Field Study on Malignant Catarrhal Fever (MCF) In Egypt

Zaki, A. A. M[1].; El-Said, H. M. [1]; Abd El-Aziz[1], A.; Iman, M. Bastawecy[2]; Shahira, A. Abd El-Wahab[3] and El-Sayed, M. M[1].

[1]Deptartment. of Internal Medicine and Infectious Diseases, Faculty of Veterinary Medicine Cairo University, Egypt
[2]Deptartment of Virology, Animal Health Research Institute, Dokki, Giza, Egypt
[3]Deptartment. of Virology, Faculty of Veterinary Medicine, Suez Canal University, Egypt
Iman_baz@yahoo.com

Abstract: Malignant catarrhal fever (MCF) is a lymphoproliferative multisystemic fatal syndrome of many domestic and wild animals. The present study was conducted on animals of different species (cattle, buffalos and sheep). The clinical examination revealed high persistent fever, corneal opacity, mucoprulent oronasal discharges, mucosal lesions and ulcerative skin lesions. Examined sheep showed no clinical signs. Peripheral blood leukocytic (pb1) samples were collected for laboratory investigations. Semi nested polymerase chain reaction (PCR) assay have been used. Sequencing and phylogenetic analysis were performed for the positive PCR products. Positive staining electron microscopy (EM) and histopathology were also performed. Ovine herpesvirus 2 (OvHV-2) DNA was detected in PBL of 25 animals (12 cattle, 2 buffaloes and 11 sheep). The phylogenetic analysis of the OvHV-2 PCR products revealed 100% identity with the OvHV-2 strains of Brazil, USA and India. The positive staining EM detected herpesviral particles. The histopathological examination showed pansystemic vasculitis with lymphocytic infiltration in lymphoid and non lymphoid organs.
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Keywords: Malignant catarrhal fever, histopathology, electron microscopy, semi-nested PCR, sequencing and phylogenetic analysis.

1. Introduction:

Malignant catarrhal fever (MCF) was localized in Europe for over a century, under the name of "Kopfkrankheit " (means head disease) which referred to a sporadic, non contagious disease to be distinguished from Rinderpest **(Plowright, 1986).**

MCF has been expressed as a lymphoroliferative multi-systemic fatal syndrome of many ungulate species **(Schock and Reid,1996).** The syndrome has a worldwide distribution and has been reported in 33 species including cattle, buffalo, swine, equine and many deer species **(Metzler,1991; Reid, 1992; Loken *et al.,* 1998 and Costa *et al.,* 2009).**

Two forms of MCF were originally illustrated. The first form was reported in Africa as outbreaks in cattle infected through the inapparent carriers, Wildebeest **(Ploweright *et al.,* 1960**). Wildebeest-derived MCF (WD-MCF) is a distinct problem with pastoralists in Africa, where wildebeest are located **(Cleaveland *et al.,* 2001).** WD-MCF is also a significant problem in zoological populations that contain wildebeest species **(Meteyer *et al.,* 1989).**

Second form is referred as sheep-associated MCF (SA-MCF). It was initially observed in Europe wherever sheep and cattle are Kept together **(Wyssmann,1934 and Giangaspero *et al.,* 2013).**

The wildebeest type (WD-MCFV) was previously designated as bovid herpesvirus-3 **(Liggitt and De Martini, 1980a and Ludwig and Hanns, 1984**). Then, the virus was classified as an Alcelaphine herpesvirus 1 (AIHV-1), as its reservoirs are wildebeest, hartebeest and topi which belong to Alcelaphinae subfamily **(Mushi and Rurangiruta, 1981).**

MCF usually appears sporadically but outbreaks can be induced by both SA-MCF and WD-MCF (**Pierson *et al.,* 1973; James *et al.,* 1975; Schultheiss *et al.,* 2000; Holliman *et al.,* 2007 and Swai *et al.,* 2013).**

The sheep associated type was reported in many countries especially those with a high sheep population such as great Britain **(Sharpe *et al.,* 1987**). Ireland (**Hamilton, 1990**), Spain **(Yus *et al.,* 1999),** Belgium (**Pardon *et al.,* 2009**), New Zealand (**Thompson and Beckett, 1987**), Australia (**Duncan,1956**); Canada (**Reid and Robinson,198**7), Japan (**Fujimoto *et al.,* 1958**). Indonesia (Daniels ***et al.,*** 1988), India (Singh et al, 1979), North and South America **(Rech *et al.,* 2005 and Li *et al.,* 2006).**

First outbreak caused by SA-MCFV in the Middle east was reported in Israel (**Brenner *et al.,* 2002**) followed by Saudi **Arabia (Abu Elzein *et al.,* 2003),** Turkey (**Dabak and Bulut, 2003**), Syria **(OIE Handistatus, 2005),** Iran (**Momtaz *et al.,***

2009), Egypt (**Bastawecy and Abd El-Samee,2012**) and Jordan **(Ababneh *et al.*, 2014).**

