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Isolation and identification of recent Bovine Herpesvirus-1 from cattle at Menoufiya governorate, Egypt 2021.

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ABSTRACT

Bovine herpesvirus-1 (BoHV-1) is responsible for different clinical findings in cattle including tracheitis, conjunctivitis, keratoconjunctivitis, vulvovaginitis, balanoposthitis, abortion, infertility, encephalitis and fatal disease in newly born calves, and causes high economic losses**.** The present study was carried out for diagnosis of BoHV-1from clinically suspected cattle in El Menoufiya governorate, Egypt during the period from September 2021 until December 2021. A total of 50 field samples including swabs (n= 35) and internal organs (n= 15) collected from clinically suspected cattle were isolated on cell culture of Madian Darby Bovine Kidney (MDBK) cell line. The isolates were identified using Antigen detection Enzyme Linked Immunosorbent Assay (ELISA) and conventional Polymerase Chain Reaction (PCR). Out of 50 tested samples, 30 samples Produce CPE on MDBK cells by third passage. The ELISA only identified BoHV-1 in 20 tissue culture isolates and confirmed by the PCR amplicon of 575 bp targeting Glycoprotein C gene. it was concluded that, isolation, antigen detection ELISA and conventional PCR based on the Glycoprotein C (gC) were efficient for the identification of BoHV-1virus. This indicates circulation of BoHV-1 on Menoufiya governorate and directed toward application of control measures.

1. INTRODUCTION

Bovine herpesvirus-1(BoHV-1) affects wild and domestic cattle. It is widely distributed all over the world, but was eradicated from some places like Denmark, Austria, Switzerland, Finland, Italy, Sweden, Norway and parts of Germany (OIE Terrestrial Manual 2010).

BoHV-1 is a members of genus Varicellovirus belonging to family Herpesviridae and order Herpesvirales. According to its genomic and antigenic characteristics there are different subtypes of the virus: BoHV1.1 and 1.2a (IBR), 1.2b (IPV / IBP) (Chettri et al., 2016). BoHV-1 genome consists of double-stranded DNA of about 140 Kbp that encodes for 33 structural proteins, among these 13 are shown to be associated with viral envelope, 14 with nucleocapsid and 6 not classified. Other than these 16 nonstructural proteins are also coded by BoHV1 genome (Esteves et al. 2008).

BoHV-1 can affect cattle of different ages and breeds; other Artiodactyla (sheep, goats, camelids and water buffaloes) can be infected with the virus (Ackermann and Engels 2006). Clinically, the disease in cattle is characterized by nasal discharge, hyperemia of the muzzle and conjunctivitis in addition to fever, in appetence, depression, reduction in milk production and abortions. BoHV-1 virus affects genital tract causing pustular vulvovaginitis (IPV) in female and balanoposthitis (IBP) in male (Nettleton and Russell 2017). The virus is excreted in nasal secretions for up to 10–17 days with a peak at the period from 4–6 days after infection (Nandi et al., 2009). Throughout the course of the primary infection, the infected cases shed large amounts of the virus that may reach to $3.8x10^6$ TCID50/ mL nasal secretion (Muylkens et al., 2007) and are considered the main sources of infection during any outbreak.

Initial diagnosis of BoHV-1 affection depends on clinical, pathological and epidemiological diagnosis. But, to make an accurate diagnosis, lab diagnosis is required (Van Oirschot, 2000). The laboratory diagnoses of BoHV-1 may be through direct identification of the virus or its components or indirectly by detecting the specific antibody response in serum samples (Muylkens et al., 2007).

For isolation of BoHV-1, different kinds of tissue cultures of bovine sources can be used, such as, primary kidney and lung cells or the Madin Darby bovine kidney cell line. BoHV-1 induces the cytopathic effects within 2-4 days (Mehrotra et al., 1994). It can be isolated from nasal or genital swabs collected from animals inducing respiratory signs, vulvovaginitis or balanoposthitis, collected during the early stage of the infection, or, in severe cases, from collected different organs at post-mortem (Tiwari et al., 2000).

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ELISA is considered to be highly useful technique for the diagnosis of BoHV-1 infection (Teixeira et al., 2001). The antigen capture ELISA showed good sensitivity and specificity when used for BoHV-1 antigen detection (Nandi et al., 2007).

Polymerase chain reaction (PCR) assay is a good method for virus detection since the results can obtained within short time whereas the virus isolation and subsequent identification method needs for longer time (Moore et al. 2000).

