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Serological evidence of Brucellosis in selected *gaushalas* of Haryana

A S SAIDU[1], N K MAHAJAN[2], MAHAVIR SINGH[2]*, DINESH MITTAL[2], BANGAR YOGESH[2] and RAJESH CHHABRA[2]

College of Veterinary Sciences, LUVAS, Hisar, Haryana 125 004 India

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ABSTRACT

Brucellosis is a neglected zoonotic disease with significant economic and public health consequences to human and animal population in developing countries. The objective of the present study was to determine the serological evidences of brucellosis in cattle reared in two *gaushalas* of Hisar and Jind districts, Haryana. The serological tests: Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT) and Enzyme Linked Immunosorbent Assay (ELISA) were employed for screening the animals for brucellosis. The overall seropositivity by RBPT, SAT and ELISA was 23.46%, 20.67% and 28.49% respectively. The logistic regression modalities concluded higher likelihood of brucellosis with age > 6 years followed by 3–6 year than cows with <3 years. The agreement between tests (RBPT and ELISA, SAT and ELISA and RBPT and SAT) was found to be 0.87 (95% CI: 0.857–0.882), 0.70 (95% CI: 0.684–0.718) and 0.82 (95% CI: 0.809–0.834) respectively by kappa statistic. This study concluded high infection rate in *gaushala* where animals were kept as closed population with more risks of brucellosis among older milching animals which poses potential public health risk through consumption of unpasteurized milk.

Keywords: Brucellosis, Cattle, Haryana, Herd-screening, Serology

Brucellosis is the second most important zoonotic disease in the world as reported by the OIE (OIE 2018). Brucellosis is currently considered as one of the highly endemic and burdened disease accounting for far reaching and deleterious effects on livestock and human health system (Deka *et al.* 2018). The most common species accountable for the disease are *Brucella abortus, B. melitensis* and *B. suis*. The disease is affecting approximately 500,000 people annually around the world (WHO 2005).

The economic impact of brucellosis on the Indian livestock industry is estimated to be about US$ 3.4 billion (Singh *et al.* 2015). The bovine brucellosis accounted for 95.6% of the total losses occurring due to the disease in livestock population in India. Fetal mortality, late term abortions, sterility, transitory infertility, repeat breeding, metritis, and loss in milk production in adult animals are the factors responsible for huge economical losses to dairy venture. Management practices and environmental conditions in a particular geographical area are accountable for the dispersal and continuation of the disease (Chand and Chhabra 2014). In Haryana, there are many *gaushalas* which provide shelter to cows and work for animal health and genetic improvement. Therefore, the aim of the present study was to investigate the serological evidence of brucellosis in *gaushalas* of Hisar and Jind districts of Haryana, India.

Present address: [1]Department of Public Health and Preventive Medicine, University of Maiduguri, Borno state, Nigeria; [2]LUVAS, Hisar, Haryana. *Corresponding author e-mail: drmahaviryadav@ gmail.com

MATERIALS AND METHODS

Study design: Two *gaushalas*; one each from Hisar and Jind districts of Haryana were selected for the study. The sero-monitoring of animals was conducted using the serological tests: Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT) and indirect Enzyme-linked immunosorbent assay (ELISA) which are OIE recommended tests for screening of brucellosis in animals (OIE 2018).

Sample collection: A total of 179 cattle were sampled and screened using the OIE recommended serological tests. The blood samples were collected from the *gaushalas* located at Hisar (n=129) and Jind (n=50) districts in the state of Haryana, respectively. Blood samples from cattle were collected in tubes and transported to the laboratory on ice for serum separation. The serum samples were labeled and stored at –20°C till further analysis.

Rose Bengal plate test: This test is a simple agglutination test that involved the use of Rose Bengal dye stained antigen and buffered to a low *p*H of 3.6±0.05. It is a qualitative test of macroscopic agglutination performed with only one dilution, and which mainly detects IgG1, but not IgG2 antibodies. An equal volume (40 µl) of RBPT antigen (procured from ICAR-IVRI, Izatnagar, Bareilly, UP) and test serum sample were mixed on glass plate thoroughly

and rotated gently for 4 min at room temperature as described by OIE (OIE 2018). The results of the test were interpreted as either: negative or no agglutination; positive for any degree of agglutination.

Serum agglutination test: The SAT has been used with a great success in surveillance and control programmes for bovine brucellosis, particularly in northern Europe. *Brucella abortus* plain antigen was procured from the ICAR-IVRI, Izatnagar. The test was carried out using the tube method as per OIE standard (OIE 2018). At least three dilutions must be prepared for each serum in order to avoid prozone phenomenon and at least agglutination up to tube 1:160 was considered as a positive serum.

