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Original Article

Epidemiological investigations of bovine brucellosis and evaluation of loop mediated isothermal amplification assay for field application

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Abstract

Brucellosis is considered as a highly contagious and zoonotic disease globally and various diagnostic tests are available for its diagnosis. Keeping in view, the limitations of currently used serological techniques, a more precise, sensitive, and reliable loop mediated isothermal amplification (LAMP) assay was evaluated as an emerging diagnostic tool. In the current study, serum samples from cows (n=1989) and buffaloes (n=1467) were collected from the study area *i.e*., District Faisalabad and Toba Tek Singh in Punjab, Pakistan. As these two districts are present around the river Ravi in Punjab, Pakistan and known as the house of Nili Ravi breed. A number of dairy farms of local and imported cows are also present in this area. Initially the samples were screened by the RBPT and then subjected to c-ELISA for confirmation. Overall, 12.16 and 9.3% cows and buffaloes were seropositive through RBPT while 11.21 and 7.70% cows and buffaloes were seropositive via c-ELISA. The positive samples from c-ELISA were further subjected to molecular amplification at 1.5% agarose gel through LAMP assay. The current study concludes that LAMP assay is more sensitive as compared to other conventional PCR techniques while detecting true positives for brucellosis, so it can be used for confirmation of *Brucella abortus* as compared to PCR. In addition to the sensitivity and specificity and qualitative results can be observed through naked eye in LAMP, which is not possible in PCR.

Keywords: Brucellosis, LAMP, Quick diagnosis, Serological techniques, Rapid point of care

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Introduction

Brucellosis is a zoonotic and transmissible disease across the globe, equally important for animals and humans. *Brucella* is main causative agent involved in clinical manifestation of disease in animals and morphologically, this microorganism is a coccobacillus, Gram negative, intracellular, non-motile and non-spore forming (Osman et al., 2016; Tulu, 2022). This organism is very specific in their

host interaction and cause the disease in specific host range like *B. abortus* specifically isolated from bovine brucellosis and also cause the disease in humans and equines as well, *B. melitensis* is mainly responsible for disease occurrence in small ruminants and human, *B. ovis* primarily affect sheep and rough in nature (Tulu, 2022). The members of class *Brucella* along with their host specificity includes *B. abortus* (Cows, buffaloes, camel, equines, and human), *B. ceti*, *B. inopinata*, *B. microti* and *B. pinnipedialis* are important in terms of marine animals. On the basis of phenotypic and genotypic feature and MLST the different other serotypes of these species have been also documented (Osman et al., 2016; González-Espinoza et al., 2021; Riaz et al., 2023).

Brucellosis has major economic impact and causing huge economic losses due to the reduced production, late term abortion, stillbirth, debilitated calves, permanent infertility, low milk production and increased inter-calving interval along with trade restrictions (Shahzad et al., 2018; Sarma and Singh, 2022; Tulu, 2022; Rabah et al., 2022; Dheyab and Abdulhameed, 2023). Brucellosis is an important disease due to wider host range, as it affects a wide-ranging mammalian species as well as domesticated animals, freshwater fish, sea mammals and wildlife species (Adamu et al., 2016; Dadar et al., 2022). Humans become infected through feeding on contaminated food like milk from the diseased animal, especially raw milk and other milk byproducts including cheese, yogurt, ice cream. Other sources include handling of aborted fetuses, fetal membranes, and uterine discharges (Godfroid et al., 2013; Gul et al., 2015; Soroka et al., 2021).

Now a days, different diagnostic techniques are present for identification of *B. abortus* but many of them have limitations. Till today, isolation and identification of the pathogen for brucellosis is the gold standard test for the accurate and confirmatory diagnosis (Freddi et al., 2021). Isolation and identification although very accurate but require sophisticated lab, skilled person and time consuming may take 2-3 days or more for growth. Another very important draw backs of this procedure includes when pathogen load is low in sample then it becomes less sensitive and due to high zoonotic potential biosafety level 3 laboratory is required (Pérez-Sancho et al., 2013). Brucellosis can be diagnosed very easily by the other serologically techniques including RBPT, STAT (Standard Tube Agglutination test) and ELISAs (Enzymes Linked Immunosorbent Assays) as compared to traditional isolation and identification of causative organism. However, these serological practices also have some limitations like cross reactivity with the other gram-negative bacteria *i.e.*, serotype 0:9 of *Yersinia enterocolitica* and false positive results have been recorded due to the cross reactivity (Freddi et al., 2021).

Different molecular assays like PCR and LAMP have been used for the confirmatory diagnosis of animal brucellosis as well as for human brucellosis. Various techniques for the detection of conserved *brucella* genome including BCSP31, 16SrRNA, IS711 and OMPs have been established (Gul et al., 2014; Hemade and Gandge, 2016; Freddi et al., 2021; Ma et al., 2021). Since the production of brucella specific antibodies in body requires many days after the onset of infection so these assays may have less significance in early-stage diagnosis of brucellosis. Molecular detection methods including PCR (Polymerase Chain Reaction) and its various types have used for diagnosis of various infectious diseases including brucellosis, as they are convenient to use, sensitive and very much accurate but required well developed lab and trained staff. Furthermore, the post amplification processes are also laborious which makes these procedures to be impractical at field level (Aguilar-Marcelino et al., 2022). Quick and confirmatory diagnosis at field level is a matter of concern because of the limitations of existing assays both serological and molecular, so it is a need of time to use an alternative tool for the quick, accurate and reliable assay for the diagnosis of brucellosis as point of care like LAMP (Loop mediated isothermal amplification assay), which fulfills the required criteria (Meurens et al., 2021).

