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Sero-epidemiology of infectious bovine rhinotracheitis and brucellosis in organised dairy farms in southern India

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ABSTRACT

The present study was conducted to know the seroprevalence of infectious bovine rhinotracheitis (IBR) and burcellosis and its epidemiology in organised dairy farms in southern India. Sera samples (559) 398 cattle and 161 buffaloes) were collected from 6 organised dairy farms in southern India. Samples were screened for IBR by avidin biotin ELISA and brucellosis by RBPT and indirect ELISA. The overall apparent prevalence were 61.54%, 10.20% and 11.63% for IBR, *Brucella* by RBPT and iELISA respectively. The state-wise seroprevalence showed highest in Andhra Pradesh for IBR and Karnataka for both IBR and brucellosis; lowest in Tamil Nadu for both the diseases. There was no significant difference in male and female in seroprevalence of these diseases. Crossbred cattle showed high seroprevalence for IBR and *Brucella* antibodies when compared to indigenous cattle breeds. Buffaloes showed increased seroprevalence for IBR and *Brucella* when compared to cattle. The IBR seropositive animals showed positive relationship with increase in age. Animals with history of abortions showed seroprevalence of 100% for IBR and 40-50% for brucellosis. The animals with history of reproductive problems showed increased seroprevalence when compared to apparently healthy bovines. The seropositivity for both IBR and brucellosis were 2.76% and 29.19% in cattle and buffaloes, respectively and with overall seropositivity of 10.38%. Thus IBR and brucellosis seroprevalence has increased over the years and there is a need to tackle these diseases effectively by zoosanitary measures and control programmes in organised dairy farms which would benefit the dairying in Southern India.

Key words: Brucellosis, Infectious bovine rhinotracheitis, Organised dairy farm, Seroepidemiology, Southern India

Abortions, repeat breeding, retained placenta, anoestrus, etc. are the main impediments for profitable dairying. Several bacterial and viral infectious agents like *Brucella abortus*, bovine herpes virus-1 (BoHV-1), *Leptospira* spp. etc. may be responsible for abortions in dairy animals (Isloor *et al.* 1998, Mariya *et al.* 2007). Infectious bovine rhinotracheitis (IBR), a viral disease caused by BoHV-1, causes abortions in bovines. A high prevalence of antibodies against BoHV-1 in cattle (50.9%) and buffaloes (52.5%) was recorded in India (Renukaradhya *et al.* 1996) and BoHV-1 was also isolated from bovine semen samples (Rana and Sharma 2004). Brucellosis is known for its zoonotic potential as it is transmitted to humans. A high seroprevalence of bovine brucellosis (17%) was reported in Indian dairy herds with the history of abortions (Isloor *et al.* 1998) and the organism could also be isolated from aborted material in cattle (Chahota *et al.* 2003). The concurrent seroprevalence of IBR and brucellosis based on sex, species, breed, age and animal health status is not available. Hence, the present study was undertaken to investigate the concurrent prevalence of IBR and brucellosis in organised dairy farms in southern India and to know the epidemiology of these diseases in bovines in such farms.

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MATERIALS AND METHODS

Six organised dairy farms located one each in Andhra Pradesh (A), Kerala (D) and 2 farms each in Karnataka (B, C) and Tamil Nadu (E, F) in southern India were selected randomly for this study during April 2012 to March 2014. The blood samples were collected randomly by using serum vacutainer tubes coated with sillicone and multi sample vacutainer needles. The serum was separated by centrifugation at 2,000 rpm for 20 min. All serum samples were stored at -20° C until used for testing. The history of the animals like sex, species, breed, age and animal health status were collected from the organised dairy farms. The management practices followed in these dairy farms were also recorded. For serological investigations, a total of 559 serum samples, from cattle (398) and buffaloes (161), were collected for screening against IBR and brucella antibodies. Based on sex, 19 male and 540 female samples were collected. Based on breeds from cattle, Jersey cross (217), Holstein Friesian cross (77), Sahiwal (43), Gir (22), Deoni (12), Kangayam (12), Rathi (5), Tharparkar (5),

Dharmavaram (3), Bargur (1), Kankarej (1) were selected. Among buffalo breeds, Murrah (133), Jaffrabadi (22), Mehsana (6) were selected. The animals were classified based on age in years for bovines having date of birth and bovines not having date of birth, number of lactations was also considered and used for analysis. Based on animal health status and reproductive history, apparently healthy (364), pregnant (90), repeat breeding (66), abortions (36), anoestrus (2) and retention of placenta (1) were classified.