MCF is a systemic disease of cattle caused by multiple virus belonging to new genus macavirus (previously known as Rhadinovirus) of the subfamily Gamma herpesvirinae, family Herpesviridae **(Li *et al.*, 2005 and Davison *et al.*, 2009).** This genus includes at least ten viruses **(Crawford *et al.*, 2002 Li *et al.*, 2005)** including the alcelaphine herpesvirus 1 (AIHV-1) which is the causative agent of wildebeest (or African)- associated MCF (**Plowright *et al.*, 1960**) and ovine herpesvirus 2 (OvHV-2) which is the causative agent of sheep-associated MCF (SA-MCF) which is worldwide because it is endemic in most sheep **(OIE,2008).**

The analysis revealed that OvHV-2 and AIHV-1 are related more distantly to other gamma-herpesvirus, such as Kaposi, sarcoma-associated herpesvirus (KSHV), herpesvirus saimiri (HVS), Murid herpesvirus 4 (MuHV-4) and Epstein- Barr virus (EBV) **(McGeoch *et al.*, 2006 and Hart *et al.*, 2007**).

The clinical picture of MCF was initially divided into four forms; peracute form, intestinal form, mild form and "eye and head" form (**Gotze *et al.*, 1930**) where "eye and head" form is considered the most frequent type (**Shih *et al.*, 1989 and Kumar *et al.*, 2014**). Other researchers consider this classification of little value as there is much overlap between clinical forms and mixed clinical symptoms could be occurred in the same affected animal (**Selman *et al.*, 1981**).

Incubation period varies from 2-3 weeks to 9-10 months with an average of 30 days (**Schofield and Bain, 1941** and **O'toole and Li, 2014)** and could be differed according to infecting viral dose **(Gailbreath *et al.*, 2008).** MCF is fatal in majority of clinical cases with a mortality rate up to 90 -100 % in most reports (**Rafoth and Bruce, 1975; Mushi and Wafula, 1983; Schuller *et al.*, 1990** and **O'toole *et al.*, 1997),** however, clinical recovery (**Milne and Reid, 1990 and O'Toole et al., 1997**), chronicity **(O'Toole *et al.*, 1997**) and latent infection (**Powers *et al.*, 2005** and **Palmer *et al.*, 2013)** have been recorded.

Given names to MCF clinical forms are usually descriptive (Cutaneous lesions "skin form", nervous signs" nervous form", gastrointestinal disorders " alimentary form", eye and head affection "eye and head form", mild clinical symptoms "mild form". However, multiple mixed signs could be developed in one or many animals in the same herd **(Robertson, 1960; Hoffmann *et al.*, 1984; Brenner *et al.*, 2002; Li *et al.*, 2006** and **OIE, 2013).**

It is recommended to use the laboratory diagnostic aid to clarify such infections, especially when recent clinical diseases have been encountered among the Egyptian livestock. The laboratory diagnosis is the key to differentiate between confusing diseases and it is the best way for accurate diagnosis.

Virological techniques, histopathology, positive staining EM, PCR, sequencing and phylogenetic analysis were used to confirm the identity of the etiological agent.

The aim of this work is to confirm the clinical occurrence of MCF, to investigate the MCF hotspots in the Egyptian governorate and to determine the most prevalent strain of SA-MCF virus (OvHV-2) among the clinical cases throughout sequencing and phylogenetic analysis.

Material and methods:

Material:

Animals:

Twelve cattle and 2 buffalos (showing symptoms simulating MCF) with 31 sheep (showing no clinical signs and of different ages) belong to Damietta, El-Mansoura, El-Gharbia, El-Sharkia, Kafr El-shiekh, Giza, Fayoum, Beni-Suef, El-Menia and Assuite governorates were observed for the presence of MCF clinical signs in ill animals (Cattle and buffalos) and detection of the virus in carriers (sheep) in the years 2013-2014.

Samples:

Whole blood samples:

Whole blood samples were taken from the tested animals in EDTA tubes (for DNA extraction) under complete aseptic conditions.

Tissue samples from a slaughtered animal (buffalo calf):

Tissue samples were taken during the post-mortem examination of the slaughtered animal; including heart, brain, lungs, spleen, lymph nodes and kidneys. All samples were placed in formalin (10%) for histopathological investigation.

Material used for DNA extraction and PCR assay:

QIAamp blood Mini Kit (Qiagen, Germany) for DNA extraction from fluids (Blood/buffy coat suspension).

Taq PCR core kit (Qiagen, Germany) was used for OvHV-2 semi-nested PCR reaction.

Ready to use PCR master mix.

Primers for (SA-MCF) virus (OvHV-2):

Three primers were designed for amplification of a specific region of tegument protein coding gene of OvHV-2 in semi-nested PCR reaction (**Baxter *et al.*, 1993).** They were synthesized by Metabion International AG (Germany). The sequence of used primers and the expected length of PCR product were described in table(1).