Qualitative LAMP assay is very much convenient to be used as it requires persistent temperature which can be maintained even by the simple water bath and requires no complicated lab instruments. Another prime feature of LAMP assay is that its results can be observed through naked eye. As compared to other molecular technique like PCR, the specificity and sensitivity of LAMP assay is much higher (Bardhan et al., 2020). In Pakistan, at national level a scarce information is available regarding the use of LAMP assay for the diagnosis of brucellosis specifically *B. abortus*. So, the present study was planned to evaluate LAMP assay for the diagnosis of *B. abortus* at field level targeting the BCSP31 gene.

Material and Methods

Samples collection

The ethical permission regarding the proposed study procedures and protocol was obtained from the Graduate study and research board (GSRB, vide letter no. DGS/17037-40 dated 14-07-2021.) and institutional biosafety committee, University of Agriculture, Faisalabad, Pakistan. A total of 3456 samples were collected bovine including cows (n=1989) and buffaloes (n=1467) residing in the different locales and cattle colonies of the study area *i.e.*, District Faisalabad and Toba Tek Singh in Punjab, Pakistan. Both the animal and herd level data including age, sex, breed, reproductive problems, parity, location, status of vaccination breed and breeding methods were collected by the candidate through a well-structured questionnaire from the animal record.

Serodiagnosis through Rose Bengal plate test (RBPT) and c-ELISA

Sera samples collected were primarily screened through RBPT by following all the principles and procedure as described by (Corbel, 1972) and antigen procured from IDEXX-USA (Porquier®, Rose Bengal Antigen, Montpellier, France). For comparison of the test samples results were compared with positive and negative control serum samples already maintained in our research laboratory. All the collected samples were screened through c-ELISA for the detection of anti-brucella antibodies through a commercially available c-ELISA kit (Savanova®, Sweden). Protocols and procedures mentioned in literature of the kit were followed accordingly. Instructions by the kit manufacturer were strictly followed for testing the sera.

DNA extraction and optimization of LAMP

All the c-ELISA positive samples were further subjected to genomic DNA isolation. DNA extraction from the *brucella* positive samples was done through the commercial DNA extraction kit provided by the Favorgen® (Taiwan, Ref no. FABGK001-2). All the manufacturers' procedures and protocols were strictly followed. LAMP primers i.e., F3, B3, BIP, FIP, LF and BF used in the current study for the LAMP Amplification were already described by Trangoni et al., 2015. Briefly, the LAMP reaction mixture were prepared as follows: a total volume of 25µl was prepared based on the commercial Loop amp® DNA Amplification Kit (New England Biolabs®, Ipswich, USA). It was composed of 1 × thermal buffer; 6 mM of $MgSO_4$; 0.8 M of betaine; 1.6 mM of deoxyribonucleotide triphosphate (dNTP); 0.2 µM of each outer primer; 1.6 µM of each inner primer; 0.8 µM of each loop primer; 8U of Bst DNA polymerase (New England Biolabs®, Ipswich, USA) and 2 µl of DNA templates. respectively. The incubation for the LAMP reaction was set at 65°C for 45 mins and carried out in water bath. All the procedures and protocols as mentioned by Trangoni et al. (2015) were followed. In this study 1.5% agarose gel was prepared and 5 µL of LAMP product was run on agarose gel to visualize results. The positive control for *B. abortus* was procured from Veterinary Research Institute (VRI), Lahore, Pakistan while as a negative control, nuclease free water (NFW) was used. Results were also confirmed by the naked eye by adding the 1µL SYBRE green dye (Thermoscientific®, USA). LAMP positive reaction was shown to a typical ladder-like pattern when subjected to electrophoresis (Fig. 4 (B)).

Statistical analysis

The obtained data was subjected to Chi-square test by statistical software through MINITAB 21 to estimate prevalence of bovine brucellosis and its association with various risk factors based upon the RBPT and c-ELISA results. The association between both variables (response & explanatory) was estimated through binary logistic regression.

Results

Serological investigations of brucellosis in bovines

Overall prevalence of brucellosis in bovines (Cows and Buffaloes) in study area was observed 10.86 and 95% through the Rose Bengal Plate Test (RBPT) and competitive enzyme linked immunosorbent assay (c-ELISA), respectively. The prevalence recorded after the initial screening through RBPT in cows and buffaloes was 12.16 and 9.13%, respectively. Highest prevalence was observed in cows as compared to the buffaloes. The prevalence in cows and buffaloes was significantly ($P \leq 0.05$) different and incidence in cows was 1.34 times higher as compared to the buffaloes (Fig 1).

