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Seroprevalence of caprine brucellosis in organised farms of Assam, India

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Abstract
Brucellosis is most contagious, often regionally neglected disease of small ruminants in the country and study aimed to estimate the sero-prevalence of caprine brucellosis in Assam state in the Indian subcontinent. A total of 1514 goat sera samples were collected from organised farms located in 19 out of 33 different districts of Assam and screened by RBPT and iELISA. In addition, 33 clinical samples including vaginal discharge (2), vaginal swab (29) and aborted foetus (02) from aborting goats were processed for isolation of *Brucella*. Out of 1514 goat sera samples screened, 26 were found to be positive by both the tests with an overall sero-positivity of 1.72%. Seroprevalence was highest in animals above 18 months (2.30%) of age and animals having history of abortion (12.99%). Isolation from 33 clinical samples yielded one *Brucella spp.* from one aborted foetus. The isolate was identified by bacteriological identification tests, *bcsp*31 genus and species specific AMOS and Bruce ladder PCRs as *Brucella abortus*.

Keywords: Seroprevalence, ruminant, I-ELISA, RBPT, brucellosis

1. Introduction
Goat rearing plays an important role in socioeconomic conditions of traditional Indian society and considered as a source of family income. Many factors that reduces reproductive performance, adversely affects the productivity of goats. Brucellosis is most widespread and economically devastating contagious disease of sexually matured animals caused by different *Brucella* spp. Caprine brucellosis mainly caused by *Brucella melitensis* which is widespread in India and a major cause of abortion in goats as well as brucellosis in humans [1-3]. Caprine brucellosis is also caused by *B. abortus* [4], it is endemic, highly transmissible to human and reported to be responsible for maximum number of human brucellosis cases [2, 5].
Different serological tests such as RBPT, SAT and iELISA are generally used for the detection of anti-*Brucella* antibodies [6]. The most incontrovertible method of diagnosis for brucellosis is by isolation and identification of the organism though it has some limitations like low sensitivity, health risk to laboratory personnel and not feasible to process large number of samples [7]. Recently, molecular diagnostic techniques are used which are safe and sensitive and confirmative tools for diagnosis of various diseases including brucellosis.
Brucellosis has been reported to be moderate to high in cattle [8, 9] and recently 2.15% prevalence in goats from Assam state [10] have been reported. In Assam, unlike bovine brucellosis, the farmers are not much aware about the occurrence of brucellosis in goat. As regular screening for the disease is not practiced and there is lack of access to timely diagnosis, spread and transmission of infection through distress sale of disease confirmed goat and use of infected buck for natural service can be ruled out. Trade and movement of improved breeds of infected goats from northern states to Assam might have contributed to the distribution of brucellosis among goat flocks in the state. National Control Programme of Brucellosis (NCPB) was undertaken in the state in the year 2012 with an objective to reduce the incidence of brucellosis in cattle through periodic surveillance and mass vaccination of female calves between 6-8 months in the areas where prevalence of the disease is high. The control programme though did not include goat and other livestock species may indirectly help in reducing the incidence of caprine brucellosis caused by *Brucella abortus*. The present study is restricted to Assam state which has 60,88,096 goat population [16] and is a continued effort to provide updates on brucellosis in goat flocks in wider area of Assam and isolate *Brucella* sp. associated with the disease in goat.

2. Materials and Methods

2.1 Sample source and collection

A total of 19 flocks located in wider geographical area of Assam were visited during October, 2012 to September, 2017. The farmers were interviewed on farm practices and animal details were recorded in a closed-ended pretested questionnaire after receiving the consent from the farm owners. A total of 1514 goat blood samples were collected from the jugular vein using vacutainers without EDTA (Becton Dickson, UK). The clotted blood in the tubes were centrifuged at 3000g for 20min to obtain clear serum and stored at -20 ^{0}C until used. Each sample was having data on age, sex and previous history of abortion.

2.2 Serological screening of serum samples

All the sera samples were tested by Rose bengal plate test (RBPT) and results rated as negative when agglutination was absent and 1+ to 3+ as positive, according to the strength of the agglutination observed within 1 to 3 min (Alton *et al.*, 1988). *B. abortus* S99 coloured antigen was procured from Institute of Animal Health and Veterinary Biologicals (IAH&VB), Hebbal, Bengaluru, India. Indirect Enzyme-Link Immunosorbent Assay (iELISA) was performed by using iELISA kit procured from ICAR-NIVEDI, Bengaluru, India as per recommended protocol.