Infectious bovine rhinotracheitis: The seroprevalence of IBR was carried out by detection of antibodies against BoHV-1 virus from serum using avidin biotin enzyme linked immunosorbent assay (AB-ELISA) kit developed by Project Directorate on Animal Disease Monitoring and Surveillance (PD_ADMAS), Bengaluru, Karnataka. The sensitivity and specificity of the AB-ELISA were 98 and 95 % respectively. This is the only kit indigenously developed and available in India for screening IBR.

Brucellosis: Rose Bengal plate test (RBPT) was performed according to the procedure described by World Organization for Animal Health (OIE 2008). RBPT antigen was procured from the Institute of Animal Health and Veterinary Biologicals, Bengaluru, Karnataka, India. Briefly, 30 µl of serum was mixed with equal volume of *brucella* antigen on microscopic glass slide circled approximately 2 cm in diameter with sterile microtips. The result was recorded after the mixture was rocked gently for 4 min at room temperature. Any sign of agglutination was considered as positive. Diagnosis of brucellosis from serum on the basis of detection of antibodies against *Brucella abortus* was carried out by using an indirect ELISA (iELISA) kit developed by PD_ADMAS, Bengaluru, Karnataka. The test was standardized using smooth lipopolysaccharide antigen from a standard strain of *Brucella abortus* S99 and recombinant protein G conjugated with Horse raddish peroxidase. The sensitivity of the test recorded as 98 and 92 % in cattle and buffaloes, respectively, whereas, specificity was 95 and 98% in cattle and buffaloes, respectively. The use of smooth lipopolysaccharide antigen from *Brucella abortus* S99 strain in indirect ELISA instead of crude antigen helps to detect antibodies against other smooth species such as *B. melitensis* and *B. suis.* The protein G conjugate is advantageous over using different conjugates for different livestock species and this can be used for screening brucellosis in cattle, buffalo, sheep, goat, pigs and humans.

Statistical analysis: The estimation of apparent prevalence with 95% confidence interval and data analysis were carried out statistically (Snedecor and Cochran 1989) using Statistical Analysis System (SAS) Software version 9.3. The apparent prevalence and true prevalence was also estimated as per the following formula (Thrushfield 2005).

Table 1. Seroprevalence of IBR and *Brucella* antibodies in organised dairy farms in southern India

Location	Species	No. tested	IBR (AB ELISA) positive	*Brucella* (RBPT) positive	*Brucella* (iELISA) positive	Both IBR and *Brucella* positive
			Andhra Pradesh			
Farm A	Cattle	100	99 (99.00)	1 (1.00)	1 (1.00)	1 (1.00)
	Buffalo	-	-	-	-	-
Subtotal		100	99 (99.00)	1 (1.00)	1 (1.00)	1 (1.00)
			Karnataka			
Farm B	Cattle	-	-	-	-	-
	Buffalo	82	42 (51.22)	7 (8.54)	15 (18.29)	12 (14.63)
Farm C	Cattle	26	26 (100.00)	10 (38.46)	10 (38.46)	10 (38.46)
	Buffalo	64	64 (100.00)	35 (54.69)	35 (54.69)	35 (54.69)
Subtotal		172	132 (76.74)	52 (30.23)	60 (34.88)	57 (33.14)
			Kerala			
Farm D	Cattle	125	80 (64.00)	-	-	-
	Buffalo	-	-	-	-	-
Subtotal		125	80 (64.00)	-	-	-
			Tamil Nadu			
Farm E	Cattle	80	18 (22.50)	3 (3.75)	3 (3.75)	-
	Buffalo	-	-	-	-	-
Farm F	Cattle	67	13(19.40)	1 (1.49)	1 (1.49)	-
	Buffalo	15	2 (13.33)	-	-	-
Subtotal		162	33 (20.37)	4 (2.47)	4 (2.47)	-
Total		559	344 (61.54)	57 (10.20)	65 (11.63)	58 (10.38)
Confidence interval at 95% level			58.40 - 64.68	7.06 - 13.34	9.77 - 13.49	4.20 - 16.54

Values in parenthesis were expressed in percentage.

(i) Apparent prevalence = number of positive animals/ number of tested animals. (ii) True prevalence = [apparent prevalence + (specificity-1)]/[(sensitivity + specificity) 1].

