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A Field Isolate of Bovine Hepesvirus-1 with Different Behavior on Cell Culture

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Abstract: Several field viral isolates (seven isolates) from cattle with respiratory clinical sings of IBR were cultured on bovine kidney cell line (IRKHBK, NCBI Code: C541). After observation CPEs on cell culture the cultures were frozen and thawed, clarified by centrifugation and the supernatants were utilized to confirmatory tests for BoHV-1 incuding Virus Neutralization Test, Dot-Blot enzyme immunoassay and PCR. Timing and shape of CPEs were followed. Pure units of each isolate were selected by Plague Forming Unit (PFU) for three consequent times. Several isolates were inoculated by different Multiplicity of Infection (MOI) in to IRKHBK cell line twenty once and the timing and shape of CPE were followed by inverted microscope. Supernatants of each pure isolate after freeze and thaw and centrifugation process (like the first stage) were utilized to confirmatory tests for BoHV-1 incuding Virus Neutralization Test, Dot-Blot enzyme immunoassay and PCR. Regarding the results two different types of CPE of BHV-1 isolates were observed on monolayer cell culture by invert microscope; one as common CPE of BHV-1, another with an unusual behavior, however, results of VNT, Dot-ELA and PCR indicated all isolates were BHV-1. To sum up, it can be said that this special isolate of BHV-1 had different behavior on its sensitive cell culture (IRKHBK, NCBI Code: C541). Discussing about main reason of that change and probably relationships between this change and pathogencity needs more research.

Key words: Bovine Herpesvirus-1(BHV-1) · IRKHBK cell line · Flame shape · Cytopathic effect · PCR · VNT

INTRODUCTION

Infectious Bovine Rhinotracheitis (IBR) is a widespread viral disease in cattle and can cause serious economic losses in dairy herds. The virus responsible, bovine herpesvirus type 1 (BoHV-1), is an alphaherpes virus and the cause of the respiratory disease, abortion, conjectivitis and other clinical forms of disease complex. Genetic analyses of various clinical isolates have found at least three distinct BoHV-1 subtype: a respiratory subtype, a genital subtype and an encephalitic subtype designated as BoHV-1.1, BoHV-1.2 and BoHV-1.3, respectively [1-3].

The growth of viruses in cell culture is often (but not always) associated with microscopically visible morphologic changes in the infected cells, referred to as cytopathic effect (CPE). The features of the CPE may allow recognition of the infecting virus. Important characteristics of CPE include (a) which cell culture types are affected, (b) the timing and rate of progression, (c) the distribution (focal, diffuse, limited to the margins of the cell culture) and (d) the nature of the morphologic changes [1].

For virus isolation, bovine cells of various origins can be used. Primary or secondary bovine kidney, lung or testis cells, cell strains derived from bovine fetal lung, turbinate or trachea and established cell lines, such as the Madin-Darby bovine kidney cell line (MDBK), are suitable for BoHV-1 propagation. CPE of BoHV-1 usually appears within 2-4 days after inoculation. It is characterized by grapelike clusters of rounded cells gathered around a hole in the monolayer; sometimes giant cells with several nuclei may be observed [4]. Infection of permissive cells or calves with BoHV-1 leads to rapid cell death, in part due to apoptosis [5-7]. The timing, rate of progression and shape of CPE are most important characteristics of virus.

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The objective of this study was to report a field Isolated of Bovine Hepesvirus-1 with different behavior on cell culture.

MATERIALS AND METHODS

Several field viral isolates (seven isolates) from cattle with respiratory clinical sings of IBR were cultured on bovine kidney cell line (IRKHBK, NCBI Code: C541). After observation CPEs on cell culture the cultures are frozen and thawed, clarified by centrifugation and the supernatants were utilized to confirmatory tests for BoHV-1incuding Virus Neutralization Test, Dot-Blot enzyme zimmunoassay and PCR. Timing and shape of CPEs were followed. Pure units of each isolate were selected by Plague Forming Unit (PFU) for three consequent times [8]. Several pure isolates were inoculated by different MOI (Multiplicity of Infection) in to IRKHBK cell line and the timing and shape of CPE were followed by inverted microscope. Appearances of CPEs in different flask were observed in macroscopic manner after Gimsa staining. Supernatants of each pure isolate after freeze and thaw and centrifugation process (like the first stage) were utilized to confirmatory tests for BoHV-1incuding Virus Neutralization Test, Dot-Blot enzyme immunoassay and PCR.

