DEMONSTRATION OF CIRCULATING ANTIBODIES OF *Coxiella burnetii* IN DAIRY CATTLE OF RUPANDEHI DISTRICT, NEPAL

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Abstract: *Coxiellosis, caused by Coxiella burnetii, causing reproductive disorders in cattle and other ruminants, is still unexplored broadly in Nepal despite its identification in neighbor countries. The main objective was to determine the seroprevalence of Coxiellosis among cattle. A cross sectional study was carried out in Rupandehi district, Nepal, a total of 184 serum samples was collected purposively. Blood was collected from jugular vein and serum was separated and stored at -20^{0}C. ELISA test was performed according to the manufacturer's protocol (ID Screen® manufactured by ID Vet, France). The overall prevalence was found to be 1.63%. Statistically, there was non-significant effect of age and BCS on seroprevalence of disease in present study (p>0.05). Prevalence in cattle of <8 years age group was found to be 1.19%, while 5.88% of serum samples of cattle of >8 years age group were positive. Out of 9 serum samples of young animals and 175 serum samples of lactating cattle, the seropositivity was 0% and 1.71% respectively. Cattle with good body condition (BCS=2.5-3.5) had more seroprevalence than thin cattle (BCS <2.5). Out of 48 serum samples of tick infested cattle, 6.25% were found positive. This study has detected seropositivity of coxiellosis in cattle for the first time in Nepal. Enhanced surveillance using confirmatory techniques with sufficient sample size is a prerequisite to confirm the findings and assess the public health risk.*

Key Words: *Coxiella burnetii, ELISA, Rupandehi, Seroprevalence.*

1. INTRODUCTION:

Q fever (for query fever), is a zoonosis due to *Coxiella burnetii,* a small intracellular bacterium. The disease has been known since the 1930s and has a worldwide distribution, with the exception in Antarctica and New Zealand [1], where its presence has not really been confirmed [2]. Q fever is a human zoonosis which is caused by an obligate intracellular Gram-negative bacterium, *Coxiella burnetii* [3]. It is a highly contagious zoonosis present in virtually all 'animal kingdoms', including arthropods, affects mostly humans, cattle, sheep and goats[4][5][6][7]. *C. burnetii* is a Gram-negative obligate intracellular bacterium, adapted to thrive within the phagolysosome of the phagocyte [8]. Only a few organisms can cause disease, although *C. burnetii* is highly infectious. It can remain viable and virulent for months because of its spore-like life cycle. [9]

Transmission of Q fever to animals and humans occurs through contact with body fluids or secretions like milk, urine, feces or birthing products (amniotic fluid, placenta) from infected animals. It may occur from direct contact, ingestion, or indirect contact through objects contaminated with these materials, while ticks (vector) also are agents of disease transmission between animals. In domestic ruminants, Q fever is mostly associated with abortions and dead or weak offspring. The infection is probably life-long or persisting for several years. Sheep, goats and cows are mainly subclinical carriers, but can shed bacteria in their secretions and excreta.

Humans usually get Q fever through contaminated barnyard dust or by direct contact with infected animals while assisting with the delivery of newborn animals, and occasionally by drinking contaminated milk or from tick bites. Affected persons develop high fever with headache, muscle pains, sore throat, nausea, vomition, chills, night sweats, fatigue, chest pains and stomach pains. In serious cases, it can lead to pneumonia and hepatitis. Low infectious dose, stability in the environment, and capability for aerosol dispersion, makes this organism a potential source for bioterrorism.

The 'Q' stands for 'query', the name being given since the cause of a 1935 outbreak of illness among abattoir workers in Australia fever was not known. The pathogen of Q fever was discovered in 1937, when Frank Macfarlane Burnet and Mavis Freeman isolated the bacterium from one of Derrick's patients [10]. It was originally identified as a species of *Rickettsia* by H.R. Cox and Gordon Davis from ticks in Montana, USA in 1938 [11]. Q fever was first described by Edward Holbrook Derrick [12] in abattoir workers in Brisbane, Queensland, Australia. Recent phylogenetic analyses suggest that *C. burnetii* is more closely related to *Legionella* and *Francisella* than to the genus *Rickettsia*, although

classically considered a rickettsial agent [13]. The main objective of this study was to find out the seroprevalence of Q fever in dairy cattle of Rupandehi district, Nepal.

2. MATERIALS AND METHODS:

A cross sectional study was carried out in Rupandehi district, Nepal, a total of 184 serum samples was collected purposively from female cows between October 2016 and December 2016. Blood was collected from jugular vein and serum was separated and stored at -20^{0}C. ELISA test was performed according to the manufacturer's protocol (ID Screen® manufactured by ID Vet, France). Data entry, management and analysis was done using program Microsoft Office Excel 2010. The difference in prevalence according to age, physiological status, body condition score (BCS) and tick infestation at sample collection time was compared statistically by a Chi-square ($\chi 2$) analysis using OpenEpi version 3 with significance level defined at the $p<0.05$.

