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Research Article

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Prevalence of *Coxiella burnetii* from Raw Milk Samples in and Around Anand

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ABSTRACT

Milk is an excellent medium for growth of microorganism due to its high nutritive value and high moisture content. Q-fever is a zoonotic disease which is caused by the obligate intracellular bacterium Coxiella burnetii and infection may cause Q fever in human and in animal species. Some human cases exhibited symptoms like headache, fever and muscle aches which usually resolve without treatment. For people with chronic C. burnetii infections, the liver and heart are usually affected. The present study was carried out to determine the prevalence of Coxiella burnetii from the raw milk in Anand district of Gujarat. The prevalence of Coxiella burnetii was studied by I-ELISA in the 104 raw milk samples which collected from 82 cattle and 22 buffaloes from organised farm in and around Anand. Overall 28.85% (30/104) prevalence was observed comprising of 28.05% (23/82) in cattle and 31.82% (07/22) in buffaloes, respectively. The detection of C. burnetii in raw milk indicates a potential health risk for human beings as well as domestic livestock, especially those who consume raw or unpasteurized milk.

Key words: *Coxiella burnetii, Q-fever, Raw milk, I-ELISA, Zoonotic disease*

INTRODUCTION

Coxiella burnetti causes Q fever (query fever) in animals and humans and is found throughout the world.[5]. *C. burnetii* is an important occupational zoonosis of worldwide significance and has been classified as OIE notifiable disease[1, 2]. This highly infective organism has been considered as a potential weapon for bioterrorism and therefore it has been classified as a Category 'B' critical biological agent by the Centre for Diseases Control and Prevention[3]. Domestic ruminants such as sheep, goats and cattle are widely used as food animals (meat and milk). These infected animals by *C. burnetii* represent source of human infection[4, 11]. The prevalence of *C. burnetii* often shows no clinical signs; the common manifestations of Q fever in ruminants are abortion, stillbirth, premature delivery, and delivery of weak offspring[9]. Indeed these clinical manifestations are usually observed in sheep and goats. Q fever is mostly asymptomatic in cattle[10].

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Consumption of raw milk is considered as suspected mode of transmission because *C. burnetti* localizes in the mammary gland. The prevalence of *C. burnetti* in bulk tank milk from dairy cattle is 21.00 %[8]. Symptoms of infection in humans vary from inapparent to severe. Some human cases show mild flu like symptoms such as headache, fever and muscle aches. In chronic *C. burnetti* infections, the liver and heart are usually affected[7]. The principal aim of this study was to use I-ELISA for the detection of *C. burnetii* in raw milk samples collected from organised farm in and around Anand.

MATERIAL AND METHODS

Sampling

Milk samples (n =104) which included 82 cattle and 22 buffaloes from organized farm in and around Anand were collected aseptically into sterile plastic tubes. All samples were brought to the laboratory of Department of Veterinary Public Health and Epidemiology, Veterinary College, AAU, Anand in Ice box and they were further processed. Milk preparation Centrifuge at 7500 rpm for 20 minutes. Take up the middle layer of liquid by means of a glass Pasteur pipette inserted through the upper layer of cream, taking care not to touch the underlying cell sediment. Use undiluted skimmed milk samples in the wells.

ELISA Analysis

The raw milk samples were tested for antibodies against *C. burnetii* using the commercial I-ELISA kit **(Monoscreen AbELISA- *Coxiella burnetti* / indirect monowell- Bio-X Diagnostics).** The reagents must be kept between +2°C and +8°C. Bring all components to 21°C +/- 3°C before use. Remove the micro plate from its wrapper. To guarantee the reliability of the results, one must follow the protocol of I-ELISA. The samples (100µl) were distributed in well along with positive control and negative control. The plate was covered with a lid and incubated for 21º+/- 3ºC for one hour then after Rinsed the plate with the washing solution three times. Add 100µl diluted conjugate solution to each well. Covered the plate with a lid and incubated for 1 hour at 21º+/- 3ºC. Again wash the plate three times. Add 100µl of the chromogen solution to each well on the plate. Incubated for 10 minutes at 21º+/- 3ºC and Protected from the light and uncovered the plate. 50µl stop solution added to micro well for stopping the reaction. The blue color changed into a yellow color. Finally, checked the optical densities in the micro well using a ELISA Reader (Thermo).

