INTERNATIONAL JOURNAL FOR INNOVATIVE RESEARCH IN MULTIDISCIPLINARY FIELD ISSN: 2455-0620 Volume - 6, Issue - 3, Mar – 2020
Monthly, Peer-Reviewed, Refereed, Indexed Journal with IC Value: 86.87 Impact Factor: 6.497
Received Date: 06/03/2020 Acceptance Date: 18/03/2020 Publication Date: 31/03/2020

Q Fever in Cattle Population of Chitwan, Nawalpur and Rupandehi Districts, Nepal: A Preliminary Study

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Abstract: *Coxiella burnetii is an important zoonotic pathogen of worldwide distribution. Cattle, including other ruminants, act as the reservoir host. Still, in Nepal, systematic epidemiological data are lacking. The study aims to determine the seroprevalence of C. burnetii among cow population in Chitwan, Nawalpur and Rupandehi districts of Nepal. A cross sectional study was performed including cow with history of reproductive disorder like abortion and stillbirth. Purposive sampling was performed in all 3 districts. Serum specimens were taken and screened for anti-C. burnetii antibodies using IDvet ID screen Q fever Indirect Multispecies (France) test kit. A total of 104 samples were tested; no results were positive. Statistical analysis for population freedom from disease revealed a likelihood of high probability that C. burnetii was absent in our study population. Thus, our work provides preliminary epidemiological data on Coxiellosis in cattle in Nepal indicating either a low epidemicity or the absence of this pathogen in the study area.*

Key Words: *Qfever, Coxiella burnetii, risk factors, cow, Chitwan, Nawalpur, Rupandehi.*

1. INTRODUCTION:

Query (Q) fever is a zoonotic disease with worldwide distribution with the exception in the Antarctic regions and possibly New Zealand (Hilbink et al., 1993; Mohammed et al., 2017), where its presence has not yet been confirmed (Greenslade et al., 2003). It was first identified as a disease in the 1930s, the causative agent was unknown and so it was named as Query fever. *Coxiella burnetii*, the causative agent of Q fever was first discovered by Frank Macfarlane Burnet and Mavis Freeman in 1937, from one of Derrick's patients (Burnet and Freeman, 1937) and was originally identified as a species of Rickettsia by H.R. Cox and Gordon Davis from Ticks in Montana (Davis and Cox, 1938). Recent phylogenetic analyses suggest that *C. burnetii* is more closely related to Legnionella and Francisella than to the genus Rickettsia (Plummer, 2015). C. burnetti is a gram negative obligate intracellular bacterium for which ruminants are the most prevalent natural reservoir (Mohammed et al., 2017; Eldin et al., 2017; Alvarez et al., 2012; Vanderburg et al., 2014). The bacterium is shed in urine, feces, and milk and birth products of infected animals and is highly infectious, as only a few organisms can cause the disease and can remain viable and virulent for months because of its spore-like lifecycle (Honarmand, 2012). Domestic livestock, particularly cattle, remain carriers of *C. burnetii* for protracted periods and sheep often excrete large numbers of organisms in their birth products at parturition (Welsh HH, Lennette EH, Albatini FR, Winn JF, 1953, cited by Marrie, 1990). Some species of ticks also act as a natural reservoir of *C. burnetii* (De Bruin et al., 2013).

In many livestock species, Q fever is frequently asymptomatic; however, clinical expression of *C. burnetii* infection in sheep and goats includes late gestation abortion, reduced reproductive efficiency because of stillbirths, delivery of weak offspring and premature delivery (Angelakis & Raoult, 2010), whereas cattle may develop metritis, mastitis and infertility (To et al., 1998). *C. burnetii* is transmitted to humans via direct contact with milk, urine, faeces, amniotic fluid or aborted tissues and placentae at birth (EFSA, 2012). As *C. burnetii* is a highly resistant bacterium, the environment itself can also serve as a reservoir for this bacterium (De Bruin et al., 2013). Inhalation of aerosolized particles from live ruminants and aborted fetuses is a major source of infection for humans (Isken et al., 2013). In human, manifestation of acute Q fever can be like flu and self-limited illness, and major clinical symptoms of these patients are fever, headache, coughing, atypical pneumonia, hepatitis, myalgia, arthralgia, cardiac involvement, skin rash and neurologic signs; while chronic Q fever is accompanied with endocarditis, vascular infection, prosthetic joint arthritis, osteoarticular infection and lymphadenitis (Raoult, 2010, 2012; Eldin et al., 2017). Numerous seroprevalance surveys of *C. burnetii* infection in cattle has been conducted worldwide, including the countries like Northern Ireland (McCaughey, et al., 2010), Germany (Psaroulaki et al., 2006), Central African Republic (Nakoune et al., 2004), Mexico (Hellenbrand et al., 2001), Cyprus (Salman et al., 1990), Turkey (Kirkan et al., 2008), Iran (Rahimi et al., 2009), Korea (Kim et al., 2014), India (Randhawa et al., 1973) Bangladesh (Chakrabartty et al., 2016; Rahman et al., 2016; Haider et al., 2015). These studies revealed that Q fever seroprevalance varies widely by animal species and geographical location. The seroprevalance rates reported in cattle population vary greatly ranging from 3.4% to 84% (McCaughey et al., 2010). Seroprevalance of Q fever in cattle in China was 15% (Mahollawy et al., 2015). Likewise,

