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Sero-prevalence of *Brucella abortus* Among Dairy Cattle and Buffaloes in Pothohar Plateau, Pakistan

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Abstract.- The sero-prevalence of brucellosis was investigated among different breeds of cattle and buffaloes in Islamabad Capital Territory (ICT), Rawalpindi and Attock regions of Pakistan. A total of 2330 milk samples (1168 cattle and 1162 buffaloes) were screened for the presence of *Brucella abortus* antibodies using the milk ring test (MRT). Information related to animal type, urbanicity, sampling area and breeds were collected with the help of a pre-tested questionnaire on the day of sampling. The overall sero-prevalence was 6.9% in cattle and 6.6% in buffaloes. More seropositive animals were found in ICT compared to the other regions. The odds of brucellosis sero-positivity were higher among cross breed cattle and Nili-ravi buffaloes. This study is the first evidence of prevalence of *Brucella abortus* up to breed level in dairy cattle and buffaloes in Pakistan.

Key words: Sero-prevalence, *Brucella abortus*, milk ring test.

INTRODUCTION

Brucella abortus is the main causative agent of brucellosis in bovines (Ali *et al*., 2013; OIE, 2008) and is reported to cause severe economic loss in farm animals in the form of various reproductive disorders like abortion, retained placenta in female animals and orchitis in male animals (Asif *et al*., 2009; Megid *et al*., 2010). Apart from animals, persons having close contact with infected animals, its products or by-products, can easily acquire infection. The infected animals excrete brucellae in milk that is the main source of infection in humans especially when it is consumed in raw form (Shimol *et al*., 2012).

For the diagnosis of brucellosis in farm animals and humans, serological, bacteriological and molecular methods are used. Among these methods, bacteriology is considered as the gold standard test (Sathyanarayanan *et al*., 2011). However, it is not widely applicable due to its time consuming nature, concerns with safety of laboratory workers and non-availability of BSL-3 laboratory for characterization and manipulation of culture. Likewise molecular tools such as polymerase chain reaction are neither suitable nor economical when used at animal level or herd level for screening purposes in resource limited countries like Pakistan. A serological test like the milk ring test (MRT) is economical and mostly used for screening and monitoring of *Brucella* infections in dairy cattle (Alton *et al*., 1988).

Available pieces of information related to brucellosis especially in dairy cattle and buffaloes are scarce. The objective of this study was to estimate the sero-prevalence of brucellosis among different breeds of cattle and buffaloes in Islamabad Capital Territory (ICT), Rawalpindi and Attock using the cost effective milk ring test (MRT).

MATERIALS AND METHODS

Study area

This study was conducted in ICT, Rawalpindi and Attock districts of Pothohar plateau. This area is surrounded by river Jhelum, river Indus, Kala Chitta Range and Margalla hills and Salt range from East, West, North and South. Different breeds of farm animals are kept in this area and pasture land having vegetation in form of grasses and herbs is source of

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feed. This area is divided into sub-humid and semi-arid zones with variable annual amount of rainfall.

Samples and data collection

Milk samples were collected from 1168 and 1162 randomly selected cattle and buffaloes respectively from 3 different localities namely: ICT, Rawalpindi and Attock. The samples were collected according to standard procedure (OIE, 2008). For this purpose, first two to three streams were discarded before collection of actual sample in 50 ml collection tube. Immediately after collection, samples were transported at 4°C in an ice box to National Veterinary Laboratories, Islamabad, Pakistan for further analysis. In the laboratory, the samples were immediately transferred to a refrigerator (4°C) and analyzed within 6 hours. During sample collection, data on animal species, urbanicity and breed (Achai, Cholistani, Dhani, Lohani, Red Sindhi, Sahiwal, Cross, Australian, Jersey, Nili-Ravi, Kundhi and Azi-Kheli) were recorded using pre-tested questionnaires.

