Journal of American Science

Websites:
http://www.jofamericanscience.org
http://www.sciencepub.net

Emails:
editor@sciencepub.net
sciencepub@gmail.com

MARSLAND PRESS
Multidisciplinary Academic Journal Publisher

Electron microscopy as a differential diagnosis technique: Serving in the diagnosis of malignant catarrhal fever in farm animals

Iman, M. Bastawecy

Dept. Of Virology, Animal Health Research Institute, Dokki, Giza, Egypt
Email: Iman_baz@yahoo.com

Abstract: Malignant catarrhal fever (MCF) is a multisystemic disease, characterized by lymphoid hyperplasia in addition to widespread vascular, epithelial, and mesothelial lesions. Ovine herpesvirus 2 (OvHV-2) is the major etiology for MCF in susceptible animals globally. Suspected cases of cattle, buffalo, sheep, and goats for infection with OvHV-2 were subjected to diagnosis by isolation in specific pathogen-free-embryonated chicken eggs (SPF-ECE), using yolk sac and chorioallantoic membrane routes, identified with electron microscopy (negative staining and thin -sectioning techniques) and real-time polymerase chain reaction (PCR) after gross pathology and field diagnosis. In conclusion, accurate diagnosis is achieved by EM to identify herpesviral family, isolation, and PCR techniques following field diagnosis using clinical symptoms of the suspected cases for MCF and gross pathology. Testing of animals infected with OvHV-2 for IL-6, CRP, iron, vitamin D, vitamin B 12, zinc, selenium, magnesium, phosphorus, calcium, and glucose measurement in addition to bilirubin, liver, and kidney function tests and C B C as well is recommended. Also, testing of animals especially cattle and equines having symptoms of vitiligo for infection with OvHV-2 is recommended. Moreover, sequencing for the whole gene of B glycoprotein for any suspected herpes virus including EBV to avoid false results is also recommended. Detection of susceptibility of other species especially avian and fish for natural infection with OvHV- 2. Considering OvHV- 2 as a cause of leukosis in susceptible species.
[Iman, M. Bastawecy **Electron microscopy as a differential diagnosis technique: Serving in the diagnosis of malignant catarrhal fever in farm animals** .*J Am Sci* 2022;18(9):32-41]. ISSN 1545-1003 (print); ISSN 2375-7264 (online). http://www.jofamericanscience.org. 06.doi:10.7537/marsjas180922.06.

Keywords: Malignant catarrhal fever, OvHV-2, EM, isolation, IL-6

1. Introduction:

Malignant catarrhal fever (MCF) is a multisystemic disease, characterized by lymphoid hyperplasia and widespread vascular, epithelial, and mesothelial lesions that are associated morphologically with lymphoid cells (1). Ovine herpesvirus 2 is considered to be the major cause of MCF in domesticated animals throughout the world. It was also reported as the major etiology for MCF among ruminants' zoos and wildlife parks (2). OvHV-2 has a broad natural host range where it can infect a wide range of animals (3). MCF has been reported in more than 33 species including cattle, buffalo, swine, equine, many deer species, camelids, sheep and goats, and laboratory animals such as guinea pigs, rabbits, and hamsters are also susceptible (4;5;6;7and 8). Lambs become usually infected rapidly following birth through horizontal transmission (9). Also, calves were detected to be infected at the age of 5 to 35 days (10).

OvHV-2 is endemic in most sheep (11). OvHV 2 is classified in the subfamily Gammaherpesvirinae of the family Herpesviridae (12). Factors affecting the incidence rate of MCF are climatic factors that influence the viral survival in the environment, vectors and fomites presence, variation of stocking densities, and the stress level due to weather or management (13). OvHV-2 has been transmitted from infected animals to susceptible hosts through nasal and ocular secretions, direct contact with poorly defined airborne routes, vectors, or contaminated feed or water (14). Also, sources of OvHV-2 are infected cells in colostrum and milk in addition to semen, the skin of sick animals (7), feces (2), urogenital tracts (9), and transplacental (15).

All breeds of sheep are thought to become infected with OvHV-2 which results in T-lymphocyte proliferation and transformation. However, in sheep, immunosuppression due to pregnancy causes reactivation of the latent virus or acute epithelial infection leading to productive infection. Latency is maintained in a proportion of B-lymphocytes (12). Morbidity varies from sporadic outbreaks, that affect single or few animals, to large outbreaks reaching 40 % of the susceptible herd (16).

Susceptibility for infection with OvHV-2increases during pregnancy and viral shedding increases in the case of recently introduced animals stressed by a new environment could be a factor in detected outbreaks (7). So, reduction of stress can help disease prevention in subclinical or mildly affected

animals (17). SA-MCF occurs all the year with increased incidence during lambing season or winter months (8). Latent infection in susceptible animals results in clinical disease even in the absence of contact with sheep (18). Cattle-to-sheep-to-cattle transmission occurs (8) although some references mentioned that cattle are a dead-end host for this disease. A chronic course of SA-MCF is possible with reported cases of recovery (19; 20) and recovery reaching up to 50% (19).

Pathogenesis of MCF depends on direct virus-cell interactions or immune-mediated responses directed towards infected cells (21). Gamma-herpesvirus such as OvHV-2 and EBV encode seven trans-membrane (TM) receptors having structural and functional homologies to host receptors (22). They are very related and at the beginning, a PCR technique for diagnosis of OvHV-2 could be performed with primers used for EBV (12). Surprisingly, EBV antibodies were detected between 76. 4% of farmers and 23 % of white collars (23).

