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Research Article

SCREENING FOR BRUCELLOSIS IN DAIRY CATTLE

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A R T I C L E I N F O

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A B S T R A C T

Brucellosis was a zoonotic disease and constitutes serious public health hazard. The study was conducted to assess the prevalence of brucellosis in dairy cattle and to compare different screening tests for detection of brucellosis in dairy cattle. About 62 crossbred dairy cattle from different private farms in Wayanad district were utilized for the study. The animal details including breed and lactation were recorded. About 5 ml each milk and blood was collected from these animals. Milk was subjected to milk ring test (MRT) and serum was subjected to different screening tests like rose bengal plate test (RBPT) and lateral flow assay (LFA). Out of 62 milk samples tested 14 samples were found to be positive for MRT. The prevalence was found to be zero (0/62), 4.8(3/62), 11.3(7/62), 6.5(4/62) and zero (0/62) per cent in 1st, 2nd, 3rd, 4th and 5th lactation respectively. One serum sample was found to be positive for both MRT and RBPT, but gave a negative result to LFA, so the sensitivity of this test might be less than that of RBPT and MRT. However, an accurate estimation of sensitivity and specificity of different diagnostic tests requires true status of the disease using gold standard test.

INTRODUCTION

Brucellosis is a zoonotic disease caused by *Brucella spp.* The disease is transmitted from animals to humans by ingestion through infected food products, direct contact with an infected animal or inhalation of aerosols. Humans are accidental host, but brucellosis continues to be a major public health concern worldwide and is the most common zoonotic infection. As a global problem brucellosis commands all attention because of its significant health and economic implications. Species of main concern in India are *B. melitens*is and *B. abortus. B. melitensis* is the most virulent and common strain for man and it causes severe and prolonged disease with a risk of disability. *B. abortus* is the dominant species in cattle. Bovine brucellosis is widespread in India and appears to be on the increase in recent times, due to increased trade and rapid movement of livestock (Renukaradhya *et al.,* 2002). Free grazing and movement with frequent mixing of flocks of sheep and goats also contribute to the wide distribution of brucellosis in these animals. Chahota *et al.* (2003) have reported a severe outbreak of brucellosis in an organized dairy farm leading to abortions, retained placenta and still birth in cows.

In India, about 80 per cent of people live within close contact to domestic livestock animals or wildlife and it is a critical risk factor for transmission of zoonotic disease such as brucellosis. The true incidence of human brucellosis is unknown, however the seroprevalence studies suggest infection may range between 0.9 to 18.1 per cent with higher risk in veterinarians and farm attenders. Progress reports of monitoring programs from 2012-2013 by the Indian Council of Agricultural Research estimate that the current national seroprevalence of brucellosis in cattle is roughly 13.5 per cent (Kang *et al*., 2014). Reddy *et al*. (2014) studied the seroprevalence of brucellosis in slaughter cattle of Kerala, showed an overall prevalence of 6.17 per cent. In Punjab, Maharashtra, Rajasthan, Karnataka, Madhya Pradesh, Tamil Nadu, Gujarat and Kerala, the true prevalence of greater than 5 per cent was recorded for cattle and it indicates a high prevalence for brucellosis.

In this context, the present study is conducted with the following objectives: (1) To study the prevalence of brucellosis in dairy cattle (2) To compare different screening tests for detection of brucellosis in dairy cattle.

MATERIALS AND METHODS

The present study was conducted to screen brucellosis in dairy cattle. About sixty two crossbred dairy cows from different private farms in Wayanad district were selected for the study. The animal details including breed, parity and lactation were recorded. About 5 ml of milk and blood was collected from these animals, separated the serum and stored at -20℃ until assayed. Milk was subjected to milk ring test and serum was subjected to different screening tests like Rose Bengal plate test and Lateral flow assay.

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Milk ring test

Sample collection and handling

Five ml of afternoon milk pooled from all the four quarters were collected from 62 cows. The breed and stage of lactation of each cow were recorded. To get a more reliable result the milk samples were mixed well to ensure an even distribution of the milk cream.