Methods:

Molecular biology diagnosis for OvHV-2 by PCR according to **Baxter *et al.*, (1993).**

This PCR protocol is based on semi-nested PCR assay. Three nucleotide primers are used to amplify a specific region of tegument protein coding gene of OvHV-2 in semi-nested PCR reaction which was performed in two amplification steps. The primary amplification step was done using primer 556 and primer 755 to produce an amplicon size 422 bp. The obtained amplicons were used as DNA template for the second step. The second amplification step was performed using primer 556 and primer 555 to produce an amplicon size 238 bp.

First amplification step (422 bp):

The PCR reaction formulation and sequence of primers are illustrated in table (2).

Table (1): Sequence of used primers and expected length of OvHv-2 PCR product.

Primers	Primer sequence	Expected length	Reference
556	5'- AGT CTG GGG TAT ATG AAT CCA GAT GGC TCTC -3'	First round (422bp)	(Baxter 1993)
775	5'- CGA TTG CGT AGC ACC AGT AG -3'	Second round (238bp)	
555	5'- TTC TGG GGT AGT GGC GAG CGA AGG CTT C -3'		

Table (2): PCR reaction formulation and sequence of primers used in primary amplification of OvHV-2

Reagent	Volume (50 µl)
10x CoralLoad PCR Buffer	5µl
dNTP mix (10mM each)	2µl
Primer 556 5'- AGT CTG GGG TAT ATG AAT CCA GAT GGC TCT C -3'	1 µl
primer 755 5'- CGA TTG CGT AGC ACC AGT AG -3'	1 µl
Q solution	10 µl
Taq DNA polymerase	0.25µl(1.25 unit/reaction)
PCR grade water	25.75 µl
DNA template	5µl

The reaction tubes of primary amplification were spun down and placed in thermo-cycler for amplification. The thermal cycling conditions of the first round are described in table (3).

Table (3): Thermal cycling conditions of first round in the semi-nested PCR assay for OvHV-2

Steps	Number of Cycles	Temperature and Time
Initial denaturation	1X	94°C for 5 min
Denaturation	35X	94°C for 1 min
Annealing		60°C for 1 min
Extension		72°C for 2min
Final Extension	1X	72°C for 7min

Second amplification step (238 bp):

PCR product made throughout the first round (422bp) was used as a template for the second one.

The reaction tubes were spun down and placed in thermal cycler. The thermal cycling conditions of the second round and sequence of used primers were described in table (4).

Agarose gel electrophoresis for visualization of the PCR products:

Each PCR product was put in wells of 1% agarose gel stained with Ethidium bromide.

Sequencing of PCR product of OvHV-2 and phylogenetic analysis:

Five PCR products of the first amplification were submitted for sequencing to confirm the semi-nested assay results. The sequencing process used the same primers (primer 556 and primer 755) which had been used in the first PCR amplification step. The PCR products were sequenced in Macrogen (South Korea).

Table (4): Thermal cycling conditions and sequence of primers used for second round PCR assay for OvHV-2

Sequence of primers used in the second round	Steps	Number of Cycles	Temperature and time
Primer 556 5' AGT CTG GGG TAT ATG AAT CCA GAT GGC TCT C- 3'	Initial denaturation	1X	94°C for 5min
primer 555 5'- TTC TGG GGT AGT GGC GAG CGA AGG CTT C -3'	Denaturation Annealing Extension	35X	94°C for 1min 60°C for 1min 72°C for 2min
	Final Extension	1X	72°C for 7min

Both of the first and second PCR products were analysed by gel electrophoresis.

Positive staining electron microscopy (EM):

Positive staining EM was conducted according to **Payment and Trudel (1993)** and **Bozzola and Russell (1991)** on 0.5 to 1cm samples from lymph nodes and lungs of the slaughtered buffalo calf that was suspected to be infected with MCF.

Histopathological examination:

Histopathological examination was performed according to **Chauhan and Agarwal (2008)** on different tissue samples collected from the slaughtered animal which was suspected to be infected with MCF. The tissue samples were taken from different body organs including lung, kidney, spleen, liver, brain and lymph nodes during post-mortem examination where gross pathological lesions were also observed in multiple organs and recorded.

3. Results:

Field diagnosis of clinical cases associated with MCF:

Field diagnosis of animals with MCF was based on clinical examination, detection of typical clinical signs and characteristic case history for the disease. History of the presence of recently lambing ewes in the same premises of the clinically affected cattle and buffaloes may imply the infection of MCF with OvHV-2. Typical clinical signs for MCF including fever, lymphadenopathy, skin lesions, mucosal lesions, corneal opacity, edema in lower parts were presented by cattle and buffaloes subjected for this study. No clinical signs were detected in the examined sheep.

Persistence of high fever (40.5 – 41.5) is a characteristic observation in all clinical cases of cattle (12) and buffaloes (2) associated with MCF. Persistent fever was noted clinically in three cases, 2 buffaloes (El-Gharbia and El-Menia governorates) and one cattle (Kafr El-Sheikh governorate). They had been kept under observation for about 6-8 weeks. It is noteworthy that fever respond to the antipyretic medication, but flare up was occurred following the cessation of the medical treatment. The three animals were slaughtered as the treatment with antibiotics and antipyretics failed to stop their deterioration. Bilateral corneal opacity was fully noted in both species, buffalo (Fig.1) and cattle (Fig.2). Skin lesions were observed in regions of the neck, back and trunk. They were appeared as hypersensitivity like reaction (Fig.3 and Fig.4) and/or ulcerative dermatitis (Fig.5). Abnormal symmetrical skin pigmentation was observed in a buffalo calf (El-Gharbia governorate). Enlargement of superficial lymph nodes especially the prescapular and the prefemoral lymph nodes were observed (Fig.6).

