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Incidence of Bovine Brucellosis in Thatta, Sindh-Pakistan

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Abstract: A study was done to investigate the incidence of Brucella abortus in cattle and buffaloes in Thatta Sindh. A total of n = 360 serum samples were randomly collected from buffaloes and cattle (130 each species). The Rose Bengal Plate Test was used to screen serum samples at first (RBPT). A B. abortus specific indirect enzyme-linked immunosorbent test was performed on RBPT positive samples (i-ELISA). An rPCR was used to investigate the efficacy of detecting Brucella in the blood of infected animals after serum samples were proven to be positive for B. abortus by serology. The effectiveness of an rPCR reported in detecting Brucella at the genus level and later at the species level (B. abortus and B. melitensis) in the serum of sick cattle and buffaloes was investigated. The samples that were verified to be positive via both immunological tests, RBPT and i-ELISA, were submitted to the rPCR for this reason. Initially, rPCR based on the Brucella genus-specific bcsp31 genomic region was utilized. The IS711 genomic region of B. abortus and B. melitensis was discovered using two species-specific rPCRs. By RBPT, 13 serum samples from cattle (10%) and 3 from buffalo (2.31%) were shown to be positive for B. abortus. 8 (6.15%) of the 13 RBPT positive cattle samples also tested positive in i-ELISA, whereas 5 tested negative. The 3 buffalo that tested positive for RBPT then 2 were tested positive for i-ELISA. All 8 seropositive samples had Brucella genus specific rPCR amplification. B. abortus was found in all of the samples using species-specific rPCR.

Keywords: Bovine Brucellosis, ELISA, Sindh, RBPT, rPCR

1. Introduction

Brucellosis is a financially important illness that affects a variety of animal species, and it is one of the diseases recognized by the Office International des Epizooties (OIE). Cattle, buffalo, swine, sheep, goats, camels, and dogs are among the animals that can contract the disease, and because it is zoonotic, it can also infect humans [1]. Bacteria can be isolated from body fluids, tissues, and aborted babies for disease diagnosis [19]. However, due to the difficulty of culturing the organism, this is not done. Instead, several serological procedures such as the Milk Ring Test (MRT), Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT), and ELISA are used to diagnose [5]. PCR, which detects Brucella DNA in serum samples from infected animals, is a faster, less expensive, sensitive, and safe method [4]. When used on milk samples for the diagnosis of bovine brucellosis, the PCR and i-ELISA were shown to have sensitivity levels of 87.5% and 98.2%, respectively [4]. Based on these findings, it is suggested that both tests be used in conjunction for herd screening in epidemiological and surveillance programmes [18]. Brucellosis has been recorded in Pakistan for a long time, and due to its rising frequency, routine screening of livestock herds and animals brought to abattoirs and livestock markets has become a priority [2, 3]. Antibodies to B. abortus were tested in cattle and buffaloes in district Thatta of Sindh in this investigation. In addition, rPCR assay was evaluated for its ability to detect Brucella in the serum of afflicted local breeds of animals.

2. Materials and Methods

The research was carried out in district Thatta, Sindh province areas. In 10 mL disposable clot activating tubes, n= 360 blood samples (130 each from cattle and buffaloes) were obtained at random. Animals from animal markets and

