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Prevalence of brucellosis and infectious bovine rhinotracheitis in organized dairy farms in India

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Abstract This study was carried out to investigate the prevalence of bovine brucellosis and infectious bovine rhinotracheitis (IBR) in organized dairy farms with history of abortion in India. ELISA and Rose Bengal Plate Test (RBPT) were used to detect the seropositive animals and the test results indicated that 22.18% and 13.78% animals were declared as seropositive by ELISA and RBPT, respectively. Milk Ring Test (MRT) was carried out only in one farm and 12.82% of the tested animals were turned positive. Culture examination analysis of milk samples, two animals revealed the presence of organisms indistinguishable from *Brucella* spp. The organism was confirmed as brucella by morphological characteristics and biochemical tests. An overall seroprevalence of antibodies against IBR was found to be 60.84%. None of the genital and nasal swab samples was found to be positive for presence of bovine herpesvirus -1 (BHV-1) on repeated passage in Madin-Darby Bovine Kidney (MDBK) cell lines. Brucella and IBR considered as the causal agent for abortions in these farms. The present study indicates the urgent need and the necessity for control of these infectious diseases which cause heavy economic losses to the organized farms.

Keywords Brucellosis · IBR · BHV-1 · ELISA · RBPT

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Introduction

In organized dairy farms, reproductive disorders, viz; infertility, retained placenta, abortions, endometritis etc. are the main impediments to profitability. There are several bacterial and viral infectious agents like *Brucella abortus,* bovine herpesvirus-1 (BHV-1), *Leptospira* spp etc. may be responsible for abortions in dairy animals (Isloor et al. 1998; Mariya et al. 2007). A high sero prevalence (17%) of bovine brucellosis has been 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). A high prevalence of antibodies against BHV-1 in cattle (50.9%) and buffaloes (52.5%) has been recorded in India (Renukaradhya et al. 1996) and BHV-1 was also isolated from bovine semen samples (Rana and Sharma 2004). In the present study an attempt was made to investigate the prevalence of brucellosis and infectious bovine rhinotracheitis (IBR) in a few organized dairy farms by serological, bacteriological

and virological studies to understand the prevalence of these diseases in India.

Materials and methods

Four organized dairy farms located in western, southern, central and northern regions of India (A, B, C and D) with history of abortion were identified for this study during the year 2007-08. For serological investigations, a total of 595 serum samples, 478 from cattle and 117 from buffaloes, were collected for screening against brucellosis and IBR. All serum samples were stored at -20°C until used for testing. Genital and nasal swabs were collected from 32 animals (12 from aborted and 20 from in-contact animals) by using sterile swabs from the Farm A located at western region. These samples were transported to the laboratory in transport medium in ice for isolation of BHV-1 virus. Towards bacteriological analysis, milk samples from 6 aborted, 9 in-contact animals of Farm A and 7 aborted animals of Farm B were processed for isolation of *Brucella* spp. In addition, post-mortem tissue samples (placenta, abomasal content, fetal heart, lung and Kidney) from five aborted fetuses were processed for isolation of *Brucella* organism.

Brucellosis

Serological tests

Rose Bengal Plate Test (RBPT) was performed according to procedure described by World Organization for Animal Health (OIE 2008a). Briefly, 30 μl of serum was mixed with equal volume of brucella antigen on white enamel plate circled approximately 2 cm in diameter with sterile glass or plastic rod. The result was recorded after the mixture was rocked gently for 4 minutes at room temperature. Any sign of agglutination was considered as positive. RBPT antigen was procured from the Indian Veterinary Research Institute (IVRI), Izatnagar, Uttar Pradesh (UP), India.

Milk Ring Test (MRT) was performed as per instruction from manufacturers of the MRT antigen. Briefly, 30–50 μl of brucella antigen was added to 1–2 ml of whole milk in a test tube. After mixing, the tube was incubated at 37°C for 1 hour. The formation of dark blue ring above the white milk column indicates positive reaction (OIE 2008a). MRT antigen was procured from IVRI, Izatnagar, UP, India.

Diagnosis of brucellosis from serum on the basis of detection antibodies against *Brucella abortus,* was carried out by using BRUCELISA®, an indirect ELISA kit from Veterinary Laboratories Agency (VLA), UK.

