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Prevalence of bovine brucellosis in Assam, India

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Abstract
The present work was conducted to investigate the prevalence of bovine brucellosis on the basis of serological test viz., MRT, RBPT, STAT, I-ELISA during the period from February, 2015 to May, 2016. The prevalence of brucellosis was recorded, 10.53%, 12.69%, 13.08% and 13.84% in bovine by MRT, RBPT, STAT and I-ELISA respectively. Sex-wise higher prevalence rate was recorded in female cattle (14.06%), Age-wise highest prevalence recorded in the age group of 3-7 year (15.09%), breed-wise higher prevalence in crossbred cattle (20.00%) and cattle from organized farm recorded higher prevalence (15.65%) than unorganized farm by I-ELISA. Prevalence rate on the basis of previous history or clinical symptoms recorded highest in case of aborted cattle (64.24%) followed by retention of placenta (47.13%) in cattle.

Keywords: Brucellosis, bovine, prevalence, zoonoses

1. Introduction
Bovine brucellosis is the most widespread and economically devastating contagious reproductive disease of different sexually matured animals [1]. Brucellosis is caused by a coccobaciliary, microaerophilic, Gram negative organism of genus *Brucella* [1]. Different species of *Brucella* are responsible for the reproductive disease of domestic animal. *Brucella abortus* is the dominant species in cattle [2]. Though bovine brucellosis mainly caused by *B. abortus* but other species of the genera viz., *B. melitensis*, *B. suis* may also cause the disease in cattle [1, 2]. Clinically, the disease is characterized by abortion, retained placenta, orchitis, epididymitis, rarely arthritis, hygroma, with excretion of organism in uterine discharge and in milk [3].
Brucellosis is the most common zoonoses in the world, accounting for annual occurrence of more than 500,000 cases [4]. Brucellosis is endemic in low socio-economic countries like India and the prevalent species causing human infection are *Brucella melitensis* and *Brucella abortus* [5]. Considering the damage done by the *Brucella* infection in animals in terms of decrease productivity that brings negative impact on the development of economy of the infected countries [6]. Losses in animal production primarily because of the decrease milk production by aborting dairy animals, weak offspring, weight loss, infertility and lameness [7]. It is also a major impediment for international trade of milk, meat and their products [8]. In India brucllosis alone has been estimated to cause an annual loss of Rupees 350 million in terms of food animals and man day of labour. The disease has been reported almost all part of the country with wide variation in different species of animals [9].
Occurrence of brucellosis had been reported from the North-eastern part of India time to time. Evidences of numbers of acute and chronic cases of brucellosis appear in cattle in Assam and its neighbouring states in investigation of the earlier workers [10, 11].
The diagnosis of brucellosis presents difficulties in spite of the fact that there is no other disease for which such a large numbers of tests are available as for brucellosis [12]. Though, diagnosis of brucellosis by bacteriological technique is the most reliable approach [13] and it provides incontrovertible diagnosis of *Brucella* infection, is a time consuming, tedious and always put the laboratory workers under great risk of infection. Apart from this, it has often been reported that recovery of *Brucella* from blood and milk culture is quite insensitive [14]. Many a times, isolation of *Brucella* from known positive cases is not possible [15]. Therefore recourse to serological techniques is taken for diagnosing the disease. The infection results in varied immune response in the host which necessitates the detection of antibodies to *Brucella.* The use of conventional assays such as the acidified antigen agglutination test like Rose Bengal Plate Test (RBPT), Milk Ring Test (MRT), Serum Tube Agglutination Test (STAT),

Indirect Enzyme Linked Immuno-sorbent Assay (I-ELISA) etc. widely used in various combinations for the diagnosis of brucellosis [16].

With consideration of the above facts the present study was carried out to investigate the prevalence of bovine brucellosis on the basis of serological test viz., MRT, RBPT, STAT, I-ELISA has been conducted.