Indirect Enzyme-Linked Immunosorbent assay: In this study, commercially available indirect ELISA kit was employed that uses S-LPS as coating antigen for the detection of anti-Brucella antibodies in cattle. The appropriate dilution of serum samples were added in the coated wells along with positive and negative serum controls. After washing, conjugate antibodies were added and finally substrate was added. The colour development was measured in terms of OD values by ELISA reader and data was analysed to make interpretations as per manufacturers' instructions.

Data analysis: The data obtained from the serological surveys were statistically analyzed using a statistical package for social sciences (SPSS V20.0). A chi-square test of association and logistic regression modalities were used to measure the possible association and likelihood of seropositivity by comparisons to the age categories, breed of cattle and location of sampling. The agreement between the serological tests employed was also determined by using a kappa statistic. Tables and charts were constructed using the Microsoft Excel version 2016. Values of $P<0.05$ and $P<0.01$ were considered significant throughout the analysis.

RESULTS AND DISCUSSION

A total of 179 cattle from two *gaushalas* at Hisar (n=129) and Jind (n=50) districts of Haryana were used in this study. The overall seropositivity by RBPT, SAT and ELISA was 23.46% (95% CI: 17.26–29.67), 20.67% (95% CI: 14.74–26.60) and 28.49% (95% CI: 21.88–35.10) respectively (Table 1). The present study found high seropositivity of brucellosis in the two *gaushalas* (Hisar and Jind) of Haryana. Previous study on brucellosis in the state revealed high prevalence and reoccurrence of the disease as endemic in the same region (Chand and Chabbra 2014). Even the backyard farms and peri-urban settlements in other districts of Haryana are at risk, due to porous borders and probable mixing of animal species. Due to zoonotic nature of disease; there is a public health risk through possible human infections (WHO 2005, Godfroid *et al*. 2011). Some earlier reports revealed the Disability Adjusted Life Years' (DALY's) estimation to be of high impact in India as an index of socio-economic losses associated with the disease (Singh *et al*. 2015, Singh *et al*. 2018). The livestock population of Haryana is about 8.81 million resulting in total milk production of 588.19 million tons (Census, 2012). This data is an indication of the contribution of livestock in dairy industries and to the national Gross Domestic Products at large. There is a need to tackle the menace associated with brucellosis to accelerate the growth of dairy sector. The cattle farms involved in this study supply milk to the majority of the local community. Udder secretions of *Brucella* positive animals may have the organism which can transmit the disease to human beings if milk is consumed raw or unpasteurized.

District-wise, the estimated seropositivity of brucellosis was appeared to be higher in Jind *gaushala* than that of Hisar. However, the statistically significant difference between districts and seropositivity was observed for RBPT test only. Prevalence of the disease also being influenced by the management practices like stocking density, hygienic practices, disposal of aborted foetus, placenta, quarantine practices, purchase of new animals etc. involved in rearing animals at a particular farm (Renukaradhya *et al.* 2002, Chand and Chhabra 2014). Furthermore, another author from India had observed inadequate floor space and lack of awareness about brucellosis as crucial risk factor for transmission of disease in animal population (Pathak *et al*. 2016). The endemic situation and mixing of animals at watering and grazing points may be another reason and risk factor for transmission of brucellosis in Hisar, Jind and other districts of Haryana. This may favour the transmission of the disease among the cattle population and even beyond a single species transmission to a cross-species transmission between cattle and other small ruminants due to spill-over host (Renukharadya *et al.* 2002, Godfroid *et al.* 2011). This scenario might be one of the reasons for the endemic situation of the disease in the study area, state of Haryana and country at large (Chand and Chhabra 2014, Pathak *et al.* 2016, Singh *et al*. 2018). Therefore, the livestock density is a determining risk factor to brucellosis among the different farms of diverse breeds and species in the same endemic situation.

Among the breeds studied, Sahiwal had the highest percentage positivity for serological tests, followed by Hariana breed and then cross-bred, but dependency between breeds and seropositivity appeared to be non-significant (Table 1). Likewise the regression analysis revealed non-significant association between breed and brucellosis status of animal. This finding is consistent with reports which stated no association between brucellosis status and breed of animal (Mai *et al*. 2012). However, in another study from organized farm of North India reported no case of abortion in pure Sahiwal breed due to *Brucella abortus* infection and indicated the possibility of breed predisposition to abortions (Mittal *et al.* 2018). The natural resistance to *Brucella abortus* in livestock had been associated with polymorphisms in microsatellites at the 3' UTR of the SLC11A1 (solute carrier family 11 member A1) gene (Hasenauer *et al.* 2013).

