Isolation of Bovine Herpesvirus-2 (Bhv-2) from a Case of Pseudo-Lumpy Skin Disease in Egypt

Iman, M. Bastawecy

Dept. of Virology, Animal Health Research Institute, Dokki, Giza, Egypt

Abstract: A bovine herpes viru-2 (BHV-2) was recently isolated for the first time from a cow with generalized BHV-2 infection which is called pseudo-lumpy skin disease. The isolated virus was identified with negative staining electron microscope (EM), Polymerase chain reaction (PCR) and sequence analysis for the PCR product. Further serological investigations are recommended to differentiate carriers from susceptible cattle because the latency of herpesvirus infections is consistently characterized by a positive serologic status. Nucleotidesequence data must be submitted to GenBank to certain the strain isolated and described in the current study.
[Iman, M. Bastawecy. **Isolation of Bovine Herpesvirus-2 (Bhv-2) from a Case of Pseudo-Lumpy Skin Disease in Egypt**] Journal of American Science 2012; 8(2):122-127]. (ISSN: 1545-1003). http://www.americanscience.org. 20

Key words: BHV-2; isolation; EM; PCR; sequence analysis.

1. Introduction

Bovine herpesvirus type 2 (BHV-2) was first isolated from generalized skin infections of cattle in Africa where a wide range of susceptible wild ruminant appear to act as subclinical reservoirs. It became apparent that the same agent can cause two distinct and well-defined conditions: a generalized benign skin infection that somewhat mimcs lumpy skin disease (LSD), or a localized ulcerative mammillitis. The occurrence of one or the other form of BHV-2 infection appears to be related to geographic variations **(Anthony and Werner, 1992)**. Generalized skin infection caused by BHV-2 called pseudolumpy skin disease **(D'Offay *et al.,* 2003).**

BHV-2 belonged to subfamily Alphaherpes virinae (**Brooks *et al.,* 1998**) with a genome composed of double stranded DNA of molecular weight 86x10^6 daltons that has a high degree of homology to herpes simplex virus (HSV) the genome is enclosed in an icosahedral capsid, which is surrounded by an amorphous protein layer called the tegument, and a lipid envelop containing glycoproteins **(Cockrell *et al.,* 2011)**. There is a close antigenic relationship between the different strains of BHV-2. Typical of herpes viruses, BHV-2 establishes a latent infection in the host following an acute infection. Serological diagnosis of BHV-2 infections can be performed by virus neutralization test (VNT), complement fixation test (CFT), agar gel immunodiffusion (AGID) or enzyme linked immunosorbent assay (ELISA) but there is a high prevalence of BHV-2 antibodies in cattle populations (Suggesting that the vast majority of infections are subclinical **(Anthony and Werner, 1992)**.

Generalized BHV-2 infection is called pseudolumpy skin disease (PLSD) **(d'Offay *et al.,* 2003)**, it may be confused with LSD (**Barnard *et al.,* 1994**). The rapid spread of the disease and the sudden appearance of lumps in the skin after an initial fever, make LSD quite unlike any other affliction of cattle **(Radostits, 2000)**. However, animals that develop only a few skin lesions and/or transient fever, laboratory diagnosis is needed.

Rapid diagnosis of BHV-2 infection can be made by direct visualization of the virus using negative staining electron microscopy (EM) of fresh lesions **(Anthony and Warner, 1992)** but EM may not always be available where the disease occurs **(Davies *et al.,* 1971)**. Isolation can be attempted by the readily propagated infectious virus contained in the vesicular fluids in both primary cell cultures and established bovine cell lines **(Anthony and Werner, 1992)**. Diagnosis of BHV-2 can be confirmed rapidly by performing polymerase chain reaction (PCR) assay **(D'Offay *et al.,* 2003)**. Genetic sequences of some specific BHV-2 proteins genes offered a rapid diagnostic test based on gene amplification by PCR **(Anthony and Werner, 1992)**.

The aim of the present study is the accurate diagnosis of suspected case of generalized infection with BHV-2 by isolation and identification with negative staining EM, PCR and nucleotide sequencing.

