Epidemiological Studies on Infectious Bovine Rhinotracheitis (IBR) in Different Parts of India

Kollannur, Justin Davis*, Syam, Radhika2 and Chauhan, R. S.3

Department of Veterinary Epidemiology and Preventive medicine, College of Veterinary and Animal Sciences, Thrissur (INDIA)

2Phd scholar, Bacteriology and Mycology Division, Indian Veterinary Research Institute, Izatnagar, UP
3Professor, Veterinary Pathology, G B Pant University of Agriculture and Technology, Pantnagar.
*Corresponding author: jestindavis@gmail.com

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Abstract
Bovine herpesvirus-1 (BHV-1), the causative agent of Infectious Bovine Rhinotracheitis (IBR), is considered to be the most common viral pathogen found in bovine semen and can cause various syndromes like infectious bovine rhinotracheitis (IBR), pustular vulvovaginitis (IPV), encephalomyelitis, mastitis and affects respiratory, ocular, reproductive, alimentary, integumentary and central nervous systems besides causing neonatal infections. The BHV-1 infection has already been reported by seroprevalence studies in India amongst exotic and crossbred cattle at some organized farms. The current work was designed to study the prevalence of IBR using serological and molecular techniques, respectively in serum and semen samples, collected from different parts of India. IBR antibodies were diagnosed from 1010 serum sample by serum neutralization test and noticed 32.25% seroprevalence with maximum prevalence in Uttar Pradesh and minimum in Himachal Pradesh. Four percentage of semen samples were found positive for BHV-1 virus.

Key words: Infectious Bovine Rhinotracheitis, Infectious Pustular Vulvovaginitis, Bovine Herpesvirus-1, Epidemiology

Introduction
By volume, India is the biggest milk producer of the world and the techniques like cryopreservation of semen and artificial insemination has assisted it to gain this status. Artificial insemination helped to use single ejaculate for inseminating more number of female animals while that of cryopreservation facilitated its wider distribution. However, it also resulted in a wider spread of some sexually transmitted infectious diseases to larger number of populations in a short span of time. To retain India’s status as biggest milk producer while improving the milk availability to its population, reproductive health maintenance of its livestock population is of paramount importance. Infectious Bovine Rhinotracheitis (IBR) is a disease which is transmitted through semen causing serious threat to reproductive health and productivity of Indian cattle.
IBR is caused by bovine herpesvirus-1 (BHV-1) which can also cause diseases like pustular vulvovaginitis (IPV), encephalomyelitis and mastitis. BHV-1 affects respiratory, ocular, reproductive, alimentary, integumentary and central nervous systems besides causing neonatal infections (Gibbs and Rweyemamu, 1977). BHV-1 is considered to be the most common viral pathogen found in bovine semen. Like other herpesviruses, BHV-1 can establish latent infection in animals which become reservoirs of the virus in the herd. After reactivation of latent infection, bulls can shed virus in semen. From epidemiological point of view, BHV-1 is perpetuated in nature by interplay of short cycle of infection, latency, resistance to environmental factors and recrudescence under various stress conditions. The incidence of BHV-1 infection has drawn considerable attention in developing countries owing to its effect on internal movement of livestock and germplasm (Gibbs, 1981).

The disease due to BHV-1 has been reported in India amongst exotic and crossbred cattle at some organized farms, as evidenced by the seroprevalence studies (Samal et al., 1978; Mehrotra et al., 1981). Investigations so far carried out indicate that it may be fairly widespread in the country and needs immediate attention of the veterinarians. Even though some data is available on seroprevalence, no useful work has been undertaken to analyze the presence of virus in semen samples in India. In this context, a study on the prevalence of infectious bovine rhinotracheitis (IBR) was done using serological and molecular techniques, respectively in serum and semen.

**Materials and Methods**

Serum samples from different parts of the country received by the General Bacteriology Laboratory (Center for Animal Disease Research and Diagnosis (CADRAD), Indian Veterinary Research Institute, Izatnagar, India), the sera from IVRI Dairy Farm and the state of Kerala were used for the analysis. In brief, blood was collected aseptically from the jugular vein of each animal in a vacuumtainer (AKÜRET Medikit, Belgium). The vacuumtainers were kept in an upright position at room temperature for about two hours. The blood samples were then centrifuged at 3000 rpm for 15 minutes. Separated sera were collected in screw capped plastic vials and transported to the laboratory. At the laboratory, sera were heat inactivated at 56°C for 30 min and subsequently stored at a temperature of -20°C until use. Collected serum samples were subjected to serum neutralization test for detection of BHV-1 antibodies.

