Prevalence of infectious bovine rhinotracheitis in southern India

G.J. RENUKARADHYA *, M. RAJASEKHAR * and R. RAGHAVAN **

Summary: The authors describe a serological survey on the prevalence of infectious bovine rhinotracheitis (IBR) among cattle and buffalo (Bubalus bubalis) in three southern states of India. A local isolate of bovine herpesvirus 1 (BHV-1) from an outbreak of the respiratory form of IBR was used as a source of virus antigen in avidin-biotin enzyme-linked immunosorbent assay (ELISA). The overall prevalence of antibodies to BHV-1 in cattle was 50.9%, and in buffalo was 52.5%. Among breeding bulls, 114/120 samples from Tamil Nadu (95%) and 41/99 samples from Karnataka (41.4%) were seropositive. The possible association of IBR with bovine abortions was recorded in 31/56 samples (55.4%) from aborted crossbred cows. However, virus isolation was not performed on these animals. The authors also highlight the economic importance of IBR to the rapidly developing livestock industry in India.


INTRODUCTION

India has massive cattle and buffalo populations (200 million and 75 million, respectively), which are frequently exposed to several endemic viral diseases. Infectious bovine rhinotracheitis (IBR), caused by bovine herpesvirus 1 (BHV-1), has been known to exist in India since 1976 (6). The isolation of BHV-1 from the respiratory and genital tracts of bovines has been reported from the states of Orissa (7), Karnataka (8) and Gujarat (16). Furthermore, widespread serological evidence of respiratory as well as genital tract infections is reported from most of the states in India (15, 18). IBR infection assumes great economic importance in India, which is the second largest milk producer in the world. Moreover, the dairy industry is poised for rapid growth with the introduction of exotic germplasm for crossbreeding with the

* Indian Council of Agricultural Research (ICAR) Project on Animal Disease Monitoring, Surveillance and Forecasting, Institute of Animal Health and Veterinary Biologicals Campus, Hebbal, Bangalore 560 024, India.

** Department of Veterinary Microbiology and Public Health, University of Agricultural Sciences, Hebbal, Bangalore 560 024, India.
indigenous cattle population. The present investigation was undertaken to establish the status of IBR prevalence in the southern states of India, using avidin-biotin enzyme-linked immunosorbent assay (A-B ELISA).

MATERIALS AND METHODS

Bovine herpesvirus 1 antigen

A local isolate of BHV-1, from an outbreak of the respiratory form of IBR in cattle in Hassan in the state of Karnataka, was used for the production of virus antigen (8). The virus-infected Madin Darby bovine kidney (MDBK) cells showing maximum cytopathic effects were pooled, frozen and thawed repeatedly. The suspension, after initial clarification at 10,000 × gravity (g) for 30 minutes, was sequentially concentrated, using cut-off filters (molecular weight: 100,000) in a fluid concentration system. The virus was then pelleted at 100,000 × g for one hour in an ultracentrifuge, followed by discontinuous sucrose gradient separation in tris-ethylene diamine-tetracetic acid (EDTA) buffer. The purified virus preparation was sonicated for three cycles of 20 seconds each. Protein was estimated by the Folin-Ciocalteau method.

Immunochemicals and equipment

The biotinylated anti-bovine immunoglobulin G (IgG) was obtained from the United States of America (USA). Avidin-horseradish peroxidase (HRP), bovine serum albumin and ortho-dianisidine dihydrochloride (ODD) were also acquired from the USA. Polystyrene flat-bottomed medium-binding microtitre plates and a microtitre plate reader were used in the assay procedures.

Reference sera

Reference positive and negative control sera were obtained from a commercial indirect ELISA kit used for serological diagnosis of IBR infection. Serum samples collected from two cows, which had recovered from natural IBR infection (confirmed by virus isolation) (8), and ten colostrum-deprived neonatal calves born to IBR-negative dams served as positive and negative controls, respectively (also confirmed by commercial IBR kit).

Source of field serum samples

In all, 2,420 field serum samples were collected from May to August 1993 from the following sources:
- ten private farms (610 sera)
- six government farms (383 sera)
- Bangalore abattoir (1,427 sera).

