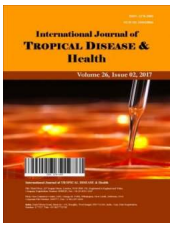

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Sero-prevalence of Brucellosis in Cattle and Its Associated Risk Factors in North East India (Meghalaya)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors HK and PP designed and planned this study. Authors LD, HK, LRD and IW collected the samples. Authors HK, LRD and IW executed the sero-diagnostic tests of all samples. Authors MK, LRD and HK analyzed the data. All authors contributed equally in preparation and revision of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: A cross-sectional sero-prevalence study was conducted from July 2008 to December 2014 in Meghalaya (India) to determine prevalence of bovine brucellosis and also for assessing the risk factors associated with the disease in cattle.
Materials and Methods: In the present study, serum samples were collected from a 1248 animals aged 1-12 years and were screened for brucella antibodies using RBPT and Indirect ELISA. The samples were collected from male and female cattle, which were reared in organized farms and smaller private holdings.
Results: Rose Bengal Plate Test (RBPT) revealed 2.8% serum positivity whereas 2.24% were detected positive by indirect ELISA. Prevalence was higher in female (2.16%) compared to male (0.08%) and cattle of age group 2-7 years old were much susceptible than others. Higher prevalent

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were detected from organized farms maintaining high number of animals (3.3%) than smaller private holdings (1.49%). Cattle with history of abortion also indicate higher and significant association with sero-positivity found in such sampling animals.

Conclusion: Brucellosis is prevalent in the hilly state of Meghalaya (India).Therefore the study give an insight into the sero-prevalence of bovine brucellosis in the state with potential risk factors for transmission of disease.

Keywords: Brucellosis; cattle; RBPT; iELISA; sero-prevalence.

1. INTRODUCTION

North Eastern Region of India particularly Meghalaya heavily depends on Agriculture and Livestock farming for livelihood and economy of the state. Meghalaya has cattle population of 9,05,733, which are reared mainly for milk, meat and meat products [1]. Brucellosis is an important health problem in many coutries. It is caused by a variety of *Brucella* species. The diseases in cattle is prevalent around the world, but there are countries such as Canada, Japan, Australia and New Zealand in which cases of *Brucella abortus* and *Brucella Melitensis* have never been reported [2]. Brucellosis caused by *Brucella abortus* is the main causative bacterial agent in dairy cattle and associated with widespread contagious reproductive disease of dairy animals and particularly in India it is highly prevalent among all bovine population [3] thus causing huge economic losses to the farmers and other vested agencies. Brucellosis is caused by genus *Brucella* which is coccobacillary shaped bacteria and is Gram-negative, facultative, intracellular bacteria, comprising of many different species when based upon biochemical reactions and also their affinity with preferred host species. Currently, there are ten spp. described in the genus Brucella. Each *Brucella* species may infects various animal hosts, but have preference for particular host animals, such as *B. abortus* for cattle, *B. suis* for pigs, *B. melitensis* for sheep and goats, *B. ovis* for rams, *B. canis* mainly infect dogs, *B. microti* infect rodents-Microtusarvalis, *B. neotomae* for rodents-Neotoma lepida, *B. Pinnipedialis* infect pinnipeds, *B. ceti* for cetacea, however *Brucella inopinata* which is first isolated from human, but so far its preferential host is not known [4,5]. In cattle, brucellosis is usually caused by *B. abortus,* but has also been attributed to *B. melitensis* and infrequently to *B. suis* [6]. In young animals and non-pregnant females, disease symptoms are usually not recognisable. Symptoms of *B. abortus* or *B. melitensis* in pregnant adult females includes placentitis usually resulting in abortion between the fifth and ninth month of pregnancy. Adult male cattle may develop orchitis and/or epididymitis. Infertility due to brucellosis may occur in both males and females. In some tropical countries, hygromas particularly of leg joints is a common manifestation of brucellosis [2,7].

