RESEARCH ARTICLE

Study on Seroprevalence of Brucellosis in Caprine in Kohat (Khyber Pakhtoon Khwa)

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Abstract. - A study was conducted on Sero prevalence of the brucellosis in goats, at Kohat District of KHYBER PAKHTOON KHWA. A total of 200 serum and 150 milk samples from goats were collected and examined through Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT) and Milk Ring Test (MRT). The overall seroprevalence of brucellosis in goats was recorded as 4%, 3% and 3.33% by RBPT, SAT and MRT respectively. In this survey, 100 serum samples each from male and female goats were collected and tested. The seroprevalence of brucellosis in females was recorded as 6%, 4% and 3.33% by RBPT, SAT and MRT respectively, while in males it was observed as 2% and 2% by RBPT and SAT respectively. The seroprevalence of brucellosis in different age groups of goats was also studied. A total of 200 adult goats, 100 from each males and females, were tested. From 100 adult females (above 12 months age), 6%, 4% and 3.33% were detected as positive by RBPT, SAT and MRT respectively while in 100 young females (up to 9 months of age), no brucellosis was detected and considered to be free from brucellosis. A similar number of 100 adult males (above 12 months age) were examined by RBPT and SAT and recorded 2% positive for brucellosis while no brucellosis was recorded by the above techniques in 100 young males (up to 9 months of age) and thus considered to be negative for brucellosis. The sera of 100 females were titrated for antibody titer, 2 sera reacted positively with antigen at 1:20, 1 at 1:40 and 1 at 1:80 dilutions; beyond these dilutions no interaction between antigen and serum antibody of positive cases was detected. Similarly antibody titration was carried out against male goats by SAT. Only 2 serum samples were found positive for Brucella antibody, from which 1 interacted with antigen at dilution 1:20 and 1 at 1:40. However, beyond these dilutions, the interaction between antigen, and antibody was not observed. The incidence of Brucella species was also investigated by different techniques. The incidence of Brucella abortus and Brucella melitensis in the sera of goats was recorded by RBPT as 3% and 4%, and by SAT as 2.50% and 3% respectively.

Key words: — Seroprevalence, Brucellosis, RBPT, SAT, MRT, Kohat

INTRODUCTION

Livestock is an important sector of agriculture in Pakistan, which accounts for 50% agricultural value, about 11% in the total GDP, of the country. The role of livestock in rural economy is realized from the fact that 30-35 million rural population is engaged in livestock raising, having household holdings of 2-3 cattle/buffaloes and 5-6 sheep/goats per family, that help them to derive 30-40% of their income from it. Rapid economic development is resulting in considerable pressure on the livestock sector to increase its output, as demand for meat and milk is increasing rapidly. In Pakistan, the livestock population was 26.3 million buffaloes, 24.2 million cattle, 56.7 million goats, 24.9 million sheep, 366.0 million poultry and 0.8 million camels. Among livestock products, the production of beef was 1,115,000 tons, mutton 740,000 tons and poultry meat 416,000 tons during the year 2004-2005 GOP (2005). According to world livestock census, the goat population of world is 446 million, while in Pakistan it is 47.3 million. The highest growth rate of goats during last two decades is recorded as 79% while that of buffaloes, cattle and sheep as 77, 37 and 27% respectively FAO (2001). Goats are very curious, and like toddlers, they explore the world by putting everything in their mouths. As for feeding is concerned, goats are not lawn mowers; they are opportunistic feeders
and they will eat whatever plant life is available, including roses, tree leaves, bark, grass and forest plants. But if they are producing anything, such as milk, as dairy goat would or are in confinement, they require some grains, as well as high quality hay at free choice. Beside other reproductive and genetic defects, goats, cattle and buffaloes their reproductive efficiency is greatly affected by several bacterial, viral and fungal diseases. The brucellosis is the disease of genital tract of male and female animals which causes infertility and transmit the bacterial agent through the copulation. The bacterial agents, Brucella is a minute non–motile small Gram-negative coccoid rods causing brucellosis, contagious abortion, Bang’s disease etc. The genus Brucella contains four species which are Brucella abortus, Brucella melitensis, Brucella suis, Brucella branchiseptica Merchant and Packar (1999). Goat suffers a lot of reproductive and non-reproductive viral, fungal and bacterial diseases. Among all the diseases, brucellosis causes infertility and transmits the bacterial agent easily from male to female through mating. Animals suffering from such disease should be isolated immediately and antibiotic treatment may be provided by Chloromycetin and Aureomycin Panhwar (2004). In most of the countries Brucellosis is a serious problem regarding public health and economic significance both. It is recognized world widely and is considered to be an important disease of cattle, buffaloes, goats, sheep and men etc. The incidence of the disease is associated with demographic and geographic factors. Seroprevalence of the disease has been reported up to 4.4% from different areas of Pakistan Ahmed et al. (1990). Abortion during late pregnancy is the most obvious sign in goats and sheep. The infection is systemic in reaction with fever, depression, loss of weight and some diarrhea, these signs may also occur in acute, natural outbreaks in goats by mastitis, laminitis, hygroma etc. Brucella infection is frequently present in people who are indirect contact with infected goat herds, manures, milk and its by-products. An increase in commercialization of goat keeping was observed during the last two decades. It reflects the interest of Pakistani farmers in goat keeping on commercial basis and as well as domestic scales Qureshi (2001). In hilly areas of Pakistan goats are mostly reared for production of milk, meat and skin Qureshi (2001) The present study was therefore designed to Determine serologically presence of brucellosis in goats of Kohat, Khyber Pukhtoon Khwa. It provided the baseline data on the prevalence of the brucellosis in the area. To compare the sensitivity among Rose Bengal, Serum Agglutination and Milk Ring tests for the diagnosis of brucellosis in the goats. To check the seroprevalence of brucellosis in male and female goats by using RBPT, SAT and MRT.

