ORIGINAL ARTICLE

Brucellosis: A Threat to Human Population of District Rawalpindi

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ABSTRACT

Objective: This study was conducted to detect cases of brucellosis among shepherds and to identify risk factors associated with human brucellosis.

Study Design: Case control study

Methodology: A descriptive study followed by case control study was conducted during the month of August, 2016 at Village Hassar tehsil Taxilla, district Rawalpindi. A case was defined as "intermittent fever, profuse night sweats, headache and positive brucella antibodies on ELISA in a resident of Hassar from August 21-25, 2016. Epidemiological information was recorded on structured questionnaire. Cases and controls were matched by age and sex (1:4). Blood samples were collected from sheep/goat handlers (n=30) and small ruminants (n=144). Rose Bengal plate test (RBPT) and indirect Enzyme linked immunosorbent assay (I-ELISA) was used for testing of serum samples. Frequencies were calculated, odd ratios were determined at 95% confidence interval with p value less than 0.05.

Results: A total of six cases of brucellosis were identified. Among cases 42% were having direct contact with small ruminants and 60% were raw milk consumers. Animal handler (OR =12 CL=1.19-123.6: p<.026) were likely to have brucellosis as compared to those who were not directly involved in animal handling. Persons consuming raw milk are more likely to have brucellosis (OR=11: CI= 1.3-95: p<0.04) as compared to those consuming pasteurized milk. Among small ruminants tested, 52% were found positive for brucellosis.

Conclusion: Animal handlers/shepherds of district Rawalpindi were infected with brucellosis. Animal handler and raw milk consumer were more likely to get brucella infection. Infected small ruminant are potential source of infection for human. Presence of brucella infection in animal handlers/shepherd of Rawalpindi is suggestive of brucella infection all across the country.

Key Words: Brucellosis, Antibodies, Pasteurized.

Introduction

Brucellosis is a zoonotic bacterial infection of livestock caused by a number of bacteria in the genus *Brucella*. Among ten members of genus Brucella, *B. melitensis* is most pathogenic and invasive species for human, followed in descending order by *Brucella abortus, Brucella suis* and *Brucella canis* (Acha and Szyfre, 2003).[1]

Brucellosis is considered an occupational hazard for people working with animals like veterinarians, farmers, animal handlers and butchers or laboratory staff working with live cultures of *Brucella*. Among general public, human infections commonly result from the consumption of raw/ unpasteurized milk, dairy products prepared from unpasteurized milk such as soft cheeses, yoghurts and ice creams. Inhalation and direct contact with sick

animals especially after abortion induced by brucellosis are important routes of disease transmission within a herd (Pappas *et al*., 2005).[2] Brucellosis has been described as an important public health problem in Pakistan (Nusrat, 2004).[3]

In livestock, the main feature of brucellosis is abortion. However, in humans, the diseases has variable clinical features. It is mainly characterized by fever, common flu known as Pyrexia of unknown origin (body temperatures of up to 38.3 °C) (Petersdorf, 1961).[4] Other symptoms include: backache, headache, chills, night sweats, weakness and weight loss (Mantur *et al*., 2007).[5] Usually these symptoms are confused with malaria, typhoid fever, tuberculosis and rheumatic fever. Now researchers are becoming more interested in brucellosis due to two main reasons. Threat of zoonotic diseases is increasing with facilitation in international travel promoting tourism and migration; secondly *Brucella* can be used as potential biologic weapon(Pappas et al., 2006)[6] Clinically, history of animal contact, travel to endemic region and identification of organism up to genus level is enough to initiate therapy. Identification of specific *Brucella* species affects the choice of therapy and it is necessary for epidemiologic surveillance. Identification requires biochemical, serological and molecular testing. Treatment is of long duration in human, treatment options include aminoglycosides, doxycycline, septran, streptomycin and rifampicin however vaccine strains are resistant to rifampicin.

Present study is related to outbreak of brucellosis in sheep herd involving human, this study was conducted to establish a link between animal disease and human infection.

