Original Research Article

Assessing the Seroprevalence and Risk Factors of Bovine Brucellosis in Meghalaya: North-East 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 serological tests such as Rose Bengal Plate Test (RBPT), Indirect ELISA (iELISA) and Milk Ring Test (MRT).

Materials and Methods: Serum samples were collected from 3193 cattle aged 1 year to 12 years and screened for Brucella antibodies using Rose Bengal Plate Test (RBPT) and Indirect ELISA (iELISA). The samples were collected from both male and female cattle reared in organized farms and smaller private holdings. Individual milk samples (n=896) from representative dairy cattle were screened for brucellosis using the Milk Ring Test (MRT).

Results: TheiELISA revealed a serum positivity rate of 9.89% while the RBPT detected a positive rate of 9.33%. The prevalence rate was higher in females (12.32%) compared to males (2.21%). Cattle aged1-4showed higher susceptibility (11.66%)than other age groups. Higher prevalencewere detected from organized farms maintaining high number of animals (13.09%) compared to smaller private holdings (9.02%). Cattle with a history of abortion and related clinical symptoms also had a higher and significant association with sero-positivity. The MRT indicated a high positivity of 17.29%among sampleddairy cattle.

Conclusion: Brucellosis is prevalentin the hilly state of Meghalaya, India. This study insight into the prevalence of bovine brucellosis in the state highlighting potential risk factors for transmission to human.The high prevalence of brucellosis highlights the urgent need for enhanced surveillance and control measures in Meghalaya such as implementing targeted vaccination programs of all eligible female calves and intense information education campaign (IEC) to all the farmers and general public which could significantly reduce the disease's impact.

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

1. INTRODUCTION

Meghalaya state located in the North East Region of India relies onagriculture and livestock farming for its livelihood and economy. Amongst the various diseases affecting bovine species, brucellosis is a significant health problem in many countries. While the disease is prevalent globally, but there are countries such as Canada, Japan, Australia and New

Zealand, cases of *Brucella abortus* and *Brucella melitensis*have never been reported [1]. The disease is endemic in many states and regions of the India. In cattle, the disease is mainly caused by *Brucella abortus* and is associated with widespread contagious reproductive disease of dairy animals. In India it is highly prevalent amongthebovine 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* [3]. *Brucella*organism is coccobacillary shaped bacteria, Gram-negative, facultative, intracellular bacteria,comprising of many different species based on biochemical reactions and host affinity.

Currently, there are ten spp. described in the genus Brucella. Each species may infectvarious animal hosts, but 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*for dogs, *B. microti*infect rodents-(Microtusarvalis), *B. neotomae*for rodents- (Neotoma lepida), *B. Pinnipedialis*for pinnipeds, *B. ceti*for cetaceans,*Brucella inopinata*which is first isolated from humans, does not yet have a known preferential host. The underlying mechanisms of intracellular survival, including the host determinants remain relatively unknown [4,5]. In young animals and non-pregnant females, symptoms of brucellosis are usually not noticeable. However, in pregnant adult females, symptoms of *B. abortus* and *B. melitensis* includes placentitisoften resulting in abortion between the fifth and ninth month of pregnancy. Adult male cattle may develop orchitis and/or epididymitis. In both males and females, may experiences infertility due to brucellosis.In some tropical countries, hygromas particularly of leg joints are a common manifestation of disease[1,6]. Additionally, the disease can cause chronic arthritis, reducing the productive lifespan of infected cattle [7].

To control the disease, many countries adopt a test and slaughter policy for infected animals, with proper disposal following confirmatory diagnostic tests[8]. Regulation of the disease often relies on vaccination and culling of infected animals to minimize the risk ofspreadingthe disease to consumers and people involved in regular animal farming activities [9]. In India, the main control programmefor bovine brucellosis is based on timely vaccination of young female cattle (4-8 months old) and isolation of positive reactors.

The Milk ring test (MRT) first described by Fleischhauer in 1937 [7], is animportant serological testto detect of brucellosis particularly at the herd level. The test can detect IgM and IgA antibodies bound to fat globules. It iswidely accepted due to its cost effective, ease of performance and ability to cover a large population in a short time [10]. However, MRT can sometimes produce false positives commonly in colostrum or milk at the end of the lactation period and milk from cows suffering from hormonal disorder or mastitis, which therefore needs confirmatory testing through more specific assays like RBPT and iELISA [11, 12].

