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Short Communication

Prevalence of Brucellosis in Hospitalized Patients in Islamabad and Rawalpindi, Pakistan

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ABSTRACT

Prevalence of human brucellosis in hospitalized patients of the twin-cities of Rawalpindi and Islamabad was studied using Rose Bengal Plate Test (RBPT) and quantitative real-time PCR (qPCR). Risk factors possibly contributing to the transmission of disease in the region were also investigated. A total of 276 serum samples were collected from hospitalized patients suspected of having brucellosis. RBPT was performed for the initial screening of serum samples and 23 samples were found to be positive. These RBPT positive serum samples were then subjected to a qPCR for *Brucella*, and out of the 23 samples 15 were confirmed positive by the qPCR. Due to the potential non-specificity of the RBPT, only those samples that were positive by both RBPT and qPCR were considered positive for brucellosis. Consequently, the combined prevalence rate of brucellosis among hospitalized patients in the regions of Rawalpindi and Islamabad was 5.43%. All the 15 positive samples were from female patients, who were from rural areas, and all of whom were animal handlers. Thirteen of the 15 patients had a history of consuming raw milk. Rural background, female gender, contact with animals, and raw milk consumption were identified as potential risk factors associated with the prevalence of the disease.

Brucellosis is a zoonotic disease affecting many species of livestock, including cattle, buffaloes, sheep, goats, pigs, dogs, and other animals. It is caused by members of the genus *Brucella*, and human brucellosis is mostly caused by *B. melitensis*, *B. abortus*, *B. suis*, and *B. canis*. The disease is globally prevalent, with the exception of a few industrialized countries. It poses an occupational health hazard, particularly for animal farmers, veterinarians, and abattoir workers, who are at high risk of contracting the infection from animals. Transmission to humans mainly occurs through inhalation of bacteria from aborted fetuses, placentas, and uterine secretions of animals, although it can also occur through wounds. Consumption of raw milk or dairy products is another source of transmission. According to the WHO, millions of brucellosis cases are reported annually worldwide, but the actual number may be 10-25 times higher than the reported cases. In developing countries, efforts to reduce brucellosis prevalence have been hampered by inadequate hygiene and sanitation, poverty, and a lack of effective administration. In humans, typical symptoms of brucellosis include undulant fever, fatigue, chills, joint pain, headaches, and myalgia (Khurana *et al*., 2011). Human brucellosis is particularly significant for women's health due to its potential harmful effects on the female reproductive system (Kurdoglu *et al*., 2015).

The serological tests most commonly used for diagnosing human brucellosis are the serum agglutination test (SAT), Rose Bengal test (RBPT), Coombs' test (CT), and enzyme-linked immunosorbent assay (ELISA). Molecular detection through PCR is also employed in diagnostic laboratories (Yagupsky *et al*., 2019).

Pakistan is an agricultural country with 60-70% of its population in direct contact with animals. Consequently, the risk of brucellosis transmission to humans is high (Abubakar *et al*., 2011). Although numerous studies have examined prevalence of brucellosis in livestock, few have focused on its occurrence in humans in Pakistan. Prevalence of brucellosis is increasing in the livestock

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population of Pakistan including the regions of Islamabad and Rawalpindi (Ali *et al*., 2017), and therefore, continuous monitoring of the local human population for prevalence of brucellosis is essential. The aim of this study was to estimate the prevalence of brucellosis in hospitalized patients in Rawalpindi and Islamabad and to identify the risk factors associated with the disease.

Materials and methods

A total of 276 blood samples (3-4 ml per sample) were collected from patients suspected of having brucellosis and admitted to seven hospitals in Islamabad and Rawalpindi. Among these, 71 patients were from the Islamabad region (58 from urban areas and 13 from rural areas). The remaining 205 patients were from the Rawalpindi region (103 from urban areas and 102 from rural areas). Oral consent was obtained from all patients before collecting samples from them. Patients were showing symptoms such as undulant fever, loss of appetite, and body-wide aches and pains. Blood samples were collected in specialized serum-separating tubes containing clot-activating factors. During sample collection, patients' age, sex, locality, and histories of contact with animals, raw milk consumption, and abortion (in females) were recorded. The serum samples were carefully transported on ice to the Department of Zoology and stored at -20 °C until further analysis.

