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

Comment [D1]: AlternativeTitle Suggestion: Prevalence of Bovine Brucellosis in Meghalaya: A Study of the North-East State of India

ABSTRACT

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

Comment [D2]: Do clarify either to use with serosurveillance study or serological test (RBPT and iELISA)

Materials and Methods: ~~In the present study, s~~Serum samples were collected from 3193 ~~animals~~ cattle aged 1 year to 12 years and ~~were~~ screened for ~~b~~Brucella antibodies using Rose Bengal Plate Test(RBPT) and Indirect ELISA (iELISA). The samples were collected from both 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).~~ MRT.

Results: ~~Test report by indirect~~ The iELISA revealed a serum positivity rate of 9.89% ~~whereas 9.33% were~~ while the RBPT detected a positivity rate of 9.33%. ~~positive by Rose Bengal Plate Test (RBPT).~~ The prevalence rate was higher in females (12.32%) compared to males (2.21%).~~and c~~Cattle ~~of age group~~ aged 1-4~~years old with positivity of~~ showed higher susceptibility (11.66%)~~were much susceptible~~ than other age groups. Higher prevalen~~t~~ce were detected from organized farms maintaining high number of animals (13.09%) ~~than~~ compared to smaller private holdings (9.02%). Cattle with a history of abortion and related clinical symptoms also had a ~~indicate~~ higher and significant association with sero-positivity.~~found in such sampling animals.~~The MRT ~~Milk Ring Test result revealed~~ indicated a high positivity of 17.29%~~tested from representative individual~~ among sampleddairy cattle.

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

Comment [D3]: Discuss the broader impact of your findings.
For example :
1.The high prevalence of brucellosis highlights the urgent need for enhanced surveillance and control measures in Meghalaya.
2.Implementing targeted vaccination programs and improving farmer education could significantly reduce the disease's impact.

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

1. INTRODUCTION

Meghalaya ~~is one of the S~~state located in the North East Region of India,~~and its population mostly depends~~ relies on ~~A~~agriculture and ~~L~~livestock farming for its livelihood and economy.Amongs~~t~~ the ~~many~~ various diseases affecting bovine species, brucellosis is an ~~important~~ significant health problem in many countries. While ~~T~~the disease ~~in cattle~~ is prevalent ~~around the world~~ globally, 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 i~~In India, it is highly prevalent among~~all~~ the bovine population [2] thus causing huge economic losses to the farmers and other stakeholders. Bovine brucellosis has also been attributed to *B. melitensis*and infrequently to *B. suis* [5]. *Brucella*organism is coccobacillaryshaped, bacteria ~~and is~~ Gram-negative, facultative, intracellular bacteria,comprising of many different species ~~when~~ based on ~~upon~~ biochemical reactions and ~~also their~~ host affinity~~with preferred host species.~~

Currently, there are ten spp. described in the genus Brucella. Each ~~*Brucella*~~ species may infectvarious animal hosts, but ~~have~~ has preferences 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~~ for dogs, *B. microti*infect rodents ~~(~~Microtusarvalis~~)~~, *B. neotomae*for rodents ~~(~~Neotoma lepida~~)~~, *B. Pinnipedialis*~~infect~~ for pinnipeds, *B. cetii*for cetaceans.~~,however~~ *Brucella inopinata*~~which is~~ first isolated from humans, ~~but so far its~~ does not yet have a known preferential host ~~is not known~~[3,4]. In young animals and non-pregnant females, ~~disease~~ symptoms of brucellosisare usually not~~recognizable~~noticable~~. Symptoms of *B. abortus* or *B. melitensis*~~However, in pregnant adult females, symptoms of B.abortus or B. melitensis include~~splacentitis~~,usually~~ often 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 occuri~~In both males and females, may experience infertility due to brucellosis. In some tropical countries, hygromas, particularly of leg joints ~~is~~ are a common manifestation of ~~brucellosis~~disease[1,6].Additionally, the disease can cause chronic arthritis, reducing the productive lifespan of infected cattle [9].

