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RESEARCH ARTICLE

SERO-EPIDEMIOLOGY OF BRUCELLOSIS AMONG HIGH RISK OCCUPATIONAL GROUPS BY CONVENTIONAL METHODS AND INDIRECT ENZYME LINKED IMMUNOSORBENT ASSAY

*Deepthy, B. J., Sreejith, K., Jisha, P. and Ravindran, P. C.

Department of Microbiology, School of Health Sciences, Palayad Campus, Kannur University, Kerala

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ABSTRACT

Brucellosis is a re-emerging bacterial zoonoses and a major health concern worldwide; most cases being undiagnosed or misdiagnosed as pyrexia of unknown origin. The occupational source of exposure predisposes the farmers, shepherds, butchers, laboratory workers, veterinarians and slaughterhouse workers to a greater risk of contracting the disease. The present work was thus undertaken to study the risk of exposure to the high risk occupational groups from different parts of Kerala, and also to compare between conventional test like Rose Bengal Plate test (RBPT) and Standard tube agglutination Test (STAT) with Indirect Immuno-sorbent assay (I-ELISA) for the serodiagnosis of human Brucellosis. A total of 240 human serum samples collected from 83 veterinary doctors, 47 livestock inspectors and 110 slaughter house workers were subjected to RBPT, STAT and Indirect Enzyme-Linked Immuno Sorbant Assay. Out of the 240 samples tested, 56 (23.3%), 2(0.83%) and 13 (5.41%) samples were found positive for antibodies to *Brucella abortus* by RBPT, STAT and I-ELISA respectively. Among Veterinary Doctors (n=83), RBPT tested positive in 17(20.48%) samples while I-ELISA tested positive in 6(7.22%), but STAT did not give any seropositivity. Among livestock inspectors, (n=47) RBPT tested positive in 8(17%). Among Slaughterhouse workers (n=110), RBPT tested positive in 31 (21.18%) while STAT tested positive in 2 (1.81%) and I-ELISA in 7 (6.36%). Seroprevalence was high in veterinary doctors (7.22%) followed by slaughter house workers (6.36%), and the incidence is high among male population and the highest age group of the affected was of 30-40 years (2.37%). The data collected from the study participants revealed that all the identified positive cases were chronic non vegetarians using all kinds of meat products. The positive cases had not underwent any kind of treatments or never attempted any specific diagnosis for brucellosis ever before. The present study suggests that brucellosis is a professional hazard in the veterinary practitioners and slaughterhouse workers. So periodic screening especially among occupationally exposed people must be done. The disease is easily misdiagnosed because of the deceptive nature of the clinical signs and symptoms. The clinicians may miss many cases of Brucellosis because it is not considered a common disease. Elimination of the infection in animals by vaccination to produce *Brucella* free animals and products will help to prevent transmission of infection.

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INTRODUCTION

Brucellosis is a re-emerging bacterial zoonoses and a major health concern world wide.¹ The *Brucella* species pathogenic to humans are *B. melitensis* from sheep and goats, *B. abortus* from cattle, *B. suis* from pigs, and *B. canis* from dogs.² Of these *B. melitensis* causes the severest form of disease in humans.³ The disease is primarily a disease of animals in which man is an accidental host.² The occupational source of exposure predisposes the farmers, shepherds, butchers, laboratory workers, veterinarians and slaughterhouse workers to a greater risk of contracting the disease through inhalation of contaminated aerosols, contact with conjunctival mucosa, cuts and abrasions in the skin as a result of contact with infected animals or their products.^{4,5}

The non-occupational sources of exposure include ingestion of infected meat, unpasteurized milk and milk products.⁴ Veterinary doctors are another high risk professional groups because of handling with infected materials such as placenta, uterine secretions of infected animals. Hence there are more chances of contracting the infection. Brucellosis is a multi system disease that may present with a broad spectrum of clinical manifestations. Hepatic, renal and cardiac involvements are reported in later stages of the disease. Central nervous system involvement in Brucellosis sometimes can cause demyelinating syndromes.⁷ The prevalence of Brucellosis in occupationally high risk groups is difficult to estimate because most of the cases are undiagnosed or misdiagnosed as pyrexia of unknown origin. Keeping these views in mind, the present work was undertaken to study the the risk of exposure to the high risk occupational groups from different parts of Kerala, and also to compare between

*Corresponding author: Deepthy, B. J.

conventional test like Rose Bengal Plate test (RBPT) and Standard tube agglutination Test (STAT) with Indirect Immuno-sorbent assay (I-ELISA) for the serodiagnosis of human Brucellosis.

