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A STUDY ON THE SEROPREVALENCE OF BRUCELLOSIS IN HUMAN AND GOAT POPULATIONS OF DISTRICT BHIMBER, AZAD JAMMU AND KASHMIR

Din, A. M. U. [1], S. A. Khan[2*], I. Ahmad[3], R. Rind[1], T. Hussain[4], M. Shahid[5] and S. Ahmed[6]

[1]Department of Microbiology, Sind Agriculture University, Tandojam; [2]Department of Pre-Clinical Studies, Faculty of Veterinary & Animal Sciences, University of Azad Jammu & Kashmir; [3]Department of Animal Health, Agriculture University Peshawar; [4]National Institute of Agriculture and Biology, Faisalabad; [5]Veterinary Research Institute, KPK, Peshawar; [6]Stockholm University Sweden
Corresponding author: mughal_161@yahoo.com

ABSTRACT

The present study was design to determine the seroprevalence of brucellosis in human and goat population. A total of 300 blood samples, comprising of 150 each from goats and humans were randomly collected from District Bhimber Azad Jmmu and Kashmir. Out of the 150 blood samples 75 were collected from each males and females. The serum samples were tested for the presence of anti-Brucella antibodies by Rose Bengal Plate Test (RBPT), Serum Plate Agglutination Test (SPAT) and Serum Tube Agglutination Test (STAT). The overall prevalence of brucellosis in goats was recorded as 13.33%, 11.33% and 9.33% by RBPT, SPAT and STAT, respectively and in humans was found to be 9.33%, 7.33% and 6.0% by RBPT, SPAT and STAT, respectively. The sex-wise prevalence of brucellosis in male goats was recorded as 10.66%, 8.0% and 6.66% by RBPT, SPAT and STAT, respectively; while in females it was 16.0%, 14.66% and 12.0% by RBPT, SPAT and STAT respectively. In males of human, the seroprevalence of brucellosis was recorded 6.66%, 5.33%, and 4.0%, whereas in females it was 12.0%, 9.33% and 8.0% by RBPT, SPAT and STAT, respectively. The prevalence of brucellosis was relatively higher in goats as compared to humans, irrespective of techniques used. It was also concluded that brucellosis was higher in females than in males in both goats and humans. Among all the serological tests applied in the present study, RBPT was found to be more sensitive and showed higher prevalence of brucellosis in goat as well as in human populations.

Key words: Brucella, Seroprevalence, Antibody Titre, Rose Bengal Plate Test, Serum Tube Agglutination Test, Serum Tube Agglutination Test.

INTRODUCTION

Brucellosis is a global zoonotic disease associated with significant morbidity that can lead to increased rates of spontaneous abortions in livestock and also in humans. In Pakistan, brucellosis is still remaining one of the major disease problems that affect animal industry as well as human health. Brucellosis is also known as Bang's disease, contagious abortion, infectious abortion, undulant fever, Malta fever and Mediterranean fever. The disease is widely distributed throughout the developing world, considered to be a serious problem in at least 86 countries (WHO, 1996). The *Brucella* species are non-motile, non-sporing, aerobic (but may require 5-10% CO_2 for growth except *Brucella suis* and *Brucella canis*), small Gram-negative rods or coccobacilli. Among the various species of *Brucella*, several strains exist and these are defined as biotypes. Nine biotypes have been recognized in bovine brucellosis. The incubation period is usually 1-6 weeks (Blood *et al.,*1983 and Brooks *et al.,* 1998). The organisms become localized in the reticulo-endothelial tissues, namely, the lymph nodes, liver, spleen, kidneys and bone marrow. Within these tissues, the organisms multiply within the macrophages. Humans become infected upon contact with animals or animal products that are contaminated with these brucellosis species. Severe infection of the central nervous system or lining of the heart may occur. Brucellosis can also cause long-lasting or chronic symptoms that include recurrent fevers, joint pain and fatigue. The symptoms of brucellosis in human includes, malaise, chills, fever (39-40°C), weakness, headache, backache, anorexia and weight loss, undulant fever can continue for weeks to years (Chamberlain, 2003). Brucellosis in goats is recognized clinically by an abortion usually taking place from 4 months and onwards i.e.; last trimester of pregnancy. There is retention of fetal membranes. The organisms are likely to get localized in the supra-mammary lymph nodes. In bull, the genital organs are affected leading to obvious manifestation of epididymitis or orchitis. These changes may be noted in seminal vesicles and ducts differentia. *Brucella* infection frequently present in peoples who are in direct contact with infected cattle and buffalo herd, manure, milk and its by products. After incubation period, non-specific symptoms such as fever, chills, fatigue, malaise, night sweat and anorexia appear. *Brucella melitensis* infections tend to be more severe and prolonged, whereas those caused by *Brucella abortus* are more self-limited.