Methodology

A descriptive study followed by case control study was conducted during the month of August,2016 at Village Hassar tehsil Taxilla, district Rawalpindi. Purpose of study was to detect cases of brucellosis among persons involved in rearing of small ruminants and to identify risk factors associated with human brucellosis. A case was defined as "intermittent fever, profuse night sweats, headache and positive brucella antibodies on ELISA in a resident of Hassar from August 21-25, 2016. Cases and controls were matched by age and sex (1:4). A total of 30 blood samples were collected from animal handles and their family member. A structured questionnaire related to demographic and epidemiological information was filled up during blood sampling. Written consent was obtained prior to sampling of each individual. From human 3ml of blood was collected using sterile needle by radial vein puncture in labeled vacutainers. Similarly 5ml of blood was collected from small ruminant (n=144) by jugular vein puncture. After overnight refrigeration at 4°C, sera were harvested by centrifugation (Sigma, Germany) at 1500×g for 10 minutes. Initial screening of the sera was carried out by Rose Bengal Plate test (RBPT) (MacMillan, 1990: John *et al.*, 2010).[7,8] Briefly; samples positions were marked on white tile and 25μl of serum sample was mixed with same quantity of RPBT antigen to produce a circular zone of about 2cm in diameter. The plate was rotated gently for 4 minutes at room temperature, any visible clumping within 4 minutes was indicative of a positive result. Any test showing agglutination beyond this time was considered negative. Positive and negative controls for RBPT were tested for reference. RBPT positive human sera were confirmed through I-ELISA technique using ELISA kit (PishtazTeb diagnostics, Iran) sample OD were measured at 450 nm. Cut off values/Cut off index(COI) for each sample was calculated. Samples having COI higher than 1.1 were consider positive and those less than 0.9 were assumed as negative and those between these two values i.e. 0.9-1.1 were supposed as suspicious. Questionnaire data combined with results of serological testing was used to identify risk factors associated with human brucellosis. Frequencies were calculated, odd ratios were determined at 95% confidence interval with p value less than 0.05. Human found serologically positive for brucellosis were referred to Health department for further investigation and medical advice.

After initial testing by RBPT, positive small ruminant sera were confirmed through I-ELISA technique using ELISA kit (IDEXX, USA) optical densities values of samples and control were measured at 450nm. Sample percentage (s/p) values were calculated to find out presence of antibodies in serum. Samples with s/p % ≤ 110% were considered negative for presence of Brucella antibodies while Sample with s/p % > 110 and <120 were

considered as suspected. Samples with s/p % ≥120 were taken as positive for Brucella antibodies.

Results

A total of six cases were identified. Among cases 42% were having direct contact with small ruminants and 60% were raw milk consumers. Analysis of various risk factors showed that persons who were directly engaged in handling of animals were more likely to get disease (OR =12 CL=1.19-123.6p<.026) as compared to those who were not directly involved in rearing/ management practices, similarly those who were habitual of drinking raw milk were more likely to get brucellosis (OR=11: CI= 1.3-95: p<0.04) as compared to those consuming pasteurized milk.

Presence of Brucellosis in small ruminants was confirmed through serological testing. Of 144 small ruminants 75 were found positive for Brucella antibodies on serological testing, indicating a higher prevalence (52%) of brucellosis in animals of village Hassar, Taxilla, Rawalpindi.

Table III: Risk factors associated with human brucellosis

Risk Factor	No. of people tested	Sera Positive by I-ELISA	Odds Ratio (OR)	CI-95%	Exact test p-value
Raw milk					
Yes	5	3	11	1.3-95	p<0.04
No	25	3			
Animal contact					
Yes	12	5	12	1.19-123.6	p<0.026
No	18	1			