2.3 Isolation and identification of *Brucella* organism from clinical samples

A total of 33 brucellosis suspected clinical samples [foetuses (2), vaginal discharge (2) and vaginal swabs (29)] were collected in *Brucella* selective broth (BBL *Brucella* Agar, BBL Becton) containing antibiotic supplements (nystatin, bacitracin, polymyxin-B, cycloheximide and nalidixic acid) processed in class II plus biosafety cabinet facility. Sample inoculated tubes were incubated with and without 10% CO_2 at 37 ^{0}C for 72 hours as per the methods described in OIE Terrestrial Manual [11] for isolation of the organism.

A loopful of broth culture from both sets were streaked onto *Brucella* selective agar containing supplements and incubated with and without 10% CO_2 at 37 ^{0}C until the appearance of growth or up to one week. Suspected colonies were purified twice, identified by Gram staining, their CO_2 requirement, biochemical characteristics viz., H_2S production, catalase, urease, oxidase tests and inhibition of growth by basic fuchsin and thionin dyes [12]. *Brucella* genus specific *bcsp*31 PCR was performed as per Baily *et al*., [13] and species specific AMOS [14] and Bruce ladder PCRs [15] PCRs were performed to identify the species.

2.4 Statistical analysis

Statistical analysis of the risk factors for brucellosis was done (Chi-square test) by SPSS Version 14 (Statistical Package for Social Science). The Chi-square value was obtained by using the formula

X^2 = $\sum (O - E)^2 / E$
X^2 = the test statistic
$\sum$ = the sum of
O = Observed frequencies
E = Expected frequencies

"P" value was generated by SPSS

3. Results

Out of 1514 goat sera samples screened, 26 were found to be positive by both the tests with an overall sero-positivity of 1.72%. Although there is variation in the sample size collected from different districts of Assam, highest sero-positivity to brucellosis was detected in goats of Udalguri district (15.00%) followed by Nalbari district (12.50%) (1). Sex-wise prevalence of brucellosis was higher in female (1.94%) than in male (1.06%) (Table 2). Statisticaliy no significant association was observed. (p>0.05). In relation to age, seroprevalence was highest in animals above 18 months (2.30%) of age but statistically insignificant (p>0.05). Prevalence of brucellosis was also found to be higher in goats with symptom or previous history of abortion (12.99%) than in animals with no clinical sign (Table 2). Statistically, there was a significant (p<0.01) association of reproductive disorders on sero-prevalence of brucellosis in goats.

Table 1: District wise results of brucellosis screening ingoatsby RBPT and IELISA

Sl no	District	No. of animals tested	No. of animals positive	Prevalence (%)
1	Kamrup-M	275	4	1.45
2	Kamrup-R	188	-	-
3	Nalbari	88	11	12.50
4	Barpeta	205	-	-
5	Dhubri	63	1	1.59
6	Baska	62	-	-
7	Morigaon	45	-	-
8	Sonitpur	15	1	6.66
9	KarbiAnglong	100	-	-
10	Lakhimpur	110	-	-
11	Cachar	35	1	2.86
12	Golaghat	22	-	-
13	Darrang	75	-	-
14	Chirang	15	-	-
15	Bongaigaon	40	3	7.50
16	Goalpara	28	-	-
17	Udalguri	20	3	15.00
18	Jorhat	98	2	2.04
19	Dibrugar	30	-	-
Total		1514	26	1.72

Table 2: Prevalence of brucellosis in goats in relation to sex, age and symptoms

Risk Factors		No. of animals	No. of Positives	Prevalence (%)	χ2	P-value*
Sex	Male	378	4	1.06	0.0471	0.8283
	Female	1136	22	1.94		
Clinical Signs	Abortion	77	10	12.99	44.2183	<.0001
	No Signs	1338	16	1.20		
Age Group	Below 9 months	361	5	1.39	1.8933	0.3811
	9-18 months	458	5	1.09		
	Above 18 months	695	16	2.30		

Only one *Brucella* isolate could be recovered from an aborted foetus of doe out of 33 clinical samples examined (Table 3). The isolate was Gram negative, oxidase and urease positive and confirmed as *Brucella* spp.by *bcsp*31 genus specific PCR which yielded a PCR amplified product of 223 bp (Fig1). The isolate was speciated as *B. abortus* by AMOS and Bruce ladder PCRs. The size of the amplicon produced by *B. abortus* isolate in AMOS PCR was 498 bp (Fig2) and five bands of 152, 450, 587, 794 and 1682 bp in size in Bruce ladder PCR (Fig3).