2. Material and Methods:

Animal:

Holestein breed cow of 2 years old, belonged to private farm in Luxor governorate, Egypt. It revealed generalized skin disease, normal body temperature. The skin lesions distributed allover the body. Their diameter ranged from 1.2 to 2.5cm, they were superficial and easily removed.

Samples:

Skin lesions comprising epidermis were collected for virus isolation and identification.

Cell culture:

Madden Derby Bovine Kidney (MDBK) cell culture was provided by Virology Department, Animal Health Research Institute, Dokki, Giza, Egypt and subjected for isolation.

Diagnostic Methods:

Samples were subjected for inoculation onto MDBK cell culture according to **Payment and Trudel (1993)**. The cells were maintained in Eagle's essential medium containing 2% fetal calf serum, 100µg of streptomycin per ml, and 100 U of penicillin per ml. The inoculated cultures are incubated at 32°C according to **Anthony and Werner (1992)**. Cell cultures should be examined for cytopathic effect (CPE) for 3 to 5 days. If no CPE is detected, cultures should be frozen and thawed 3 times and used for inoculation up to 3 blind-passages.

Negative staining electron microscopy:

Negative staining EM was conducted according to **Payment and Trudel (1993)**. Supernatant of inoculated MDBK cell culture showing CPE is mixed with a droplet of 3% phosphotungestic acid (PTA). A copper grid coated with carbon formvar was dipped into the mixture. After drying, the grid was examined by electron microscope.

Polymerase Chain Reaction and Sequencing of PCR Product:

The PCR product is constructed and sequenced by **Macrogen, South Korea**. Viral DNA is extracted from infected MDBK cells as described by **Inoshima *et al.* (2000)**. PCR study is performed according to **Egyed and Bartha (1998)** and **Bremer (2010)**. A set of primers for BHV-2 gB were:

BHV-2/2F5'-tgtcctcctttctgtccaaccc-3'
BHV-2/2R5'-caaagaacgcaactcgggtgac-3'

Reaction conditions for PCR were 94°C/2 min (1X) followed by 94°C/30 s, 50°C/30 s, 72°C/30 s (45X). The PCR product is electrophoresed in 1.5% agarose gel and stained with ethidium bromide.

3. Results:

Isolation:

The inoculated MDBK cell culture revealed CPE at first passage, characterized by early focus of swollen cells and extensive multinucleated syncytia (Fig. 1).

Negative staining EM:

Bovine herpesviral particles were detected in supernatant of inoculated MDBK cell culture, revealing CPE and subjected for EM. (Fig. 2).

PCR and sequence analysis of PCR product:

Supernatant of inoculated MDBK cell culture (which showed CPE characteristic for BHV-2 and detecting bovine herpes viral particles in negative staining EM), revealed PCR and sequence analysis of PCR product for BHV-2 (Figs. 3 and 4, respectively).

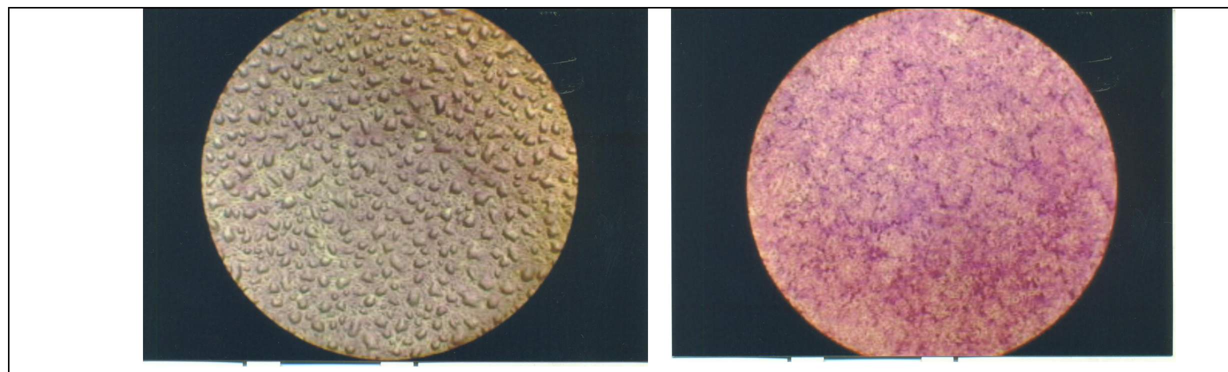


Fig. (1): Right: Inoculated MDBK cell culture showed CPE characterized by swollen cells which are multinucleated giant cells or syncytia.
Left: Uninoculated MDBK cell culture (control)

