ORIGINAL RESEARCH

Seroprevalence studies of bovine brucellosis using indirect-enzyme-linked immunosorbent assay (i-ELISA) at organized and unorganized farms in three different states of India

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Abstract

Aim: The present study was undertaken to know the seroprevalence of brucellosis in cattle and buffaloes. The work was carried out during the period 2011 through 2013 at Centre for Animal Diseases, Research and Diagnostics, IVRI, Izatnagar, Uttar Pradesh, India.

Materials and Methods: A total of 1005 sera samples were collected and tested for bovine brucellosis using ELISA Kit; IDEXX, CHEKIT, Brucellose serum, *Brucella abortus* Antibody Test Kit

Results: A total of 1005 serum samples were collected from Karnataka, Uttar Pradesh and Uttarakhand during the period of 2011 to 2013 and were screened for bovine brucellosis using i-ELISA (indirect enzyme linked immunosorbent assay). The prevalence of bovine brucellosis was compared between organized and unorganized farms in order to find the epidemiology of the disease among the animal population. Sera from 5 organized farms in Karnataka were collected for seroprevalence studies. Out of 417 animals, 191 (45.80%) animals were found positive by i-ELISA. A total of 361 serum samples were collected from 5 unorganized farms or villages, of which 82 (22.71%) were positive. From Uttar Pradesh, bovine serum samples were collected from 3 organized farms. Out of 192 animals, 43 (22.39%) animals were found positive for brucellosis. Similarly, sera collected from a single organized farm from Uttarakhand, showed 3 (8.57%) positivity among 35 animals. On the whole, 319 (31.74%) animals were found positive for brucellosis among the 3 states taken for study, which includes 138 (27.21%) cattle and 181 (36.34%) buffaloes.

Conclusion: It has been found that *Brucella* infections are widely prevalent in organized and unorganized dairy farms in investigated states of India.

Keywords: brucellosis, buffaloes, cattle, i-ELISA, seroprevalance

Introduction

Bovine brucellosis is a major zoonotic disease caused by bacteria which affects both cattle and buffaloes equally. More prevalent in adults while young ones do not show infection till maturity. The bacteria commonly associated with the bovine brucellosis are mainly *Brucella abortus*, less frequently *Brucella melitensis* and rarely by *B. suis* [1].

The prevalence of bovine brucellosis in dairy farms has long been reported and studies have confirmed widespread prevalence in different states of India [2, 3].

The control of bovine brucellosis is possible only by accurate diagnosis of the disease at the appropriate time. Diagnostic methods include direct tests, involving isolation of bacteria or DNA detection by polymerase chain reaction (PCR) or loop mediated isothermal amplification (LAMP) methods. Indirect test includes serological tests, which are applied *in vitro* using milk or plasma or serum and allergic test done *in vivo* in suspected animals. The gold standard for the diagnosis of brucellosis is isolation, which is the confirmative test [4]. However, it is time consuming, requires biosafety level-3 facilities and skilled persons to handle samples. The alternative methods involving serological tests are easy, safe, and less time and resource intensive.

The aim of the present study was to study the prevalence of brucellosis in cattle and buffaloes of Karnataka, Uttar Pradesh and Uttarakhand in order to take an effective control measures against contagious infection which causes heavy economic losses.

Materials and Methods

Ethical consideration: The study was approved by the Institute Animal Ethics Committee. Adequate measures were taken to minimize pain or discomfort to animals in accordance with the Institute Animal Ethics Committee, while collection of samples. The researchers obtained oral consent from dairy farmers during the survey visits.

Study area: The samples were collected from states of Karnataka, Uttar Pradesh and Uttarakhand during the period 2011 through 2013. The laboratory analysis was conducted at Centre for Animal Diseases, Research and Diagnostics (CADRAD), IVRI, Izatnagar (UP).

ELISA kit: The commercial ELISA kit was procured

Table-1. Prevalence of brucellosis in cattle and buffaloes of three different states of India, assayed using i-ELISA

State	Type of Farm	Cattle	Buffalo	Total
Karnataka	Organized	37/79 (46.83%)	154/338 (45.56%)	191/417 (45.80%)
	Unorganized	55/201 (27.36%)	27/160 (16.87%)	82/361 (22.71%)
Uttar Pradesh	Organized	43/192 (22.39%)	-	43/192 (22.39%)
Uttarakhand	Organized	3/35 (8.57%)	-	3/35 (8.57%)
Total		138/507 (27.21%)	181/498 (36.34%)	319/1005 (31.74%)

from IDEXX-CHEKIT, Brucellose serum (Netherland) to screen bovine brucellosis.

Preparation of sera samples and i-ELISA: The i-ELISA test was validated using positive and negative control sera. After collection of blood from cattle and buffaloes the serum was separated, inactivated at 56°C for 30 min and stored at -20°C till the test was performed. The serum samples were subjected to i-ELISA using manufacturer's protocol.

Results and Discussion

A total of 1005 serum samples were analysed from 3 states which includes 778, 192 and 35 respectively from Karnataka, Uttar Pradesh and Uttarakhand (Table-1). The low specificity of RBPT and Standard Tube Agglutination Test (STAT) [5-8] made us to use highly specific test like i-ELISA for screening brucellosis.