2. Material and Methods

2.1 Sources and places of samples/materials

The present investigation was carried out during the period from February, 2015 to May, 2016. The samples were collected randomly from unvaccinated (*Brucella*) animals having history of abortion, repeat breeding, retention of placenta, mastitis, hygroma, as well as from apparently healthy animals of different age groups. The milk and serum samples has been collected from Lakhimpur, Dhemaji, Sonitpur, Darang, Marigaon, Golaghat, Kamrup Metro, Dhubri, Kokrajhar districts of Assam. The study protocol was approved by IAEC (Approval No. 770/ac/CPCSEA/FVSc/AAU/IAEC/15-16/351 Dated. 10.04.2015).

2.2 Prevalence based on different serological tests

The milk samples were screened for the presence of *Brucella* specific antibody by Milk Ring Test according to the method described by OIE. (2008) [17]. The antigen for the test was procured from the Institute of Animal Husbandry and Veterinary Biologicals, Hebbal, Bangalore (IAHVB). RBPT was performed as described by Alton *et al*. (1975) [18].The serum samples were screened for the presence of *Brucella* specific antibody by Rose Bengal Plate Test using *Brucella* Coloured Antigen (Institute of Animal Husbandry and Veterinary Biologicals, Hebbal, Bangalore). In the present research work, protein-G based indirect ELISA kit (ICAR NIVEDI, Yelahanka, Bengaluru, Karnataka) was used to detect the antibody against the *Brucella* infection in the serum samples collected from cattle according to their protocol. Similarly, STAT was performed using the procedure as described by Stemshorm *et al*. (1985) [19]. The collected data were subjected to preparation of master sheet in MS Excel. The empirical percentage of disease were estimated with 95% CI, using calculator for computing sample summery information available in JMP 10 of SAS 4.3 version.

3. Results and Discussion

3.1 Prevalence of brucellosis based on different tests

The present study showed that *Brucella* antibodies were present in the milk of cattle. Based on the screening test by MRT, the prevalence found in cattle ranged from 0.00% to 18.75% with an overall prevalence of 10.53% (CI:5.65%,15.65%) (Table 1). Similar observation was recorded by Barman *et al*. [20] recorded a prevalence of 54.7% in Assam which was higher than the present study. The present findings are in consistent with observation made by Kushwaha *et al*. [21] and Basit *et al*. [22] who have observed a prevalence rate ranged from 17% to 42.68% among the lactating animals. The higher prevalence recorded in the present study might be due to the pathogenesis of the disease, as in the chronic stage of the disease, organism is harboured intracellularly often localized in the supramammary lymph nodes and the udder. In this situation antibody titre may remain in the diagnostic threshold and some such animals may shed *Brucella* organism in their milk [23, 24]. Among the districts highest prevalence showed in Kamrup Metro (18.75%) followed by Kokrajhar (15.38%), Marigaon (13.33%) district. The highest prevalence showed in Kamrup Metro district might be due to large portion of the milk samples collected from Crossbred animals from organized farm.

The RBPT test revealed an overall prevalence rate of 12.69% which ranges from 0.0- 28.57% in case of cattle by RBPT (Table 2). The present findings corroborated with the findings of Chakravarty *et al*. [25], Barman *et al*. [20] and Chakraborty *et al*. [10] in Assam who have recorded prevalence rate of about 29.07% by RBPT. Similar reports were observed by Ghodasara *et al*. [26] and Varasada [27] ranges from 11.21% to 16.80% in central Gujarat. Mangi *et al*. [28], found prevalence of 25.00% by RBPT in Hyderabad district of Pakistan. The high prevalence of brucella reactive animals recorded in the present study, in spite of organized or domestic courtyard rearing might be due to the wide distribution of brucellosis in animals by introduction of new high yielding animals in to the farm without proper serological test [29]. On the contrary, some workers suggested that serological test have higher sensitivities, but their specificities are generally low [30]. Also serological tests can be non-specific due to cross reaction or high immunity reaction, depending on the subclinical or endemic prevalence of the disease [31].