abattoirs, as well as animals kept in small animal holdings, were used to gather samples. A history of abortion or a long calving interval was also recorded when collecting samples. Animals brought in for treatment at veterinary institutions in District Thatta Sindh province the areas also provided samples. The samples were sent to Government Veterinary Diagnostic Laboratory at 4°C. To speed up the serum separation process, samples were centrifuged at 10,000 rpm for 1 minute and stored at -20°C until further use. The RBPT was carried out according to the instructions in the manual published by the Office International des Epizooties (OIE) (www.oie.int/fileadmin/Home/eng/Healthstandards/tahm/2.01.04_ Brucellosis.pdf). On a slide, a drop of test serum was mixed with 30µL of RBPT antigen (obtained from the SB Lab Rawalpindi) for 4 minutes. A positive control reaction utilizing positive serum was performed alongside each test sample. Agglutination was tested in each test sample by comparing it to the positive control. Following the manufacturer's instructions, serum samples reported to be positive by the RBPT were submitted to a B. abortus specific i-ELISA using a commercially available kit (Cat. No. C561, IDEXX Switzerland). The i-ELISA was carried out at the SB Lab. The effectiveness of an rPCR reported [10] in detecting Brucella at the genus level and later at the species level (B. abortus and B. melitensis) in the serum of sick cattle and buffaloes was investigated. The samples that were verified to be positive via both immunological tests, RBPT and i-ELISA, were submitted to the rPCR for this reason. A widely available kit was used to extract genomic DNA from serum samples (Cat No. FABGK001, Favorgen, Taiwan). On genomic DNA, a Brucella genus-specific rPCR targeting the bcsp31 gene was done using the primers 5'GCTCGGTTGCCAATATCAATGC 3' and 5'GGGTAAAGCGTCGCCAGAAG 3', as well as the genus-specific probe 5' 6FAMAAATCTTCCACCTTGCCCTTGCCATCABHQ1 3' and the genus-specific probe 5' 6FAMAAATCTTCCACC (Tibmolbiol, Berlin, Germany). The following ingredients were used in the reaction: 10µL Taq-Man^{TM} Universal Master Mix (Applied Biosystems, New Jersey), 200 nM primers, 100 nM probe, 4µL template DNA, and water up to a total volume of 20µL. No-template-control (NTC) and positive control reactions containing Brucella DNA were amplified alongside each test reaction. The amplification regimen consisted of 10 minutes at 95°C, followed by 45 cycles of 15s at 95°C and 1 minute at 57°C. The following species-specific rPCR primers were used to target the IS711 element downstream of the alkB gene in B. abortus and insertion of the same element downstream of the BMEI1162 locus in B. melitensis: 5'GCGGCTTTTCTATCACGGTATC3' and reverse 5'CATGCGCTATGATCTGGTTACG3' 5'AACAAG CGGCACCCCTAAAA3' and reverse CATGCGCTA HEXCGCTCATGCTCGCCAGACTTCAATGBHQ1 and CY5CAGGAGTGTTTCGGCT CAGAATAATCCACABHQ2 were the probes employed for B. abortus and B. melitensis, respectively. The reaction mixture was the same as in the genus-specific rPCR, but the amplification parameters were changed: initial denaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, and annealing/extension at 60°C for 60 seconds. For both the genus specific and species specific rPCRs, samples with a cycle threshold (Ct) value of 40 were declared positive. The depiction of a graphical representation of cycle numbers versus fluorescence values was used to record positive data.

3. Results and Discussion

The prevalence of B. abortus in cattle and buffaloes as determined by RBPT, i-ELISA, and rPCR. 13 (10%) out of 130 cow samples and 3 (2.31%) out of 130 buffalo samples tested positive in RBPT (Table 1).

Table 1. *Prevalence of brucellosis in cattle and buffalo In District Thatta, Sindh.*

Parameter	Cattle	Percentage	Buffalo	Percentage
Total samples (n)	130		130	
RBPT positive (n)	13	10	3	2.31
i-ELISA positive (n)	8	6.15	2	1.54
rPCR positive (n)	8	6.15	2	1.54

When RBPT positive samples were retested using the i-ELISA method, 8 out of 13 cow samples were found to be positive, while the 2 RBPT positive out of 3 buffalo samples was also confirmed to be positive. The discrepancy in the two tests' results can be explained to the RBPT's tendency to produce false positives as a result of the antigen's cross-reaction with other gram negative bacteria. By using a genus-specific rPCR, the 8 cows and 2 buffalo samples were likewise shown to be positive for Brucella. B. abortus was detected in all 13 samples using species-specific rPCR. Cattle had a prevalence of 6.15%, while buffaloes had a prevalence of 1.54%. MRT was utilized to determine the prevalence in a prior study conducted in the same location. In comparison to our findings, cattle had a somewhat higher prevalence and buffaloes had a slightly higher prevalence [3]. Several research on the prevalence of bovine brucellosis have been undertaken in Pakistan, with the majority of publications relying on serological approaches. Though early research showed low prevalence rates of 0.33 to 0.65% [15], more recent studies in the provinces of Punjab and Khyber Pukhtunkhwa (KPK) have reported substantially higher prevalence rates (21.05 to 26.1%) [2, 11]. Various degrees of prevalence have been recorded in various investigations. The incidence of brucellosis was found to be 14.70% in cattle and 15.38% in buffaloes at government livestock farms, and 18.53% in cattle and 35.40% in buffaloes at private livestock farms in various districts of Punjab, according to a study conducted on animals at livestock farms [9]. Prevalence rates of 8.5% in buffaloes and 3% in cattle have been reported in Quetta [12]. The frequency of brucellosis varies greatly between countries and continents around the world [8]. The isolation and culture of the causative organism is the gold standard for diagnosing brucellosis, however the processes

for isolation and cultivation of Brucella are arduous, time-consuming, and expensive [7]. Working with Brucella is also dangerous, necessitating biosafety level 3 certification [6]. As a result, molecular diagnostics such as rPCR are a fast and safe technique to detect Brucella. Another advantage of rPCR is that it can identify DNA from bacteria that have been damaged and cannot be grown [13]. For detecting Brucella species, several single-primer and multiplex PCRs and rPCRs have been developed [14]. A multiplex PCR for simultaneous detection of B. abortus and B. melitensis in a single tube was described [10]. They tested it on Brucella genomic DNA and discovered that it correctly identified both Brucella species. We tested the efficiency of the same assay on genomic DNA isolated from serum to see if it could detect the organism directly in the serum of infected animals. The i-ELISA positive samples were likewise positive by the rPCR, demonstrating that this rPCR technique was capable of detecting Brucella in the serum of diseased cattle and buffaloes from the local breeds. 75 of the 130 cattle studied had a history of abortion, and 13 of the 75 samples tested positive for brucellosis (Table 2).