In Karnataka a total of 5 organized farms with the history of bovine brucellosis were selected for sero-prevalence study. Among 417 animals (79 were cattle and 338 were buffaloes), 191 (45.80%) were found positive. Among the tested animals, 46.83% (n=37) cattle and 45.56% (n=154) buffaloes showed reactivity for brucellosis (Table-1). Previously, higher bovine prevalence of 29.0% was reported, with 38.29% in cattle and 26.63% in buffaloes in Central Gujarat using ELISA [9], 33.33% in cattle by employing ELISA [10], 38.95% in buffaloes of Northern Gujarat [11] and 26.50% by ELISA in cattle and buffaloes from 3 different states [12]. However, some studies have reported a lower seroprevalence [13,14] in Uttaranchal (4.6%) and Gorakhpur district of Uttar Pradesh (3.11% cows and 4.18% buffaloes) by i-ELISA. Also, a low seroprevalence of 1.9% in cattle and 1.8% in buffalo were reported on serological survey of 19 states in India [15]. The high seroprevalence of brucellosis was recorded in our study, because the samples were collected at the phase of out break, when the heard showing signs of abortion, retained placenta and infertility in the farms.

A total of 361 sera samples (201 cattle and 160 buffaloes) were collected from 5 unorganized farms or villages of Karnataka. Of this, total of 82 (22.71%) were positive for brucellosis reactivity. This included 27.36% (n=55) cattle and 16.87% (27) buffaloes (Table-1).

The over all prevalence of 22.71% observed in unorganized farms of Karnataka (Table-1) was comparable with previous reports of average 25% seroprevalence overall and 24.30% in cows and 26.03% in buffaloes as detected by i-ELISA [5]. However, a prevalence 21.74 % was reported in endemic areas [16]. Similar reports were shown with 19.12% (n=48) positive out of 251 sera tested for brucellosis with i-ELISA in Anand, Vadodara and Kaira districts [3]. Gender wise, 18.70 % (n=43) were found positive out of 230 females and 23.81 % (n=5) were found positive among 21 males by i-ELISA [3]. Also, prevalence of 23.79% was found in 374 yaks using AB -ELISA in seroprevalence studies of north-eastern hilly yak tracts of Arunachal Pradesh [17].

The prevalence of brucellosis in unorganized farms was noticed less compared to organized farms; it may due to break in chain of disease spread among discrete populations.

A total of 192 bovine serum samples were collected from 3 organized farms of Uttar Pradesh, 22.39% (n=43) animals were found positive for brucellosis using i-ELISA. However earlier study using i-ELISA had observeda lower prevalence of 3.11% in cows and 4.18% in buffaloes from Gorakhpur district of Uttar Pradesh[10]. A single organized dairy farm was taken for seroprevalence studies in Uttarakhand. Out of 35 animals, n=3 (8.57%) animals showed positive reaction by i-ELISA.

Overall, in our study, 319 (31.74%) animals were found positive for brucellosis out of total 1005 in all the 3 states taken together, that includes 138 (27.21%) cattle and 181 (36.34%) buffaloes (Table-1). The high prevalence rate of brucellosis in buffaloes compared to cattle could be due to the use of infected buffalo bulls in natural service and rare use of artificial insemination in the farms. Similar findings were observed by other researchers [10,11].

Comparison of the prevalence between organized and unorganized farms in Karnataka showed 45.80% (n=191 out of 417 samples) and 22.71% (n=82 out of 361 samples) (Table-1). However, previous reports indicated 22.18% animals sero-positive by i-ELISA in organized dairy farms with history of abortion in India [18]. Also, in contrary to our work, a study in Punjab revealed that organized farms had lower prevalence in well managed farms (5.2%) when compared to unorganized poorly managed farms (14.81%) [19].

The lower prevalence of brucellosis can be achieved or it can be eliminated from an organized farm with regular screening and removal of reactive animals with highly specific screening test like i-ELISA. Also, false positive reactions shown by Rose Bengal Precipitation Test (RBPT) can be avoided in i-ELISA [20]. The i-ELISA was found to be the more sensitive among the tests used for screening brucellosis [3,8] and it detected 96 (14.46 %) sera samples positive for brucellosis [21]. It is considered as gold standard

test by many workers to compare the results of other tests in case of brucellosis [22]. ELISA has been reported to be more sensitive compared to other conventional tests [23]. It suggests that i-ELISA could replace not only the currently used confirmatory CFT (Complement Fixation Test) [24], but also other two routine screening tests, namely the RBPT and STAT [25].

The i-ELISA, under laboratory conditions is sensitive as radio immunoassay but can also be adopted as simple field screening procedures [26]. Similarly higher efficacy of i-ELISA was found in cattle [27] and buffaloes [5]. It may be concluded that i-ELISA is a better serological test compared to RBPT and STAT and it could be advocated for screening of brucellosis. The validation and extensive use of primary binding assays like i-ELISA has made diagnosis of bovine brucellosis more manageable [28].

Conclusion

Present finding, reveals high prevalence of bovine brucellosis in organized farms as compared to unorganized farms, which is due to spread of infection from one animal to other by contact between the females or during natural service with infected bull. The highly sensitive and specific diagnostic test i.e. ELISA helps in screening of bovine brucellosis at the low titre compared to RBPT, STAT and other diagnostic tests.

Authors' contribution

This work was carried out by RJ under the guidance of RR with technical and administrative support of RS. KK collected material for manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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