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Prevalence and risk factors of brucellosis among veterinary health care professionals

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ABSTRACT

The study describes prevalence, clinical symptoms and risk factors for brucellosis in personnel engaged in veterinary health care in Karnataka, India. A total of 1050 sera samples were collected from animal handlers, veterinarians, veterinary students, para-veterinarians and persons engaged in artificial insemination of animals. The sera samples were tested for brucellosis by Rose Bengal plate test (RBPT), serum agglutination test (SAT), IgG and IgM indirect ELISA and PCR. Age, sex, clinical symptoms and risk factors were recorded in structured questionnaire. Of the 1050 samples tested, 6.76, 6.38, 3.90, 2.67 and 2.0% were positive by IgG ELISA, RBPT, SAT, IgM ELISA and PCR, respectively and overall prevalence recorded was 7.04%. The prominent clinical symptoms observed were intermittent fever (71.62%) followed by joint pain and body aches. A high degree of suspicion, awareness and multimodal diagnostic approach is suggested for early diagnosis, treatment and disease follow up.

KEYWORDS

Brucellosis; PCR; risk factors; serological tests; veterinary professionals

Introduction

More than 60% of emerging human pathogens are zoonotic [1] and brucellosis is the most widespread zoonosis in the world caused by bacteria belonging to genus *Brucella* [2–5]. Among the recognized 12 *Brucella* species, *Brucella melitensis, Brucella suis* and *Brucella abortus* are the major human pathogens resulting in considerable disability and morbidity [6,7]. Protean clinical manifestations, diagnostic limitations, prolonged treatment courses and recurrent febrile episodes make brucellosis a complicated infection in both livestock and humans. The disease manifests with diverse symptoms such as fever, sweating, malaise, anorexia, headache, joint and back pain [5]. Transmission of infection from infected animals to humans is mainly by contact with infected animals, consumption of unsterilized milk, milk and meat products. Human to human transmission can occur through blood transfusion [8], bone marrow transplantation [9], sexual contact [10,11] transplacental transmission, exposure to mother's fomites during delivery or through breast breeding [12,13].

Though brucellosis is diagnosed by cultural, serological and molecular techniques, timely and accurate diagnosis of human brucellosis continues to be a challenge to clinicians because of its non-specific clinical features, slow growth rate in blood cultures and the complexity associated with serodiagnosis [1]. Isolation of brucellae from blood, bone marrow or other tissues of the patient is a gold standard and confirmatory diagnosis but it is time-consuming and hazardous to laboratory workers [4]. Serological tests are preferred to avoid false positive and negative results and amplification of DNA targets through different PCR methods instantly abbreviating the time required for multiple tests [4,5].

India is recognized as geographical hotspot for brucellosis [2] and increased prevalence of brucellosis in livestock has been reported in recent years [5]. In India, brucellosis in livestock is responsible for a median loss of US $3.4 billion per year [14]. High brucellosis prevalence in animals has greater risk of disease transmission to animal handlers, veterinarians, veterinary staff, vaccinators and students [15,16]. In Karnataka, the sero prevalence ranging from 1–3% have been reported [17]. However, the epidemiological data is lacking due to lack of awareness, laboratory facilities and misdiagnosis due to overlapping clinical spectra. Therefore, the objective of the present study was to determine the prevalence, clinical symptoms and risk factors for brucellosis by multimodal differential diagnostic approaches. This information may be useful for clinicians for providing accurate diagnosis

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to initiate treatment and disease follow up and planners to implement control measures in animals.

Materials and methods

India is the highest milk producing country in the world and Karnataka state is the second largest milk producer after Gujarat. The state has 13,000 cooperative milk societies in 29,406 villages and is situated in the Deccan Plateau bordered by 6 Indian states and the Arabian Sea to the west with an area of 191,976 square kilometres (74,122 sq mi) or 5.83% of the total geographical area of India [18].