Lymphadenopathy was milder in buffalo than in cattle. Mucosal lesions were observed as ulcerative lesions with severe hyperemia. They were detected in labial mucosa, gum and below the tongue of clinically affected animals (Figs 7,8).

Semi-nested PCR assay for the diagnosis of SA-MCF:

DNA samples were tested throughout the first round of these semi-nested PCR assay to generate the desired bands at 422 bp (Fig. 9A products yielded from the first PCR reaction were tested through the second round to generate the specific diagnostic DNA band at 238 bp (Fig.9B).

The PCR results of semi-nested PCR assay for detection of OvHV-2 DNA are illustrated in table (5) and the positive –PCR animals include sheep, cattle and buffalos were geographically distributed in chart (1) where 60% of positive- PCR animals were located in governorates of upper Egypt (El-Menia 44%, Giza 12% and Fayoum 40%) while the lower Egyptian governorates represent 40% of the positive cases. Chart (2) represents percentage of clinical findings of the positive cases where all affected animals suffered from persistent fever (100%). Opacity of cornea represented by 50% of the clinical affected cattle and buffalos. Symptoms of skin affection were not commonly encountered in the clinical cases (35.7%). Mucosal lesions were presented by most of clinically affected animals (57.1%).

Post-mortem examination, histopathological findings and positive staining EM of MCF in buffalo calf:

A six months old buffalo calf suffered from acute MCF, as indicated by the clinical signs, and the PCR positive reactions, was put under observation for 6 weeks. The animal showed the typical MCF signs including persistent fever (40.5-41.5), bilateral corneal opacity, abnormal symmetrical skin pigmentation, loss of appetite with mild enlargement in lymph nodes. The buffalo calf was slaughtered for the examination and detection of (a) post – mortem lesions and the associated (b) histological changes in different organs and EM was used to confirm these histological changes:

a- Post – mortem examination:

Degrees from mild to severe congestion were marked in different body parts (including mucous membrane, lungs, brain, kidney, heart and lymphnodes). Both lungs showed mixed area of severe congestion and grey hepatization (Fig.10). Emphysematous reaction was obviously detected in scattered regions of lung tissue, this was indicated by floating in a water tank. Cut sections in lung tissues revealed mucopurulent exudates filling the alveoli. The heart muscle suffered from degenerative changes and appeared flabby in texture. The pericardial sac was filled with a straw yellow fluid. Also, fibrinous inflammatory thickening in the pericardium was obviously detected (Fig.11).

Mixed areas of hemorrhagic patches and congestion were detected on the endocardial wall (Fig.12). Both kidneys were enclosed in yellow (high bilirubin) stained fat (Fig.14). Renal capsule was not easily stripped from attached parenchyma. Sub capsular several pinpoint hemorrhages (Fig.13) were detected in both kidneys. Pinpoint hemorrhages were obviously detected on surface of the cerebral gyri (Fig.15). Enlargement of liver with mixed parts of pale, congested and yellowish stained areas were obviously detected (Fig.16) Mild congestion with swelling was detected in superficially and deeply located lymph nodes.

(b) Histological changes:

Generalized systemic vasculitis, lymphocytic aggregations and lymphoproliferative reaction were the most prominent remarks in the histopathology of lymphoid and non lymphoid organs. Lungs with infiltration of bronchiolar and peri-bronchiolar inflammatory cells (mainly lymphocytes) and edema (Fig17 and Fig.18). Infiltration of perivascular tissues with inflammatory cells (mainly lymphocytes) mixed by fibrinoid necrosis in most of blood vessels specially the renal ones (Fig.19 and Fig.20). Some renal tubules suffered from degeneration and interstitial nephritis with mononuclear inflammatory cells infiltrates specially around blood vessels (Fig.21) were detected. Brain showed neuronal degeneration (darkly stained neurons) with lympohocytic perivascular cuffing. (Fig.22). Lymphoid hyperplasia was clear in lymphoid organs as lymph nodes (Fig.23) and spleen (Fig.24).

Positive staining EM:

Characterestic viral particles were detected in preparations made from lymph nodes (Fig.25) and lung tissues (Fig. 26-28). The main 3 components of typical herpesvirus particle (envelop, tegument and core "nucleocapsid ") were cleared obviously.

Sequencing of the PCR product and phylogenetic analysis:

The sequencing results and phylogenetic analysis of the PCR products of the Egyptian strain of OvHV-2 (El-Said strain, Accession number: **KT725443**), Fig. (29) revealed 100% identity with OvHV-2 strains of India, USA and Brazil (Equine strain).