The test was performed by adding 30 µL (0.03 mL) of *B. abortus* bang ring antigen (hematoxylin-stained antigen manufactured by the State Biological Laboratory, Institute of Veterinary Preventive Medicine, Ranipet, India) to 1mL of milk taken in a test tube. Then incubated at 37°C for 1 h, together with positive and negative control samples. Agglutinated *Brucella* cells were picked up by fat globules as they rose, forming a dark cream layer on the top of the sample. A strongly positive reaction was indicated by formation of a dark blue ring above a white milk column. The test was considered negative if the colour of the underlying milk exceeded that of the cream layer and when the cream layer was normal. Samples were read as negative, 1+, 2+, 3+ and 4+ depending on the intensity of colour in the cream layer (Najibullah *et al*., 2014).

Rose Bengal plate test

The test was performed according to the reference cited by (Morgan *et al*., 1978). The formation of clear clumps was considered a positive test while the absence of clear clumps was considered a negative reaction.

Lateral flow assay

The test was performed by the addition of 20 µL serum to the sample well followed by two to three drops of sterile phosphate-buffered saline (pH 7.6) containing 1.67 per cent bovine serum albumin and 3 per cent Tween 20. Test results were read within 3-5 min by visual inspection for staining of the test and control lines. Tests were scored negative when no stained band was observed at the test line and scored positive when the test line stained band was observed along with control line. The test was rejected or retested if control line in the test was absent (Shome *et al*., 2018).

RESULTS

Milk ring test

Out of 62 milk samples tested overall 22.6 per cent (14/62) of the milk samples were found to be positive for MRT. The cows were divided in to five groups based on lactation number 1st, 2nd, 3rd, 4th and 5th lactation. The prevalence was found to be zero (0/62), 4.8 (3/62), 11.3 (7/62), 6.5 (4/62) and zero (0/62) per cent respectively in the corresponding lactation.

Rose Bengal plate test

Clump formation indicated a positive reaction. Out of 62 samples tested only one sample (1/62) or 1.6 per cent was found to be positive for RBPT.

Lateral flow assay

Tests were scored negative in all the samples since no stained band was observed at the test line.

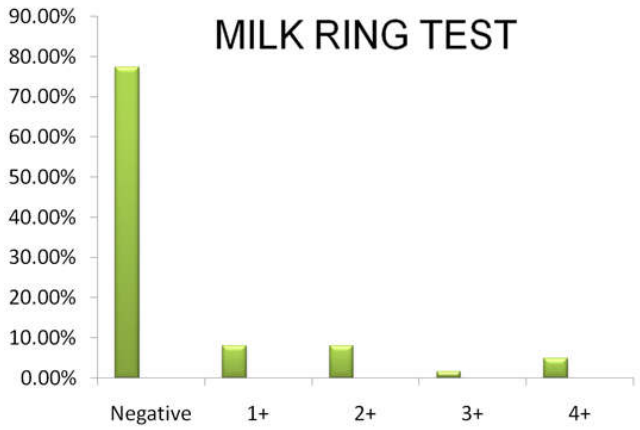

Fig 1 Milk ring test and the percentage positive

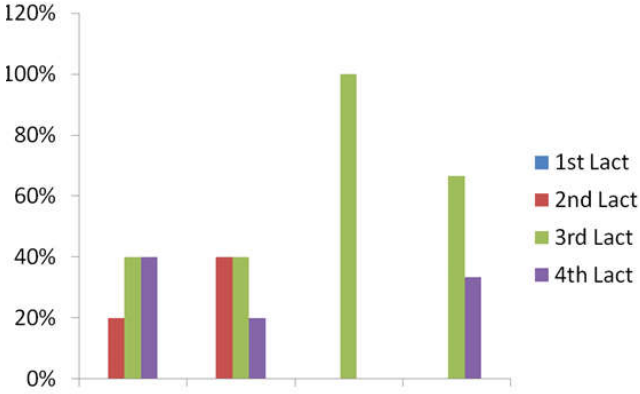

Fig 2 Influence of lactation status on milk ring test results

Fig 3 Milk ring test positive, control and negative samples

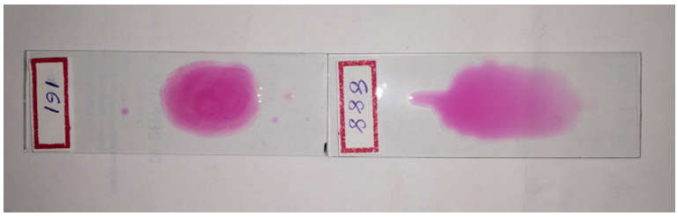

Fig 4 Rose bengal plate test results (sample 161-test positive & sample 888-test negative)