Calculate milk's coefficient by means of the following formula:

$$\text{Sample's Coeff.} = \frac{\text{OD Sample} - \text{OD negative control}}{\text{OD positive control} - \text{OD negative control}} \times 100$$

- A sample is negative if its coefficient is < 37%
- A sample is positive if its coefficient is > / = 37%

RESULTS

Out of 104 raw milk samples; 30 samples were found positive for *C. burnetii* antibodies by I-ELISA. Overall 28.85% (30/104) prevalence was observed comprising of 28.05% (23/82) in cattle and 31.82% (07/22) in buffaloes, respectively.

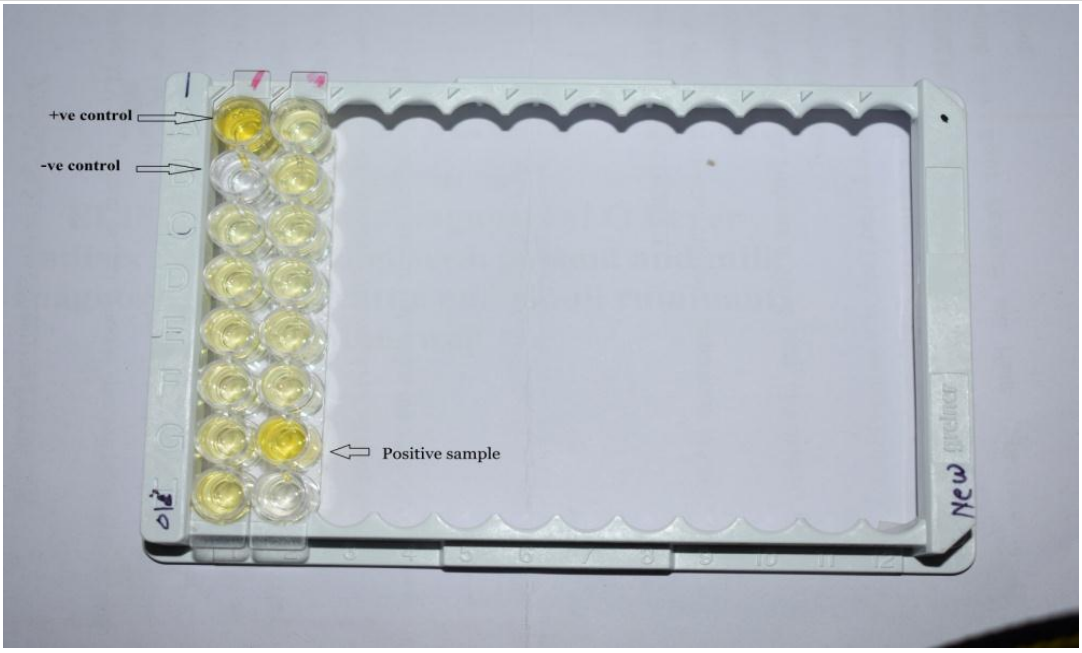

Fig. 1: I-ELISA Test Result

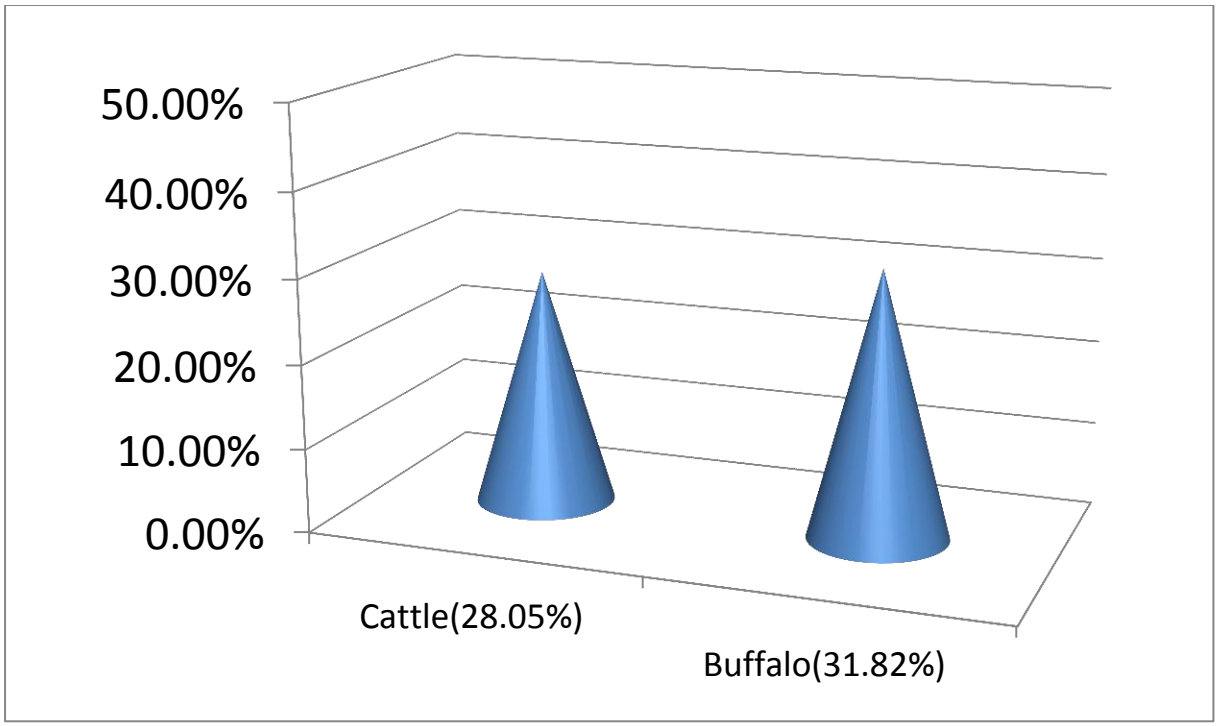

Fig. 2: Prevalence of *C. burnetii*

DISCUSSION

Very few studies have been conducted on Q – fever in India and this study in raw milk samples analysis by I-ELISA in Anand district of Gujarat. The present study included 104 raw milk samples which collected from 82 cattle and 22 buffaloes from organised farm in and around Anand and was sufficiently large to estimate the prevalence of *C. burnetii* antibodies.

According to the results, 28.85% (30/104) were positive by I-ELISA in this study. The preliminary studies were conducted by authors indicate people and animals in India are exposed to *C. burnetii*. It was detected in 4.63 per cent milk samples in India by trans –PCR[7]. As compared to present findings higher prevalence of *C. burnetii* antibodies reported by Khalili *et al*.[6] 45.4 per cent of prevalence of *C. burnetii* antibodies from dairy cattle

farms based on Bulk tank milk samples were tested for antibodies against *C. burnetii* using the commercial CHEKIT® Q -fever antibody ELISA test kit.

Results of this study confirm that antibody positive samples are very prevalent in this area and possibly other province of India. In the previous study used BTM samples from 44 dairy herds included near 12000 dairy cattle but in this study we used only (104) raw milk samples from organised farm in and around Anand. Lower prevalence recorded in the present work might be due to relatively smaller sample size.

However, comparatively lower prevalence of *C. burnetii* from milk than the findings of present study, appeared in the literature cited earlier Rahman *et al*. [9] determined the herd level prevalence of *C. burnetii* in cattle based on bulk milk was estimated by using indirect ELISA test. The overall prevalence of *C. burnetii* in bulk cow milk was 15.6% indicating that Q-fever is an existing disease in dairy cattle population.