To control the disease , many countries adopt a ~~T~~test and slaughter policy ~~of~~ for infected animals, with proper disposal ~~of animals~~ following confirmatory diagnostic tests~~is usually adoptedto control the disease in many countries~~ [7]. ~~In many countries r~~Regulation of the disease ~~depends~~ often relies on vaccination and culling of infected animals ~~in order~~ to minimize ~~chances~~ the risk of ~~for~~ spread~~of~~ the disease to consumers and people ~~that are associated with~~ involved in regular animals~~s~~farming activities [8]. In India, the main control programme~~of~~ for bovine brucellosis is based on ~~proper and~~ timely vaccination of young female cattle ~~of (~~4-8 months old)~~age group~~ and isolation of positive reactors.

The Milk ring test (MRT) first described by Fleischhauer in 1937 [9], is ~~one of the~~ an important serological tests used ~~in detection of~~ to detect brucellosis particularly at the herd level. The test can detect IgM and IgA antibodies bound to fat globules. It is widely accepted

Comment [D4]: Could you provide more details on how Brucella species are adapted to their preferred hosts? Are there specific factors or mechanisms that make certain Brucella species more pathogenic to particular animals?
For example, the species-specific adaptation is partly due to differences in the receptors and immune responses of the host species, which Brucella species exploit for survival and reproduction.

due to its~~It has a wide acceptability as it is~~ cost-effective, eas~~yc~~to~~ of~~ performance, and ~~can~~ ability to cover a large population in a short time [10].MRT can sometimes produce false positives despite its widespread use, which needs confirmatory testing through more specific assays like RBPT and iELISA[28, 29].

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~~ this study ~~was~~ aimed to determine seroprevalence of bovine brucellosis ~~by~~ using RBPT and iELISA tests, as well as ~~detection of~~ to detect infection ~~by through the MRT Milk Ring Test~~ in individual milked cattle.Understanding the prevalence and risk factors of brucellosis in this region is criticalto develop targeted intervention strategies for controlling the disease [30].

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~~ across different districts of Meghalaya~~ State~~, India between January 2015 and December 2018. The serum samples ~~from different Districts of Meghalaya~~ were collected by the Disease Investigation team, A.H & Veterinary Department. Meghalaya, Shillong~~,~~. The samples werebased on representative sampling with reference to age, sex, production and also ~~by~~ through a purposive sample approach based on farmer's requests. The samples were collected from Holstein Friesian, Jersey and various crossbreeds. Approximately 7 ml of blood ~~sample~~ was collected from the jugular vein of each animal using vacutainers (Becton Dickson, USA). The ~~S~~samples were properly labelled and all the clear serum samples were stored at −20°C until tested. Herd and animal level data were recorded ~~comprising~~ including ~~of~~ age group, sex, farm size, and history of abortion or repeat breeding. Of the 3193 animals sampled, 2425 were females (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 e to~~ were from cattle ~~of more~~ older than 8 years of age. ~~Again, out of 3193 animals,~~Additionally, 1420 (44.47%) of the samples were from organized farms (including ~~G~~government run farms) ~~maintaining~~ with more than 20 lactating dairy cattle, while ~~and~~ 1773 (55.52%) were from private holdings~~ maintaining~~ with fewer than 20 cattle ~~mainly less than 20 nos.~~

2.2. Serological tests

Comment [D5]: Recommending an additional statement for this section along with references.

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Comment [D6]: Recommending an additional statement for this section along with reference.

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Comment [D7]: Were there any specific protocols followed during the collection, labeling, and storage of serum samples to ensure their integrity?
For example, information on handling procedures and any quality control measures like providing details on protocols for ensuring sample integrity during collection, transportation, and storage would be useful.