Furthermore, there was a statistically significant ($P<0.01$)

Table 1. Brucellosis seropositivity based on cattle farms location, breed and age of animals

Variable	Category	RBPT N (%, 95% CI)	SAT N (%, 95% CI)	ELISA N (%, 95% CI)
Overall	N=179	42 (23.46; 17.26–29.67)	37(20.67; 14.74–26.60)	51(28.49; 21.88–35.10)
Location	Hisar (N=129)	25 (19.38; 12.56–26.20)	24 (18.60; 11.9–25.3)	32(24.81; 17.4–32.3)
	Jind (N=50)	17 (34.00, 20.87–47.13)	13 (26.00; 13.8–38.2)	19(38.00; 24.6–51.5)
Chi-square		4.29*	1.20^{NS}	3.08^{NS}
Breed	Crossbred (N=48)	9 (18.75; 7.71–29.79)	11 (22.92; 11.03–34.8)	12 (25.00; 12.75–37.3)
	Hariana (N=51)	11 (21.57; (10.28–32.86)	9 (17.65; 7.18–28.11)	13 (25.49; 13.5–37.5)
	Sahiwal (N=80)	22 (27.50; 17.72–37.28)	17 (21.25; 12.29–30.2)	26 (32.50; 22.24–42.8)
Chi-square		1.42^{NS}	0.45^{NS}	1.14^{NS}
Age group	<3 (N=51)	3 (5.88; –0.5–12.34)	2 (3.92; –1.41–9.25)	5 (9.80; 1.64–17.97)
	3 – 6 (N=76)	23 (30.26; 19.93–40.6)	18 (23.68; 14.13–33.2)	26 (34.21; 23.54–44.9)
	>6 (N=52)	16 (30.77; 18.22–43.3)	17 (32.69; 19.94–45.4)	25 (38.46; 25.24–51.7)
Chi-square		12.28 **	13.73**	12.49**

N (n), number of animals; Figures in parenthesis indicates per cent positivity along with 95% confidence interval; **, significant at 1% level; NS, Non-significant; *, significant at 5% level .

association among age groups (<3years, 3–6 years and >6 years) for all the three serological tests employed in the present study. The results of logistic regression revealed that the higher likelihood of sero-positivity of brucellosis by RBPT, SAT and ELISA for cows with age > 6 years (OR= 6.17; 95% CI: 1.58–24.13) and 3–6 year (OR= 5.8; 95% CI: 1.54–21.86) than cows with age < 3 years (OR= 1.0) respectively. Statistically significant association between probability of being brucellosis positive and the age groups reported in this study was consistent with the earlier findings (Asgedom *et al*. 2016, Awah-Ndukum *et al*. 2018, Jain *et al*. 2019), who linked the chronicity of brucellosis progresses with age of the animal. Moreover, there are higher chances of the animals likely to be exposed to the disease as the age of animal progresses. Additionally, the kappa statistic results revealed agreement between the tests (RBPT and ELISA, SAT and ELISA and RBPT and SAT) to be 0.87 (95% CI: 0.857–0.882), 0.70 (95% CI: 0.684–0.718) and 0.82 (95% CI: 0.809–0.834) respectively, which indicated strong agreement between serological tests. Strong agreements among the serological tests (RBPT, SAT and ELISA) employed in this study is in line with the earlier reports (Salman and El-Nasri 2012, Chisi *et al*. 2017). Analytically ELISA appeared to be the most promising serological technique to ensure the health status of animals regarding brucellosis. However, it is quite possible to detect false positive due to vaccination or cross-reactivity. In the current study, there was no history of calfhood vaccination of these animals. There was a statistically significant (P<0.01) association between age groups and serological status of the animals as revealed by RBPT (χ^2=12.28), SAT (χ^2= 13.73) and iELISA (χ^2 =12.49) respectively. Similarly, yester studies had reported ELISA as an alternate test to both RBPT and SAT for diagnosis of brucellosis in animals with high sensitivity and specificity (Shome *et al*. 2014, Gurbilek *et al*. 2017). This is in line with the recommendations set by the OIE, considering these tests as standard for screening of cattle and other ruminants for international interests (OIE 2018). However, as per OIE rule at least two tests at a time are recommended in screening for brucellosis for import and export of animals and animals' products across the borders. Serological tests like RBPT remain the immediate and simple to apply in the field for screening of animals for brucellosis.

The present study found high seropositivity of brucellosis in the two *gaushalas* of Hisar and Jind districts of Haryana. Animals in these farms were kept as closed population with higher likelihood of brucellosis among the older age groups, which represents the milching category in our study. This is a public health risk of major concern due to risk of intermittent excretion of *Brucella* organisms in udder secretions and consumption of milk by local populace. This burden requires a policy at the level of Government towards segregation of brucella positive animals to safeguard livestock as well as human health.

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REFERENCES

Asgedom H, Damena D and Duguma R. 2016. Seroprevalence of bovine brucellosis and associated risk factors in and around Algae district, Ethiopia. *Springerplus* **5**: 851–58.

