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Detection of Bovine Herpesvirus-1 Infection in Bovine Clinical Samples by Direct Fluorescent Antibody Test

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A B S T R A C T

Detection of viral antigen by direct fluorescent antibody test (FAT) from clinical samples collected from cattle and buffaloes of Navsari, Surat and Valsad Districts of Southern Gujarat were carried out. Out of a total of 116 clinical samples (44-cattle, 72-buffaloes) tested, fluorescence was observed in 14 (12.09%) samples. Species-wise, eight cattle (11.11%) and 6 buffaloes (13.64%) were found to be positive by the test. The highest number of animals were detected from Surat (14.29%) followed by Valsad (12.07%) and least in Navsari (10.81%) district. Viral antigen detected in 8.00% (4/50), 7.14% (1/14), 28.57% (2/7) in cattle and 16.67%, (2/12), 16.67% (1/6), 11.547% (3/26) in buffaloes from nasal, conjunctival and vaginal swabs, respectively. Only placental cotyledon sample collected from cattle showed positive reaction by direct FAT.

Introduction

Bovine herpesvirus-1 (BoHV-1), a member of the subfamily *Alphaherpesvirinae*, is responsible for causing rhinotracheitis, pustular vulvovaginitis, abortion, mastitis, balanoposthitis, infertility, keratoconjunctivitis and encephalitis in bovines (Gibbs and Rweyemamu, 1977). In India, the infection was first reported by Mehrotra *et al.,* (1976) and since then the disease has been prevalent and reported by many workers from various parts of the country (Samal *et al.,* 1981; Renukaradhya *et al.,* 1996; Trangadia *et al.,* 2012; Krishnamoorthy *et al.*, 2015). Currently, virus isolation, ELISA, PCR etc. are used for the diagnosis of BoHV-1. The present study was designed to detect BoHV-1 virus in various clinical samples collected from bovine of Southern Gujarat by direct fluorescence antibody test (FAT).

Materials and Methods

Collection of clinical samples

A total of 116 clinical samples comprised of 72 samples (50 nasal swabs, 14 conjunctiva swabs, 07 vaginal swabs and one placental cotyledon from aborted case) from cattle and 44 from buffaloes (12 nasal swabs, 06 conjunctiva swabs and 26 vaginal swabs) were

collected from Valsad, Navsari and Surat districts of Southern Gujarat for detection of BoHV-1 antigen by direct FAT. These samples were collected in viral transport media (VTM) made from Dulbecco's modified Eagles media (DMEM) with 2% foetal calf serum (FCS) with kanamycin from animals exhibiting clinical signs such as conjunctivitis, lacrimation, serous/ mucopurulant nasal discharge, abortion and vulvo-vaginitis. Location-wise, species-wise and breed-wise details of sample collection are shown in Table 1.

Direct FAT

For standardization of test, infectious bovine rhinotracheitis (IBR) seed virus was procured from Veterinary Type Culture Collection (VTCC), National Research Centre on Equines (NRCE), Hisar (Haryana) under Material Transfer Agreement (MTA) and used as reference virus. Reference virus (100µl) was inoculated in 25ml tissue culture flask containing MDBK (Madin Derby Bovine Kidney) cell monolayer. The flask incubated at 37ºC for 1 hour and 20ml of DMEM medium was added to it. Further the flask was incubated at 37ºC for 24-36 hours till 50% CPE (Cytopathic Effect) was observed. Then DMEM medium was discarded and MDBK cell monolayer was fixed in acetone-methanol (3:1) solution and further processed as per the test protocol and reagents supplied by VMRD Inc., Pullman, WA, USA. In brief, smears were prepared from 50µl of clinical samples on clean glass slide. These smears were air dried overnight at room temperature and fixed in acetone-methanol (3:1) at room temperature for 20 minutes. Then the slides were stained with 50-75µl of direct FA conjugate (Catalog no.: 210-69-IBR) for 30 min at 37ºC in humid chamber. Further, the slides were gently rinsed in FA rinse buffer and soaked for 10 min in the same buffer. The slides were dried with paper towel without allowing stained surface to dry. Slides were mounted with FA Mounting Fluid and scanned with fluorescent microscope. Slides showed green fluorescence were considered as positive (Fig. 1c) While Figure 1a and 1b indicate the negative and positive control, respectively.