In case of STAT the overall prevalence was found to be 13.08% with a range from 9.09% to 30.16% in case of cattle (Table 3). The results of present study was in agreement with the findings of Rahman *et al*. [32] and Boro *et al*. [33] in Assam and Maiti *et al*. [34] in Nagaland. A higher prevalence was recorded by Chakravarty *et al*. [25] and Barman *et al*. [20] in cattle having history of various reproductive diseases using STAT. Kushwaha *et al*. [21], Senthil *et al*. [35] and Mangi *et al*. [28] recorded higher prevalence from various part of India. This variation in prevalence might be due to variation in the managemental practices adopted, breed and outbreak condition of disease [36]. It was observed that all the animals found positive showed a STAT titre of 80 to 640 IU against plain antigen of *Brucella abortus*. This difference might be due to the variation of degree of infection. The appearance of antibody is related to many factors such as size and method of exposure, virulence of organism, stage of pregnancy and previous exposure. Antibody titre usually reach diagnostic level by four weeks after exposure during four to sixth month of gestation and at 10 weeks after exposure in non-pregnant or in the first trimester gestation [37]. The highest prevalence was found 30.16% in Kamrup Metro district followed by Sonitpur district 22.73%.

In I-ELISA an overall prevalence rate of 13.84% in cattle (Table 4). Similar findings were also observed by Jagapur *et al*. [38] who have observed a higher prevalence rate of 22.39% - 46.83% in different districts of Karnataka, Uttar Pradesh and Uttarakhand in case of cattle. In the present study highest prevalence was observed in Kamrup Metro (34.92%) followed by Sonitpur (18.18%). The present findings corroborated with the findings of several earlier workers [10, 39-41] who have recorded the prevalence rate ranged from 17.5% - 63.90% in lactating, pregnant Jersey crossbred cows from individual farms from Guwahati and nearby areas. The variation in prevalence might be due to variation in sample size, breeding method, animal procurement.

3.2 Evaluation of host factors associated with prevalence of bovine brucellosis

A high association between age and prevalence of brucellosis was recorded in the present study. Highest percentage of

Brucella positive reactors among cattle recorded in the age group of 3 to 7 years (16.98%) followed by more than 7 years (12.86%) of age group (Fig. 1), whereas lowest prevalence was recorded in the age group of less than 3 years (16.98%) by I-ELISA. The results of present study was in agreement with the earlier workers [25, 41] from Assam who have found highest sero-prevalence in the age group of 2-4 and under 7 years of age in different organized farms. According to Blood *et al*. [42], low percentage of brucella reactors in heifers might be due to less numbers of samples as well as insufficient exposer of their immune system to the organism. Young animals remain sero-negative until its first parturition. Chettri *et al*. [41] opined that older animals had more chances of exposer to bacteria and contacting the diseases and there was concurrent decrease in immunity and increase in stress in older age group. Susceptibility to brucellosis increases with age; it seems to be commonly associated with sexual maturity than age [7].

Sex-wise higher prevalence of brucellosis was found in female (15.38%) than in male (3.07%) (Fig. 2). The present findings are in consistent with observation made by the former worker Barman *et al*. [20] who have detected highest percentage (60.30%) of *Brucella* agglutinin in aborted cows than prevalence in case of breeding bulls. According to Basit *et al*. [22] the bulls may act as carrier of *Brucella* infection to other female animals through natural breeding practices. Disease can be transferred to all those animals which were mounted by infected bulls. On the other hand female animals are kept for longer in a particular herd and are stocked together compared to male animals which are individually housed, thereby increasing chances of exposure in females [6].

Higher percentage of *Brucella* positive reactors found in crossbred cattle (22.08%) than Local cattle breed (6.79%) (Fig.3). The present findings corroborated with the findings of Chettri *et al*. [41] who found highest prevalence in Holstein Friesian (21.95%). Yohannes *et al*. [43] reported high prevalence of brucellosis in cross breed animals 3.64% than indigenous animals 1.7% and opined as brucellosis can also be transferred through artificial insemination by using contaminated needles or poor management practices. Islam *et al*. [44] opined that crossbred cattle are more susceptible to stress conditions than local bred of cattle. In their view genetic difference especially in innate immune system may also be a possible reason for variation in sero-prevalence between the two breeds.