IBR virus adapted in Maudin Derby bovine kidney (MDBK) cell line and maintained in virus laboratory, CADRAD, IVRI, Izatnagar was used for serum neutralization test. MDBK cell line was received from National Center for Cell Sciences (NCCS), Pune and maintained in virus laboratory, CADRAD, IVRI, Izatnagar. Dulbecco’s Modified Eagle Medium (Gibco, USA) supplemented with 10% fetal calf serum (FCS), 2mM L-glutamine, 100 IU benzyl penicillin / ml and 100 µg /ml Streptomycin Sulphate was used.
as growth medium. Media with 2% FCS were used as maintenance medium. Serum neutralization test was conducted as prescribed in OIE manual (Terrestrial Manual, 2008).

Semen samples collected from the Germplasm Center of IVRI and samples received from different parts of the country by CADRAD were subjected to molecular analysis for detection of viral genome. In brief, DNA from semen samples were extracted by phenol cholorform method with SDS and Proteinase K. Polymerase chain reaction was carried out in 200 µl capacity thin-walled PCR tubes to a final volume of 25 µl using specific upstream and downstream primers for gI glycoprotein (Rocha et al., 1998). The product was visualized by standard Agarose Gel electrophoresis by using 1% Agarose containing Ethidium Bromide (at the rate of 0.5µl per ml).

**Result and Discussion**

**Seroprevalence**

A total of 1010 serum samples were collected for screening of IBR from various parts of the country. Total number of 326 positive cases was discovered by SNT with a prevalence rate of 32.25%. Higher prevalence rate was noticed in Uttar Pradesh (55.42%) followed by Uttarakhand (40.71%). Least prevalence was noticed in Madhya Pradesh and Himachal Pradesh. The details of the samples with results are given below Table 1.

**Table 1- State wise prevalence of Infectious Bovine Rhinotracheitis by using Serum Neutralization test**

<table>
<thead>
<tr>
<th>States</th>
<th>Total</th>
<th>Positive</th>
<th>Sero-prevalence (%)</th>
<th>Prevalence with 95% CI</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chhattisgarh</td>
<td>99</td>
<td>34</td>
<td>34.34</td>
<td>24.99, 43.69</td>
<td>34.34</td>
</tr>
<tr>
<td>Himachal Pradesh</td>
<td>43</td>
<td>3</td>
<td>6.98</td>
<td>-0.64, 14.6</td>
<td>Himachal Pradesh</td>
</tr>
<tr>
<td>Kerala</td>
<td>200</td>
<td>45</td>
<td>22.5</td>
<td>16.71, 28.29</td>
<td>Kerala</td>
</tr>
<tr>
<td>Karnataka</td>
<td>130</td>
<td>26</td>
<td>20</td>
<td>13.12, 26.88</td>
<td>Karnataka</td>
</tr>
<tr>
<td>Maharashtra</td>
<td>315</td>
<td>115</td>
<td>36.51</td>
<td>31.19, 41.83</td>
<td>Maharashtra</td>
</tr>
<tr>
<td>Punjab</td>
<td>83</td>
<td>46</td>
<td>55.42</td>
<td>44.73, 66.11</td>
<td>Punjab</td>
</tr>
<tr>
<td>Uttar Pradesh</td>
<td>140</td>
<td>57</td>
<td>40.71</td>
<td>32.57, 48.85</td>
<td>Uttar Pradesh</td>
</tr>
<tr>
<td>Uttarakhand</td>
<td>1010</td>
<td>326</td>
<td>32.25</td>
<td>29.37, 35.13</td>
<td>Average</td>
</tr>
</tbody>
</table>

**Detection of Viral Genome In Semen Sample**

Out of 50 semen samples, 2 (4%) were found positive for BHV-1 genome by published sequence of gI glycoprotein gene region of BHV-1, which was predicted to produce a PCR product of size 468 base pairs (bp) (Figure 1).

