

SEROPREVALENCE OF BOVINE HERPESVIRUS-1 AMONG CATTLE AND BUFFALOES IN PAKISTAN

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ABSTRACT

Seroprevalence and virus isolation studies were carried out to determine the role of Bovine herpesvirus-1 (BHV-1) in reproductive ailments of cattle and buffaloes. Serum samples collected from slaughter house Rawalpindi and Livestock Research Station (LRS) were subjected to the indirect immunofluorescence to detect the antibodies against BHV-1. Out of 268 serum samples from slaughter house, 58(21.64%) were seropositive for BHV-1 antibodies. Out of 185 samples from LRS, 53(28.65%) were positive for BHV-1 antibodies. The sample from aborted animals & their fetuses were also tested for the presence of IBRV. No virus could be detected from field cases of aborted fetuses both by cell culture inoculation and by direct fluorescent antibody test on the tissue samples. The study revealed the seroprevalence of Infectious bovine rhinotracheitis (IBR) in cattle & buffaloes in Pakistan particularly in animals with reproductive disorders.

INTRODUCTION

The herpesvirus infections in domestic livestock and exotic ruminants are regarded as some of the most costly forms of viral infections in terms of producing disease and requiring efforts towards prevention and control (Everman and Henry, 1989). Bovine herpesvirus infections are particularly of considerable economic importance. The most significant of these infections are those associated with bovine herpesvirus-1 (BHV-1) which is one of the members of bovine herpesvirus group.

Bovine herpesvirus-1 group comprises of at least two major subtypes i.e., infectious bronchitis virus (IBRV) and infectious pustularvulvo-vaginitis virus (IPV). The respiratory form IBR, first reported in the USA in 1955 seems to be of comparatively greater significance as far as economic losses linked to the infection are concerned. However, the reproductive form of the disease is also known to cause many problems related to reproductive health of the animal. Low sporadic to enzootic disease occurrence is reported from many countries and clinical reports indicating infections with IBRV has also appeared in records at various veterinary hospital in this country. However, its presence through seroconversion or virus isolation has not been documented in this country. This study was designed to record seroprevalence of BHV-1 and/or isolate the pathogen from field to

generate information regarding rate of viral presence and its relationship with the reproductive disorders in cattle.

MATERIALS AND METHODS

Virus Cell Culture

Reference BHV-1 strain 50265 received from Institute of Animal Science and Health, Lelystad, Netherlands was propagated and maintained in MDBK cells. For virus propagation and indirect fluorescent antibody (IFA) test MDBK cells were used. However, for the isolation of virus from field cases primary foetal bovine kidney (FBK) cells were used. The cells were propagated in Eagle's Modified Minimal Essential Medium (MEM). The medium was supplemented with 10% foetal calf serum, 1% non-essential amino acids, 0.6% L-glutamine and antibiotics (Freshney, 1980).

Quantification Of Stock Virus

To determine viral concentration of the stock virus used for IFA, the method of calculating plaque forming units/ml was employed, using MDBK cell monolayer in 12-well tissue culture plates. For this purpose, MDBK cells with a concentration of 4×10^6 cells / well were used. Here tenfold serial dilutions of the reference virus were prepared in PBS. From each dilution 100 μ l was dispensed in quadruplicate

monolayered wells. A set of four wells was left uninfected as control. Virus was allowed to adsorb for one hour at 37° C after which the inoculum was discarded and agar overlay was poured on to it (Lennette and Schmidt, 1979). The plate was incubated in the CO₂ incubator at 37° C upto 5 days. The plaques were observed and titer of the virus was determined (Reed and Muench, 1938).

Serum Sample Collection

For this purpose blood was collected from 268 cattle at Sehala Slaughter House, Rawalpindi and from 185 animals at Livestock Research Station (LRS), of the National Agricultural Research centre, Islamabad. Out of 268 animals selected from the Sehala Slaughter House, 125 were more than 2 years of age, whereas 143 animals aged less than 2 years. Out of 185 animals selected at LRS, 110 had a history of some reproductive disorder (identified as problem animals) whereas 75 animals were considered normal and healthy. The animals were bled, serum was collected and stored at -20°C till tested.