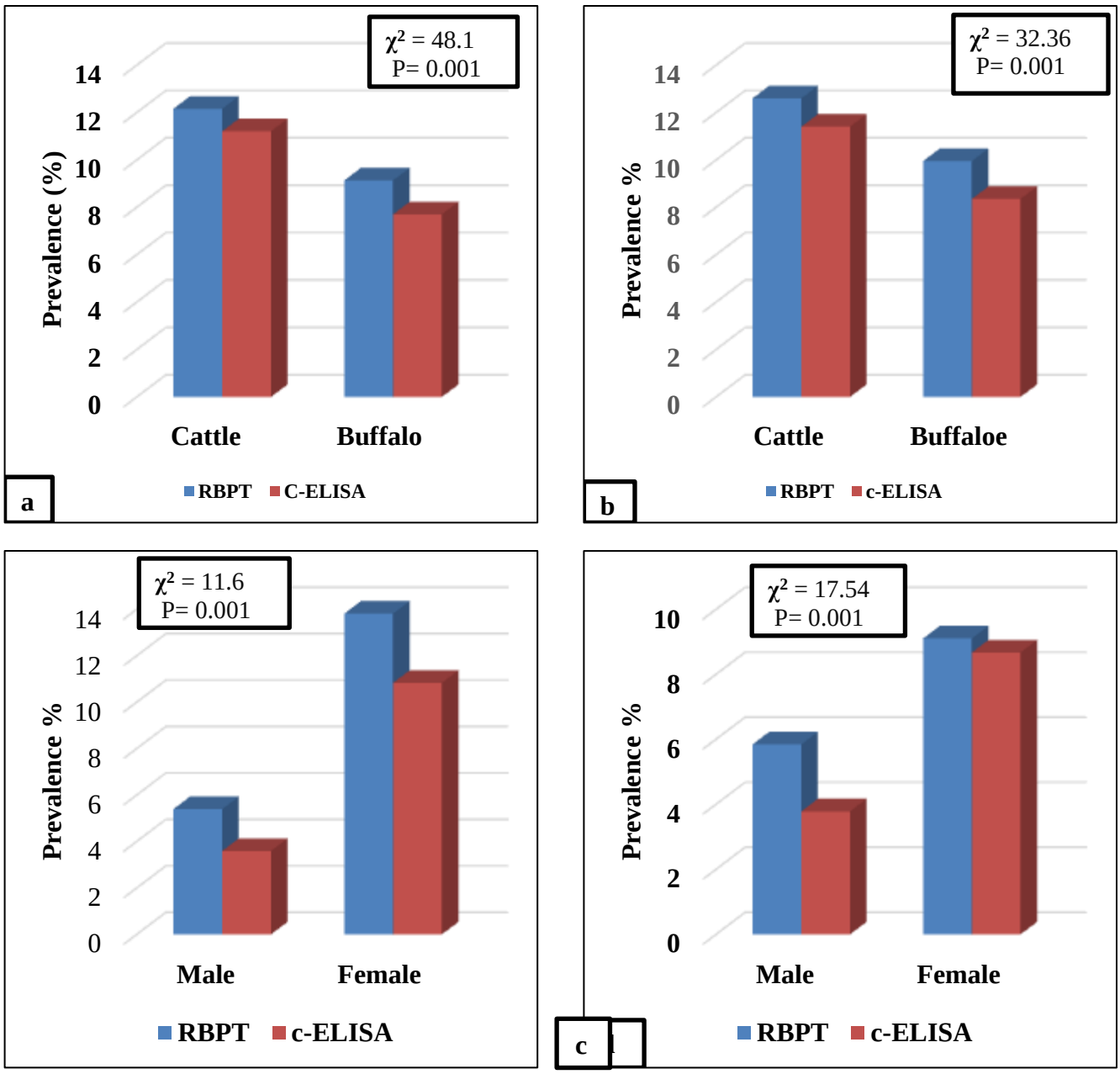

Figure-1. Prevalence of Bovine Brucellosis in dairy herds associated with risk factors (a) Overall prevalence cattle vs buffalo (b) District based prevalence in cattle vs buffalo (c) Sex-based prevalence in cattle (d) Sex-based prevalence in buffalo.

In Faisalabad, the prevalence of brucellosis is based upon RBPT and c-ELISA was observed was 12.60 and 11.4%, and in the buffaloes the prevalence observed was 9.95 and 8.35% through the RBPT and c-ELISA, respectively. High prevalence was observed in cows through both tests i.e., RBPT (12.60%) and c-ELISA (11.4%) as compared to the buffaloes, and it was significantly ($P \leq 0.05$) higher statistically (Fig 1).

In short, the prevalence of brucellosis observed in Faisalabad in cows and buffaloes through both tests was higher as compared to that of the prevalence of brucellosis observed in Toba Tek Sigh and this difference in prevalence in both districts was statistically significant ($P \leq 0.05$). The chances of brucellosis were 2.36 times higher in district Faisalabad as compared to that of Toba Tek Singh (Fig 1).

