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Research Article

Seroprevalence of *Chlamydia abortus* Among Cattle of Selected Areas in Terai Belt of Nepal

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Abstract

A cross-sectional study was carried out to determine the seroprevalence of *Chlamydia abortus* in dairy cattle of selected areas in Chitwan, Nawalpur, and Rupandehi districts, Nepal from November to December 2018. A total of 92 serum samples were collected and screened by Indirect ELISA from ID vet. Out of 92 samples, three samples were positive resulting overall prevalence of 3.27%. As there is no practice of vaccination, overall seroprevalence was from natural infection. Significantly (p=0.034) all samples were seropositive from the Rupandehi district. Sero-prevalence was higher in Jersey crosses (4.22%) as compared to Holstein Friesian (HF) crosses (0%) and cattle of age >3 years were found to be more seropositive (6.97%) as compared to cattle of age ≤3 years (0%). There was no statistically significant association of seroprevalence with breed and age group. Thus, the cattle population of Rupandehi District are at high risk of acquiring *Chlamydia* infection. Strict quarantine and good biosecurity can prevent the possibility of a disease outbreak.

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Keywords: Antibodies; Cattle; *Chlamydia abortus*; ELISA; Seroprevalence

Introduction

Chlamydia abortus is gram-negative obligate, intracellular bacteria responsible for economic losses due to infection, and subsequent induction of abortion in several animal species and possesses zoonotic threat (Gokce *et al*., 2007). *C. abortus* is endemic in ruminants throughout the world (Li *et al*., 2015) and it causes epizootic bovine abortion in cattle (Gokce *et al*., 2007). This bacterium causes abortion, infertility, weak calves, fetal loss, and mastitis in cattle and also in sheep and goat (Chahota *et al*., 2015). Animals can become infected at any time of the year, especially via the ingestion of elemental bodies from an aborted fetus, placentas, and uterine discharge from infected individuals that contaminate drinking water or food (Longbottom and Coulter, 2003). Infected heifers are latent carriers until the next gestation period where risks of abortion are high. These

animals remain carriers for the rest of their productive life (Koehler *et al*., 1997).

Livestock sector including fisheries contributes nearly 12.5% to the total Gross Domestic Products (GDP) and 25.7% of the Agricultural GDP (AGDP) (MoALD, 2018; Lamsal *et al*., 2020). There are about 7.37 million cattle in Nepal with an increment of 2.80% in the last 10 years (Poudel *et al*., 2020). Increase in trend of rearing improved cattle, farmers are purchasing crossbred cattle from other districts or even importing from India and other places but crossbred cattle are more susceptible to infection than indigenous one. There are no sufficient investigations on seropositivity and associated risk factors of the *Chlamydia* infection in Nepal, which is necessary for the areas where cattle farming is commercializing. The objectives of our study were to evaluate the seroprevalence of *Chlamydia abortus* among cattle of terai belts of Nepal and determine associated risk factors with *Chlamydia abortus* seropositivity.

Materials and Method

The cross-sectional descriptive study was conducted from November 2018 to December 2018 to detect the presence of antibodies against *Chlamydia abortus* in the serum sample of cattle of Chitwan, Nawalpur, and Rupandehi districts, in a mid-tropical region of Nepal (Table 1). Chitwan (27˚35' N 84˚30' E), Nawalpur (27˚32' N 83˚40' E) and Rupandehi (27˚30' N83˚27'E) are three different districts out of 77 districts of Nepal located in Bagmati Province, Gandaki Province and Province 5 respectively. These three districts have a similar geographical background, climatic conditions, and pattern of livelihood (Fig. 1).

The sample size was calculated using Daniel's Formula (Daniel, 1999) $n= [Z^2*P (1-P)]/e^2$

Where, Z= value from standard normal distribution corresponding to desired confidence level (Z=1.96 for 95% CI), P= expected true proportion, e= desired precision

Assumption of 5.80% prevalence of *Chlamydia abortus* in improved and crossbred cattle of Nepal (NCRP, 2018) with an expected precision of 5% and 95% confidence level, the required sample size was 84. However, we took 92 samples from 51 herds by purposive sampling among cattle having a history of reproductive problems like abortion, repeat breeding, and infertility.

Selected animals were restrained properly and 5 ml blood was collected from a jugular vein with the help of a 10ml syringe and 18-gauge needle giving minimum pain to the animal. Immediately after collection, blood was transferred into Serum Separating Gel Tube (SSGT) (Human Liuyang Medical Instrument Factory, China) with proper labeling and recording. Collected samples were brought to the National Cattle Research Program (NCRP), Rampur, Chitwan laboratory in an icebox, and serum was extracted by centrifugation at 3000 rpm for 10 minutes. Extracted serum was transferred into the Eppendorf tube and was stored in a deep freezer at -20˚C until laboratory analysis was performed. Collected samples were screened by Indirect LISA from ID vet.