Although *B. melitensis* accounts for most recorded cases, *B. abortus* and *B. suis* cause substantial morbidity in countries in which they persist in domestic animals, notably in Asia and Latin America. *B. canis* rarely causes overt human disease, and *B. neotomae* and *B. ovis* have not been identified as causes of infection in humans. The presence of brucellosis in wild animals, with a potential for continuous transfer to domestic animals and from them to humans is another epidemiological issue (Cutler *et al.,* 2005). The species that may infect man are *B. melitensis, B. suis, B.abortus* and *B. canis*. *B. melitensis* colonizes in ovine stock and is the frequent cause of brucellosis, in humans. Bhimber is the fourth largest district of Azad Jammu and Kashmir having a total area of 1516 km square divided into three subdivisions / Tehsils (Bhimber, Barnala, Smahni,) and a total population of more than 343000 (0.343 million). Different breeds of goats are reared by the people of this area for milk and meat production. The exact current situation about the prevalence of brucellosis in the area is unknown. Therefore, the current study will provide the latest status of brucellosis in human and goat populations of District Bhimber, Azad Jammu and Kashmir and to know the species wise and sex wise prevalence of brucellosis in the area. Similarly it was also designed to compare the sensitivity of Rose Bengal, Serum Tube Agglutination Test and Serum Plate Agglutination Test for the diagnosis of brucellosis in humans and goats as these are more simple, reliable and economical tests. This study also shows the zoonotic ratio of brucellosis between human and goat populations and provides good information about the prevalence of disease and planning strategies for its control.

MATERIALS AND METHODS

Collection of Blood samples: A total of 300 blood samples were collected from goats and humans in District Bhimber, Azad Jammu and Kashmir. Out of total samples 150 were collected from each species including 75 each from both sexes. After collection, the blood samples were labeled accordingly. The blood samples were kept in refrigerator overnight and then the serum was isolated from each sample and transferred to other tubes and labeled accordingly. After separated, the serum samples were freezed and then shifted to Veterinary Research Institute, Khyber Pakhtunkhwa, Peshawar.

Samples processing: All the serum samples were investigated for anti-*Brucella* antibodies using three different serological techniques including Rose Bengal Plate Test (RBPT), Serum Plate Agglutination Test (SPAT) and Serum Tube Agglutination Test (STAT). A questionnaire was also filled for getting information about the animal age, sex, breed, management system, reproductive problems, number and species of animals in the flock and owners/human subjects name, sex, age, living condition, utilization of raw goat milk etc by either interviewing the animals owner or the human individual by him/herself.

Antibody Titration: Two fold dilutions of all serum samples were made and antigen for *Brucella abortus* and *Brucella melitensis* was added to all dilutions. Antibodies were serially diluted in the test tubes, a 0.1ml of the test serum was added to the first tube. Mixed well and 1.0 ml of the diluted serum was then transferred from first tube to the second tube. After mixing thoroughly, 1.0 ml of the diluted serum was transferred from the second tube to third tube. This procedure was repeated till tube number ten. And then 1.0 ml of the diluted serum was discarded from tube ten and left 1 ml in tube ten. The series of ten tubes then contained 1.0 ml each of serial two fold dilution of 1:20 to 1:1024. However, tube number one was considered as a 1:20 dilution.

RESULTS

The overall seroprevalence of brucellosis in goats was recorded as 13.33%, 11.33% and 9.33%, while in humans was founded to 9.33%, 7.33% and 6.0% by RBPT, SPAT and STAT respectively. Seroprevalence of brucellosis was recorded higher in goats as compared to human populations, irrespective of the techniques (Table-1). Among these serological tests, the RBPT showed higher (13.33% and 9.33%) prevalence of brucellosis in both goats and humans as compared to SPAT and STAT. The STAT showed lower (9.33% and 6.0%) incidence of brucellosis in both, goat and human populations.