Samples analysis

The antigen to *B. abortus* for MRT was purchased from the Veterinary Research Institute, Lahore, Pakistan. Milk samples were analyzed according to standard procedure (OIE, 2008). Briefly, milk and sufficient amount of antigen was brought at room temperature before performing the test. Antigen was shaken gently to ensure homogeneity. After acquiring room temperature, 1ml milk sample was transferred into a tube and mixed well after addition of 30-50 µl of antigen. Samples were incubated for 1 hour at 37°C. If specific antibody is present in the milk, it will bind to the antigen. A sample was considered positive following the presence of a blue ring above milk column indicating the presence of agglutinins in the milk and negative otherwise.

Data analysis

Descriptive statistics were computed for cattle and buffaloes separately to study the variations in brucellosis sero-positivity across district, urbanity and breed. The apparent seroprevalence of brucellosis was calculated at the individual animal level as the ratio of the number of seropositive animals to the total number of animals tested across the different potential risk/indicator factors. On the other hand, the true animal prevalence (TP) was estimated using the Rogan-Gladen formula (Rogan and Gladen, 1978) which uses the apparent prevalence and accounts for imperfect sensitivity (Se) and specificity (Sp) of MRT as:

$$TP \quad \frac{AP \quad Sp \quad 1}{Se \quad Sp \quad 1}$$

Where *AP* is the apparent animal level seroprevalence, Se and Sp are the animal sensitivity and specificity based the paper by Aggad and Boukaa (2006). From this paper, the value of the sensitivity of MRT was 87.5% and the specificity was 98.6%. In addition, a univariate analysis based on the Chi-squared test or Fishers exact test as appropriate was conducted to determine the association between brucellosis sero-positivity and the indicator factors: breed, urbanicity and district. Finally, based on the univariate analysis, all indicator factors that were significant at the 20% level were subjected to a multivariate logistic regression analysis. A backward stepwise selection strategy was used to identify important indicator variables and the Hosmer-Lemeshow goodness of fit ^{2} test statistic (Lemeshow and Hosmer, 1982) was used to assess overall model fit. Only factors that were significant at the 5% level were retained in the final model. The effects of confounding were investigated by observing the change in the estimated odds ratios of the variables that remain in the model once a non-significant variable was removed. When the removal of a variable led to a change of more than 25% in estimated odds ratios, that variable was considered a confounder and was not removed from the model. All statistical analyses were performed using STATA, version 12, software (SataCorp LP, College station, Texas).

RESULTS

The apparent and true sero-prevalence of *Brucella abortus* antibodies determined for cattle and buffaloes for each urban region (urban and rural), sampling area (ICT, Rawalpindi and Attock) and breed using MRT is given in Table I. Out of

Table I.- Sero-prevalence of *Brucella abortus* antibodies with respect to sampling area (District), urbanicity and breed for both cattle and buffaloes.

Indicator factor	Samples examined	Samples positive	Apparent prevalence (95% CI)	True prevalence (95% CI)
		Cattle		
District*				
Attock	274	19	6.9 (4.2-10.6)	6.4 (3.5-9.3)
Islamabad	398	46	11.6 (8.6-15.1)	11.8 (8.7-15.0)
Rawalpindi	496	15	3.0 (1.7-4.9)	1.9 (0.6-3.0)
Urbanicity				
Urban	538	41	7.6 (5.5-10.2)	7.2(5.0-9.4)
Rural	630	39	6.2 (4.4-8.4)	5.6(3.8-7.4)
Breed*				
Local	248	11	4.4 (2.2-7.8)	3.5 (1.2-5.8)
Exotic	149	9	6.0 (2.8-11.2)	5.3 (1.7-9.0)
Cross	771	60	7.8 (6.0-9.9)	7.4 (5.6-9.3)
		Buffaloes		
District*				
Attock	410	21 (5.1)	5.1 (3.2-7.7)	4.3 (2.3-6.3)
Islamabad	196	31 (15.8)	15.8 (11.0-21.7)	16.7 (11.5-21.9)
Rawalpindi	556	25 (4.5)	4.5 (2.9-6.6)	3.6 (2.1-5.1)
Urbanicity*				
Urban	567	44 (7.8)	7.8 (5.7-10.3)	7.4 (5.3-9.6)
Rural	595	33 (5.5)	5.5 (3.8-7.7)	4.8 (3.1-6.5)
Breed				
Azi-Kheli	13	0	0 (0-26.5)	0 (N.C.)
Kundhi	72	3	4.2 (0.9-11.7)	3.3(0-7.3)
Nili-ravi	1077	74	6.9 (5.4-8.5)	6.4 (4.9-7.8)

*Confidence intervals are exact binomial confidence intervals. N.C., not computed.