Susceptible species develop clinical signs ranging from mild to severe and may be sudden death mainly in calves as MCF appears in different forms (8). However, some researchers found that the classification of MCF into four forms (peracute, head and eye, intestinal and mild) is of little value due to there being an overlap between clinical forms and mixed signs that can appear in the same animal (26). Not all OvHV-2 positive animals develop the disease (27) and a lot of animals may have an infection without developing clinical signs (28). So, symptoms can be mild to dramatic with multiple organ failures. However, the disease may begin with depression and high fever of 40 to 42, tachycardia of 100 to 120 beats/minutes, anorexia, and agalactia (29). Some animals develop lymphocytic myocarditis with necrosis of the heart muscle (30), persistent leucoma, or generalized chronic obliterative arteriosclerosis following recovery from acute lymphoid pan arteritis (19). There is leukopenia and neutropenia at earlier stages and leucocytosis at later stages. Nervous symptoms can occur with terminal encephalitis. Necrotizing vasculitis, thrombosis, and tissue infiltration with dividing lymphocytes and macrophages are pathognomonic lesions in MCF (8).

OvHV-2 and EBV possess many proteins homologous to each other and have sequence and functional homology with host proteins (24) including cytokines which increase if the number of cells producing them increases or viral copies increase (25and 24) and due to cytokines mediate the innate immune response and their action if sustained and/or dysregulated, the pathological condition occurs.

Ov 4. 5 encodes a protein that works as B cell lymphoma-2 and acts as an anti-apoptosis protein to regulate cell death (31 ;16) and it can prevent important host antiviral mechanisms. Ov 2.5 encodes a protein that works as IL-10 where it is homologous with the host IL-10 and stimulates the proliferation of mast cells (32; 33). IL-15 (and consequently TN F-alpha) is produced in excess in case of MCF to maintain and stimulate the proliferation of cytotoxic cells resulting in tissue damage (34: 35). Ov 5 ORF which is related to EBV BILF1 may produce its similar role in OvHV-2 infected cells (31) by functioning as a constitutively signaling (ligand-independent) G protein-coupled receptor (G PCR) which alters intracellular signaling and also it can cause cell transformation (36). BILF1 is essential for EBV-mediated immunosuppression and oncogenesis (37).

G-protein coupled receptors are known as integral membrane protein that is used by cells to convert extracellular signals into intracellular responses, including responses to vision, olfaction and taste signals, hormones, and neurotransmitters (38).

OvHV-2 and EBV, therefore, are deemed to be similar in their symptoms, genes, miro RNAs and TATA boxes (39; 8; 31; 40 and 41) in addition to (42) who stated that the EBV genome was 135 kb and (43) who mentioned that EBV genome was 172 kb including repeats that were up to 12 each were 3 kb, which if subtracted, the genome became about 135 kb, the same size of OvHV-2.

The aim of this study is the accurate diagnosis of suspected cases of cattle, buffalos, sheep, and goats to be infected with OvHV-2 by isolation, EM, gross pathology, and real-time polymerase chain reaction (rt-PCR) technique.

2. Material and Methods:

Animals:

Foreign and native breeds of cattle, buffalos, sheep, and goats of different ages and both sexes were subjected for the present study. They belonged to El-Kalyobia, Monufia, New Valley, and Dakahlia governorates, Egypt.

Samples:

Tongue epithelia (cattle and buffalos), skin lesions (sheep and goats), spleen, heart, lung, small intestine, kidney, liver, and lymph nodes (dead or slaughtered cattle, buffalos, and sheep and goats). Samples were submitted to the laboratory on ice without delay for isolation and identification.

Specific pathogen-free embryonated chicken eggs (SPF-ECE):

SPF-ECE were obtained from Poultry Department, Animal Health Research Institute (AHRI), Dokki, Giza, Egypt and subjected to inoculation with the suspension of prepared samples.

Diagnostic Methods:

1. Isolation in SPF-ECE

Suspension of the tested samples was subjected to inoculation in SPF-ECE (6-8) days old via the yolk sac

route according to (8) in addition to inoculation of (10 -12) days old via the CAM route according to (44). Eggs were examined daily for stunted growth, oedematous, hemorrhagic, or macerated embryos (yolk sac route), and pock lesions or thickened (oedematous) CAM for 3 to 7 days.

***2.* Identification**

***2.1.* Electron microscopy (EM):**

2.1.1. Negative staining -transmission electron microscopy (T E M):

Negative staining TEM was conducted according to (45). Suspensions from prepared samples and their isolates were mixed with droplets of 3 % phosphotungstic acid (PTA). A copper grid coated with carbon formvar was dipped into the mixture and after drying, the grid was examined.

2.1.2. Thin-sectioning electron microscopy:

Thin-sectioning EM was conducted according to (45) on the skin lesions (scabs) and CAM inoculated with a prepared suspension of samples.

2.2. Real time-Polymerase chain reaction (rt-PCR) technique:

rt-PCR was performed on all the samples in AHRI.

3. Gross pathological examination:

A gross pathological examination was performed on organs and tissues collected from the dead or slaughtered animals, suspected to be infected with MCF during post-mortem examination.