Results and Discussion

Location-wise, species-wise and breed-wise incidence of BoHV-1 infection is depicted in Table 1. The highest incidence recorded in Surat (14.29%) followed by Valsad (12.07%) and Navsari (10.81%) with an overall incidence of 12.09%. Percent positivity in cattle and buffaloes were 11.11% and 13.64%, respectively. Comparatively, higher incidence of BoHV-1 was observed in crossbred cattle (11.54%) than Gir animals (10.00%), whereas among buffaloes highest incidence observed in Mehsani (16.67%) followed by Surti (14.29%) and Jafarabadi (9.09%) breed. Sample-wise incidence of BoHV-1 infection is depicted in Table 2.

Earlier workers (Rissi and Barros; 2013; Gould *et al.,* 2013) used FAT as a diagnostic tool for detection of BoHV-1 viral antigen. In Gujarat, Jain (2006) and Jain *et al.,* (2008) supported our findings and detected BoHV-1 antigen in 32.67% of semen samples of cattle and buffaloes by FAT. Further Jain *et al.,* (2008b) recorded highest incidence of BoHV-1 by direct FAT in Mehsani buffalo bulls (36.84%) followed by Jafrabadi buffalo bulls (28.57%) and Surti buffalo bulls (14.28%).

In Odisha, Mishra *et al.,* (1982) found an incidence of 18% by immunofluorescence technique. Misra and Mishra (1987) cultured 15 various tissues samples in primary bovine kidney cells and isolated BoHV-1 from 5 samples and confirmed by direct FAT. However, Elazhary *et al.,* (1980) demonstrated BHV-1 in sperm heads from the bull by direct FAT.

Table.1 Incidence of BoHV-1 antigen in bovine by direct FAT

Attributes	Number tested	Number positive	Percent positive
A) Location-wise			
Valsad	58	07	12.07
Navsari	37	04	10.81
Surat	21	03	14.29
Total	**116**	**14**	**12.09**
B) Species-wise			
Cattle	72	08	11.11
Buffalo	44	06	13.64
Total	**116**	**14**	**12.09**
C) Breed-wise (Cattle)			
Crossbred	52	06	11.54
Gir	20	02	10.00
Total	72	08	11.11
D) Breed-wise (Buffalo)			
Surti	21	03	14.29
Mehsani	12	02	16.67
Jafarabadi	11	01	9.09
Total	**44**	**06**	**13.64**

Table.2 Sample-wise detection of BHV-1 antigen by direct FAT

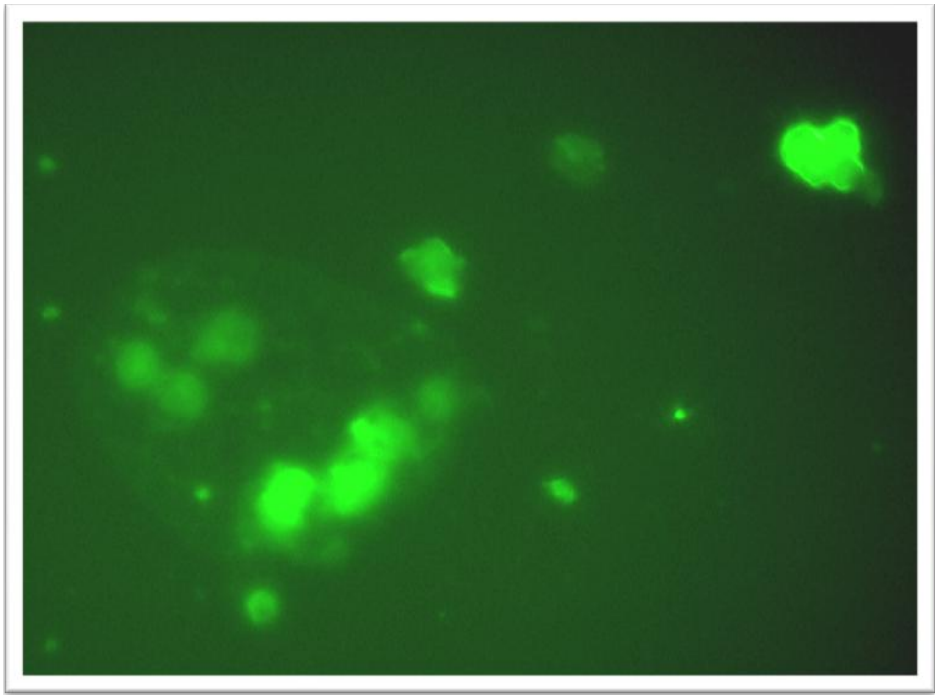
Sr. No.	Type of sample	Species of the animals				Total	
		Cattle		Buffaloes			
		Tested	Positive	Tested	Positive	Tested	Positive
01	Nasal swabs	50	04 (8.00)	12	02 (16.67)	62	06 (9.68)
02	Conjunctival swabs	14	01 (7.14)	06	01(16.67)	20	02(10.00)
03	Vaginal swab	07	02 (28.57)	26	03 (11.54)	33	05 (15.15)
04	Placental cotyledons	01	01(100)	-	-	01	01(100)
Total		**72**	**08 (11.11)**	**44**	**06 (13.64)**	**116**	**14 (12.09)**