Table 3: Isolation of *Brucella* from clinical samples

Sl No.	Nature of samples	No of samples examined	No. of *Brucella* isolated
2	Vaginal discharges	02	Nil
3	Aborted foetuses	02	01
	Total	33	01

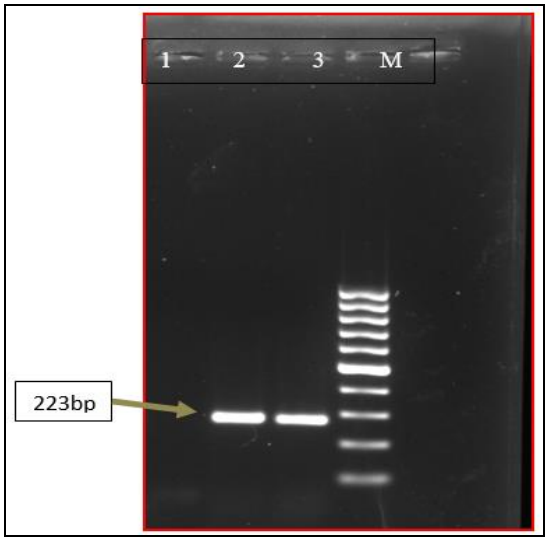

Fig 1: *Brucella* genus specific PCR Lane 1: negative control; lane 2: positive control (*B. abortus* S99); lane 3: *Brucella* culture DNA and Lane M: 100 bp ladder

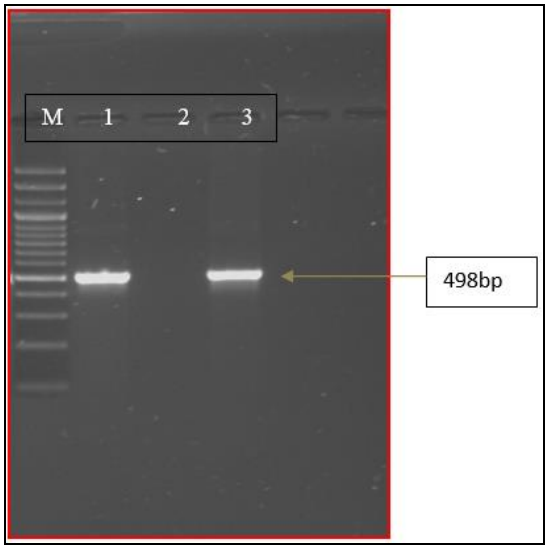

Fig 2: AMOS PCR for identification of *Brucella* species Lane 1: positive control (*B. abortus* S99); Lane 2: negative control; Lane 3: *Brucella* culture DNA and Lane M: 100 bp ladder

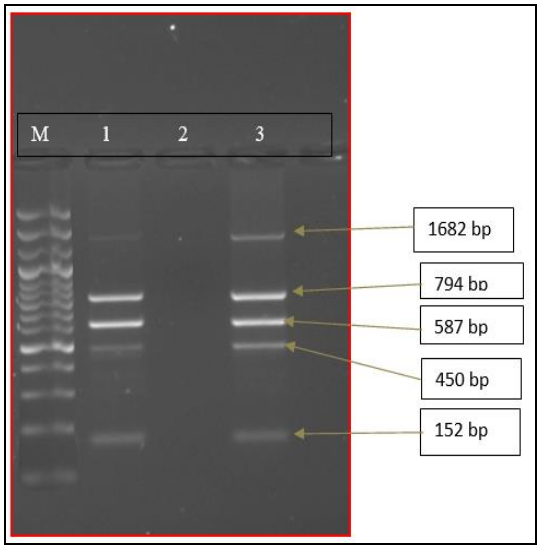

Fig 3: Bruce Ladder PCR for identification of *Brucella* species Lane 1: positive control (*B. abortus* S99); lane 2: negative control; lane3: *Brucella* culture DNA and Lane M: 100bp ladder

4. Discussion

Knowledge on spatiotemporal distribution of brucellosis is of paramount importance for prioritizing the geographical regions for vaccination and implementation of control strategies. The top five states of India (Bihar, Andhra Pradesh, Madhya Pradesh, Manipur and Rajasthan) are already prioritized for control of small ruminant brucellosis [3]. Overall brucellosis prevalence was found to be 1.72% among 1514 sera samples based on RBPT and iELISA test results taken together. Highest seropositivity to brucellosis was detected in goats of Udalguri (15.00%) and Nalbari districts (12.50%). The present finding is more or less in agreement with recent report of Gogoi *et al*. [10] who have recorded a slightly lower prevalence (1.42%) in goats of Assam. Elsewhere in India, seroprevalence of 5.7% from Kerala [17]; 2.20% [5], 9.95% [18], 5.15% [19] and 2.3% [3] from Karnataka; l4.55% and 10.62% in goats of Uttar Pradesh and [20,21]; 5.30% [22] and 5.78% [23] from Punjab state have been reported. From neighbouring Pakistan 9.80% [24] and bordering country Bangladesh seroprevalence of 9.53% [25]

