

Serological Investigation of Bovine Brucellosis, Johne's Disease and Infectious Bovine Rhinotracheitis in Two States of IndiaB.J. Trangadia^{1*}, S.K. Rana¹, K. Nagmani², V.A. Srinivasan²¹Research and Development Centre, National Dairy Development Board, C/o Indian Immunologicals Ltd. Gachibowli, Hyderabad-500032, India.²Research and Development Centre, Indian Immunologicals Ltd. Gachibowli, Hyderabad- 500032, India.

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Abstract

In the present study, serological diagnosis of brucellosis, Johne's Disease (JD) and infectious bovine rhinotracheitis (IBR) was carried out in the states of Gujarat and Andhra Pradesh of India. Apparent prevalence of brucellosis was 7.57 % (true prevalence, 5.66%) in Gujarat and 12.27 % (true prevalence, 10.60%) in Andhra Pradesh. For JD apparent prevalence in Gujarat and Andhra Pradesh was 13.39% (true prevalence, 15.68%) and 16.26% (true prevalence, 19.31%) respectively. Apparent prevalence of IBR was found to be 23.94% (true prevalence, 18.20%) and 26.49% (true prevalence, 21.03%) in Gujarat and Andhra Pradesh respectively. The results of the study revealed that these diseases are highly prevalent in both the states; hence, there is an urgent need for adopting suitable control measures.

Keywords: Andhra Pradesh; Brucellosis; ELISA; Gujarat; IBR; India; JD; Serology

Introduction

Bovine brucellosis, Johne's disease (JD) and infectious bovine rhinotracheitis (IBR) are economically important diseases of cattle and buffaloes caused by *Brucella abortus*, *Mycobacterium avium* subspecies paratuberculosis (MAP) and Bovine herpesvirus-1 (BHV-1), respectively. A considerably high sero-prevalence (17.00 to 22.18%) of bovine brucellosis has been reported in Indian dairy herds with the history of abortions concomitant with the isolation of the organism from aborted fetal material and milk (Isloor *et al.*, 1998, Chahota *et al.*, 2003, Trangadia *et al.*, 2009). Limited reports available on the prevalence of the MAP in the country indicate a sero-prevalence of 15.60 to 22.50 % in cattle and buffaloes (Garg *et al.*, 2007, Tripathi *et al.*, 2007). Prevalence of IBR in cattle ranges from 50.9 to 60.46% and in buffaloes from 2.75 to 81.0% in India (Renukaradhya *et al.*, 1998; Renukaradhya *et al.*, 2002, Malmurugan *et al.*, 2004, Trangadia *et al.*, 2009).

Animals in organized herd in India are generally tested for these infectious diseases as per OIE

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Corresponding author: B. J. Trangadia
Address: Department of Veterinary Pathology, Vanbandhu College of Veterinary Science and Animal Husbandry, Navsari Agricultural University, Navsari-396450, Gujarat, INDIA
E-mail address: Srini@indimmune.com

(2008) guidelines. The results of serological investigation for brucellosis, JD and IBR by ELISA in cattle and buffaloes from the states of Gujarat and Andhra Pradesh of India are presented in this article.

Materials and methods*Source of the serum samples*

Sampling was carried out from cattle and buffaloes maintained in the herd of seven districts of Gujarat and five districts of Andhra Pradesh states. For investigation of these diseases, 77 and 29 herd were sampled from Gujarat and Andhra Pradesh respectively, representing approximately 10 per cent of the animals from each herd. However, number of samples tested for each disease varied either due to insufficient quantity of samples or limited availability of test kits. None of the animals were vaccinated against any of these three diseases.

Serology

Testing for antibodies against brucellosis, JD and IBR was carried out by commercial ELISA kits as per manufacturers' instructions.

Brucellosis:

Diagnosis of brucellosis on the basis of detection serum antibodies against *Brucella abortus*, was carried out by an indirect ELISA BRUCELISA® (Veterinary Laboratories Agency, UK). A cut-off was considered as 10% of the mean of the optical density (OD) of the positive control wells. As recommended by the manufacturer, any test sample showing an OD equal to or above the cut-off value was considered as positive. Sensitivity (Se) and specificity (Sp) of the kit was reported to be 97.2% and 97.8% respectively (McGiven et al., 2003).

