Journal of Animal Research: v.8 n.5, p. 759-766. October 2018

DOI: 10.30954/2277-940X.10.2018.4

Sero-Surveillance and Control of Bovine Brucellosis in Akshayakalpa Dairy Farms in Karnataka, India

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Received: 25 April, 2018 **Revised:** 30 July, 2018 **Accepted:** 08 Aug., 2018

ABSTRACT

Brucellosis is a disease of domestic animals with serious zoonotic implications in humans, causing huge economic losses to the livestock industry. The present study was conducted to find the prevalence of brucellosis in Akshayakalpa organic dairy farms. Seventeen pooled milk samples from different herds contributing to the farm were collected and subjected to milk ring test (MRT), among these seven samples were found to be positive. Individual animal milk samples were collected from the positive herds and subjected to MRT again. Further, serum samples of all MRT positive animals were screened by Rose bengal plate agglutination test (RBPT) and indirect ELISA. Prevalence rate of brucellosis was found to be 11.08%, 20.40% and 38.77% by MRT, RBPT and indirect ELISA, respectively. Farmers were educated about the risk of disease and it's public health significance and all the affected animals were isolated. By the consent of farmers the management of the farm culled all the ten infected animals which were positive by all the above tests and financial compensation was provided to the affected farmers. Since then, the farm has adapted regular screening of new animals before introducing it to the herd and all the animals contributing to farms are free from brucellosis. In conclusion, MRT, RBPT and indirect ELISA in combination can be used for diagnosis of brucellosis and test and slaughter policy is the best method of choice to control the infection if the sizes of the positive reactors are less.

Keywords: Sero surveillance, Brucellosis, Dairy farm, Karnataka, India

Brucellosis is an important zoonotic disease, causing considerable economic losses to dairy farmers and a significant health hazard in India (Gill *et al.,* 2000). It is considered as the second most important zoonotic disease next to Rabies (FAO, 2005) with an estimate of five million new cases every year according to OIE (Pappas *et al.,* 2006a; Pappas *et al.,* 2006b). In cattle brucellosis is caused by *Brucella abortus* with major clinical signs of abortion, retained placenta, infertility and reduced milk production (Dricot *et al.,* 2004). Transmission of brucellosis in cattle is by ingestion of organisms from feed and water contaminated with aborted fetuses, fetal membranes, vaginal discharges (Hong *et al.,* 2000). In humans, brucella organisms are transmitted through direct contact with infected material and by consumption of unpasteurized milk and milk products from infected cows, sheep and goat (Gall and Nielsen, 2004). The present study was taken up to find the prevalence of brucellosis in Akshayakalpa organic dairy farms, Karnataka and initiate suitable control measures.

MATERIALS AND METHODS

Farm details

Akshayakalpa organic dairy farms comprises of seventeen herds (Routes or locations) in Tiptur taluka, Tumkur district of Karnataka state, India. All farms are located within the radius of 60 km from Tiptur town and operated by 160 farmers with good animal husbandry practices. A total of

1112 lactating (2nd to 5th lactation) cross breed Holstein Friesian & Jersey cattle (Table 1) contributed to the farm and these cattle were managed in stress free environment by allowing them to stay in large area of paddocks. On an average, 10,000 liters of milk per day is produced by this farm and chilling of the milk immediately after collection is mandatory at farm level. Every day, this farm is supplying raw chilled clean milk and milk products to the doorsteps of consumers all over the Bangalore city, the capital of Karnataka state, India.

Antigens

Brucella abortus strain 99 - based milk ring test (MRT) and Rose Bengal plate agglutination (RBPT) antigens were procured from Institute of Animal Health and Veterinary Biologicals (IAH & VB), Bangalore, KVAFSU, India. These antigens were stored at 4 °C and routinely checked for the presence of autoagglutination, if any, prior to use.

Samples

Seventeen pooled milk samples, 442 milk samples from individual animals and 49 serum samples were collected under sterile conditions from various herds contributing to the farm, transported and stored in laboratory at 4°C.

All the pooled milk samples were subjected to MRT as a preliminary screening test. For those samples which were found to be positive by MRT, milk and serum samples were collected from all the animals contributing to that particular pooled sample and were further tested by MRT and RBPT. All the serum samples which were found to be positive by RBPT were also tested by indirect ELISA for confirmation of IgG antibodies against brucella organisms.