The RBPT was performed at the Department of Zoology, Quaid-i-Azam University, Islamabad, using RBPT antigen obtained from the Veterinary Research Institute, Lahore, Pakistan. Positive and negative control sera were provided by the Friedrich-Loeffler Institute, Jena, Germany. Briefly, 30 µl of test serum were placed on a white slide. Then, 30 µl of the RBPT antigen were mixed with the serum using a sterile toothpick. The mixture was examined for agglutination after 4 minutes, and the results were compared with the positive and negative controls (Diaz *et al*., 2011).

RBPT-positive serum samples were subjected to qPCR for the molecular detection of *Brucella*. The qPCR was conducted at the Brucella Biosafety Laboratory, University of Veterinary and Animal Sciences, Lahore – Jhang Campus, Jhang. Serum samples were processed for genomic DNA extraction using the JetFlex™ Genomic DNA Purification Kit (Invitrogen, USA, Cat No. A30701) following the manufacturer's guidelines. *Brucella* genus-specific and species-specific (*B. abortus* and *B. melitensis*) qPCR assays were performed as previously described (Saddique *et al*., 2019).

The association of potential risk factors (locality, gender, animal contact, and raw milk consumption) with brucellosis in the local population was analyzed using the chi-square test with R-GUI software (R Foundation for Statistical Computing, Austria). Factors with p-values ≤ 0.05 were considered significant.

Results

Out of 276 serum samples, 23 were found to be positive by the RBPT. Fifteen of these 23 RBPT-positive samples were confirmed positive for *Brucella* by qPCR (Table I). Only these 15 samples that were positive both by the RBPT and the qPCR, were declared positive for brucellosis, as suggested in previous studies (Gwida *et al*., 2011). Thus, the combined prevalence rate in Islamabad and Rawalpindi was 5.43%. In all 15 samples, only *B. abortus* was detected through species-specific qPCR. As a check, 40 randomly selected RBPT-negative samples were tested using qPCR, and all 40 were confirmed negative for *Brucella*.

Table I. Region-wise prevalence of human brucellosis in Rawalpindi and Islamabad.

Region	Total samples	Urban	Positive Urban	Rural	Positive rural
Islamabad	71	58	0	13	2
Rawalpindi	205	103	0	102	13
Total	276	161	0	115	15

Table I shows the regional distribution of samples. In Islamabad, 2 out of 71 samples (2.81%) were positive, while in Rawalpindi, 13 out of 205 (6.34%) samples were positive. All positive samples (13.04%) were from patients with a rural background.

Table II. Prevalence in terms of risk factors possibly associated with human brucellosis in Rawalpindi and Islamabad.

Risk factors	Category	Samples tested	Total positive	Positive %
Locality	Rural	115	15	13.04
	Urban	161	0	0.00
Gender	Male	94	0	0.00
	Female	182	15	8.24
Animal contact	Yes	104	15	14.42
	No	172	0	0.00
Raw milk consumption	Yes	135	13	9.62
	No	141	2	1.41

Prevalence in terms of possible risk factors associated with bovine brucellosis in the twin cities is presented in Table II. All 15 positive cases were from the rural community (13.04%) and were female patients (8.24%).

Out of these 15 cases, 12 had a history of abortion. The positive cases also had a history of regular contact with animals (14.42%). Thirteen (9.62%) patients reported consuming raw milk. Statistical analysis using the chi-square test showed that rural background ($p = 0.000005$), female gender ($p = 0.005644$), contact with animals ($p = 0.0000006$), and raw milk consumption ($p = 0.004483$) were significant risk factors associated with the prevalence of brucellosis.

Discussion

Brucellosis is an important zoonotic disease. Its prevalence is increasing in the livestock in Pakistan (Abubakar *et al*., 2011). Human-to-human transmission of the disease is also possible (Tuon *et al*., 2017). Therefore, it is crucial to monitor the prevalence of this disease in animal handlers and the general human population.