Prevalence of brucellosis in bovines in relation to different risk factors

Sex: In cows, the prevalence of brucellosis through RBPT was 13.81 in female and in male was 5.39% while in case of buffaloes, prevalence was 9.01% in female and in male was 5.84%. Higher prevalence of disease was recorded in females as compared to males and similar results were also observed through c-ELISA. In cows, it was recorded that the prevalence of brucellosis in female was 13.06 and in males was 3.59% while in case of buffaloes the prevalence in females was 8.67 and in males was 3.78% as shown in Fig. 1.

Age: The prevalence of brucellosis based on RBPT in cows with reference to age groups was 12.08, 7.67, 14.14 and 15.74% in 1-2, 3-4, 5-6 years and more than 6 years of age, respectively. Highest prevalence was recorded in more than 6 years (15.74%) of age group followed by 5-6 years (14.14%), 1-2 years (12.08%) and 3-4 years (7.67%), while in buffaloes the prevalence was 5.26, 3.70, 14.90 and 6.00% in 1-2, 3-4, 5-6 and more than 6 years, respectively. Highest prevalence was recorded in 5-6 years (14.90%) of age group followed by more than 6 years (6.00%), 1-2 years (5.26%) and 3-4 years (3.70%). The difference in prevalence in different age groups was statistically significant ($P \leq 0.05$) as shown in Fig. 2.

The prevalence of brucellosis based on c-ELISA in cows was 11.77, 6.81, 11.75 and 14.46% in 1-2, 3-4, 3-6 and more than 6 years, respectively. Highest prevalence was recorded in more than 6 years (14.46%) of age group followed by 5-6 years (11.75%), 1-2 years (11.77%) and 3-4 years (6.81%) while in buffaloes the observed prevalence was 3.34, 2.27, 14.10 and 3.53% in 1-2, 3-4, 5-6 years and more than 6 years, respectively. Highest prevalence was recorded in 5-6 years (14.10%) of age group followed by more than 6 years (3.53%), 1-2 years (3.34%) and 3-4 years (2.27%) as shown in Fig. 2.

Reproductive disorder

Serological diagnosis through RBPT and c-ELISA indicated that the prevalence of brucellosis varies significantly ($P \leq 0.05$) between animals having any reproductive disorder as compared to that of clinically healthy animals. In current study, it was observed that prevalence of brucellosis based on RBPT in diseased (reproductive problem) cows (19.37%) and buffaloes (10.14%) was higher as compared to healthy cows (3.53%) and buffaloes (6.26%). While the observed prevalence in cows based upon the c-ELISA was 1.76 and 19.09% and in buffaloes 3.91 and 9.04% in healthy and diseased animals with reproductive disorder was observed. The difference in both situations was statistically significant ($P \leq 0.05$) as shown in Fig. 2.

Pregnancy status

Highest prevalence was recorded in pregnant cows as compared to non-pregnant. The current findings based upon the RBPT were 13.90 and 8.15% in pregnant and non-pregnant cows, respectively. While in case of buffaloes the observed prevalence through RBPT was 9.31 and 7.82% in pregnant and non-pregnant buffaloes, respectively. Based on c-ELISA the prevalence of brucellosis in cows was 13.04 and 6.82% and in buffaloes was 7.91 and 6.14% in case of pregnant and non-pregnant buffaloes, respectively. Statistically, the difference in prevalence between these two groups was significant ($P \leq 0.05$) as shown in Fig. 2.