3. RESULTS:

Table 1: Overall Seroprevalence of Coxiellosis

Total Cases	Positive Cases	Negative Cases	Seropositivity %
184	3	181	1.63%

Table 2: Animal Level prevalence of antibody to *Coxiella burnetii* with respect to age

Variables	Positive Cases	Negative Cases	Seropositivity %	P-value*
<8 years	2	165	1.19 %	**0.50 (NS)**
>8 years	1	16	5.88 %	
Total	3	181	1.63%	

Table 3: Animal Level prevalence of antibody to Co*xiella burnetii* with respect to Physiological Status

Variables	Positive Cases	Negative Cases	Seropositivity %
Heifer	0	9	0%
Lactating	3	172	1.71 %
Total	3	181	1.63%

Table 4: Animal Level prevalence of antibody to *Coxiella burnetii* with respect to Body Condition Score (BCS)

Variables	Positive Cases	Negative Cases	Seropositivity %	P-value*
Thin (BCS <2.5)	1	121	0.81 %	**0.5258 (NS)**
Good (BCS 2.5-3.5)	2	60	3.22 %	
Total	3	181	1.63%	

Table 5: Animal Level prevalence of antibody to *Coxiella burnetii* with respect to Tick Infestation at sample collection time

Variables	Positive Cases	Negative Cases	Seropositivity %
Yes	3	45	6.25%
No	0	136	0%
Total	3	181	1.63%

NS= Non-significant; P≥0.05 S= Significant; P<0.05 *= Fisher Exact P-value

The overall prevalence was found to be 1.63% out of 184 serum samples. Statistically, there was non-significant effect of age and BCS on seroprevalence of disease in present study ($p>0.05$). Prevalence in cattle of <8 years age group was found to be 1.19%, while 5.88% of serum samples of cattle of >8 years age group were positive. Out of 9 serum samples of young animals and 175 serum samples of lactating cattle, the seropositivity was 0% and 1.71% respectively. Cattle with good body condition (BCS=2.5-3.5) had more seroprevalence than thin cattle (BCS <2.5). Out of 48 serum samples of tick infested cattle, 6.25% were found positive.

4. DISCUSSION:

The study shows 1.63% of cattle having circulating antibodies against *C. burnetii* in their blood. This is the first seroprevalence study of *C. burnetii* in cattle in Nepal, to our knowledge. The prevalence rate of 1.63% coincides with 1.7% seropositivity among 3087 Korean native cattle from eight provinces in South Korea [14] and 1.5 % seropositivity

in Malawian zebu cattle [15]. However, majority of the studies show higher prevalence rates in comparison to ours i.e., 3.57% in Bangladesh [16]; 6.5% in India [17]; 6.92% in Thailand [18]; 10.4% in Pakistan [19]; 24.29% in Delhi and Uttar Pradesh, India [20]; 50% in Anhui Province, China [21].
These differences in the prevalence rates of *C. burnetii* infection in animals between present study and another studies in variant areas of world are attributed to number of samples taken, type of sample collection, season, geographic location, assay type, as well as possible differences among laboratories and testing procedures and criteria used to define positive results.

Statisitically, there was non-significant effect of age and BCS on seroprevalence of disease in present study.
In present study, there is high prevalence of Q fever in cattle infested with ticks and no prevalence in cattle without tick infestation, which is similar to Q-fever study in Dutch dairy cattle herds by van Engelen *et al.,* 2014 [22] (28.4% in tick infested cattle compared to 14.9% in cattle with no tick infestation). Cantas *et al.,* 2011[23] also found that dairy cattle in Cyprus with ticks had increased risk of *C. burnetii* positivity. Sprong *et al.*, 2012 [24], however found no *C. burnetii* in ticks originated from dairy cattle in Netherlands in 2008.

The high prevalence in lactating animals is similar to study in Dutch dairy herds based on testing bulk tank milk and individual samples by PCR and ELISA by Muskens *et al.,* 2011 [25], which showed prevalence of 16% in lactating cows and 1% in young animals and results of Ruiz-Fons *et al.,* 2010[26] (6.2% in heifer versus 6.7% in adult).
The cattle with age >8 years has higher seroprevalence than cattle below 8 years of age in our study, which is similar to Na *et al.*, 2016 [27] in Gwanju area of Korea.

5. SUMMARY AND CONCLUSION:

The serological evidence of Q fever in cattle suggests that Q fever exists in Nepal. Although the significant effect of age and BCS in the prevalence of the disease is not found, the study shows there is higher risk of being seropositive in cattle infested with ticks. Similarly, seroprevalence in only tick infested cattle supports that *C. burnetii* is transmitted by ticks.
However, the validity and accuracy of this research could be challenged through increased sample sizes. Enhanced surveillance using confirmatory techniques with sufficient sample size is a prerequisite to confirm the findings. Further epizootiological investigations on Q fever in other farm animals and man at the country level is important to monitor and determine the magnitude of Q fever infection in order to estimate its economic impact on animal industry and its public health hazard.

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