In Pakistan, brucellosis in humans was first identified in 1979 with a seroprevalence rate of 0.6% (Mohiyudin, 1979). Since then, the prevalence of brucellosis has continued to rise in various regions of Pakistan. For instance, a high seroprevalence rate of 21.7% was observed in abattoir workers in Lahore (Mukhtar and Kokab, 2008). In District Bhimber, the seroprevalence rates were 6.66% in males and 12.0% in females (Din *et al*., 2013). In a study using SAT and PCR on veterinary professionals, livestock farmers, and butchers, overall prevalence rates of 38.9% (SAT) and 14.7% (PCR) were noted in these workers (Asif *et al*., 2014).

In the present study, the overall prevalence of brucellosis was found to be 5.43% in hospitalized patients in Rawalpindi and Islamabad. Statistical analysis revealed that rural background, female gender, contact with animals, and raw milk consumption were linked with the occurrence of brucellosis.

Similar findings have been reported in previous studies conducted in Pakistan. An overall seroprevalence rate of 6.9% in high-risk individuals (veterinary personnel, milkers, abattoir workers, and livestock farmers) was found in the Potohar region. Individuals consuming raw milk were at higher risk of developing brucellosis (Ali *et al*., 2013). In Rawalpindi, 5.8% of pregnant women were found to be seropositive for brucellosis, with risk factors including animal contact, raw milk consumption, a history of abortion, and intrauterine fetal death (Ali *et al*., 2016). In a recent study on patients with acute febrile illness, *Brucella* DNA was detected in 5.8% of cases, with the prevalence associated with contact with infected animals, raw milk consumption, socioeconomic status, older age, and female gender (Saddique *et al*., 2019). In a cross-sectional study of the general population in Punjab, exposure to animals and raw milk consumption were strongly associated with brucellosis (Arif *et al*., 2017).

Brucella has been shown to multiply in human trophoblasts (Salcedo *et al*., 2013), and it might have the potential to cause abortion in women (Kurdoglu *et al*., 2015). Therefore, proper education and awareness, especially for pregnant women, are advised.

Milk is a vital part of the daily diet for people of all ages in rural Pakistan. Milk is usually consumed boiled, as pasteurized milk, or as ultra-high-temperature-treated milk. However, in rural areas, some people consume raw milk. In a large-scale risk assessment study, 66% of livestock farming families in Punjab and Sindh provinces of Pakistan reported consuming raw milk or its products (Arif *et al*., 2017). Since *Brucella* can be shed in the milk of infected animals, raw milk may contain the organism. Hence, the practice of consuming raw milk should be discouraged, and milk should always be boiled or pasteurized before consumption.

Out of the 23 RBPT-positive samples, 15 were confirmed positive by qPCR, suggesting low specificity of the RBPT. This, along with findings from previous studies (Gwida *et al*., 2013; Saddique *et al*., 2019), indicates that while RBPT is a cost-effective method for initial brucellosis screening, other tests such as qPCR and ELISA etc. should be used for confirmation of results. The World Organization for Animal Health (WOAH) also recommends that no single serological test, including RBPT, should be solely relied upon for brucellosis confirmation, and multiple tests should be performed to confirm a positive case (WOAH, 2022).

Conclusion

The prevalence rate of human brucellosis in the Rawalpindi and Islamabad regions was found to be 5.43% in this study. Thus, brucellosis should not be overlooked in patients exhibiting symptoms such as fever, body and joint pain, headaches, sweating, and fatigue. Rural background, contact with animals, female gender, and raw milk consumption were identified as significant risk factors associated with the occurrence of this disease. Additionally, due to the low specificity of RBPT, any serum samples testing positive for brucellosis with RBPT should be confirmed using some other molecular or serological method.

DECLARATIONS

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IRB approval

This study was approved by the IRB of the Quaid-i-Azam University and the IRB of University of Veterinary and Animal Sciences, Lahore – Jhang Campus, Jhang.

Ethical statement

This study was approved by the Institutional Bioethics Committees of Quaid-i-Azam University and the University of Veterinary and Animal Sciences, Lahore–Jhang Campus, Jhang.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

References

Abubakar, M., Mansoor, M. and Arshed, M.J., 2011. *Pak. Vet. J.,* **32**: 147-155. http://www.pvj.com.pk/pdf-files/32_2/147-155.pdf

Ali, S., Akhter, S., Neubauer, H., Melzer, F., Khan, I., Abatih, E.N., El-Adawy, H., Irfan, M., Muhammad, A., Akbar, M.W., Umar, S., Ali, Q., Iqbal, M.N., Mahmood, A. and Ahmed, H., 2017. *BMC Res. Notes*, **10**: 73. https://doi.org/10.1186/s13104-017-2394-2