Virus Culture: The isolates of BoHV-1 were inoculated by different MOI (0.01-0.1) into the IRKHBK cell line in Dulbecco's modified Eagle's medium containing 3% Fetal Bovine Serum (GIBCO, Australia, Ref: 10099-141).

PCR: Briefly, DNA of virus was extracts by Invitek Extraction of DNA. Next a part of gI of BHV-1 was amplificated by Sina Gen PCR Master Kit. The production of PCR was appeared by irritating of X ray on Agarose gel.

VNT: This test was done according to OIE procedure, briefly, to identify the recovered virus as BoHV-1, the supernatant of the culture were neutralized with a BoHV-1 antiserum or. For this purpose, serial tenfold dilutions of the test supernatant are made and to each dilution BoHV-1 antiserum or negative control serum is added. Following incubation at 37°C for 1 hour, the mixtures were inoculated into cell cultures; 3-5 days later, the neutralization index was calculated. The neutralization index was the virus titer (in log10) in the presence of negative control serum minus the virus titer in the presence of specific antiserum. If the neutralization index was greater than 1.5, the isolate likely considered being BoHV-1. To shorten the virus isolation procedure, two specimens may be inoculated into cell culture: one that had been preincubated with antiserum and another that had been preincubated with negative control serum. If the CPE was inhibited by the monospecific antiserum, the isolate could be considered to be BoHV-1 [4, 8].

Dot-Blot ELA Test: Viral antigen prepared as described above was spotted on to1×1cm nitrocellulose (NC) membranes at 20 µL per spot and then allowed to air dry. Each NC membrane was transferred into a well of a 6 well fill at bottom, cell culture plate (utilized and washed) and incubated in blocking solution (1% non fat dry milk in TBS) for 1 h at room temperature with occasional hand shaking. The membranes were then washed for 3×5 min in TTBS, overlaid with 1/40 dilution of monoclonal anti IBR serum and incubated for 1 h at 37 C and washed 3×5 min in TTBS. All the membranes were overlaid with 1/1000 dilution of HRP conjugated (Dako, Denmark) incubated and washed as described before and developed using 4-chloronaphtole (Merk, Germany) as substrate. The color reaction was allowed to proceed for 10 min and then stopped with several washes with distilled water. The membranes were air dried in the dark before being read [9-11].

RESULT

Results of VNT (VN Index = 3.1>1.5), Dot-Blot enzyme immunoassay test (Fig. 1) and PCR (Fig. 2) approved that all isolates were BHV-1.

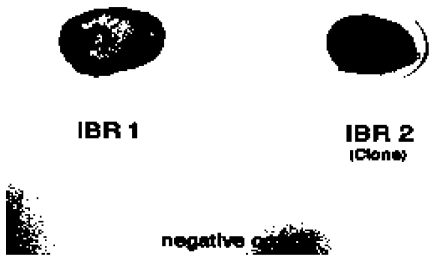
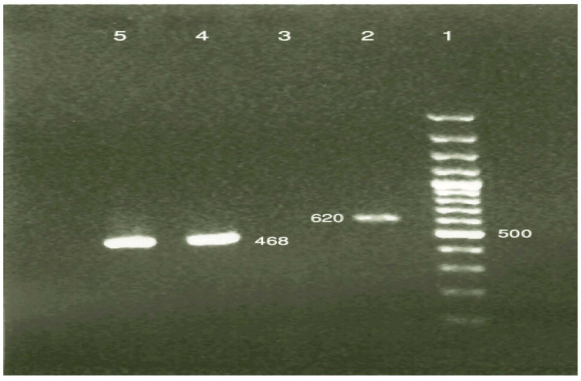


Fig.1: Dot-Blot ELA of two different Isolates of IBR (IBR1: isolate with flam shape CPE, IBR2: isolate with common CPE)



1. DNA Ladder
2. Positive control of kit
3. Negative control
4. Isolate 2(with common CPE)
5. Isolate 1(with flame shape CPE)

Figure 2: PCR of two different Isolates of IBR.