These sources were located in the states of Karnataka (2,027 sera), Tamil Nadu (328 sera) and Andhra Pradesh (65 sera). All samples were screened for antibodies to BHV-1.
The origins of the samples included the following:
- 993 dairy cows
- 219 breeding bulls
- 417 slaughtered cattle (*Bos indicus* and crossbred cattle)
- 1,010 slaughtered buffalo (*Bubalus bubalis*).

Of the 219 breeding bulls, 120 originated from Tamil Nadu and the remaining 99 came from the state of Karnataka. A detailed history of abortions and respiratory infections was collected from organised farms. Disease status of slaughtered animals was not available.

**Standardisation of avidin-biotin enzyme-linked immunosorbent assay**

The A-B ELISA technique, previously described for the assay of rinderpest antibodies in large and small ruminants (2, 14, 17), was employed in this study, with some modifications, as detailed below. Optimisation protocols indicated that working dilutions of 1 in 100 each of virus antigen and test serum, 1 in 20,000 of anti-bovine IgG and 1 in 15,000 of avidin-HRP were adequate for monitoring antibodies to BHV-1 (4).

**Procedure for avidin-biotin enzyme-linked immunosorbent assay**

BHV-1 antigen-coated microtitre plates (1:100 dilution, 100 µl) were incubated at 37°C for one hour, then thoroughly washed five times with phosphate-buffered saline (PBS)-Tween 20 washing buffer, using an autowasher. Each well was then treated with 200 µl of the blocking solution (3% bistrimethyl silylacetamide (BSA)-PBS-Tween 20) and incubated for one hour at 37°C. The plates were emptied and each filled with 100 µl of each test serum (1:100 dilution) in duplicate, and further incubated for one hour at 37°C. After washing the plates as described earlier, biotinylated anti-bovine IgG (1:20,000, 100 µl) was added to each well and incubated for one hour at 37°C. After washing the plates as described earlier, biotinylated anti-bovine IgG (1:20,000, 100 µl) was added to each well and incubated for one hour at 37°C. The plates were washed and avidin-HRP (1:15,000, 100 µl) was added to each well. After incubation for 20 minutes at 37°C, the plates were washed and treated with 100 µl of freshly prepared ODD solution for 30 minutes. Finally, the enzyme substrate reaction was arrested by adding 2.5 normal hydrochloric acid (25 µl). The absorbance values were recorded at 405 nm. During the assay, a panel of duplicate control sera was included regularly. The test plates showing absorbance values outside the range of the control panel of negative and positive sera were rejected and retesting was performed.

**Interpretation of avidin-biotin enzyme-linked immunosorbent assay**

The absorbance values of ten negative control sera ranged from 0.062 to 0.093. The values of the two positive sera from animals recovered from IBR were 0.51 to 0.53. In addition, the negative absorbance value of the control sera included in the commercial IBR kit ranged from 0.045 to 0.09, and the value of the positive sera ranged from 0.42 to 0.52. Keeping these absorbance values as the basis for determining the positive/negative cut-off, any field serum sample showing an absorbance value above 0.142 (the sum of the mean of ten negative control sera [0.0862] plus 3 × standard deviations [0.0558]) was considered positive for the
presence of BHV-1 antibodies. This critical cut-off absorbance limit was found to be acceptable when a number of control and field sera were re-examined, using a commercial IBR indirect ELISA kit.

RESULTS

Optimisation of avidin-biotin enzyme-linked immunosorbent assay technique

The density gradient purified virus preparation contained 3.6 mg per ml of protein. The mean absorbance of the negative control sera was 0.0862 and that of the positive control sera was 0.521. The BHV-1 antibody levels, as indicated by absorbance values, ranged from 0.142 to 0.744 in the field serum samples, and no significant differences were observed in the spectrum of absorbance values among cattle or buffalo sera. This suggested that the anti-bovine IgG reagent was also suitable for use on buffalo samples.

Prevalence of antibodies to bovine herpesvirus 1

The results of this study confirmed widespread prevalence of antibodies to BHV-1 in the three southern states of India, as follows:
- 45% in Tamil Nadu
- 50% in Karnataka
- 64% in Andhra Pradesh (Table I).