Isolation of organism

Briefly, isolation of *Brucella spp.* was attempted by inoculation of morbid material and milk samples on Serum Dextrose Agar (SDA) and Tryptose Soya Agar (TSA) with antibiotic cocktail containing polymyxin B sulphate, bacitracin, nystatin, cycloheximide, nalidixic acid, vancomycin (Brucella selective supplement FD 005 supplied by Hi-Media Laboratories Ltd., Mumbai, India) and incubated in an atmosphere of 5% CO_2 for 5–7 days at 37°C as per the protocol of Alton et al. (1988). The bacterial isolate was confirmed on the basis of cultural, morphological characteristic (Modified Z-N staining) along with biochemical characterization and by agglutination with National Standard *Brucella abortus* S99 antiserum (IVRI, India) and a panel of known *Brucella abortus* S99 positive rabbit and bovine field sera.

Infectious bovine rhinotracheitis

Serological test

Diagnosis of IBR was carried out by detection of antibodies against BHV-1 virus from serum by using CHEKIT- Trachitest Serum- Screening, an ELISA kit from IDEXX Europe, B.V., The Netherlands.

Isolation of BHV-1

Genital and nasal swabs were agitated in the transport medium to elute BHV-1 virus and left at room temperature for 30 minutes. After centrifugation, supernatants were filtered through 0.45 μm filters and inoculated into MDBK monolayer cells (Cat No. CCL-22, ATCC, USA). After one hour adsorption period, the monolayer cells were rinsed with tissue culture (TC) medium and TC maintenance medium with 10 % calf serum was added. The cells were observed daily under microscope for cytopathic effect (CPE), which usually appears within 3 days after

inoculation. When after 7 days, no CPE has appeared, three blind passages were made. The cell cultures were freeze–thawed and clarified by centrifugation, and the supernatant was used for inoculation of fresh monolayer (OIE 2008b).

Results

The highest prevalence of brucellosis by indirect ELISA was recorded in Farm C (56.41%) followed by Farm B (45.71%), Farm D (41.46%) and Farm A (6.20%). (Table 1). Similarly, the prevalence by RBPT also found to be highest in Farm C (42.31%) followed by Farm B (30.48%), Farm D (17.07%) and Farm A (2.70%). MRT was carried out with the samples collected at Farm C and five out of 39 samples (12.82%) turned positive. An overall sero-prevalence of brucellosis in organized farms was 22.18%, 13.78% and 12.82% by ELISA, RBPT and MRT respectively (Table 1).

Out of 22 milk samples processed for isolation, two milk samples (9.09%), one each from aborted animals of Farm A and B indicated the presence of *Brucella* spp. The organism was confirmed on the basis of morphological and biochemical characterizations. Brucella organism could not be isolated from postmortem material of any of the aborted foetal tissues.

Out of a total of 595 animals screened for IBR by ELISA, 362 (60.84%) animals were found to be sero-positive for BHV-1. The highest prevalence of IBR was recorded in Farm C (92.31%), followed by Farm B (70.48%), Farm A (55.26%) and Farm D (26.83%) (Table 1). For isolation of BHV-1 virus, genital and nasal swabs collected from 32 IBR sero-positive animals were also processed and none of the samples were found to be positive for BHV-1 virus by isolation on repeated passage in MDBK monolayer cells.

Discussion

In organized dairy farms, high economic losses are attributed mainly due to reproductive disorders, caused by infectious agents like *Brucella* spp., *Leptospira* spp., BHV-1, *Campylobacter* spp. etc. Hence, in the present study animals in the farms which had the history of abortions were screened against the sexually transmitted diseases viz. bovine brucellosis and IBR, so as to pinpoint the etiology of symptoms as well as to develop a disease control strategy.