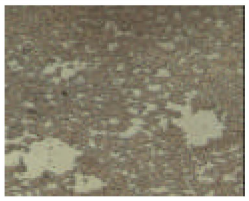
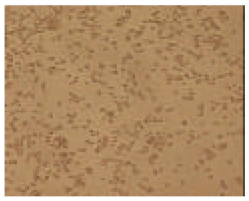
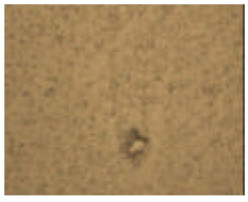
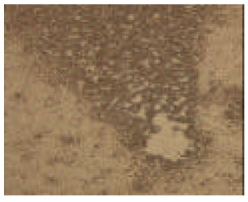
CPEs of all isolates (MOI=0.01) were started 24h after inoculation and a common CPE of IBR were observed within 48-72h after inoculation apart from one isolate (Table1). CPEs of pure isolates of that different isolate were started after 24 hours and a particular behavior was observed after 72 hours. Those CPEs were flame shape (microscopic and macroscopic views) and all of them were similar and they were progressed on same side in different part of cell culture flask (Fig 3); whereas, pure isolates of other have a common behavior, as a normal CPE of BHV-1, progressed in all sides on cell culture (Table 1).

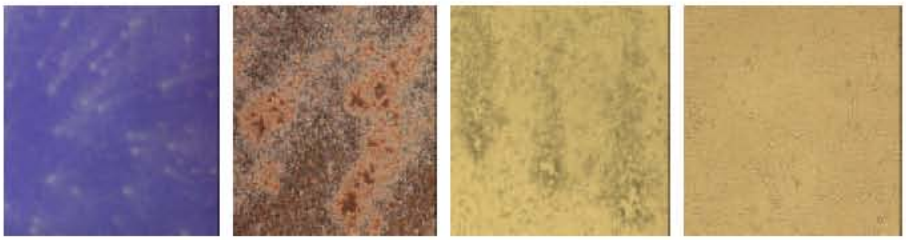
DISCUSSION

Herpesviruses enter cells by fusing with the cell plasma membrane in a complex process of attachment and penetration. Virus entry requires the presence of complementary binding partners on the virus and on the host cell. Studies show that the BoHV-1 glycoproteins gB, gC, gD, gE, gH, gK and gL are required for virus entry [12-15]. BoHV-1.1 and BoHV-1.2 subtypes differ in gC epitopes, which may alter viral attachment and account for subtype differences of viral virulence [16]. Although the host cell proteins required for BoHV-1 entry are not fully understood, the virus initially binds to cell surface heparan sulphate [17] via BoHV-1 gB and gC. After this initial binding, BoHV-1 gB and gD then bind further cell surface receptors (that have yet to be identified) with high affinity [14].

After entry into the host cell, BoHV-1 is transported along microtubules to the nucleus for replication using host cell proteins. The virus becomes enveloped as it buds through the nuclear envelope and is then transported within intracellular vesicles to the cytoplasmic membrane and released from the cell [1]. BoHV-1 replication starts within two hours of infection in cattle with cell surface antigen expression within 3-4 hours after infection and viral release and spread starting at 8 hours after infection [18]. CPE of BHV-1 usually appears within 2-4 days after inoculation. It is characterized by grapelike clusters of rounded cells gathered around a hole in the monolayer; sometimes giant cells with several nuclei may be observed.

Table1: Timing, rate of progression and shape of 2 different Isolates of BHV-1

	24h	48h	72h
Isolate2 (common CPE) MOI=0.01			
Isolate1 (Flame shape CP E) MOI=0.01			



A B C D

Fig. 3: A; Macroscopic view (Gimsa stained) of flame shape CPE of Isolate 1(72h), B; Microscopic view of flame shape CPE of Isolate 1(72h), C; Microscopic view of flame shape CPE of Isolate 1(72h), D; Microscopic view of IRKHBK cell line(72h)

Several Investigations on Infectious Bovine Rhinotracheitis indicate CPE changes began to appear at the fourth passage on the second day post inoculation. [4].

Regarding the result of present research, as can clearly seen, two different types of CPE of BHV-1 isolates were observed on monolayer cell culture by invert microscope; One as common CPE of BHV-1, another with an unusual behavior, however, results of VNT, Dot-ELA and PCR indicated all isolates were BHV-1. Some little changes in whether virus entry mechanisms or viral release process, likely, cause this particular behavior. Molecular researches in both entrance and release mechanisms of this different isolate of BHV-1 perhaps help us to figure out the main reason of this change.