3. Results:

Field diagnosis of clinical cases suspected as MCF:

Examined animals showed variations in types and severity in symptoms such as high fever which sometimes is persistent and fluctuated, ulcerative stomatitis, tachycardia, nasal and ocular discharges, severe dyspnea with stertor, fragile mucosa, slippery mouth and tongue, bubbly saliva which sometimes is ropey, smacking sound, erosive mucosal lesions, hemorrhagic and sometimes eroded check papillae, lymphoid hyperplasia, vasculitis, erosions in interdigital space, lameness, adipsia, synovitis especially in the tibiotarsal joint, the skin of teats, vulva, and scrotum sometimes is slough on touching or make dry scab, trismus, lymph nodes may be enlarged, fecal consistency varies from constipation to profuse diarrhea which sometimes is bloody, cystitis and sometimes with hematuria, swollen limb joints, recumbency, sometimes liver and/ or spleen are enlarged, blue or eye opaqueness, leucoma, blindness, abortion, reproduction failure or stiff-legged gait. Some animals were detected to have tumors in different parts that resemble Burkitt's lymphoma.

***1.* Results of isolation in SPF-ECE:**

1.1. Yolk sac route:

Embryos were stunted, oedematous, macerated, or hemorrhagic (Fig. 1).

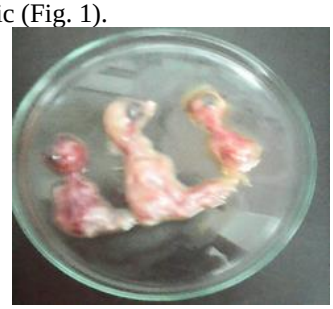

Figure 1. Control embryo in the middle. Right and left embryos are stunted and hemorrhagic in case of yolk sac inoculation.

1.2. CAM route:

Typical pock lesions in the CAM (Fig. 2) on the third day of inoculation. Pock lesions were small, white, and enlarged on prolonged incubation for 7 days. Also, the CAM was oedematous.An embryo showed arthrogryposis which is one of the congenital anomalies (Fig. 3).

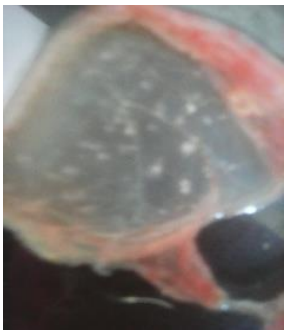

Figure 2. White and scuttered pocks on CAM in case of CAM inoculation.

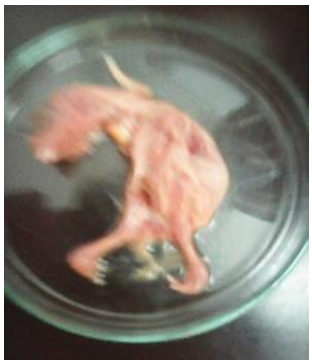

Figure 3. An embryo has arthrogryposis in case of CAM inoculation.

2. Results of EM:

***2.1.* Results of negative staining EM:**

Enveloped herpesviral particles were detected in the suspension of detached tongue epithelial and isolates (Fig. 4, 5, 6)

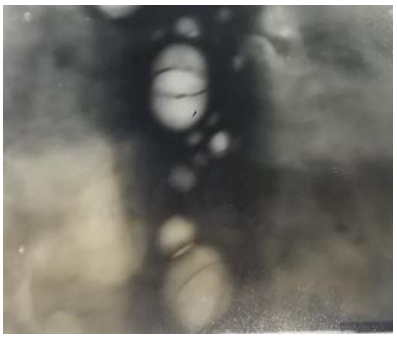

Figure 4. Herpesviral particles in tongue epithelium, showed, envelops, tegument, nucleocapsid and proteins of different sizes by negative staining technique.

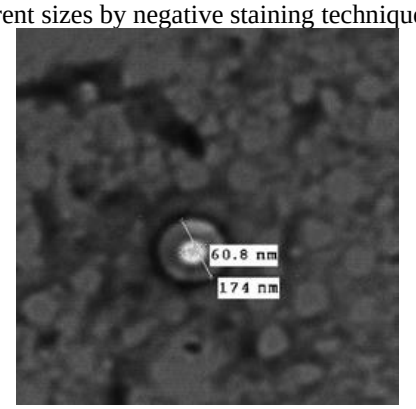

Figure 5. Herpesviral particle (nucleocapsid, envelope and tegument in between) in suspension of isolates by negative staining technique.

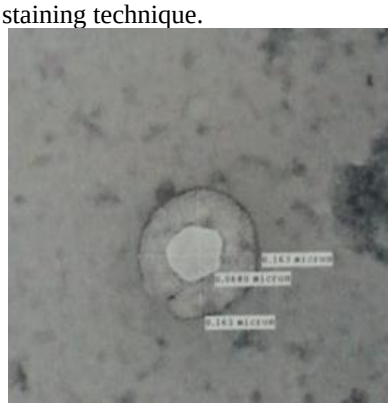

Figure 6 . Typical herpesviral particle after isolation showed irregular tegument between icosahedral capsid and envelop by negative staining technique.

2.2. Results of thin-sectioning technique EM:

Cross section 0f herpes virions were detected in thin sectioning of skin lesions (Fig. 7) and CAM (Fig. 8). The electron micrograph shows irregular tegument lies between the icosahedral capsid and the envelope (Fig. 8).