MATERIALS AND METHODS

Collection of Blood Samples

A total of 200 blood samples, 100 from each male and female goat were randomly collected for the present investigation from NWA. Bannu, Karack, Kohat Districts of KHYBER PAKHTOON KHWA province. Blood samples from goats were obtained through jugular vein by disposable sterilized plastic syringes. Before collection, the site of the collection was properly shaved and disinfected. The blood was collected and then left it in slanting position to clot at least for half an hour. After that, blood was then kept in refrigerator for overnight. On the following day, the serum was harvested in clean screw caped plastic bottles and brought to the disease investigation laboratory (D.I. Lab) Kohat and stored at –20°C in deep freezer till used.

Collection of Milk Samples

A total of 150 milk samples were collected from the same animals (female) from which blood samples were already collected. Before collection, the teats of the goats were cleaned with antiseptic and then first few drops of milk were discarded and then collected in screw caped bottles. After collection of milk samples in the bottles, the same were caped tightly and brought to the disease investigation laboratory, Kohat for further investigation.

Analysis Methods

Rose Bengal Plate Test (Slide Agglutination Test) as described Zahid et al. 2002. Rose Bengal stained antigen containing Brucella abortus cells (strain 99) were suspended in buffer with pH 3.6. The anitigen was prepared
Fig. 1: Map showing the study areas of District Kohat

and standardized as recommended by Veterinary Research Institute, Lahore. Test procedure
Before conduct of test, the antigen and serum samples were kept at room temperature to bring
them in their normal physiological state.

A drop of 0.03ml of the serum, using serological pipette was placed on the centre of a
square of clear transparent glass slide for Brucella abortus while two drops of serum were
placed on another square of the slide for Brucella melitensis. Similarly, a drop of negative control and a drop of positive serum
were placed separately on the squares of the slide. Before taking the antigen, the vial was
shaken gently to make a uniform suspension and then a drop of 0.03 ml quantity of antigen
suspension was taken from the vial and placed near to the drops of serum to the squares. Using
an applicator stick, the serum and antigen were thoroughly mixed and each mixture was spread
in the form of a circle over an area of approximately 1.5 cm radius. A separate applicator stick was used for each serum on the
squares of the glass slide. The slide was then gently rocked back and forth for no longer than
four minutes. The slide was then macroscopically examined, using magnifying glass for agglutination under a good source of
light against a dark background field. The positive interaction between antigen and serum,
the appearance of granules with different intensity indicated the level of antibodies in the
serum of the animal infected with specific species of bacterial organisms.