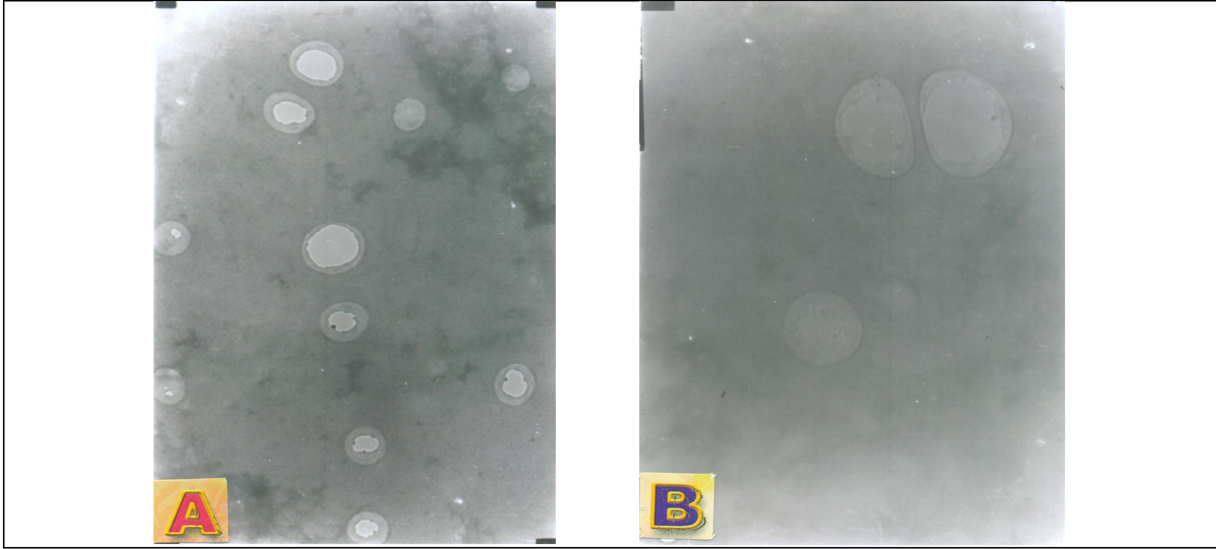


Fig. (2): Ultrastructure of herpes viral particles shown by negative staining. Capsid exposed to negative stain and structural details are visible where irregular tegument lies between the icosahedral capsid and the envelop. A: (42.000 x) B: (70.000 x)