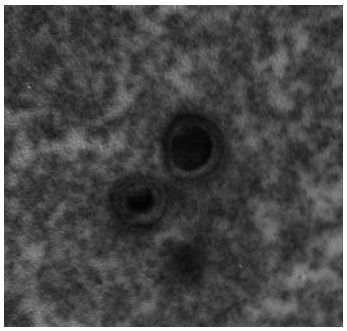

Figure 7. Cross section in herpesvirus particles in skin lesion by thin-sectioning technique.

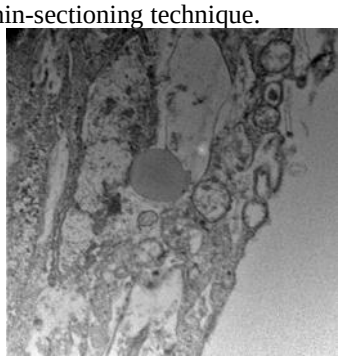

Figure 8 . Cross section in herpesviral particles in CAM by thin-sectioning technique.

3. Results of Real-time PCR:

Results of Real-Time PCR are positive for OvHV-2 and negative for the other suspected pathogens.

4. Results of gross pathology:

Vasculitis (arteritis and phlebitis) might be present which were tortuous, very prominent and had thickened walls (Fig. 9). Congestion degraded from mild to severe appeared in different tissues including mucous membranes and skeletal muscles (Fig. 10), lymph nodes and different organs including lung (Fig. 11) which might have also severe congestion in addition to grey hepatization and sometimes showed thrombosis (Fig. 12), liver is congested (Fig. 13) sometimes was enlarged, showed inflammation, friable or had tiny white flat or elevated spots (Fig. 14), spleen may be enlarged and, heart muscle have degeneration and may appeared flabby, pericardium has thickening of fibrinous inflammation, lymphocytic infiltration, thrombosis , or highly congested (Fig.15), tongue is haemorrhagic and ulcerated (Fig. 16), haemorrhagic and / or ulcerative oesophagitis, abomasum is congested (Fig. 17), kidneys sometimes showed infarction and / or white spots, urinary bladder is highly congested(Fig. 18), intestine is congested and dilated (Fig. 19) and testicles were shown congested in some cases in addition to haemorrhages in the parenchyma of the two testicles. Some cases showed cyanosed organs and muscles (Fig.20).

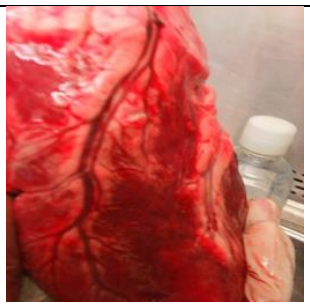

Figure 6. Prominent and thickened wall blood vessels and tortuous.

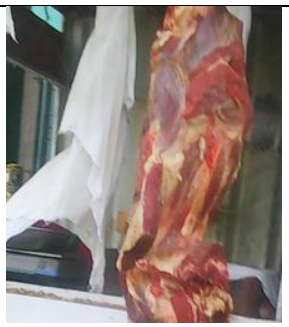

Figure 7. Congested skeletal muscles with yellowish coloration of fascia and fat.

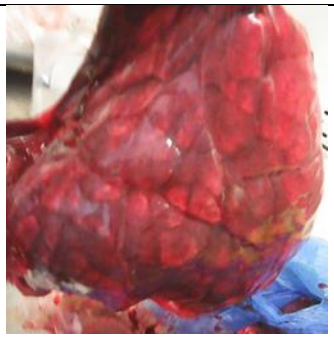

Figure11. Lung with grey hepatization

Figure 8. Severely congested lung with thrombosis.

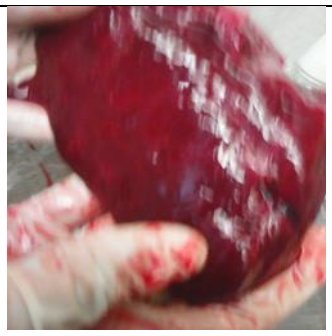

Figure13. Congested liver.

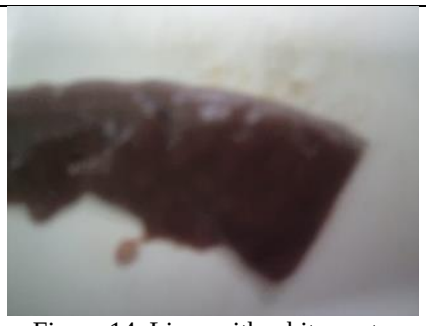

Figure 14. Liver with white spots.

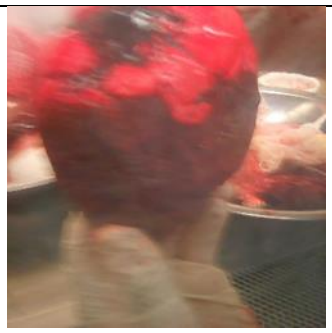

Figure 15. Highly congested heart.

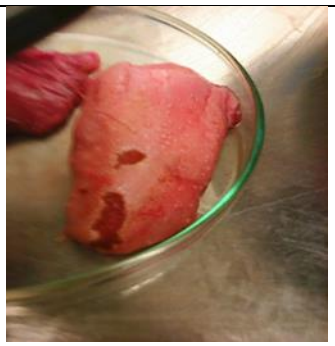

Figure 16 . Congested and ulcerated tongue.