The present study was applied for diagnosis of BoHV-1 that causes respiratory and genital disorders in different herds of cattle in Menoufiya governorate during the period from September 2021 until December 2021 and may causing mortality, using accurate, rapid, and sensitive techniques like virus isolation in cell culture, characterization of the isolates by commercial sandwich ELISA kit and gpC based PCR ssay.

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2. MATERIAL AND METHODS

2.1. Samples collection:

A total of 50 field samples from clinically, unvaccinated, BoHV-1 suspected cattle during the early stage of affection during 2021 from different districts of El Menoufiya Governorate were collected including 35 swabs (15 nasal, 5 conjunctival, 7 vaginal and 8 preputial swabs) and 15 tissue samples (5 lung, 3 respiratory tract mucosa, 3 tonsil, 2 bronchial lymph nodes, 2 liver tissue samples) as shown in table (1).

Table 1 Number and type of collected samples from Menoufiya governorate.

governorate	samples			
	swabs		Internal organs	
	type	number	type	number
Menoufiya	nasal swabs	15	lung tissue	5
	Conjunctival swabs	5	respiratory tract mucosa	3
	vaginal swabs	7	tonsil	3
	preputial swabs	8	bronchial lymph nodes	2
			liver	2
Total number		35		15

The collected samples were rapidly transmitted to the laboratory in viral transport medium (phosphate buffered gelatin saline containing antibiotics – PBGS), under cooled conditions and preserved at -20 ºC for further diagnosis.

2.2. Preparation of collected samples:

Samples were properly prepared for virus isolation and antigen detection according to OIE Terrestrial Manual (2010). Swabs were shacked well for half an hour at room temperature in the transport medium for elution of the virus then the swabs were removed and the transport medium then clarified by using centrifuge at 1500 rpm at 4ºC for ten minutes. Tissues were homogenized to a 10% (w/v) suspension in cell culture medium then centrifuged at 1500 rpm at 4ºC for ten minutes. The supernatants of these samples were filtered using 0.45 µm filters and kept for using in virus isolation.

2.3. BoHV-1 reference strain:

A standard strain of BVH-1 (Colorado strain) was kindly supplied by Virology Department, Animal Health Research Institute, Dokki, Egypt. The Eagle's Minimum Essential Medium (EMEM) without serum was used as a virus growth medium. The infectious power of the viral strain was calculated as $TCID_{50} = 10^{-5.25}/0.1$ ml as a result of the microtitration test conducted on Madin-Darby bovine kidney (MDBK) cell culture. The virus strain has been stored at -80ºC. It was used as a positive control for PCR technique.

2.4. Cell Culture:

Medin-Darby bovine kidney (MDBK) cell line was supplied and propagated at Virology Dep., Animal Health Research Institute, Egypt. For cell growth Eagle's Minimum Essential Medium supplemented with 10% fetal calf serum (supplied by Sigma) was used.

2.5. Virus isolation:

According to Mehrotra *et al.,* (1994), the isolation of BHV-1 from suspected specimens was applied on Madin Darby Bovine Kidney (MDBK) cell line for three successive blind passages. The cells were grown on flat bottom 24well plates. Monolayer of MDBK cells was inoculated by 200 µl of the sample/well. The plates were incubated at 37ºC for 1 h for adsorption then the inoculums was replaced with Eagle's MEM supplemented with 2% foetal calf serum (maintenance medium) and incubated in 5% CO2 atmosphere at 37ºC, control non-infected cells were involved, each sample was inoculated in cell culture for a three blind passages, infected cell lines were microscopically examined daily for cytopathic effect induction, in each passage the cells were observed for changes. By the third passage, samples that did not show any cytopathic effect were discarded and treated as negative samples. The virus isolates were identified and confirmed by using direct sandwich ELISA and PCR.

2.6. Antigen detection ELISA:

Commercial ELISA kit (Monoscreen Ag ELISA BoHV-1) was supplied by (Bio X Diagnostics, Belgium) and used for identification of BoHV-1 isolates (as recommended by the manufacturer). ELISA reader with a 450 nm filter was used for reading the optical density. The test is validated only when positive control antigen gives a difference in the optical density that is more than the value given on the quality control (QC) data sheet at ten minutes.

