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Seroprevalence Studies on Caprine Brucellosis in Punjab (India)

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A B S T R A C T

The present study was carried out to determine the seroprevalence and risk factors associated with caprine brucellosis in Punjab, India. A total of 277 serum samples collected randomly from goats were tested by RBPT, MAT, STAT and i-ELISA. Out of 277 sera samples, 31 (11.19%), 44(15.88%), 49(17.68) and 52 (18.77%) samples were positive by RBPT, MAT, STAT and i-ELISA. Overall prevalence was 18.7%. The seroprevalence was found significantly ($p<0.05$) higher in females, non-descript breeds, animals in age group of 3-5 years, mixed flock, animals bred on farm, unorganized rearing system and animals kept in unhygienic conditions. Further analysis was done using multivariate logistic regression model to assess the association between the potential individual and management risk factors at $p\leq0.01$ at 5% level. Upon odds ratio analysis, parity, breeding practices, flock type, contact with other herd and lack of disinfection were considered the major risk factors associated with spread of brucellosis in animals.

Introduction

Brucellosis is a highly contagious and zoonotic disease of livestock caused by *Brucella* spp. The disease is associated with significant morbidity, leading to spontaneous abortions in livestock (Baek *et al.*, 2003 and Kakoma *et al.*, 2003). Brucellosis in goats is primarily caused by one of the three biovars of *Brucella melitensis* and rarely by *B. abortus* and *B. suis* (Corbel and Brinley, 1984 and Paolicchi *et al.*, 1993). The disease is characterized by abortion during the last trimester, retained placenta, weak offspring, metritis, drop in milk production and infertility. Disease can be transmitted by direct as well as indirect contact. Oral and venereal routes are considered to be the important

routes of transmission (Alton, 1990). During last 3-4 decades, *B. melitensis* is getting widespread in India causing abortion in small ruminants and imposing a great economic loss and a severe hazard to human health (Abeer *et al.,* 2003).

Diagnosis can be carried out by isolation of *Brucella*spp. from clinical samples and is considered the "gold standard" and confirmative test in diagnosis of brucellosis (Godfroid *et al.,*2010*).* Many serological tests like Standard tube Agglutination test (STAT), Rose Bengal Plate test (RBPT), Micro titre Plate Agglutination test (MAT) and Enzyme linked immune-sorbent assay (ELISA) have been used for detection of antibodies against *Brucella*. Nucleic acid amplification has been used for the rapid detection and confirmation of *Brucella* spp. But due to varying sensitivities/specificities of individual tests, it is advised to use a combination of serological tests for diagnosis of brucellosis. To the best of our knowledge, no systematic study on seroprevalence of caprine brucellosis along with detection of risk factors has been reported till date in the region under study.

The present study was designed with an aim to estimate seroprevalence of *B.melitensis* in goats and to assess the risk factors associated with occurrence of brucellosis in goats.

Materials and Methods

Study area and design

The assessment of seroprevalence and risk factors associated with brucellosis in goats was carried out in various districts of Punjab (India). Punjab is situated in northwest India. A systematic questionnaire was designed and variables like herd size, breed, sex, age, parity, animal rearing practices and herd management were included in the questionnaire.

Sample collection

A total of 277 serum samples irrespective of breed, sex and age of goats were collected from the various parts of Punjab. The sampling was done in a randomized form and samples were collected from healthy animals as well as animals suffering from abortions and other reproductive disorders.

Serological testing

The collected serum samples were screened using RBPT, MAT, STAT and commercially available ELISA kit. The RBPT, STAT and MAT were performed as per (Alton *et al.,* 1988). I-ELISA was carried out using IDEXX Brucellosis Ovine/Caprine antibody test kit (Iddex Laboratories).

Statistical analysis

The Statistical analysis was performed using SAS Version 9.3. The data was entered in the Microsoft Excel and analysed using Chi-square test. This test is used to assess the association among various risk factors associated with brucellosis. The test is considered significant at p-value ≤ 0.05 at 5% level. All variables with $p \leq 0.05$ were further analyzed using multivariate logistic regression model to assess the association between the potential individual and management risk factors at $p \leq 0.01$ at 5% level.

