Prevalence study of Bovine Brucellosis in Meghalaya: North-EastState of India

ABSTRACT

Aim: A cross-sectional prevalence study was conducted from 2015 to 2018 in Meghalaya state (India) to determine prevalence of bovine brucellosis by serosurveillance study and Milk Ring Test.

Materials and Methods: In the present study, serum samples were collected from 3193 animals aged 1 year to 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. Individual milk samples (n=896) from representative dairy cattle were also screened for brucellosis using the Milk Ring Test (MRT).

Results: Test report by indirect ELISA revealed serum positivity of 9.89% whereas 9.33% were detected positive by Rose Bengal Plate Test (RBPT). The prevalence rate was higher in female (12.32%) compared to male (2.21%) and cattle of age group 1-4 years old with positivity of 11.66% were much susceptible than other age groups. Higher prevalent were detected from organized farms maintaining high number of animals (13.09%) than smaller private holdings (9.02%). Cattle with history of abortion and related clinical symptoms also indicate higher and significant association with sero-positivity found in such sampling animals. Milk Ring Test result revealed a high positivity of 17.29% tested from representative individual dairy cattle.

Conclusion: Brucellosis is very much prevalentin the hilly state of Meghalaya, India. The study gives an insight into the prevalence of bovine brucellosis in the State with potential risk factors for transmission of disease to human.

Keywords: Brucellosis, Seroprevalence, Cattle, RBPT, iELISA.

1. INTRODUCTION

Meghalaya is one of the State located in the North East Region of India and its population mostly depends on Agriculture and Livestock farming for livelihood and economy. Amongst the many diseases affecting bovine species, brucellosis is an important health problem in many countries. The disease 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 [1]. The disease is endemic in many states and regions of the India. Brucellosis is caused by a variety of *Brucella* species. In cattle, the disease is mainly caused by *Brucella abortus* and is generally associated with widespread

contagious reproductive disease of dairy animals and in India it is highly prevalent amongall bovine population [2] thus causing huge economic losses to the farmers and other stake holders. Bovine brucellosis has also been attributed to *B. melitensis*and infrequently to *B. suis* [5]. *Brucella*organism 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 infectvarious 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 farits preferential host is not known [3,4]. In young animals and non-pregnant females, disease symptoms are usually not recognizable. 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 [1,6].

Test and slaughter policy of infected animals with proper disposal of animals following confirmatory diagnostic tests is usually adoptedto control the disease in many countries [7]. 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 [8]. In India, the main control programme of bovine brucellosis is based on proper and timely vaccination of young female cattle of 4-8 months age group and isolation of positive reactors.

The Milk ring test (MRT) first described by Fleischhauer in 1937 [9], is one of the important serological tests used in detection of brucellosis particularly at the herd level. The test can detect IgM and IgA antibodies bound to fat globules. It has a wide acceptability as it is cost effective, easy to perform and can cover a large population in a short time [10].

There is limited information on the status of bovine brucellosis in North East region of India particularly on prevalence study of bovine brucellosis in a hilly and tribal state of Meghalaya (India). Therefore, the objective of the study was to determine seroprevalence of bovine brucellosis by RBPT and iELISA tests as well as detection of infection by Milk Ring Test in individual milked cattle.

2. MATERIALS AND METHODS

2.1. Collection of Samples

A total of 3193 serum samples were collected during the period from January2015 to December 2018 from cattle of various organized farms (n=28) and private holdings (n=96) in different districts of Meghalaya State, India. The serum samples from different Districts of Meghalaya were collected by the Disease Investigation team, A.H & Veterinary Department. Meghalaya, Shillong, based on representative sampling with reference to age, sex, production and also by purposive sample approach based on farmer's 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. Herd and animal level data were recorded comprising of age group, sex, farm size, history of abortion or repeat breeding. Of 3193 animals sampled, 2425 (75.94%) and 768 (24.05%) were females and males, respectively. A total of 1937 sera were from cattle aged 1 to 4 years, 789 sera were from cattle aged 5 to 8 years and 467 sera belonging to cattle of more than 8 years of age. Again, out of 3193 animals, 1420 (44.47%) were from organized farms (including Government run farms) maintaining more than 20 lactating dairy cattle and 1773 (55.52%) from private holdings maintaining fewer cattle mainly less than 20 nos.