<table>
<thead>
<tr>
<th>TABLE I</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prevalence of antibodies to bovine herpesvirus 1 among bovines in the southern states of India</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>State</th>
<th>No. tested</th>
<th>No. positive</th>
<th>Percentage positive</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Karnataka</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organised farms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cows</td>
<td>501</td>
<td>272</td>
<td>54.3</td>
</tr>
<tr>
<td>Bulls</td>
<td>99</td>
<td>41</td>
<td>41.4</td>
</tr>
<tr>
<td>Abattoir</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cows</td>
<td>417</td>
<td>167</td>
<td>40.0</td>
</tr>
<tr>
<td>Buffalo</td>
<td>1,010</td>
<td>530</td>
<td>52.5</td>
</tr>
<tr>
<td><strong>Tamil Nadu</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organised farms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cows</td>
<td>208</td>
<td>95</td>
<td>45.7</td>
</tr>
<tr>
<td>Bulls</td>
<td>120</td>
<td>114</td>
<td>95.0</td>
</tr>
<tr>
<td><strong>Andhra Pradesh</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organised farms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cows</td>
<td>65</td>
<td>29</td>
<td>44.6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2,420</td>
<td>1,248</td>
<td>51.6</td>
</tr>
</tbody>
</table>
The overall presence of BHV-1 antibodies in cattle and buffalo was 50.9% and 52.5%, respectively. Among the breeding bulls examined, 114/120 samples from Tamil Nadu (95%) and 41/99 samples from Karnataka (41.4%) were found positive for BHV-1 antibodies. In an organised dairy herd in Karnataka (i.e. on a farm in which basic husbandry practices and records are maintained), 59/81 samples were positive following a severe respiratory infection (72.8%). In another herd, 31/56 samples from aborted crossbred cows were positive for BHV-1 antibodies (55.4%). However, this could only be considered as circumstantial evidence of BHV-1 infection, in the absence of virus isolation from these seropositive animals.

DISCUSSION

Serological procedures, such as the serum neutralisation test (9), passive haemagglutination test (11) and indirect ELISA (10, 12) have been used for the assay of BHV-1 antibodies in the sera of animals. An A-B ELISA was specifically developed for the present study, as the technique is highly sensitive, specific and very economical, since it allows the use of immunoconjugates at very high dilution, as compared to indirect ELISA (2). Furthermore, the use of A-B ELISA in studies to monitor antibodies to rinderpest, peste des petits ruminants (2, 14, 17), tropical theileriosis (13, 19) and Newcastle disease (3) suggests that the technique could also be extended to BHV-1 infection. Several research workers have reported the seroprevalence of antibodies to BHV-1 in India, and a survey of the bovine population of Andhra Pradesh, Haryana, Karnataka, Orissa, Tamil Nadu, Uttar Pradesh and West Bengal states indicated a high prevalence of BHV-1 antibodies (47% to 74%), using the passive haemagglutination technique (5). The moderate prevalence figures reported in the present study (51.6%), despite the use of a more sensitive technique, such as A-B ELISA, could be attributed to the large sample size and the inclusion of a significant number of abattoir serum samples (59%), without any clinical history of IBR. It appears, from the clinical experience of field veterinarians, that IBR infection, particularly the respiratory form, is often mild and may go unnoticed, particularly in the indigenous bovine population. However, clinical symptoms such as nasal and ocular discharge, rise in body temperature, moderate to severe cough, dyspnoea and dry cracked muzzle have been reported in crossbred cattle in Karnataka (8). An isolate of BHV-1 from one such outbreak was used as a source of virus antigen in this survey.

The present study has confirmed the widespread prevalence of IBR in the southern states of India and the association of the disease with respiratory and genital tract infections. In an organised dairy herd, 59/81 samples from crossbred heifers (72.8%) tested positive for the presence of BHV-1 antibodies following a respiratory infection and, in another herd, 31/56 samples from aborted crossbred cows (55.4%) were positive for BHV-1 but remained negative for brucellosis or leptospirosis. However, samples from these animals were not used to conduct virus isolation.