1168 milk samples from cattle and 1162 from Buffaloes, 80 and 77 were found to be seropositive, respectively. Sero-prevalence was found to be slightly higher among cattle (6.9%) as compared to buffaloes (6.6%). However, the difference in sero-prevalence was not statistically significant since the confidence interval of the difference includes zero (difference = 0.22; CI: (-1.80-2.26)). Animals kept in urban areas appeared to have higher sero-prevalence (7.6% for cattle and 7.8% for buffaloes respectively) compared to those in rural areas (6.2% for cattle and 5.5% for buffaloes). The sero-prevalence of *Brucella abortus* antibodies was higher in ICT for both cattle and buffaloes (11.6% and 15.8% respectively) followed by Attock (6.9% and 5.1% respectively) compared to Rawalpindi (3.0% and 4.5%, respectively). Among cattle, cross bred were found to be more seropositive (7.8%) compared to exotic and local breeds (6.0% and 4.4%, respectively). All buffaloes were of the local breed type with the highest sero-prevalence for the Nili-ravi breed (6.9%) followed by the Kundhi breed (4.2%). Overall, the observed differences between apparent and true prevalence were not significant since their confidence intervals overlapped.

The results of the univariate analysis indicated that only location (District) was significant at the 5% level for both cattle and buffaloes. Breed among cattle and urbanicity among female buffaloes were not significant at the 5% level but their p-values were <20% implying that they qualify as potential variables to be submitted to the multivariate logistic regression analysis. The final model for cattle included location (District) and Breed type whereas the final model for female

buffaloes included only location (Table II). The Hosmer-Lemeshow goodness-of-fit ^{2} test statistics (2.5-6 d.f and 0.00-1 d.f respectively) indicated that overall, the models fit the data well (P = 0.644 and 1, respectively).

Table II.- Results of the multivariate logistic regression analysis of risk factors for brucellosis sero-positivity among cattle and buffaloes in the Pothohar Plateau

Variable	Odds Ratio	95% C. I	P-value
		Cattle	
District			
Attock	1	-	-
Islamabad	1.4	0.77-2.54	0.272
Rawalpindi	0.3	0.16-0.69	0.003
Breed			
Local	1	-	-
Exotic	2.0	0.97-4.01	0.060
Cross	1.5	0.61-3.86	0.368
		Buffaloes	
District			
Attock	1	-	-
Islamabad	3.5	1.94-6.24	<0.001
Rawalpindi	0.9	0.48-1.58	0.652

According to the final model, cattle in Rawalpindi appeared to have significantly lower odds of brucellosis sero-positivity compared to cattle in Attock. Breed type was a confounder and was thus kept in the final model. There was weak evidence that exotic cattle breeds had higher odds of brucellosis positivity compared to cattle of the local breeds. For buffaloes, the odds of brucellosis sero-positivity were significantly higher for those in Islamabad compared to those in Attock.

DISCUSSION

In developing countries, it is a good strategy to use conventional tests like the Rose Bengal test (RBT and MRT) for screening of individual animals and herds in an effective way. The milk ring test, commonly used for serodiagnosis of brucellosis in dairy herds is easy to use and more economical (Alton *et al*., 1988). This strategy has been successfully used for the surveillance of brucellosis in different countries *e.g.* Sudan and Chile (Rivera *et al*., 2002; Abdalla and Mohamed, 2011). Moreover, viability of milk ring test was found to be better than for other serological tests (Al-Mariri *et al*., 2011).