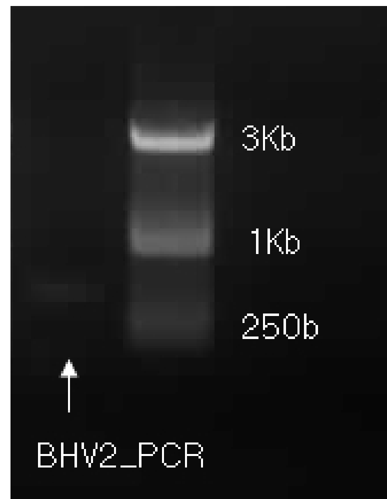


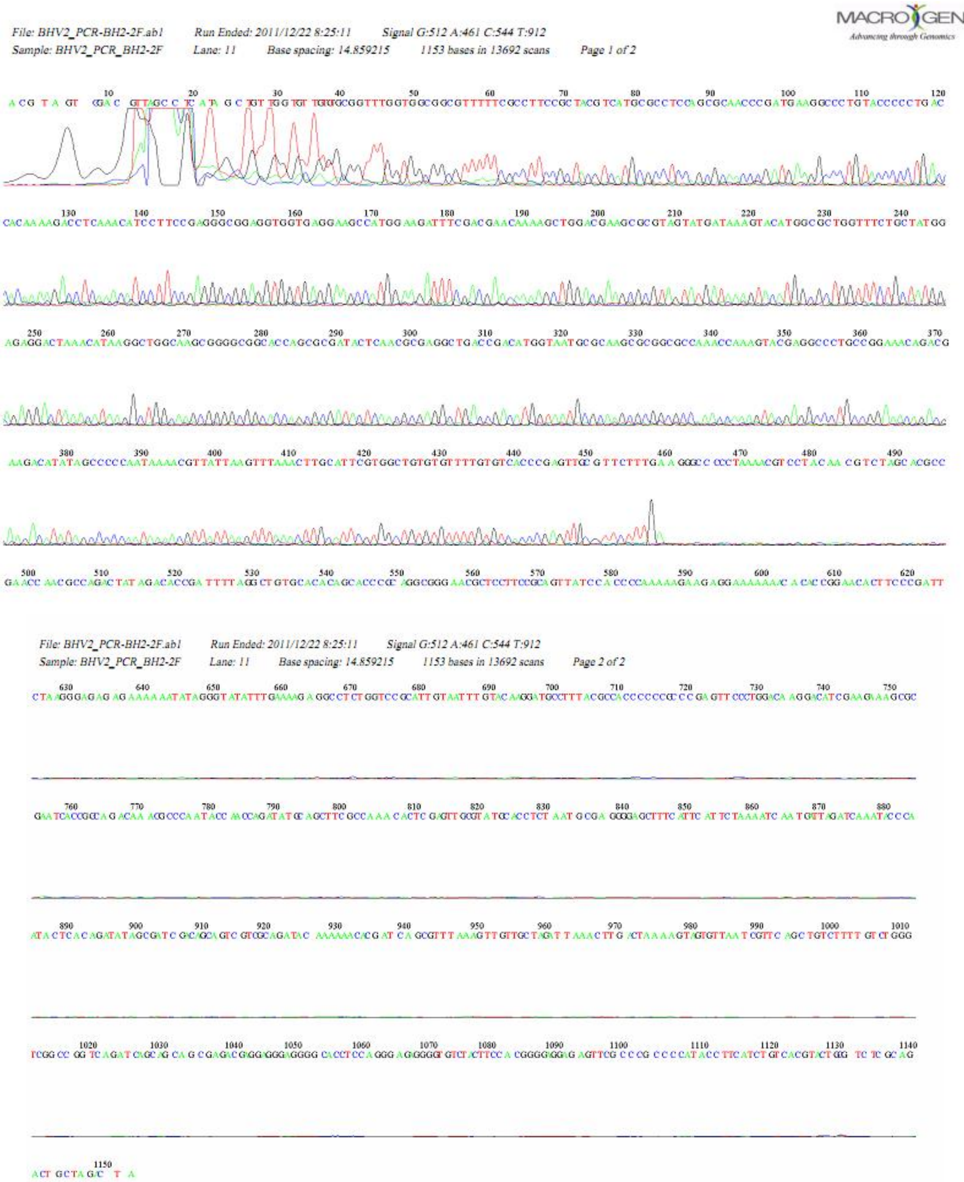
Fig. (3):PCR result for the isolated herpesvirus BHV-2 positive isolate (Lane 1) and a marker (Lane 2).