Data entry and management was done using MS Excel 2016 and analysis was done in SPSS version 16. Chi-square test and Fisher exact test whenever required was applied to check the association of risk factors and laboratory result. Data were analyzed at 95% level of confidence ($p < 0.05$).

Oral consent was sought from farmers before commencing blood sampling from each farm or herd. Blood samples were taken with minimal harm to animals as far as possible. Ethical approval for the study was obtained from the National Cattle Research Program (NCRP), Rampur, Chitwan.

Table 1: Table showing the areas of sample collection for the study.

Districts	Selected areas	Number of samples collected	Total
Chitwan	Ratnanagar	06	32
	Gajurel Tole	08	
	Jirauna	08	
	Neupane Tole	10	
Nawalpur	Bhedabari	03	31
	Pitauji	09	
	Harkapur	06	
	Kawasoti	06	
	Sadh	07	
Rupandehi	Siddharthanagar	05	29
	Kailashnagar	08	
	Devdaha	06	
	Siyari	10	
Total			92

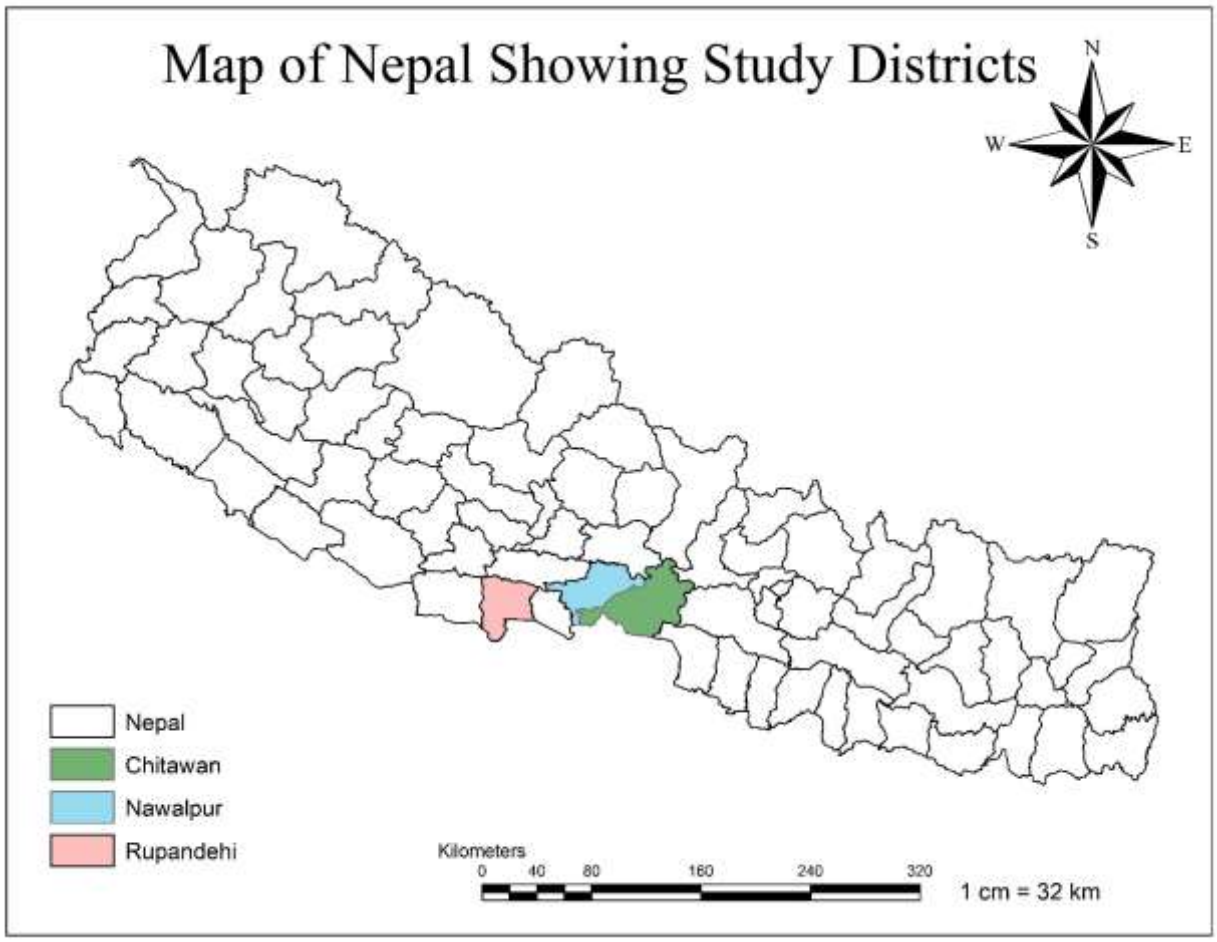

Fig. 1: Study area in the terai belt of Nepal.

