

Original Research Article

Sero-Prevalence of Bovine Brucellosis and its Associated Risk Factors in North East India (Meghalaya)

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 assess the potential risk factors.

Materials and Methods: In this study, a total of 1248 animals aged from 1-12 years 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 were detected from organized farms maintaining high number of animals (3.3%) than smaller private holdings (1.49%). There is significant association of sero-positivity of bovine brucellosis with history of abortion.

Conclusion: Brucellosis is very much prevalent in the hilly state of Meghalaya (India). 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, caused by a variety of *Brucella* species, is an important public health problem in many countries and infection with *Brucella* in cattle is widespread globally but there are many countries in northern and central Europe, Canada, Japan, Australia and New Zealand which are believed to be free from both *B. abortus* and *B. Melitensis* [2]. Brucellosis is one of the highly contagious ubiquitous reproductive diseases of dairy animals and highly prevalent among bovine population of the India [3] thus causing huge economic losses. It is an infectious bacterial disease caused by genus *Brucella* which is Gram-negative, facultative, intracellular coccobacillary comprised of species based upon biochemical features and their correlation with preferred host species. Currently, there are ten spp. described in the genus *Brucella*. Each one may infect different host spp., but each *Brucella* spp. has a preference for its host spp., *B. melitensis* (sheep and goats), *B. abortus* (cattle), *B. suis* (pigs), *B. ovis* (rams), *B. canis*

(dogs), *B. microti* (rodents-Microtus arvalis), *B. neotomae* (rodents-Neotoma lepida), *B. Pinnipedialis* (pinnipeds), *B. ceti* (cetacea), and *B. inopinata* (originally isolated from a human patient, but 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]. The disease is usually asymptomatic in young animals and non-pregnant females. Following infection with *B. abortus* or *B. melitensis*, pregnant adult females develop a placentitis usually resulting in abortion between the fifth and ninth month of pregnancy. Adult male cattle may develop orchitis/epididymitis and brucellosis may be a cause of infertility in both sexes. Hygromas, usually involving leg joints, are a common manifestation of brucellosis in some tropical countries [2,7].

Control program for the disease include vaccination of young and mature animals and slaughter of infected animals and disposal of animals following serological test [8]. Regulation of the disease in many countries relies on vaccination and culling of infected animals in order to minimize chances for perpetuation of the infection and for protection of consumers and people that are associated with animals keeping [9]. Limited study has been done on prevalence of bovine brucellosis in hilly state of North East India (Meghalaya) and there is limited information available on the status of bovine brucellosis in the state. Therefore the objective of the paper was to assess the prevalence of bovine brucellosis and its associated risk factors.

2. MATERIALS AND METHODS

Sample collection

A total of 1248 serum samples were collected during the period from 2008 to 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°C 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 calfhod vaccination.

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

71 protocol [10]. The *B. abortus* S99 colored antigen was procured from Institute of Animal Health and
72 Veterinary Biologicals (IAH&VB), Kolkata, India. The serum samples were also tested for sero-
73 positivity by indirect ELISA diagnostic kit (NIVEDI, Bengaluru, India). Samples were also sent to
74 IAH&VB, Kolkata and ICAR NEH Region, Umiam for further analysis.

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

82 3. RESULTS AND DISCUSSION

83 Out of 1248 serum samples, 35 (2.8%) were detected positive by RBPT test, whereas 28
84 (2.24%) were detected as positive serum by iELISA. Comparative evaluation of tests revealed that 25
85 (2.0%) samples were positive by both RBPT and iELISA tests. Due to the absence of gold standard
86 isolation and PCR method, paired samples positive by iELISA test were considered to calculate
87 prevalence of disease. Hence, the overall prevalence of bovine brucellosis was found to be 2.24%
88 (28/1248). The result agreed with the study conducted by [11] who reported prevalence of 2.46% in
89 Southern Ethiopia. In another study from Peninsular Malaysia [12] reported *Brucella* antibodies
90 detected in 2.5% of sampled cattle. However, [13] reported only 0.7% prevalence of brucellosis in
91 cattle of Central Ethiopia during 2013-14. The present study on prevalence of bovine brucellosis in
92 Meghalaya (2008-2014) was lower compared to bovine brucellosis in India by [14] who reported that
93 5.22% serum samples were positive by RBPT and 6.03% by iELISA. The seroprevalence of 2.24% in
94 the present study is lower compared to other countries such as Egypt (11%) and Nigeria (19.7%) as
95 reported by [15,16]. The prevalence disparity by different studies could be attributed to various
96 factors such as surveillance activities, the type of cattle-rearing management system, and the level of
97 stringency in disease-control measures in the country.

98 Comparison of both the serological tests (RBPT and iELISA) revealed that RBPT could
99 detect more number of serum samples than iELISA. Though RBPT is the definite test for *Brucella*
100 screening in many countries; but it is not a specific test to detect brucella antibodies and has several
101 limitations [17]. The persistence of IgG antibody for longer period in recovered or vaccinated animals
102 are being detected by the high sensitivity of iELISA. Hence, the seroprevalence by iELISA could
103 reflect either past or present exposure to *Brucella* organisms. Since any type of brucellosis vaccination
104 has not done in the farms investigated during 2008-2014, the vaccinal antibody is ruled out. Molecular
105 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 prevalent of brucellosis is higher in female with 2.37% (27/1138) compared to male (0.9%; 1/110). The low prevalence in males is in agreement with the study of [13,18] Lack of periodical screenings in large female bovine population and undiagnosed infected females causes increased spreading of the disease in the herds. [13,14,] reported that 1% and 6.63% female cattle were found to be positive reactors. This was also explained by [19] who stated clearly that sex has been one of the 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] which in small farms may be attributed to 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. Age category prevalence of brucellosis 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 (Table 1). According to [19] The susceptibility to disease increases with age and more commonly associated with sexual maturity than age. Few seropositives were detected in cattle less than two year age group (0.09%) and it may be due to exposure to brucellosis infected animals in the farms. The study revealed that disease is more prevalent in those farms where abortion and repeat breeding problems are reported. Hence, the prevalence of brucellosis was much associated with their history of abortion on those examined animals.

