

Diagnosis of brucellosis in the equines by serological tests and PCR: A clinical report

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Brucellosis, an infectious disease caused by various Gram-negative bacteria of the genus *Brucella*, is the cause of significant economic losses in livestock production due to reproductive disorders and reduced production of affected animals (Radostits *et al.* 2002). Almost all domestic species can be affected with brucellosis except cats, which are resistant to *Brucella* infection (OIE 2009). *Brucella* species and their major hosts are *B. abortus* (cattle), *B. melitensis* (goats), *B. suis* (swine) and *B. ovis* (sheep). Equine brucellosis is caused by *B. abortus* and most commonly manifests as fistulous withers in horses, which can be a source of exposure to humans. Clinically, brucellosis may also be associated with poll evil, nonspecific lameness due to joint infection or, rarely, late abortions in mares. Infected horses may not show signs of the disease for as long as 2 years post-exposure. The present communication deals with the diagnosis of brucellosis in horses by serological and polymerase chain reaction (PCR).

Sera samples (7 mares and 6 stallions) received from Udaipur, Rajasthan, India with history of abortions and infertility in mares and limping in stallions (characteristic symptoms of brucellosis) were analyzed for brucellosis. The sera samples were subjected for the detection of anti *Brucella* antibodies by three serological tests, viz. Rose Bengal plate test (RBPT), Standard tube agglutination test (STAT) and indirect ELISA. Sera samples were initially subjected to RBPT according to standard procedures (Alton *et al.* 1988) and sera negative for RBPT are not tested further. RBPT positive samples were subjected to STAT according to

Weybridge technique (Alton *et al.* 1988) and end point titre of $\geq 1:160$ (320 IU/ml) was considered positive for equine brucellosis (Alton *et al.* 1988, Smits and Kadri 2005). *B. abortus* S₉₉ colored and plain antigens were procured from Institute of Animal Health and Veterinary Biologicals (IAH&VB), Hebbal, Bengaluru, India. All sera samples were screened by recombinant protein G based iELISA standardized in the laboratory for cattle, sheep, goat, humans and pig as per Shome *et al.* (2010). The optical density (OD) obtained for the negative and positive samples were interpreted by 3 standard deviations above the arithmetical mean of the OD obtained for the healthy controls (Daniel and Debanne 1987). To confirm seropositivity and the active infection, PCR was carried out as per Baily *et al.* (1992).

In the study, out of 13 horses tested 3, 2, 6 and 4 sera samples were positive by RBPT, STAT, iELISA and PCR respectively (Table 1). A seroprevalence of 12.89% and 22.7% was recorded by STAT/RBPT in horses from India (Kulshershta *et al.* 1977 and Sharma *et al.* 1979). Sen *et al.* (2010) have recorded *Brucella*-agglutinins in 3.5% out of 424 random sera samples from equines by conventional tests. The prevalence of disease when compared sex wise, out of 7 mares, 2/7 were positive by RBPT and these 2 RBPT positive sera had significant STAT titres (1:160) indicating *Brucella* infection. Similarly, iELISA and PCR tests detected 6/7 and 4/7 in mares respectively. Among 6 stallions, only 1/6, 2/6 and 1/6 were positive by RBPT, iELISA and PCR. The high prevalence in mares reported in our study is in accordance

Table 1. Diagnosis of brucellosis in horses by serological tests and PCR

Sex	No. samples	RBPT+ve	STAT titre >1: 160	Protein G iELISA	PCR
Mares	7	2(28.57%)	2(28.57%)	4(57.14%)	3(42.85%)
Stallion	6	1(16.66%)	0	2(33.33%)	1(16.66%)
Total	13	3(23.07%)	2(15.38%)	6(46.15%)	4(30.76%)

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with Ahmed and Munir (1995) and Solmaz *et al.* (2004). The higher prevalence in mares is attributed to close association of infected mares, which can infect the healthy

ones by discharges passed after abortion or parturition or susceptible to infection might be due to physiological stress during pregnancy and lactation.

Among 3 serological tests conducted, RBPT, STAT and iELISA, latter detected higher positives than other 2 tests (46.15%). The high positivity in iELISA is due to the ability of ELISA to detect very low levels of antibodies present in the early stage of infection, while RBPT and STAT fails to detect the same (Guarino *et al.* 2001). A problem with ELISA is that each species requires its own anti-immunoglobulin detection reagent. The universal reagent such as recombinant protein G which reacts with immunoglobulins of a large number of different species can overcome this problem. A very strong affinity of recombinant protein G to horse immunoglobulins was cited in pierce product catalogue. The 3 RBPT positive samples were also found positive by protein G iELISA, showing 100% agreement of the assay with the classical serological tests.