Milk Ring Test (MRT). Antigen and milk samples Hematoxylin stained Brucella abortus
strain 99 antigen was used for abortus Bang Ring test for the diagnosis of Brucellosis,
obtained from Veterinary Research Institute (VRI), Lahore. Test Procedure described by
Abbas and Aldeewan, (2009). A 1 ml quantity of milk sample was distributed on to each test tube,
while other two test tubes were also placed in the same rack as a control antigen, one of which
contained Brucella positive milk sample and other Brucella negative milk sample (control). A
0.05 ml quantity of Milk Ring Test stained antigen (hematoxylin stained antigen) was added
to each tube by using graduated pipette. The rack in which the tubes were placed shaken
gently. The antigen and test milk samples were mixed thoroughly and allowed to stand for about
2 minutes. Then the rack was placed in an incubator at 37 °C for 1 hour. After incubation,
the test tubes were taken-out from the incubator, examined and results were recorded.

Note: A deep blue coloration ring appears on the top of milk is considered to be positive result.
No ring formation appears on the top of milk sample considered to be negative result. Serum Agglutination Test (SAT) antigen and serum Antigen was obtained from Veterinary Research Institute Lahore prepared and standardized according to the procedure given by (Chachra et al., 2009). Test Procedure Sodium chloride solution 0.9% containing 0.5% phenol (normal physiological solution) test tube rack, serological pipette, and an incubator capable of maintaining a temperature of 37 °C. Five conical agglutination tubes were placed in the rack for each serum sample.

0.8 ml of normal saline solution containing 0.5% phenol was added to the first tube and 0.5 ml in the remaining four tubes.

0.2 ml of the serum to be tested was added to the first tube and mixed thoroughly, this will make 1/5 dilution.

0.5 ml was withdrawn from the first tube and transferred into the 2nd tube. After mixing well, 0.5 ml was discarded. Now the dilution in each tube left 1/5, 1/10, 1/20, 1/40, and 1/80 respectively.

A 5 ml of the standardized Brucella abortus concentrate antigen diluted 1:10 were added to each tube, containing serum dilution, giving a series of final dilutions 1/10, 1/20, 1/40, 1/80 and 1/160 respectively. Serum and antigen suspensions were then mixed thoroughly from the highest dilution to the lowest i.e. 1/60 to downward. The known positive and negative sera were kept as controls. The rack containing tubes was then incubated at 37°C for 18-20 hours. The time could be reduced by keeping rack in a water bath at 60°C for 1 hour.

After incubation, the results were recorded based on clearing of the suspension along with clumping of the organisms and persistency of the sediments upon gentle shaking.

RESULT

A total of 200 serum and 150 milk samples from goats were collected and examined through Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT) and Milk Ring Test (MRT). It seems that both, SAT and MRT techniques detected a little difference in the percentage prevalence of brucellosis in goats. The Rose Bengal Plate Test showed relatively higher prevalence 4% as compared to Milk Ring Test and Serum Agglutination Tests, both of these generated 3.33% and 3% respectively. However, the Rose Bengal Plate Test was considered to be the best technique that exhibited higher prevalence. From the present results obtained by different techniques, the Serum Agglutination Technique was found the best method that recognized antibody titers in the sera of infected goats. In the present survey, 96%, 97% and 96.66 goats were recorded serologically negative by Rose Bengal Plate Test, Serum Agglutination Test and Milk Ring Test respectively (Table I).

The seroprevalence of brucellosis in male and female goats.

Another parameter investigated was to check the seroprevalence of brucellosis in male and female goats by using RBPT, SAT and MRT. During present survey, the prevalence of brucellosis in females was recorded as 6.00%, 4% and 3.33% by RBPT, SAT and MRT respectively. While in the prevalence of males brucellosis was recorded or 2% and 2% by RBPT and SAT respectively. Whereas 94%, 96% and 96.66 female goats were found seronegative for brucellosis by RBPT, SAT and MRT respectively. However 94% and 98% male goats were found negative for brucellosis by RBPT and SAT respectively. It is concluded that a relatively higher prevalence of brucellosis was recorded in females by Serum Agglutination Test as compared to males by any other techniques used during present study. Furthermore, when a compression was made between the sexes of higher seroprevalence of brucellosis, 6% was recorded in females. It was obvious that irrespective of any techniques applied, a risk of higher prevalence of brucellosis was evidence in females as compared to any other category of goats investigated during present survey (Table II).

The seroprevalence of brucellosis in different age groups of goats

During present investigation, 100 adult goats (above 12 months) were examined by Rose Bengal Plate Test (RBPT), Serum Agglutination (SAT) and milk of 150 adult female goats were also examined by Milk Ring Test (MRT). A total of 100 young goats (upto 9 month) were...
tested by RBPT and SAT, all were found for brucellosis negative (Table III). While the adult goats (above the 12 months) were tested for brucellosis, 8, 6 and 3.33% were found positive for brucellosis by RBPT, SAT and MRT respectively. Finally seronegative percentage was also observed in adult goats which were recorded as 92%, 94% and 96.66% respectively by RBPT, SAT and MRT. While in young male goats (upto 9 months) no any single case was found positive for brucellosis by RBPT and SAT (Table III).