To sum up, it can be said that this special isolate of BHV-1 had different behavior on its sensitive cell culture (IRKHBK, NCBI Code: C541). Discussing about main reason of that and probably relationships between this change and pathogen city and other it's subsequent need more research.

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Conflict of Interest Statement: Authors have agreed that there is no conflict of interest between them.

REFERENCES

1. Karron, R.A., P.L. Collins, D.M. Knipe and P.M. Howley, 2007. Fields virology. 10th edition, Lippincott Williams and Wilkins, Philadelphia.
2. Radostits, O.M., Gay-CC, K.W. Hinchcliff and P.D. Constale, 2008. Veterinary medicine, A text book of the diseases of cattle, sheep, pigs, goats and horses. Saunders, London.
3. Smith, B.P., 2009. large animal internal medicine. Mosby, USA.
4. OIE manual, 2010. chapter 2.3.5 Infectious bovine rhinotracheitis/Infectious pustular vulvovaginitis.
5. Devireddy, L.R. and C.J. Jones, 1999. Activation of caspases and p53 by bovine herpesvirus 1 infection results in programmed cell death and efficient virus release. J. Virol., 73: 3778-3788.
6. Jones, C. and S. Chowdhury, 2007. A review of the biology of bovine herpesvirus type 1 (BHV-1). Anim. Health Res. Rev., 8: 187-205.
7. Winkler, M.T., A. Doster and C. Jones, 1999. Bovine herpesvirus 1 can infect CD4 T lymphocytes and induce programmed cell death during acute infection of cattle. J. Virol., 73: 8657-8668.
8. Cann, A.J., 1999. Virus culture a practical approach, Oxford University Press.
9. Bollage, D.M. and S.J. Edelstein, 1991. Protein Methods, Wiley-liss, New York, pp: 181-208.
10. Hemmatzadeh, F. and F. Amini, 2009. Dot-blot enzyme immunoassay for the detection of bovine viral diarrhea virus antibodies. Veterinarski Archive, 79: 343-350.
11. ROY, P. and A.T. Venugopalan, 1999. Dot-enzyme linked immunosorbent assay for demonstration of Newcastle disease virus infection. Comp. Immunol. Microbiol. Infect. Dis., 1: 27-31.
12. Dasika, G.K. and G.J. Letchworth, 1999. Cellular expression of bovine herpesvirus 1 gD inhibits cell-to-cell spread of two closely related viruses without blocking their primary infection. Virology, 254: 24-36.

13. Hanon, E., G. Keil, P. Griebel, A. Vanderplasschen, F.A. Rijsewijk, L. Babiuk and P.P. Pastoret, 1999. Bovine herpesvirus 1-induced apoptotic cell death: role of glycoprotein D. Virology, 257: 191-197.
14. Li, Y., L.A. Babiuk and X. Liang, 1995. Characterization of cell-binding properties of bovine herpesvirus 1 glycoproteins B, C and D: identification of a dual cell-binding function of gB. Journal of Virology, 69: 4758-4768.
15. Schroder, C. and G.M. Keil, 1999. Bovine herpesvirus 1 requires glycoprotein H for infectivity and direct spreading and glycoproteins gH(W450) and gB for glycoprotein D-independent cell-to-cell spread. Journal of General Virology, 80: 57-61.
16. Rijsewijk, F.A., M.J. Kaashoek, J.P. Langeveld, R. Meloen, J. Judek, K. Bienkowska-Szewczyk, Maris-M.A. Veldhuis and J.T. Van Oirschot, 1999. Epitopes on glycoprotein C of bovine.
17. Hanon, E., G. Meyer, A. Vanderplasschen, Dessy-C. Doize, E. Thiry and P.P. Pastoret, 1998. Attachment but not penetration of bovine herpesvirus 1 is necessary to induce apoptosis in target cells. Journal of Virology, 72: 7638-7641.
18. Babiuk, L.A., van Drunen Littel-van den Hurk and S.K. Tikoo, 1996. Immunology of bovine herpesvirus 1 infection. Veterinary Microbiology, 53: 31-42.