One of the many control programme for brucellosis is proper and timely vaccination of animals of different age groups. Test and slaughter policy of infected animals with proper disposal of animals following confirmatory diagnostic tests is usually adopted to control the disease [8]. In many countries regulation of the disease depends on vaccination and culling of infected animals in order to minimize chances for spread of the disease to consumers and people that are associated with regular animals farming activities [9]. Limited information is available on the status of bovine brucellosis as limited study has been done on prevalence of bovine brucellosis in hilly state of Meghalaya (India). Therefore the objective of the paper was to determine prevalence of bovine brucellosis and for assessing the potential risk factors associated with the disease.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 1248 serum samples were collected during the period from July 2008 to December 2014 from cattle of various organized farms (n=36) and private holdings (n=82) in different districts of Meghalaya. The serum samples were collected by the Disease Investigation team, A.H & Veterinary Deptt. Meghalaya, Shillong, based on representative sampling with reference to age, sex, production and also by purposive sample approach based on farmers request. The samples were collected from Holstein Friesian, Jersey and various cross breeds. Approximately 7 ml of blood sample was collected from the jugular vein of each animal using vacutainers (Becton Dickson, USA). Samples were properly labelled and all the clear serum were stored at

−20℃ until tested. Of 1248 animals, 1138 (91.18%) and 110 (8.81%) were females and males, respectively and belonged to different age groups from 1 to 12 years. Again, out of 1248 animals, 512 (41%) were from organized farms (including Government run farms) maintaining more than 20 lactating dairy cattle and 736 (58.97%) from private holdings maintaining fewer cattle (<20 nos.). Herd and animal level data were recorded comprising of age, sex, farm size, history of abortion or repeat breeding and live S19 calfhood vaccination.

2.2 Serological Tests

All the serum samples were used to evaluate the disease prevalence by serological tests, viz., Rose Bengal Plate test (RBPT) and indirect Enzyme Linked Immunosorbent Assay (iELISA). The serum samples (n=1248) were analysed by rose bengal plate test (RBPT) according to standard protocol [10]. The *B. abortus* S99 colored antigen was procured from Institute of Animal Health and Veterinary Biologicals (IAH&VB), Kolkata, India. The serum samples were also tested for sero-positivity by indirect ELISA diagnostic kit (NIVEDI, Bengaluru, India). Samples were also sent to IAH&VB, Kolkata and ICAR NEH Region, Umiam for further analysis.

All the procedures for sero-positivity diagnosis of the serum by ELISA diagnostic kit were followed as per manufacturer's protocol. Any colour development in the form of the optical density (OD) was read at 492 nm using an ELISA microplate reader (Infinite F50, Tecan, Austria). Percent positivity (PP) values which were used for the diagnostic interpretations were calculated as per manufacturer's calculation. Any sample of Percent Positivity (PP) value below 55% is taken as negative, between 55-65% as moderate positive, more than 65% as strong positive and sample with only 55% PP are recommended for re-sampling for confirmation.

3. RESULTS AND DISCUSSION

Out of 1248 serum samples, 35 (2.8%) were detected positive by RBPT test, whereas 28 (2.24%) were detected as positive serum by iELISA. Comparative evaluation of tests revealed that 25 (2.0%) samples were positive by both RBPT and iELISA tests. Absence of gold standard methods like isolation of the bacteria and polymerase chain reaction technique, calculation for prevalence of the disease is based on test conducted by screening of paired samples which are positive by iELISA test. Hence, the overall prevalence of bovine brucellosis was found to be 2.24% (28/1248). The result agreed with the studyconducted by [11] who reported prevalence of 2.46% in Southern Ethiopia. In another study from Peninsular Malaysia [7] reported Brucella antibodies detected in 2.5% of sampled cattle. However, [12] reported only 0.7% prevalence of brucellosis in cattle of Central Ethiopia during 2013-14. The present study on prevalence of bovine brucellosis in Meghalaya (2008-2014) was lower compared to bovine brucellosis in India by [13] who reported that 5.22% serum samples were positive by RBPT and 6.03% by iELISA. However, another study by [14] reported higher seroprevalence of brucellosis in cattle of Meghalaya with 5.91% by RBPT and 11.29% by ELISA, particularly from border areas with Assam. The seroprevalence of 2.24% in the present study is lower compared to other countries such as Egypt (11%) and Nigeria (19.7%) as reported by [15,16] respectively. There is disparity in prevalence of the disease by different studies which may be due to various extrinsic factors such as the type of surveillance activities, farm management system including cattle-rearing, and finally the level of stringency regarding disease-control measures adapted in different countries.