DISCUSSION

Prevalence of bovine brucellosis

In this present study the prevalence of *Brucella* infection from milk samples were 22.6 per cent by MRT. These findings were contradicted with Priyadarshini *et al*. (2013) who observed (3.44 %), a lower prevalence than the present study. Out of 62 samples tested only one sample (1/62) or 1.60 per cent was found to be positive for RBPT. However, Seroprevalence of brucellosis in slaughter cattle of Kerala using RBPT test was found to be 7.74 per cent (Reddy *et al*., 2014). In the present study, only one serum sample was found to be positive for RBPT and it was also positive to MRT, but gave a negative result to LFA, so the sensitivity of this test might be less than that of RBPT, MRT and this finding is contradictory to the observation made by Elshemey *et al*. (2014). Salman *et al*. (2012) stated 85 per cent and 95 per cent sensitivity and specificity to MRT. According to the work done by Chisi *et al*. (2017) the estimated sensitivity and specificity of the RBPT were 95.8 per cent and 100 per cent respectively. However, both MRT and RBPT showed false positive results under certain circumstances, so an accurate estimation of sensitivity and specificity of different diagnostic tests requires true status of the disease using gold standard test.

Age wise prevalence of bovine brucellosis

The cows were divided in to five groups based on lactation number 1st, 2nd, 3rd, 4th and 5th lactation. Overall 22.6 per cent (14/62) of the milk samples were positive for MRT. The prevalence was found to be zero per cent (0/62), 4.8 per cent (3/62), 11.3 per cent (7/62), 6.5 per cent (4/62) and zero per cent (0/62) respectively. Brucellosis is more prevalent in 2nd to 4th lactation compared to 1st and 5th lactation. A similar prevalence of brucellosis was observed by Mohamand *et al*. (2014), 0.92 per cent, 15.60 per cent and 1.83 per cent in 1st, 2nd to 4th and $\geq$ 5th lactation, respectively. However, these findings were contradicted with Kumar *et al*. (2017) who found a higher prevalence in animals above seven years of age group (8.12 %) followed by four to seven years (1.81 %) and two to four years (1.67 %). Low prevalence is noticed in calves when compared to mature and old animals which might be due to passive immunization of calves through feeding of dam's colostrum (Silva *et al.,* 2000 and Mohammed *et al*., 2011). Sexually matured and adult cattle have increased sex hormones and erythritol which favours the growth and multiplication of *Brucella* organisms in the adult animals also play a major role in age advances with *Brucella* infection (Radostits *et al.,* 2010).

Breed wise prevalence of bovine brucellosis

The present study was conducted in crossbred cattle. In breed-wise prevalence, crossbred cattle were more susceptible than non-descript cattle. The low prevalence in Non-Descript breeds might be due to natural genetic resistant pattern, adoption in field environment and innate immunity (Aulakh *et al.,* 2008).

Present findings support high prevalence of brucellosis in unknown history of animals which might be due to lack of appropriate diagnostic facility at field level and screening of animals for brucellosis prior to purchase. These findings support that, proper screening, correction of identified risk factors and elimination of infected animals by using confirmatory test which will be useful in reduce the incidence of bovine brucellosis from the study area.

CONCLUSION

The results of present study indicated that a combination of MRT and RBPT could be used for primary screening of brucellosis in dairy cattle and has to be later confirmed with more specific tests like ELISA and CFT. Implementation of control measures like test and removal of affected animals, calf-hood vaccination, use of semen from brucella screened bulls and general hygienic measures helps in control of brucellosis in farms.