**Discussion**

Bovine herpesvirus-1 (BHV-1), the causative agent of IBR, is considered as the most common viral
pathogen found in bovine semen (Kendrick et al., 1958; Miller et al., 1991). Latent virus may be established in the nerve ganglia of infected but clinically normal animals after primary infection despite the development of neutralizing antibody. Vaccines, although capable of preventing clinical disease, are unable to prevent the establishment of latency. BHV-1 can spread through artificial insemination (AI), causing a variety of genital tract disorders such as endometritis, infertility and abortion. Control by vaccination may, therefore, be contraindicated in bulls producing semen for export due to the risk of disease spread by latent virus. Moreover, bovine semen is stored and handled in conditions that are ideal for preserving the viral pathogen. Therefore, contaminated semen presents a potential threat to the cattle industry.

In this study, overall seropositivity of 32.25% was found by SNT from a total of 1010 sera samples from different parts of the country. It is similar to the result by Singh et al. (1986) with 32.34% seropositivity against IBR virus by applying SNT while 60.74% seropositivity was reported Singh et al. (1983) in buffaloes. More or less similar results were obtained by Manickam and Mohan (1987) and Singh et al. (1985), respectively by applying SNT. Higher seropositivity of 60.74%, 52.31% and 70.6% were recorded by using SNT, respectively by Singh et al. (1983), Babu et al. (1984) and Pandey et al. (2000). The present study has confirmed the widespread prevalence of IBR in different states of India and the

[Image: PCR amplification of g1 glycoprotein
Lane M- 100 bp DNA ladder
Lane 1- negative sample
Lane 2- Positive sample with 468 bp amplicon
Lane 3- Positive Control with 468 bp amplicon]
association of the disease with respiratory and genital tract infections

Table 2 - Comparison of previous prevalent studies of Infectious Bovine Rhinotracheitis with present work

<table>
<thead>
<tr>
<th>Previous Studies</th>
<th>Present Study</th>
<th>State</th>
</tr>
</thead>
<tbody>
<tr>
<td>56.5% (Samal et al., 1981)</td>
<td>55.42%</td>
<td>Uttar Pradesh</td>
</tr>
<tr>
<td>64.47% (Vaid et al., 1991)</td>
<td>6.97%</td>
<td>Himachal Pradesh</td>
</tr>
<tr>
<td>10.39% (Jain et al., 2006)</td>
<td>40.71%</td>
<td>Uttarakhand</td>
</tr>
<tr>
<td>22.22% (Sweta-Raghuvanshi et al., 2006)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29.78% (Gill et al., 1987)</td>
<td>36.50%</td>
<td>Punjab</td>
</tr>
<tr>
<td>34.16% (Dhand et al., 2002)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45.01% (Deka et al., 2005)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.88% (Rajesh et al., 2003)</td>
<td>22.5%</td>
<td>Kerala</td>
</tr>
<tr>
<td>49.86% (Sulochana et al., 1981)</td>
<td></td>
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</tr>
</tbody>
</table>

Out of 50 semen samples, only 2 were positive with a prevalence rate of 4%. The seropositivity of IBR was found to be 32.24% from a total of 1011 serum samples in this study. Previous reports (Samal, 1978; Sulochana et al., 1981; Singh et al., 1983; Singh et al., 1985; Tongaonkar et al., 1986; Manickam and Mohan, 1987; Pandita and Srivastva, 1993; Sarumathi et al., 2002; Rajesh et al., 2003; Aradhana et al., 2005; Jain et al., 2006; Raghuvanshi et al., 2006) stated a seropositivity between 14.88 to 79% in various Indian states. The prevalence in semen shows considerable reduction as far as seropositivity is concerned. This might be due to the previous exposure and possibly attributed to the fact that the herpesviruses establish life-long latent infection with periodic reactivation, and shedding of virus may be periodic or continuous. In general, BHV-1 is excreted in much higher concentration in the primary phase of the infection compared to the subsequent phases where shedding is often intermittent (Bitsch, 1973). Huck et al. (1971) reported that bulls start shedding BHV-1 from the prepuce between 2 and 7 days after primary intrapreputial infection and it lasted several weeks. After the primary phase of infection, virus can often no longer be isolated.

Thus the present study suggests that IBR is still a major cause of concern in India. However, the pattern of infection seems to be changing in different part of the country, it is imperative to take preventive measures to contain this disease in India.

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