Results and Discussion

Out of a total of 277 serum samples tested, 31 (11.1%), 49 (17.6%), 44 (15.88%) and 52 (18.7%) samples were positive by RBPT, STAT, MAT and i-ELISA respectively. Contrary to the present study, Suryawanshi *et al.,* (2014) found an overall seroprevalence of 7.32% in goats from Maharashtra, Sharma *et al.,* (1979) found lesser seroprevalence of 5.53% in goats from Uttar Pradesh and Delhi

whereas Islam *et al.,* (2013) reviewed an overall seroprevalence of 3.6% in goats Valarmathy and co-workers in (2007) recorded seroprevalence of brucellosis in goats as. Shome *et al.,* (2006) evaluated 1005 sera samples from Rajasthan, Gujarat, Karnataka and recorded an overall seroprevalence of 9.95%, 5.67%, 7.36% by RBPT, STAT and i-ELISA respectively. Valarmathy and co-workers in 2007 recorded seroprevalence of brucellosis in goats as 14.55%, 9.85%, 30.04% by RBPT, STAT and indirect ELISA, respectively in Uttrakhand. In the present study, the seropositivity was highest by i-ELISA, intermediate by STAT and MAT and lowest by RBPT. Although RBPT is observed as a quick method but (Nielsen, 2002) opined that this type of test was susceptible to false positive reactions resulting from exposure of cross reacting microorganisms.

The epidemiological patterns and risk factors associated with brucellosis were estimated using records on breed, age, sex, parity, breeding practices etc. Breed was classified into Non-descript Beetal and a subpopulation known as Amritsari which may or may not be similar to Beetal. According to results obtained, the seroprevalence was higher in non-descript breed (9.03% by RBPT, 12.64% by MAT, 13.72% by STAT, 14.80% by i-ELISA) followed by Amritsari (1.44% by RBPT, 2.53% by MAT, STAT and i-ELISA) and lowest in Beetal (0.72% by RBPT and MAT and 1.44% by STAT and i-ELISA). The result indicated that difference in seroprevalence among breeds was significant at P<0.05%.But according to Ajogi *et al.,* (2002), brucellosis is known to be neither breed nor sex specific. The difference may be either due to sample size variation or management of animals. A relatively higher prevalence of brucellosis was found in crossbred Black Bengal goat than pure Black Bengal goat by Rahman *et al*., (2012). Sadhu *et al.,* (2016) found a statistically significant difference among various breed of goats whereas no significant association was found among breeds (Brisibe *et al.,* 1996 and Junaidu *et al.,* 2010) by Brisibe *et al*., (1996) and Junaidu *et al*., (2010).

Animals were classified into three age groups i.e. >3year, 3-5 year and <5. In 3-5 year age group animals, the prevalence of brucellosis by RBPT, MAT, STAT and i-ELISA was 5.05%, 9.39%, 11.19% and 11.91% respectively, followed by 6.14% by RBPT, 6.50% by MAT and STAT and 6.86% by i-ELISA in more than 5 year age group. No prevalence was recorded below 3 year of age group.$\chi^2$was found significant at p= 0.05% indicating that there was a significant differential distribution and association between brucellosis and age of animal. Rahman *et al.,* (2011) and Maheshwari *et al.,* (2012) also found higher seroprevalence in adults as compared to young animals. This could be due to the fact that susceptibility to brucellosis increases after sexual maturity and especially with pregnancy (European Commission, 2001). Contrary to the present study, Ogugua *et al.,* (2014) found a higher sero-positivity in young animals than adults, however there was no significant association between *Brucella* infection and age of animals. Singh *et al.,* (2010) reported higher prevalence rates in 1-2 yr. age group in goats which is in contrast to the results obtained in present study.

The effects of gender was not found significant using χ^2 test and further supported by Fisher's exact test. Non significant difference in seroprevalence in females (10.83% by RBPT, 15.52% by MAT, 17.3% by STAT and 18.41% by i-ELISA) was seen and there was no association between seropositivity to *Brucella* antibodies and sex of the animal. Similar results where seroprevalence was insignificantly (p>0.05)

higher in female were obtained by Rahman *et al*. (2012) and Ogugua *et al*. (2014). According to Ajogi *et al.*, (2002) brucellosis is not sex specific and both males and females are known to be equally susceptible to *Brucella* infection (European Commission, 2001). The higher infection rate of brucellosis in females than males reported by various workers might be due to the fact that only few bucks are needed for breeding purposes hence fewer males are reared by the goat farmers (Oguagua *et al.*, 2015).