On the basis of husbandry practice animals from organized farms were showing a higher prevalence (17.83%) than animals from unorganized farms (10.69%) in case of cattle. The results of present study was in agreement with the earlier worker Jagapur *et al*. [38] who have recorded higher prevalence of 45.80% in animals from organized farm than unorganized farms in three different states of India. According to Jagapur *et al*. [38] the prevalence of brucellosis in unorganized farms was noticed less in comparison to organized farms and this might be due to break in chain of disease spread among discrete population. As opined by Hellman *et al*. [45] the organized dairy farms tend to have more animals per unit area. Large herd size enhances the exposer potential through increased contact between infected and non-infected animals, thereby prompting transmission of the organism.

The highest percentage of *Brucella* positive reactors found among the animals having previous history of abortion (64.21%) followed by retention of placenta (47.13%) in case of cattle (Fig. 4). Similar findings were also reported by Barman *et al*. [20] where they have recorded 53.8% of *Brucella* positive reactors in animals aborted between 7 and 8 months of age of pregnancy and retention of placenta was common sequel. Barman *et al*. [20] opined that among the aborted animals, non-pregnant infected animals develop repeat breeding condition. Roberts [46] stated that, multiplication of the *Brucella* organism is enhanced with the increased concentration of erythritol in gravid uterus leads to increase percentage of abortion. Placentitis and adhesion usually accompanied with sever endometritis leading to retention of placenta after birth in aborted animals due to brucellosis [47].