All the serum samples were used to evaluate ~~the~~ disease prevalence ~~by~~ using serological tests~~, viz.,~~ namely theRose Bengal Plate test (RBPT), and the indirect Enzyme Linked Immunosorbent Assay (iELISA). The 3193 serum samples ~~(n=3193)~~ were analysed by ~~Rose Bengal plate test (~~RBPT~~)~~ according to standard protocol [11]. A known positive and negative serum ~~were~~ was included in each day's tests. The test was read by examining for agglutination in a good light, ~~and is~~ best observed~~facilitated~~ as ~~when~~ the mixture ~~is observed as it~~ flows ~~away~~ on the slide. Results were recorded ~~The test was read~~ as positive (any degree of agglutination) ~~and~~ or 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~~ iELISA~~)~~was performed on the~~sampled~~ serum samples using a commercial kit(IDEXX Brucellosis Serum X2 Ab Test, USA). The test ~~was~~ followed the manufacturer's ~~performed 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~~ were 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). According to manufacturer, a ~~A~~ positive result was defined ~~by themanufacturer~~ as an S/P ratio of ≥80%, and negativeresults was for an S/P ratio of <80%

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

The ~~milk ring test~~ MRT is based on agglutination of antibodies secreted into the milk. ~~This test allows~~ allowing 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~~ and is classified as surveillance or monitoring test [12]. The ~~milk ring test~~ MRT is also the most practical method for locating infected dairy animals. In ~~the~~ this 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

Comment [D8]: What are the sensitivity and specificity of the Rose Bengal Plate Test (RBPT) and Indirect Enzyme-Linked Immunosorbent Assay (iELISA) used in this study?

Comment [D9]: What statistical methods were used to analyze the serological and milk ring test results, and how were the results interpreted?
For example, comparisons between different groups, sex and age, etc.

tube. The milk (antigen) mixtures were incubated at 37°C for 1 hour, together along 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 appeared normal.

3. RESULTS AND DISCUSSION

Out of 3193 serum samples, 298 (9.33%)samplewere 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 iELISAtests. Details of the prevalence rateThe year-wise prevalence rate is depicted in Fig. 1. Due to the Aabsence of gold standard methodslike bacterialisolation of the bacteria and polymerase chain reaction technique,the prevalence calculation for prevalence of the disease is based on samples that tested test conducted by screening of paired samples which are positive by iELISA test. Hence, the overall prevalence of bovine brucellosis was recordedto be at 9.89% (316/3193). In comparison, there were many other several researchers have who reported lower prevalence rates.of bovine brucella antibodies of 2.46% in Southern Ethiopia For instance, in SouthernEthiopia, a prevalence of 2.46% was reported [13],then in aAnother study from Peninsular Malaysia [6] reported Brucella antibodies detected in 2.5% of sampled cattle[6] and[14] in Central Ethopia, a reported only 0.7% prevalence was reportedof brucellosis in cattle of Central Ethiopia during 2013-14[14]. In India, antibodies to Brucella wereas reported by [15] antibodies to brucella were reported to be have a positivity rate of 5.22% positivity by RBPT and 6.03% by iELISA[15]. In a Astudy in Meghalayaby [16] reported aseroprevalence of brucellosis in cattle of Meghalaya as at 5.91% by RBPT and 11.29% by ELISA, particularly from inneighbouringborder areas with Assam State[16].

Comment [D10]: How was the prevalence of bovine brucellosis calculated based on the serological test results and the absence of gold standard methods?Were statistical analyses conducted to compare the performance of RBPT and iELISA in this study?

Comment [D11]: What factors might explain the differences in prevalence rates reported in various studies across different regions?

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 The disparityin prevalence of the disease prevalence reported by different studies which may be due attributed to various extrinsic factors such as the type of surveillance activities, farm management systems including cattle-rearing practices, and finally the level of stringency regarding disease-control measures adapted in different countries.

By comparing both the the two commonly used serological tests, i.e., Rose Bengal Plate test (RBPT)and indirect iELISA, it shows that RBPT could also can detect accountable number of sero-positive samples as similar to iELISA. Even though its limitation and lack of specificity in detecting Brucella antibodies, RBPTit is not a specific test to detect brucella

antibodies and has several limitations, is still widely used forpreliminary screening for brucellosis in many countriesis still usually done by RBPT[19]. TheHhigh sensitivity of indirect iELISA is detected in recovered or vaccinated animals due to the persistence of IgG antibody for longer period. Hence, seroprevalence detected by iELISAcould may reflect either past or present exposure to *Brucella* organisms. However, since no brucella vaccination has not been done in was administrated to the sampled animals of on different farms in Meghalaya state during the study period, the presence of vaccinal antibody is can be ruled out. Molecular detection by PCR using serum and blood DNA may could be used to further validated the results.