Animals were divided in four groups i.e. no parity, 1-3 parturition, 3-5 times parturition and more than five parturitions. Seroprevalence was significantly (p=0.05) higher in animals having parity of 3-5 times (6.50% by RBPT, 6.86% by MAT, 7.94% by STAT and 8.30% by i-ELISA).

Seroprevalence of animals that bred on farm was found to be 10.83% by RBPT, 15.52% by MAT, 17.33% by STAT and 18.41% by i-ELISA and animals that were purchased have seroprevalence as 0.36% by all the four tests and results showed that breeding practice was significantly associated with brucellosis seropositivity for goats at p value <0.05. This was due to fact that some farmers borrow or lend breeding males to other farms through which brucellosis free animal get infected followed by spread of the infection to females of other herd during breeding although the venereal route is not considered as an important factor for transmission of *B.melitensis* (Elzer, 1998). Also, the variation may be perceived due to less number of animals purchased from the market in the present study. However, Kabagambe *et al.*, (2001) suggested that animals purchased from the market and introduced into herd can introduce brucellosis.

Flock was divided into mixed and single group. Seroprevalence was significantly higher in mixed flock as 11.19% by RBPT, 15.88% by MAT, 17.69 % by STAT and 18.41% by i-ELISA than single type flock as 0.36 % by i-ELISA. The results showed that animals that were kept together with other livestock have higher prevalence of brucellosis.

Table.1 Multivariable logistic regression model of risk factors for *Brucella* seropositivity in goats, as tested by i-ELISA

Variables	Levels	Odds ratio	P-value
Parity	<1 1-3 3-5 >5	0.142 0.152 0.34 -	0.08
Breeding practices	Bred on farm Purchased	14.15 -	0.01
Flock type	Mixed Single	0.15 -	0.09
Contact with other herd	Yes No	0.27 -	0.03
Disinfection	No Yes	2.27 -	0.07

Table.2 Multivariable logistic regression model of risk factors for *Brucella* seropositivity in goats, as tested by STAT

Variables	Levels	Odds ratio	P- value
Parity	<1 1-3 3-5 >5	0.18 0.60 1.54 -	0.07
Breeding practices	Bred on farm Purchased	11.9 -	0.01
Contact with other herd	Yes No	0.1 -	0.0002
Disinfection	No Yes	2.2 -	0.08

Table.3 Multivariable logistic regression model of risk factors for *Brucella* seropositivity in goats, as tested by MAT

Variables	Levels	Odds ratio	P- value
Breeding practices	Bred on farm Purchased	10.2 -	0.02 -
Contact with other herd	Yes No	0.04 -	<0.0001

Table.4 Multivariable logistic regression model of risk factors for *Brucella* seropositivity in goats, as tested by RBPT

Variables	Levels	Odds ratio	P- value
Parity	<1 1-3 3-5 >5	<0.001 0.61 4.17 -	0.009
Breeding practices	Bred on farm Purchased	6.63 -	0.07
Contact with other herd	Yes No	0.084 -	0.001

The results showed that animals that were kept together with other livestock had higher prevalence of brucellosis. Asmare *et al.*, (2013) and Sadhu *et al.*, (2016) observed higher seroprevalence rates of brucellosis in mixed flock. Mikolon *et al.*, (1998) and Megersa *et al.*, (2011) reported higher prevalence of brucellosis in mixed and large herd. Kabagambe *et al.*, (2001) stated that keeping sheep in addition to goats and lack of

veterinary care were important risk factors for brucellosis. This is due to fact that all species were kept together so infected animal leads to increase in frequency and rate of contact with healthy flock (Sadhu *et al.,* 2016). Also effect of herd size might be due to increased number of animals tested in large herd.