the prevalence of antibodies to *C. burnetii* was observed 24.29% among 490 cattle in Delhi and Uttar Pradesh, India, among which 20% of 55 dairy cows were found positive for *C. burnetii* antibodies in their milk (Yadav and Sethi, 1979). However, in Nepal, there are only few documented studies and facts associated to Q fever. In the study by Panth et al. (2017), it was found that 1.63% among 184 serum samples of cattle in Rupandehi district of Nepal are having circulating antibodies in their blood which was the first study in Nepal regarding seroprevalance of *C. burnetii* in cattle. Some studies has been conducted in small ruminants in few districts which shows the overall seroprevalance rate to be 1.45% (3.03% in sheep and 0% in goats) among 276 serum samples of goat and sheep in Bardiya and Surkhet district (Koirala, 2015), while 10% of 100 serum samples from goats with history of abortion and stillbirths were seropositive in Chitwan district (Acharya, 2015). Similarly, risk factors underlying this variability in infection rate are poorly understood (Vanderburg et al., 2014). The economic and public health impacts of Q fever is a major concern in developing countries (like Nepal): a zoonotic importance and causing significant loss of animal productivity (Mostafavi et al., 2012; Van Asseldonk et al., 2015). Also, Q fever is an occupational hazard for veterinarians, abattoir workers, dairy farmers and anyone with regular contact with livestock or their products (Khalili et al., 2010). Therefore, this study was conducted with the aim to explore the seroprevalance of *C. burnetii* among the cattle population, using the blood serum sample, in three districts of central Terai region of Nepal: Chitwan, Nawalpur and Rupandehi districts and to investigate the risk factors potentially associated with the disease seropositivity in this area.

2. METHOD:

Cross-sectional survey was conducted between July, 2018 to September, 2018 in Chitwan, Nawalpur and Rupandehi districts of Nepal, from where households were purposively selected from areas where farmers predominantly rear cattle. The sample size required for the detection of *C. burnetii* antibodies was calculated from EpiTools Epidemiological Calculators by Ausvet, assuming 1.63% prevalence, as reported by Panth et al., 2017 in his study conducted in Rupandehi district of Nepal, and computed with expected precision of 5% and 95% confidence level. The sensitivity and specificity of this ELISA kit was 100% and 97.8% respectively as used by Mohammad et al., 2017. The calculated minimum sample size was 63 for each district which implies, altogether 189 samples from 3 districts. But, a total of only 104 samples were collected (35 from Chitwan district, 37 from Nawalpur district and 32 from Rupandehi district), due to limitation of ELISA kit availability for required sample size. Purposive two stage sampling, for samples with history of reproductive disorders like abortion, repeat breeding, anestrous and retention of placenta, was done. In the first stage, pocket areas from each district were selected by convenience sampling procedure which was taken as primary sampling unit (PSU). In the second stage, blood samples from jugular vein of cows with the history of reproductive disorders were collected, by visiting the farms purposively in the preselected pocket areas, which was taken as secondary sampling unit (SSU). Epidemiological data was collected using sample data collection sheet during blood sampling for individual animal level data and herd level data. A structured questionnaire containing 11 variables potentially associated with *C. burnetii* seropositivity was inquired using both closed and open-ended questions. Questions pertaining to individual cattle's age, breed (indigenous/ cross), BCS, history of abortion and presence of ticks were included. Additional data was gathered for general herd and management data which comprised of geographical location of herd, herd size, husbandry system, contact with small ruminants (yes or no), introduction of new individual in herd (yes or no) and herd size were categorized into two groups: small (< 5 head), medium (5-10 head) and large herds (>10 head). The questionnaire was completed by face-to-face interviews with the farm owner.

Laboratory analysis was performed at National Agriculture Research Council, Khumaltar in the Animal Health Research Division (AHRD). Sera were confirmed using Q Fever Indirect Multi-species ELISA (IDvet) kit, which uses a *C. burnetii* phase I and II strain isolated in France from an aborted bovine placenta and detects anti-C burnetii antibodies in serum, plasma and milk of cattle, goats and sheep. ELISA was done as per the principles and procedures provided in IDvet ID Screen Q Fever Indirect Multi-species protocol. Data collected in the field using individual data sheet and data from laboratory analysis were entered and coded in MS Excel Spreadsheet. Ausvet Epitools was used to test the survey result for true prevalence of the disease in the study population.