Earlier literature lower prevalence revealed Vaidya *et al,*[11] determined Antibodies against *C. burnetii* were detected in 23 of 217(10.59%) sera samples tested from 10 (11.36%) cattle, 6 (18.18%) buffaloes, 4 (9.30%) sheep and 3 (5.66%) goats by ELISA. This study indicates that the occurrence of *C. burnetii* in bovine milk in India might be common and therefore, a large scale screening is needed for evaluation and implementation of necessary legal regulations with regard to coxiellosis in dairy herds.

The findings of the present studies indicating overall prevalence of 28.85 per cent, which is in approximation with the findings of Paiba *et al.*[8] reported prevalence of 21.00 per cent from raw milk, indicating similar ecological possibilities of its occurrence in the milk.

The variation in the recovery rate may be due to unhygienic conditions prevailing at the time of sample collection, handling and sample processing may add into the number of microorganisms.

The frequency estimates are apparent prevalence. This implies that the true prevalence may be different if the test sensitivity and test specificity are less than 100%. However, the sensitivity and the specificity are unknown. ELISA test is generally considered better than the Complement Fixation test. Raw milk sample is easy and inexpensive to collect, could be used to assess, on a larger scale at a low cost, the efficiency of control schemes aimed at controlling or preventing *Coxiella* shedding in organised farm. In common with all zoonotic diseases, control of the disease in animals will influence the level of disease seen in Man. Raw milk samples is good samples for screening of dairy animal for *C. burnetii* antibodies.

CONCLUSION

C. burnetii in the raw milk indicates a potential health risk for domestic livestock as well as human beings, especially those who consume raw or unpasteurized milk. The high excretion rate of pathogen particularly in milk is a potential public health threat. So, it is recommended that milk should be consumed only after pasteurization.

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