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=3193) were analysed by Rose Bengal plate test (RBPT) according to standard protocol [11]. A known positive and negative serum were included in each day's tests. The test was read by examining for agglutination in a good light and is best facilitated when the mixture is observed as it flows away on the slide. The test was read as positive (any degree of agglutination) and negative with no agglutination. The *B. abortus* S99 colored antigen used in the study was procured from the Institute of Animal Health and Veterinary Biologicals (IAH&VB), Kolkata, India.

The Indirect enzyme-linked immunosorbent assay(I-ELISA)was performed on sampled serum using a commercial kit(IDEXX Brucellosis Serum X2 Ab Test, USA). The test wasperformed according to the recommendation of themanufacturer. Colourdevelopment in the form of the optical density (OD) was read at 450 nm using an ELISA microplate reader (Infinite F50, Tecan, Austria).Results are expressed as the ratio ofthe sample optical density (OD) minus the mean kitnegative control OD to the mean kit positive controlOD minus the mean kit negative control OD (S/Pratio). A positive result was defined by themanufacturer as an S/P ratio of ≥80%, and negativefor an S/P ratio of <80%

A total of 82 sampling units (cattle herds) were also sampled during the period from different farms of the State and in total 896 individual lactating cows of different age groups were sampled. Approximately 10ml milk pooled from 4 quarters were collected and properly labelled. Milk samples obtained from the animals were kept refrigerated at 4°C overnight prior to examination by milk ring test [11]. The milk samples were mixed well to ensure an even distribution of the milk cream.

The milk ring test is based on agglutination of antibodies secreted into the milk. This test allows screening of large number of cattle by using milk samples from tanks or pools from several cows. This test is useful for monitoring cattle herds or areas free of brucellosis so it is classified as surveillance or monitoring test [12]. The milk ring test is also the most practical method for locating infected dairy animals. In the study, 896 milk samples were analysed by MRT according to manufacturer's instructions. The *B. abortus* Bang Ring Antigen was procured from Institute of Animal Health and Veterinary Biologicals (IAH&VB), Kolkata, West Bengal, India. The test was performed by adding 30µl of *B. abortus* Bang Ring Antigen (hematoxylin-stained antigen) to 5ml milk sample in a test tube. The milk (antigen) mixtures were incubated at 37°C for 1 hour, together with positive and negative control samples. A strongly positive reaction was indicated by formation of a dark blue ring above a white milk column. The test was considered negative if the color of the underlying milk exceeded that of the cream layer and when the cream layer was normal.

3. RESULTS AND DISCUSSION

Out of 3193 serum samples, 298 (9.33%) samples were detected positive by RBPT test, whereas 316 (9.89%) samples were detected as positive by iELISA. Comparative evaluation of tests revealed that 287 (8.98%) samples were positive by both RBPT and iELISA tests. Details of the prevalence rate year-wise is depicted in Fig. 1. Absence of gold standard methodslike 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 recorded to be 9.89% (316/3193). In comparison, there were many other researchers who reported lower prevalence of bovine brucella antibodies of 2.46% in Southern Ethiopia [13], then in another study from Peninsular Malaysia [6] reported Brucella antibodies detected in 2.5% of sampled cattle and [14] reported only 0.7% prevalence of brucellosis in cattle of Central Ethiopia during 2013-14. In India as reported by [15] antibodies to brucella were reported to be 5.22% positivity by RBPT and 6.03% by iELISA. In a study by [16] reported seroprevalence of brucellosis in cattle of Meghalaya as 5.91% by RBPT and 11.29% by ELISA, particularly from neighbouringborder areas with Assam State.