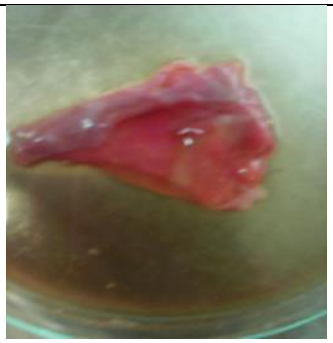

Figure 17. Highly congested abomasum.

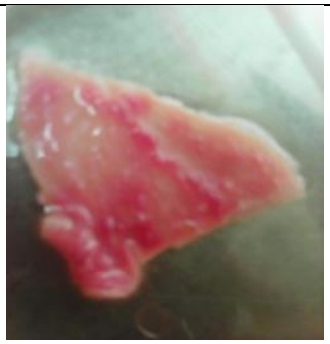

Figure 18. Highly congested urinary bladder.

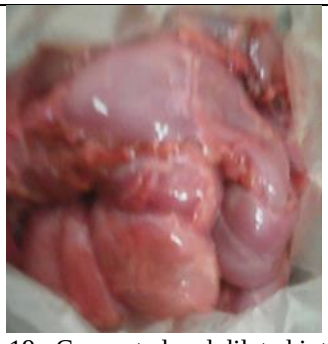

Figure 19 . Congested and dilated intestine.

Figure 20 . Cyanosed skeletal muscles.

4. **Discussion:**

Ovine herpesvirus 2 caused an outbreak in Egypt, in 2012 and it still causes sporadic, outbreak with sometimes mortalities in different ages in addition to mouth, hoof, or skin lesions, respiratory, ocular, or nervous symptoms, abortions, and diarrhea as well.

Our results denoted that newborn lambs were susceptible to infection with OvHV-2 like adults and this result disagreed with results obtained by (46) who mentioned that lambs do not become infected before 3 months. Adult sheep in our study showed symptoms and this result disagrees with (11) who mentioned that sheep are carriers only. Necrotizing Vasculitis could explain blood sepsis, cyanosis (dark purple) of muscles and organs in some cases may be due to low oxygen percent in cases with pulmonary problems or congestive heart failure which may explain also detected cases of hypothermia. Hemorrhagic muscles and organs could explain hepatic problems and increase in the vascular permeability which may be caused by IL-6. At the same time, some organs were very pale may be due to some pathological conditions such as liver cirrhosis, nephrotic syndrome, acute myocardium infarction, or malignancies that may be the cause of decreased transferrin and dysregulated IL-6 (47).

Due to all cells are susceptible to infection with OvHV-2, red blood cells could be infected, and the virus produces in them vacuoles (48) and the virus causes their destruction resulting in hemophilia which may be misdiagnosed as Babesia, but this organism has a chractrestic shape.

Vitamin D causes bone mineralization and controls the absorption of calcium and phosphorus from the intestine as well as normal parathormone functions where parathyroid hormones and mineralocorticoids control the metabolism of calcium and phosphorus in addition to maintaining electrolyte balance (49). However, the sun's ultraviolet rays convert-7 dehydrocholesterol present in the skin into vitamin D 3 which is not an active form. 1, 25-dihydrocholecaciferol is the active form, which is achieved by hydroxylation in 1, 25-dihydrocholecaciferol by adding two hydroxyl groups in position 25 in the liver and the other in position 1 in the kidney (49). So, abnormalities related to this factor can result from an infection of the thyroid and consequently parathyroid, liver, and kidney by the MCF-causing virus (50; 8).

Isolation could be achieved by using tissue culture cells, laboratory animals, and embryonated chicken eggs (45). Although most papers mentioned that OvHV-2 is difficult to be isolated, it could be isolated easily depending on the number of enveloped viral particles present in a sample where glycoproteins embedded in the envelope are responsible for attachment to cell receptors. I found that baby hamster kidney (BHK 21) cell line is the most sensitive cells and OvHV-2 increases acidity of tissue culture i.e., turns the maintenance medium acidic (unpublished data).

SPF-ECE is considered to be the most suitable, specific, and sensitive vivo for viral isolation because cell cultures (primary, secondary, and established cell lines) and sera of animal or human origin may be latently infected with a herpes virus and there was an observation that in the last years, control cells sometimes showed cytopathic effect (CPE). Also, laboratory animals are susceptible to infection with herpesvirus. So, they may have latency and their use for viral isolation, in general, can give a false result. However, SPF-ECE, yolk sac, and chorioallantoic routes were used for isolation (8; 44) in this study and were sensitive and arthrogryposis detected in an embryo was a surprise. Isolation is noticed to be more sensitive if cells are associated with infected lymphocytes and this result agrees with those obtained by (51)

MCF could be identified using EM as a typical herpes virus (50 and 4). OvHV-2 when detected with EM shows the presence of enveloped herpesviral particles, capsids, and proteins (which are more than 70 proteins) of different sizes. EM is known as the method of choice to show details of the virus up to the molecular level because of its very high resolution. The negative staining EM technique permits complete viral particle detection in the shape of three-dimensional interpretation. Embedding and thin-sectioning EM technique completes negative staining results from details obtained from sections of the virus. (45).