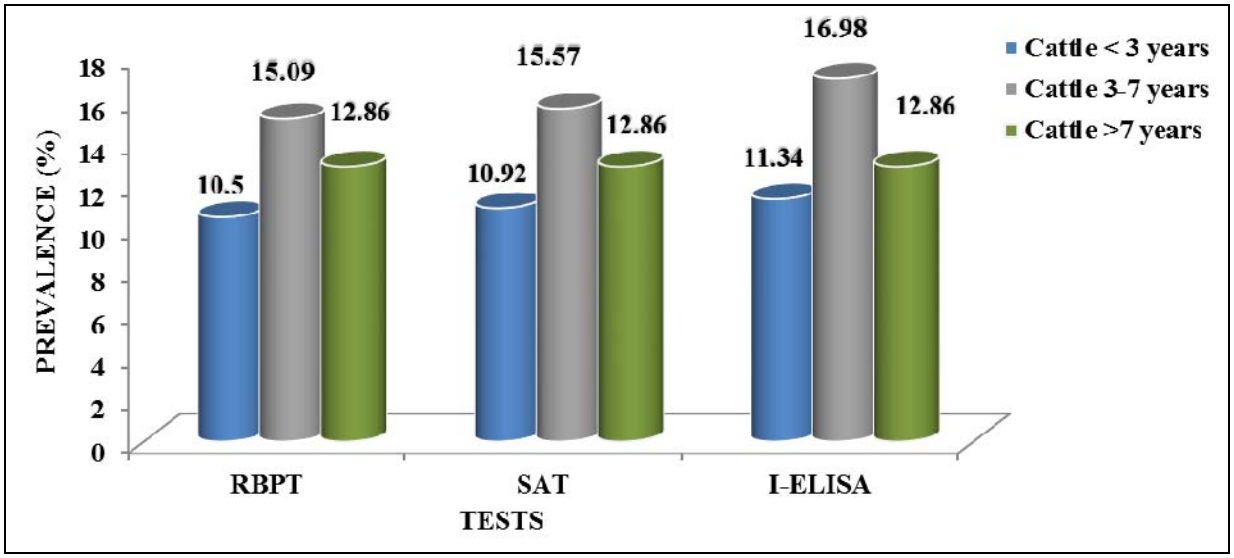

Fig 1: Graphical Representation of Age Wise Prevalence of Brucellosis in Cattle

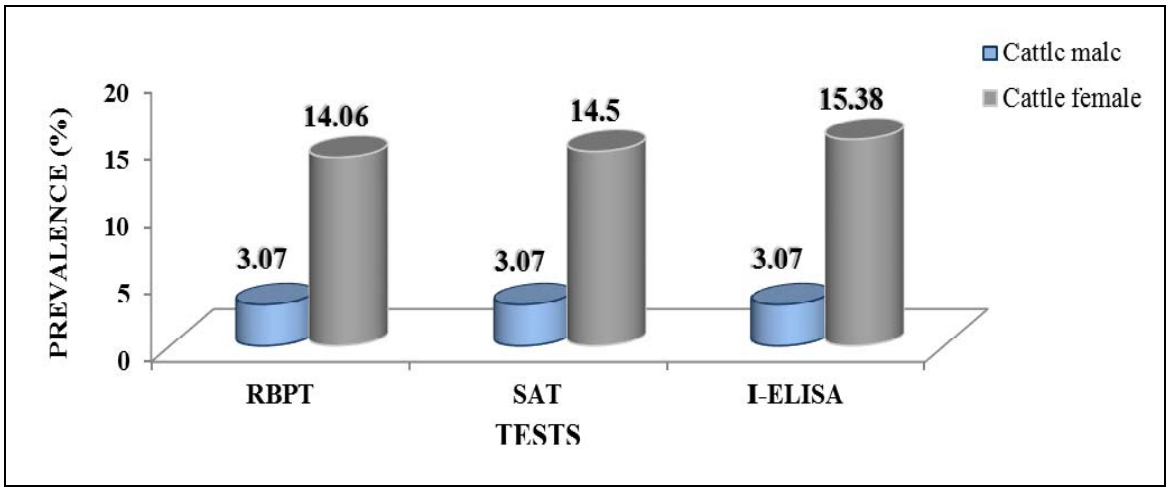

Fig 2: Graphical Representation of Sex Wise Prevalence of Brucellosis in Cattle

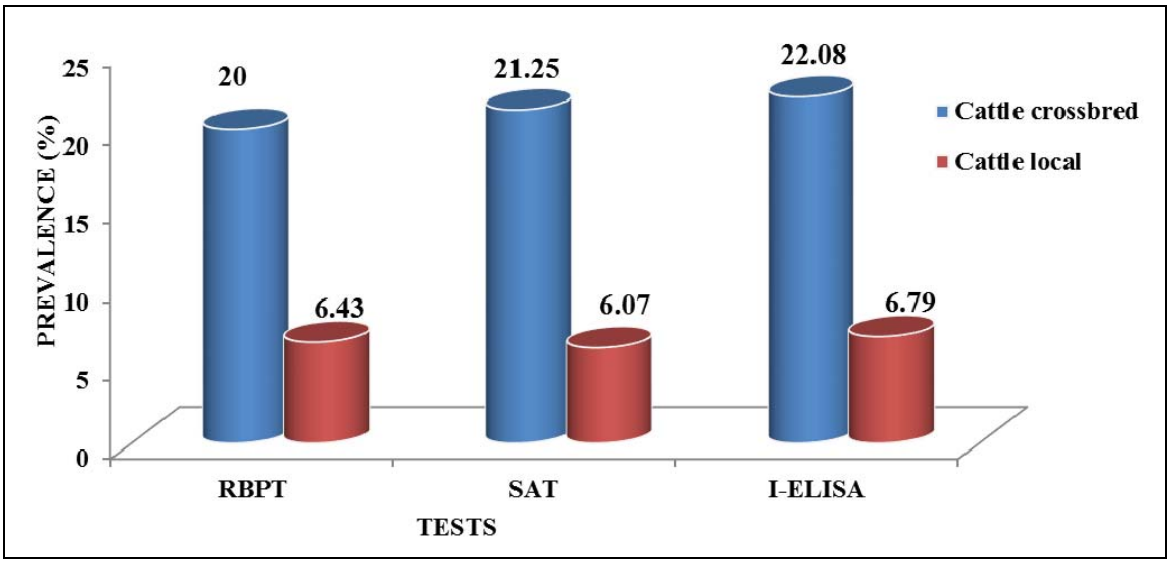

Fig 3: Graphical Representation of Breed-Wise Prevalence of Brucellosis in Cattle