The seroprevalence of 9.89% in the present study is lower compared to brucellosis seroprevalence findings of other researchers in other countries such as Egypt (11%) and Nigeria (19.7%) as reported by [17,18] respectively. There is disparityin 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 also detect accountable number of sero-positive samples as iELISA. Even though RBPT it is not a specific test to detect brucella antibodies and has several limitations, preliminary screening for brucellosis in many countries is still usually done by RBPT[19]. 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. However, brucella vaccination has not been done in the sampled animals of different farms in Meghalaya state during the study period, 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 and results were depicted in Table 1. The sex wise prevalence showed that prevalent of brucellosis is higher in female with 12.32% (299/2425) compared to male (2.21%; 17/768). In the present investigation, there is low prevalence of brucellosis in male cattle which agreed with previous findings of other investigators [14,20]. Spread of the disease in the herds is mainly due to lack of periodical screenings in large female bovine population and undiagnosed infected females.Researchers[14,15] reported that 1% and 6.63% female cattle were found to be sero-positive and the same was alsoextensively reported by worker [21] who clearly concluded that sex of the susceptible animal species is one of the many risk factorsaffecting susceptibility of cattle to *Brucella abortus* infection.

Higher prevalence was detected from organized farms maintaining higher number of lactating animals (13.09%; 186/1420) than smaller private holdings (9.02%; 160/1773). In case of bovine brucellosis, the greater chances of spreading of infection have been found especially in organized herds than in marginal herds [22] 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 1to 4 years old compared to the rest of the age groups (Fig. 2). According to [21] susceptibility of animals to

disease is in factmore commonly associated with sexual maturity of the host. Higher percentage of prevalence in age group of less than 4 years old may be attributed to new exposure to brucellosis infected animals in the farms.The present study also revealed that brucellosis is mostly prevalent(62.50%) 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 in which infected animals may act as carriers of infection. Representative individual dairy cattle's milk sampled during 2015-2018 when tested by Milk ring test (MRT) revealed a high positivity of 17.29% (155/896) regardless of age groups of animals as depicted in Fig.3. The finding comparatively corelated with the research of [23] who detected 18.35% positivity against Brucella and [24] in Iran who detected 14% positive reaction for *B. abortus* using MRT and taking many age groups into consideration. Another researcher [25] in his study observed that 12.5% of animals were positive for brucellosis by MRT. The MRT is preferred due to its simplicity to detect *B. abortus* infection, however, the test has also been reported for a number of false positive results of the samples [26].

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 [27]. 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 farmswith 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 seroprevalence of bovine brucellosis (9.89%) is comparatively higherin the hilly state of Meghalaya and various potential risk factors were involved that need proper attention to reduce the disease and prevent production loss. Cases of bovine brucellosis have been increased in certain areas of MeghalayaState, possibly due to increased cattle trade and demand, undetected movement of cattle from other states, and possibly from neighbouring border countries. Milk Ring test detected a high positivity of 17.29%, however, it is suggested that other confirmatory tests are to be used in conjunction. 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 byall concerned stakeholders and various sector in the State is urgently required particularly on educational awareness programmesfocusing on

preventive measuresto safe guard and prevent transmission risk of the infection to human population.

CONSENT

It is not applicable

References

1. OIE Terrestial Manual. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals: Bovine brucellosis. 2016.

2. Patel MD, Patel PR, Prajapati MG, Kanani AN, Tyagi KK, Fulsoundar AB. 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.

3. de Jong MF, Tsolis RM. Brucellosis and type IV secretion. Future Microbiol. 2012; 7: 47-58.

4. Hadush A, Pal M. Brucellosis: An infectious re-emerging bacterial zoonosis of global importance. Int J Livestock Health. 2013; 3: 28-34.

5. 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.

6. 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.

7. 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.

8. 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 orogorodistricts in eastern Tanzania. J of Vet Med Series. 2004; 51: 123-128.

9. Fleischhauer, G.Die Abortus-Bang-Ring-probe (ABR) zur Festellung von bangverdächtigen Vollmilchproben. Berl Tierarztl Wochenschr.1937;**53:** 527-528.

10. Cadmus, S.I.B., Adesokan, H.K. and Stack, J. (2008) The use of the milk ring test and Rose Bengal test in brucellosis control and eradication in Nigeria. J. of the South African. Vet. Asso. 2008;**79:** 113-115.