Out of 150 goats the seroprevalence of brucellosis in male goats was recorded as 10.66%, 8.0% and 6.66% by RBPT, SPAT and STAT respectively, while in female goats it was 16.0%, 14.66% and 12.0% by RBPT, SPAT and STAT respectively. It is obvious from the data that a higher prevalence of brucellosis was recorded in female goats as compared to male goats (Table 2). Similarly out of 150 human samples, the seroprevalence of brucellosis in males was recorded as 6.66%, 5.33% and 4.0%, while in females it was recorded as 12.0%, 9.33% and 8.0% by RBPT, SPAT and STAT respectively. Generally it was observed that irrespective of any techniques used in the present study, a higher prevalence of brucellosis was recorded in female as compared to male.

Antibody titre: During present study serum samples were also examined for antibody titre by using Serum Plate Agglutination Test (SPAT). Out of 150 goat serum samples, 17 (11.33%) were reactive and showed antibody titre at dilutions of 1:40, 1:80 and 1:160. Of these 17 positive reactors, 9 samples showed antibody titre at dilution of 1:40, 5 samples at dilution of 1:80 while 3 samples at dilution of 1:160 (Table 4). Similarly in

humans, out of 150 samples, only 11 (7.33%) sera were reacted and showed antibody titre at dilutions of 1:40 and 1:80. Among 11 positive reactors, 6 showed antibody titre at dilution 1:40 while remaining 5 sera showed antibody titre at dilution of 1:80 (Table 4).

The antibody titre examined by STAT: From 150 goat serum samples, 14 (9.33%) interacted with antigen and showed antibody titre at dilutions of 1:40, 1:80 and 1:160. Out of 14 positive samples, 9 showed antibody titre at dilution of 1:40, while 4 samples at dilution of 1:80, however, 1 sample showed antibody titre at dilution of 1:160 (Table 5). In the same way, out of 150 human serum samples 9 (6.0%) were reactive and showed antibody titre at dilutions of 1:40 and 1:80. However, from 9 positive reactors, 6 showed antibody titre at dilution of 1:40 while 3 samples at dilution of 1:80 (Table 5).

Comparative seroprevalence of *Brucella:* A total of 150 serum samples, obtained from goat, the prevalence of *Brucella abortus* was recorded as 10 (6.66%) by SPAT and 8 (5.33%) by STAT. This was the total prevalence of *Brucella abortus* either in separate form or in combined form with *Brucella melitensis.* While the seroprevalence of *Brucella melitensis* was found to be 13 (8.66%) by SPAT and 11 (7.33%) by STAT. The number of those positive samples containing reactive antibodies against both *Brucella abortus* and *Brucella melitensis* antigens was found to be 6 (4.0%) by SPAT and 5 (3.33%) by STAT. However, similar pattern of the prevalence of *Brucella abortus* and *Brucella melitensis* was demonstrated in 150 samples of humans using the same serological techniques. The prevalence of *Brucella abortus* was recorded as 7 (4.66%) by SPAT and 5 (3.33%) by STAT. While prevalence of *Brucella melitensis* in human samples was found to be 9 (6.0%) by SPAT and 7 (4.66%) by STAT. Furthermore some of the samples were found to be reactive to both *Brucella abortus* and *Brucella melitensis* antigens. In humans, the number of samples were demonstrated as positive for both, *Brucella abortus* and *Brucella melitensis* and recorded as 5 (3.33%) by SPAT and 3 (2.0%) by STAT.

The comparative sensitivity of tests: Of 300 serum samples, 34 (11.33%) were found positive by RBPT, 28 (9.33%) by SPAT and 23 (7.66%) by STAT, respectively as shown in Table-7. The RBPT was found to be more sensitive than other two techniques and showed a higher prevalence of brucellosis in human as well as in goat populations. The Serum Tube Agglutination Test (STAT) was found less sensitive but its results were found to be more reliable because it consists of a proper dilution method and showed qualitative as well as quantitative results about the antibody titer against brucellosis.