Understanding the prevalence and risk factors of brucellosis in a region is critical to develop targeted intervention strategies for controlling the disease [13]. Information on the status of bovine brucellosis is limited in North East region of India particularly in a hilly and tribal state of Meghalaya. Therefore, thisstudy aimed to determine seroprevalence of bovine brucellosis usingRBPT and iELISA tests, as well as to detectinfection through the MRT in individual milked cattle.

2. MATERIALS AND METHODS

2.1. Collection of Samples

A total of 3193 serum samples were collected from cattle of various organized farms (n=28) and private holdings (n=96) across different districts of Meghalaya, Indiabetween January 2015 and December 2018. The serum samples were collected by the Disease Investigation team, A.H & Veterinary Department. Meghalaya, Shillong. The samples were based on representative sampling with reference to age, sex, production and also through a purposive sample approach based on farmer's requests. The samples were collected from Holstein Friesian, Jersey and various cross breeds. Approximately 7 ml of blood was collected from the jugular vein of each animal using individual sterile syringes and commercially available serum separator vacutainers (Becton Dickson, USA). Samples were properly labelled, maintaining a cold chain at 4°C during transportation till serum separation process in the laboratory and all the clear serum samples were stored at −20°C until tested. Herd and animal level data were recorded including 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 males. 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 were from cattle older than 8 years of age. Additionally, 1420 (44.47%) of the samples were from organized farms (including government run farms) with more than 20 lactating dairy cattle, while 1773 (55.52%) were from private holdings with fewer than 20 cattle.

2.2. Serological tests

All the serum samples were used to evaluate disease prevalence using serological tests, namely theRose Bengal Plate test (RBPT) and the indirect Enzyme Linked Immunosorbent Assay (iELISA). According to [14], sensitivity and specificity were 96.8% and 96.3% respectively for iELISA, whereas estimates of RBPT were 89.6% and 84.5% respectively. The 3193 serum samples were analysed by RBPT according to standard protocol [15]. A known positive and negative serum was included in each day's tests. Briefly, 30µl of serum sample

was mixed thoroughly using a clean tip for each sample with 30µl of Rose Bengal antigen on the clean slide. The mixture was mixed and agitated softly for 4 min. The test was read by examining for agglutination in a good light, best observed as the mixture flows on the slide. Results were recorded as positive (any degree of agglutination) or negative (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 iELISAwas performed on the serum samples using a commercial kit (IDEXX Brucellosis Serum X2 Ab Test, USA). The test followed the manufacturer's recommendation. Colourdevelopment in the form of the optical density (OD) was read at 450 nm using an ELISA microplate reader (Infinite F50, Tecan, Austria). Results were expressed as the ratio of the sample (OD) minus the mean kit negative control OD to the mean kit positive control OD minus the mean kit negative control OD (S/P ratio). According to manufacturer, a positive result was defined as an S/P ratio of ≥80%, and negative results was for an S/P ratio of <80%.

A total of 82 sampling units (cattle herds) from different farms in the state were sampled, totaling 896 individual lactating cows of various age groups. Approximately 10ml milk pooled from 4 quarters was collected and properly labelled. Milk sampleswere kept refrigerated at 4°C overnight prior to examination by Milk Ring Test (MRT) [15]. The milk samples were mixed well to ensure an even distribution of the milk cream.

The MRT is based on agglutination of antibodies secreted into the milkallowing 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 and is classified as surveillance or monitoring test [16]. The MRT is also the most practical method for locating infected dairy animals. In 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 in a test tube. The milk-antigen mixtures were incubated at 37°C for 1 hour, 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 layerand the cream layer appeared normal.

3. RESULTS AND DISCUSSION

Out of 3193 serum samples, 298 (9.33%) were detected positive by RBPT test, whereas 316 (9.89%) samples were positive by iELISA. The year-wise prevalence rate is depicted in Fig. 1. Indirect ELISA is a confirmatory diagnostic test for bovine brucellosis; hence, the

overall sero-prevalence of bovine brucellosis was recorded at 9.89% based on serum samples that tested positive by iELISA. In comparison, severalresearchers have reported lower prevalence rate. For instance, in Southern Ethiopia, a prevalence of 2.46% was reported[17]. Another study from Peninsular Malaysia reported Brucella antibodies in 2.5% of sampled cattle [6] andin Central Ethiopia 0.7% prevalence was reported during 2013-14 [18]. In India, antibodies to Brucella were reported to have a positivity rate of 5.22% by RBPT and 6.03% by iELISA. Astudy in Meghalaya reported a seroprevalence in cattle at 5.91% by RBPT and 11.29% by ELISA, particularly inneighbouringborder areas with Assam State [20].The seroprevalence of 9.89% in the present study is lower compared to findings of other countries such as Egypt (11%) and Nigeria (19.7%) [21,22]. The disparityin disease prevalence report by different studies indicate the variability of brucellosis infection which may be attributed to various extrinsic factors such as the type of surveillance activities, farm management and production systems including cattle-rearing practices, and the level of stringency regarding disease-control measures adapted in different countries; infection status of the sampled animals and number of samples collected during the study period.

