JOURNAL OF CURRENT TRENDS IN CLINICAL MEDICINE & LABORATORY BIOCHEMISTRY

CASE REPORT

Prevalence of brucellosis among high-risk people in South India

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ABSTRACT

Brucellosis was a zoonotic disease causing serious public health and economic problems. Total 780 samples were collected between July 2013 and August 2015 from veterinarians, farmers and cattle handlers who at high-risk individuals. The diagnosis was based upon clinical symptoms, serological and bacteriological test. 144 individuals (18%) were diagnosed as having been infected with brucellosis. The positive rate of brucellosis diagnosis was correlated with gender, ethnic group and clinical symptoms. High prevalence is predominantly increases of brucellosis in South India. An improved healthcare system and preventive measures is needed to minimize the infection of brucellosis.

KEY WORDS : Brucellosis, high-risk groups, prevalence, serological test, bacteriological test.

Introduction

Brucellosis was a zoonotic infection caused by Brucella resulting in reproductive failure in animals and febrile diseases in humans [1]. Based on sole phenotypic characterization using a range of bacteriological and biochemical tests, Brucella was generally classified into six species including Brucella melitensis, Brucella abortus, Brucella suis, Brucella canis, Brucella ovis and Brucella neotomae, respectively [2].

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Over recent years, the number of species has increased to more than 10 as several new Brucella strains had been isolated from marine mammals, rodents, and infected human breast implant, respectively [3,4,5]. An important characteristic of Brucella was that they could penetrate the endothelial reticulum and infect macrophages, acting as facultative intracellular parasites [6,7]. Human being can be infected with Brucellosis through various routes like consumption of contaminated dairy products, microbial inoculation through cuts or abrasions in the skin surface, the conjunctiva inoculation, inhalation of infectious aerosols, accidental human contact with infected animals and consumption of contaminated meat [8]. The common clinical symptoms included weakness, lethargy, chill, fever, sweating, decreased appetite, arthralgia, myalgia, weight loss, headache, back pain and psychological symptoms [6].

The most important first step for control of the disease was accurate diagnosis of brucellosis [9]. Bacteriological detection of brucellosis in humans was the confirming method and depended on the isolation of the bacteria from blood and bacteriological tests and its biotype, which would take at least one week [10]. Due to the adequate sensitivity, simplicity and lower cost, serological tests such as standard agglutination test (SAT) and enzyme-linked immunosorbent assay (ELISA). The commonly used for brucellosis screening in laboratory [11]. Many ethnic groups are intermingled and most people are engaged in agriculture and animal husbandry in India. Traditional habits, limited veterinary support services and husbandry practices aggravated the spread of this disease. In this study, the incidence of brucellosis was screened and its correlation with gender, ethnic group and clinical symptoms has been investigated. Moreover, the diagnosis value of serological and bacteriological detection on brucellosis has also been evaluated.

Materials And Methods

Patients And Samples

All samples of 780 high-risk individuals were mainly collected between July 2013 and August 2015 from veterinarians, farmers and cattle handlers who at high-risk individuals. About 144 peoples had clinical symptoms suggestive of brucellosis such as fever, sweating, arthralgia, myalgia and weakness. The blood samples were taken and it was processed immediately for the various tests.

Inclusion Criteria

Individuals residing in the study villages for more than 1 year irrespective of symptoms of brucellosis.

Exclusion Criteria

Individuals staying in the study villages for less than 1 year were excluded. Consent from each study subject, eligible and willing to participate in the study, was obtained. All the participants were interviewed with a pre-designed questionnaire regarding age, sex, occupation, contact with animal/animal products, type of animal, duration of contact, raw milk ingestion, knowledge regarding animal and human brucellosis as a disease, its transmission, clinical symptoms, prevention, etc.

Brucella Standard Agglutination Test (Sat)

The SAT was carried out by double- dilution of serum from 1:40 to 1:5120 according to previous report [12]. Brucella antigen and control sera were used according to the instruction of the manufacturer. Positive reactions were determined using compound microscope, and the titre given indicated the highest dilution in which 50% or more agglutination occurred in the tube.

Culture And Identification Of Organism

A minimum of 5 ml of blood (for children 1-3 ml) was taken through vein puncture and was injected into the culture vials BACTEC 9240 (BD Diagnostics, USA system). Standard aerobic/F and Peds plus/F media (BD Diagnostics, USA) were used for the adult and the paediatric patients, respectively. Inoculated bottle were monitored for 5 days before vials with negative results were removed according to the procedures outlined by the Clinical and Laboratory Standards Institute. If a vial shows positive result were subjected to Gram stained smear microscopy and subcultured on sheep blood agar and MacConkey agar, incubated at 37°C. Brucella was identified and differentiated

from other Gram negative genera on the basis of small, translucent, soft and easily emulsifiable colonies on MacConkey and blood agar (non-pigmented and non-haemolytic) with absence of X and V factor dependence; Gram-negative tiny coccobacilli, non-encapsulated, non-motile, oxidase, catalase and urease positive, producing acid from xylose in oxidative fermentative medium [13]. The results of culture were compared with standard agglutination tube test (SAT). The result was reported by the software automatically and well knowledged microbiologist.

Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) for Windows, version 22.0. The significance of differences between groups was determined using Chi-square. A value of $P \leq 0.03$ was considered as statistically significant.

Results

Demographic Findings

A total of 780 individuals of outpatients distributed in south india were registered. Among 144 individuals (18%) were diagnosed as infected with brucellosis with ages ranging from 10 to 89 years old. In the 40-61 years-old group, the infection rate of brucellosis reached 68 (9%) and significant difference was found between various age. In all suspected cases, the incidence of brucellosis in male 96 (12%), individuals with clinical symptoms was statistically higher than that in female 48 (6%). (Table 1).

Table 1. Brucellosis distribution on Demographic characteristics and common clinical symptoms.

Parameters	Cases(n)	Individuals with Non-brucellosis	Individuals with Brucellosis (%)
Age			
0-20	145	123 (16%)	22 (3%)
21-40	320	284 (36%)	36 (5%)
41-60	217	149 (19%)	68 (9%)
61 or older	98	80 (10%)	18 (2%)
Gender			
Female	230	182 (23%)	48 (6%)
Male	550	454 (58%)	96 (12%)
Common clinical symptoms of brucellosis			
Yes	335	229 (29%)	106 (14%)
No	445	407 (52%)	38 (5%)

The overall distribution of SAT results found in the surveyed individuals and the percentages of individuals for SAT were shown in (Table 2). The symptomatic brucellosis shows 94 (14%).The Culture shows positive of about 84 (66%).

Table 2. Prevalence of SAT and identification in individuals with and without clinical symptoms of brucellosis.

Test	Result	Cases(n)	Individuals with clinical of brucellosis (%)	Individuals without Clinical symptoms of brucellosis (%)
SAT	Negative	636	278 (44%)	358 (46%)
	1:40	33	21(63%)	12 (36%)
	1:80	19	12(63%)	07 (37%)
	1:160	27	18 (67%)	09 (33%)
	1:320	48	32 (66%)	16 (34%)
	1:640	11	07 (63%)	04 (34%)
	1: >640	06	04 (67%)	02 (33%)
	Total positive	144	94 (14%)	50 (5%)
Microbial culture and identification				
Negative		652	158 (24%)	494 (76%)
Positive		128	84 (66%)	44 (34%)

Discussion

Brucellosis continues to be a major public and animal health problem in many regions of the world. According to World Health Organization (WHO), every year 500,000 people were infected [14]. In the USA, 4 to 10% of cases were diagnosed and reported [15,16]. A total of 189,226 cases of human brucellosis were reported officially, about 90,000 of these were registered between 2000 and 2005 (approximately 15,000 cases per year) in Turkey [17]. The prevalence rate of brucellosis in different provinces of Iran varies from 1.5 up to 107.5 per 100,000 persons in 2003 [18].

People were they are mostly engaged in agriculture and animal husbandry, and the direct contact with animals and animal products was common. Taking unpasteurized dairy products was usual. Furthermore, keeping, slaughtering, and taking of dairy and non-dairy products from livestock were carried out traditionally. The main livestock of the region were sheep, goat, and cattle. Brucellosis was a zoonosis and, with few exceptions, infection in humans resulted from direct or indirect contact with animal sources. The main source of infection for the general population was dairy products prepared from milk from infected livestock. The milk of infected sheep, goats or cattle may contain large numbers of viable organisms, which become concentrated in products such as yogurt, milk tea, cheese, and etc [19]. For good-taste of food, the meat was usually barbecued and half-baked. In this study, the incidence of brucellosis in south India people was higher than previous studies and highest in patients aged between 40 and 60 years (19%). It may attribute to different habits between both ethnic groups. The positive rates of male and individuals with clinical symptoms were significantly higher than that of female and those without clinical symptoms. The brucellosis in human has a diverse range of clinical symptoms, and the most important of them was undulant fever and arthrodynia [20,21]. When the disease became chronic, it would affect almost all organs and resulting in a complicated syndrome including spondylitis, endocarditis, and meningoencephalitis [20]. Antibiotics regimens eg Doxycycline 1 g twice a day and rifampicin 600 mg daily for 3-6 months was usually used in the treatment of acute patients with brucellosis.The diagnosis of human brucellosis was not difficult if the level of suspicion was high and the symptom was typical, but the varied manifestations for localized, sub-acute, or chronic infection made it prone to be misdiagnosed [22-24]. As the symptoms of brucellosis were non-specific, the diagnosis could be done based upon laboratory finding only [25].

In conclusion, although brucellosis had been, or was close to being eradicated from a number of developed countries, it continued to be a

major public and animal health problem in many regions in the developing countries of the world. Among south India, human cases continued to occur due to their traditional use of raw milk products or eating the half-baked meat and having close contact with infected animals or people. A well developed healthcare system and preventive measures would help to reduce potential infection risks and decrease incidence of brucellosis. Government should make awareness about the brucella and its clinical manifestation.

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