Table 2. *Prevalence of brucellosis in animals with risk factors.*

Parameters	Cattle	Buffalo
Total samples (n)	130	130
Animals with abortion history	75	35
Brucella positive samples	13	3
Brucella positive samples (%)	17.33	8.57
Animals with long calving interval	35	32
Brucella positive samples	6	2
Brucella positive samples (%)	17.14	6.25

35 of the 130 buffalo serum samples came from animals that had previously suffered an abortion. Only 3 of the 35 samples tested positive for Brucella. 35 of the 130 cattle samples came from animals with lengthy calving intervals, and 6 of the 35 samples tested positive for brucellosis. The 2 buffalos that tested positive for brucellosis had a history of extended calving intervals. Univariate analysis using the programme Minitab 12.22 (Minitab Inc, PA, USA) was used to see if brucellosis was a risk factor for abortions and lengthy calving intervals (Table 2). The upper bound of the 95% confidence interval was found to be 0.02 and the lower bound was found to be 50.939 using risk analysis. The odd ratio was found to be 1. These findings suggest that abortion and a long calving gap were risk factors for brucellosis in the animals studied [16]. Abortion in cattle can be caused by a variety of factors. Genetic factors, vitamin A deficiency, heat stress, and trauma are examples of non-infectious causes. Neospora caninum infection, bovine viral diarrhea, infectious bovine rhinotracheitis, leptospirosis, mycotic abortion, Trueperella pyogenes infection, trichomoniasis, listeriosis, chlamydiosis, and Bluetongue are some of the infectious causes other than brucellosis. Any of the reasons listed above might have caused abortion in the remaining cattle and buffaloes with a history of abortion. Brucella infection may potentially cause extended calving intervals [17]. 6 cattle and 2 buffalo tested positive for brucellosis, which could explain why these animals have such a long calving interval. Lengthy inter-calving intervals in the rest of the animals with long calving intervals could have been caused by a nutritional or management component, or another infection. The importance of accurate diagnosis, immunization, and screening of animals at farms, livestock markets, and abattoirs has been highlighted in order to control the disease. In addition, quarantine measures have been proposed. It's also a good idea to avoid mixing diseased and sensitive animals. It is also critical that farmers, livestock owners, and public health officials are aware of the disease.

4. Conclusion

The prevalence of brucellosis in cattle and buffaloes was determined in the district Thatta, Sindh in this study. Cattle had a prevalence of 10%, while buffaloes had a prevalence of 2.31%. The RBPT is a screening test that can be performed as a first step. The results must, however, be validated by i-ELISA. The rPCR used in this work correctly detected Brucella in the serum of all of the animals who were serologically positive for the disease. As a result, this rPCR provides a reliable, quick, and safe method for detecting Brucella in indigenous cattle and buffalo breeds.

Conflict of Interest

All the authors do not have any possible conflicts of interest.

References

[1] Abubakar, M., Arshed, M. J., Hussain, M., Ehtisham, U. H. and Ali, Q., (2010). Prevalence of Bovine Brucellosis in Islamabad and Rawalpindi Districts of Pakistan Transbound. Emerg. Dis., 57: 443-447. https://doi.org/10.1111/j.1865-1682.2010.01171

[2] Akhtar, S., Afzal, M., Ali, S. and Khan, M. I., (1990). Vaccination of adult animals with a reduced dose of Brucella abortus S19 vaccine to control brucellosis on dairy farms in endemic areas of India. Rev-Off Int. Epizootol., 9: 1179-1185.

[3] Ali, S., Ali, Q., Abatih, E. N., Ullah, N., Muhammad, A., Khan, I. and Akhter, S., (2013). Sero-prevalence of Brucella abortus among dairy cattle and Buffaloes in Pothohar Plateau, Pakistan. Pakistan J. Zool., 45: 1041-1046.

[4] Bricker, B. J., (2004). In: Brucella: molecular and cellular biology (eds. I. Lopez-Goni and I. Moriyon). Horizon Bioscience, Norfolk, UK, pp. 24-51.