In the present this study, important risk factors associated to bovine brucellosis were also analysed and results were depicted in Table 1. The sex wise prevalence by sex showed that prevalent of brucellosis is higher in females, with a rate of12.32% (299/2425) compared to males which had a prevalence of (2.21%; 17/768).In the present investigation, there is Thislow prevalence of brucellosis in male cattle which agreed aligns with previous findingsby of other investigators [14,20]. TheSspread 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 Additionally, it was alsoextensively reported by worker [21] who clearly concludedthat the sex of the susceptible animal species is one of the many risk factorsaffecting the susceptibility of cattle to *Brucella abortus* infection.

A Hhigher prevalence was detected from organized farms maintaining higher a larger number of lactating animals (13.09%; 186/1420) than compared to smaller private holdings (9.02%; 160/1773). In the case of bovine brucellosis, the greater chances of spreading of infection have been is found especially in organized herds than in marginal herds [22].whereas iIn 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 from the farmer himselfare the factors which attribute contribute to thereduced spread of infection. As per age category,the prevalence of brucellosis is indicated that it was higher in those cattle whose agedranged from 1to 4 years old compared to the rest of the other age groups (Fig. 2). According to [21] susceptibility of animals to disease is in factmore commonly associated with sexual maturityof the host. The Hhigher percentage of prevalence in the 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

Comment [D12]: How were the identified risk factors (e.g., sex, farm size, age, history of reproductive issues) quantified in their impact on brucellosis prevalence?
For example, this sectiondescribes associations between risk factors and brucellosis prevalence but does not quantify their impact. Details on how these risk factors were measured and analyzed to assess their influence on prevalence rates would provide a clearer understanding of their significance.

Comment [D13]: This sentence is most likely discussed on the factors that could reduce the spread of infection. Do rephrase the sentence.

Comment [D14]: "Prevailed" typically means to gain ascendancy or to become dominant, which seems less fitting in this context. Could you please clarify your choice of wording here and explain if there is a specific nuance you intended to convey with "prevailed"?

was ~~much~~ closely associated with their history of abortion ~~on those~~ in the examinedanimals, ~~in~~ which ~~infected animals~~ may act as carriers of the infection. Representative individual dairy cattle~~'s~~ milk sample~~ds~~during collected between 2015-2018 ~~when~~ and tested by ~~Milk ring test (~~MRT~~)~~ revealed a high positivity rate of 17.29% (155/896) regardless of the animals' age groups,~~of animals~~as depicted in Fig.3.~~The~~ This finding ~~comparatively~~ corelate~~d~~s with the research of [23] who detected 18.35% positivity ~~against~~ for Brucella and [24] in Iran, who detected a 14% positive reaction for *B. abortus* using MRT ~~and taking many~~ across various age groups ~~into consideration.~~ Another researcher [25] in ~~his study~~ observed that 12.5% of animals were positive for brucellosis by MRT. While T~~t~~he MRT is preferred ~~due to its~~ for its simplicity ~~to detect~~ in detecting *B. abortus* infection, ~~however, the test~~ it has also been reported ~~for~~ ,to produce a number of false positive results~~of the samples~~ [26].

Despite of various preventive and control measures being followed in India, there ~~is still~~ remains a high potential for the transmission and spread of *Brucella abortus* due to its widespread prevalence [27]. Timely confirmatory laboratory testing of ~~the~~ animals alongwith 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~~ using recommended disinfectants. Careful selection of animals before purchase particularly from ~~farms free of brucella infection, then~~ brucella-free farm, along with pre-purchase tests and quarantine ~~needs to~~ measures, should be judiciously followed to keep the animals free of brucellosis.Current studies highlight the importance of surveillance and biosecurity measures in controlling brucellosis in cattle populations. For example, [31] emphasized the need for improved vaccination strategies and diagnostic capabilities in endemic regions to reduce the spread of the disease. Furthermore, the utilization of molecular diagnostics, such as conventional or real-time PCR, has more accuracy in identifying *Brucella* spp. in various samples, providing a more precise method for disease management [32]. These findings suggest that integrating traditional serological methods with molecular techniques, along with biosecurity protocols, could significantly decrease the burden of brucellosis in cattle populations.