Infectious bovine rhinotracheitis

Detection of antibodies against IBR from serum was done by an indirect Enzyme Immunoassay kit, SVANOVIR® IBR-Ab ELISA (Svanova Biotech AB, Uppsala, Sweden). The sample showing percent positivity values (PP) ≥ 20 was considered as positive for IBR. Se and Sp of this kit were 97.4% and 92.4% respectively in relation to serum neutralization test

$$PP = \frac{\text{OD Test sample or negative control}}{\text{OD Positive control}} \times 100$$

Johne's Disease:

Screening of serum samples was carried out to de-

Table 1. Sero-positivity of brucellosis, Johne's Disease (JD) and infectious bovine Rhinotracheitis (IBR) in Indian states of Gujarat and Andhra Pradesh

States	Brucellosis		IBR		JD	
	No. Tested	Positive (%)	No. Tested	Positive (%)	No. Tested	Positive (%)
Gujarat	2443	7.57	1994	13.39	1817	23.94
Andhra Pradesh	1687	12.27	1458	16.26	1495	26.49
Total	4130	9.49	3452	14.60	3312	25.09

The apparent prevalence of brucellosis in the herd of Gujarat and Andhra Pradesh was 7.57 % (95% CI 4.78-10.37) and 12.27 % (95% CI 8.75-15.79) respectively with an overall prevalence of 9.49% (95% CI 7.15-11.83). Considering the Se (97.2 %) and Sp (97.8 %) of the ELISA, the true prevalence of was calculated as 5.66 % (95% CI 2.47-8.84) in Gujarat and 10.60 % (95% CI 6.89-14.31) in Andhra Pradesh with an overall prevalence of 7.68% (95% CI 5.24-10.11).

tect antibodies against MAP by using an in vitro solid phase indirect enzyme immunoassay, PARACHEK® (Biocor Animal Health, Omaha, NE, USA). Sample with OD values greater than negative control plus 0.100 absorbance units were considered positive, as recommended by the manufacturer. Se of the assay was 80% whereas Sp was 99.0% as per manufacturer of the kit (Cox et al., 1991).

Statistical analysis:

A t -test at 95% level of significance was used to compare the prevalence of diseases between the states. True prevalence (TP) of the diseases was calculated on the basis of apparent prevalence (AP) as per the following formula:

$$TP = \frac{AP - (1 - Sp)}{Se - (1 - Sp)}$$

Results

In the present study, serum samples of cattle and buffaloes from the states of Gujarat and Andhra Pradesh of India were screened for antibodies against brucellosis, JD and IBR by ELISA and the results are depicted in Table 1.

Apparent prevalence of JD in Gujarat and Andhra Pradesh was 13.39% (95% CI 10.10-16.68) and 16.26% (95% CI 13.08-19.43) respectively with an overall prevalence of 14.60% (95% CI 12.08-17.12). Considering the Se (80.0 %) and Sp (99.0%) of the ELISA, the true prevalence of was calculated to be 15.68% (95% CI 12.29-19.08) in Gujarat and 19.31% (95% CI 15.74-22.88) in Andhra Pradesh with an overall prevalence of 17.22% (95% CI 14.53-19.90).

Apparent prevalence of IBR in Gujarat and Andhra Pradesh was 23.94 % (95% CI 17.92-29.96) and 26.49% (95% CI 21.79-31.18) respectively with an overall prevalence of 25.09% (95% CI 20.72-29.46). Considering the Se (97.4 %) and Sp (92.4%) of the ELISA, the true prevalence of was calculated as 18.2% (95% CI 11.77-24.62) in Gujarat and 21.03% (95% CI 16.55-25.51) in Andhra Pradesh with an overall prevalence of 19.48% (95% CI 14.95-24.00).

The results indicated that sero-positivity of all these diseases were higher in the state of Andhra Pradesh as compared to Gujarat. Estimation of t test on the apparent prevalence of Bovine brucellosis, JD and IBR of Gujarat and Andhra Pradesh revealed statistically significant ($P < 0.05$, 0.019).