Table-1. The seroprevalence of brucellosis in goat and human populations examined by various serological techniques

Techniques used	Goats			Humans		
	Total No. of serum samples examined	No. of positive samples	%age of positive samples	Total No. of serum samples examined	No. of positive samples	%age of positive samples
RBPT	150	20	13.33%	150	14	9.33%
SPAT	150	17	11.33%	150	11	7.33%
STAT	150	14	9.33%	150	9	6.0%

Table-2. The seroprevalence of brucellosis in male and female of goats investigated

Techniques used	Male			Female		
	Total No. of serum samples examined	No. of positive samples	%age of positive samples	Total No. of serum samples examined	No. of positive samples	%age of positive samples
RBPT	75	8	10.66%	75	12	16.0%
SPAT	75	6	8.0%	75	11	14.66%
STAT	75	5	6.66%	75	9	12.0%

Table-3. The seroprevalence of brucellosis in male and female of human populations

Techniques used	Male			Female		
	Total No. of serum samples examined	No. of positive samples	%age of positive samples	Total No. of serum samples examined	No. of positive samples	%age of positive samples
RBPT	75	5	6.66%	75	9	12.0%
SPAT	75	4	5.33%	75	7	9.33%
STAT	75	3	4.0%	75	6	8.0%

Table-4. The antibody titre against brucellosis in positive reactors of goat and human populations examined by SPAT.

Species examined	Sex	Total No. of serum samples examined	Total No.of positive samples	SPAT Agglutination Titres				
				1:40	1:80	1:160	1:320	1:640
Goats	Male	75	6	3	2	1	0	0
	Female	75	11	6	3	2	0	0
Humans	Male	75	4	2	2	0	0	0
	Female	75	7	4	3	0	0	0

Table-5. The antibody titre against brucellosis in positive reactors of goat and human populations examined by STAT.

Species examined	Sex	Total No. of serum samples examined	Total No.of positive samples	STAT Agglutination Titres				
				1:40	1:80	1:160	1:320	1:640
Goats	Male	75	5	4	1	0	0	0
	Female	75	9	5	3	1	0	0
Humans	Male	75	3	2	1	0	0	0
	Female	75	6	4	2	0	0	0

Table-6. The seroprevalence of *Brucella* abortus and *Brucella melitensis* in goat and human populations.

Species examined	Techniques used	Total No. of serum samples examined	Total No. of positive samples	Samples positive for *B. abortus*	%age of positive samples	Samples positive for *B. melitensis*	%age of positive samples	Samples positive for both, *B. abortus* and *B.melitensis*	%age of positive samples
Goats	SPAT	150	17	10	6.66%	13	8.66%	6	4.0%
	STAT	150	14	8	5.33%	11	7.33%	5	3.33%
Humans	SPAT	150	11	7	4.66%	9	6.0%	5	3.33%
	STAT	150	9	5	3.33%	7	4.66%	3	2.0%

Table-7. The comparative sensitivity of different serological techniques applied in present the study.

Techniques used	Total No. of serum samples examined	No. of positive samples	%age of positive samples
RBPT	300	34	11.33%
SPAT	300	28	9.33%
STAT	300	23	7.66%

DISCUSSION

The overall seroprevalence of brucellosis in goats was recorded to be 13.33% by RBPT, 11.33% by SPAT and 9.33% by STAT respectively. While in humans the prevalence was recorded as 9.33% by RBPT, 7.33% by SPAT and 6.0% by STAT, respectively. Generally, all serological tests showed similar results, however, it seems that RBPT recorded relatively higher prevalence of brucellosis in both humans and goats as compared to other two serological tests and was found in line with the results of Omer *et al.* (2000) who screened-out the samples from goats and other domestic animals for *Brucella* infection by Rose Bengal Test at the rate of14.3% by RBPT in goats. The results of the present study are also in line to that of Jakson *et al.* (2004) who investigated the seroprevalence of brucellosis by using the Rose Bengal test as 7·24%. The mean value of prevalence as obtained in the present study was in accordance to the range reported by Mahboob (2005) 4.0, 4.67 and 10.0% , while Riaz (2006) also obtained similar results an overall seroprevalence of brucellosis as 9.88% by SPAT and 5.88% by STAT.