Note: Figures in parentheses indicate percentage.

Fig.1a Normal uninfected MDBK cell monolayer as negative control at 36 hrs stained with direct FA conjugate viewed under fluorescent light microscope (100X)

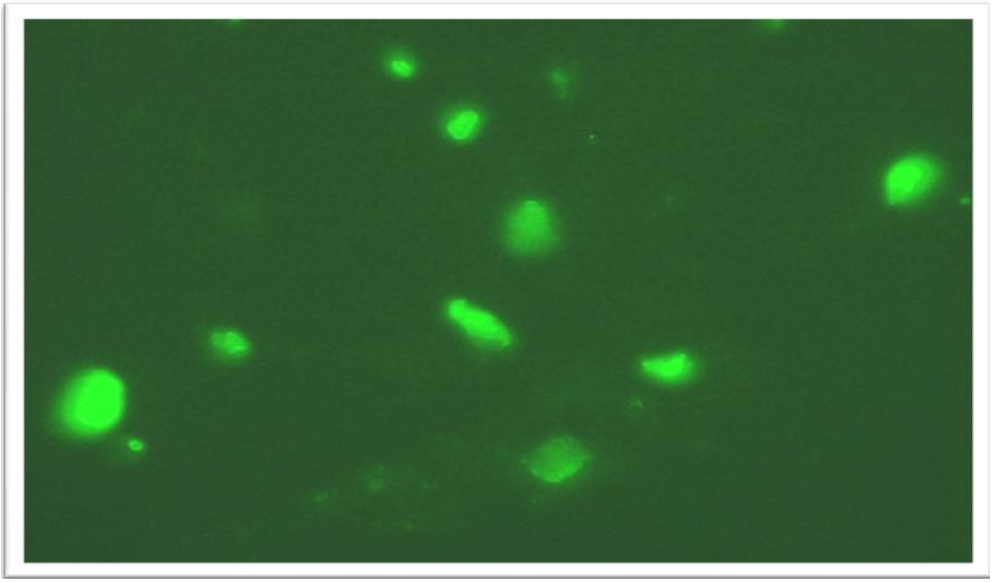
Note: The absence of fluorescence

Fig.1b BoHV-1 reference virus infected MDBK cell monolayer as positive control after 36 hrs stained with direct FA conjugate viewed under fluorescent light microscope (400X)



Note: The presence of fluorescence at the place previously CPE observed

Fig.1c Immunofluorescent staining reaction with free BoHV-1 antigen cluster and direct FA conjugate in smear prepared from clinical sample



Note: The brightly fluorescing cluster of BoHV-1 (400X)

Due to the lack of published literature on detection of BHV-1 infection in semen samples using direct FAT, it is difficult to compare this study and to reach meaningful conclusions. However, it is deduced that the direct FAT can be a valuable test for detection of BoHV-1 antigen within a short period of time. Considering the fact that BoHV-1 can be transmitted through various routes, the findings of this study should be taken as an indicator of evidence of infection in the South Gujarat region of Gujarat. It warrants large scale systemic surveillance using appropriate sampling techniques to assist in planning of state level disease control programme.

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