Blood samples ($n = 1050$) were collected during brucellosis awareness programmes conducted for animal health care personnel at different districts of Karnataka. The veterinarians, paraveterinarians, persons engaged in artificial insemination, animal handlers and veterinary students who gave the consent to share the demographic data (age, sex, location), occupation and history of association with animals, clinical symptoms and other details in questionnaire were part of the study. The study protocol designed as per the guidelines of Indian Council of Medical Research (ICMR) and approved by institutional ethics committee. Verbal and written consents were obtained for collection of 5 ml of blood in vaccutainers without anticoagulants (Bencton Dickinson, UK). The serum was separated by centrifuging at 2500 rpm for 5 min and stored at 4 °C until tested.

All the serum samples were initially tested by rapid screening test (Rose Bengal Plate Test, RBPT) [19] and results rated as negative when agglutination was absent and 1+ to 3+ as positive, according to the strength of the agglutination noticed within 1 to 3 min. *B. abortus* S99 colored and plain antigens for RBPT and SAT, respectively were procured from Institute of Animal Health and Veterinary Biologicals, Hebbal, Bengaluru, India. RBPT positive samples were further evaluated by serum agglutination test (SAT) by preparing twofold serial dilutions of the serum starting at 1:10 to 1:1280 dilution according to Weybridge technique [19]. The highest dilution of the serum which showed 50 percent agglutination was considered end point titre and 1:160 titre (320 IU/ml) and above was declared positive for human brucellosis [20]. For detection of anti-*Brucella* IgM and IgG antibodies, sera samples were screened by laboratory standardized iELISA protocols. The smooth lipopolysaccharide (sLPS) antigen was extracted from *B. abortus* S99 strain procured from Indian Veterinary Research Institute, Izatnagar, India. Human convalescent sera positive by RBPT, 2-ME-SAT (2-mercaptoethanol) titer of 1:640 (1280 IU /ml) and SAT titre of 1:1280 (2560 IU /ml) were included as IgM and IgG iELISA positive sera controls in the iELISA, respectively [21]. For negative control in ELISA, serum sample from healthy donor negative by all tests for brucellosis was used. To determine the optimum concentration of antigen for iELISA, checkerboard titration of antigen against 1:100 and 1:200 dilutions of strong positive and negative sera samples in blocking buffer (2% gelatin in phosphate buffer saline with 0.05% tween-20) were titrated. Rabbit anti-human IgG and IgM HRP conjugates (Sigma, Germany) diluted 1:8000 and 1:6000, respectively in blocking buffer were used as detection antibodies. The color was developed by using o-phenylenediamine dihydrochloride (OPD) as chromogen and hydrogen peroxide (H_2O_2) as substrate and optical densities (OD) were read at 492 nm using an ELISA micro plate reader (Biorad, Japan) and percent positivity (PP) values were calculated as per given formula.

$$PP = \frac{\text{Average OD values of test sera}}{\text{Median OD value of strong positive control sera}} \times 100$$

The PP values thus obtained in the iELISA were compared with 2- ME -SAT and SAT titres using the MedCalc software and the positive cut-off ≥ 50% PP was selected based on the maximum sensitivity and specificity value obtained in the ROC curve [10,22].

DNA was extracted from all the sera samples using QIAamp DNA kit, as per the instructions of the manufacturer (Qiagen, USA) and quantified by NanoDrop 2000c (Thermo Scientific, USA). The primer sequences were B4 (F): TGGCTCGGTTGCCAATATCAA and B5 (R): CGCGCTTGCCTTTCAGGTCTG. The genus-specific PCR was carried out in 25 µl reaction volume containing approximately 30 ng DNA from serum, 0.2 µM of B4 and B5 primers with 2X Go Taq green master mix (Promega, USA) and cycled (1 min at 93 °C, 30 s at 60 °C, 45 s at 72 °C) 35 times in a thermal cycler (Eppendorf, Germany) [23]. The amplified PCR products were analysed on 1.5% agarose gel electrophoresis and stained with ethidium bromide (1.0 µg/ml). DNA extracted from serum sample spiked with *B. abortus* S99 and DNA from healthy individual were used as PCR positive and negative controls, respectively. The association between risk factors and seropositivity were analyzed using Chi-square test for the variables such as sex, age, profession, education and region using SPSS 22 program and p-value of ≤ 0.05 was defined as statistically significant.