2.7. Polymerase chain reaction (PCR):

DNA from nasal and ocular swabs and lung specimens was extracted by using QIAamp DNA Mini kit (Qiagen, Germany, GmbH) as recommended by the manufacturer (Fuchs *et. al.,* 1999).Samples were amplified using EmeraldAmp Max PCR Master Mix (Takara, Japan). The primers were selected on the basis of the published sequence of gc glycoprotein gene region of BoHV-1 (Esteves *et al*., 2008), which was predicted to produce a PCR product of 575 base pairs (bp). Primers Synthesized by Metabion Germany as mentioned in **Table (2)** were used for PCR amplification. The PCR was performed in 35 cycles; each temperature cycle consisted of 60 s at 95°C, 60 s at 57°C and 60 s at 72°C. A final extension time of six minutes at 72°C was included at the end of last cycle. The PCR products were analyzed by agarose gel electrophoresis (2% agarose) using 5 µL of the each PCR product mixed with 10 µL of loading dye.

Table 2 sequences, target genes and amplicon sizes of used primers.

Target virus	Target gene	sequence of primer (5'-3')	size of amplified product	Reference
BOHV-1	gc	F CGGCCACGACGCTGACGA R CGCCGCCGAGTACTACCC	575 bp	Esteves *et al.*, 2008

3. RESULTS

3.1. Isolation of BoHV-1 on MDBK cell lines:

A total of 50 suspected samples (35 swabs and 15 internal organs) were inoculated in MDBK cell culture for three successive blind passages. It was found that out of 50 inoculated samples, 30 samples (8 nasal, 2 conjunctival, 5 vaginal, 6 preputial swabs and 3 lung, 2 respiratory tract mucosa, 2 tonsil, 1 bronchial lymph nodes, 1 liver tissue samples) induced a clear Cytopathic effect in inoculated cells up to 3rd passage in form of rounding and aggregation of the infected cell endings with dispatched areas of infected cells in the cell sheath as shown in **t**able (3) and figures (1and 2).

Table 3 Isolation of prepared collected specimens on MDBK cell culture.

inoculated specimen		number of positive specimens *			Total number of positive specimens
type	number	1st passage	2nd passage	3rd passage	
nasal swabs	15	2	5	1	8
Conjunctival swabs	5	1	1	1	2
vaginal swabs	7	1	2	2	5
preputial swabs	8	1	3	2	6
Lung tissue	5		1	2	3
respiratory tract mucosa	3			1	2
tonsil	3			2	2
bronchial lymph nodes	2			1	1
liver	2			1	1
Total number	50	5	12	13	30

*Positive result represented by rounding and aggregation of the infected cell endings with dispatched areas of infected cells in the cell sheath.

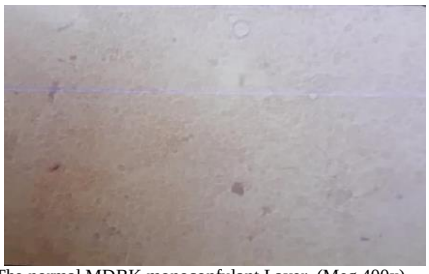

Figure 1 The normal MDBK monoconfulant Layer. (Meg 400x)

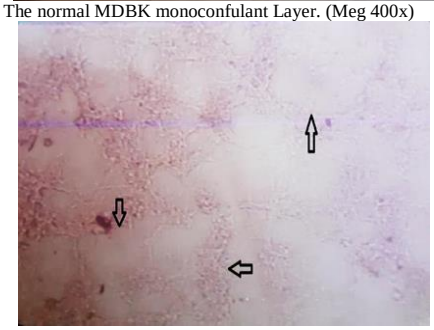

Figure 2 characteristic CPE of BoHV-1 by cell round in cell aggregation, coalesce together to form clusters within 72 hr post inoculation. (Meg 400x).

3.2. Identification of BoHV-1 using Antigen detection ELISA:

It was done using of Monoscreen Ag ELISA BoHV-1for identification of BoHV-1in positively isolated samples, we found that out of 30 examined samples there are 20 samples (6 nasal, 2 conjunctival, 4 vaginal, 3 preputial swabs and 2 lung, 1 respiratory tract mucosa, 1 tonsil, 1 bronchial lymph nodes) gave positive results as shown in table (4).

