Indian Journal of Animal Sciences **85** (10): 1077–1080, October 2015/Short communication

Milk ring test from lab to field: A surveillance strategy for states under brucellosis control program

R SHOME[1], M NAGALINGAM[2], B R SHOME[3], JYOTI MISRI[4], B S PADMASHREE[5], A KAMAL[6], R G BAMBAL[7] and H RAHMAN[8]

National Institute of Veterinary Epidemiology and Disease Informatics, Bengaluru, Karnataka 560 064 India

Received: 7 July 2014; Accepted: 10 June 2015

Key words: Brucellosis, Control program, ELISA, Milk, MRT

Bovine brucellosis, a contagious disease of domestic cattle with humans as accidental host caused by the bacteria *Brucella abortus,* is widely prevalent in India causing economic losses to the tune of US $ 58.8 million (Kollannur *et al.* 2007, Shome *et al.* 2014). Surveillance and vaccination are the 2 effective approaches to control the disease. Surveillance consists of the systematic collection, collation, analysis, interpretation and prompt dissemination of the data. The components of bovine brucellosis surveillance include detecting brucellosis in domestic cattle, estimating the magnitude of brucellosis infection (i.e., prevalence), measuring progress towards regulatory goals, providing metrics to aid in evaluating compliance with program standards, giving stakeholders and decision-makers timely and relevant actionable information (National Bovine Brucellosis Surveillance Plan 2012). Brucellosis surveillance may be adopted in different ways, viz. slaughter surveillance, on farm surveillance, livestock market surveillance, enhanced passive surveillance etc.

Several serological tests such as rose bengal plate test (RBPT), complement fixation test (CFT) and enzyme-linked immunosorbent assay (ELISA) are used for diagnosis of brucellosis. Tests for detection of *Brucella* antibodies in milk from bulk milk tanks are considered the principal methods for detecting infected herds (Noviello 2004). There are many reports of screening and diagnosis of brucellosis in the organised dairy herds by MRT, which is simple to perform and serves as an alternative to serum or milk based ELISA. The MRT works on the principle that antibodies to *B. abortus* attach themselves to fat globule which rise to the surface of the milk, cluster in the cream layer and bind with tetrazolium/ haematoxylin stained *B. abortus* antigen to form a ring in the creamy layer of milk (Sutra *et al.* 1986, Huber and Nicoletti 1986).

Department of Animal Husbandry, Dairying and Fisheries, Government of India, has initiated Brucellosis Control Program (Brucellosis-CP) during 2011–2012 by providing grants-in-aid under the Livestock Health and Disease Control (LH and DC). As part of the program, surveillance and vaccination activities were started in many states throughout the country. This study aimed at evaluating the feasibility of conducting the MRT test for pooled milk samples in the veterinary dispensary/hospitals for the surveillance of bovine brucellosis so as to facilitate states to conduct regular surveillance of the diseases throghout the 5 years program.

Training: To appraise the importance of Brucellosis-CP, three levels of trainings were advocated to Officers of Dept. of Animal Husbandry, Govt of Karnataka. Initially, Deputy Directors (DDs) at the state level were trained at NIVEDI (master trainers), DDs in turn trained Assitant Directors(ADs) at the District level and subsequently ADs trained Junior Veterinary Officers (VOs) and Veterinary Livestock Inspectors (VLIs) at the taluk level.

Sample collection: A pilot study was conducted in the institute using 1,624 bulk milk samples drawn from 1,900 milk cooperative societies under the administration of 8 taluks of Bengaluru rural and urban districts. Milk samples were transported on ice within 3 h of collection to the institute and the test was conducted on the same day.

Subsequently, 64,818 bulk milk samples were tested in 364 veterinary hospitals/dispensaries from 30 districts of Karnataka state in 2 rounds (first round in July 2012 and second round in March 2013). All the logistics like milk collection tubes, test tubes, stands, antigen and other disposable materials were centrally procured and distributed to the veterinary hospitals/ veterinary dispensaries through DDs of the each district by Department of Animal Husbandry, Government of Karnataka in control program grants. The proper test was conducted by the concerned veterinarian in the ear-marked months as per the targets

Present address: [1,3]Principal Scientist (raji_shome1 @rediffmail.com, brshome@gmail.com), [2]Scientist (nagar75 @gmail.com), [5]Senior Research Fellow (padmashreebioscience @gmail.com), [8]Director (hricar@gmail.com), NIVEDI. [4]Principal Scientist (AH) (jmisri.icar@gmail.com), Division of Animal Science, Krishi Bhavan, New Delhi. [6]Director, NPRE. [7]Assistant Commissioner (LH),GOI, Ministry of Agriculture, Department of Animal Husbandry, Dairying and Fisheries, Krishi Bhavan, New Delhi.

provided based on number of villages, number of milk societies and quantity of milk poured in each society/day. The MRT results from veterinary dispensary/ hospital were communicated to State Nodal Officer via DDs in devised formats.