Interestingly, 114/120 samples from breeding bulls in Tamil Nadu (95%) and 41/99 samples from Karnataka (41.4%) were also seropositive. The transmission of BHV-1 from these bulls through semen used for artificial insemination is a distinct possibility. Contamination by BHV-1 and the spread of the disease through semen has been well documented (1). This situation assumes great importance in the field, as artificial insemination programmes to improve the indigenous cattle population are now
Conducted across the country. At present, there are no specific IBR control programmes in India, and vaccination is not practised. The impact of this disease on the development of the livestock industry needs to be reviewed, considering the vast bovine population in India, with frequent and unrestricted livestock migration and poor animal health conditions.

**CONCLUSIONS**

Serological evidence of IBR infection in 51.6% of 2,420 bovines was recorded by A-B ELISA in the three southern states of India. Evidence of infection of breeding bulls is significant, as infected semen could be a potential source for the rapid spread of this disease in India.

**ACKNOWLEDGEMENTS**

The authors thank Professor M.S. Shaila, Indian Institute of Science, Bangalore, for her help in the purification of virus antigen and Professor U. Khim, Institute for Virology and Immunoprophylaxis, Basle, Switzerland, for providing the commercial ELISA kit.

* * *

**PRÉVALENCE DE LA RHINOTRACHÉITE INFECTIEUSE BOVINE DANS LE SUD DE L’INDE.** – G.J. Renukaradhya, M. Rajasekhar et R. Raghavan.

Résumé : Les auteurs décrivent une enquête sérologique visant à déterminer la prévalence de la rhinotrachéite infectieuse bovine chez les bovins et les buffles (Bubalus bubalis) dans trois Etats méridionaux de l’Inde. Un isolat local de l’herpèsvirus 1 bovin (bovine herpesvirus 1 : BHV-1) provenant d’une forme respiratoire de la rhinotrachéite infectieuse bovine a été utilisé comme source d’antigène du virus dans l’épreuve immuno-enzymatique (enzyme-linked immunosorbent assay : ELISA) avidine-biotine. La prévalence globale des anticorps vis-à-vis du virus de la rhinotrachéite infectieuse bovine était de 50,9 % chez les bovins et de 52,5 % chez les buffles. Chez les taureaux reproducteurs, 114 prélèvements sur 120 provenant de Tamil Nadu (95 %) et 41 sur 99 provenant de Karnataka (41,4 %) possédaient des anticorps. La rhinotrachéite infectieuse bovine a été considérée comme une cause possible de l’avortement de 55,4 % (31/56) femelles de race croisée. Toutefois, le virus n’a pas été isolé chez ces animaux. Les auteurs mettent également l’accent sur l’importance économique de cette maladie pour le secteur de l’élevage qui connaît une rapide expansion en Inde.

Resumen: Los autores describen un estudio serológico sobre la prevalencia de la rinotraqueítis infecciosa bovina (infectious bovine rhinotrachitis: IBR) en el ganado bovino y los búfalos (Bubalus bubalis) de tres estados del sur de la India. Una cepa local de herpesvirus bovino 1 (bovine herpesvirus 1: BHV-1), aislada en el curso de un brote respiratorio de IBR, fue utilizada como fuente del antígeno vírico para la realización de un ensayo inmunosorbente asociado con enzimas (enzyme-linked immunosorbent assay, ELISA) con empleo del complejo avidina-biotina como amplificador. La prevalencia global de anticuerpos IBR en el ganado bovino fue de un 50,9%, mientras que en el búfalo era de un 52,5%. Entre los sementales, 114/120 muestras de Tamil Nadu (95%) y 41/99 muestras de Karnataka (41,4%) resultaron seropositivas. La posible relación de la IBR con los abortos bovinos se verificó en 31/56 de las muestras (un 55,4%) tomadas de hembras de raza cruzada que habían abortado. No obstante, no se realizó en estos animales el aislamiento del virus. Los autores subrayan asimismo la trascendencia económica de la IBR para la industria ganadera de la India, actualmente en pleno desarrollo.


REFERENCES


