Brucelosis is recognized worldwide as an important bacterial disease of cattle, buffaloes, goats etc., that causes infertility in both male and female animals. The present study was carried out to determine the prevalence of brucellosis in aborted cattle and buffaloes in district Hyderabad, Sindh, Pakistan. Amongst 69 and 89 milk samples of cattle and buffaloes, the positive reactors for Brucella abortus antibodies examined by milk ring test (MRT) were, 22 (31.88%) and 42 (47.19%), respectively. The main constraints of the prevalence of brucellosis in cattle and buffaloes recorded through interviewing the farmers of the study area were recorded as: addition of animals through an auction (25% and 91%), improper disposal of aborted material (44% and 68%), < 3 kilometer distance between herds (40% and 65%), contact with stray cattle (36% and 49%) and stray dogs (60% and 36%), respectively. It was concluded that brucellosis in cattle and buffaloes is prevalent in dairy and household farms in district Hyderabad, moreover, Brucella positive reactors were predominantly higher in buffaloes than cattle. Veterinary services with improved level should be maintained in the area to reduce the burden of brucellosis in cattle and buffaloes in district Hyderabad.

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INTRODUCTION
Brucelosis is a serious problem for both public health and economic significance of most developing countries. It is recognized worldwide and considered to be an important disease of cattle, buffaloes, goats and man etc. The incidence of the disease is related to several factors including demographic and geographical factors. Seroprevalence of the disease has been reported from 3.25 to 4.4% in different areas of Pakistan (Ahmed et al., 1990).

Humans become infected by coming in contact with animals or animal products that carries brucellosis. In humans, brucellosis can cause a range of symptoms that are similar to the flu and may include fever (39-40°C), sweats, headaches, back pains and physical weakness. Severe infection of the central nervous system or the lining of the heart may also occur (Chamberlain, 2003). Brucella infection frequently presents in peoples who are in direct contact with infected cattle and buffalo herd, manure, milk and its by products.

Brucelosis in cattle and buffaloes has been recognized clinically by an abortion usually taking place for 6 months and onwards i.e.; last trimester of pregnancy. Grayish white mucoid or mucopurulent discharges from the vagina, prior to parturition of cow, may show the clinical patterns of normal parturition like swelling of the vulva, relaxation of pelvic ligament, enlargement of udder and discharge from the vulva. There is a retention of fetal membranes. The organisms are likely to get localized in the supra-mammary lymph nodes. In the bull, the genital organs are affected, leading to obvious manifestation of epididymitis or orchitis. These changes may be noted in the seminal vesicles and ducts differentia (Gul et al., 2013; Kamboh et al., 2007).

The present study was, therefore, designed to determine the prevalence of brucellosis in cattle and buffaloes in and around district Hyderabad using milk ring test (MRT). The present study would provide baseline data on the prevalence of the brucellosis in the area. Moreover, the purpose of the present study was to analyze the risk factors regarding contracting and dissemination of brucellosis among the herds of cattle and buffaloes. In addition, this study would also be helpful in devising an eradication strategy for brucellosis, which would ultimately contribute to the socioeconomic of livestock farmers in Sindh, Pakistan.
MATERIALS AND METHODS

Data Collection
A questionnaire format was designed for the collection of information regarding aborted cattle and buffaloes in District Hyderabad, Sindh, Pakistan; to appraise the various constraints associated with brucellosis, these include: abortion (%age), source of addition, disposal of aborted material, distance between herds, contact with stray animals, frequently visits of peoples and physiological disorders/ status in aborted cattle and buffaloes. A total of 200 animals (100 cattle and 100 buffaloes) was included in this data collection.

A collection of milk samples
A total of 158 milk samples was collected from the lactating cattle (n = 69) and buffaloes (n = 89). Before collection, the teats were cleaned with an antiseptic solution and first few drops of milk were discarded, then 3 - 5ml of milk was collected in sterilized screw capped bottles (Bijous). The samples were brought to the laboratory and stored at -5°C until analyzed. The MRT was performed according to the standard method (OIE, 2008). The antigen to B. abortus was purchased from the Veterinary Research Institute, Lahore, Pakistan.