3. RESULT:

A total of 104 female cattle serum samples were tested. Of those, 71(68.3%) were HF cross, 31(29.8%) were Jersey cross, 1 was local and 1 was Brown Swiss cross; 9 (8.7%) were of age less than 3 years, 82 (78.8%) were of age 3 to 7 years, 10 (9.6%) were of age 7-11 years and 3 (2.9%) were of age more than 11 years. Similarly, 26 (25%) had history of abortion cases and 53 (51%) were tick infested. Screening by *C. burnetii* IDvet Indirect ELISA revealed all 104 cattle serum samples as negative. True prevalence and predictive values were estimated from the result of the test with kit sensitivity 100% and specificity 97.8%

using Ausvet Epitools. True prevalence was estimated 0% with confidence interval (95%) between 0 to 0.0139 and the negative predictive value to be 1 which are shown in Table 1.

Table 1: Overall Seroprevalence of C. burnetii in Cattle of Chitwan, Nawalpur and Rupandehi District based on Ausvet Epitools

Test Assay	District	Classification
ELISA	**Chitwan**	Positive: 0 Negative: 35
	Nawalpur	Positive: 0 Negative: 37
	Rupandehi	Positive: 0 Negative: 32
Total		Positive: 0 Negative: 104
Apparent Prevalence (%)		0
Wilson CI (95% CI)		0 – 0.0356
True Prevalence (%)		0
Blaker CI (95% CI)		0 – 0.0139
Positive Predictive Value		-inf
Negative Predictive Value		1

3.1. DISCUSSION:

Coxiella burnetii is considered to occur worldwide (except New Zealand and French Polynesia); however, its incidence rates vary considerably from country to country (Norlander, 2000). Because of its clinical polymorphism, the diagnosis of Q fever is challenging (Angelakis et al., 2010). With an increasing awareness and diagnostic advances, the infection has emerged in various regions including the tropics (Million et al., 2015). In addition, there are ample wild and domestic animals in the study area that could act as hosts for *C. burnetii*, and the study area being connected with the open border of Uttar Pradesh, India from where cattle and other livestock are directly imported and where the seroprevalence of Q fever was found 24.29% (Yadav and Sethi, 1979); despite the policy of trying to prevent entry of the organism by quarantine of imported animals, there are much chances and opportunities for introduction of the bacteria. So, in view of its known epidemiological features, it is difficult to explain the absence of Q fever in this study population. The most plausible explanation seems to be that, for the organism to be maintained in wild or domestic animals, an animal-tick cycle must operate (Worthington, 2001). The most abundant livestock tick present in Chitwan, Nawalpur and Rupandehi districts of Nepal is *Boophilus (Riphicephalus) microplus* (Shrestha et al., 2005) which, according to the review done by Cooper (2011), is not a tick species that is known to transmit the causative agent. Therefore, if it is a poor vector for C burnetii, this could explain the failure of the organism to establish in the study area (Anon, 1997).

To examine the likelihood that *C. burnetii* was absent in our study population, a "Freecalc: result analysis of freedom testing" by Ausvet Epitools was used for this imperfect test, which showed that the results were adequate to conclude that the study population is free from disease (at an expected minimum prevalence of 1.63% and the ELISA test sensitivity and specificity of 100% and 97.8% respectively) at the 0.9821 confidence level. The negative predictive value is also 1 (Table 1), so we can be 95% confident that the negative results are actually negative, which further supports the absence of disease in our study population. Early epidemiological evidences and serological investigations shows 1.63% seropositivity in 184 cattle in Rupandehi district (Pantha et al., 2017) and 1.45% in 276 serum samples of goat and sheep in Bardiya and Surkhet district, Nepal (Koirala, 2015), which are quite a low epidemicity and supports a high probability of the absence of disease in the study population. Likewise, if the organism were present in farm livestock, disease would be expected to have occurred regularly in people, particularly farmers and abattoir workers. No case has ever been reported of anybody contracting the disease in Nepal. The human population in effect acts as a sentinel system for the presence of infection (Worthington, 2001).. The history of reproductive disorders in the sample animals may most probably be due to any other infectious or non-infectious cause that results in reproductive disorders. Limitation of our study design were that the sites were not systematically chosen

(e.g. to represent different biogeo-climatic zones) and the sample size taken was small due to lack of time and budget. Therefore, the study with adequate cattle sample size by random sampling from different eco-climatic zones, where cattle farming are important agricultural sectors, would be more reliable and informative regarding the situation of *C. burnetii* among cattle in Nepal.

4. CONCLUSION:

Our study provides a preliminary epidemiological data on C. burnettii infection among cattle in Nepal indicating that this pathogen might be of very low epidemicity or non-endemic in the study areas. This information is a step forward in understanding the epidemiology of Q fever in mid Terai (Tropical) area of Nepal. Still, to confirm the very low rate or absence of this zoonotic disease in Nepal, further systematic serological surveys including larger sample size and/or molecular studies in other ruminants, non-ruminants, humans, and potential vectors should be performed.

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