By comparing the two commonly used serological tests, i.e., RBPT and iELISA, it shows that RBPT canalso detect accountable number of sero-positive samples similar toiELISA. Even thoughits limitation and lack of specificity in detecting Brucella antibodies, RBPT is still widely used forpreliminary screening for brucellosis in many countries[23]. The high sensitivity of iELISA is detected in recovered or vaccinated animals due to the persistence of IgG antibody for longer period. Hence, seroprevalence detected by iELISAmay reflect either past or present exposure to *Brucella* organisms. However, since nobrucella vaccination was administratedto the sampled animals on different farms in Meghalaya during the study period, the presence of vaccinal antibody can be ruled out. Molecular detection by polymerase chain reaction (PCR) using serum and blood DNA could be to further validate the results.

In this study, important risk factors associated to bovine brucellosis were also analysed and results were depicted in Table 1. The prevalence by sex showed that brucellosis is higher in females with a rate of 12.32% (299/2425) compared to maleswhich had a prevalence of 2.21%; 17/768. This low prevalence of brucellosis in male cattle aligns with previous finding by other investigators[18,24]. The 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[18,19] reported that 1% and 6.63% female cattle were found to be sero-positive. Additionally, it was extensively reported by [25] that the sex of the susceptible animal species is one of the many risk factorsaffecting the susceptibility of cattle to *Brucella abortus* infection.

A higher prevalence was detected from organized farms maintaining a larger number of lactating animals (13.09%; 186/1420) compared to smaller private holdings (9.02%; 160/1773). In the case of bovine brucellosis, the greater chances of spreading of infection is found especially in organized herds than in marginal herds [26]. In small farms various factors like sufficient unit floor space for each animal; stall feeding that minimizes contact with other infected animals and more personnel attention to the animals from the farmer himselfcontribute to the reduced spread of infection. As per age category, the prevalence of brucellosis was higher in those cattle whose age ranged from 1to 4 years compared to other age groups (Fig. 2). According to [25] susceptibility of animals to disease is in factmore commonly associated with sexual maturity. The higher 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 reported. Hence, the prevalence of brucellosis was closely associated with their history of abortion in the examined animals, which infected animals may act as carriers of the infection. Representative individual dairy cattle milk sample collected between 2015-2018 and tested by MRT revealed a high positivity rate of 17.29% (155/896) regardless of the animal' age groups,as depicted in Fig.3. This finding corelates with the research of [27] who detected 18.35% positivity for Brucella and [28] in Iran, who detected a 14% positive reaction for *B. abortus* using MRT across various age groups into consideration. Another researcher [29] observed that 12.5% of animals were positive for brucellosis by MRT. While the MRT is preferred for its simplicity in detecting*B. abortus* infection, however, it has also been reported to producea number of false positive results [30].

Despite of various preventive and control measures being followed in India, there remains a high potential for the transmission and spread of *Brucella abortus* due to its widespread prevalence [31]. Timely confirmatory laboratory testing of animalsalongwith 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 farmsusing recommended disinfectants. Careful selection of animals before purchase particularly from brucella-free farm, along withpre-purchase tests and quarantine measures, should bejudiciously followed to keep the animals free of brucellosis**[**32].Current studies highlighted the importance of surveillance and biosecurity measures in controlling brucellosis in cattle populations. For example, [33] 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 [34]. 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.

4. CONCLUSION

The study reveals that seroprevalence of bovine brucellosis (9.89%) is comparatively higherin the hilly state of Meghalaya with various potential risk factors 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 foci of infection that leads to the spread of the disease. Hence, greater attention byconcerned stakeholders in the State is urgently required,particularly focusing on mass vaccination programme of eligible animals, information educational campaign of general public and farmers on preventive measures to safe guard and prevent transmission risk of the infection to human population.