Data analysis
Descriptive statistics were applied to cattle and buffaloes separately to study the variations in prevalence of brucellosis. The results of MRT were expressed in percentages that were calculated by dividing the number of positive samples with total number of samples x100. All statistical analyses were carried out using STATA, version 12, software (SataCorp LP, College station, Texas, USA).

RESULTS
A total of 100 each of aborted cattle and buffaloes were included in the study and the results collected through questionnaire were presented in Figure 1. It reveals that the abortion (%age) in cattle and buffaloes was 8 and 10 in the 2nd month of pregnancy, 10 and 8 in the 3rd month, 20 and 16 in the 4th month, 17 and 12 in the 5th month, 20 and 19 in the 6th month, 10 and 15 in the 7th month, 15 and 14 in the 8th month, and 0 and 6 in the 9th month of pregnancy, respectively. It was further noted that the minimum abortion percentage (0 and 6%) was in the 9th month, while the maximum (20 and 19%) in the 6th month of pregnancy in cattle and buffaloes, respectively. Information regarding various constraints of abortion in cattle and buffaloes was obtained from the owners/dairy farmers interviewed and results are depicted in Figure 2. It was observed that, among 100 each of aborted cattle and buffaloes, 75% and 9% of animals were added in their herd by self raising, while 25% and 91% through auction, respectively. On enquiry regarding the disposal of aborted materials, 56% and 32% of farmers reported a proper disposal of materials, while 44% and 68% improper from cattle and buffalo farms, respectively. Forty percent
aborted cattle and 65% of aborted buffaloes were at a distance of < 3 km, while 60 and 33% aborted cattle and buffalo herds, respectively were at a distance of > 5 km and more. It was further observed that 36 and 49% aborted cattle and buffalo herd, respectively was in contact with stray cattle, while 60 and 36% aborted cattle and buffalo herds respectively was in contact with stray dogs, whereas 4 and 15% of cattle and buffalo herd, respectively were in contact with other zoo animals. It was revealed that 36 and 48%, 45 and 28%, 5 and 7%, 4 and 9% and 10 and 8% of cattle and buffalo farms, respectively, were visited frequently by veterinarians, stock assistants, butchers, animal attendant from other herds and vet, quacks, respectively.

![Figure 2: Constraints of abortion in cattle/buffaloes in dairy farms in District Hyderabad.](image2)

![Figure 3: Physiological disorders/status in aborted cattle/buffaloes in dairy farms in District Hyderabad.](image3)
During analysis of risk factors related to the physiological status of aborted animals, it was revealed that infertility 3 and 8%, Hygroma 2 and 8% and retained after birth 95 and 82% were the common disorders in aborted cattle and buffaloes, respectively (Figure 3). Moreover, it was observed that 33% of cattle and 30% of buffaloes were pregnant and 67% of cattle and 70% of buffaloes were non pregnant. However, of the pregnant animals, 8% cattle and 10% of buffaloes were dry and 29% of cattle and 20% of buffaloes were wet. Where as non-pregnant animals showed 7% cattle and 15% of buffaloes in dried status and 60% cattle and 55% of buffaloes in wet status (Figure 3).

Figure 4 shows the positive reactors (%age) for Brucella antibodies in cattle and buffaloes diagnosed by MRT, and 69 and 89 milk samples of cattle and buffaloes, respectively, were examined. Of these, 22 (31.88%) in cattle and 42 (47.19%) in buffaloes, respectively were observed positive reactors of Brucella antibodies.

DISCUSSION

In the present study, month-wise abortion in cattle and buffalo have been reported first time in Hyderabad, Sindh, Pakistan. Our results are closely related to Munir et al., (2011), who recorded the abortion rate of 8.9 to 17.86% in different private and government bovine dairy farms of Punjab, Pakistan.