Table 4 Results of direct sandwich ELISA for identification of BoHV-1 Antigen in isolated specimens.

number of examined specimens		number of positive specimens
type	Number	
nasal swabs	8	6
Conjunctival swabs	2	2
vaginal swabs	5	4
preputial swabs	6	3
Lung tissue	3	2
respiratory tract mucosa	2	1
tonsil	2	1
bronchial lymph nodes	1	1
liver	1	0
Total number	30	20

3.3. Identification of BoHV-1 using PCR:-

Detection of nucleic acids in isolated samples using conventional PCR was recorded in all prepared samples. The amplification and running of characteristic 575 bp fragments of BoHV-1viral DNA were shown in Figure (3).

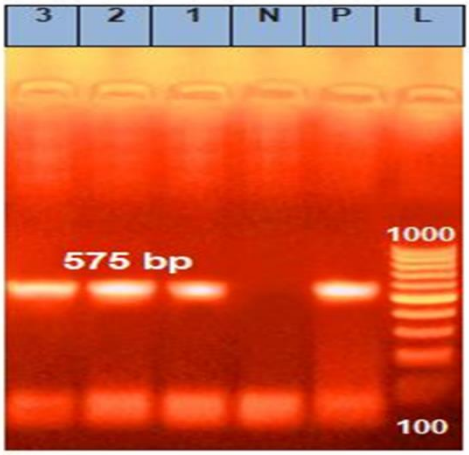

Figure 3 Detection of BoHV-1viral DNA after amplification by PCR. L: 100bp molecular marker. P: Positive PCR products from BoHV-1reference strain. N: negative PCR control. Lanes (1-3): positive PCR products from BoHV-1isolate.

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4. DISCUSSION

Bovine herpesvirus-1 is responsible for two diseases in cattle, Infectious bovine rhinotracheitis and Infectious pustular vulvovaginitis/or balanoposthitis (IPV/IPB). It causes variety of clinical conditions including tracheitis, conjunctivitis–keratoconjunctivitis, balanoposthitis of bulls, pustular vulvovaginitis, abortion, mastitis, infertility, encephalitis and fatal disease in newborn calves, and thus causes high economic losses (Graham, 2013).Although clinical signs may be highly suggestive of BoHV-1infection, no real characteristic signs provide a clinical

diagnosis of BoHV-1. So, laboratory confirmation is essential for the diagnosis of BoHV-1infection (Zhou *et al.*, 1999). There are numerous laboratory techniques for BoHV-1diagnosis as isolation of the virus, direct fluorescent antibody technique, antigen and antibody detection by ELISA and viral nucleic acid detection by PCR (Van Oirschot *et al.*, 1997).

The PCR is an excellent technique for fast and sensitive identification of viral genomes in biological and clinical samples (Dehkordi *et al.*, 2013). Different PCR assays have been described for the identification of BoHV-1. Primers were selected to amplify parts of gC gene, gB gene, gD gene, and thymidine kinase gene of BoHV-1with different sensitivities (Kibenge *et al.*, 1994; Engelenburg *et al.*, 1995; Gee *et al.*, 1996 and Lyaku *et al.*, 1996). Also, BoHV-1infections in animals are usually diagnosed using serological tests such as ELISA or serum neutralization assays, these tests are generally efficient and sensitive (Tiwari *et al.*, 2000).

The BoHV-1is excreted in nasal and ocular discharges, placenta of aborted animals and in semen of infected animals (OIE, 2010). Virus can be diagnosed from nasal or genital swabs from animals with respiratory signs, balanoposthitis or vulvovaginitis, collected during early stage of infection and in severe cases, collected organs at post-mortem by antigen capture ELISA (Collins *et al*., 1988). BoHV-1can be identified in the nasal swabs for 14 days post-infection (Nandi *et al*., 2009). Also, the contents of abomasum of aborted fetuses can be used for BoHV-1 detection (Dehkordi *et al.*, 2013).

In present study, 50 samples in form of 35 nasal, conjunctival and genital (vaginal and Preputeal) swabs plus 15 internal organs of lung, mucous membranes of the respiratory tract, tonsil, bronchial lymph nodes, liver and spleen were collected (Table 1).

The isolation of viruses is considered as a 'gold standard' for several viruses but the most important disadvantages of this technique are that it is time-consuming than other techniques and it needs at least 2-3 passages. Also, following these procedures, isolated viruses have to be identified by other methods (Murphy *et al.*, 1999). For BoHV-1isolation, different cell cultures of bovine sources are used, like secondary lung, kidney cells and Madin–Darby bovine kidney cell line where the cytopathic effects were produced through 2 - 4 days by the virus (Mehrotra *et al*., 1994).