Similarly, the 3 RBPT positive samples were also positive by PCR and in addition 1 sample found negative by RBPT and positive by protein G ELISA was detected in PCR (Fig. 1). Using the *bcsp* 31 primers, confirmation of *Brucella* isolates and clinical samples was reported for humans and other species of livestock in the laboratory (Shome *et al.* 2010). Though all the samples were suspected for the disease, lesser number of PCR positivity in serum was observed. This might be due to the intracellular nature of *Brucella* or normally they reside in various joints and internal organs or due to the absence of bacteria in serum during chronic infection (Morata *et al.* 1998). Owing to difficulty in identifying the stage of brucellosis infection at the time of sample collection, multiple samples like blood, serum, vaginal samples and aborted material should be investigated for the diagnosis of brucellosis (Vivekananda 2008). In horses, *B. abortus* is commonly found in chronic bursal

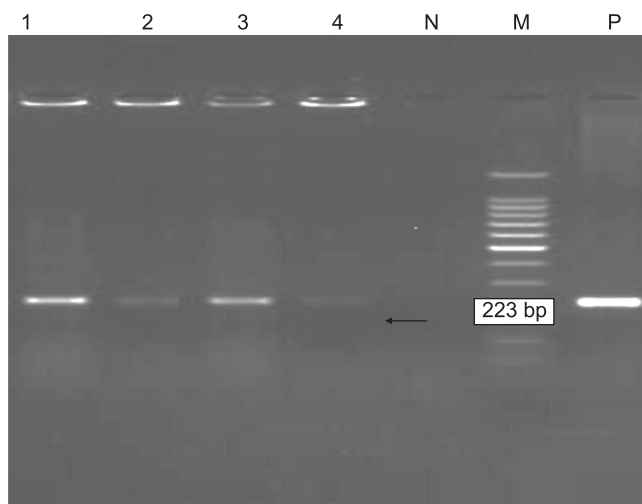
enlargements as a secondary invader rather than a primary pathogen (Acha and Szyfer 1987) and hence appropriate clinical samples are prerequisite for the diagnosis.

SUMMARY

In the present study, in the absence of samples for isolation, serological and PCR tests were utilized for the diagnosis. The different approaches for diagnosis of brucellosis in horses used in the present study are of value as both anti *Brucella* antibodies and antigen were detected in horses confirming the disease in the stud farm. As the test values of protein G based iELISA showed 100% agreement with conventional tests and PCR, the test which is under validation for livestock species and humans in the laboratory, the same test can be utilized for diagnosis of brucellosis in horses.

REFERENCES

- Ahmed R and Munir M. 1995. Epidemiological investigations of brucellosis in horses, dogs, cats and poultry. *Pakistan Veterinary Journal* **15**: 85–88.
- Alton G G, Jones L M, Angus R D, Verger J M. 1988. *Techniques for the Brucellosis Laboratory*. 1st edn, pp. 174. Institute Nationale de le Rech, France, Paris.
- Baily G, Krahn G, Drasar J B and Stoker N G. 1992. Detection of *Brucella melitensis* and *Brucella abortus* by DNA amplification. *Journal of Tropical Medicine and Hygiene* **95**: 271–75.
- Daniel T M and Debanne S M. 1987. Sero diagnosis of tuberculosis and other mycobacterial diseases by enzyme linked immunosorbant assay. *American Review of Respiratory Diseases* **137**: 1137–51.
- Guarino A, Serpe L, Fusco G, Scaramuzza A, Gallo P. 2001. Detection of *Brucella* species in buffalo whole blood by gene-specific PCR. *Veterinary Record* **147**: 634–36.
- Kulshrestha R, Arora C. and Kalra D S. 1977. Brucellosis in camels and horses in Haryana State, India. *Indian Journal of Animal Sciences* **45**: 675–77.
- Morata P, Queipo-Ortuno M I, De Dios and Colmenero J. 1998. Strategy for optimizing DNA amplification in a peripheral blood PCR assay used for diagnosis of human brucellosis. *Journal of Clinical Microbiology* **36**: 2443–46.
- OIE. 2009. *Porcine brucellosis*. Chapter 2. OIE Terrestrial Manual. 8. 5. Version adopted by the World Assembly of Delegates of the OIE in May 2009.
- Radostits O M, Gay C, Blood D C and Hinchcliff K W. 2000. *Veterinary Medicine*. 9th edn. W. B. Saunders Company Ltd. London, UK.
- Shome R, Narayana Rao, M. Nagalingam and Prabhudas K. 2010. Development of common ELISA kit for diagnosis of brucellosis in livestock and humans. *XVI Annual convention of ISVIB and national symposium*. Namakkal (TANUVAS), during 8–10th April, 2010. page No. 102.
- Sen M R, Shukla B N, Goyal R K. 2010. Sero prevalence of brucellosis in and around Varanasi. *Journal of Communicable Diseases* **34**: 226–27.
- Sharma V D, Sethi M S, Yadav M P and Dube D C. 1979. Sero-epidemiologic investigations on brucellosis in the states of Uttar



M, Marker; N, negative control; P, *B. abortus* S₉₉ positive control; Lane, 1,2,3, mare sera; Lane, 4 stallion sera.

Fig. 1. Detection of *Brucella* genus in horse sera samples by PCR

- Pradesh (U.P.) and Delhi (India). *International Journal of Zoonoses* **6**: 75–81.
- Smits H L and Kadri S M. 2005. Brucellosis in India: A deceptive infectious disease. *Indian Journal of Medical Research* **122**: 375–84.
- Solmaz H, Tutuncu M, H., Akhan A, Aksakal A, Gulhan T. and Boynukara B. 2004. Brucellosis in horses around Van, Turkey. *Indian Veterinary Journal* **81**: 748–49.
- Vivekananda. 2008. 'Molecular detection of *Brucella* infection in livestock and risk group.' Ph. D Thesis submitted to Department of Microbiology, Veterinary College, KVAFSU, Hebbal, Bengaluru 560 024.