Criteria followed for the diagnosis of brucellosis as positive case:

(a) Clinical history of association with animals, symptoms such as fever, sweating, chills, joint/body/ back aches more than 2 weeks and significant antibody titre (>1:160) in SAT.
(b) Detection of anti-*Brucella* antibodies by at least two serological tests with clinical history of association with animals and persistent clinical symptoms characteristic of brucellosis for more than 2 weeks.
(c) DNA amplification in PCR irrespective of seropositive status with clinical history of association with animals and persistent clinical symptoms characteristic of brucellosis for more than 2 weeks.

Table 1. Analysis of risk factors for human brucellosis.

Risk factors		No. of samples	No. of Positives	No. of Negatives	χ^2	*p*-value
Sex	Male	992 (94.47%)	74 (7.45%)	918 (92.54%)	4.73	0.029*
	Female	58 (5.52%)	00 (0.00%)	58 (100.00%)		
Age (Years)	<10	01 (0.09%)	00 (0.00%)	01 (100.0.00%)	3.2	0.524
	11–20	11 (1.04%)	00 (0.00%)	11 (100.00%)		
	21–30	168 (16.00%)	15 (8.90%)	153 (90.20%)		
	31–40	637 (60.60%)	43 (6.75%)	594 (93.04%)		
	41–50	191 (18.19%)	15 (7.85%)	176 (92.15%)		
	51–60	39 (3.71%)	01 (2.56%)	38 (97.44%)		
	>60	03 (0.28%)	00 (0.00%)	03 (100.00%)		
Profession	Animal handlers	93 (8.85%)	15(16.12%)	78 (83.87%)		
	Veterinarians	833 (79.34%)	50 (6.00%)	783(94.00%)		
	Para-veterinarians	49 (4.67%)	08(16.32%)	41 (83.67%)	23.92	<0.0001*
	Artificial inseminators	18 (1.71%)	01 (5.56%)	17 (83.34%)		
	Veterinary students	57 (5.42%)	00 (0.00%)	57 (100.00%)		
Education	Illiterate	23 (2.19%)	2 (8.69%)	21 (91.30%)		
	Below higher secondary	24 (2.28%)	2 (8.34%)	22 (91.66%)	2.87	0.579
	Higher secondary	46 (4.38%)	4 (8.69%)	42 (91.30%)		
	Graduates	769 (7.32%)	58 (7.54%)	711(92.45%)		
	Post graduates	188 (17.90%)	8 (4.25%)	180 (95.74%)		
Region	Urban	174 (16.57%)	06 (3.44%)	168 (96.56%)	4.12	0.042*
	Rural	876 (83.42%)	68 (7.76%)	808 (92.23%)		

*p-value < 0.05.

Results

In the present study, a total of 1050 sera samples collected from veterinary health care persons were tested for antibodies against brucellosis using different tests. Of these, 992 (94.47%) and 58 (5.52%) were males and females, respectively. The disease status was noticed only in males (7.46%) and was found statistically significant (*p*-value: 0.029) compared to females. The age of the individuals ranged from 7 to 69 years and the highest number of samples were collected from 31–40 years (60.6%) followed by the age group of 41–50 years (18.19%) and 16% of the samples were from 21–30 years age group. Less number of samples were obtained from young (<10 and 11–20) and older age group individuals (51–60 and >60 years). In the age groups 21–30 (8.90%), 41–50 (7.85%) and 31–40 (6.75%) high brucellosis seroprevalences was observed (Table 1).