Serological tests employed in the present study for diagnosis of brucellosis are ELISA, RBPT and MRT. An overall sero-prevalance of bovine brucellosis was recorded to be 22.18% (132/595) and 13.78% (82/595) by ELISA and RBPT, respectively. Whereas MRT was carried only in Farm-C and 12.82% (5/39) of the tested animals were turned positive. Species wise analysis by ELISA, revealed that sero-prevalence in cattle and buffaloes was 18.20% (87/478) and 38.46% (45/117), respectively. More number of animals was detected positive for brucellosis by ELISA compared to RBPT, may be due to higher sensitivity of ELISA (Sahin et al. 2008). As brucellosis is self-limiting infection, its prevalence in the organized 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 sero-positive animals. Perusal of the literature indicated prevalence of brucellosis in western region (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 central region (Madhya Pradesh state) (Mehra et al. 2000) and 12.9% in northern region (Punjab state) (Dhand et al. 2005). In other countries, sero-prevalence of brucellosis was reported to be 3.1% in Ethiopia (Ibrahim et al. 2009), 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 this study, attempts were made to confirm brucellosis by isolation of organisms from the aborted material as well as from milk in addition to serological analysis. Out of 22 milk samples tested, *Brucella* spp. has been isolated from two samples and both the animals were serologically positive. Earlier, in Himachal Pradesh, India Verma et al. (2000), Nagal et al. (1994) and Sahin et al. (2008) reported the involvement of *Brucella* spp. based on isolation and monitoring serological responses in aborted cows. In the present study, although a high percentage of animals exhibited sero-positivity, bacterial isolation

Table 1 Seroprevalence of brucellosis and infectious bovine rhinotracheitis in organized farms

Location	Species	No. Tested	Brucellosis				IBR	
			RBPT		ELISA		ELISA	
			No. Pos	% Pos	No. Pos	% Pos	No. Pos	% Pos
Western	Cattle	322	10	3.11	23	7.14	185	57.45
Region	Buffalo	49	-	-	-	-	20	40.82
(Farm A)	Sub-total	371	10	2.70	23	6.20	205	55.26
Southern	Cattle	105	32	30.48	48	45.71	74	70.48
Region	Sub-total	105	32	30.48	48	45.71	74	70.48
(Farm B)								
Central	Cattle	29	1	3.45	8	27.59	24	82.76
Region	Buffalo	49	32	65.31	36	73.47	48	97.96
(Farm C)	Sub-total	78	33	42.31	44	56.41	72	92.31
Northern	Cattle	22	3	13.64	8	36.36	6	27.27
Region	Buffalo	19	4	21.05	9	47.37	5	26.32
(Farm D)	Sub-total	41	7	17.07	17	41.46	11	26.83
TOTAL		595	82	13.78	132	22.18	362	60.84

Pos = Positive

was low which may be attributed to the slow growth of the *Brucella* spp., low sensitivity depending on stage of the disease, type of culture medium, putrefaction of specimen, overgrowth of contaminants, quantity of bacteria and culture technique employed. However, by both isolation and sero-positivity indicated that *Brucella* spp. may be one of the agents responsible for abortions and other reproductive problems in the dairy herd in the country.

As BHV-1 infection is also responsible for abortions and other reproductive disorders in dairy animals, serological and virological screenings were carried out for IBR, which indicated an overall seroprevalence of 60.84% by ELISA. Sero-prevalence in cattle and buffaloes was found 60.46% (289/478) and 62.39% (73/117), respectively. In India, seroprevalence of antibodies against BHV-1 in cattle has been reported to be 50.9% and in buffaloes 2.75 to 81.0% (Renukaradhya et al. 1996; Sinha et al. 2003; Malmurugan et al. 2004). Possible association of IBR with bovine abortion was recorded as 55.4% from aborted crossbred cows (Renukaradhya et al. 1996). 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.

Although a high prevalence of antibodies against IBR was recorded in this study, no BHV-1 virus could be isolated from any of the genital and nasal swab samples in cell culture. In India, BHV-1 virus was isolated from bull semen samples (Rana and Sharma 2004; Deka et al. 2005) and nasal swabs and blood samples of cattle collected during outbreak of respiratory form of IBR (Singh et al. 1989). In a similar kind of study in Egypt, IBR virus could be identified by culture isolation in vaginal, nasal and ocular swabs. (Mahmoud et al. 2009).

After BHV-1 infection the virus becomes latent in ganglions and the animals remain sero-positive 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 (OIE 2008b). This phenomenon might have resulted in low percentage of positive samples in virus isolation.

The wide distribution and high prevalence of bovine brucellosis and IBR in organized farms warrants immediate attention and preventive measures should be developed and implemented. 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 etc. To overcome this situation, 1. Regular screening of animals against these diseases and culling of positive reactors, 2. Adaption of proper prophylactic measures by vaccination, 3. Screening of animals at the time of procurement and quarantine thereafter, 4. Use of semen doses for artificial insemination free from infectious agents, 5. Implementation of strict zoo-sanitary and bio-security measures, including increased awareness among the farm authorities, would be mandatory to control diseases.

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