Similar findings regarding the seroprevalence were reported by Wali (2005) who recorded seroprevalence in 2.0% males by SPAT and 13.38% by STAT in female Goats. A study from Pakistan made by Riaz (2006) regarding the seroprevalence as 5.71% males and 12.73% females by SPAT, while by STAT it was recorded as 1.43% and 8.79% in males and females, respectively. Similarly in male of human the seroprevalence of brucellosis was found to be 6.66, 5.33

and 4.0% by RBPT, SPAT and STAT respectively. While in female of humans was observed as 12.0, 9.33 and 8.0% by RBPT, SPAT and STAT respectively. These results are also in close agreement with those of Fevziye (2005) who recorded higher prevalence in females (3.7%) as compared to males (2.9%) however, similar results were recorded by Azhar *et al.* (2009) who analyzed a higher seroprevalence in females than males. Though, the above mentioned authors did not use other serological tests.

Hunduma and Regassa (2009) studied the prevalence in 12.2% female and 9.8% in male goats using the Rose Bengal Plate Test. While Junaidu *et al.* (2010) also recorded a higher prevalence of brucellosis in female as compared to male goats by using Rose Bengal Plate Test (RBPT), the Serum Agglutination Test (SAT) and the Competitive ELISA (complisa) for brucellosis as in goats, the prevalence of *Brucella abortus* was found to be 6.66 and 5.33% by SPAT and STAT, respectively. The prevalence of *Brucella melitensis* was recorded to be 8.66 and 7.33% by SPAT and STAT, respectively. While 4.0 and 3.33% goats were found to be positive by SPAT and STAT for both *Brucella abortus* and *Brucella melitensis* respectively. While in humans, the prevalence of *Brucella abortus* was found to be 4.66 and 3.33% by SPAT and STAT, respectively while that of *Brucella melitensis* it was reacted as 6.0 and 4.66% by SPAT and STAT, respectively. The *Brucella abortus* and *Brucella melitensis* were found in 3.33, and 2.0% by SPAT and STAT, respectively. In both, goat and human populations, a higher prevalence of *Brucella melitensis* was observed as compared to *Brucella abortus.* Similar pattern of results were observed by Jakson *et al.* (2004) Whereas, Mehboob (2005) recorded similar pattern of results on the seroprevalence of brucellosis in goats. The incidence of *Brucella abortus* and *Brucella melitensis* through the sera of goats was recorded as 3.33% and 4.0%, respectively, however, the results of the present study are in close agreement to the findings of the above worker.

Conclusion: From present investigation it is concluded that the brucellosis is prevailing in goat and human of the areas as determined by different techniques. The bacterial species *Brucella abortus* and *Brucella melitensis* were identified as the only species causing brucellosis in goat and human, respectively. The higher incidence of brucellosis was recorded in goat (13.33%) and in human (8.66%) by Rose Bengal Plate Test (RBPT). It is also recorded that brucellosis was relatively higher in females than in males in both goats and humans. The serum antibody titre of *Brucella abortus* and *Brucella melitensis* was also determined which interacted with antigen at dilutions of 1:40, 1:80 and 1:160, however, beyond these dilutions, no interaction between antigen and serum antibodies was observed. Furthermore, the prevalence of *Brucella melitensis* was recorded to be higher than *Brucella abortus.* It is also concluded that Rose Bengal Plate Test (RBPT) was found to be more sensitive; however, it was observed that this test some times may produce false results. During study it is also observed that the Serum Tube Agglutination Test (STAT) was found to be highly specific, and accurate which produced more accurate qualitative and quantitative results as compared to other serological techniques used in the present study.

Recommendations: Keeping in view the facts and figures, the author recommended the following measures should be adopted to reduce the prevalence of brucellosis in humans and goats in the area in particular and in the country in general. A frequent serological examination must be carried-out to know the prevalence of brucellosis in all animals and the suspected/infected should be separated/slaughtered immediately. While dealing with brucellosis, veterinarian should take care and adopt all protective measures so as to make sure that all contaminated or infected materials such placenta, aborted fetus etc should be destroyed/buried properly to reduce chances of spreading the infection in animals as well as in humans. Milk must be pasteurized before consumption as it is a potential source of infection from animals to human beings.Proper awareness regarding brucellosis in animals and infection related risk factors to human health, and its economical losses in terms of reduced livestock production should be provided through mass media to public to help in its eradication from the area.

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