Among high risk groups, 833 (79.34%), 93 (8.85%), 57 (5.42%), 49 (4.67%) and 18 (1.71%) were veterinarians, animal handlers, veterinary students, paraveterinarian staff and persons engaged in artificial insemination, respectively. High brucellosis prevalence was recorded in paraveterinarians (16.32%) and animal handlers (16.12%) compared to veterinarians (6.0%) and persons engaged in artificial insemination (5.56%). Statistical analysis indicated paraveterinarians, animal handlers and veterinarians (*p*-value < 0.05) to be significantly at the greater risk of acquiring infection compared to persons engaged in artificial insemination and veterinary students. The intermittent fever was recorded as the most predominant symptom (71.62%) followed by spondyloarthropathy in more than half of the cases (52.70%), epididymo-orchitis in 12.16% and problem of infertility in 8.10% of cases (Table 2).

Of the samples tested, 6.76, 6.38, 3.90, 2.67 and 2.0% were positive by individual serological tests, IgG iELISA, RBPT, SAT, IgM iELISA and PCR, respectively. Overall, 26 (2.47%), 20 (1.90%), 18 (1.71%), cases declared positive by two, three and four serological tests, respectively. With respect to ELISA test results, 7 (0.67%) cases were positive by both IgM and IgG iELISA and 3 (0.28%) by combination of IgM ELISA and PCR assay. Overall positivity recorded was 7.04% and comparative analysis of serological tests and PCR is presented in Table 3.

Table 2. Clinical symptoms reported in patients tested positive for brucellosis.

Sl. No	Clinical complaints	No. of cases
1	Intermittent fever	53 (71.62%)
2	Joint pain	39 (52.70%)
3	Body aches	33 (44.59%)
4	Sweating	31 (41.89%)
5	Weight loss	23 (31.08%)
6	Head ache	16 (21.62%)
7	Abdominal pain	13 (17.56%)
8	Pain in testicles	9 (12.16%)
9	Infertility	6 (8.10%)
10	Depression	4 (5.40%)

Discussion

Brucellosis is known as an occupational disease. Globally, brucellosis is regarded as a significant threat for public health and food security [24]. The true incidence of human brucellosis however, is unknown in many of the countries including India. Epidemiological pattern of human brucellosis is constantly changing with new foci of emerging and re-emerging over the period of last 25 years due to extensive livestock farming, sanitary, socioeconomic and political reasons [24–26]. Brucellosis has also been identified as a major cause of travel associated morbidity [7]. Although human brucellosis is observed as a febrile illness with low mortality rates but can lead to debilitating complications. Very high prevalence of brucellosis was recorded in para-veterinarians (16.32%)

Table 3. Comparative analysis of serological tests and PCR assay.

Sl. No	RBPT	SAT	IgMELISA	IgGELISA	PCR	Total	Status
1	–	–	–	–	–	976 (92.95%)	Negative
2	+	–	–	+	–	26 (2.47%)	Positive
3	–	–	+	+	–	7 (0.67%)	Positive
4	+	+	–	+	–	20 (1.90%)	Positive
5	+	+	+	–	+	3 (0.28%)	Positive
6	+	+	+	+	+	18 (1.71%)	Positive
Total	67 (6.38%)	41 (3.90%)	28 (2.67%)	71 (6.76%)	21 (2.00%)	1050	

+: positive; –: negative.

and animal handlers (16.12%) compared to veterinarians (6.0%) and artificial inseminators (5.56%). Statistically significant association to brucellosis was recorded among animal health care workers ($p < 0.0001$).