Awah-Ndukum J, Mouiche M M M, Bayang H N, Ngwa V N, Assana E, Feussom K J M, Manchang T K and Zoli P A. 2018. Seroprevalence and associated risk factors of brucellosis among indigenous cattle in the Adamawa and North regions of Cameroon. *Veterinary Medicine International* **18**: 1–10.

Census 2012. The 19th Livestock Census-2012. *All India Report*. Ministry of Agriculture Department of Animal Husbandry,

Dairying and Fisheries Krishi Bhawan, New Delhi.

Chand P and Chhabra R. 2014. Herd and individual animal prevalence of bovine brucellosis with associated risk factors on dairy farms in Haryana and Punjab in India. *Tropical Animal Health Production* **45**: 1313–19.

Chisi S L, Marageni Y, Naidoo P, Zulu G, Akol G W and Van Heerden H. 2017. An evaluation of serological tests in the diagnosis of bovine brucellosis in naturally infected cattle in KwaZulu-Natal Province in South Africa. *Journal of South African Veterinary Association* **88**: 1–7.

Deka R P, Magnusson U, Grace D and Lindahl J. 2018. Bovine brucellosis: prevalence, risk factors, economic cost and control options with particular reference to India- A review. *Infections, Ecology and Epidemiology* **8**: 556–48.

Godfroid J, Scholz H C, Barbier T, Nicolas C, Wattiau P, Fretin D, Whatmore A M, Cloeckaert A, Blasco J M, Moriyon I, Saegerman C, Muma J B, Al Dahouk S, Neubauer H and Letesson J J. 2011. Brucellosis at the animal/ecosystem/human interface at the beginning of the 21st century. *Preventive Veterinary Medicine* **102**: 118–31.

Gurbilek S E, Tel O Y and Keskin O. 2017. Comparative evaluation of three serological tests for the detection of *Brucella* antibodies from infected cattle herds. *Journal of Applied Animal Research* **45**: 557–59.

Hasenauer F C, Caffaro M E, Czibener C, Comerci, D, Poli M A and Rossetti C A. 2013. Genetic analysis of the 3' untranslated region of the bovine SLC11A1 gene reveals novel polymorphisms. *Molecular Biology Reproduction* **40**: 545–552.

Jain L, Kumar V, Chaturvedi S, Roy G and Barbuddhe S B. 2019. Seroprevalence of brucellosis in bovines of Chhattisgarh, India. *Indian Journal of Animal Research* **53**: 255–59.

Mai H M, Irons P C, Kabir J and Thompson P N. 2012. A large seroprevalence survey of brucellosis in cattle herds under diverse production systems in northern Nigeria. *BMC Veterinary Research* **8**: 144–57.

Mittal M, Sharma V, Nehra K, Chakravarti S, Kundu K, Bansal V K, Churamani C P and Kumar A. 2018. Abortions in an organized dairy farm from North India reveal the possibility of breed susceptibility to bovine brucellosis. *One Health* **5**: 1–5.

OIE. 2018. Manual of Diagnostic tests and vaccines for terrestrial animals. *World Organization for Animal Health* 355–98.

Pathak A D, Dubal Z B, Karunakaran M, Doijad S P, Raorane A V, Dhuri R B, Bale M A, Chakurkar E B, Kalorey D R, Kurkure N V and Barbuddhe S B. 2016. Apparent seroprevalence, isolation and identification of risk factors for brucellosis among dairy cattle in Goa, India. *Comparative Immunology Microbiology Infectious Disease* **47**: 1–6.

Renukaradhya G J, Isloor S and Rajasekhar M. 2002. Epidemiology, zoonotic aspects, vaccination and control/ eradication of brucellosis in India. *Veterinary Microbiology* **90**: 183–95.

Salman A M A and El-Nasri H A. 2012. Evaluation of four serological tests to detect prevalence of bovine brucellosis in Khartoum State. *Journal of Cell and Animal Biology* **6**: 140–43.

Shome R, Padmashree B S, Krithiga N, Triveni K M, Sahay S, Shome B R, Singh P and Rahman H. 2014. Bovine brucellosis in organized farms of India—An assessment of diagnostic assays and risk factors. *Advances in Animal and Veterinary Sciences* **2**: 557–64.

Singh B B, Dhand N K and Gill J P S. 2015. Economic losses occurring due to brucellosis in Indian livestock populations. *Preventive Veterinary Medicine* **119**: 211–15.

Singh B B, Kostoulas P, Gill J P S and Dhand N K. 2018. Cost-benefit analysis of intervention policies for prevention and control of brucellosis in India. *PLoS Neglected Tropical Disease* **12**: e0006488.

WHO. 2005. Brucellosis in humans and animals. World Health Organization. Control of communicable diseases manual: An official report of the American Public Health Association. 18th ed. (Ed.) Heymann D L. Washington DC, WHO/APHA.