

## Seroprevalence of Brucellosis among Pigs of Commercial Farms in Chitwan District of Nepal

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### ABSTRACT

**Objectives:** This study aimed to determine the seroprevalence of brucellosis among pigs of commercial farms in Chitwan district of Nepal.

**Methods:** This cross sectional prospective study was conducted among 100 pigs of commercial farms located in western region of Chitwan district of Nepal. Blood sample was collected from each pig by the trained veterinarians and serum was extracted. Each serum sample was processed for Rose Bengal plate test (RBPT) and ELISA for the detection of *Brucella* spp. Data was analyzed using SPSS software version 21.0 and a p-value of less than 0.05 was considered as significant.

**Results:** Prevalence of brucellosis in pigs was found to be 15% (15/100) by RBPT and 10% (10/100) by ELISA. There was statistically insignificant difference (p=0.98) in gender wise prevalence of brucellosis among pigs. Younger pigs below one year of age were more susceptible to brucellosis than the older pigs. Landrace breed showed more positive test results compared to other breeds.

**Conclusion:** Pigs can be the potential source of transmission of brucellosis to humans. Considering the high economic loss on livestock sector and possible transmission to humans, awareness program and appropriate control strategies is warranted. Breed and age factors should be considered while adopting the control measures of brucellosis among pigs.

**Keywords:** *Brucella*, pigs, commercial farms, Nepal

### INTRODUCTION

Brucellosis is an infectious bacterial disease that primarily affects livestock and humans (Pappas et al., 2006). The etiological agent of brucellosis is a Gram negative bacterium of the genus *Brucella*. Of the nine recognized species of *Brucella*, three species namely *B. melitensis*, *B. abortus* and *B. suis* are of economic importance. Porcine brucellosis is an infection caused by biovar 1, 2 or 3 of *Brucella suis* (CDC 2021). The disease affects a wide range of animals including ruminants in

which it is characterized by abortion (Corbel 1997). Brucellosis in pigs is also characterized by stillbirths or weak piglets. It occurs in many countries where pigs are raised. The mode of transmission among animals is through exposure of mucous membranes, inhalation of aerosols or direct contact with infected materials (Kolo et al. 2019). Humans contract brucellosis from animals through ingestion of contaminated, unboiled or unpasteurized milk and by direct contact with infected animals, animal carcasses and aborted materials (CDC 2021).

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Brucellosis occurs worldwide but is much controlled in developed countries due to routine screening of domestic animals and animal vaccination program. Though some European and Asian countries have been declared free of *Brucella*, it is still endemic in Asian countries, such as Sri Lanka, India, China, Pakistan, Mongolia and Nepal (Acharya 2016). Brucellosis in animals has already been reported from different districts of Nepal (Jackson et al. 2014). Most of the people are engaged in agriculture and livestock sector is the major contributor for livelihood in Nepal. Considering the animal and human health disorders, occupational risks, and the economic burden it imparts, knowledge on the status of brucellosis infection in animals and establishing the epidemiology could be valuable for farmers, veterinarians, researchers, consumers, disease prevention and control program planners and any others concerned with better animal and human health. The epidemiology of brucellosis varies markedly with region and over time. Most of the published studies from Nepal have focused on bovine brucellosis with sparse information on brucellosis among small ruminants. Hence, this study aimed to determine the seroprevalence of brucellosis among pigs of commercial farms in Chitwan district of Nepal.

## METHODOLOGY

### Study population

This cross-sectional prospective study was conducted among pigs of commercial farms located at western region of Chitwan district, Nepal. Altogether 100 pigs were selected randomly. Blood samples from age 1 month to 5 years were taken from different pig farms. The males and females of Landrace, Tamworth, Hampshire and Pakhribas black breeds of pigs were noted.

### Sample collection and processing

Five ml of blood sample was collected from each pig from ear vein at the respective farms by the trained veterinarian. The samples were transported immediately to the Theriogenology laboratory of Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal. Serum sample was extracted from each blood sample by letting it to clot and centrifugation at 5000 rpm for 5-10 minutes (Cheesbrough 2009).

### Rose Bengal plate test (RBPT)

Each serum sample was processed for RBPT following manufacturer's instruction (prioCheck Rose Bengal Test®). Briefly, 30µl of test serum was mixed with an equal volume of antigen on a white tile or enamel plate to produce a zone approximately 2 cm in diameter. The mixture was agitated gently for four minutes at ambient

temperature, and then observed for agglutination. Any visible reaction was considered to be positive.

### ELISA

Each sample was also processed for ELISA following manufacturer's instruction (IDVet®) Briefly, 10 µl of each of the negative control, positive control and test serum were added to 190 µl of dilution buffer in the respective wells and incubated at 21°C (± 5°C) for 45 min (± 4 min). After washing with wash solution, 100 µl of the conjugate 1x was added to each well and incubated at 21°C (± 5°C) for 30 min ± 3 min. After washing, 100 µl of the substrate solution was added to each well and again incubated at 21°C (± 5°C) for 15 min ± 2 min in the dark. Then, 100 µl of the stop solution was added to each well and the absorbance was read at 450 nm. The test was considered to be valid if the mean value of the absorbance of positive control is greater than 0.350 and the ratio of the mean values of the positive and negative controls is greater than 3.

### Data analysis

Data were analyzed using SPSS software version 21.0 and a p-value of less than 0.05 was considered significant for the statistical analysis of gender wise, age wise prevalence, breed wise prevalence, prevalence of brucellosis on the basis of vaccination.