Milk ring test may yield false-positive reactions in cattle vaccinated less than 4 months before testing, abnormal milk samples or in cases of mastitis. Therefore, it is not recommended for use in small farms where these problems can have a greater impact on test results. The milk I-ELISA is more sensitive and specific for testing large herds. However MRT is a suitable alternative if the ELISA is not available (OIE, 2008). Due to the imperfect nature of MRT, we estimated true prevalence of *B. abortus* antibodies in present study taking into account the sensitivity (Se) and specificity (Sp) of the MRT.

In the present study, prevalence of *Brucella abortus* antibodies in cattle was found to be similar to that in buffaloes. Similar findings have been reported from Sri Lanka, where 4.6% cattle and 4.2% buffaloes were found to be positive for *Brucella abortus* antibodies (Silva *et al*., 2000). Previously, a high sero-prevalence was reported among cattle (4.6%) compared to buffaloes (1.7%) based on MRT from Pakistan (Shafee *et al*., 2011). Likewise, a higher prevalence of brucellosis was found in cattle (51%) compared to buffaloes (49.8%) using MRT in Egypt (Ibrahim *et al*., 2012). In another study, about 20% dairy cattle and 12% milking goats were found to be seropositive for *Brucella* antibodies using MRT in Turkey (Terzi, 2006).

Cattle kept in urban areas were found to have a higher sero-positivity compared to those in rural areas. However, this observed difference was not statistically significant. In a relevant study, a high prevalence of *Brucella abortus* antibodies was found in household milk samples from urban areas (4.7%) compared to those from rural area (3.2%) in Kenya (Kangethe *et al*., 2000). Similarly, among buffaloes, the observed difference in sero-positivity was not statistically significant.

In the present study, it was surprising to find high sero-prevalence of *Brucella abortus* antibodies in ICT and Attock compared to Rawalpindi. One of the possible reasons of higher sero-prevalence of

brucellosis in ICT might be due to differences in farming structure. As in ICT the majority of the cattle and buffaloes are reared for dairy use to provide milk to individuals of Islamabad and Rawalpindi. Where, dry cattle and buffaloes are without delay replaced with lactating ones to maintain the supply chain to the mentioned areas. Non-lactating cattle and buffaloes are mostly sent to the local villages far away from urban region ICT and in these areas frequently feed on available green fodder or on grazing. Moreover, Due to lack of green fodder in ICT, lactating cattle and buffaloes are fed on seed cake, wheat straw and other supplements. So we can say that the high turnover caused by repeated replacement may be a plausible reason for the higher sero-prevalence of brucellosis in ICT as compared to the other two regions. Similarly, variation in sero-positivity of brucellosis in different regions of Urmia, Iran was reported using MRT (Maadi *et al*., 2011).

When we compared sero-prevalence of *Brucella abortus* antibodies in different breeds of cattle, the highest sero-positivity was found in cross bred and exotic cattle (7.8% and 6.0% respectively). In a recent study conducted in Bangladesh, a high sero-prevalence of *Brucella abortus* antibodies was found in cross breed cattle (6.28%) compared to local breeds (0.85%) (Sikder *et al.*, 2012). A study conducted in Asmara region of Eritrea reported a higher sero-prevalence of *Brucella abortus* among mixed breeds of cattle compared to exotic breeds (Omer *et al*., 2000).

Sero-prevalence of *Brucella abortus* antibodies in three breeds of buffalo showed that Nili-Ravi and Kundhi breeds were affected with brucellosis. No animal of Azi-Kheli breed was found positive which might be due to low number of animals available for this study. Nili-Ravi and Kundhi are major breeds of buffaloes in Pakistan and this study shows that both breeds are affected by brucella infection.

CONCLUSIONS

This study adds some information related to brucellosis prevalence in available breeds of cattle and buffaloes in Pakistan using MRT as a screening tool. Being a zoonotic disease, it is very much important to develop strategies for the control of the disease in animals especially in common food producing animals like cattle and buffaloes. Milk from these animals is a very common source of our food and used for production of dairy products. Milk from infected livestock, especially unpasteurized milk, is a potential source of brucellosis infection for humans which may in turn cause health complications. Individuals in ICT are especially at a higher risk of acquiring the infection. Therefore brucellosis control programs should be initiated for effective control of this disease in ICT in particular and Pakistan as a whole.

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