RESULTS AND DISCUSSION

The state and farm wise apparent seroprevalence of IBR and *Brucella* antibodies are presented in Table 1. The overall true prevalence was 66.12, 11.16 and 12.73 %for IBR by AB-ELISA, Brucella by RBPT and iELISA, respectively. The overall apparent prevalence was 61.54, 10.20 and 11.63 %for IBR by AB-ELISA, *Brucella* by RBPT and iELISA respectively. The highest prevalence of IBR was recorded in the Farm C (100%) followed by Farm A (99%) and Farm D (64%). The highest prevalence of brucellosis was recorded in Farm C (54.69% for cattle and 38.46% for buffalo) followed by Farm B (18.29% for buffalo) by iELISA. The state wise seroprevalence showed high prevalence in Andhra Pradesh and low in Tamil Nadu for IBR and high in Karnataka and no positive animals in Kerala for *Brucella* antibodies. Previous report indicated that the seroprevalence of IBR was 64.76 %in organised dairy farms in India (Trangadia *et al.* 2010) and concurred with the present study. The serological evidence of IBR infection 51.6% in bovines was recorded by AB-ELISA in three southern states of India (Renukaradhya *et al.* 1996). In the present study, the seroprevalence of IBR was increased which might be due to lack of control measures for IBR in India. The seroprevalence was 4.5 and 6.7 % for *Brucella* antibodies by RBPT and iELISA respectively in an organised dairy farm with cattle in Chennai as reported earlier (Bhanu Rekha *et al*. 2013). In this study, more number of animals was detected positive for brucellosis by iELISA compared to RBPT, which may be due to higher sensitivity of ELISA method (Sahin *et al.* 2008). Persual of the literature indicated prevalence of brucellosis in Gujarat state was ranging from 8.98 to 44.00% (Sutariya *et al.* 2005, Chauhan *et al.* 2000), 17% in southern region (Isloor *et al.* 1998), 6.3% in Madhya Pradesh state (Mehra *et al.* 2000) and 12.9% in Punjab state (Dhand *et al.* 2005). In other countries, seroprevalence of brucellosis was reported to be 3.1% in Ethiopia (Ibrahim *et al*. 2010), 6.5% in Jordan (Al-Majali *et al.* 2009), 8.4% in Cameroon (Bayemi *et al.* 2009) and 32.92 to 39.45% in Turkey (Sahin *et al.* 2008). In China, an overall seroprevalence was reported as 35.8% (Yan *et al.* 2008), whereas in England and in Egypt, seroprevalence was 42.5% (Woodbine *et al.* 2009) and 62.5 to 85.7% (Mahmoud *et al.* 2009) respectively. In the present study, the brucellosis seroprevalence decreased and might be due to awareness of management practices by dairy farm owners and also by implementation of disease control programmes by central and state Governments. As brucellosis is self-limiting infection, its prevalence in the organised dairy farms mainly depends upon the protocol adapted for procuring animals for the farms as well as zoo-sanitary measures followed. Variation in the incidence of brucellosis in different farms indicates the level of bio-security measures adopted by farm authorities. Lower incidence indicates the effective implementation of regular testing and culling of seropositive animals, especially in Kerala where slaughter of cattle is allowed.

The seroprevalence of IBR and brucellosis based on sex, species, breed and age is presented in Table 2. The male and female animals showed no significant difference in % positivity for IBR and brucella antibodies and indicated that there was no much difference in males and females in susceptibility to these diseases. The true prevalence of IBR were 63.54 and 74.97% in cattle and buffaloes respectively. The apparent prevalence of IBR antibodies were 59.15 and 67.50% in cattle and buffaloes, respectively. The true prevalence of brucella antibodies by RBPT, iELISA were 4.26, 3.98% and 28.45, 34.7% in cattle and buffaloes, respectively. The apparent seroprevalence of brucella antibodies by RBPT, iELISA were 4.01, 3.76 and 25.63, 31.25 in cattle and buffaloes, respectively. Seroprevalence of IBR in cattle and buffaloes were 50.9% and 52.5%; and 60.46% (289/478) and 62.39% (73/117), respectively as reported earlier (Renukaradhya *et al.* 1996, Trangadia *et al*. 2010). The highest number of seropositivity was observed in buffaloes for both IBR and brucellosis when compared to cattle and concurred with previous report (Trangadia *et al*. 2010). In India, seroprevalence of antibodies against BoHV-1was reported to be 50.9% in cattle and 2.75 to 81.0% in buffaloes (Renukaradhya *et al.* 1996, Sinha *et al.* 2003, Malmurugan *et al.* 2004).