Discussion

Results of present study indicated that persons consuming infected raw milk had more probability of getting brucellosis. This finding is in agreement with other studies. A study conducted in district Lahore stated that raw milk consumption was a statistically significant (OR =2.25: 95% CI =1.04-4.87: p=0.039) risk factor for Brucella sero-positivity among slaughterhouse workers (Mukhtar et al., 2010).[9] Another study conducted in agro-pastoralist communities of south western Uganda also found consumption of unpasteurized milk a statistically significant risk factor (p = 0.02) (Benon *et al*., 2015).[10] Similarly, Nasinyama *et al*. (2014)[11] observed significantly higher prevalence (p<0.004) of brucellosis among humans of Mabarara district, Uganda consuming raw milk. The possible explanation for higher probability of being infected with brucellosis is that brucellosis is typical example of milk-borne infection. Brucellae are sheded in milk of infected animal (Ebrahimi et al, 2014).[12] and the appearance and taste of the milk are seldom affected by the presence of the brucellae Therefore, consumption of raw milk infected with Brucella may cause brucellosis in humans (Tumwine et al., 2015).[13] Moreover in rural areas of Pakistan consumption of raw goat/sheep milk is considered beneficial for health. Once humans become infected, *Brucella* causes an acute febrile illness that is often confused with other diseases. Untreated brucellosis at times, persist and progress to a chronically debilitating disease with severe outcome (Corbel, 2006).[14]

However, studies have indicated that pasteurization may effectively kill Brucella reducing the risk of transmission of brucellosis to humans (Albala, 1995).[15]

Present study depicted animal contact another significant risk factor related to brucella sero-positivity in human. These results are in agreement with previous studies. A study conducted in Lahore, Pakistan reported higher prevalence of brucllosis in slaughterers (27.1%) compared to cleaners (18%) and drivers (0%) (Mukhtar, 2010).[9] Another study conducted in India reported animal contact a significant factor (<0.01) related to Brucella sero-positivity (Agasthya 2007).[16] Tsend *et al*. (2014)[17] also reported animal contact as a significant risk factor (OR = 2.8: 95% CI= 1.5–5.0: p<0.001) for brucellosis among people of Mongolia.[15] Gemechu *et al*. (2011)[18] in a study conducted in India also noticed animal contact a statistically significant factor (OR =4.636: CI =1.202–17.883: p<0.026) associated with brucellosis. In a study performed at Goa, India study, serum samples from cases of pyrexia of unknown origin (PUO) and occupationally exposed individuals were collected and tested for brucellosis showed a high prevalence (6.02%) of brucellosis among tested population (Ajay *et al*., 2014). [19] Similar findings have been reported by Nusrat (2004)[3]

Infected animals become carrier and excrete brucellae in milk, urine, vaginal secretions throughout their life. In addition to this aborted fetuses are rich source of organisms. Brucella gain entry into the human body

through breaks in the skin, mucous membranes, conjunctivae, and respiratory and gastrointestinal (GI) tracts. Conjunctival exposure through eye splash and inhalation are the most common routes of entry. Minimum infective dose of Brucellae required to induce infection through respiratory route is low (Bossi, 2004)[20] compared to oral infection (Izadjoo, 2004).[21] The possible explanation for high likeliness of becoming infected with brucellosis in persons having contact with animals, may be that these workers may have prolonged and direct exposure to Brucella infected animals at the time of parturition, milking, slaughtering thereby increasing chances of getting infection not only through skin cuts and abrasions but also through conjunctival route or by inhalation of organism in heavily saturated air.

Conclusion

Animal handlers of district Rawalpindi were infected with brucellosis. Animal handlers and persons consuming raw milk are more likely to get brucellosis. Small ruminants of district Rawalpindi have brucellosis. Presence of brucella infection in small ruminants of Rawalpindi is suggestive of brucellosis all across the country. Infected small ruminants are source of infection for human therefore awareness at public level is necessary to limit transmission of disease from infected animals to human. Up till now no human vaccine is available, treatment of brucellosis in human is prolonged and in most of cases chances of relapse are higher so control of disease in animal is the only way to prevent brucella infection in human. A control strategy for brucellosis in small ruminants using Rev 1 vaccination is recommended to prevent transmission of disease to human.

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