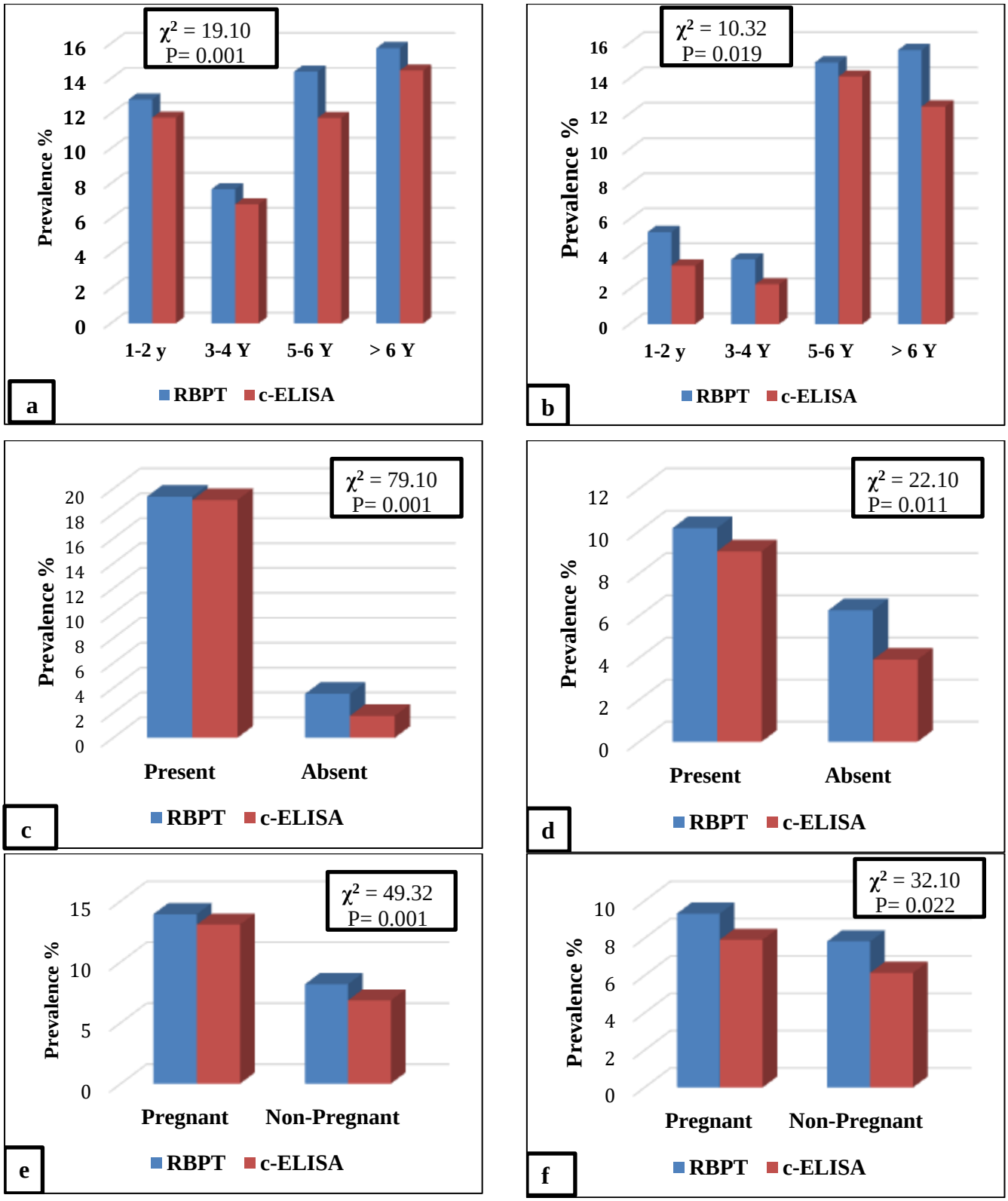

Figure-2. Prevalence (%) of bovine brucellosis in association to (a) Age based prevalence in cattle, (b) Age based prevalence in buffalo (c) Reproductive disorder based prevalence in cattle (d) Reproductive disorders based prevalence in buffalo (e) Pregnancy status based prevalence in cattle (f) Pregnancy status based prevalence in buffalo.

Parity No

Animals were divided into 5 groups depending upon their parity number i.e., 0, 1-2, 2-3, 3-4, 4-5 and 5 or more. Prevalence of brucellosis significantly varied ($P \leq 0.05$) among these groups. Through RBPT, the prevalence observed in cows was 6.57, 7.54, 14.68, 15.11 and 15.40% in 0, 1-2, 2-3, 3-4, 4-5 and 5 or more, respectively. In buffaloes, prevalence was 7.32, 6.58, 8.88, 15.71 and 9.54% in 0, 1-2, 2-3, 3-4, 4-5 and 5 or more, respectively. The prevalence of brucellosis based upon c-ELISA in cows was 5.71, 6.70, 14.02, 13.82 and 14.56 in 0, 1-2, 2-3, 3-4, 4-5 and 5 or more, respectively and in buffaloes was 5.23, 4.93, 8.08, 12.85 and 8.29% in 0, 1-2, 2-3, 3-4, 4-5 and 5 or more, respectively. The difference in prevalence in different parity numbers through c-ELISA was statistically significant ($P \leq 0.05$) as shown in Fig. 3.

Breeding system

Commonly, two types of breeding systems are used in local and corporate dairy sector i.e., Artificial insemination and natural breeding through common sire for whole herd. In the present study, prevalence of brucellosis was significantly different in both breeding systems through both diagnostic tests including RBPT and c-ELISA ($P \leq 0.05$). Prevalence of brucellosis in cows based on the RBPT was 10.66 and 20.59% and in buffaloes was 9.05 and 9.26% in artificial insemination and natural breeding, respectively. Higher prevalence was recorded through natural breeding as compared to artificial insemination. Prevalence through c-ELISA observed in cows was 10.18 and 16.94% and in buffaloes was 7.03 and 8.74% in artificial insemination and natural breeding, respectively as shown in Fig. 3.

Vaccination status

According to the current study disease was significantly different regarding the vaccination status of animals ($P<0.05$), the prevalence of brucellosis depending upon the RBPT observed was 3.32% and 18.64% in vaccinated and non-vaccinated cows and in case of buffaloes the observed prevalence was 7.40% and 9.69% in vaccinated and non-vaccinated animals. Similar findings were also observed through c-ELISA, and the prevalence observed in cows was 1.78 and 18.11% and in buffaloes was 4.62 and 8.55% in vaccinated and non-vaccinated animals, respectively. Higher prevalence was observed in non-vaccinated herds as compared to the vaccinated as shown in Fig. 3.