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References

Aulakh, H.K., Patil, P.K., Sharma, S., Kumar, H., Mahajan, V *et al*., 2008. A study on the epidemiology of bovine brucellosis in Punjab (India) using milk-ELISA. Acta Veterinaria Brno.77(3): pp.393-399

Chahotal, R., Sharma, M., Katochl, R.C., Verma, S., Singh, M.M *et al*., 2003. Brucellosis outbreak in an organized dairy farm involving cows and in contact human beings, in Himachal Pradesh, India. *Vet. arhiv*. *73*(2): pp.95-102.

Chisi, S.L., Marageni, Y., Naidoo, P., Zulu, G., Akol, G.W *et al*., 2017. An evaluation of serological tests in the diagnosis of bovine brucellosis in naturally infected cattle in KwaZulu-Natal province in South Africa. J. of the South African Vet. Assoc. 88(1): pp.1-7

Elshemey, T.M. and Abd-Elrahman, A.H. 2014. Evaluation of a rapid Immunochromatographic test for detection of *Brucella abortus* antibodies in Egyptian cattle sera and milk. Alex. J. Pharm. Sci. 40: 24-28

Kang, G.J., Gunaseelan, L. and Abbas, K.M. 2014. October. Epidemiological modeling of bovine brucellosis in India. *IEEE*. pp. 6-10

Kumar, V.N., Bharathi, M.V. and Porteen. K. 2017. Risk factors associated with prevalence of bovine brucellosis in milk from Tamil Nadu, India. Int. J. of Current Microbiol and Appl. Sci. 6(7): pp.2604-2609

Mohamand, N., Gunaseelan, L., Sukumar, B. and Porteen. 2014. Milk Ring Test for spot identification of Brucella abortus infection in single cow herds. J. of Adv. Vet and Anim. Res. 1(2): pp.70-72

Mohammed, F.U., Ibrahim, S., Ajogi, I. and Olaniyi, B.J. 2011. Prevalence of bovine brucellosis and risk factors assessment in cattle herds in Jigawa State. ISRN Vet. Sci. pp 1-4

Morgan, W.J.B., Mackinnon, D.T., Gill, K.P.W., Gower, S.G.M. and Norris, P.I.W. 1978. Brucellosis diagnosis: Standard Laboratory Techniques Report Series no. 1, Weybridge, England

Najibullah, Mohamand, Lakshmanasami, Gunaseelan, Bharathy *et al*., 2014. Milk ring test for spot identification of Brucella abortus infection in single cow herds. *J. of. Adv. Vet. Anim. Res*. 1(2): pp.70-72.

Priyadarshini, A., Sarangi, L.N., Palai, T.K., Panda, H.K., Mishra, R *et al*., 2013. Brucellosis in cattle and occupationally exposed human beings: A Serosurvey in Odisha, India. J. Pure Appl. Microbiol. 7. pp.3255-60

Radostits, O.M., Gay, C.C., Hinchliff, K.W. and Constale, P.D. 2010. In a medicine textbook of diseases of cattle, horses, sheep, pigs and goats, 10th ed. W.B. Saunder. Co., United Kingdom

Reddy, R.R., Prejit, S.B., Vinod, V.K. and Asha, K. 2014. Seroprevalence of brucellosis in slaughter cattle of Kerala, India. J. Foodborne Zoonotic Dis. 2(2): pp.27-29

Renukaradhya. G.J., Isloor, S. and Rajasekhar, M. 2002. Epidemiology, zoonotic aspects, vaccination and control/eradication of brucellosis in India. Vet. Microbiol. *90*(1-4): pp.183-195

Salman, A.M. and El Nasri, H.A. 2012. Evaluation of four serological tests to detect prevalence of bovine brucellosis in Khartoum State. *J. of Cell and Anim. Biology*. *6*(9): pp.140-143.

Shome, R., Kalleshamurthy, T., Shome, B.R., Sahay, S., Natesan, K *et al*., 2018. Lateral flow assay for brucellosis testing in multiple livestock species. J. of microbiological methods. pp.93-96

Silva, I., Dangolla, A. and Kulachelvy, K. 2000. Seroepidemiology of Brucella abortus infection in bovids in Sri Lanka. Preventive Vet. Med. 46(1): pp.51-59

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