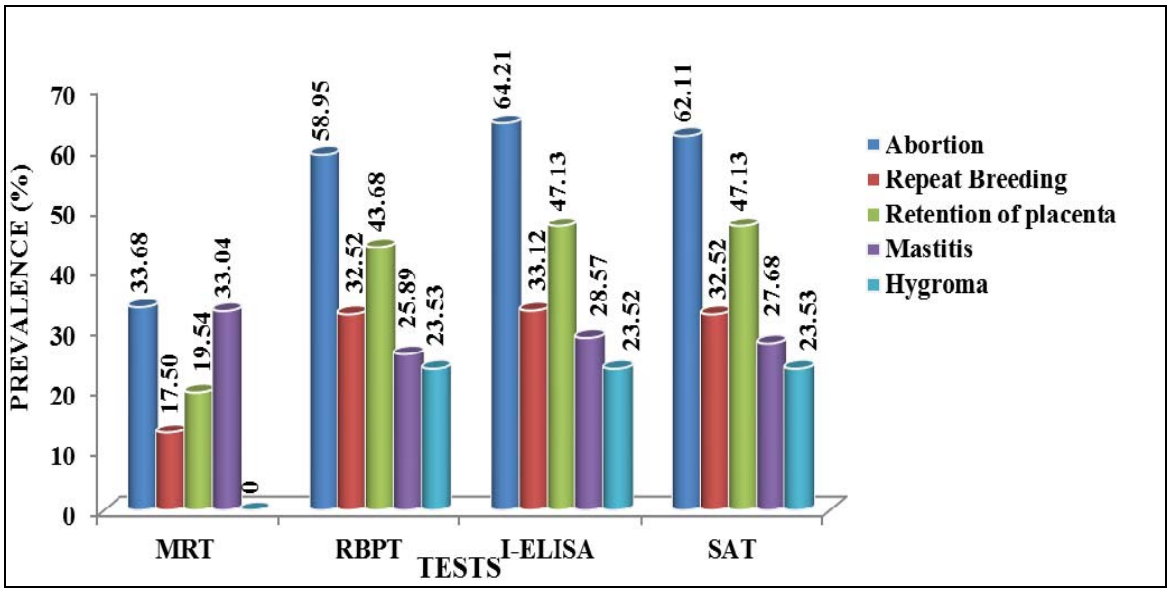

Fig 4: Graphical Representation of Prevalence of Brucellosis in Cattle Based On Previous History/Clinical Symptoms.

Table 1: District-Wise Prevalence of Brucellosis in Different Animals by Milk Ring Test (Mrt).

Districts	No. of samples collected from different animals		Positive in MRT					
			Cattle				Goat	
	Cattle	Goat	No. +ve	Prevalence (%)	95% CI Lower	95% CI Upper	No. +ve	Prevalence (%)
Lakhimpur	40	9	4	10.00	0.70%	19.30%	0	0.00%
Dhemaji	32	7	2	6.25	0.00%	14.64%	0	0.00%
Kokrajhar	13	0	2	15.38	0.00%	35.00%	0	0.00%
Dhubri	9	3	0	0.00	0.00%	0.00%	0	0.00%
Golaghat	7	0	0	0.00	0.00%	0.00%	0	0.00%
Marigaon	15	0	2	13.33	0.00%	30.54%	0	0.00%
Kamrup (M)	32	3	6	18.75	5.23%	32.27%	0	0.00%
Sonitpur	4	0	0	0.00	0.00%	0.00%	0	0.00%
Darang	0	0	0	0.00	0.00%	0.00%	0	0.00%
Total	152	22	16	10.53	5.65%	15.41%	0	0.00%

Table 2: District-Wise Prevalence of Brucellosis in Different Animals by Rose Bengal Plate Test (Rbpt).

District	No. of samples collected from different animals			Positive in RBPT									
				Cattle				Goat				Pig	
	Cattle	Goat	Pig	No. +ve	Prev. (%)	95% CI Lower	95% CI Upper	No. +ve	Prev. (%)	95% CI Lower	95% CI Upper	No. +ve	Prev. (%)
Lakhimpur	183	35	29	20	10.93	6.41%	15.45%	0	0	0.00%	0.00%	0	0.00%
Dhemaji	124	22	34	12	9.68	4.47%	14.88%	0	0	0.00%	0.00%	0	0.00%
Kokrajhar	22	12	18	1	4.55	0.00%	13.25%	0	0	0.00%	0.00%	0	0.00%
Dhubri	31	23	0	5	16.13	3.18%	29.08%	2	8.70	0.00%	20.21%	0	0.00%
Golaghat	25	10	9	0	0.00	0.00%	0.00%	0	0	0.00%	0.00%	0	0.00%
Marigaon	35	15	15	6	17.14	4.66%	29.63%	0	0	0.00%	0.00%	0	0.00%
Kamrup (M)	63	13	7	18	28.57	17.42%	39.73%	0	0	0.00%	0.00%	0	0.00%
Sonitpur	22	0	0	4	18.18	2.06%	34.30%	0	0	0.00%	0.00%	0	0.00%
Darang	15	8	3	0	0.00	0.00%	0.00%	0	0	0.00%	0.00%	0	0.00%
Total	520	138	115	66	12.69	9.83%	15.55%	2	1.45	0.00%	3.44%	0	0.00%