Table I.- The seroprevalence of brucellosis in goats determined by various conventional techniques applied during present survey.

<table>
<thead>
<tr>
<th>Techniques used</th>
<th>Total No. of samples examined</th>
<th>No. of positive reactors</th>
<th>% of positive reactors</th>
<th>No. of negative reactors</th>
<th>% of negative reactors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rose Bengal Plate Test (RBPT)</td>
<td>200</td>
<td>8</td>
<td>4</td>
<td>192</td>
<td>96</td>
</tr>
<tr>
<td>Serum Agglutination Test (SAT)</td>
<td>200</td>
<td>6</td>
<td>3</td>
<td>194</td>
<td>97</td>
</tr>
<tr>
<td>Milk Ring Test (MRT)</td>
<td>150</td>
<td>5</td>
<td>3.33</td>
<td>145</td>
<td>96.66</td>
</tr>
</tbody>
</table>

Table II.- The seroprevalence of brucellosis in different sexes of goats determined by various conventional techniques used during present study.

<table>
<thead>
<tr>
<th>Techniques used</th>
<th>Female goats</th>
<th>Male goats</th>
<th>Total No. of samples examined</th>
<th>No. of positive reactors</th>
<th>% of positive reactors</th>
<th>No. of negative reactors</th>
<th>% of negative reactors</th>
<th>Total No. of samples examined</th>
<th>No. of positive reactors</th>
<th>% of positive reactors</th>
<th>No. of negative reactors</th>
<th>% of negative reactors</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBPT</td>
<td>100</td>
<td>6</td>
<td>94</td>
<td>94</td>
<td>2</td>
<td>2</td>
<td>98</td>
<td>98</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAT</td>
<td>100</td>
<td>4</td>
<td>96</td>
<td>96</td>
<td>2</td>
<td>2</td>
<td>98</td>
<td>98</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>MRT</td>
<td>150</td>
<td>5</td>
<td>145</td>
<td>96.66</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Table III.- The seroprevalence of brucellosis in different age groups of goats determined by various conventional techniques used during present investigation.

<table>
<thead>
<tr>
<th>Techniques used</th>
<th>Adult goats (above 12 months)</th>
<th>Young goats (up to 9 months)</th>
<th>Total No. of samples examined</th>
<th>No. of positive reactors</th>
<th>% of positive reactors</th>
<th>No. of negative reactors</th>
<th>% of negative reactors</th>
<th>Total No. of samples examined</th>
<th>No. of positive reactors</th>
<th>% of positive reactors</th>
<th>No. of negative reactors</th>
<th>% of negative reactors</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBPT</td>
<td>100</td>
<td>8</td>
<td>92</td>
<td>92</td>
<td>00</td>
<td>00</td>
<td>100</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAT</td>
<td>100</td>
<td>6</td>
<td>94</td>
<td>94</td>
<td>00</td>
<td>00</td>
<td>100</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRT</td>
<td>150</td>
<td>5</td>
<td>145</td>
<td>96.66</td>
<td>-</td>
<td>-</td>
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</table>

DISCUSSIONS

The seroprevalence of brucellosis in 200 goats was recorded to as 8 (4%), 6 (3%) and 5 (3.33%) by RBPT, SAT and MRT respectively. Overall, it seems that all test techniques produced similar percentage of prevalence of brucellosis in goats. However, the Rose Bengal Plate Test showed relatively somewhat higher prevalence than other two tests, which recorded 4%, as compared to SAT and MRT, both, detected 3 and 3.33% respectively (Table I).

The data recorded for the seroprevalence of brucellosis in goats by Milk Ring Test in the present survey are similar to that of Desai and
Krishnappa (1997), they recorded 4% seroprevalence of Brucellosis in goats. By Rose Bengal Plate Test, they recorded positive cases, which are also very close to the findings observed in this survey by the same methods. Generally, the Serum Agglutination Test applied for brucellosis in goats and results recorded are comparable to Mirza et al. (1998) who reported similar results of the seroprevalence of brucellosis in goats. They detected 8.6% by Rose Bengal Plate Test (RBPT). It is clear from the present study that our results are in conformity and accordance with the above authors. Furthermore, similar findings were also recorded by Russo et al. (1998) who recorded higher prevalence in female (7.4%) than male goats (4.5%) by Serum Agglutination Test which is also very close to the present findings. Nevertheless, the results observed by other tests are not in line to the results of the present study, as they recorded relatively some higher seroprevalence in goats. The seroprevalence of brucellosis in goats and other domestic animals obtained by various workers through different techniques, which are also applied in this study, are not comparable because they detected higher prevalence as compared to the present study Klevezas et al. (2000), Grewal and Kaur (2000).