Optimization of LAMP assay for brucellosis

The positive cases during the initial serological screening from both (cows and buffaloes) were further subjected to Loop Mediated Isothermal Amplification (LAMP) Assay by using the specie specific primers targeting the region of the sequence encoding a 31kDa periplasmic immunogenic *bcsp*31 gene. The optimum LAMP assay results were observed at 65°C for 45 mins and in current study detection limit of LAMP assay was 100-fold more recorded as compared to conventional PCR which is commonly used DNA based diagnostic tool for *B. abortus*. The overall observed molecular prevalence based on the LAMP assay was 27.38% (92/336), while in cows and buffaloes was 27.47% (61/222) and 27.19% (31/114), respectively. Qualitive results observed through color change and on agar rose gel have been shown in Fig. 4 (A & B).

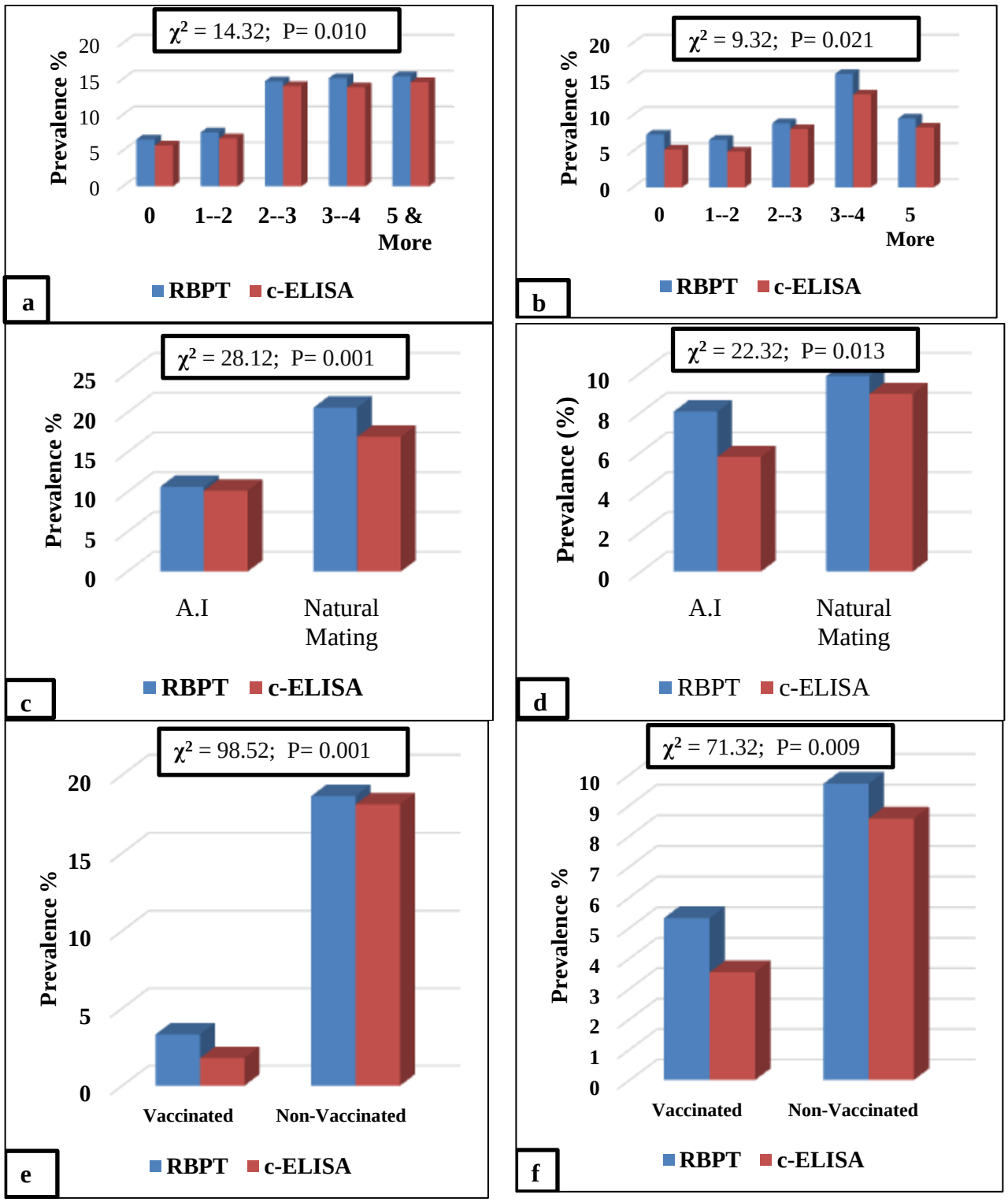

Figure-3. Prevalence (%) of bovine brucellosis in association to (a) parity based prevalence in cattle (b) parity based prevalence in buffalo (c) breeding method based prevalence in cattle (d) breeding method based prevalence in buffalo (e) vaccination status based prevalence in cattle (f) vaccination status based prevalence in buffalo.