Trials for isolation of BoHV-1from 50 samples in form of 35 nasal, vaginal swabs and 15 internal organs of clinically suspected cattle at different localities of El Menoufiya governorate during 2021 by three blind passages through MDBK showed that only 30 from 50 isolated samples induced characteristic Cytopathic effect in the form of rounding, granulation, shrinkage and aggregation of cells that lead to the grape like cluster appearance "bunch of grapes" within 2-4 days post inoculation that become more pronounced and reduced in time of appearance by advanced propagation on the cells while the other isolates (20 samples) did not showed any changes on the inoculated cells as shown in (table 3 and photo1,2). The finding of cytopathic effect of BoHV-1with the appearance of the virus infected cells was in correlation with previous studies of BoHV-1isolation (Madbouly and Hussien 1997 and Murphy *et al.*, 1999) and that the diagnosis of BoHV-1usually occurs by isolation of the virus.

The ELISA test had been widely used to detect antibodies and antigens in a variety of test systems. The assays were found to be specific, sensitive and relatively inexpensive to set up (Sontakke *et al.* 2002). The reagents involved were stable and much safer than the techniques in which radioisotopes were used (Kramps *et al.*1994).

Monoscreen Ag ELISA BoHV-1 kit was used for identification of BoHV-1 in isolated samples. Out of 30 examined samples, there are 20 samples gave positive results with a percentage of 66.66% of examined samples while 10 samples were negative (Table 4). These results were in agree with Sarumathi *et al.* (2004) who recorded that ELISA is very effective in diagnosis of BoHV-1 infection with high specificity and sensitivity when the specimens were tested for the presence of BoHV-1 antigen.

The genome of BoHV-1consists of 10 genes encodes for glycoproteins (gB, gC, gD, gE, gI, gH, gL, gG, gK and gM) (Wellenberg, *et al.*, 1998). The molecular weights of the corresponding gene products are ranged from 17 to 101 kDa. However, their sizes observed larger because they contain O - as well as N-linked oligosaccharides and may form structures similar to those specified by HSV-1(Schwyzer and Ackermann, 1996). Due to the location of these glycoproteins in the virion envelope and on the surface of infected cells, they are important targets for the host immune response (Sivarama, *et al.*, 1999).

PCR is more specific, sensitive and relatively rapid in comparison to virus isolation (results can be obtained within 12h) (Dehkordi *et al.*, 2013). BoHV-1viral glycoprotein C can be detected in the aborted fetus and semen using PCR. Beside this target, other viral glycoproteins like gD, gB and thymidine kinase genes of BoHV-1 can also be detected using PCR with variable sensitivities (Majumder *et al.*, 2015).

PCR was used for BoHV-1detection in a wide range of clinical specimens such as blood, semen, nasal, fecal and vaginal swabs (Fuchs *et al*., 1999; Grom *et al*., 2006; Mahmoud and Ahmed, 2009 and Saha *et al*., 2010). The Polymerase chain reaction was found to be accurate, fast and sensitive method for BoHV-1detection (Moore *et al.*, 2000).

In this study, we detected the BoHV-1in positively MDBK using conventional PCR by using Primers that bind and amplify the partial gC gene, the Agarose gel electrophoresis of amplification products showed the presence of bands with the size of 575 bp fragment for BoHV-1 (Fig. 1). These results agreed with results by Deka *et al.*, 2005; Grom *et al.*, 2006 and Jain *et al.*, 2009 for BoHV-1identification.

5. CONCLUSION

A field BoHV-1was successfully isolated from clinically diseased cattle in 2021 in El Menoufiya Governorate, Egypt. Using a limited number of samples indicated that the infection was associated with BoHV-1. Virus isolation in combination with ELISA and The gc gene based PCR assay can be used for identification BoHV-1infection.

6. REFERENCES

1. Ackermann, M. and Engels, M., 2006. Pro and contra IBR-eradication, Veterinary Microbiology 113:293–302.
2. Chettri, S., Ahmed, K., Bora, D., Dutta, L. J., Bora, M. and Sharma, D., 2016. Reproductive Status in Bovine Herpes Virus 1 (BHV-1) sero-positive Dairy Cattle. Ind J Ani Repro. 36(2):53-56.
3. Collins, J., Ayers, V. and Carman, J., 1988. Evaluation of an antigen-capture ELISA for the detection of bovine herpesvirus type 1 shedding from feedlot cattle. Vet Microbiol, 16: 101–107.
4. Dehkordi, F., Haghigh, N., Momtaz, H., Rafsanjani, M. and Momeni, M. 2013. Conventional vs real –

time PCR for detection of bovine herpes virus type 1 in aborted bovine, buffalo and camel fetuses. Bulgarian J. Vet. Med. 16(2): 102−111.