Due to tegument being an amorphous substance, there is variability in the size of the herpesviral particles. Typical herpesviral particles were detected as stated by (4, 44, and 50). In case of outbreaks or cases of unclear diagnosis by other screening methods, negative staining EM can be used for preparing and detecting a specimen in less than an hour in addition to observation of all viral particles found in the specimen in addition to unsuspected ones. In some situations when the viral particles are not opened to detect their internal structure, thin-sectioning EM is applied (52).

Widespread PCRs (conventional and real-time) were used, for years using primers from G-protein-coupled chemokine receptor gene, as for molecular diagnosis of pox virus, (53) although gamma herpesvirus such as OvHV-2 and EBV could be diagnosed by PCR with the same primers where gamma herpesvirus as poxvirus obtain their chemokine receptor genes from their hosts during evolution (54), so fallacy may result if we do not identify the viral family with EM at first. Real-time PCR was positive for all suspected samples because it is highly sensitive and

specific for the detection of low copy numbers of OvHV-2 as mentioned by (55).

In this study, I want to through light on the role of IL-6 which may be produced in some conditions of MCF because of the similarity between OvHV-2 and EBV as mentioned before. IL-6 is increased because of cortisol elevation as a response to stressors, it can inhibit insulin receptor signal transduction and the action of insulin. Also, IL-6 is produced as infection and tissue injuries response like host defence and goes to the liver to produce acute phase protein such as C reactive protein (CRP), hepcidin, serum amyloid A, fibrinogen, haptoglobin, and alpha 1-antichymotrypsin. IL-6 reduces the production of fibronectin, albumin, and transferrin. Also, it can cause the induction of malignant B cells. So, if it is dysregulated by continuous synthesis, chronic inflammation and immune disorders will result. (56)

In conclusion, an accurate diagnosis for OvHV-2 could be achieved by using EM to identify the herpesviral family. Clinical symptoms, gross pathology, isolation, and PCR techniques after EM examination could complete its diagnosis.

Recommendations:

Due to OvHV-2 and EBV being extremely near to each other and maybe the same virus, IL-6, CRP, vitamin D, and B12, zinc, selenium, magnesium, phosphorous, calcium, iron, and glucose measurements in addition to bilirubin, liver, and kidney function tests and CBC as well. Testing of animals having vitiligo especially cattle and equines for OvHV-2. Special attention and hard work must be concentrated on Ov5 ORF and BILF1 which are highly suspected of having transformation potentiality and tumor development and metastasis. Detection of all genes or ORFs for OvHV-2 and EBV, their coupled m RNAs in addition to their coupled proteins and know which of them possess sequence and function homology with the host proteins. Retrospective studies on the previously diagnosed RNA viruses and comparing their sequences with all m RNAs of OvHV-2 and EBV transcribed on their different genes or ORFs as well as retrospective studies on the previously detected DNA viruses and comparing their sequences with all genes or ORFs of OvHV-2 and EBV to exclude fallacy in their discovery. Sequencing for the whole gene of the B glycoprotein of any suspected herpesvirus to avoid false results. Examination of other species, specially avian and fish for natural infection with OvHV- 2. Considering OvHV – 2 as a cause of leukosis in susceptible species.

References

[1]. Liggitt, H. D., & DeMartini, J. C. (1980). The Pathomorphology of Malignant Catarrhal Fever: II. Multisystemic Epithelial Lesions. *Veterinary pathology*, *17*(1), 73-83.

[2]. OIE (2020): World organization for animal health, malignant catarrhal fever.

[3]. Meier-Trummer, C. S., Ryf, B., & Ackermann, M. (2010). Identification of peripheral blood mononuclear cells targeted by Ovine herpesvirus-2 in sheep. Veterinary microbiology, 141(3-4), 199-207.

[4]. Zaki, A. A. M., El-Said, H. M., Abd El-Aziz, A., Bastawecy, I. M., Abd El-Wahab, S. A., & El-Sayed, M. M. (2016). Field study on malignant catarrhal fever (MCF) in Egypt. Life Sci. J, 13, 83-97.

[5]. Costa, É. A., Bomfim, M. R. Q., Da Fonseca, F. G., Drumond, B. A., Coelho, F. M., Vasconcelos, A. C., & Resende, M. (2009). Ovine herpesvirus 2 infection in Foal, Brazil. Emerging Infectious Diseases, 15(5), 844.

[6]. Goerigk, D., & Merbach, S. (2012). Clinical and pathological findings in an alpaca suffering from malignant catarrhal fever. Tierarztliche Praxis. Ausgabe G, Grosstiere/nutztiere, 40(2), 112-118.

[7]. CSFPH (2012): Malignant catarrhal fever, the center for food security and public health, last updated: November 2012. IOWAState University.

[8]. Blood, D.C.; O.M. Radostits, and J. A.Henderson, (1983): Veterinary Medicine, sixthedition.pp 750-754.

[9]. Saura Martinez, H. (2020). Insights into the role of latent virus in the vasculitis in sheep-associated malignant catarrhal fever (Doctoral dissertation, University of Zurich).

[10]. Abu Elzein, E. M. E., Housawi, F. M. T., Gameel, A. A., Al-Afaleq, A. I., & El-Bashir, A. M. (2003). Sheep-Associated Malignant Catarrhal Fever Involving 3–5-Week-Old Calves in Saudi Arabia. Journal of Veterinary Medicine, Series B, 50(2), 53-59.