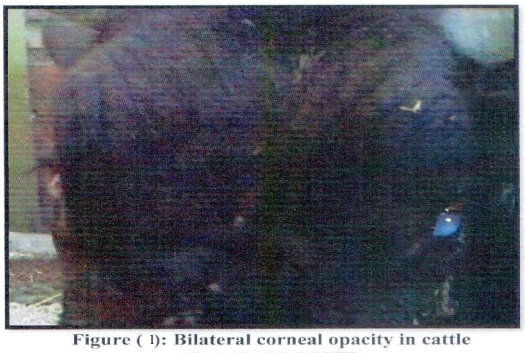


Figure (1): Bilateral corneal opacity in cattle affected with MCFD.

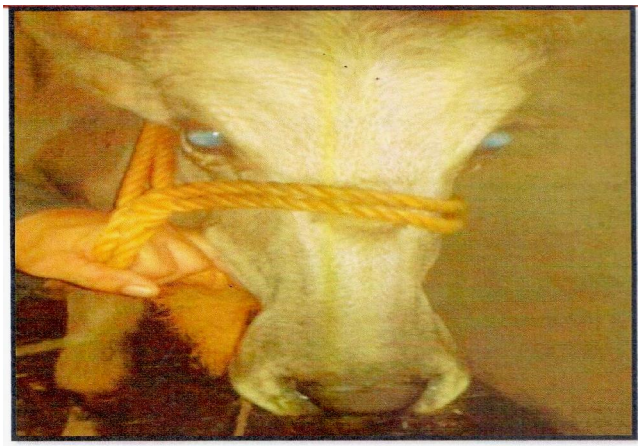


Figure (3): Bilateral corneal opacity in a buffalo affected with MCFD.

Figure ( ): Mild skin lesion due to MCF presented in the neck of Frisian cattle .

Figure ( ): Skin lesions of MCF which appeared as hypersensitivity like reaction in an affected cow.

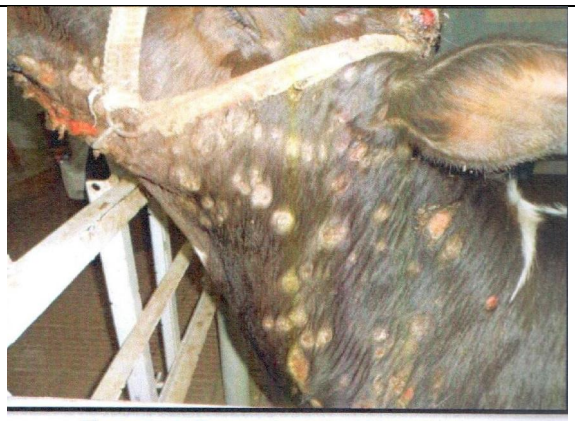


Figure (5): Severe ulcerative skin lesion in the head and neck of a bull with MCF.

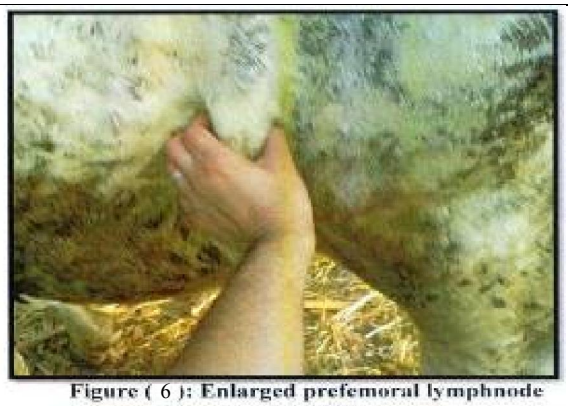


Figure (6): Enlarged prefemoral lymphnode in a cow with MCF disease.

Figure (7): Mucosal lesions of MCFD appeared as ulcerative lesions on lips of clinically affected cow.

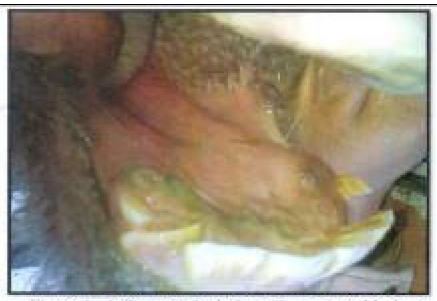


Figure (8): Symmetrical ulcerative mucosal lesions on floor of the buccal cavity of a buffalo with MCFD.