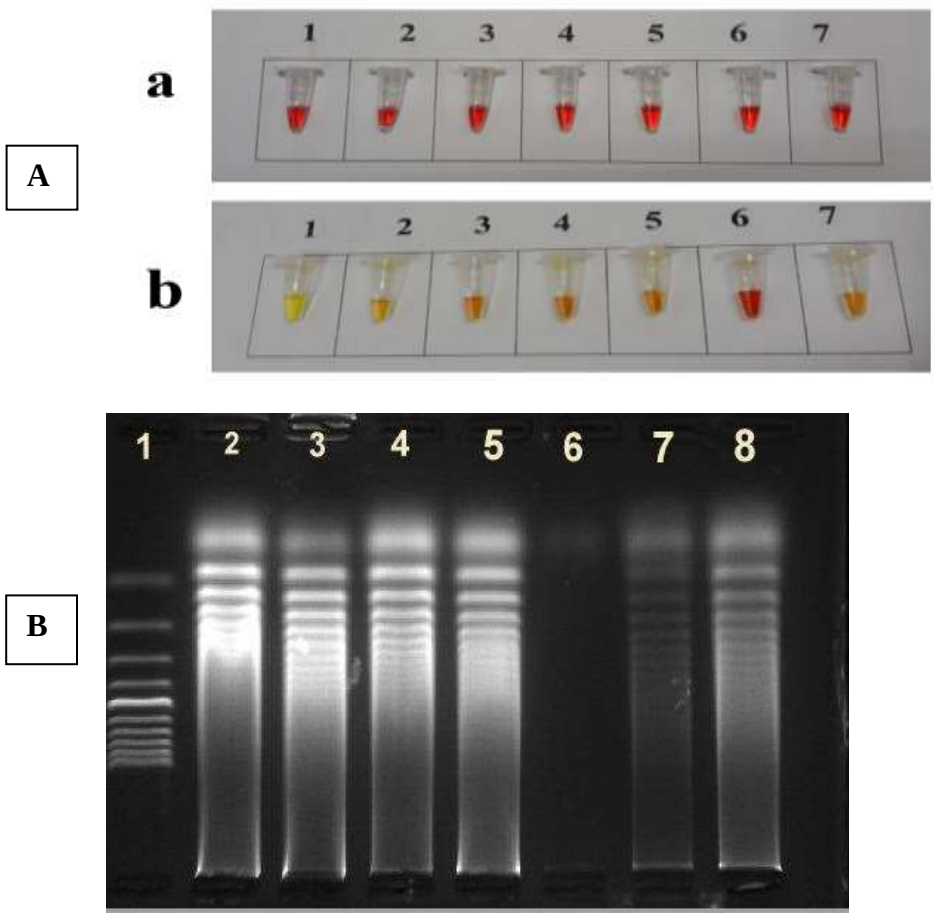

Figure4: A) Photograph of selected samples positive for *Brucella* (LAMP results) a) showed samples mixed with master mix before incubation in water bath. b) Photo of samples after water bath incubation color changed (Red to yellow) indication of positive results naked eye. B) LAMP results: photograph showing positive cases for *B. abortus* Lanes explanation: 1, ladder (50bp); 2, control positive; 6, control negative; 3, 4, 5, 7 and 8 positive test samples.

Discussion

Brucellosis is a considered among highly contagious and zoonotic diseases around the globe. It causes massive economic losses due to reduced milk production, replacement of dairy herds, which also has been attributed to many other pathogens leading to mastitis and repeat breeding. However major concern is about the trade restrictions on animals and animal-origin food products due to endemicity of brucellosis in Asian and sub-Saharan African regions (Saleem et al., 2020; Babar et al., 2021). The disease affects variety of livestock species including farm/food animals, pet animal aquatic mammals, freshwater fishes and many other wild species including humans (Ducrotoy et al., 2017; Gemechu, 2017).

Early diagnosis in this disease is very difficult because of lacking characteristic signs and symptoms (Jiang et al., 2020). Initial and economical test used in the sero-diagnosis of disease is RBPT which is fast and simple, is executed mainly for *B. abortus* strain in serum. According to OIE guidelines, results of RBPT should be confirmed by more accurate assays like CFT or Enzyme Linked Immunosorbent Assay (ELISA) for true positivity (Mahmood et al., 2016).
Researchers have reported prevalence of brucellosis in cattle and buffaloes in Pakistan. These reports are regarding the epidemiology of the diseases through already available serological tests, but no such application of rapid and quick diagnosis of brucellosis based on DNA and easier to perform have been reported previously (Gul et al., 2014; Gul et al., 2015; Jiang et al., 2020; Ullah et al., 2020; Jamil et

al., 2021; Hussain et al., 2022).

Overall sero-prevalence of brucellosis in bovines in study area was highest in cows as compared to the buffaloes. The prevalence based upon the RBPT in cows and buffaloes was statistically significant (*P≤0.05*). These findings are in agreement with the previously documented literature (Gul et al., 2015; Khan et al., 2020; Mustafa et al., 2023). The high incidence rate of brucellosis in cows and buffaloes is due to persistence abortions at farms which may be the source of contamination and leads to storms of abortion at infected farms, intensive farming and malpractices (mode of insemination, management etc) are other major reasons of high prevalence of brucellosis in this region. In this study, the prevalence observed in cows through RBPT, and c-ELISA was in agreement with the previously documented prevalence (10-23.4% and prime reasons are same as mentioned above like carrier animals at farm along with the intensive farming and poor husbandry practices (Kaltungo et al., 2014; Gul et al., 2015).