Rearing system was classified into unorganized farm where animals graze outside the farm and organized farm where animals were kept tethered in a particular area**.** The seroprevalence was found higher in unorganized farm as 10.47% by RBPT, 15.16% by MAT, 16.25% by STAT, 17.33 by i-ELISA than organized farm as 0.72% by RBPT and MAT and 1.44% by STAT and i-ELISA. The result of the non-parametric test for independence of factors for rearing system indicated that difference in seroprevalence among unorganized and organized farms were significant at P≤0.05% and the results showed that grazing outside farm (unorganized sector) were considered as more susceptible to brucellosis. This could be due to animals grazing over long distances in search of pasture, frequent mixing of flock and lack of management. Also, the lesser incidence of brucellosis in organized may be due to good and controlled management inside farm. Reviriego *et al.,* (2000) and Kabagambe *et al.,* (2001) reported that animals which graze in communal pastures were at higher risk for brucellosis infection and farms using free browsing management system were more likely to have brucellosis in the herd. Similar to the results obtained in present study, Sadhu *et al.,* (2016) and Lone *et al.,* (2013) recorded higher prevalence of brucellosis in unorganized farm whereas Singh *et al.,* (1998) found higher incidence of brucellosis in organized farm. Similar to the present study, Marin *et al.,* (2016) also stated that common grazing of infected flocks with brucellosis free flocks leads to intermingling and spread of the disease.

Seroprevalence was higher in an animals who had contact with other neighboring herds as 10.47% by RBPT, 15.15% by MAT, 16.25% by STAT and 16.96% by i-ELISA. Seroprevalence was significantly associated at p≤0.05 and contact with other herds can be considered as an essential risk factor for occurrence of brucellosis. Similar findings have been reported by Reviriego *et al.,* (2000) and Al-Majali (2005).

According to present study, seroprevalence was significantly (p≤ 0.05) higher in animals that were sold to the market (9.03% by RBPT, 12.64% by MAT, 13.72% by STAT and 15.16% by i-ELISA). Animals that were kept in the farm had less prevalence as (2.17%, 3.25%, 3.97% and 3.61%) by RBPT, MAT, STAT and i-ELISA respectively. Farmers have tendency to sell their animals that are mostly underperforming reproductively. Thus, selling of diseased animal and introduction of diseased animal to the other brucellosis free herd can be a potential risk factor. Similar results were obtained by Kabagame *et al.,* (2001), and Refai (2002). According to Refai (2002) one of the main reasons for spread of brucellosis was lack of control in the displacement of animals. Ogugua *et al.,* (2014) showed significantly (p < 0.0001) higher seropositive cases among trade animals in comparison to animals kept in the farms and households.

Disinfection of farm after abortion was classified according to question whether workers clean the farm or not. Seroprevalence was significantly (p≤0.05) high (7.58% by RBPT, 9.75% by MAT, 10.83% by STAT and 11.19% by i-ELISA) higher in farm where there was lack of hygiene and no disinfection was done after abortion.

This might be due to the fact that most of the goat farmers were poor and uneducated so there was lack of knowledge regarding

sanitation and hygiene. Since infected animals excrete high concentrations of pathogens during abortion, in placenta and uterine fluids therefore, a high risk of contamination and transmission of *Brucella* spp. to animals and humans was there. Thus, disinfection of farm after abortion is considered to be a high risk factor.

Similar study was done by Al-Majali (2005) and Boukary *et al.,* (2013) and they suggested that an effective method to control brucellosis is through disinfection of farm regularly as well as during abortion.

For Multivariate logistic regression analysis, variables that had $p \leq 0.05$ were evaluated using multivariate logistic regression model (Table 1, 2, 3, 4). Five statistically significant ($P < 0.05$) risk factors (parity, breeding practices, flock type, contact with other herd and disinfection) were considered significant by i-ELISA for *Brucella* seropositivity, four variables by STAT (parity, breeding practices, contact with other herd and disinfection), two by MAT (Breeding practices, Contact with other herd) and three by RBPT (Parity, Breeding practices, Contact with other herd)having odds ratio significant at $p<0.01$.

The seroprevalence for brucellosis in goats was 18.7%. Prevalence of brucellosis was high in female goats of age-group 3-5 years, in non descript breeds, in mixed flock among animals that were bred on farm in unorganized rearing system and kept in unhygienic conditions.

Upon odds ratio analysis, parity, breeding practices, flock type, contact with other herd and disinfection were considered the major risk factors associated with prevalence of brucellosis in animals.

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