MATERIALS AND METHODS

The study was conducted in the Department of Microbiology, School of Health Sciences, Kannur University, Kerala over a period of two years. A total of 240 human serum samples were tested for Brucella antibodies which include serum samples collected from 83 veterinary doctors, 47 livestock inspectors and 110 slaughter house workers and were subjected to RBPT, STAT and Indirect Enzyme-Linked Immuno Sorbant Assay. The whole study population was grouped into three, based on the level of occupational risk of exposure to zoonotic infections. The first group was labeled as group I which Included Veterinary Doctors; group II consisted of Live stock Inspectors and group III with Slaughterhouse workers. The serum samples were collected from study participants after sufficient awareness programme regarding importance of the study. The data regarding demographic and socioeconomic variables and on exposure to animals and animal products was collected by using a structured questionnaire which included their age, occupation, nature of work, history of consumption of raw milk, history of fever (nature and duration) in the past, and complaints of joint pain, if any. All the 240 serum samples collected were appropriately labelled and transported to the laboratory for analysis. Samples were subjected to Brucella antibody detection by RBPT, STAT and Indirect ELISA. The antigens required for both tests were procured from Institute of Veterinary Research Institute, Bareilly. Data were recorded and analysed for the interpretation of the results to know the influence of age and sex in contracting brucellosis using Statistical Package for Social Sciences (SPSS) version 16.0. The data collected from the study participants was used to assess the association between exposure variables and seroprevalence of brucellosis and to find out which factor(s) best predicted the likelihood of Brucella seropositivity.

RESULTS

Out of the 240 samples tested, 56 (23.3%), 2(0.83%) and 13 (5.41%) samples were found positive for antibodies to Brucella by RBPT, STAT and I-ELISA respectively. Among Veterinary Doctors (n=83), RBPT tested positive in 17(20.48%) samples while I-ELISA tested positive in 6(7.22%), but STAT did not give any seropositivity. Among livestock inspectors, (n=47) RBPT tested positive in 8(17%). Among Slaughterhouse workers (n=110), RBPT tested positive in 31 (21.18%) while STAT tested positive in 2 (1.81%) and I-ELISA in 7 (6.36%). By keeping IELISA as the standard diagnostic test, the prevalence rate of brucellosis was calculated in all the three groups. Among the veterinary doctors the prevalence rate identified was 7.22%, in the slaughter house workers the prevalence rate was 6.36% and there were no positive cases identified among livestock inspectors. Among the 6 positive cases identified among veterinary doctors 5 were male and 1 was female. Two of the positive cases had reported with complaints of frequent myalgia. The majority of positive is identified were in the age group of 35-43 years and the duration of exposure recorded was calculated as 6 to 20 years.

Among the slaughter house workers the majority of the affected were in the age group of 42-48 years. All of them were from male gender and 2 of the positive cases (28.6%) had the habit of drinking raw milk, one had relapsing cycles of fever, five reported frequent arthralgia and one had orchitis. The duration of exposure among the positive cases were ranged from 8 years to 20 years. The results of Statistical analysis of the samples have shown that the seroprevalence is high in veterinary doctors (7.22%) followed by slaughter house workers (6.36%), and the incidence is high among male population and the highest age group of the affected was of 30-40 years (2.37%). The data collected from the study participants reveals that all the identified positive cases were chronic non vegetarians using all kinds of meat products. The positive cases were not underwent any kind of treatments or never attempted any specific diagnosis for brucellosis ever before.

DISCUSSIONS

Brucellosis is one of the most common zoonotic diseases in the world. It has emerged as a major public health concern worldwide with implications for the economic prosperity of many nations.⁹ The general public can be infected by the ingestion of contaminated milk and milk products, but certain occupations are considered carrying high risk such as abattoir workers, veterinarians, butchers, meat inspectors and farmers.¹⁰ In the present study, the exposure rate noted was based on the result of I-ELISA. The prevalence estimated was high among veterinary practitioners (7.22%) which are closely followed by slaughterhouse workers (6.36%). It is difficult to compare seroprevalence of brucellosis in different studies as it varies from place to place and time to time. Magnitude of problem differs from state to state in India. Even with in the states in which prevalence is known it differs from place to place and the diagnosis is also depends upon type of antigen, diagnostic techniques used, and on levels of antibody titers. Selection criteria used for selection of cases for laboratory investigation for brucellosis also play an important role in determining seroprevalence of brucellosis in particular geographical area.