One of the important critical control points of brucellosis is addition of animals in the herd (Earhart et al., 2009). In the present study, 75% of cattle and 9% of buffaloes were added in the herd through self raising, while 25% of cattle and 91% of buffaloes by auction. Herds consisting of replacement were bought from regular auction sales have been found to be at higher risk of contracting brucellosis (Richey and Harrell, 1997).

Distance between herds is also a risk of contracting brucellosis, during the present study. It was recorded that 40 and 69% cattle and buffalo herds, respectively, were found with a distance of < 3km, whereas 60 and 53% cattle and buffalo herds with a distance of 5km or more. The distance between a herd and brucellosis infected herd increases the risk of contracting brucellosis. Herds at half a mile to one mile away are at risk of brucellosis contracting through the cattle community. However, the spread of disease in a herd is much less likely to occur when the herd is located over one mile from the brucellosis infected herd (Omer, et al., 2000; Richey and Harrell, 1997).

During the present study the proper disposal of aborted material was observed in 56% cattle and 32% buffaloes herds. Whereas improper disposal was recorded in 44% cattle and 68% buffaloes herds. Moreover, the cattle ingested contaminated forages by aborted fetuses and licking of calves born from infected cattle and buffaloes are at higher risk of brucellosis that might be due to improper disposal of infected materials (Richey and Harrell, 1997).

It has been reported that cattle and buffaloes, where the intrusion of stray cattle into the herds and contact with stray dogs and other wild animals and birds, were also at higher risk of contracting brucellosis. Moreover, these were spreading brucellosis by dragging dead /aborted calves and after birth (placenta) between neighboring herds (Richey and Harrell, 1997). While during present study of the constraints, it was investigated that 36 and 49% cattle and buffaloes were in contact with stray cattle, further more 60 and 36% were in contact with stray dogs and 4 and 15% cattle and buffaloes were found involved in contact with other zoo animals.

Likewise, some other factors also contributed in risk of brucellosis including, the frequent visits by veterinarians,
stock assistants, butchers, animal attendant from other herds and veterinary quacks. During present study it was observed that 36 and 45%, 45 and 48%, 15 and 7%, 4 and 9% and 10 and 8% farms of cattle and buffaloes, respectively, were frequently visited by veterinarians, stock assistants, butchers, animal attendants from other herds and veterinary quacks, respectively. A recent study indicated the relative risk (RR) of 1.14 in the spread of brucellosis for the visitors to bovine farms (Kaoud et al., 2010).

The positive reactor percentage for brucellosis in aborted cattle and buffaloes was recorded as 31.88 and 47.19%, respectively, by MRT that is closely related (31 and 47%), in cattle and buffaloes, respectively) to a previous study done in our laboratory using the Rose Bengal Plate Test (Kamboh, et al., 2007). Our results for the prevalence of brucellosis in cattle and buffaloes by MRT during the present studies are also in close agreement with Rathore et al., (2002), who recorded the prevalence of brucellosis in organized dairy farms in Quetta as 4.6% and 1.7% in cattle and buffalo respectively. These little prevalence rates might be due to low environmental temperatures in these areas, as it is well known that pathogenic bacteria are inhibited at low temperature (Tyagi et al., 2013). The results observed for prevalence of brucellosis in cattle and buffaloes by MRT, during the present investigation are not in agreement of Oloffs et al., (1998), who detected 10% of bovine brucellosis. Whereas in the present study, 31.88% in cattle and 47.19% in buffaloes were confirmed positive reactors for Brucella antibodies by MRT. It could be concluded that, the main constraints of brucellosis in the risk factor analysis were; the source of addition of cattle/buffaloes, disposal of aborted material, distance between herds and contact with other animals etc. Moreover, MRT assay indicates that bovine brucellosis is relatively higher in buffaloes than cattle. These results addressed that veterinary practice should be improved in the area to reduce the disease burden and risk of infection in cattle and buffaloes.

REFERENCES


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