have been reported. This variation in prevalence of brucellosis may be attributed to animal production system, susceptibility of different breeds of goat to stress conditions and infection, screening tests used and number of goats tested for the disease. Highest seroprevalence of brucellosis in goats in Udalguri district might be due to the fact that district wise population of goat was highest (9,84,337) in that district of Assam which allowed contact of healthy goats with infected animals in farms or goats reared under free grazing system. Serorevalence of brucellosis was higher in females (1.94%) than in males (1.06%) but statistically insignificant ($p>0.05$). This finding supports the observations made by Chandra *et al*. [26] and Rahman *et al*. [27]. In relation to age, seroprevalence of brucellosis was highest in goats of more than 18 months of age (2.30%) than other age groups and no significant association was observed ($p>0.05$). This observation is consistent with that of Ashenafi *et al*. [28] who have recorded a prevalence of 5.3% in adult Black Bengal goats. The high prevalence of brucellosis among older animals might be related to sexual maturity with advancing age with increased susceptibility [29]. Although susceptibility to the disease increases with age, it seems to be more commonly associated with sexual maturity than age [30]. Lower prevalence of brucellosis in young animals might also be attributed to passive immunization of kids through colostrums of the infected does. Prevalence of brucellosis was found to be higher in goats with history of abortion (12.99%) than in animals with no clinical signs and statistical significance ($p<0.01$) was observed. Similar observations were also made by Gogoi *et al*. [10] and also stated by Radostits *et al*. [30] that there is higher incidence of abortion in cattle in third trimester of pregnancy due to brucellosis. The study indicates that chances of abortion in goat increases due to brucellosis and higher incidence of abortion in the advance stage of pregnancy is due to the uterine environment conducive for multiplication of the bacteria which leads to placentitis and destruction of villi [31].

Only one *Brucella* isolate could be recovered from an aborted foetus of doe of 33 clinical samples examined. The isolate was Gram negative, oxidase and urease positive and confirmed as *Brucella* spp. by *bcsp*31 genus specific PCR which yielded a PCR amplified product of 223 bp. Ghodasara *et al*. [32] detected *Brucella* DNA by using three pairs of primers (B4/B5, F4/R2, JFP/JPR) yielding three different fragments of 223bp, 905bp and 193bp in size respectively and opined superiority of B4/B5 over other two pairs of primers. Roseliza *et al*. [33] substantiated usefulness of B4/B5 primers for identification of *Brucella* DNA. The isolate was speciated as *B. abortus* by AMOS and Bruce ladder PCRs. The size of the amplicon produced by *B. abortus* isolate in AMOS PCR was 498 bp and five bands of 152, 450, 587, 794 and 1682 bp in size in Bruce ladder PCR. Morales-Estrada *et al*., [34] and Roseliza *et al*. [33] have identified *B. abortus* from goat by Bruce ladder PCR. Similarly, Nagalingam *et al*. [35] have identified 47 field isolates of *Brucella* by AMOS and Bruce ladder PCRs (28 *B. abortus*, 14 *B. melitensis* and 5 *B. suis*). Isolation of *B. abortus* from goat raises the possibility of transmission from cattle due to traditional system of animal husbandry in Indian villages where cattle, sheep and goat are reared and kept together. Isolation of *B. abortus* from goat is also a public health concern as the organism may be transmitted to human through handling of infected goats.

The present study provides brucellosis seroprevalence from 19 districts of Assam state which is useful information in prioritizing the regions for carrying out further clinical surveillance. The age, sex and clinical history very well relates to brucellosis risk. Isolation of *B. abortus* from goat substantiates brucellosis transmission among different livestock species in mixed farming conditions existed in the state. Periodical evidence-based brucellosis studies from all the districts and at human- goat interface are needed to generate potential impact estimates and to suggest control strategies of demonstrated efficacy.

5. Conclusion

The present study provides estimate of brucellosis seroprevalence in goat from 19 districts of Assam state which formed the basis for carrying out further periodic state-wide surveillance to know the actual status of the disease and suggest effective control strategies for sustainable management of the disease.

6. Acknowledgement

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