Hyperimmune Serum

Hyperimmune serum against BHV-1 was raised in New Zealand white rabbits which were kept during experiment in metallic cages at a room temperature of 25°C (± 1°C). The virus was first inactivated with formaline and then emulsified with Freund's complete adjuvant and injected to each rabbit. A booster using incomplete Freund's Adjuvant in the antigen was injected 15 days later. The animals were bled and serum was collected and stored at -20° C till further use (Harlow and David, 1988).

Virus Isolation

Uterine swabs were collected from freshly aborted animals in viral transport medium. The fluid was centrifuged at 1500 rpm (8°C) for 15 minutes. Supernatant was aspirated and saved for inoculation onto the cells.

Liver, spleen, cotyledons and lungs from aborted foetuses were also collected. Sections sizing 4 microns were prepared from these organs using cryostat microtome (Miles. USA). The sections were mounted on Poly-L Lysine coated slides. The slides were fixed in acetone and saved at -20°C till used for DFA. A part of frozen sample was triturated in PBS to get a final 10% preparation and centrifuged at 1500 rpm for 10 minutes. The supernatant was inoculated in 100 µL quantity onto monolayer of FBK cells in multiwell plate. The plate was examined for CPE. At day 6 post inoculation the cells were scrapped, transferred on to

glass slides, fixed in cold acetone and processed for DFA.

Fresh semen samples were collected from the bulls. The samples were diluted with sterile PBS and centrifuged. Supernatant was aspirated and subsequently inoculated onto FBK cells in multi-well plate and processed as discussed above.

Direct Fluorescent Assay (DFA)

The cells scrapped from inoculated cells culture were placed on a multiwell Teflon-coated slide, it was air dried and fixed in chilled acetone for 5 minutes. Anti BHV-1 conjugate (1:80) was placed onto the slide and was allowed to react for 30 minutes in a humid atmosphere at 37°C. The slides were then washed three times with PBS (pH 8.5), and then dipped in 0.1% Evan's Blue solution for 30 seconds. Slide was again washed with distilled water as above and air dried. Mounting medium was placed onto the slide and examined under fluorescent microscope for the presence of intranuclear inclusions in the infected cells. The above mentioned tissues collected from the aborted foetuses were also subjected to the IFA following the above described procedures.

Indirect Fluorescent Antibody Assay (IFA)

In order to test the serum samples for the detection of BHV-1 antibodies, IFA was conducted. For this purpose MDBK cells were trypsinized and infected with BHV-1 with a multiplicity of infection (moi) of 0.1. The cells were grown in 12 well Teflon-coated slides and probed using the following protocol.

Medium in the multiwell slides was discarded and the cells were fixed with acetone. Anti-BHV-1 serum diluted to 1:20 was placed in each well and slides were incubated in humid chamber at 37°C for 30 minutes. The wells were washed thrice with PBS (pH 7.5) and air dried. Now FITC labeled antibody conjugate was diluted to 1:1000 and placed in each well. The slides were again incubated and washed as described above. The slides were gently washed with distilled water and air dried. Mounting medium and cover slip were placed and the slides were examined under fluorescent microscope. The fluorescence was observed in intranuclear area of the infected cells in positive samples.

RESULTS

Table 1 shows 58 positive samples for BHV-1 antibodies from 268 samples of Slaughter House animals tested by IFA. In this group among 143 animals below 2 years of age 21 animals (14.6%) were

found positive whereas among 125 animals of more than two years of age, 37 animals (29.6%) were found positive.

At LRS, out of 110 problem animals tested, 43 animals (39%) were seropositive for BHV-1 while among normal animals, 10 out of 75 animals (13.3%) were found positive (Table-2).

The organ samples from aborted fetuses and semen & uterine discharge from the animals suffering from reproductive disorders examined for the isolation of BHV-1 did not show viral presence after three blind passages of the cell culture (Table-3). The cell culture inoculated for virus isolation when tested by IFA were also found negative. The organ samples of aborted fetuses when tested by DFA were also found negative for BHV-1.