On the basis of breeds, Holstein Friesian crossbred showed increased percent positivity for both IBR and brucellosis when compared to other cattle breeds. In buffaloes, all the three breeds showed similar percent positivity for IBR but Murrah breed showed increased positivity for brucellosis. The crossbred cattle showed increased seroprevalence for IBR and *Brucella* antibodies when compared to indigenous breeds of cattle. This might be due to increased susceptibility to infections by crossbred cattle and also chance of spread of diseases by infected bull semen. Based on age, the IBR seroprevalence increased as the age increases, i.e. after 6 years of age was observed. Based on the both IBR and brucellosis positivity, there was 2 (10.52%) male and 56 (10.37%) female animals are positive. The breed-wise seropositivity was 10 (12.98%) and 2 (33.33%) in Holstein Friesian cross and Mehsana breeds respectively. The 10 years, above 10 years and first calving, 6 calving animals showed increased number of positivity for both IBR and brucellosis. The 6 dairy farms screened in this study practiced artificial insemination, procured from government agencies except Farm C. The Farm C, has the increased number of buffaloes regularly practiced natural breeding which may be the reason for spread of these diseases within the farm easily. The higher prevalence might be due to the purchasing of Murrah buffaloes from Haryana and Punjab state which have higher prevalence of brucellosis. The serological response in cattle immunized with inactivated oil and aluminium hydroxide gel adjuvant vaccines against IBR showed anti BoHV-1 antibodies up to 180 days post vaccination both by ELISA

Table 2. Seroprevalence of IBR and *Brucella* antibodies based on sex, breed, age and lactation no.

	No. tested		*Brucella*		Both IBR and *Brucella*
		IBR	RBPT	iELISA	
		Positive	Positive	Positive	Positive
Sex					
Male	19	11 (57.89)	3 (15.79)	3 (15.79)	2 (10.53)
Female	540	333 (61.67)	54 (10)	62 (11.48)	56 (10.37)
Total	559	344 (61.54)	57 (10.20)	65 (11.63)	58 (10.38)
Breed		Positive	Positive	Positive	Positive
Cattle					
Jersey cross	217	116 (53.46)	2 (0.92)	2 (0.92)	1 (0.46)
Holstein Friesian cross	77	77 (100.00)	10 (12.99)	10 (12.99)	10 (12.99)
Sahiwal	43	20 (46.51)	-	-	-
Gir	22	9 (40.91)	2 (9.09)	2 (9.09)	-
Deoni	12	1 (8.33)	1 (8.33)	1 (8.33)	-
Kangayam	12	4 (33.33)	-	-	-
Rathi	5	1 (20.00)	-	-	-
Tharparkar	5	3 (60.00)	-	-	-
Dharmavaram	3	3 (100.00)	-	-	-
Bargur	1	1 (100.00)	-	-	-
Kangarej	1	1 (100.00)	-	-	-
Subtotal	398	236 (59.30)	15 (3.77)	15 (3.77)	11 (2.76)
Buffalo					
Murrah	133	92 (69.18)	40 (30.08)	44 (33.08)	42 (31.58)
Jaffrabadi	22	12 (54.54)	2 (9.09)	4 (18.18)	3 (13.64)
Mehsana	6	4 (66.67)	-	2 (33.33)	2 (33.33)
Subtotal	161	108 (67.08)	42 (26.09)	50 (31.05)	47 (29.19)
Total	559	344 (61.54)	57 (10.20)	65 (11.63)	58 (10.38)
Age		Positive	Positive	Positive	Positive
0-2 years	86	23 (26.74)	3 (3.49)	3 (3.49)	2 (2.32)
3 years	48	14 (29.17)	2 (4.17)	1 (2.08)	-
4 years	48	12 (25.00)	-	-	-
5 years	25	7 (28.00)	-	-	-
6 years	37	21 (56.76)	2 (5.40)	2 (5.40)	2 (5.40)
7 years	24	15 (62.50)	1 (4.17)	1 (4.17)	-
8 years	12	7 (58.33)	-	-	-
9 years	8	5 (62.50)	-	-	-
10 years	21	12 (57.14)	1 (4.76)	3 (14.29)	2 (9.52)
Above 10 years	25	19 (76.00)	-	-	-
Subtotal	334	135 (40.42)	9 (2.69)	10 (2.99)	6 (1.80)
Lactation no.		Positive	Positive	Positive	Positive
1C	107	104 (97.20)	42 (39.25)	42 (39.25)	42 (39.25)
2C	36	34 (94.44)	-	1 (2.78)	1 (2.78)
3C	39	37 (94.87)	2 (5.13)	5 (12.82)	5 (12.82)
4C	24	20 (83.33)	4 (16.67)	3 (12.50)	1 (4.17)
5C	11	9 (81.82)	-	1 (9.09)	1 (9.09)
6C	3	2 (66.67)	-	1 (33.33)	1 (33.33)
7C	2	-	-	1 (50.00)	-
8C	2	2 (100.00)	-	1 (50.00)	1 (50.00)
9C	1	1 (100.00)	-	-	-
Subtotal	225	209 (92.89)	48 (21.33)	55 (24.44)	52 (23.11)
Total	559	344 (61.54)	57 (10.20)	65 (11.63)	58 (10.38)