Ali, S., Akhter, S., Neubauer, H., Scherag, A., Kesselmeier, M., Melzer, F., Khan, I., El-Adawy, H., Azam, A., Qadeer, S. and Ali, Q., 2016. *BMC Infect. Dis*., **16**: 468. https://doi.org/10.1186/s12879-016-1799-1

Ali, S., Ali, Q., Neubauer, H., Melzer, F., Elschner, M., Khan, I., Abatih, E.N., Ullah, N., Irfan, M. and Akhter, S., 2013. *Foodb. Pathog. Dis*., **10**: 500-505. https://doi.org/10.1089/fpd.2012.1360

Arif, S., Thomson, P.C., Hernandez-Jover, M., McGill, D.M., Warriach, H.M. and Heller, J., 2017. *PLoS One,* **12**: e0173365. https://doi.org/10.1371/journal.pone.0173365

Asif, M., Waheed, U., Farooq, M., Ali, T. and Khan, Q.M., 2014. *Int. J. Agric. Biol.,* **16**: 986-990.

Díaz, R., Casanova, A., Ariza, J. and Moriyón, I., 2011. *PLoS Negl. Trop. Dis.*, **5**: e950. https://doi.org/10.1371/journal.pntd.0000950

Din, A.M.U., Khan, S.A., Ahmad, I., Rind, R., Hussain, T., Shahid, M. and Ahmed, S., 2013. *J. Anim. Pl. Sci*., **23**: 113-118.

Gwida, M.M., El-Gohary, A.H., Melzer, F., Tomaso, H., Rösler, U., Wernery, U., Wernery, R., Elschner, M.C., Khan, I., Eickhoff, M., Schöner, D. and Neubauer, H., 2011. *BMC Res. Notes*, **4**: 525. https://doi.org/10.1186/1756-0500-4-525

Khurana, S.K., Sehrawat, A., Tiwari, R., Prasad, M., Gulati, B., Shabbir, M.Z, Chhabra, R., Karthik, K., Patel, S.K., Pathak, M., Yatoo, M.I., Gupta, V.K., Dhama, K., Sah, R. and Chaicumpa, W., 2021. *Vet. Q*., **41**: 61-88. https://doi.org/10.1080/01652176.2020.1868616

Kurdoglu, M., Cetin, O., Kurdoglu, Z. and Akdeniz, H., 2015. *Int. J. Women's Hlth. Reprod. Sci.*, **3**: 176-183. https://doi.org/10.15296/ijwhr.2015.38

Mohiyudin, M.A.Z., 1979. *Pak. J. Med. Res.*, **18**: 1-7.

Mukhtar, F. and Kokab, F., 2008. *J. Ayub Med. Coll. Abbottabad*, **20**: 56-61.

Saddique A., Ali S., Akhter S., Khan I., Neubauer H., Melzer F., Khan A.U., Azam A. and El-Adawy H., 2019. *Int. J. Environ. Res. Publ. Hlth.*, **16**: 4071. https://doi.org/10.3390/ijerph16214071

Salcedo, S.P., Chevrier, N., Lacerda, T.L., Ben Amara, A., Gerart, S., Gorvel, V.A., de Chastellier, C., Blasco, J.M., Mege, J.L. and Gorvel, J.P., 2013. *J. Infect. Dis*., **207**: 1075-1083. https://doi.org/10.1093/infdis/jit007

Tuon, F.F., Gondolfo, R.B. and Cerchiari, N., 2017. *Trop. Med. Int. Hlth.*, **22**: 539-546. https://doi.org/10.1111/tmi.12856

WOAH, 2022. Brucellosis (Infection with *B. abortus*, *B. melitensis* and *B. suis*) (version adopted in 2022). In: *WOAH manual of diagnostic tests and vaccines for terrestrial animals, 12th edn*. Available at: https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.01.04_BRUCELLOSIS.pdf (accessed 27 Sep 2024).

Yagupsky, P., Morata, P. and Colmenero, J.D., 2019. *Clin. Microbiol. Rev*., **33**: e00073-19. https://doi.org/10.1128/CMR.00073-19