[11]. OIE, A. (2008). Manual of diagnostic tests and vaccines for terrestrial animals. Office international des epizooties, Paris, France, 1092-1106.

[12]. Baxter, S. I. F., Pow, I., Bridgen, A., & Reid, H. W. (1993). PCR detection of the sheep-associated agent of malignant catarrhal fever. Archives of virology, 132(1), 145-159.

[13]. Li, H., McGuire, T. C., Müller-Doblies, U. U., & Crawford, T. B. (2001). A simpler, more sensitive competitive inhibition enzyme-linked immunosorbent assay for detection of antibody to malignant catarrhal fever viruses. Journal of veterinary diagnostic investigation, 13(4), 361-364.

[14]. Brown, C. and A Torres (2008): Foreign Animal Diseases (gray book). Seventh Edition
[15]. Headley, S. A., Pimentel, L. A., Oliveira, V. H. S., Toma, H. S., Alfieri, A. F., Carvalho, A. M., ... & Alfieri, A. A. (2015). Transplacental transmission of ovine herpesvirus 2 in cattle with sheep-associated malignant catarrhal fever. Journal of Comparative Pathology, 153(4), 206-211.
[16]. Russell, G. C., Stewart, J. P., & Haig, D. M. (2009). Malignant catarrhal fever: a review. The Veterinary Journal, 179(3), 324-335.
[17]. OIE (2009): Technical Disease Cards.
[18]. Luvizotto, M. C. R., Ferrari, H. F., & Cardoso, T. C. (2010). Malignant catarrhal fever-like lesions associated with ovine herpesvirus-2 infection in young calves (Bos indicus): a case report. Journal of Venomous Animals and Toxins including Tropical Diseases, 16, 178-185.
[19]. O'Toole, D., Li, H., Miller, D., Williams, W. R., & Crawford, T. B. (1997). Chronic and recovered cases of sheep-associated malignant catarrhal fever in cattle. Veterinary Record, 140(20), 519-524.
[20]. Penny, C. (1998). Recovery of cattle from malignant catarrhal fever. Veterinary Record, 142(9).
[21]. Simon, S., Li, H., O'Toole, D., Crawford, T. B., & Oaks, J. L. (2003). The vascular lesions of a cow and bison with sheep-associated malignant catarrhal fever contain ovine herpesvirus 2-infected CD8+ T lymphocytes. Journal of General Virology, 84(8), 2009-2013.
[22]. Rosenkilde, M. M., Smit, M. J., & Waldhoer, M. (2008). Structure, function and physiological consequences of virally encoded chemokine seven transmembrane receptors. British journal of pharmacology, 153(S1), S154-S166.
[23]. Wozniakowski, G., & Samorek-Salamonowicz, E. (2015). Animal herpesviruses and their zoonotic potential for cross-species infection. Annals of Agricultural and Environmental Medicine, 22(2).
[24]. Bastawecy, I. M. (2018). The most important herpesviral infections in animals and humans. J Hum Virol Retrovirol, 6(2), 66-67.
[25]. Tseng, C. K., Ho, C. T., Hsu, H. S., Lin, C. H., Li, C. I., Li, T. C., ... & Lin, W. Y. (2013). Selenium is inversely associated with interleukin-6 in the elderly. The Journal of nutrition, health & aging, 17(3), 280-284.
[26]. Stomatitis, B. P., Fever, M. C., Fever, R. V., Fever, E., Rhinotracheitis, I. B., & Vibriosis, B. G. (1981). Diseases of Cattle in the Tropics. Current Topics in Veterinary Medicine and Animal Science, 6.
[27]. Dunowska, M., Letchworth, G. J., Collins, J. K., & DeMartini, J. C. (2001). Ovine herpesvirus-2 glycoprotein B sequences from tissues of ruminant malignant catarrhal fever cases and healthy sheep are highly conserved. Journal of General Virology, 82(11), 2785-2790.
[28]. OIE (2013). Malignant catarrhal fever: Manual ofdiagnostic tests and vaccines for terrestrial animals, chapter 2.4.15. office international des Epizooties(OIE), paris.
[29]. Blood, D. C., Radostits, O. M., Arundel, J. H., & Gay, C. C. (1989). Veterinary Medicine, a textbook of the diseases of cattle, sheep, pigs, goats and horses. Bailliere Tindall.
[30]. Hoffmann, D., Soeripto, S., Sobironingsih, S., Campbell, R. S. F., & Clarke, B. C. (1984). The clinico-pathology of a malignant catarrhal fever syndrome in the Indonesian swamp buffalo (Bubalus bubalis). Australian veterinary journal, 61(4), 108-112.
[31]. Hart, J., Ackermann, M., Jayawardane, G., Russell, G., Haig, D. M., Reid, H., & Stewart, J. P. (2007). Complete sequence and analysis of the ovine herpesvirus 2 genome. Journal of General Virology, 88(Pt 1), 28-39.
[32]. Jayawardane, G., Russell, G. C., Thomson, J., Deane, D., Cox, H., Gatherer, D., ... & Stewart, J. P. (2008). A captured viral interleukin 10 gene with cellular exon structure. Journal of General Virology, 89(10), 2447-2455.
[33]. Moore, K. W., Vieira, P., Fiorentino, D. F., Trounstine, M. L., Khan, T. A., & Mosmann, T. R. (1990). Homology of cytokine synthesis inhibitory factor (IL-10) to the Epstein-Barr virus gene BCRFI. Science, 248(4960), 1230-1234.
[34]. Haig, D. M., Grant, D., Deane, D., Campbell, I., Thomson, J., Jepson, C., ... & Russell, G. C. (2008). An immunisation strategy for the protection of cattle against alcelaphine herpesvirus-1-induced malignant catarrhal fever. Vaccine, 26(35), 4461-4468.
[35]. Cohen, J. I. (2009). Optimal treatment for chronic active Epstein-Barr virus disease. Pediatric transplantation, 13(4), 393.
[36]. Lyngaa, R., Nørregaard, K., Kristensen, M., Kubale, V., Rosenkilde, M. M., & Kledal, T. N. (2010). Cell transformation mediated by the Epstein–Barr virus G protein-coupled receptor BILF1 is dependent on constitutive signaling. Oncogene, 29(31), 4388-4398.
[37]. Tsutsumi, N., Qu, Q., Mavri, M., Baggesen, M. S., Maeda, S., Waghray, D., ... & Garcia, K. C. (2021). Structural basis for the constitutive activity

