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ISOLATION OF INFECTIOUS BOVINE RHINOTRACHEITIS (IBR) VIRUS FROM BREEDING BULL SEMEN OF NORTH INDIA

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ABSTRACT

The present investigation was conducted with the objective of isolating IBR virus from semen of breeding bulls of Gsushala and Farms of U.P.IBR virus was isolated from semen samples using MDBK Cell line. The positive samples showed characteristic CPE i.e. grape-like clustering 72 hours post- infection which were subsequently used for isolation of IBR virus as per the method described by O.I.E. Out of the 58 semen samples tested, only 1 sample (1.7 %) showed the characteristic CPE for the presence of IBR virus. The less number of IBR virus isolates from semen samples could be due to low shedding of virus in secretions, small sample size and better hygienic practices in Farms as compared to Gaushalas under study. The present study indicated that semen samples should be routinely tested for the presence of IBR virus before artificial insemination or natural service in order to prevent the economic losses in dairy herds due to this contagious disease.

Keywords: MDBK, CPE, IBR, Cell Line, Semen, Insemination

INTRODUCTION

Bovine herpesvirus-1 (BHV-1), a member of the Alphaherpesvirinae is the aetiological agent of Infectious Bovine Rhinotracheitis (IBR) and it is the most frequently occurring viral pathogen in semen of breeding bulls.BHV-1 is responsible for a variety of clinical conditions in cattle and buffaloes, including pustular vulvovaginitis, abortion, mastitis, balanoposthitis of bulls, infertility, tracheitis, conjunctivitis–

keratoconjunctivitis, encephalitis and fatal disease in newborn calves, and thus causes great economic losses to the livestock industry **[1, 2]**. In BHV-1 genital tract infections of bulls, the virus replicates in the mucosae of the prepuce, penis and possibly in the distant part of the urethra. There are chances of semen contamination during ejaculation by the virus that sheds from the infected mucosae. Insemination of cows with such contaminated semen reduces the conception rate and may cause endometritis, abortion and infertility **[3, 4]**. During the primary infection the virus is transported along the axons and becomes latent in the sacral ganglion where it persists for the life of the animal **[5],** and is reactivated under stress conditions, making the animal a carrier for life and a potential shedder of the virus. To prevent transmission of BHV-1 through semen, only BHV-1 free semen should be used **[6]**. Vaccines, although capable of preventing clinical disease, are unable to prevent the establishment of latency **[7]**.

Because, the bulls of Uttar Pradesh especially Goshalas of Vrindavan, Mathura have not been screened so far for the presence of IBR virus and considering the seriousness of the disease and its economic impact on a developing country like India, the present investigation was carried out to isolate the IBR virus from breeding bull semen of U.P. so that early treatment could be started to prevent its further spread.

MATERIALS AND METHODS

Location of Experiment

The experiment was performed under stringent aseptic conditions in virology laboratory, Department of Microbiology, Uttar Pradesh, Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura. Uttar Pradeshstate, North India is situated between 23°52´Nand 31°28'N latitude and 77°3´E and 84°39´E longitude. The climate of the state is of tropical monsoon type, but variations exist because of differences in altitudes.The average temperature varies in the plains from 3°C to 4°C in January to 43°C to 45°C in May and June

Collection of Semen Samples

58 semen samples were collected from breeding bulls of Goshalas and Farms of Uttar Pradesh **(Table 1).** Neat semen was collected in Macro centifuge tube containing Eagle minimum essential media (EMEM) with 2% foetal calf serum and antibiotics and transported to the laboratory in cool ice pack. Semen samples were then stored at–70°C until tested.

Cell Lines

Bovine Madin Darby Bovine Kidney (MDBK) cells obtained from CADRAD,

I.V.R.I., Bareilly, India supplemented with 5% FCS, were utilized for virus isolation.

Virus Isolation

All the semen samples were diluted 1:10 in growth medium, processed and inoculated separately onto the confluent MDBK cell monolayer, as per the method described by O.I.E. Tubes that did not show a cytopathic effect (CPE) by the third passage were discarded and treated as negative. Samples that produced CPE by the third passage were further passaged for virus propagation **[8]**.

RESULTS

The various results of IBR virus isolation are presented in **Table 1**. The table shows that out of the 58 semen samples tested, only 1sample (1.7 %) showed the characteristic CPE i.e., grape-like clustering 72 hours post- inoculation.In present investigation, only one IBR virus could be isolated from Pagal Baba Gaushala while no BHV-1 could be detected in semen samples from bulls of Farms. All the virus isolates were completely neutralised by BHV-1 antiserum and hence confirmed as BHV-1.

Table 1: Isolation of IBR Virus From Semen Samples of Bulls of North-West Uttar Pradesh

S. No.	Location of sample collection	No. Tested	Virus Isolated
1.	Pagal Baba Goshala, Vrindavan	1	0
2.	Maduri Kund Farm, DUVASU, Mathura	5	1
3.	Farm, DUVASU, Mathura	16	0
4.	State Livestock Cum Agricultural Farm Babugarh,Ghaziabad	24	0
5.	Chak Gazaria Farm, Lucknow	12	
	TOTAL	58	1

DISCUSSION

The semen samples underwent tremendous destruction of cells during the first passage of virus isolation, which could be due to the presence of lytic enzymes in the seminal fluids and this finding was in consensus with Anonymous **[8]**. The finding of CPE of BHV-1 i.e., the "bunch of grapes" appearance of the virus infected cells was similar to previous BHV-1 isolation studies **[9]**. The previous published reports **[10, 11]** also support our findings of low detection of BHV-1 in semen, viz., Chintu *et al*., **[10]** also could not isolate any IBR virus from 40 qPCR positive semen samples of breeding bull from Uttar Pradesh. Similarly, Basavegowdanadoddi *et al.,* **[11]** could find only0.8% IBR positive bulls in his virus isolation study out of the 523 semen samples collected from Tamil Nadu, Kerala, Karnataka, and Andhra Pradesh. However, in Punjab out of the 24 semen samples tested 11 showed CPE for IBR in virus isolation study **[12]**. The low detection of IBR virus from semen samples under study could be due to low shedding of virus in secretions and small sample size. Only one semen sample of Gaushala showed CPE for

IBR virus while no BHV-1 could be detected in semen of bulls of Farms under study. This could be partly due to the fact that animals of Gaushala are grazed in open fields and contamination of pasture is likely to occur which in turn could become the source of BHV-1 infection in bulls and partly due to better hygienic practices in Farms as compared to Gaushala under study.

CONCLUSION

TheBHV-1 could be detected by virus isolation and other methods like PCR, ELISA etc. but only the sero-negative status of bulls does not completely eliminate the risk of virus transmission through semen. The greatest problem of herpes viral infections is the carrier status they induce in the animals consequent to which the presence of antibodies in an animal may not indicate the clinical disease. Considering this major drawback in serum based tests, detection of virus or its antigen becomes mandatory to designate any animal as positive for BHV-1**[8]**.

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