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]. Immediate veterinary care and timely laboratory testing assistance should be utilized to diagnose any related abortions cases, premature births and other clinical signs. Careful selection of animals before purchase from *Brucella*-free herds, pre-purchase tests and quarantine needs to be followed to keep the herd 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 Meghalaya, possibly due to increased trade and rapid movement of cattle from other states of the country. The presence of sero-positive reactors for the disease indicated the presence of foci of infection that need greater attention of the dairy industry in the State to safe guard the public health associated with brucellosis.

References

1. Livestock Census. The Livestock Census 12th edition. Animal Husbandry and Veterinary Department. Govt. of Meghalaya, Shillong. 2012, 12.
2. OIE Terrestrial Manual. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals: Bovine brucellosis. 2016.
3. Patel MD, Patel PR, Prajapati MG, Kanani AN, Tyagi KK, Fulsoundar AB. (2014) Prevalence and risk factor's analysis of bovine brucellosis in peri-urban areas under intensive system of production in Gujarat, India. *Vet World*. 2014, 7(7): 509-16.
4. de Jong MF, Tsoilis RM. (2012) Brucellosis and type IV secretion. *Future Microbiol*. 2012, 7: 47-58.
5. Hadush A, Pal M. Brucellosis: An infectious re-emerging bacterial zoonosis of global importance. *Int J Livestock Health*. 2013, 3: 28-34.
6. Sanogo M, Abatih E, Thys E, Fretin D, Berkvens D, et al. Risk factors associated with brucellosis seropositivity among cattle in the central savannah forest area of Ivory Coast. *Prev Vet Med*. 2012, 107: 51-56.
7. Anka MS, Hassan L, Adzhar A, Khairani-Bejo S, Mohamad RB, et al. Bovine brucellosis trends in Malaysia between 2000 and 2008. *BMC Vet Res*. 2013, 9: 230.
8. Taleski V, Zerva L, Kantardjiev T, Cvetnic Z, Erski-Biljic M, et al. An overview of the epidemiology and epizootiology of brucellosis in selected countries of Central and Southeast Europe. *Vet Microbiol*. 2002, 90: 147-156.
9. Mdegela RH, Kusiluk LMJ, Kapaga AM, Karimuuribo ED, Turuka FM, et al. Prevalence and determination of mastitis and milk born zoonosis in small holder farming sector in kibaha and morogoro distericts in eastern Tanzania. *J of Vet Med Series*. 2004, 51: 123-128.
10. Alton GG, Jones LM, Angus RD, Verger JM. Techniques for the brucellosis laboratory. 1st edition. Institute Nationale de le Rech. France, Paris. 1988, 174.
11. Kassahun A, Shiv P, Yilkal A, Esayas G, Gelagaye A, Aschalew Z. Seroprevalence of brucellosis in cattle and high risk professionals in Sidama Zone, Southern Ethiopia. *Ethiopia Vet J*. 2007, 11: 69-84.
12. Anka MS, Hassan L, Adzhar A, Bejo1 SK, Mohamad RB, Zainal MA. Bovine brucellosis trends in Malaysia between 2000 and 2008. *BMC Vet Res*. 2013, 9: 230.
13. Bashitu L, Afera B, Tuli G, Aklilu F. Sero-Prevalence Study of Bovine Brucellosis and its Associated Risk Factors in Debrebirhan and Ambo Towns. *J Adv Dairy Res*. 2015, 3: 131.
14. Shome R, Padmashree BS, Krithiga N, Triveni K, Sahay S, Shome BR, Singh P, Rahman H. Bovine Brucellosis in organized farms of India – An assessment of diagnostic assays and risk factors. *Adv Anim Vet Sci*. 2014, 2 (10): 557-564.
15. Holt HR, Eltholth MM, Hegazy YM, El-Tras WF, Tayel AA, Guitian, J. *Brucella* spp. infection in large ruminants in an endemic area of Egypt: crosssectional study investigating seroprevalence, risk factors and livestock owner's knowledge, attitudes and practices (KAPs). *BMC Public Health*. 2011, 11:341.
16. Mai H, Irons P, Thompson P. A large seroprevalence survey of brucellosis in cattle herds under diverse production systems in northern Nigeria. *BMC Vet Res*. 2012, 8:144.
17. Poester FP, Nielsen K, Samartino LE, Yu WL. Diagnosis of brucellosis. *The Open Vet Sci J*. 2010, 4: 46-60.
18. Kubuafor DK, Awumbila B, Akanmori BD. Seroprevalence of brucellosis in cattle and humans in the Akwapim-South district of Ghana: Public health implications. *Acta Tropica*. 2000, 76(1): 45-48.

19. Radostits OM, Gay CC, Blood DC, Hinchcliff KW. Veterinary Medicine: A textbook of the diseases of cattle, sheep, pigs, goats and horses. 9th edition. New York: Saunders, W.B. 2000, 867-882.
20. Jain U, Bist B, Sahzad P, Dwivedi K. Outbreak of brucellosis in buffaloes aborted in village Mahuan, district Mainpuri, U.P., India- A case report. Vet World. 2013, 6(1): 51-52.
21. Renukaradhya GJ, Isloor S, Rajasekhar M. Epidemiology, zoonotic aspects, vaccination and control/eradication of brucellosis in India. Vet Microbiol. 2002, 90: 183-195.

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%)