Note: Values in parenthesis were expressed in percentage.

Table 3. Seroprevalence of IBR and *Brucella* antibodies based on animal health status

Animal health status	No. tested	IBR	*Brucella*		Both IBR and *Brucella*
			RBPT	iELISA	
		positive	positive	positive	positive
Apparently healthy	364	194 (53.30)	30 (8.24)	34 (9.34)	27 (7.42)
Pregnant	90	69 (76.67)	7 (7.78)	12 (13.33)	12 (13.33)
Repeat breeding	66	43 (65.15)	2 (3.03)	4 (6.06)	4 (6.06)
Abortions	36	36 (100.00)	17 (47.22)	15 (41.67)	15 (41.67)
Anoestrus	2	1 (50.00)	1 (50.00)	-	-
Retention of placenta	1	1 (100.00)	-	-	-
Total	559	344 (61.54)	57 (10.20)	65 (11.63)	58 (10.38)

Values in parenthesis were expressed in percentage.

and micro serum neutralization test (Kamaraj *et al.* 2009) and indicating the future prospects of IBR vaccine in India.

The seroprevalence of IBR and brucella antibodies based on animal health status was presented in Table 3. The animals with reproductive problems showed increased seroprevalence for IBR and brucella when compared to apparently healthy bovines. The animals having history of abortions showed cent percent seropositivity for IBR and higher seroprevalence of brucella antibodies when compared to other animals. The overall seropositivity was 10.38% for both IBR and brucellosis in bovines of southern India. The sixteen (42%) animals with history of abortions showed both IBR and brucellosis seropositivity. There was positive relationship with increasing age in years and IBR positivity which might be due to the reduction in immunity levels as the age advances. Increased seroprevalence of IBR and brucellosis was observed in animals with history of abortions indicating that the animals might have been infected with these diseases and led to abortions. Possible association of IBR with bovine abortion was recorded as 55.4% from aborted crossbred cows (Renukaradhya *et al.* 1996) and concurred with the present study. The bovines in these farms are reared by intensive system and head to head system of housing which may also favour the spread of these disease in the bovines. After BoHV-1 infection the virus becomes latent in ganglions and the animals remain seropositive for rest of the life, the virus can be reactivated upon stress and such animals are likely to shed the virus intermittently into the environment (Trangadia *et al.* 2010). There is no vaccination for IBR in India and the seropositivity indicates the presence of latent infection in animals. A previous study evaluating the status of brucellosis in organised dairy farms with a history of abortions using RBPT and ELISA revealed seropositive animals were 13.78 and 22.18 % respectively with these diagnostic test (Trangadia *et al.* 2010) and higher prevalence was observed in this study. There are several factors playing a possible role for the spread of disease, viz. unrestricted movement of animals, procurement of animals without proper screening, absence of quarantine before entry to the main herd, lack of prophylactic measures like vaccination, etc.

The wide distribution and high prevalence of IBR in organised farms warrants immediate attention to adopt preventive measures. In addition, complementary measures should be implemented like regular screening of animals against these diseases, culling of positive reactors, strict vaccination, quarantine of animals at the time of procurement, use of semen free from infectious agents and strict implementation of zoosanitary and biosecurity measures to control these diseases. There is a need for vaccination of animals against IBR to reduce the prevalence of this disease in bovines in India. For successful brucellosis control programme, implementation of various control regimens including test and removal of affected animals, calfhood vaccination, use of semen obtained from brucella free bull and general hygienic measures will help in the control of brucellosis in organised farms. Now, the Department of Animal Husbandry and Dairying, Ministry of Agriculture, Government of India, New Delhi has initiated the National Control Programme for Brucellosis from the year 2011 which also follows similar methods for the control of the brucellosis under field conditions. Thus, IBR and brucellosis have to be effectively controlled for more profitable dairying by organised dairy farm owners in India.

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