Consent

It is not applicable

Disclaimer (Artificial intelligence)

The authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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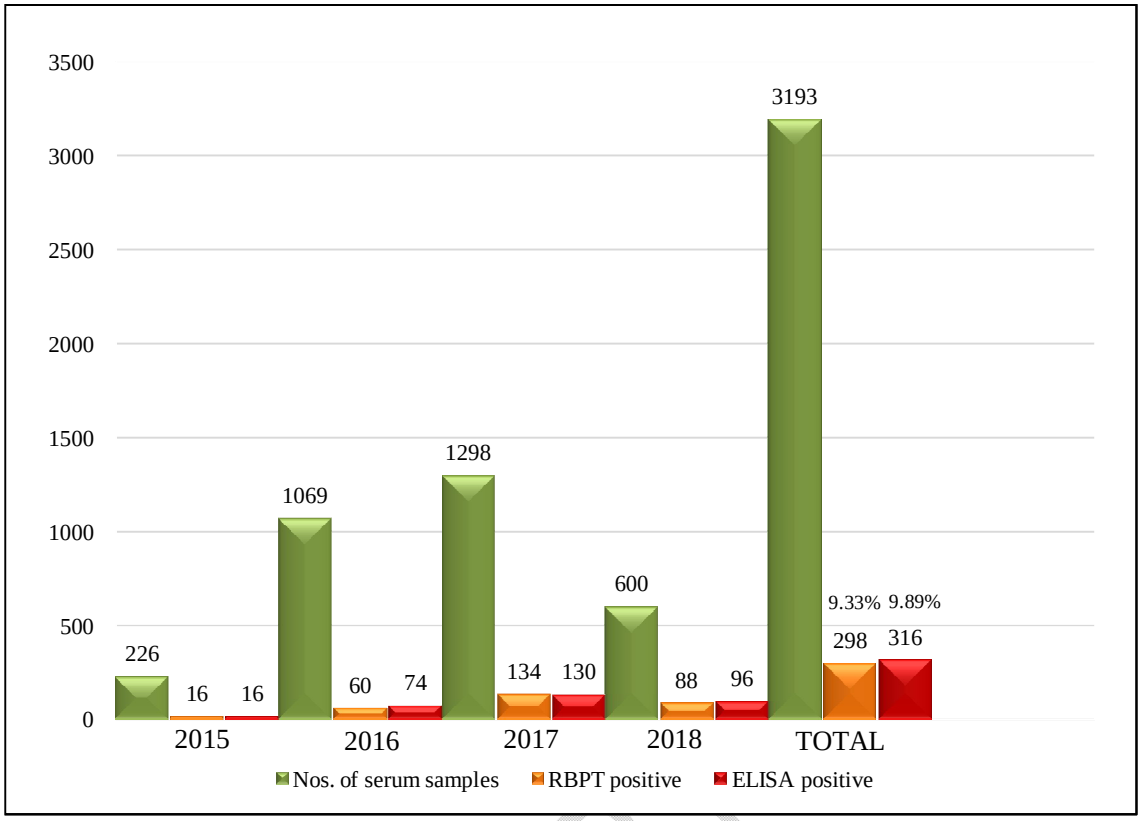