The prevalence of brucellosis observed in Faisalabad in cows and buffaloes through the both tests RBPT and c-ELISA was higher as compared to that of the prevalence of brucellosis observed in Toba Tek Sigh though both tests RBPT and c-ELISA and the prevalence recorded in this study in bovines in district Toba Tek Singh is higher as compared to the previously documented reports (Kaltungo et al., 2014; Gul et al., 2015; Khan et al., 2020). High incidence of brucellosis in female is because of the fact that, females are kept for longer period of time as compared to male so more time is available for expose to bacterium and female are likely to be in more stress during pregnancy, lactation and hormonal changes as compared to males, so animal related resistance is less in female (Shahzad et al., 2018).

Bovines may suffer from brucellosis at any age group, but the infection is more prevalent and persistent in adult animals (Shahzad et al., 2018; Di Bonaventura et al., 2021). As described in the previous literature, this study observed same prevalence of brucellosis, which is significantly higher in aged animals as compared to the younger animals (Saleem et al., 2020; Babar et al., 2021). Animals are more at risk to brucellosis with advancement in age due to the higher levels of erythritol in sexually mature animals which boosts the growth of *Brucella* (Pérez-Sancho et al., 2013) However, younger animals have less prevalent though latent infection may be present. Animals goes outside for grazing on contaminated pasture are more at risk to be suffer from brucellosis as compared to the younger animals which are not sexually matured (Shahzad et al., 2018).

Serological analysis through RBPT and c-ELISA indicated that the prevalence of brucellosis varies significantly (P≤0.005) between animals having any reproductive disorder as compared to that of clinically healthy animals. In this study, it was observed that the seropositivity of brucellosis was higher in animals suffered from reproductive disorders as compared to healthy ones. Different other reports also revealed strong association between incidence of brucellosis with that of reproductive health status (Abubakar et al., 2010). In current study significant prevalence was recorded in pregnant animals as compared to non-pregnant animals. Pregnant and sexually mature animals may be easily infected with brucella as compared to non-pregnant animals (Sharun et al., 2021). In present study likewise as in age groups the high prevalence of disease was observed in animals with more parity number as compared to lower parity number. These findings are in accordance with the previously documented literature (Adamu et al., 2016; Bardhan et al., 2020).

Commonly, two types of breeding systems are used in local and corporate dairy sector i.e., Artificial insemination and natural breeding through common sire for whole herd. In the present study, it was recorded that the prevalence of brucellosis was significantly different in both breeding systems and higher disease rates were observed in natural breeding as compared to artificial insemination. It seems to be that the infected bull is responsible for the spread of disease in entire herd. (Freddi et al., 2021).

There are two types of the vaccines i.e., S19 and RB51 for *Brucella abortus* those are commercially available and vaccination to young stock is done at the age of 3-6 months along with the booster dose annually. In current study, less outbreak of brucellosis was recorded at vaccinated herds as compared to non-vaccinated herds (Godfroid et al., 2013; Di Bonaventura et al., 2021).

Many of serological diagnostic techniques lack specificity and molecular assay like PCR need sophisticated instruments (Sharun et al., 2021). Recommended test to screen brucellosis at field level. By keeping in view, the limitations of currently used

serological techniques a specific, sensitive, quick and reliable LAMP assay was evaluated in current study for the mass confirmation of brucellosis samples at field level.

Because of the swiftness, specificity and sensitivity the LAMP assay modernized the field of diagnostics for the detection of infectious pathogens as rapid point of care (Meurens et al., 2021; Abdalhamed et al., 2023). Detection of *Brucella* at genus level by LAMP assay has been reported previously but detecting of *Brucella* specifically at specie level is missing (Hemade and Gandge, 2016). The newly evaluated LAMP assay targeting the single *Brucella* specie may the landmark for the development of multiplex LAMP assay which can be used to differentiate different Brucella spp. in a single reaction. According to the current study the field samples for *B. abortus* investigated by the newly evaluated LAMP indicated that LAMP assay detects more positive samples as compared to other serological techniques used in this study which showed that LAMP is more accurate, quick and sensitive to be used for mass screening of *Brucella abortus* as compared to PCR and these finding were in agreement to the previously documented reports (Hemade and Gandge, 2016).

Conclusion

It has been concluded from the study that the evaluated LAMP assay detecting the *Brucella* at specie level can be specifically used for diagnosis and screening of samples for *B. abortus*. In future, currently evaluated *B. abortus* LAMP may be used for advancement of multiplex LAMP assay that can be a hallmark in the diagnosis of brucellosis as well as can be valuable in differentiating *Brucella* spp. The test being rapid, reliable, specific and sensitive, the results can be viewed and interpreted by the naked eye by adding SYBR green and thus requirement of complex instruments may not be necessary for qualitative results.

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Contribution of Authors

Bilal M: Actively involved in the execution of the project, data analysis, write up of the manuscript, read and approved the final manuscript.
Gul ST: Actively involved in the execution of the project, data analysis, write up of the manuscript, read and approved the final manuscript.
Javed MT: Actively involved in the execution of the project, data analysis, write up of the manuscript, read and approved the final manuscript.
Saqib M: Actively involved in the execution of the project, data analysis, write up of the manuscript, read and approved the final manuscript.

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