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Research Article

SEROSURVEILLANCE OF INFECTIOUS BOVINE RHINOTRACHEITIS AND BRUCELLOSIS IN A PRIVATE COMMERCIAL CATTLE FARM IN KERALA

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A R T I C L E I N F O

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A B S T R A C T

Infectious Bovine Rhinotracheitis (IBRT) and brucellosis are two economically important disease listed by OIE affecting reproduction and having great impact on international trade. IBRT and brucellosis are highly contagious and spreads quickly through direct contact. Both diseases are not life threatening but predisposes to secondary bacterial pneumonia, which may result in death if untreated. The present study aims to understand the seroprevalence of infectious bovine rhinotracheitis and brucellosis in a private commercial farm in Palakkad district of Kerala with a history of frequent last trimester abortions. Sera samples were collected randomly from 50 cows that include both apparently healthy animals and animals with history of abortions. These samples were screened for presence of antibodies to IBR by using gB competitive ELISA and antibodies to brucella by using indirect ELISA. In the present study,16 out of 50 serum samples (32 %) were found to be positive for IBRT but all of them were negative for brucellosis. This indicates the need for screening of animals during international trade and implementation of proper vaccination strategy for IBRT in Kerala along with the use of disease free semen.

INTRODUCTION

Reproductive disorders mainly abortions and repeat breeding are major reason for decreased reproductive efficiency which is a major determinant of lifetime productivity of cows. 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 (Maiya *et al*., 2007). Infectious bovine rhinotracheitis (IBR), a viral disease caused by BoHV-1, causes abortions in cattle and fetal deformities in pregnant animals. In India, more than 45 per cent of apparently healthy breeding bulls were reported to be seropositive for IBR and around 50 per cent of the semen produced by seropositive bulls tested positive for IBR virus (Deka *et al.,* 2005). Brucellosis is a major zoonotic disease as well as it cause heavy economic losses through reproductive problems. Based on the epidemiological data of active surveillance programme, it was estimated that there is a loss of US$58.8 million per year in India due to brucellosis (Kollannur *et al*., 2007). Screening of 1020 serum samples from slaughter cattle of Kerala showed overall prevalence of 6.17 percent for brucellosis by c ELISA (Reddy *et al*., 2014).

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The seropevalence of IBR and brucellosis in organized dairy farms in southern India showed prevalence of 61.54%, 10.2% and 11.63% for IBR by avidin biotin ELISA and brucellosis by BPT and indirect ELISA (Krishnamoothy *et al*., 2015). 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. The present study aims at screening of a private commercial dairy farm of Kerala that had abortions in nine cows for IBR and brucellosis.

MATERIALS AND METHODS

Frequent last trimester abortions were reported in nine cows in a herd of 70 cattle in a private commercial farm of Palakkad district of Kerala. Crossbred HF and Crossbred Jersey were the breeds maintained in the farm. Cows were either naturally inseminated with their own bull or artificially inseminated. History of parity revealed an average of three. Anamnesis revealed no stages of off feed, nasal discharge or general weakness noticed in any animal. On general clinical examination, no signs of fever or respiratory distress were evident. These animals were not vaccinated against brucellosis. Serum samples were collected from 50 cows randomly, which included cows showing abortion and a bull used for natural mating, from a herd of 70 animals. The Serum samples were stored for a week in -20˚C. The samples were screened for IBR by gB competitive ELISA.

Procedure

Briefly 50µl of the dilution buffer was added to all the wells, 50µl of positive control added to wells A1 and B1,50µl of negative control added to wells C1 and D1 and 50µl of samples to remaining wells. Incubated at 37˚C for 2 h ± 5min. The wells were emptied and washed 3 times with 300µl of wash solution. Care was taken to avoid drying of wells between washings. 100µl of Ready-to-use conjugate was added to each wells and incubated at 37˚C for 30 ± 3min. The wells were emptied and washed 3 times with wash solution. 100µl of the Substrate solution to each wells and incubated at 21˚C (± 5˚C) for 15 ± 2min in the dark. 100µl of the Stop solution was added to each well and the OD value was read at 450nm.

Interpretation of Results

OD value of Negative Control(NC) > 0.7
OD value of Positive Control/OD value of Negative Control < 0.3
S/N% less than or equal to 45% indicate positivity.

The samples were screened for brucellosis by indirect ELISA (Bionote, Korea).

