.80%		
Overall Prevalence	184	34	18.40%	150	81.50%	P- Value <0.0001

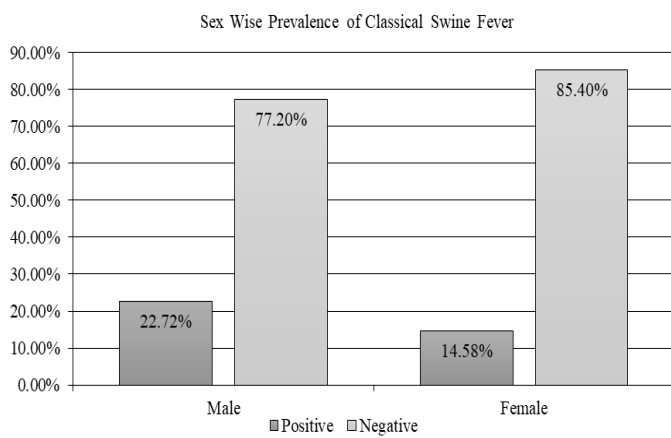


Figure 2. Sex wise prevalence of classical swine fever.

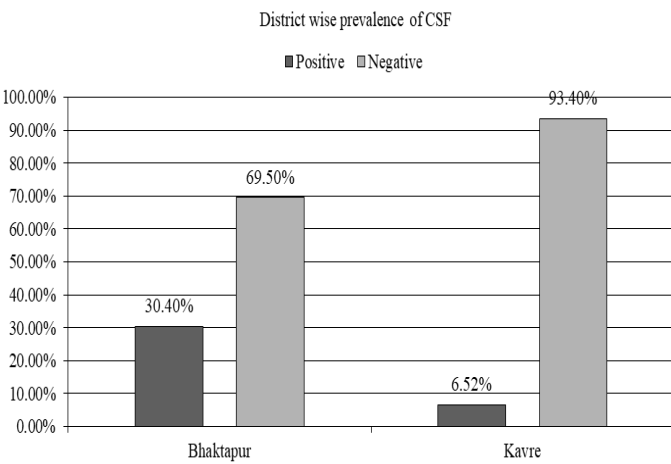


Figure 3. District wise prevalence of classical swine fever.

tested positive (22.72%), while 68 tested negative (77.20%). In females, 14 samples were positive (14.58%), while 82 were negative (85.40%). The seroprevalence was significantly ($P < 0.0001$) higher in males compared to females (Table 1 and Figure 2). Regarding sex-based differences, male pigs showed a higher seroprevalence compared to females (22.72% vs. 14.58%). This could be indicative of behavioral or physiological differences between the sexes that influence susceptibility to CSF. Additionally, factors related to management practices may also play a role in this observed variation (Chaudhary et al., 2019).

Regarding age groups, 64 samples were collected from pigs below six months old, with 10 testing positive (15.63%) and 54 testing negative (84.30%). Above six months of age, 120 samples were collected, out of which 24 were positive (20%), while 96 were negative (80%). The seroprevalence was significantly ($P < 0.0001$) higher in pigs above six months old (Table 1). Age was identified as a significant factor influencing seroprevalence. Pigs above six months old exhibited a higher seroprevalence (20%) compared to those below six months old (15.63%). This finding may be associated with the longer duration of potential exposure to CSF in older pigs. Additionally, immune maturation over time may contribute to the observed age-related differences in seroprevalence (Eshima et al., 2011). In terms of housing systems, 104 samples were collected from pigs in intensive systems, with 18 testing positive (17.30%) and 86 testing negative (82.70%). In semi-intensive systems, 66 samples were collected, with 10 testing positive (15.15%) and 56 testing negative (84.80%). Finally, in free-range systems, 14 samples were collected, with 6 testing positive (42.8%) and 8 testing negative (57.10%). The seroprevalence was significantly ($P < 0.0001$) higher in free-range systems compared to the other two (Table 1). The housing system also played a crucial role in the seroprevalence of CSF. Free-range systems demonstrated a significantly higher seroprevalence (42.8%) compared to both intensive (17.30%) and semi-intensive systems (15.15%). This could be attributed to increased contact with potential vectors or reservoirs of the virus in free-range environments. Enhanced biosecurity measures may be warranted in free-range systems to mitigate the risk of CSF transmission (Brookes et al., 2021). Thus, this study provides valuable insights into the seroprevalence of classical swine fever in Bhaktapur and Kavrepalanchok districts of Nepal. The results highlight the importance of considering district, sex, age, and housing system as crucial factors influencing the prevalence of CSF. Further research is needed to identify specific risk factors and develop targeted interventions to control and prevent the spread of this disease in the studied regions.

Conclusion

This study provides critical insights into the seroprevalence of Classical Swine Fever (CSF) in Bhaktapur and Kavrepalanchok districts of Nepal. The overall seroprevalence of 18.40% indicates a significant exposure of swine populations to CSFV. The variation in seroprevalence between the two districts underscores the need for tailored control measures. Male pigs showed higher susceptibility, suggesting potential behavioral or physiological factors. Age was a significant determinant, with older pigs exhibiting higher seroprevalence, likely due to prolonged exposure. Free-range systems presented a higher risk, emphasizing the importance of enhanced biosecurity. This research forms a foundation for targeted interventions to control and prevent CSF in these regions.

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