Milk ring test: Milk (5 ml) was collected from each society with the milk procurement capacity up to 300 liter/ time/day. Additional sample was collected, if the milk capacity of the particular society exceeded 300 liter. 50 µl of MRT antigen was added to 1 ml volume of whole milk if the milk procurement capacity of society is less than 100 litre/ time/day. Similarly, 75 µl MRT antigen was used if the milk procurement capacity of society is between 200 to 300 litre/ society/ time/day. The milk/antigen mixtures were mixed and incubated at room temperature for 1 h, together with positive and negative working controls (OIE 2009) in the veterinary hospital. A strong positive reaction is indicated by dark pink ring in the fat layer above a white milk column and negative if the colour of the antigen diffuses throughout the milk column with absence of any pink ring in the fat layers. The vitally stained tetrazolium or red MRT antigen was procured from Institute of Animal Health and Veterinary Biologicals (IAH and VB), Hebbal, Bengaluru, India.

Further, all the districts of Karnataka were categorized based on MRT positive percentage into 2 groups, viz. less than or equal to 5% and greater than 5%. It was represented using QGIS 2.2 version.

In the brucellosis sensitization training provided to Department of Animal Husbandry, Government of Karnataka, initially 76 DDs were trained in the institute and these master trainers trained 1,600 veterinary doctors at District Head Quarters. Similarly, paraveterinarians of the taluks were trained by the senior most veterinary officer at the taluk level. With this 3 tier training model, entire state veterinary machinery was sensitised on surveillance and vaccination in the control program within 1 week. The same training model was advocated to 12 other states in the country (Goa, Tamil Nadu, Madhya Pradesh, Delhi, Uttar Pradesh, Chhattisgarh, Meghalaya, West Bengal, Rajasthan, Punjab and Asom) and 730 officers working in various capacities in the veterinary departments were trained on brucellosis disease diagnosis and vaccination.

In the MRT pilot study, overall, 109 (6.71%) were detected positive out of 1,624 milk samples tested from 1900 urban and rural milk cooperatives which were having the total milk procurement capacity of 7–8 lakh liters of milk /day. The prevalence of anti *brucella* antibody in milk ranged from 2.83 to 15.62% and the prevalence rate of more than 5% was recorded in 6 taluks (Table 1).

In village level screening, the overall positivity was 2.55% (1657/ 64,818). The test was carried out twice in a year in the earmarked months (biannual screening) in the 364 veterinary hospitals. Based on the MRT test results, all the 30 districts of Karnataka were divided into 2 categories, viz. < 5% and > 5% prevalence status. In all, 23 out of 30 districts showed low prevalence (< 5%) while 7 districts have shown high prevalence (Table 2). As the vaccine availability in India is not sufficient to provide coverage to the entire calf population, classifying as high and low prevalence status will help in prioritizing the districts/states for vaccination. Accordingly, in Karnataka, vaccination was started in high prevalence districts first and gradually covering the entire state as per the vaccine availability.

Table 1. Screening of anti-*brucella* antibodies in the bulk milk samples of Bengaluru urban and rural districts

MPCS name	No of samples received and tested	No. of positive samples	% Positivity
Hoskote	268	15	5.59
Anekal	145	11	7.58
Doddaballapur	201	21	10.44
Doddaballapur bulk milk cooler	96	15	15.62
Vijayapura	180	12	6.66
Byrapatna	198	10	5.05
Solur	296	Nil	Nil
Kanakapura	218	16	7.33
Bulk milk coolers	318	9	2.83
	1624	109	6.71

Earlier, 209 out of 6,203 (3.37%) milk samples tested were positive by indirect milk ELISA from Karnataka (AICRP annual report 2000). Mahajan *et al*. (2011) recorded an apparent prevalence of 33.34% and 12.82% by Trangadia *et al.* (2010) in organized dairy farms by MRT. Chand *et al.* (2004) recorded 27.27% and 9.09% in organized and 19.29% and 7.89% in unorganized dairy farms by ELISA and MRT, respectively. Our results supported the findings of other authors on the value of the MRT as an alternative for screening of brucellosis. The anti-*brucella* antibody prevalence in the urban and rural cooperatives differed in the present study and this may be attributed to mostly unorganized dairy sector dominance in rural areas and organized dairy farms are common in urban areas. MRT is biannual screening test recommended by OIE and the test is dependent on lactating animals, migration and sale of animals. This periodical screening will help in identifying areas transforming from low prevalent areas to high prevalent areas for the purpose of vaccination and areas transforming from high prevalent areas to low prevalent areas will serve as indicator of vaccination success.