Table 1: Seroprevalence of BHV-1 in cattle (slaughter house samples)

Category of samples	Positive cases/ Total examined	Percent
Animals more than 2 years of age	37/125	29.60
Animals less than 2 years of age	21/143	14.60

Table 2: Seroprevalence of BHV-1 in cattle (LRS samples)

Category of samples	Positive cases/ Total examined	Percent
Animals with reproductive diseases	43/110	39.00
Normal Animals	10/75	13.30

Table 3: Isolation of BHV-1 from animal samples by cell culture inoculation

Kind of Specimen	Number	Virus isolation on FBK cells
Aborted foetus	2	All negative
Semen samples	10	All negative
Vaginal swabs	6	All negative

DISCUSSION

Bovine herpes virus-1 (BHV-1) is responsible for many clinical conditions in cattle, sometimes producing distressing and life threatening symptoms, but its significance has been recognized mostly due to its great economic importance. Loss of animals, abortion, decreased milk production and emaciation are the IBR induced conditions which may cause heavy economic losses to the farmers. Due to the lack of

viral diagnostic facilities in this country, no information was earlier available regarding incidence of viral infectious rhinotracheitis in cattle. In the present study, a high percentage of dairy animals have been found positive against IBRV infection. The organism has been reported on many occasions to cause infection in dairy animals in other countries (Rosadio *et al.*, 1993).

The reported seroprevalence of BHV-1 in the animals of more than two years of age, was twice as compared to that in the animals aging less than two years (29.6% and 14.6%, respectively). The higher incidence in animals aging more than two years is an indication that the virus may be responsible for reproductive disorders due to the stage of reproductive maturity of these animals. Some previous studies conducted by Suresh *et al.* (1992) also indicated the highest seroprevalence of BHV-1 in female buffaloes aging more than 3 years.

The high incidence of IBR virus antibodies reflected the prevalence of the virus in local cattle population in Pakistan. As there is no serologic difference in the presence of respiratory and reproductive strains of IBRV, it may not be possible to confirm the role of IBRV in reproductive ailments until the virus is isolated from the relevant organs among sick animals.

The virus isolation was also attempted in the organs from aborted animals and in semen samples of suspected bulls. Although BHV-1 is known to shed in reproductive secretions of infected cattle particularly in acute stage of the disease, yet no virus could be isolated from uterine discharges of aborted cows or from semen samples of bulls. This could be due to the reason that abortion in these two animals might have occurred a few days late after the foetal death because in the period between death of the foetus and abortion, the levels of virus shedding decreases and in some instances virus cannot be isolated from the aborted foetus (Kendrick, 1973). As far as viral presence in semen is concerned, this can be only seen if viremia prevails at the time of sample collection. As the virus becomes latent in the animals body so during latency it is not detectable by conventional virological procedures. Attempts, therefore, need to be made to induce immunosuppression by corticosteroid therapy to get virus shedding induced experimentally. However, there is a need to employ more sensitive techniques of virus detection to determine viral presence in the field samples. Further more an intensive effort in terms of testing more samples for virus isolation need to be attempted.

Transmission of BHV-1 infection from one animal to another can occur mainly by two ways either through direct physical contact, or through indirect semen transfer (coitus/artificial insemination). Owing to the introduction of intensive farming at government institutes and wide spread use of artificial insemination in this country, it appears that spread of IBRV may increase in Pakistan. It would be, therefore, necessary to plan control measures against IBR at this stage through detailed investigation regarding the presence of IBRV in bulls used for producing semen for AI.

A countrywide IBR eradication if not impossible, at least is much difficult mainly due to costly implementation of control program and potential for reactivation of latent infections, but there is one example of Switzerland where inspite of all the difficulties, BHV-1 as an infectious agent is practically eliminated. In Pakistan, keeping in view the small size of herd with most of the farmers and the absence of any compensation scheme by the government, it would be more appropriate to introduce mass vaccination at this stage. However, before selecting any specific vaccine, it would be necessary to undertake more work on the characterization of IBRV and its comparison with other isolates prevalent in other countries.

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