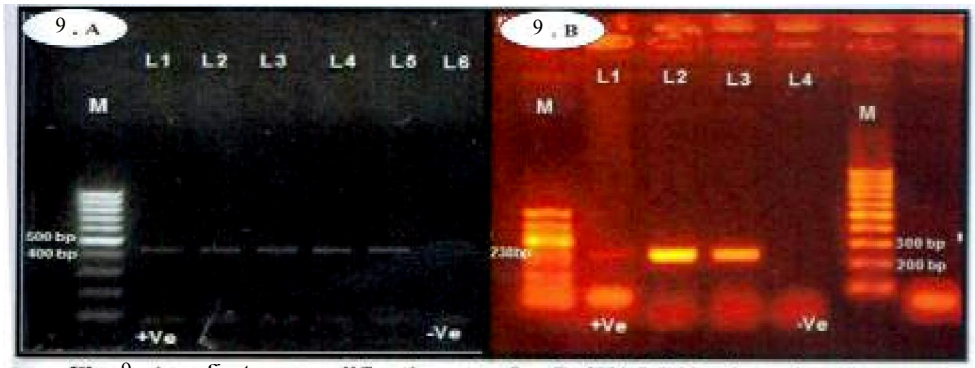


Fig. 9 .A: first amplification step for OvHV-2 DNA detection. Lanes from L2 to L5 : MCFV-PCR positive samples. Lane 1: Positive control. Lane 6: negative control. M: molecular weight marker (100bp). Notice PCR product (first round) from positive control and positive DNA samples produced at specific 422 bp band.

Fig. 9 .B: Second amplification step for OvHV-2 DNA detection. Lanes from L2 and L3 : MCFV-PCR positive samples. Lane 1: Positive control. Lane 4: negative control. M: molecular weight markers (50 bp and 100bp). Notice PCR product (second round) from positive control and positive DNA samples produced at specific 238 bp band.

Table (5): Results of semi-nested PCR assay detection OvH-2A DNA in cattle, buffaloes and sheep

PCR results / Animals	Positive PCR Results	Total number of tested animals
Cattle	12	12
Buffalo	2	2
Sheep	11	31
Total	25	45

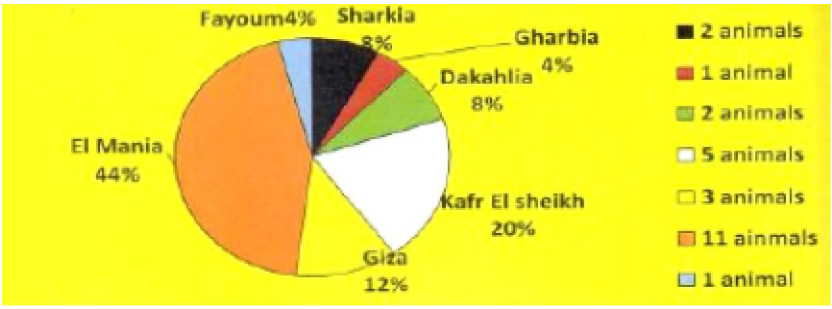


Chart (1): Geographical distribution of SA-MCFA-PCR positive animals (25 animals)

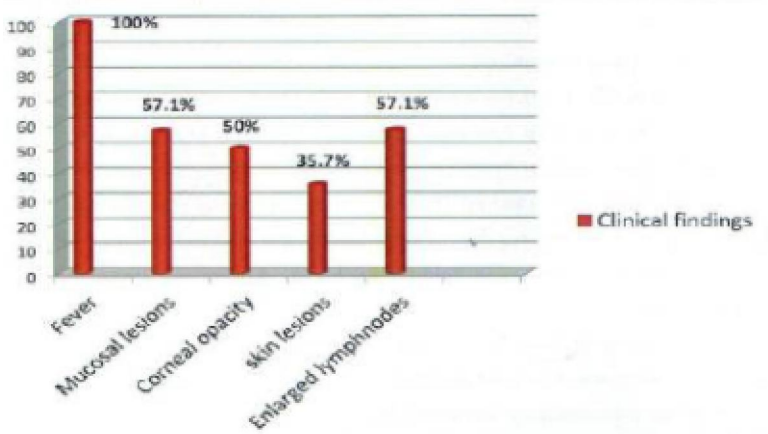


Chart (2): Clinical findings of positive –PCR MCF clinical cases (n=14, 12 cattle, 2 buffaloes)

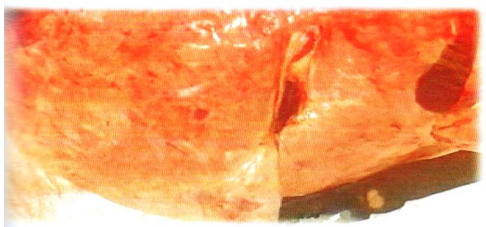


Figure (10): Both lungs showed mixed areas of severe congestion and hepatization (consolidation).

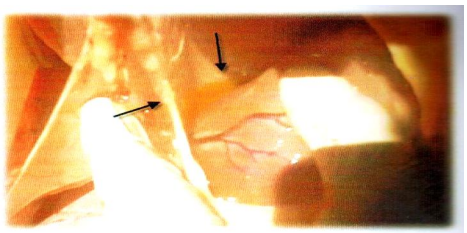


Figure (11): Fibrinous reaction associated with increased thickness of pericardium, large amount of a straw yellow fluid was obvious inside the pericardial sac.