Fig. 1.Year-wise RBPT and iELISA Results for Sampled Cattle Population

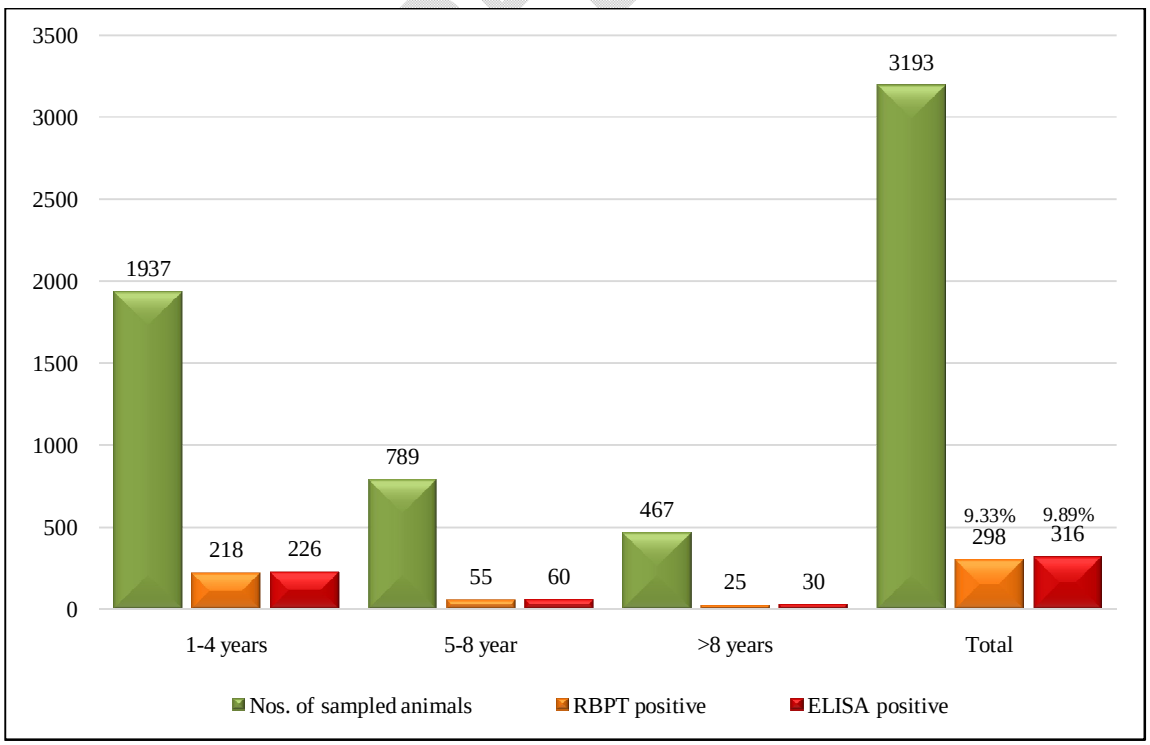

Fig. 2. RBPT and iELISA Results by Age Group in Sampled Cattle Population

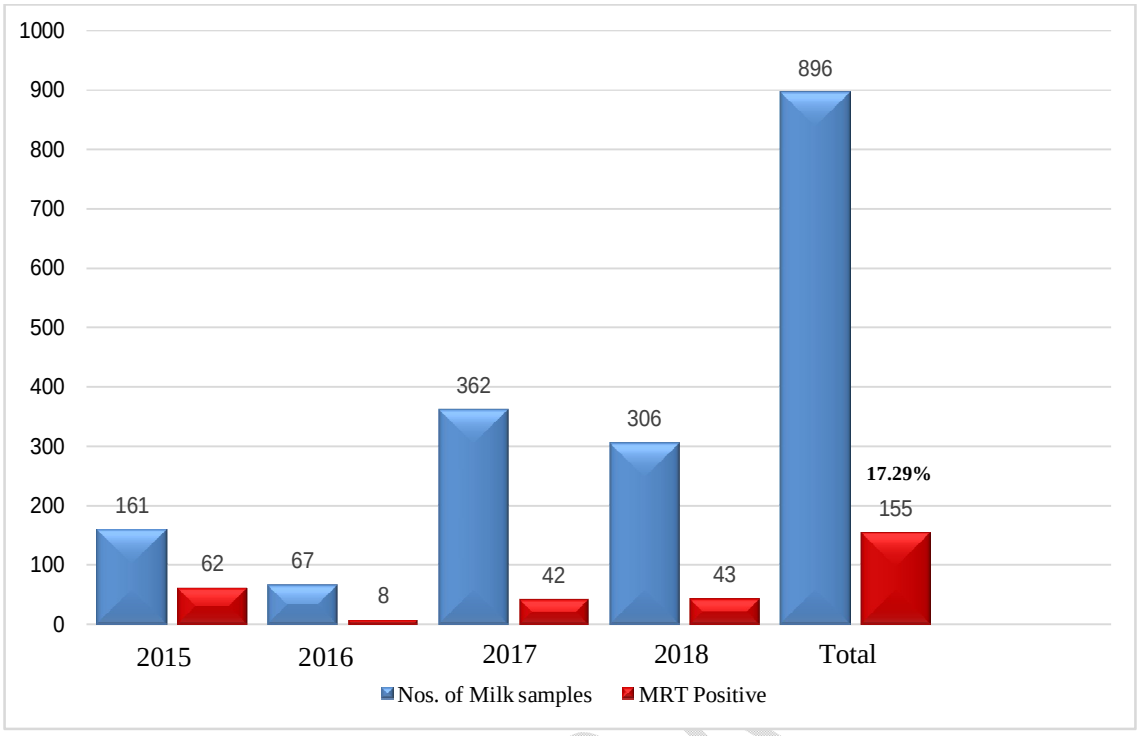

Fig.3. Year-wise Results of the Milk Ring Test with Total Prevalence

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%)