Milk Ring Test

The antigens and samples were kept at room temperature and mixed well before use. Two milliliter of milk sample was taken in sterile test tubes and to which one drop (50 µl) of MRT antigen was added, mixed well and incubated at 37 °C for one hour. The appearance of a blue stained cream layer ring over the clear white column of milk was considered as positive (OIE, 2008 and 2011).

Rose Bengal Plate Agglutination Test

On a clean glass slide equal quantities (30 µl) of test serum and colored RBPT antigen was added and mixed well. The results were interpreted as either positive or negative based on presence or absence of agglutination/clumping reaction (Alton *et al.,* 1988; OIE 2008 and 2011). Along with the samples, positive and negative serum controls were included in the test.

Enzyme Linked Immunosorbent Assay

The ELISA kit procured from SVANOVIR® Brucella Ab i-ELISA, Sweden was used in this study and all the serum samples were subjected to indirect ELISA for detection of IgG antibodies against brucella organisms as per the manufacturer`s protocol. Interpretation of results was done based on the colour development, optical density (OD) values by using BioRad® microplate absorbance reader at 450nm and percent positivity.

RESULTS AND DISCUSSION

Brucellosis is a highly contagious reproductive disease with higher prevalence among dairy cattle in India. Brucella organisms localize in supra mammary lymph nodes and mammary glands of 80% of infected animal, and these continues to be secreted in milk throughout the life as carriers but intermittently (Eisencheck *et al*., 1995). In human, brucella organisms enter the body mainly through ingestion of unpasteurized milk and milk products (Hasanjani, 2006; Young, 2009). Milk acts as a important source for spread of infection to humans, therefore in this study initially the pooled milk samples were screened for brucella organisms by using MRT. The MRT was first described in Germany by Fleischhauer (1937) and is routinely used as a screening test for diagnosis of brucellosis often in pooled milk of a herd. It is simple, cost effective and easy to perform and is conducted on fresh milk but it does not work on pasteurized or homogenized milk (Fleischhauer, 1937). Milk being a non-invasive sample, sampling of large population can be done in short time (Kumar *et al*., 2016). MRT detects IgM and IgA antibodies against Brucella infection in fresh milk (Cadmus *et al.,* 2006).

The preliminary screening of 17 pooled milk samples by MRT revealed that, seven (41.17%) were positive. Out of 442 milk samples collected from individual animals of the positive herds, 49 (11.08%) were found to be positive

by MRT (Table 1). Our results were almost similar with the study of Rehman *et al.* 1983 (11.4%). In contrast to our findings, different studies showed lower and higher prevalence rate of 3% (Shafee *et al.,* 2011), 3.9% (Kang'ethe *et al.,* 2000), 4.35% (Kumar *et al.,* 2016), 7.7% (Mbaire, 2016), 7.9% (Chand and Sharma, 2004), 10.53% (Gogoi *et al.,* 2017) and 12.82% (Trangadia *et al.,* 2010), 17% (Kushwaha *et al.,* 2015), 18.35% (Mohamand *et al.,* 2014), 18.61% (Cadmus *et al.,* 2008), 24.2% (Abbas and Aldeewan, 2009), 25.21% (Zowghi *et al.,* 1990), 25.25% (Junaidu *et al.,* 2011), 32.5% (Salman and Nasri, 2012), 35.82% (Mahato *et al.,* 2004), 38.2% (Salman *et al.,* 2014), 42.68% (Basit *et al.,* 2015), 46% (Gloria Ivy Mensah *et al.,* 2011), 54.7% (Barman *et al.,* 1989), respectively.

Table 1: Details of the samples screened by different tests

Sample (Screened number)	Positive with MRT (%)	Positive with RBPT (%)	Positive with indirect ELISA (%)
Pooled milk sample (17)	07 (41.17%)	—	—
Individual milk sample (442)	49 (11.08%)	—	—
Serum sample (49)	—	10 (20.40%)	19 (38.77%)

Herd wise prevalence of brucellosis by MRT was 19.69% (13/66), 12.90% (08/62), 1.19% (01/84), 22.72% (10/44), 21.25% (17/80) in KUP, PAT, SAR, SOR and in KUN, respectively (Table 2). In two herds (HEG, NAG), which were positive when pooled milk sample was used, all individual animal milk samples were found to negative by MRT. However, MRT can also be used to test individual milk samples but, it may give false positive results shortly after parturition, near the end of lactation, during hormonal imbalance and when subclinical mastitis is present (OIE, 2009). The higher positive results by MRT are because of false positives due to the above reasons (Morgan, 1967; Bercovich and Moerman, 1979 and Macmillan, 1990) and further MRT lacks specificity (Cadmus *et al.,* 2008). In view of the possible chances of false positive reactions in MRT, all the serum samples collected from MRT positive animals were subjected to RBPT.