11. Alton GG, Jones LM, Angus RD, Verger JM. Techniques for the brucellosis laboratory. 1st edition. Institute Nationale de le Rech. France, Paris. 1988; 174.

12. Office International Epizootic. Bovine brucellosis in terrestrial manual. Office International Epizootic Manual. 2009.

13. 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.

14. 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.

15. 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.

16. Shakuntala I, Ghatak S, Sanjukta R, Sen A, Das A, Puro AK, Dutta A, Kakoty K. Incidence of brucellosis in Livestock in North-Eastern India. Int J of Infect Dis. 2016; 45S: 1-477.

17. 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: cross-sectional study investigating seroprevalence, risk factors and livestock owner's knowledge, attitudes and practices (KAPs). BMC Public Health. 2011; 11:341.

18. 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.

19. Poester FP, Nielsen K, Samartino LE, Yu WL. Diagnosis of brucellosis. The Open Vet Sci J. 2010; 4: 46-60.

20. 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.

21. 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.

22. 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.

23. Mohamand N, Gunaseelan L, Sukumar B, Porteen K. Milk Ring Test for spot identification of Brucella abortus infection in single cow herds. J Adv Vet Anim Res. 2014; 1(2):70-72.

24. Rezaei, M., Mohebali, S.H., Abadi, Y.K., Suri, E., Zare, A., Malamir, S.H., Rasuli, E.S. and Maadi, H. (2010) Investigation on the seroprevalence and pollution severity to *Brucella abortus* and *Brucella melitensis*bacteria in cows and sheeps living in the villager region of Toyserkan city, Hamedan, Iran. *J. of. Ani. and Vet. Adv.,***9:** 2870-2872.

25. Sukumar, K., Tamilselvan, P. and Dorairajan, N. (2012) Studies on infertility in cattle and buffaloes caused by *Brucella abortus. Tamil Nadu. J. Vet. and Ani. Sci.,* **8:** 235-237.

26. MacMillan, A. Conventional serological tests. Animal Brucellosis. 206:153-197.

27. Renukaradhya GJ, Isloor S, Rajasekhar M. Epidemiology, zoonotic aspects, vaccination and control/eradication of brucellosis in India. Vet Microbiol. 2002; 90: 183-195.