Table 3: District-Wise Prevalence of Brucellosis in Different Animals by Serum Tube Agglutination Test (Stat).

District	No. of samples collected from different animals			Positive in STAT									
				Cattle				Goat				Pig	
	Cattle	Goat	Pig	No +ve	Prev. (%)	95% CI Lower	95% CI Upper	No +ve	Prev. (%)	95% CI Lower	95% CI Upper	No +ve	Prev. (%)
Lakhimpur	183	35	29	20	10.93	6.41%	15.45%	0	0.00	0.00%	0.00%	0	0.00
Dhemaji	124	22	34	13	10.48	5.09%	15.88%	0	0.00	0.00%	0.00%	0	0.00
Kokrajhar	22	12	18	2	9.09	0.00%	21.10%	0	0.00	0.00%	0.00%	0	0.00
Dhubri	31	23	0	4	12.90	1.10%	24.70%	2	8.70	0.00%	20.21%	0	0.00
Golaghat	25	10	9	0	0.00	0.00%	0.00%	0	0.00	0.00%	0.00%	0	0.00
Marigaon	35	15	15	5	14.29	2.69%	25.88%	0	0.00	0.00%	0.00%	0	0.00
Kamrup (M)	63	13	7	19	30.16	18.83%	41.49%	0	0.00	0.00%	0.00%	0	0.00
Sonitpur	22	0	0	5	22.73	5.22%	40.24%	0	0.00	0.00%	0.00%	0	0.00
Darang	15	8	3	0	0.00	0.00%	0.00%	0	0.00	0.00%	0.00%	0	0.00
Total	520	138	115	68	13.08	10.18%	15.97%	2	1.45	0.00%	3.44%	0	0.00

Table 4: District-Wise Prevalence of Brucellosis in Different Animals By indirect-Enzyme Linked Immunosorbent Assay (I-Elisa).

District	No. of samples collected from different animals		Positive in I-ELISA							
			Cattle				Goat			
	Cattle	Goat	No +ve	Prev. (%)	95% CI Lower	95% CI Upper	No +ve	Prev. (%)	95% CI Lower	95% CI Upper
Lakhimpur	183	35	21	11.47	6.86%	16.09%	0	0	0.00%	0.00%
Dhemaji	124	22	12	10.48	5.09%	15.88%	0	0	0.00%	0.00%
Kokrajhar	22	12	2	9.09	0.00%	21.10%	0	0	0.00%	0.00%
Dhubri	31	23	5	16.12	3.18%	29.08%	2	8.70	0.00%	20.21%
Golaghat	25	10	0	0.00	0.00%	0.00%	0	0	0.00%	0.00%
Marigaon	35	15	6	17.14	4.66%	29.63%	0	0	0.00%	0.00%
Kamrup(M)	63	13	22	34.92	23.15%	46.69%	0	0	0.00%	0.00%
Sonitpur	22	0	4	18.18	2.06%	34.30%	0	0	0.00%	0.00%
Darang	15	8	0	0.00	0.00%	0.00%	0	0	0.00%	0.00%
Total	520	138	72	13.84	10.88%	16.81%	2	1.45	0.00%	3.44%

4. Conclusion

In the present study, on the basis of serological test prevalence of brucellosis was recorded as 10.53%, 12.69%, 13.08% and 13.84% in bovine by MRT, RBPT, STAT and I-ELISA respectively. The higher prevalence of the disease increases the risk of zoonotic transmission and it implies a serious threat to the human population as well as the productivity of the livestock.

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