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Short Communication

Evidence of *Brucella abortus* in Non-Preferred Caprine and Ovine Hosts by Real-time PCR Assay

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Authors' Contributions
ZIC designed the study. MZS performed the experimental work and wrote the manuscript. RA and AA supervised the study. MIR helped in data analysis. MY and BAS helped in sample collection. MAM helped in samples screening.

Key words
***Brucella abortus*, Goat, Real-time PCR, RBPT, Sheep.**

ABSTRACT

The present study was conducted in Sheikhupura and Kasur districts for molecular based confirmation of *B. abortus* in both sheep and goats. Brucellosis in sheep and goats is caused by *B. ovis* and *B. melitensis,* respectively but *B. abortus* (causative agent of bovine) may cross the species barrier to infect the small ruminants which may complicate the control measures of brucellosis because most of the control programs rely on serological screening of brucellosis rather than molecular assay which could confirm the particular species circulating in ruminants. In this study 960 and 471 serum samples of goats and sheep were collected, respectively. After screening with Rose Bengal test (RBT), all seropositive samples were subjected to real-time PCR assay. RBT confirmed the seroprevalences of 19.32% ± 0.289 and 12.29% ± 0.0105 brucellosis in sheep and goats, respectively and real-time PCR confirmed the *B. abortus* in 74 samples (62.71% ± 0.044) out of 118 seropositive samples in goats while 63 samples (69.23% ± 0.048) out of 91 seropositive samples of sheep. The presence of *B. abortus* in small ruminants could be due to mixed farming of small and large ruminants, sharing of same pasture, presence of reservoirs host in a farm, which might be the main risk factors for cross-infection of *Brucella* species in their non-specific hosts. *B. abortus* could be identified as causative agent of caprine and ovine brucellosis in Pakistan. Results of this study can be used for the development of effective eradication and control strategies for brucellosis in small ruminants.

Brucellosis is an important zoonotic disease which is prevalent in both human and ruminants. Eradication and control of the disease is imperative for public health. But from last few years its prevalence is increasing day by day (Ali *et al*., 2015). Brucellosis has been eradicated in developed countries but it is still prevalent in tropical and developing countries (Pappas *et al*., 2006). It is also prevalent in Pakistan (Ahmad *et al*., 2017). In tropical areas where no strict biosafety measures are monitored, mix farming and same housing of small and large ruminants may lead to cross transmission of *Brucella* species to their non-preferred host which might complicate the control measure of brucellosis. There are eleven reported species of *Brucella*. Each specie has its preferred host. In past host specificity of *Brucella* pathogen has been recognized for phenotype isolates. But due to mix farming, sharing of same pasture of small and large ruminants, mixed livestock shelters, presence of reservoirs host in a farm, and uncontrolled animals movements may lead to cross infection of *Brucella* species in their non-preferred hosts. Eco-plasticity and polypathogenicity enables the *Brucella* to cross the species barrier. This transmission called as inter-species transmission which is the main barrier for control and eradication of brucellosis (Ali *et al*., 2015). Even dogs on farm can act as carrier of brucellosis in farm (Baek *et al*., 2003). Not only dogs but wild animals, cats and Chinese water deer can be a carrier of *B. abortus* (Truong *et al*., 2011). Avian species have also been reported for brucellosis (Mushi *et al*., 2008). Antibodies of *Brucella* pathogen were detected in

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poultry birds maintained at seropositive farm (Cadmus *et al*., 2010). The control and eradication of brucellosis is mostly based on strict enforcement of test and slaughter policy, movement control, sanitation and vaccination but cross species transmission can be a reason of vaccination failure (Akhtar *et al*., 2017). The projects on detection of prevalent species of *Brucella* in small and large ruminants, reservoirs host, fomites and wild life species are essential for effective implementation of control and eradication strategies (Muendo *et al*., 2012). It is important to inspect the *Brucella* from its outside non preferred host species in field condition. It is also imperative need of time to find out the interspecies transmission of *Brucella* which may occur naturally and cause clinical disease in non-preferred hosts. The present study was designed to find out the seroprevalences of brucellosis in small ruminants and detection of *B. abortus* in both sheep and goats by real-time PCR assay.

Materials and methods

Blood samples were collected from 471 sheep and 960 goats using a convenient sampling procedure from Kasur (Latitude: 31.0896° N, Longitude: 74.1240° E) and Sheikhupura (Latitude: 31.6243° N, Longitude: 74.1240° E) because these areas have been reported for brucellosis (Ahmed *et al*., 2017). The small ruminants of theses herds were also having mixed farming, sharing of pasture, same housing, history of abortion and close contact with seropositive large ruminants. The blood samples were collected in a vacutainer without anticoagulant then serum was separated and stored at -20°C.

All samples were serologically screened through Rose Bengal antigen obtained from Veterinary Research Institute Lahore (Baloch *et al*., 2017). All seropositive samples were used for DNA by Exgene™ Blood SV-mini Kit (GeneALL® Biotechnology Co. Ltd, Songpa-gu, Korea) according to manufacturer's instruction. Genomic samples were stored after quantification at -20°C till further investigation .