Further, the serum samples collected from the animals whose milk samples were positive by MRT were also subjected to RBPT & indirect ELISA. The RBPT is a simple agglutination test which is mostly widely used as a rapid screening test for brucellosis (Ruiz-Mesa *et al.,* 2005). The prevalence rate of brucellosis in this study by RBPT was 20.40% (10/49) (Table 1) which was in accordance with the prevalence of 20.47% by Chand and Sharma (2004). Whereas, other studies showed the prevalence rate of 1.9 % (Mbaire, 2016), 3.97% (Faqir, 1991), 4.5% (Bhanurekha *et al.,* 2013), 5.22% (Shome *et al.,* 2014), 9.77% (Cadmus *et al.,* 2008), 11.21% (Ghodasara *et al.,* 2010), 12.69% (Gogoi *et al.,* 2017), 13.78% (Trangadia *et al.,* 2010), 16.80% (Varasada, 2003), 25.00% (Mangi *et al.,* 2015), 29.07% (Chakravarty *et al.,* 2007; Barman *et al.,* 1989 and Chakraborty *et al.,* 2000), 32% (Salman *et al.,* 2014), 36.6% (Mai *et al.,* 2012), 42% (Vandana *et al.,* 2017), 50 % (Chachra *et al.,* 2009) which is not in accordance with our results. In KUP herd prevalence rate by RBPT was 76.92% (10/13) (Table 2).

Though RBPT is widely used test for *Brucella* screening in many countries; but the test has several limitations (Munoz *et al.,* 2005; Poester *et al.,* 2010) like lack of specificity (Barroso-Garcia *et al.,* 2002; Kiel and Khan, 1987). The MRT and RBPT tests are biased specifically towards the detection of IgM antibodies, which indicates the (will be seen only in) acute phase of active infection and is thus not confirmatory for diagnosis of brucellosis because a number of other microorganisms also contains antigens with epitopes similar to Brucella antigen and measurement of IgM antibody sometimes results in false positive reactions leading to low specificity of the assay (Corbel, 1985). The IgG antibody shows persistence for longer period in animals with chronic or convalescent stage of infection (Shome *et al.,* 2014). Therefore, samples have to be further confirmed by detection of positive IgG response (Godfroid *et al.,* 2010; Butler *et al.,* 1986). ELISA is a more sensitive and specific when compared to the other conventional tests like MRT, RBPT and STAT used for diagnosis of brucellosis (Hunter *et al.,* 1986; Kostoula *et al.,* 2001; Vanzini *et al.,* 1998; Nielsen *et al.,* 2004). Therefore, in this study all the serum samples were subjected to indirect ELISA for detection of IgG antibodies against brucella organisms. Since none of the animals in this farm were vaccinated against brucellosis possibility of detection of IgG antibodies due to vaccination was ruled out. Nineteen serum samples were found to be positive for brucellosis by indirect ELISA with a prevalence rate of 38.77% (19/49) (Table 1) which was similar with the studies of Salman *et al.* (2014) who showed the prevalence rate of 38.8%.

Table 2: Farm wise and herd wise prevalence of brucellosis by different tests

Sl. No.	Herd Code	Number of Animals	Positive by MRT (%)		Positive by RBPT (%)	Positive by iELISA (%)
			Pooled milk sample	Individual milk sample		
1	BAN	111	Negative	00	00	00
2	BOM	36	Negative	00	00	00
3	MAL	45	Negative	00	00	00
4	SIN	61	Negative	00	00	00
5	KUP	66	Positive	13 (19.69%)	10 (76.92%)	10 (76.92%)
6	PAT	62	Positive	08 (12.90%)	00	00
7	HEG	15	Positive	00	00	00
8	SAR	84	Positive	01 (1.19%)	00	01 (100%)
9	BGH	73	Negative	00	00	00
10	KAR	100	Negative	00	00	00
11	SOR	44	Positive	10 (22.72%)	00	03 (30%)
12	NAG	91	Positive	00	00	00
13	KUN	80	Positive	17 (21.25%)	00	05 (29.41%)
14	MAD	123	Negative	00	00	00
15	ALU	51	Negative	00	00	00
16	BYA	22	Negative	00	00	00
17	PHO	48	Negative	00	00	00
	Total	**1112**	**07 (41.17%)**	**49 (11.08%)**	**10 (20.40%)**	**19 (38.77%)**