Discussion

Bovine brucellosis, Johne's disease (JD) and infectious bovine rhinotracheitis (IBR) are infectious diseases of cattle and buffaloes and cause heavy economic losses to the farmers.

Brucellosis causes abortions, reproductive failure and also has public health significance as it can be transmitted to human from infected animals. In this study, prevalence of brucellosis in Gujarat and Andhra Pradesh was found to be 7.57% and 12.27% respectively. Renukaradhya *et al.* (2002) reported a lower seroprevalence of the disease in Gujarat (6.6% in cattle and 6.3% in buffaloes) and in Andhra Pradesh (4.3% in cattle and 2.4% in buffaloes). Perusal of the literature indicated prevalence of brucellosis in Gujarat state was ranging from 8.98 to 44.00% (Chauhan *et al.*, 2000, Sutariya *et al.*, 2005, Trangadia *et al.*, 2009) and 17% in southern region (Isloor *et al.*, 1998).

The difference in prevalence among these studies could be due to the different survey techniques employed by various researchers. Also the Rose Bengal Test and Milk Ring Test was used for analysis of samples in most of the previous studies rather than highly sensitive and specific ELISA from VLA, an OIE reference laboratory for bovine brucellosis, in this study.

Johne's disease is fatal chronic granulomatous enteritis of ruminants characterized by progressive wasting, emaciation and death. The disease is prevalent in domestic animals worldwide. Moreover, it represents a potential source of infection to humans through consumption of contaminated

milk and milk products (Slana *et al.*, 2008) and has been linked to Crohn's disease in humans (Chamberlin *et al.*, 2001). In this study, prevalence of JD in Gujarat and Andhra Pradesh was recorded to be 13.39% and 16.26% respectively. Limited reports available on the prevalence of the disease in various states of the country, indicates 15.60 to 22.50% sero-positivity in cattle and buffaloes (Garg *et al.*, 2007, Tripathi *et al.*, 2007). The prevalence of the disease recorded in the present study was marginally lower than the available reports, which may be due to screening of different sample population in different studies.

Infectious bovine rhinotracheitis is an emerging disease of cattle and buffaloes and existence of the virus was first reported by Mehrotra *et al.* (1976). The infection has serious economic implications as India has the world's largest cattle and buffalo population. Prevalence of IBR was found to be 23.94% and 26.49% in Gujarat and Andhra Pradesh respectively. Trangadia *et al.* (2009) reported a higher sero-prevalence of IBR in organized farm, 55.26% in Western region and 70.48% in Southern region of the country. Literature indicated, prevalence of antibodies against IBR varied from 50.9% in cattle and 2.75 to 81.0% in buffaloes (Renukaradhya *et al.*, 1996; Sinha *et al.*, 2002, Malmurugan *et al.*, 2004) from various parts of the country.

The results of present study indicated that sero-positivity of all these diseases under investigation were higher in the state of Andhra Pradesh as compared to Gujarat. The high prevalence found in the study is of great significance as the diseases cause huge economic losses. Variation in the prevalence of these diseases between states may be due to several factors viz. methods of sampling, source of samples, intensity of dairy farming, inter-mixing of animals under same husbandry practices, unrestricted movement of infected animals and the extend of control measures adopted.

More numbers of animals are slaughtered in Andhra Pradesh as compared to Gujarat and it leads to movement of infected animals from various parts of the state to the slaughter house. This may be responsible for spreading infectious diseases in the state.

This higher rate could be due to agroecological and climatological factors as Gujarat is situated on the Western part where as Andhra Pradesh is situated on the Southern part of the country. Such factors may favor perpetuation of the infectious

agents. In addition, Gujarat has well developed co-operative dairy sectors compared to Andhra Pradesh. Co-operatives provide animal health services to the farmers at nominal cost and door step. It may help in quick and economical treatment for animals and it is well known that delivering adequate animal health services resulted results in a low incidence of the infectious diseases It could be concluded that, high sero-positivity of brucellosis, JD and IBR, recorded in this study, perhaps due to absence of early and accurate diagnosis, lack of culling of positive reactors and unrestricted movement of infected livestock. To overcome this situation, a comprehensive and systematic study would be essential to pinpoint the more precise distribution of these diseases and a strategic disease control programme equipped with prompt and accurate disease diagnosis practice.

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