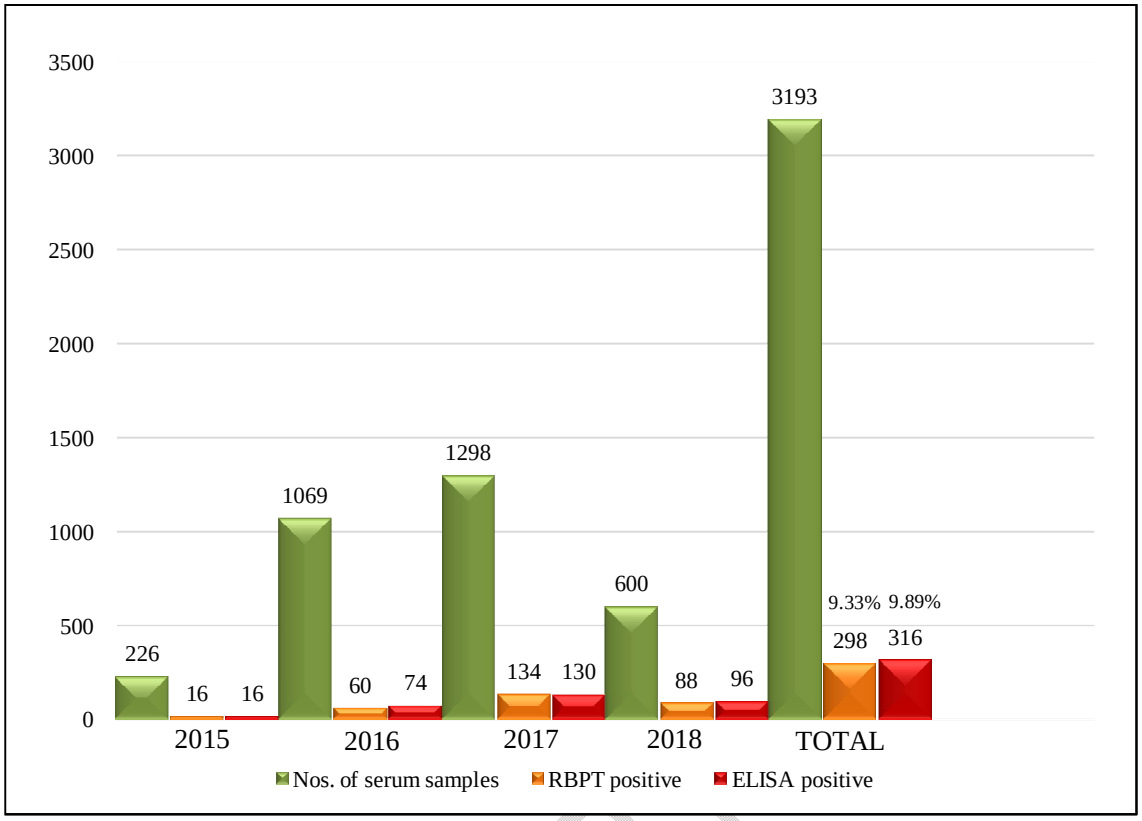

Fig. 1.Overall results of RBPT and iELISA indicated year-wise of sampled cattle population

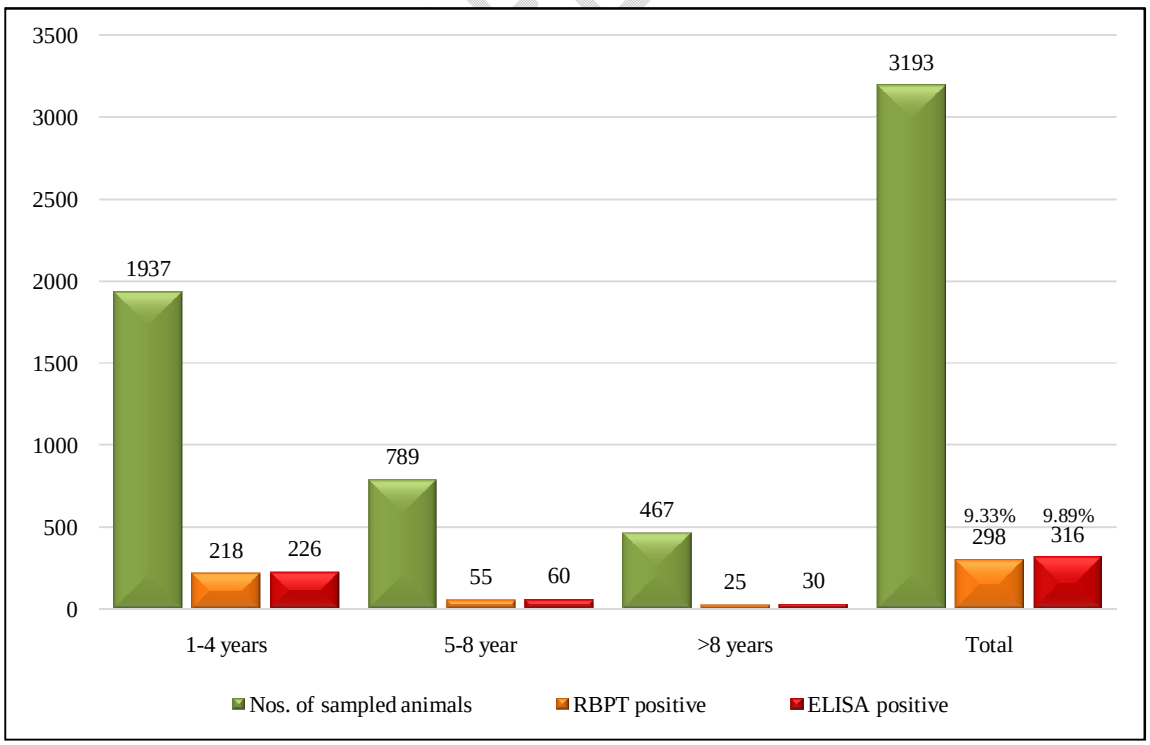

Fig. 2. Results of RBPT and iELISAaccording to age group of sampled cattle population