By comparing both the commonly used serological tests, i.e., Rose Bengal Plate test (RBPT) and indirect ELISA, it shows that RBPT could detect more number of sero-positive samples than iELISA. Even though RBPT it is not a specific test to detect brucella antibodies and has several limitations, screening for brucellosis in many countries is still usually done by by RBPT [17]. High sensitivity of indirect ELISA is detected in recovered or vaccinated animals due to the persistence of IgG antibody for longer period. Hence, seroprevalence by iELISA could reflect either past or present exposure to *Brucella* organisms. Since as per investigation, brucellosis vaccination has not been done in the any animals of different farms in Meghalaya state during 2008-2014, the vaccinal antibody is ruled out. Molecular detection by PCR using serum and blood DNA may be used to further validated results.

In the present study, important risk factors associated to bovine brucellosis were also analysed. The sex wise prevalence showed that

Table 1. Risk factors associated with bovine brucellosis in Meghalaya State (India)

Risk factors		No. of animals	No. of positive
Sex	Female	1138	27 (2.37%)
	Male	110	1 (0.9%)
Size of herd	Organized (>20)	512	17 (3.3%)
	Small holdings (<20)	736	11 (1.49%)
Age (Yrs)	1 - 2	110	1 (0.9%)
	2.1 - 4	471	13 (2.76%)
	4.1 – 6	344	9 (2.61%)
	6.1 – 8	241	4 (1.6%)
	Above 8	82	1 (1.21%)
History of abortion and repeat breeding	Yes	98	11 (11.22%)
	No	1150	17 (1.47%)

prevalent of brucellosis is higher in female with 2.37% (27/1138) compared to male (0.9%; 1/110). In the present investigation, there is significant low prevalence of brucellosis in male cattle which agreed with previous findings of other investigators [12,18]. Spread of the disease in the herds is mainly due to lack of periodical screenings in large female bovine population and undiagnosed infected females. Investigators [12,13] reported that 1% and 6.63% female cattle were found to be sero-positive and the same was also extensively reported by worker [19] who clearly concluded that sex of the susceptible animal species is one of the many risk factors affecting susceptibility of cattle to *Brucella abortus* infection.

Higher prevalence were detected from organized farms maintaining higher number of lactating animals (3.3%; 17/512) than smaller private holdings (1.49%; 11/736). In case of bovine brucellosis, the greater chances of spreading of infection have been found especially in organized herds than in marginal herds [20] whereas in small farms various factors like sufficient unit floor space for each animal; stall feeding that minimizes contact with other infected animals and possibly more personnel attention to the animals by the farmer himself are the factors which attribute to the spread of infection. As per age category, prevalence of brucellosis is indicated that it was higher in those cattle whose age ranged from 2 yrs to 6 years old compared to the rest of the age groups (above Table 1). According to [19] susceptibility of animals to disease increases with age and infact more commonly associated with sexual maturity of the host. There were only few sero-positives samples detected from cattle aged less than two year group (0.9%) which may be attributed to exposure to brucellosis infected animals in the farms. The present study also revealed that brucellosis is more prevalent in areas and farms where abortion, repeat breeding problems and other reproductive complications are prevailed and reported. Hence, the prevalence of brucellosis was much associated with their history of abortion on those examined.

Despite of various preventive and control measures being followed in India, there is still a high potential for the transmission and spread of *Brucella abortus* due to its widespread prevalence [21]. Timely confirmatory laboratory testing of the animals with emergency attentive animal health care should be utilized to diagnose any related abortions cases, premature births and other clinical signs. This should be followed by total disinfection of the farms with recommended disinfectants. Careful selection of animals before purchase particularly from farms free of brucella infection, then pre-purchase tests and quarantine needs to be judiciously followed to keep the animals free of brucellosis.

4. CONCLUSION

The study reveals that bovine brucellosis is very much prevalent in the hilly state of Meghalaya and various potential risk factors were involved that need proper attention to reduce the disease and prevent production loss. In recent years, cases of bovine brucellosis have been increased in areas of Meghalaya state, possibly due to increased trade and rapid movement of cattle from other states and possibly from neighbouring border countries. The presence of sero-positive reactors for brucellosis indicates the presence of foci of infection that leads to the spread of the disease. Therefore, greater attention of the Dairy industry and Animal Husbandry sector in the State is urgently required to safe guard and prevent transmission risk of the infection to human population.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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