In this study, 100 male goats were examined by RBPT and SAT while similar number of female goats was also investigated by RBPT and SAT techniques. Furthermore, 150 milk samples from female goats were also examined by MRT. The prevalence of brucellosis in females was recorded as 6%, 4% and 3.33% by RBPT, SAT and MRT respectively. While in males, it was recorded as 2% and 2% by RBPT and SAT respectively. Singh et al. (2000) conducted and compared a dot ELISA Test with SAT, CFT and P- ELISA against Brucellosis. A significant brucellosis in 13 and 21 goats was detected by SAT and CFT respectively. However, the seroprevalence of brucellosis recorded by above workers in males and females in their investigations are in line to the findings of the present study, although they used SAT and CFT Tests which were also carried out in this study. The results are to some extent very similar to the findings recorded by above worker. Furthermore, irrespective of any analysis carried out by different workers, the higher prevalence of brucellosis was recorded in females as compared to males (El-Hafeez et al. 2001 and Esendal, et al. (2001).

From the present survey, it was also observed that higher prevalence is evident in females as compared to males. However, throughout the world, many workers recorded similar findings of prevalence of brucellosis in different sexes of goats is also recorded in the present investigation. So, the results of the present survey regarding the seroprevalence of brucellosis obtained do justify to the findings of other workers.

Many workers recorded seroprevalence in different sexes of goats which is relatively somewhat lower than the present study, therefore, present findings could not be compared to other seroprevalence recorded in male and female goats under different managemental and husbandry conditions where animals are being reared and kept for their source of income Mirza et al. (1998), Esendal et al. (2001) and Martinez et al. (2001).

During present study, 100 adult goats (above 12 months) were examined by RBPT and SAT and 150 adult female goats by MRT while 100 young goats (up to 9 months) by RBPT and SAT. The positive cases of brucellosis in adult goats were recorded as 8%, 6% and 3.33% by RBPT, SAT and MRT respectively while all young goats were found to be negative (Table III). The study clearly demonstrates that Brucella antibodies are evidence in the sera of goats of different ages. Furthermore, that the techniques, Serum Agglutination and Milk Ring Tests (for lactating animals) are the techniques could be applied for investigation of brucellosis in all age animals.

Sobrinho et al. (2000) conducted a serological study on 4 areas of Ceará State in Brazil, to determine the prevalence of brucellosis in goats. For this purpose a sera from 3244 goats were analyzed by Serum Agglutination Test (SAT) and Card Test (CT) for the diagnosis of brucellosis. The results showed that 3.14% of the samples were found reactive by SAT and 0.25% was observed positive by CT. However, our results do agree with the results of above authors who recorded the presence of Brucella in different ages.
Three techniques were used to monitor the seroprevalence of brucellosis in different categories of goats. A total of 100 adult female goats were investigated through Rose Bengal, Serum Agglutination and 150 milk samples by Milk Ring Test that detected 6, 4 and 3.33% brucellosis in adult female goats respectively.

Bale et al. (2003) recorded higher prevalence of brucellosis in adult goats of more than 4 years of age. Similarly in this study, the higher figures of a prevalence in adult males and females. While Martinez et al. (2003) divided the animals in five groups, the group-I and II were recorded as young goats, III and IV were considered to be adult goats and V were also adults; all the goats of the groups were vaccinated. Final observation was made in group I, 15%, in group II, 10%, in group III, 5%, in group IV, 15% and in group V, 40% of brucellosis in different categories of the goats was higher than the sera studied in this investigation.

CONCLUSIONS

From the present study it is concluded that the Brucellosis is prevailing in the area of Kohat, KPK. It was also observed that brucellosis was relatively higher in females as compared to males as determined by various techniques during the present study. Further, a higher prevalence of brucellosis was present in adult females (above 12 months) than in males. Overall study showed that the incidence of brucellosis was higher in adult of both sexes as compared to young animals of both sexes.

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