The prevalence rate of 7.2% as detected by ELISA in our study agrees with the findings of other workers. A study conducted by Thakur and Thapliyal, from India revealed a Prevalence rate of 4.97% in samples obtained from persons exposed to animals 27 which is slightly less than what we observed in this study. Another study by Mathur from north India reported seroprevalence of 8.5% among dairy workers in contact with infected animals¹. A study conducted by Agasthya, *et al.* examined 618 serum samples from veterinary Personnel and found 15.69% tested as positive¹². Among the 47 Livestock Inspectors and Attenders studied, prevalence of brucellosis was 17.02% as detected by RBPT, while there were no positives detected by STAT and I-ELISA. The seroprevalence of brucellosis in the slaughterhouse workers was estimated by the detection of anti- Brucella antibodies in the serum samples. In this study three serological tests like RBPT, STAT AND I-ELISA were used to screen the antibodies. In India Seroprevalence of Brucellosis among slaughterhouse workers were estimated as (25.45%), 13 Saudi Arabia (35%), 14 Brazil (4.1%), 15 and in Algeria it was found to be 37.6% among abattoir workers, butchers, breeders and veterinarians.¹⁶

Among the 110 slaughter house workers studied, prevalence of brucellosis was 28.18% as detected by RBPT, 1.81% the by STAT and 6.36% by I-ELISA. RBPT was the rapid test and the results indicate that RBPT can be used as a screening test when large amount of serum is needed to be tested because it is easy to perform and provides results in few minutes. STAT gave positive results only in 2 samples and this may be due to blocking effect of incomplete antibodies present in serum. These same positive samples were also tested positive by RBPT. I-ELISA was the most specific test among the three methods. Paweska *et al.*, suggested that ELISA could replace not only the confirmatory CFT, but also other two screening tests, namely the RBPT and STAT.²⁶ The slaughterhouse workers are generally more prone to contract brucellosis by virtue of their direct exposure to viscera, gravid uterus and fetal membranes of infected animals which are the preferred sites of localization of the bacteria.^{13,15} The workers are not only in contact with carcasses and viscera of infected animal, but they may also get infected through cuts on bare hands, splashing of infected fluid in the conjunctiva and inhalation of aerosols in the slaughtering area.^{14,17} The minimum infective dose of *Brucella* species required to induce active brucellosis is less through the respiratory route than the oral route, increasing the vulnerability of the slaughterhouse workers.¹⁸

As brucellosis is an occupational disease, individuals in this work category would be expected to be at a greater risk because of prolonged exposure. Mishal J *et al.* have also highlighted the risk of *Brucella* seropositivity with assistance in parturition of animals. In their study, conducted in Israel, brucellosis was found to be more common among individuals who had been involved in calf deliveries and had handled placentae.¹⁹ Lim *et al.* reported contact with infected animals' placentae as the route of transmission of the disease.²⁰ Similarly, Daniela has also reported the habitual intake of raw milk as the probable cause of brucellosis.¹⁷ Although definite diagnosis of brucellosis requires isolation of the organism from blood or other body fluids but brucellae are slow growing organisms and require special culture conditions², for avoiding the delay in reporting, serological methods are used for a rapid diagnosis. The antibodies detected by serological testing are directed against the lipopolysaccharide component of the bacterial cell wall.²⁵

Though in comparison to ELISA test RBPT and STAT are less sensitive and have their own importance and prove to be effective for screening of brucellosis in many countries. However, the findings of the present study are in agreement with those stated by Chopra *et al.*²² and Chachra *et al.*, who described RBPT to be more reliable and useful than STAT for screening of brucellosis although a combination of RBPT and ELISA would be more useful in cases of samples found negative either by RBPT or STAT used singly or in combination.²³ The present study suggest that brucellosis is a professional hazard in the veterinary practitioners and slaughterhouse workers. So periodic screening especially among occupationally exposed people must be done. The disease is easily misdiagnosed because of the deceptive nature of the clinical signs and symptoms. The clinicians may miss many cases of Brucellosis because it is not considered a common disease. Elimination of the infection in animals by vaccination to produce *Brucella* free animals and products will help to prevent transmission of infection.

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