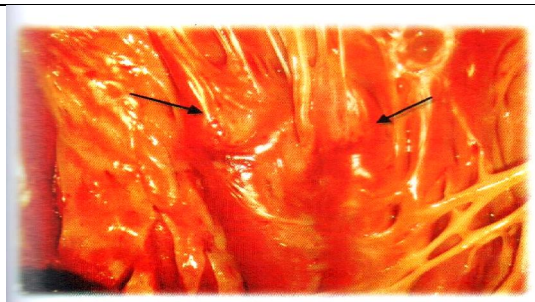


Figure (12): Hemorrhagic patches (black arrow) admixed with congestion were noted in the endocardial wall due to MCF infection.

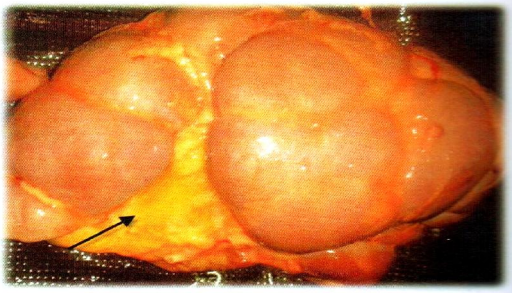


Figure (13): Both kidneys were enclosed in yellow stained fat due to high bilirubin.

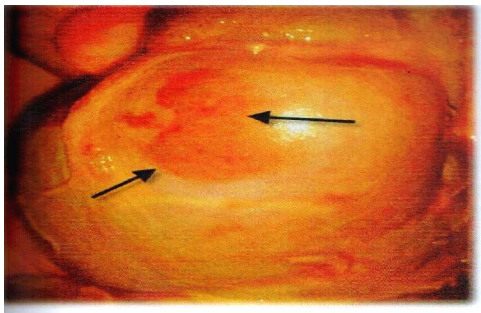


Figure (14): Sub-capsular aggregations of echymosed areas were detected in different renal lobules in each kidney.

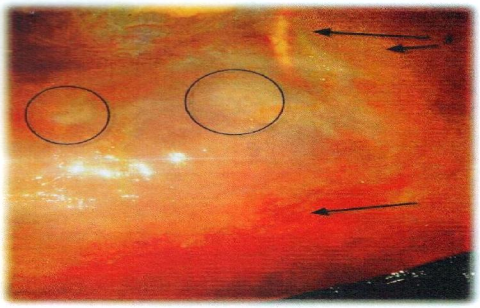


Figure (16): Mixed parts of pale, congested and yellowish stained areas were obviously detected in hepatic surface.

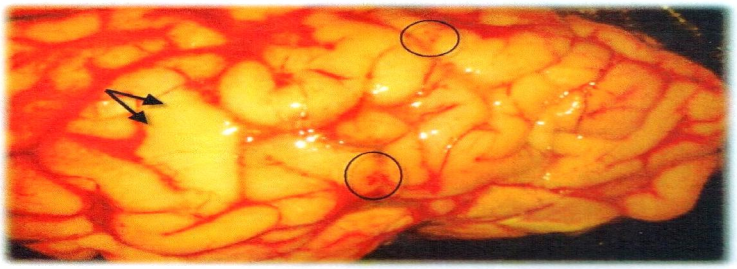


Figure (1): Brain showed edematous inflammation especially in the cerebral gyri. Engorgement of the cerebral blood vessels was clearly observed. In large parts of the cerebral gyri, pinpoint hemorrhages were obviously detected.

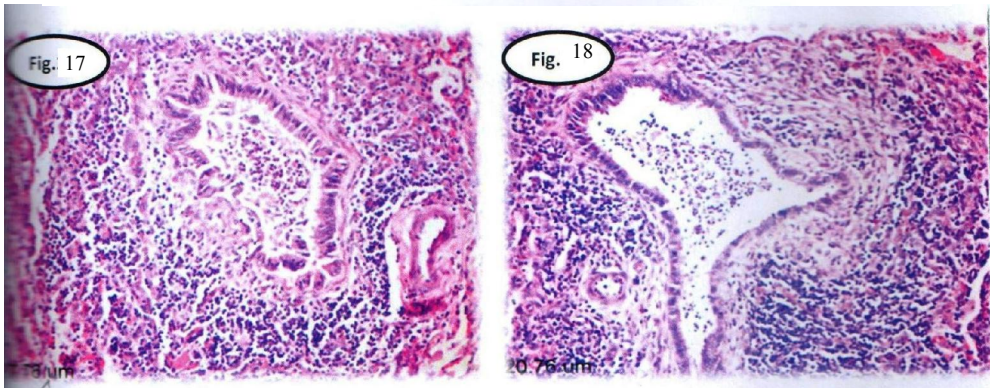


Figure (17,18): Massive infiltration of peri-bronchial tissues and bronchial lumen with inflammatory cells and tissue debris (mainly lymphocytes). .

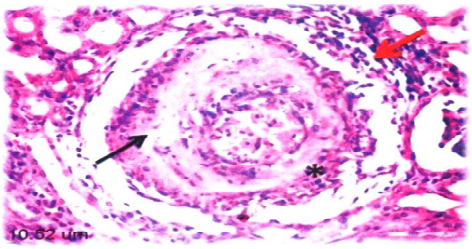


Figure (1): Vasculitis (black arrow) with perivascular lymphocytic aggregation in renal blood vessels (red arrow).