Genomic amplification was performed by using prepared Real-Amp™ SYBR qPCR master mix (Cat# 801-020, GeneALL® Biotechnology Co. Ltd, Songpa-gu, Korea). A reaction mixture of 20µL containing 4µL of master mix, 0.5µL (500nmol) of each species specific primers (Newby *et al*., 2003) forward: 5′ CCATTGAAGTCTGGCGAGC 3′ and reverse: 5′ CGATGCGAGAAAACATTGACCG 3′, 1µL of DNA and 14µL of nuclease free water were used for amplification. The cycle threshold (*Ct*-value) below 40 was considered as positive. A reference strain of *B. abortus* (BA-544) obtained from Veterinary Research Institute (VRI) Lahore, Pakistan was used as positive control. Amplification of desired DNA was done in 96-well microplate (Thermo Fischer Scientific Inc., Waltham, USA) using a 7500 Real Time PCR System, Thermo cycler system of ABI. Initial denaturation at 94°C for 10 min followed by 40 cycles of each consisting denaturation at 95°C for 10 sec, annealing at 60°C for 10 sec and extension at 72°C for 10 sec. Final extension was done at 72°C for 3 min. Double stranded PCR product was detected by fluorescent dye associated with SYBR green-I at each extension step. An amplification curve of PCR product was analyzed and recorded through computerized software.

Data was analyzed risk estimation of odds ratio (95% CI: confidence interval) by using Statistical Package for Social Science (SPSS for Windows version 20, SPSS Inc., Chicago, IL, USA). Standard error sample proportion of standard deviation was calculated by using SE_p formula as given. SE_p = Square root [P (1 – P)/ n].

Results and Discussion

Through RBPT it was found out that, 118 (12.29% ± 0.0105) samples of goats out of 960 and 91 (19.32% ± 0.289) serum samples of sheep out of 471 were seropositive. After quantitative PCR, the PCR product size of *B. abortus* was found to be 156bp which confirmed that 74 samples (62.71% ± 0.044) out of 118 seropositive samples in goats and 63 samples (69.23% ± 0.048) out of 91 seropositive samples of sheep had *B. abortus*.

The present study was conducted in Sheikhupura and Kasur districts because the disease is prevalent not only in ruminants but soil is also niche for *Brucella* infection (Ahmed *et al*., 2017). Previously *B. abortus* biovar-1 was detected in 86% blood samples and 64% milk samples of goats by PCR assay (Leal-Klevezas *et al*., 2000). In Nigeria an abortion due to *B. abortus* was confirmed in ewes. The same biovar of *Brucella* was isolated from cattle which were kept in close contact with sheep, confirmed the cross-species transmission (Ocholi *et al*., 2005). Junaidu *et al*. (2010) used the serum samples of goats after slaughtering. They found out 12.03% samples positive for *B. abortus*. It was due to grazing habit in Nigeria where cattle graze with sheep and goats. In Egypt, despite eradication program of brucellosis, the disease was endemic in animals. The study revealed the cross-species infection of *B. abortus* from cattle to non-preferred host. Close farming could be the risk factor for continuous presence of brucellosis among cattle, buffalo, sheep and goats (Wareth *et al*., 2015). Previously, a study was conducted in Pakistan, in which all the seropositive serum samples of small ruminants were positive for *B. abortus* by real-time PCR but none of the *B. melitensis* was detected. *B. abortus* was identified as a causative agent for abortion in small ruminants (Ali *et al*., 2015). There could be number of risk factors which

might have role in cross infection of species. These risk factors should be analyzed for effective planning. In a country like Pakistan where no strict biosafety measures are present, this could be the main reason of control failure. Mixed farming could be the reason of transmission of *B. abortus* to small ruminants where both small and large animals share the same pasture. Animals may secrete the infection (Samad *et al*., 2018). *Brucella* could survive in soil and other fomites (Ahmed *et al*., 2017). Same housing and herd presence of seropositive animals in mix farming system could play a role in transmission (Ali *et al*., 2015). Ectoparasites have been recently investigated as responsible for *Brucella* transmission. Ticks, mites and lice have also role in *Brucella* transmission (Wang *et al*., 2018). These ectoparasites with fomites, water and soil should be included in investigation of cross transmission of *Brucella* species. Due to cross infection of *Brucella* species in their non-preferred host brucellosis is increasing day by day. Abortion due to *B. abortus* in small ruminants cannot be identified on serogical basis. The serological based screening may lead to misleading planning for control program. Species specific identification of *Brucella* is significant in control measures (Shahzad *et al*., 2017). Early and accurate detection of *Brucella* species and its biovar/ biotype are fundamental for the control and eradication of brucellosis.

Conclusion

Little attentions was paid to inter-species transmission in previous studies. In the present study we used the molecular assay for confirmation of *B. abortus* in sheep and goats. It can be concluded that *B. abortus* could be a problem of small ruminants.

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Statement of conflict of interest

There is no conflict of authors of this paper.

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