The MRT and RBPT are generally useful for screening of brucellosis especially in developing countries where other tests are cumbersome to perform on a large scale and/or require special equipment and expertise (Alton *et al*. 1988). The sensitivity and specificity of MRT were 62.96 and 97.40%, respectively, and the low sensitivity of MRT is due to positive reactions from samples taken shortly after parturition, near the end of lactation period or from mastitic quarters (MacMillan 1990, Bercovich and Moerman 1979). However, in pooled/ bulk milk samples, these factors have limited interference. If MRT is positive, serum ELISA is performed in the herds to provide a confirmatory diagnosis

Table 2. Screening of anti-*brucella* antibodies in the bulk milk samples of 30 districts under Department of Animal Husbandry, Government of Karnataka

Name of the district	No of milk samples tested for MRT		Total	No of milk samples +ve for MRT		Total samples positive	Total percentage
	Ist Round (July 2012)	2nd Round (Mar. 2013)		Ist Round	2nd round		
Bengaluru Urban	400	493	893	31	21	52	3.14
Bengaluru Rural	1100	859	1959	71	41	112	6.76
Chitradurga	0	0	0	0	0	0	0
Kolar	1703	1928	3631	55	12	67	4.04
Shivamogga	2810	1761	4571	169	36	205	12.37
Tumakuru	2810	2987	5797	169	34	203	12.25
Davanagere	1037	1040	2077	37	23	60	3.62
Ramanagara	650	0	650	30	0	30	1.81
Chikkaballapura	1500	1500	3000	41	25	66	3.98
Chikkamagaluru	780	1000	1780	25	0	25	1.51
Dakshina Kannada	990	1500	2490	0	44	44	2.66
Hassan	1766	2197	3963	49	3	52	3.14
Kodagu	221	300	521	6	0	6	0.36
Mandya	1500	1500	3000	22	42	64	3.86
Mysuru	1001	778	1779	29	70	99	5.97
Chamarajanagara	391	500	891	8	9	17	1.03
Udupi	423	1200	1623	5	0	5	0.30
Belagavi	2001	1996	3997	64	41	105	6.34
Vijayapura	551	979	1530	50	18	68	4.10
Dharwad	480	592	1072	73	36	109	6.58
Uttara Kannada	1051	1260	2311	11	0	11	0.66
Bagalakote	2000	0	2000	43	0	43	2.6
Gadag	623	575	1198	15	20	35	2.11
Haveri	847	930	1777	36	48	84	5.07
Ballai	751	1500	2251	13	16	29	1.75
Bidar	730	730	1460	6	2	8	0.48
Kalaburgi	1335	1686	3021	30	5	35	2.11
Yadagiri	790	867	1657	0	0	0	0
Raichur	898	1621	2519	14	5	19	1.15
Koppal	800	600	1400	4	0	4	0.24
Total	31939	32879	64818	1106	551	1657	2.55%

(Hunter and Allan 1972). Hence, serum based ELISA testing was suggested in-conjunction with MRT (Tanwani and Pathak 1971, Beh 1973) by providing iELISA kits from the institute in the control program.

Milk based fluorescence polarization assay (FPA) has high sensitivity and is suggested for detection of antibodies to *B. abortus* in bulk tank milk samples at the farm levels and dairies (Gall *et al*. 2002). But in India, instrument for conducting FPA is not available. Similarly, performing milk ELISA require procuring milk samples from far flung villages/milk societies to district diagnostic laboratories was one of the detrimental factors apart from requirement of trained manpower, reagents, equipment and infrastructure support to implement the test for field use. Hence, MRT, which does not require specialised equipment was found suitable to perform at veterinary hospitals where milk can be transported easily in shortest time for the test.

This study clearly demonstrated the sucessful conduct of MRT at veterinary hospitals/ dispensaries biannually by the state departments without depending on national and state diagnostic laboratories as an effective tool for surveillance of disease and vacciantion as per the status of the disease. It is the responsibility of the administration to ensure that *Brucella* control program is adequately staffed with trained veterinarians, technicians and support staff so as to be able to carry out the surveillance and vaccination within the planned time frame.

SUMMARY

The study was conducted to evaluate suitability of milk ring test (MRT) for the bulk sample testing in veterinary hospitals. Out of 1,624 milk samples tested, 109 (6.71%)

were detected positive with prevalence ranging from 2.83 to 15.62% in the Bengaluru urban and rural taluks. After successful completion of pilot study, 64,818 bulk milk samples were tested in the 363 veterinary hospitals of 30 districts in Karnataka state and overall positivity of 2.55% (1,657/ 64,818) was recorded.

ACKNOWLEDGEMENT

Our special thanks to all the Veterinarians of Bengaluru Milk Union Limited (BAMUL), Bengaluru for facilitating the collection of the milk samples for testing and for financial support. Our thanks to Dr T Sreenivasa Reddy and A S Veena for initiating the milk testing in the veterinary hospitals of Karnataka state in the Brucellosis Control Program. We are indebted to Dept. of Animal Husbandry, Dairying and Fisheries, GOI for providing grants-in-aid under the Livestock Health and Disease Control (LH and DC) to NIVEDI to coordinate the surveillance and vaccination under control program.

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