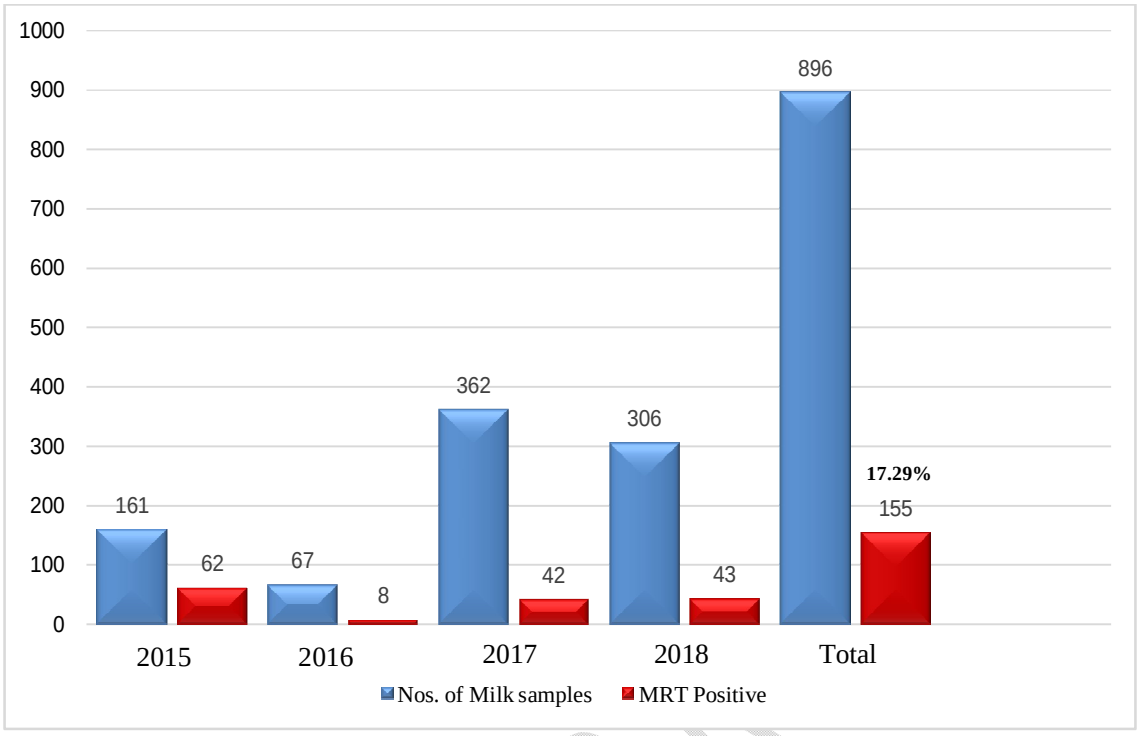

Fig.3. Results of the Milk Ring Test indicated year-wise along with total prevalence

Table 1: Risk factors associated with bovine brucellosis in Meghalaya State, India

Risk factors		No. of animals	RBPT positive	ELISA positive
Sex	Female	2425	286 (11.79%)	299 (12.32%)
	Male	768	12 (1.56%)	17 (2.21%)
Size of herd	Organized (>20)	1420	172 (12.11%)	186 (13.09%)
	Small holdings (<20)	1773	150 (8.46%)	160 (9.02%)
Age (Yrs)	1-4 years	1937	218 (11.25%)	226 (11.66%)
	5-8 year	789	55 (6.97%)	60 (7.60%)
	>8 years	467	25 (5.35%)	30 (6.42%)
History of abortion and repeat breeding	Yes	16	10 (62.5%)	10 (62.50%)
	No	3177	288 (9.06%)	306 (9.63%)