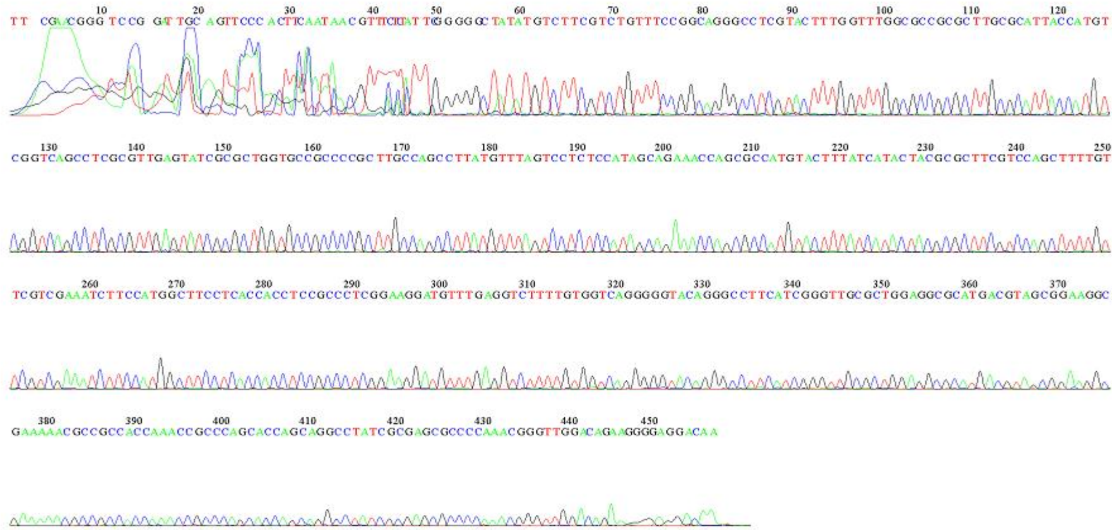
>111221-28_E01_BHV2_PCR-BH2-2F.ab1 1153

ACGTAGTCGACGTTAGCCTCATAGCTGTTGGTGTTGTGTGCGGTTTGGTGGCGGCGTTTTCGCCTTCC
GCTACGTCATGCGCCTCCAGCGCAACCCGATGAAGGCCCTGTACCCCCTGACCACAAAAGACCTCAAA
CATCCTTCCGAGGGCGGAGGTGGTGAGGAAGCCATGGAAGATTTCGACGAACAAAAGCTGGACGAAG
CGCGTAGTATGATAAAGTACATGGCGCTGGTTTCTGCTATGGAGAGGACTAAACATAAGGCTGGCAAG
CGGGGCGGCACCAGCGCGATACTCACGCGAGGCTGACCGACATGGTAATGCGCAAGCGCGGCGCCAA
ACCAAAGTACGAGGCCCTGCCGGAAACAGACGAAGACATATAGCCCCCAATAAAACGTTATTAAGTT
TAAACTTGCATTCGTGGCTGTGTGTTTTGTGTCACCCGAGTTGCGTTCTTTGAAGGGCCCCCTAAAACG
TCCTACAACGTCTAGCACGCCGAACCAACGCCAGACTATAGACACCGATTTTAGGCTGTGCACACAGC
ACCCGCAGGCGGGAACGCTCCTTCCGCAGTTATCCACCCCAAAAAGAAGAGGAAAAAAACACACCGG
AACACTTCCCGATTCTAAGGGAGAGAGAAAAAATATAGGGTATATTTGAAAAGAGGCCTCTGGTCCGC
ATTGTAATTTGTACAAGGATGCCTTTACGCCACCCCCCGCCCGAGTTCCCTGGACAAGGACATCGAAG
AAAGCGCGAATCACCGGCAGACAAACGCCCAATACCAACCAGATATGCAGCTTCGCCAAACACTCGA
GTTGCGTATGCACCTCTAATGCGAGGGGAGCTTTCATTCATTCTAAAATCAATGATTAGATCAAATACC
CAATACTCACAGATATAGCGATCGACAGCAGTCGTCGCAGATACAAAAAACACGATCAGCGTTTAAA
GTTGTTGCTAGATTAAACTTGACTAAAAGTAGTGTTAATCGTTCAGCTGTCTTTTGTCTGGGTCGGCCG

GTCAGATCAGCAGCAGCGAGACGAGGAGGGAGGGGCACCTCCAGGGAGAGGGGTGTCTACTTCCACG
GGGAGGAGAGTTCGCCCGCCCCATACCTTCATCTGTCACGTACTGCGTCTCGCAGACTGCTAGACTA

>111221-28_G01_BHV2_PCR-BH2-2R.ab1 458
TTCGAACGGGTCCGGATTGCAGTTCCCACTTCAATAACGTTTCTCTATTCGGGGGCTATATGTCTTCGT
CTGTTTCCGGCAGGGCCTCGTACTTTGGTTTGGCGCCGCGCTTGCGCATTACCATGTCGGTCAGCCTCG
CGTTGAGTATCGCGCTGGTGCCGCCCCGCTTGCCAGCCTTATGTTTAGTCCTCTCCATAGCAGAAACCA
GCGCCATGTACTTTATCATACTACGCGCTTCGTCCAGCTTTTGTTCGTCGAAATCTTCCATGGCTTCCTC
ACCACCTCCGCCCTCGGAAGGATGTTTGAGGTCTTTTGTGGTCAGGGGGTACAGGGCCTTCATCGGGTT
GCGCTGGAGGCGCATGACGTAGCGGAAGGCGAAAAACGCCGCCACCAAACCGCCCAGCACCAGCAGG
CCTATCGCGAGCGCCCCAAACGGGTTGGACAGAAGGGGAGGACAA