Procedure

Reagents and samples were allowed to come to room temperature(18-25˚c).Serum samples were diluted to 1:49 with sample diluents.100 µl of prepared sample were added into each sample well.100 µl of controls were added into appropriate wells. The microplates were covered with adhesive plate sealer and mixed well ona vibrating mixer.The wells were incubated for 60 minutes at 37˚C ± 1˚C.Liquid was aspirated and rinsed with 350µl of wash solution.100 µl of diluted enzyme conjugate was added to each well.The plates were covered with plate sealer and incubated for 30 minutes at 37˚C ± 1˚C. The wells were washed and 100µl of substrate was added to each well.The wells were covered with plate sealer and incubated for 15 minutes at 18-25˚c in the dark.100 µl of stopping solution was added to each solution and mixed by gentle shaking.The absorbance values were read at 450nm in a biochrome spectrophotometer (with reference wavelength at 620nm) right after the end of assay within 30 minute.

Interpretation of Results

The optical density value of negative control serum should be ranged from -0.005 to 0.200. The OD value of strongly positive control serum should be above 1.00. The OD value of weakly positive serum should be above 0.500.

$$\text{Percentage Positivity} = \frac{\text{OD of samples}}{\text{Average OD of SPC serum}} \times 100$$

The results were determined based on the following %P criteria

	Serum/Plasma
Positive	≥ 25
Negative	< 25

Fig 1

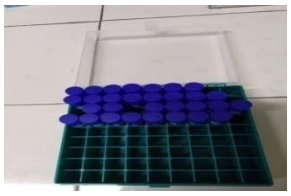
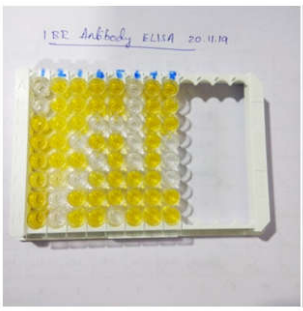


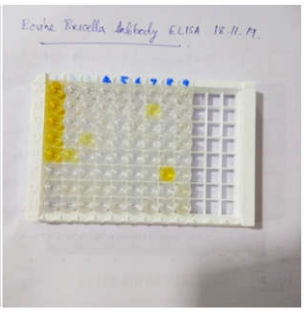
Fig 2

RESULTS

Results showed 16 positive samples out of 50, depicting 32% seroprevalence for IBRT. IgG ELISA was done for brucellosis and was found to be negative for all sample. Serum sample of bull was tested negative for both brucellosis and IBRT.



IBRT antibody ELISA
Fig:3



Brucellosis antibody ELISA
Fig:4

DISCUSSION

IBRT is caused by Bovine Herpes Virus 1(BHV1) which is associated with infectious pustular vulvovaginitis, infectious balanoposthitis, conjunctivitis, abortion, encephalomyelitis and mastitis. The disease is characterised by inflammation of upper respiratory tract. The clinical signs range from mild to severe, depending on secondary bacterial pneumonia, fever, anorexia, coughing, excessive salivation, nasal discharge.

The disease has been recorded from Kerala by Sulochana *et al* (1982). According to Laveso *et al* (1984),the rate of conception decrease in artificially inseminated cows with infectious vulvovaginitis from 80% to 45-50%.Crossbred cows are most affected in agreement with the studies of Mallick (1986) who studied the seroprevalence of the disease in seven states and observed that 65.3% exotic,62% indigenous and 73% cross bred cattle were seropositive. In the present study, most of the animals affected were in the age group 3-6 years which is in concordance with previous study by Saravanajayam *et al* (2015).

The present study mainly aims in screening of IBRT in Kerala state where it is not given much importance as the cases are not identified properly. Identification of the disease from symptoms is not confirmative, thus serological studies should be performed. Effective vaccination strategies along with currently followed brucellosis vaccination should be followed in Kerala to reduce the economic impact of the disease. The losses are from market value of culled and fatal cases, cost of feeding for 1-6 weeks, treatment cost and value of lost milk production as opinioned by Wiseman *et al*.(1979).Abortions are also taken into serious consideration as the success of a cattle farm, whether big or small, depends on the production of a calf per year.

CONCLUSION

IBRT is not a much fatal disease, but it can cause huge economic loss that can disturb the overall stability of the country. If a cow is affected, no treatment is effective. Culling is the only practical solution to avoid further losses. In Kerala, IBRT is a disease not considered serious or remains unidentified. As it is a disease listed by OIE, effective vaccination strategies should be followed in Kerala. Screening of recently introduced cattle along with the use of disease free semen should be ensured.

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