Comment [D15]: Add the reference to explain on disinfection protocols, animal selection criteria and other preventive and control measure for Brucella.

Comment [D16]: Recommending an additional statement for this section along with references.

4. CONCLUSION

Comment [D17]: Emphasize the need for specific actions, such as "Implementing regular screening programs and vaccination campaigns.".

The study reveals that seroprevalence of bovine brucellosis (9.89%) is comparatively higherin the hilly state of Meghalaya ~~and~~ with 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 lead~~s~~ to the spread of the disease. Therefore, greater attention byall concerned stakeholders and various sector in the State is urgently required,particularly focusing on educational awareness programmes~~focusing~~ on preventive measuresto safe guard and prevent transmission risk of the infection to human population.

CONSENT

It is not applicable

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Comment [D18]: Suggest to use "Harvard Referencing" style for each reference. The format is not standardized.

Comment [D19]: Pls find the latest version reference. OIE known as WOAH.

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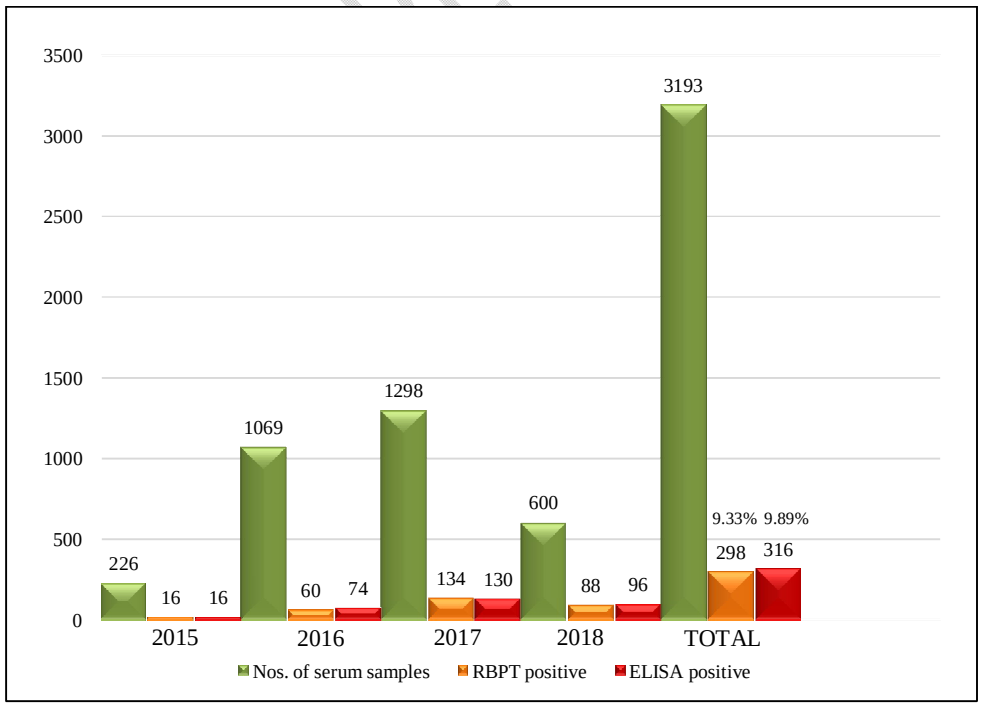

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Fig. 1.Overall results of RBPT and iELISA indicated year-wise of sampled cattle population

Comment [D20]: Revised Title:
Year-wise RBPT and iELISA Results for Sampled Cattle Population