and immunomodulatory properties of the Epstein-Barr virus-encoded G protein-coupled receptor BILF1. Immunity, 54(7), 1405-1416.

[38]. Zhao, J., Deng, Y., Jiang, Z., & Qing, H. (2016). G protein-coupled receptors (GPCRs) in Alzheimer's disease: a focus on BACE1 related GPCRs. Frontiers in aging neuroscience, 8, 58.

[39]. Bastawecy, I. M. (2021). Mode of infection and some diseases caused by Epstein Barr virus: The most ubiquitous, common malignant and characteristic virus. J Hum Virol Retrovirology, 9(1), 6-9.

[40]. Bastawecy, I.M.; FFreeg, M.A. and Abdelfattah, M.A. (2022): Role of Epstein-Barr viral genes in lytic and latent states. J Am Sci 2022; 18 (5): 10-15.

[41]. McKnight, S. L., & Kingsbury, R. (1982). Transcriptional control signals of a eukaryotic protein-coding gene. Science, 217(4557), 316-324.

[42]. Kriegler, M. (1990). Gene transfer and expression: a laboratory manual. Springer.

[43]. Taus, N. S., Herndon, D. R., Traul, D. L., Stewart, J. P., Ackermann, M., Li, H., ... & Brayton, K. A. (2007). Comparison of ovine herpesvirus 2 genomes isolated from domestic sheep (Ovis aries) and a clinically affected cow (Bos bovis). Journal of general virology, 88(1), 40-45.

[44]. Versteeg, J. (1990). A colour Atlas of Virology Wolfe Medical Publications Itd. London, England.

[45]. Payment, P., & Trudel, M. (1993). Methods and techniques in virology. Marcel Dekker.

[46]. Li, H., Cunha, C. W., Davies, C. J., Gailbreath, K. L., Knowles, D. P., Oaks, J. L., & Taus, N. S. (2008). Ovine herpesvirus 2 replicates initially in the lung of experimentally infected sheep. Journal of General Virology, 89(7), 1699-1708.

[47]. Nemeth, E., Rivera, S., Gabayan, V., Keller, C., Taudorf, S., Pedersen, B. K., & Ganz, T. (2004). IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. The Journal of clinical investigation, 113(9), 1271-1276.

[48]. Mushi, E. Z., & Rurangirwa, F. R. (1981). Epidemiology of bovine malignant catarrhal fevers, a review. Veterinary research communications, 5(1), 127-142.

[49]. Singh SP (2003): Viva in Biochemistry. IncludingMolecular Biology and Biotechnology, secondedition, New Delhi, India

[50]. Castro, A. E., & Heuschele, W. P. (1992). Veterinary diagnostic virology: a practitioner's guide.

[51]. Sangueza-Acosta, M., & Sandoval-Romero, E. (2018). Epstein-Barr virus and skin. Anais brasileiros de dermatologia, 93, 786-799.

[52]. Möller, L., Schünadel, L., Nitsche, A., Schwebke, I., Hanisch, M., & Laue, M. (2015). Evaluation of virus inactivation by formaldehyde to enhance biosafety of diagnostic electron microscopy. Viruses, 7(2), 666-679.

[53]. Le Goff, C., Lamien, C. E., Fakhfakh, E., Chadeyras, A., Aba-Adulugba, E., Libeau, G., ... & Albina, E. (2009). Capripoxvirus G-protein-coupled chemokine receptor: a host-range gene suitable for virus animal origin discrimination. Journal of general virology, 90(8), 1967-1977.

[54]. Rosenkilde, M. M. (2005). Virus-encoded chemokine receptors–putative novel antiviral drug targets. Neuropharmacology, 48(1), 1-13.

[55]. Pinheiro de Oliveira, T. F., Laguardia-Nascimento, M., Xavier, F. G., do Amaral Pinto, C., Ferreira, L. R., de Castro Campos de Souza, I., ... & Fonseca Junior, A. A. (2019). Quantification of ovine herpesvirus 2 by digital PCR in an outbreak of malignant catarrhal fever. Archives of Virology, 164(12), 3045-3050.

[56]. Bastawecy, I.M.; and Abdelfattah, M.A. (2021): Cytokines and their roles in pathological conditions. J Am Sci 2021; 17 (4): 19-25.

9/22/2022