4. Discussion:

The causative virus for pseudolumpy skin disease is BHV-2, it is identical with the Allerton bovine herpesvirus in the United Kingdom and with the dermotrophic bovine herpesvirus in the United States. The difference in clinical manifestations between the two diseases may be due to the strain of the virus, or the method of infection **(Blood *et al.,* 1983)**. Generalized skin infections caused by BHV-2 tend to occur in tropical and subtropical areas. The mammary gland syndrome affects dairy and beef cattle that are under intensive production in temperate and cold areas of the world. **(Anthony and Werner, 1992)**.

The characteristic lesions involving the superficial layers of skin along with normal body temperature in contrast to the lesions of lumpy skin disease which are often deep enough to expose underlying tissue and elevated body temperature, presumptive diagnosis and suspicion for BHV-2 infection were obtained.

Isolation in MDBK cell cultures were attempted to obtain a preliminary diagnosis **(Yedloutsching *et al.,* 1970)**. CPE characterized by early focus of swollen cells and multinucleated giant cells and these agreed with **Brooks *et al.* (1998)** and **Anthony and Werner (1992)** who stated that a rapid CPE results which is notorious for the production of extensive multinucleated syncytia. The identity of the isolate confirmed by negative staining EM because this is the first isolation for BHV-2 in Egypt and I have no reference or internal control virus or antiserum. Negative staining EM has the advantages of ease for sample preparation and rapid analysis (same day results) and high specificity if sufficiently high particles concentration exist **(Bastawecy *et al.,* 2009)**. Therefore, negative staining EM was performed following isolation to obtain considerable number of herpesviral particles.

PCR was conducted in the current study as a confirmatory test. It is also suitable for use in those countries in which the disease is not endemic and live virus is not available **(Heine *et al.,* 1999)**. Primers designed according to the sequence of gB glycoprotein gene included in the gene cluster of BHV-2. gB glycoprotein is a major target for the cellular and humoral immune response. Sequences could aid the development of a rapid diagnostic test based on gene amplification of PCR. The significant value of such procedure is in the detection of BHV-2 in clinical material e.g. semen, in which the toxicity of the specimen significantly limits virus isolation **(Anthony and Werner, 1992)**.

The present study illustrated the first isolation of BHV-2 in Egypt (Egy-1, Iman strain). The resulted sequence should be submitted to officially recognized web sites. The web sites provide phylogenetic tree analysis of the isolate based on the sequence of DNA segments. This has provided a unique opportunity to complement studies of BHV-2 epidemiology by providing information on the potential geographical origin of virus isolate, a process termed genotyping or topotyping. Thus, determination of the sequence may provide information on where the virus came from. The disease may be present before this study and mistaken as mild infection with LSD. Therefore the

this isolation is not a surprise because herpesviral particles were detected in negatively stained skin biopsy of LSD infected animal **(Bastawecy *et al.*, 2007)** in previous study and the authors failed to isolate the virus which was present in such numbers that they mask the large poxviruses which may be present **(Davies, 1991)**. Those herpesviral particles previously detected may be BHV-2 and failure of isolation due to its assembly and maturation are inhibited at higher body temperature resulted from infection with LSD. Such conditions favor the local production of large amounts of highly active interferon which efficiently inhibits BHV-2 replication **(Anthony and Werner, 1992).**

In conclusion further studies for BHV-2 are recommended to prepare antisera and diagnostic reagents. Nucleotide sequence data must be submitted to GenBank to certain the strain isolated and described in this paper. I also recommend serological investigations because serology is the method of choice when it is necessary to differentiate carriers from susceptible cattle, because the latency of herpesvirus infections is consistently characterized by a positive serologic status.

Acknowledgement:

The author is very grateful to Prof. Dr. Abd El-Samee, A.A. (Dept. of Int. Med. And Inf. Dis., Faculty of Vet. Med. Cairo University) for his collaboration and assistance.