## RESULTS

### Demographic characteristics of pigs tested for brucellosis

A total of 100 pig serum samples were collected from different commercial farms of western Chitwan, Nepal. Of the total samples, 67 were from females and 33 were from male pigs (Table 1).

About 63% of pigs were vaccinated with routine vaccines. Blood samples from age 1 month to 5 years were taken from different pig farms. Among total samples, 74% samples were collected from pigs below one year of age.

### RBPT and ELISA results

Prevalence of brucellosis in pigs was found to be 15% (15/100) by RBPT and 10% (10/100) by indirect ELISA. Out of the 15 samples positive for RBPT, only 5 samples showed positivity also for ELISA. Remaining 5 samples were positive by ELISA but negative by RBPT. Correlation between the RBPT and ELISA of the same sample was 0.327 (Spearman's rho correlation coefficient).

Out of total samples, 14.92% (10/67) of females and 15.15% (5/33) of males were positive for *Brucella*. However, there was statistically insignificant difference (p=0.98) in gender wise prevalence of brucellosis in pig. Younger pigs below one year of age 26.08% (6/23) were more susceptible to brucellosis than the older pigs

11.68% (9/77). Landrace breed showed more positive test results compared to other breeds.

**Table 1. Demographic characteristics of pigs tested for brucellosis**

Breeds of pigs	Male (No.)	Female (No.)	Total (No.) tested
Landrace	20	40	60
Tamworth	6	10	16
Hampshire	5	15	20
Pakhribas black	2	2	4
<b>Total</b>	<b>33</b>	<b>67</b>	<b>100</b>

## DISCUSSION

The seropositivity for brucellosis was 21.58% among 190 samples of pigs in Kathmandu valley which is higher than the present finding (Rana 2005). The discrepancy may be due to the use of different diagnostic techniques as the later study used. The seropositivity of 7.18% for brucellosis using the Brewer Diagnostic Card (BDC) was found in 153 samples of pigs from Itahari, Nepal (Shrestha, 2008). The present study result (15%) shows that it is quite convincing to a similar study by Shrestha et al. (2008). A much higher seroprevalence to *Brucella* has recently been found in France: overall, 31.6% of 2313 wild boars were positive between 1994 and 2000 (Garin-Bastuji et al. 2014). The overall seroprevalence rate found in the present study was lower as compared with the findings of 6.7% from Bangladesh (Islam et al. 2013). The variations in the seroprevalence might have been due to the disparity in geographical locations, climatic conditions and management practices in the different study areas. Other studies have also indicated that the rate of brucellosis infection varies among pig herds, from farm to farm or by country by origin of tested pigs (wild or domesticated) and by testing method used (Godfroid and Kasbohrer 2002). Furthermore, contact with other animals was also reported to be major risk factors that were influencing the occurrence brucellosis (Yang et al. 2021).

Among the total samples, females showed high prevalence 14.92% (10/67) than that of males 15.15% (5/33) with insignificant association ( $P < 0.005$ ). This finding was in disagreement with a study done by Kebeta et al. (2015) showed that higher seroprevalence of brucellosis in female 8.2% than male 1.6% with significant association ( $P < 0.005$ ) (Kebeta 2015). This finding was also in disagreement with the observation of Rahman et al. (2012) who found a high prevalence of brucellosis (7%) in female and 5.9% in male pigs in

Bangladesh (Rahman 2012). Similar observation was also recorded by other investigator in wild boar (*Sus scrofa*) from Republic of Croatia (Cvetnic et al. 2009). The higher rate of infection in females might be due to infection within the female reproductive tract providing a potential reservoir for the organism to propagate. In the study of Shrestha et al. (2008), samples from female showed high prevalence 9.23% (6/65) than that of males 5.7% (5/88) which is less than the present study of which samples of the ages below 1 year showed 26.08% (6/23) and ages above 1 year showed 11.68% (9/77) positive reaction for RBPT.

With regard to the age of the animal higher seroprevalence was observed in young (< 12months) (5.9%) as compared to adult ( $\geq 12$ months) pigs (3.6%) by Kebeta et al. (2015). This result was in disagreement with the findings of Rahman et al. (2012) who found higher prevalence of brucellosis in aged animal (8.1%) than young (0.0%). Previous study by Leite et al. (2014) identified that young age of the animals as risk factor that was influencing the occurrence of brucellosis (Leite 2014).

The *B. suis* are usually transmitted between animals by contact with the placenta, fetus, fetal fluids, aerosol route, ingestion of contaminated raw meat through mucus membrane, broken skin and vaginal discharges from an infected animal (Ngbede 2013). Brucellosis is a major public and animal health problem in areas like Nepal with intensive mixed types of farming and where owners cohabit with their animals during night (Acharya 2016). Studies have shown that inclusion of different tests could increase the detection rate of infectious disease other than brucellosis as well (Sharma 2021). The major limitations of our study lies in the fact that our detection technique was based on serological methods. Hence, the seroprevalence might have been underestimated. Inclusion of molecular methods like PCR might increase

the detection rate.

## CONCLUSION

Pigs can be the potential source transmission of brucellosis to humans. Considering the high economic loss on livestock sector and possible transmission to humans, awareness program and appropriate control strategies is warranted. Breed and age factors should be considered while adopting the control measures of brucellosis among pigs.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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