5. Deka, D., Matti, R. and Oberoi, M., 2005. Detection of bovine herpesvirus- 1 infection in breeding bull semen by virus isolation and polymerase chain reaction. Review of Science and Technology Office International des Epizooties, 24, 1085–1094.
6. Engelenburg, F., Schie, F., Rijsewijk F. and Oirschot J., 1995. Excretion of bovine herpesvirus 1 in semen is detected much longer by PCR than by virus isolation. J. Clin. Microbiol. 33: 308-312.
7. Esteves, P., Dellagostin, O., Pinto, L., Silva, D., Spilki, F., Ciacci-Zanella, J., Hübner, S., Puentes, R., Maisonnave, J., Franco, A., Rijsewijk, F., Batista, H., Teixeira, T., Dezen, D., Oliveira, A., David, C. and Arns, P., 2008. Phylogenetic comparison of the carboxy terminal region ofglycoprotein C (gC) of bovine herpesviruses (BoHV) 1.1., 1.2 and 5 from South America (SA). Virus Res 131, 16–22.
8. Fuchs, M., Hübert, P., Detterer, J., Rziha, H., 1999. Detection of bovine herpesvirus type 1 in blood from naturally infected cattle by using a sensitive PCR that discriminates between wild-type virus and virus lacking glycoprotein. J. Clin. Microbiol. 37, 2498-2507.
9. Gee, A., Wagter, L. and Hage, J., 1996. The use of a polymerase chain reaction assay for the detection of bovine herpesvirus 1 in semen during a natural outbreak of infectious bovine rhinotracheitis. Vet. Microbiol. 53: 163-168.
10. Graham, D. 2013. Bovine herpes virus-1 (BoHV-1) in cattle-a review with emphasis on reproductive impacts and the emergence of infection in Ireland and the United Kingdom. Ir Vet J 66:1.
11. Grom, J., Hostnik, I., Toplak, D. and Barlic-Maganja, A., 2006. Molecular detection of BHV-1 in artificially inoculated semen and in the semen of a latently infected bull treated with dexamethasone. Veterinary Journal, 171, 539–544.
12. Jain, L., Kanani, A., Purohit, J., Joshi, C. and Rank, D., 2009. Detection of bovine herpes virus-1 (BHV-1) infection in breeding bulls by serological and molecular methods and its characterization by sequencing of PCR products. Buffalo Bulletin, 28(2): 76-84.
13. Kibenge, F., Harris, L., Mckenna, P., Wadowska, D. and Yason C., 1994. Amplification of strains of bovine herpesvirus 1 by use of polymerase chain reaction with primers in the thymidine kinase region. Am. J. Vet. Res. 55: 1206-1212.
14. Kramps, J., Magdalena, J., Quak, J., Weerdmeester, K., Kaashoek, M., Maris-Veldhuis, M., Rijsewijk, F., Keil, G. and van Oirschot, J., 1994. A simple, specific, and highly sensitive blocking enzyme-linked immunosorbent assay for detection of antibodies to bovine herpesvirus 1. J. Clin. Mcrobiol. 32: 2175-2181.
15. Lyaku, J., Nettleton, P., Msolla, P. and Scott, C., 1996. Prevalence of antibody to bovine herpesvirus-1 (BHV-1) in Tanzanian Cattle. Trop. Anim. Hlth. Prod. 23: 106-107.
16. Madbouly, H. and Hussien, M., 1997. Isolation of BHV-1, from Dairy herd Genital form of infection. Alex J Vet.Scien., 13 (4):439-446.
17. Mahmoud, M. A. and Ahmed, S. A. 2009. Prevalence of bovine herpesvirus-1 in sheep and goats in Egypt. Global Veterinaria, 3, 472–479.
18. Majumder, S., Ramakrishnan, M. and Nandi, S. 2015. Infectious Bovine Rhinotrachitis: An Indian Perspective. Int J Cur Mic App Sci4:844-858.
19. Mehrotra, M., Singh, K., Khanna, P. and Shukla, D., 1994. Isolation of BHV-1 from an outbreak of abortion in an organized herd. Indian J Anim Sci, 64 (5): 341-35.