Corresponding author
Iman, M. Bastawecy
Dept. of Virology, Animal Health Research Institute, Dokki, Giza, Egypt
imanbaz@hotmail.com

References

1. Anthony, E.C. and Werner, P.H.E. (1992): Veterinary Diagnostic Virology, A practitioner's guide. Mosby Year Book. pp 83-85.
2. Bastawecy, M. Iman; Aly, W.F.; Aly, O. Nelly and Saad, A.A. (2007): Accurate and rapid techniques for laboratory diagnosis of lumpy skin disease. J. Egypt. Vet. Med. Assoc., 67(1): 133-147.
3. Bastawecy, M. Iman; El-Sayed, A.A.A.; Mohamed, A.E.A.; Abd El-Samee, A.A. and Fayed, A.A. (2009): Rapid diagnosis of foot and mouth disease in acute and carrier states. Egypt. J. Comp. Path. & Clinic. Path., 22 (2): 155-173.
4. Blood, D.C.; Radostits. O.M. and Henderson, J.A. (1983): Veterinary Medicine, sixth edition. pp 845-847.
5. Bremer, C.W. (2010): The prevalence of ovine herpesvirus-2 in 4 sheep breeds from different regions in South Africa. S. Afr. Vet. Ver., 81 (2): 93-96.
6. Brnard, B.H.J.; Munz, E.; Bumbell, K. and Prozesky, L. (1994): Lumpy skin disease. In: Infectious Diseases of Livestock with special reference to Southern Africa, Vol. 1(Coetzer, J.A.W.; Thomson, G.R.; Tustin, R.C. and Kriek, N.P.J.): 604-612. Oxford University Press, Cape Town.
7. Brooks, G.F.; Bute, J.S. and Morse, S.A. (1998): Medical Microbiology. Twenty First Editions.
8. Cockrell, S.K.; Huffman, J.B.; Torpova, K.; Conway, J.F. and Homa, F.L. (2011): Residues of the UL25 protein of herpes simplex virus that are required for its stable interaction with capsids. Journal of Virology, 85(10): 4875-4887.
9. Davies, F.G. (1991): Lumpy skin disease of cattle: A growing problem in Africa and the Near East World Animal Review, 68 (3): 37-42.
10. Davies, F.G.; Krauss, H.; Lund, J. and Taylor, M. (1971): The laboratory diagnosis of lumpy skin disease. Research in Veterinary Science, 12 : 123-127.
11. D'Offay, J.M.; Floyd, J.G.Jr; Eberle, R.; Salikl, J.T.; Brock, K. V. D'Andrea, G.H. and McMillan, K.L. (2003): Use of a polymerase chain reaction assay to detect bovine herpesvirus type 2 DNA in skin lesions from cattle suspected to have pseudo-lumpy skin disease. Journal of the American Veterinary Medical Association, 222:1404-1407.
12. Egyed, L. and Bartha, A. (1998): PCR studies on the potential sites for latency of BHV-4 in calves. Vet. Res. Commun., 22:209-216.
13. Hiene, H.G.; Stevens, M.P. Foord, A.J. and Boyle, D.B. (1991): A capripoxvirus detection PCR and antibody ELISA based on the major antigen P32 homologous of the vaccinia H3L gene. Journal of Immunological Methods. 227:187-196.
14. Inoshima, Y.; Morooka, A.; Murakami, K. and Sentsui, H. (2000): Simple preparation of parapoxvirus genome DNA for endonuclease analysis. Microbiol. Immunol., 44: 69-72.
15. Payment, P. and Trudel, M. (1993): Methods and Techniques in Virology. Marcel Dekker, New York.
16. Radostits, O.M.; Gay, C.C.; Blood; D.C. and Hinchcliff, K.W. (2000): Veterinary Medicine. 9th Edition.
17. Yedloutsching, R. J.; Breese, S.S. Jr; Hess, W.R.; Dardidi, A.H.; Taylor, W.D.; Barnes, D.M.; Page, R.W. and Ruebke, H.I. (1971): Bovine herpes mammillitis-like disease diagnosed in the United States. Proc. 74th Ann Meet U.S. Anim. Health Assoc., 208-212.

1/22/2012