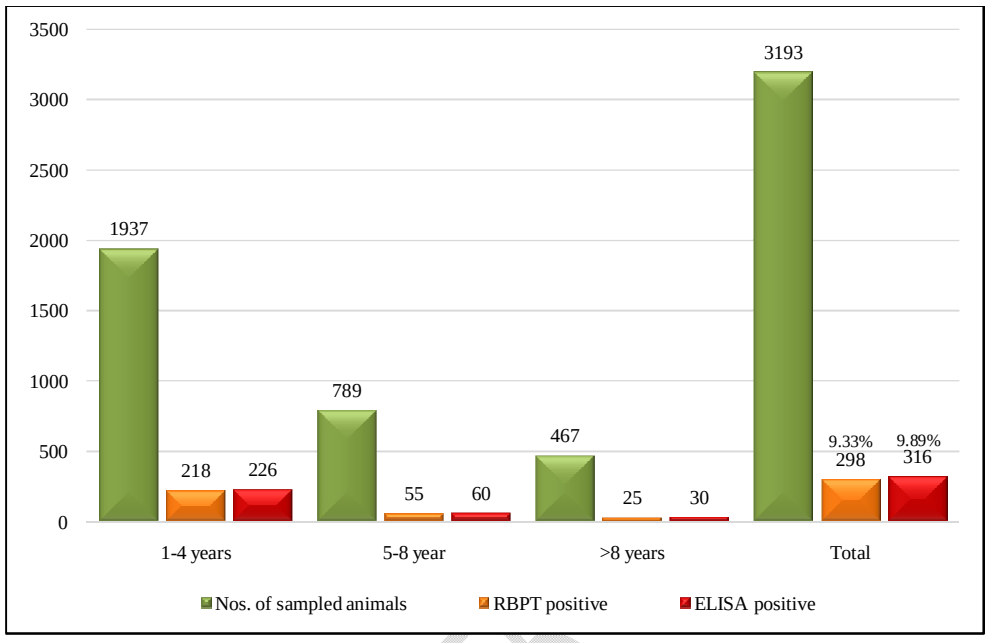

Fig. 2. Results of RBPT and iELISAaccording to age group of sampled cattle population

Comment [D21]: Revised Title:
RBPT and iELISA Results by Age Group in Sampled Cattle Population

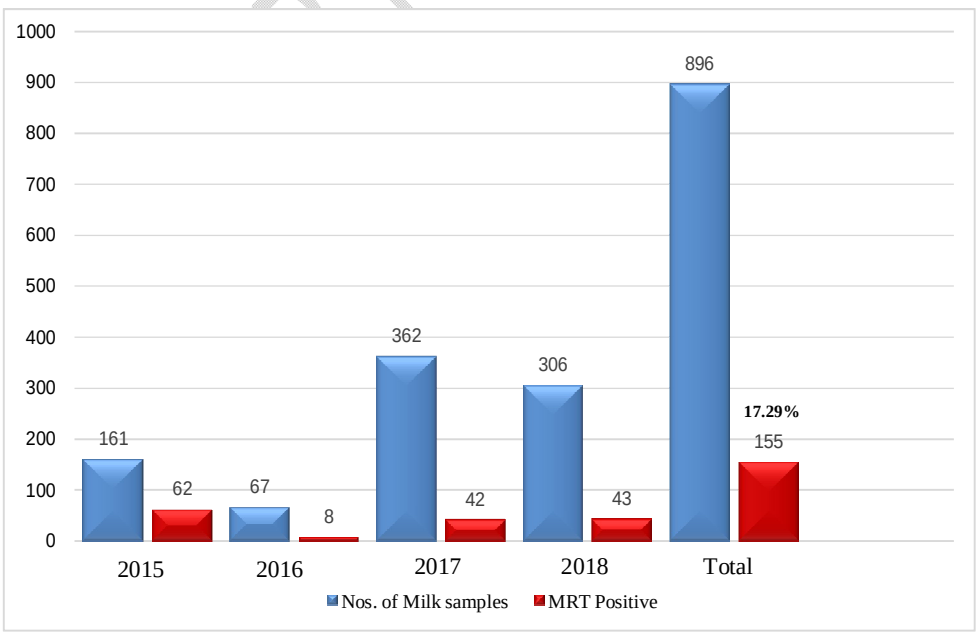

Fig.3. Results of the Milk Ring Test indicated year-wise along with total prevalence

Comment [D22]: Revised Title:
Year-wise Results of the Milk Ring Test with Total Prevalence

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

Comment [D23]: Revised Title:
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%)