20. Moore, S., Gunn, M. and Walls, D., 2000. A rapid and sensitive PCR-based diagnostic assay to detect bovine herpesvirus 1 in routine diagnostic submissions. Veterinary Microbiology, 75, 145-153.
21. Murphy, F., Gibbs, E., Horzinek, M. and Studdert, M., 1999. VeterinaryVirology. 3rd ed., Academic Press. London, Boston, New York, Sydney, Tokyo, Toronto.
22. Muylkens, B., Thiry, J., Kirten, P., Schynts, F. and Thiry, E., 2007. Bovine herpesvirus 1 infection and infectious bovine rhinotracheitis. Vet Res 38:181–209.
23. Nandi, S., Kumar, M., Manohar, M. and Chauhan, R., 2009. Bovine herpes virus infections in cattle. Anim Health Res Rev 10:85–98.
24. Nandi, S., Pandey, A.B., Sharma, K., Audarya, S. and Chauhan, R., 2007. Seroprevalence of infectious bovine rhinotracheitis in cattle of an organized farm by indirect ELISA. The Indian cow: The Scientific and Economic Journal 4:09729585.
25. Nettleton, P. and Russell, G. 2017. Update on infectious bovine rhinotracheitis. Pract 39:255–272.
26. Office International des Epizootés (OIE) 2010. Infectious bovine rhinotracheitis/infectious pusular vulvovaginitis. In : Manual of Standards Diagnostic test and Vaccine. Office International des Epizootics, Chapter 2.4.13.
27. Saha, T., Guha, C., Chakraborty, D., Pa, B., Biswas, U., Chaterjee, A, Koenig, P. and Beer, M., 2010. Isolation and characterization of BoHV-1 from cattle in West Bengal, India. Iranian Journal of Veterinary Science Technology, 2, 1–8.
28. Sarumathi, C., Reddy, T., Sreedevi, B. and Rao, U., 2004. Comparison of serum and milk based avidin-biotin ELISA for detection of antibodies to infectious bovine rhinotracheitis. Indian Vet. J. 81: 9-11.
29. Schwyzer, M. and Ackermann, M., 1996. Molecular virology of ruminant herpesviruses. Vet. Microbiol. 53: 17-29.
30. Sivarama, K., Sreekumar, E. and Rasool, T., 1999. Cloning and sequencing of truncated gIV glycoprotein gene of an Indian isolate of bovine herpesvirus 1. Acta Virol. 43:387-9.
31. Sontakke, S., Deshmukh, V. and Gujar, M., 2002. Serological investigations of clinical cases of infectious bovine rhinotracheitis. Indian J. Anim. Res. 36: 137-140.
32. Teixeira, M., Esteves, P., Schmidt, C., Spilki, F., Silva, T., Dotta, M. and Roehe, P., 2001. A monoclonal blocking ELISA for the serological diagnosis of bovine herpesvirus type 1 (BHV-1) infections. Pesquisa Veterinaria Brasileira. 21: 33-37.
33. Tiwari, A., Kataria, R., Butchaiah, G. and Prasad, N., 2000. A simple method for the detection of BHV-1 from infected MDBK cells by polymerase chain reaction Indian Vet J, 77: 98-102.
34. Van Oirschot, J., 2000. Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis. In: Manual of Standards for Diagnostic Tests and Vaccines. 4th ed. Paris: Office International des Epizooties 381–391.

35. Van Oirschot, J., Kaashoek, M., Marisveldhuis, M., Weerdmeester, K. and Rijsewijk, F., 1997. An enzyme-linked immunosorbent assay to detect antibodies against glycoprotein gE of bovine herpesvirus 1 allows differentiation between infected and vaccinated cattle. J. Virol. Methods 67, 23–34.
36. Wellenberg, G., Verstraten, E., Mars, M. and Oirschot, J., 1998. Detection of Bovine Herpesvirus 1 Glycoprotein E Antibodies in Individual Milk Samples by Enzyme-Linked Immunosorbent Assays. Journal of Clinical Microbiology 36: 409–413.
37. Z hou, J., Lyaku, J., Fredrickson, R. and Kibenge, F., 1999. Improved detection of bovine herpesvirus 1 in artificially infected bovine semen by protein amplification. J. virol. Meth, 79 (2), 181-189.