Result and Discussion

The seroprevalence of *Chlamydia abortus* among cattle of study districts is presented in Table 2. Out of 92 serum samples, three samples were found to be positive and the overall seroprevalence of 3.27%. All three positive samples were from Rupandehi districts and there was statistically significant between districts and seropositivity ($p \leq 0.05$). Out of 71 samples from Jersey Cross and 21 samples from Holstein Friesian Cross, 3 (4.22%) Jersey cross was found to be positive whereas no samples from the HF cross were positive. All cattle age group greater than three years were positive but was not statistically significant.

In our study, overall prevalence was found to be 3.27% (total samples = 92) which is lower than previous findings by NCRP (2018) i.e. 5.80% (total samples = 155) in Rupandehi, Parasi, Chitwan, and Makawanpur districts of Nepal. But prevalence was higher than the study of NCRP (2019) i.e. 2.17% (total sample=92) in Nawalpur and Chitwan District. Seroprevalence of *Chlamydia abortus* in ruminants varies widely from one country to another, 0.82% in Iraq (Majed *et al*., 2018), 4.75% in Ireland (Wilson *et al*., 2012) and 4.65% in five states of India (Chahota *et al*., 2015).

The variation in prevalence in different studies might be due to temporal and spatial variations and contrast in sampling procedure and sample size. The difference in season, location, rearing pattern, climate, breed of cattle might as well contribute to the difference of seroprevalence of *Chlamydia abortus* in different studies. Lower in seroprevalence than the study of NCRP (2018) might be due to a lower sample size. Higher in seroprevalence than the study of NCRP (2019) might be due to the inclusion of the Rupendehi district in our study. In our study, all positive samples were from the Rupandehi district. This could be due to the trans-boundary movement of cattle between Nepal and India.

Regarding breed, all samples were positive among Jersey cross but was not statistically significant. This could be due to a high number of samples from Jersey cross than HF cross. All positive samples were from cattle age group above three years. This finding was in contrast with the finding of Majed *et al*., (2018), where seroprevalence was 1.47% among calves. Feeding colostrum to neonates can boost immunity (Yang *et al*., 2015) and there is such practice in Nepal. This might be a reason for no seropositive among cattle below 3 years in our study. Also, the number of samples, sampling techniques, and geographical variation could lead to the difference in our study.

Table 2: Seroprevalence of *Chlamydia abortus* among cattle of the Terai belt of Nepal.

	Sample Number	Positive	Negative	Prevalence %	χ^2 Value	p-value
Location						
Chitwan	32	0	32	0	6.737	0.034*
Nawalpur	31	0	31	0		
Rupandehi	29	3	26	10.34%		
Breed						
Jersey Cross	71	3	68	4.22%	0.917	0.338
HF cross	21	0	21	0		
Age						
≤ 3 years	49	0	49	0	3.534	0.060
> 3 years	43	3	40	6.97%		

Since there was no vaccination against *Chlamydia abortus*, circulating antibodies can only due to natural infection of the causative agent. (Sharma *et al.*, 2003). Aborted fetuses and placentae were usually disposed of at the backyard without burial or left on the field for dogs to come and eat, and dogs can carry such fetuses and placentae far distant places. These practices might contaminate the environment with abortifacient organisms, and thus, may spread the disease.

Conclusion

Out of 92 serum samples, three were found positive for *Chlamydia abortus* i.e. (3.27%) in the present study which clearly showed that chlamydial infection is prevalent in these study areas. Since there was no vaccination against *Chlamydia abortus*, seropositivity results were from natural infection of the causative agent. Despite some limitations, seroprevalence estimated in this study provides a basis for future monitoring and surveillance of disease. Previous studies and this study urged to further planned research on *Chlamydia abortus* at the national level to protect our dairy sector from possible economic losses due to *Chlamydia* infection.

Limitation of the Study

Simple random sampling could not be done due to time and monetary constraints and only 92 samples were taken into consideration due to the unavailability of the ELISA kit.

Author's Contribution

R. Kandel and C. Kharel designed the research, performed experimental works, collected and analyzed the required data. R. Kandel and D. Subedi prepared the manuscript. All authors revised, finalized, and approved the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest with the present publication.

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