Several reports have indicated prevalence of brucellosis ranging from 2.26 to 34% in veterinarians and para-veterinarians from high milk producing states in northern, western and southern regions of India [27–29]. Worldwide, reported incidence of human brucellosis varies widely, from <0.01 to >200 per 100,000 population in endemic disease areas [2]. Human brucellosis has been extensively reported among pyrexia of unknown origin (PUO) cases, veterinarians, animal handlers and slaughterhouse workers in India [17,30–32]. Various case reports, seroprevalence studies and hospital based surveillance reports have been published [17,30,33]. Brucellosis was noticed only in males (7.46%) and sex was found to be statistically significant factor for the disease. This may be due to occupational risk as there are more males in veterinary service than the females in the country. Similarly, high seroprevalence of brucellosis was observed in 21–30, 41–50 and 31–40 age groups. Para-veterinarians, veterinarians and persons engaged in artificial insemination are recruited to the jobs beyond the age of 21 years and actively involved in animal health care activities up to 40 years of age. Brucellosis prevalence was low (1.69%) in younger individuals less than 20 years age and few cases recorded here may be due to close association with animal husbandry activities as part of the family occupation. The number of samples obtained for the study and occurrence of the disease in the older (51–60) age group was low (2.56%). This may attributed to their lesser involvement in animal health care activities. Among the other two variables, animal health care personnel from the rural region showed significantly higher seropositivity ($p \leq 0.042$) compared to urban region. Whereas, literacy to brucellosis was found statistically non-significant.

Brucellosis exhibits variable clinical symptoms this makes the diagnosis difficult for physicians. Among the symptoms, intermittent fever was recorded in 71.62% patients followed by spondyloarthropathy in 52.70% [34,35] and epididymo-orchitis in 12.16% of males [32]. The problem of infertility was stated in 8.10% cases. Reports demonstrating variable clinical symptoms are available [30,33,36]. Central nervous system involvement is a most serious and rare complication of human brucellosis has been noted in 5.40% positive cases [37,38]. Similarly, depression, mood swings and dullness were expressed by the family members of the infected patients. Mantur and Amarnath [17] studied clinical symptoms of these 792 adult patients infected with brucellosis. The most prominent symptom was fever (>37.5 °C) which was observed in 78.9% cases followed by convolutions (15.2%), joint pain and weakness (23.1%), weight loss and low backache (14.8%), papulae/mouth ulcers (11.3%).

Serological techniques used for the diagnosis of brucellosis have problems of false positive and false negatives however, serology remains the mainstay of laboratory diagnosis and a numbers of techniques are in use [39]. In the present study, in addition to routine serological tests (RBPT and SAT), IgM and IgG iELISA and PCR have been used for the diagnosis of brucellosis. RBPT can detect persistent anti-*Brucella* antibodies in persons who are associated with infected animals. In such situation, IgM and IgG iELISA assays are useful to differentiate acute and chronic phases of the disease based on dominance of IgM and IgG anti-*Brucella* antibodies [39,40]. Recently PCR assays have been extensively used in conjunction with serological tests for diagnosis of brucellosis [41,42]. In this study, overall 2.67% of cases were positive by both IgM and IgG iELISA and 1.99% by combination of serological tests and PCR assay. In the absence of real-time PCR, conventional PCR was performed and of the 7.04% seropositive samples, only 2% of the samples were detected as PCR positive. This reduced sensitivity may be due to the low number of *Brucella* organisms in the peripheral blood or serum [43].

Treatment was suggested based on symptoms in correlation with laboratory test results as per the standard guidelines (Combination of doxycycline, 200 mg and rifampicin, 600 mg oral for six weeks) [4].

It is well established fact that animals infected with brucellosis act as source of infection to humans through contact and food. The endemic nature of disease and diversification and mixed farming of livestock makes animal health care workers vulnerable to the disease. Hence, a massive campaign to use adequate protective measures while handling infected animals will help to reduce the prevalence in animal handlers and veterinary health care persons. A well defined test protocol for the differentiation of acute and chronic infection in population at risk and serological follow-up are essential to curtail the

disease and to prevent high morbidity associated with the disease. The importance of screening of household members for acute brucellosis cases in endemic areas need to be emphasized.

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Disclosure statement

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