Some studies showed a lesser prevalence of brucellosis, 6.03% (Shome *et al.,* 2014), 6.7% (Bhanurekha *et al.,* 2013), 8.5% (Shafee *et al.,* 2011), 13.84% (Gogoi *et al.,* 2017), 17.5% (Chettri *et al.,* 2015), 22.18% (Trangadia *et al.,* 2010). The nine samples from different herds (SAR, SOR and KUN) (Table 2) which were negative by RBPT were detected as positive by indirect ELISA indicating the higher sensitivity of the assay compared with RBPT which was in accordance with the studies of Sahin *et al*. (2008) and Shome *et al.* (2014). Herd wise prevalence rate by indirect ELISA was 76.92% (10/13), 100% (1/1), 30% (3/10) and 29.41% (5/17) in KUP, SAR, SOR and KUN, respectively (Table 2). From the herd PAT, out of eight serum samples none were positive by both serological tests (Table 2).

In comparison with results of our study the difference in prevalence rate of brucellosis in the above mentioned various studies might be due to sample size, variation in the sampling methods, interpretation of results and also clinical conditions of the animals under study.

Strategies for eradication of brucellosis depend on implementation of various strict control regimens like test and slaughter of infected animals, vaccination and good management practices. Test and slaughter is a proven strategy for elimination of this disease especially in brucellosis free countries (Ebel *et al.,* 2008; Kang *et al.,* 2014) and most of the southeast asian countries are currently following this method to eradicate brucellosis (Zamri-Saad and Kamarudin, 2016). Test and slaughter is expensive and only recommended and feasible in countries where prevalence rate is not exceeding 2% (Alton, 1987 and WHO, 1986). Therefore, in many instances screening and culling of seropositive reactors (positive by more than two serological tests) was considered as a better way to control the spread of brucellosis at herd level (Bhanurekha *et al.,* 2013). However in India, the major problem for effective control of brucellosis has been disposal or culling of infected animals due to poor economic status of the farmers (Kollannur *et al.,* 2007).

Though the management of Akshayakalpa farms decided to cull the infected animals owners of the positive animals were initially reluctant and claiming that uninfected (apparently healthy) animals were being eliminated. The farmers were educated about the impact of the brucellosis on livestock when it spreads from infected animals to healthy animals. Awareness was also created about the zoonotic potential risk of brucellosis to the human community. Further, with the consent of owner of the KUP herd ten infected animals which were positive by all three tests (MRT, RBPT & indirect ELISA) were euthanized by humane method and measures were taken for proper disposal of carcass under the supervision of experts. The farmers were provided compensation to meet the loss and financially supported by the management of the farm. Purchase of animals without prior screening, lack of awareness about the disease are considered as major risk factors for the transmission of brucellosis at both herd and individual animal level (Shome *et al.,* 2014). Therefore, prior screening of animals for brucellosis before introducing a new animal into the herd has to be made mandatory to prevent the spread of infection to the herd (Chand and Chhabra, 2013).

CONCLUSION

Based on our results, we suggest using MRT, RBPT and indirect ELISA in combination for successful diagnosis of brucellosis in dairy farms. If the positive reactors are less in number, test and slaughter policy is best method to control the further spread of the disease. Regular screening of all animals of the dairy farms and thorough investigation of the new animals before introducing them into the herd is off prime importance. In this study, after culling positive reactors now all the animals contributing to Akshayakalpa farms are free from brucellosis and the farm has adapted regular screening tests as a criteria for introduction of any new animals to the farm.

ACKNOWLEDGEMENTS

Authors are very thankful to the Dean (Professor L. Ranganath), Convenor of SCP-TSP cell (Dr. Ravikumar, C) Veterinary College, Hassan, KVAFSU, Bidar and Mr. Shashi Kumar, CEO of Akshyakalpa Dairy farms and staff & management of the farm for their kind help and support.

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