[5] Godfroid, J., Nielsen, K. and Saegerman, C., (2010). Evaluation of three serum i-ELISAs using monoclonal antibodies and protein G as peroxidase conjugate for the diagnosis of bovine brucellosis. Croat. Med. J., 51: 296-305. https://doi.org/10.3325/cmj.2010.51.296

[6] Hinic, V., Brodard, I., Thomann, A., Holub, M., Miserez, R. and Abril, C., (2009). IS711-based real-time PCR assay as a tool for detection of Brucella spp. in wild boars and comparison with bacterial isolation and serology. BMC. Vet. Res., 14: 5-22.

[7] Kattar, M. M., Zalloua, P. A., Araj, G. F., Samaha-Kfoury, J., Shbaklo, H., Kanj, S. S. and Deeb, M., (2007). Serological and molecular techniques for the diagnosis of Brucellosis. Diagn. Microbiol. Infect. Dis., 59: 23-32. https://doi.org/10.1016/j.diagmicrobio.2007.04.002

[8] Lopes, L. B., Nicolino, R. and Haddad, J. P. A., (2010). Brucellosis - Risk Factors and Prevalence: A Review Open. Vet. J., 4: 72-84. https://doi.org/10.2174/1874318801004010072

[9] Mangi, M. H., Kamboh, A. A., Rind, R., Dewani, P., Nizamani, Z. A., Mangi, A. R., Nizamani, A. R. and Vistro, W. A., 2015. Molecular Identification of Bovine Brucellosis Causing Organisms at Selected Private Farms in Pothohar Plateau, Pakistan J. Anim. Hlth. Prod., 3: 82 -87. https://doi.org/10.14737/journal. jahp/2015/3.4.82.87

[10] Nasir, A. A., Parveen, Z., Shah, M. A. and Rashid, M., (2004). Seroprevalence of brucellosis in animals at government and private livestock farms in Punjab. Pak. Vet. J., 24: 144-146.

[11] Probert, W. S., Schrader, K. N., Khuong, N. Y., Bystrom, S. L. and Graves, M. H. (2004). Rapid detection of *Brucella* spp. in Blood Cultures by Fluorescence In Situ Hybridization J. clin. Microbiol. 42: 1290-1293. https://doi.org/10.1128/JCM.42.3.1290-1293

[12] Ramazan, M., (1996). Incidence of brucellosis in farm animals based on serology. M. Phil thesis, Quid-iAzam University, Islamabad, Pakistan.

[13] Shafee, M., Rabbani, M., Sheikh, A. A., Ahmad, M. D. and Razzaq, A., (2011). Vet. Med. Int., 2011: Prevalence of bovine brucellosis in organized dairy farms, using milk ELISA, in Quetta City, Balochistan, Pakistan. 358950. http://dx.doi.org/10.4061/2011/358950

[14] Sheikh, S. A., Shah, M. A. and Khan, S. A. (2015). Prevalence of Bovine Brucellosis in Islamabad and Rawalpindi Districts of Pakistan. Pak. J. Sci., 19: 189-192.

[15] Yu W. L. and Nielsen, K., (2010). Review of Detection of Brucella spp. by Polymerase Chain Reaction. Croat. Med. J., 51: 306- 313. https://doi.org/10.3325/cmj.2010.51.306

[16] Ullah, S., Jamil, T., Mushtaq, M. H. and Saleem, M. H., (2015). Epidemiology and Associated Risk Factors for Brucellosis in Small Ruminants Kept at Institutional Livestock Farms in Punjab, Pakistan. J. Infect. Mol. Biol., 3: 52-56. https://doi.org/10.14737/journal.jimb/2015/3.2.52.56-1

[17] Wareth, G., Melzer, F., El-Diasty, M., Schmoock, G., Elbauomy, E., Abdel-Hamid, N., Sayour, A. and Neubauer, H., (2016). Isolation of Brucella abortus from a dog and a cat confirms their biological role in re-emergence and dissemination of bovine brucellosis on dairy farms. Transbound. Emerg. Dis., https://doi.org/10.1111/tbed.12535

[18] Wareth, G., Melzer, F., Elschner, M. C., Neubauer, H. and Roesler, U., (2014). Detection of Brucella melitensis in bovine milk and milk products from apparently healthy animals in Egypt by real-time PCR. J. Infect. Dev. Ctries., 8: 1339-1343. https://doi.org/10.3855/jidc.4810

[19] Wareth, G., Melzer, F., Tomaso, H., Roesler, U. and Neubauer, H., (2015). Detection of *Brucella abortus* DNA in aborted goats and sheep in Egypt by real-time PCR BMC Res. Notes, 8: 212. https://doi.org/10.1186/s13104-015-1173-1