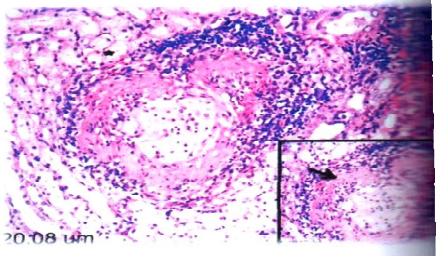


Figure (2): Fibrinoid necrotizing vasculitis with marked increase in vacsular wall thickness is marked observation in clinical cases with MCFD.

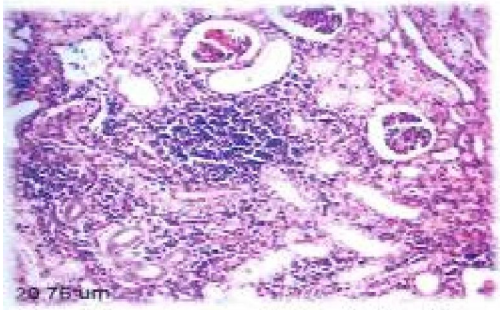


Figure (21): Interstitial nephritis with degeneration of some renal tubules in kidney section of a buffalo with MCFD.

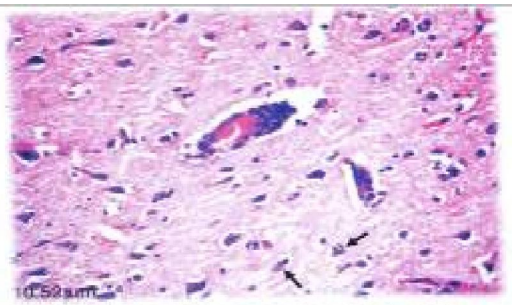


Figure (22): Perivascular cuffing with neuronal degeneration (black arrow) in brain section of a buffalo with MCFD.

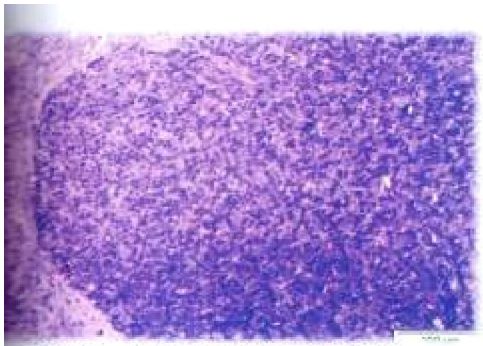


Figure (23): Lymphoid hyperplasia (lymph follicles) in lymphnodes of a buffalo with MCFD.

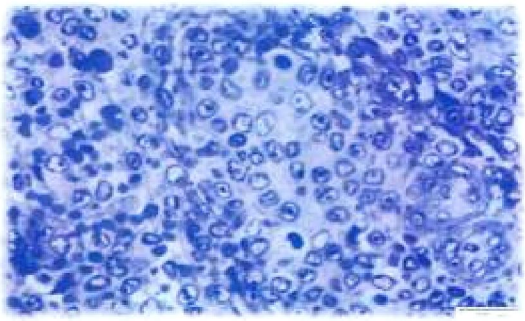


Figure (24): Lymphoid hyperplasia in Spleen (Malpighian corpuscles) of a buffalo with MCFD.

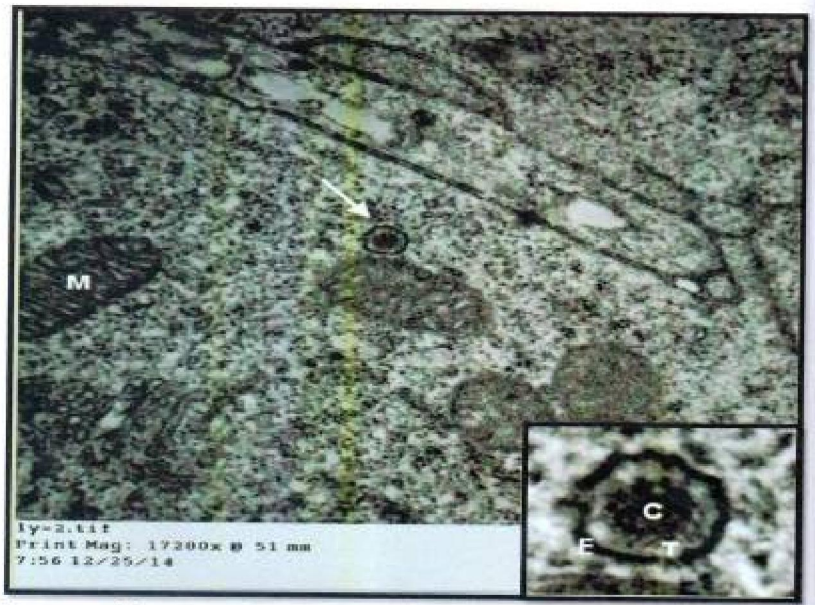
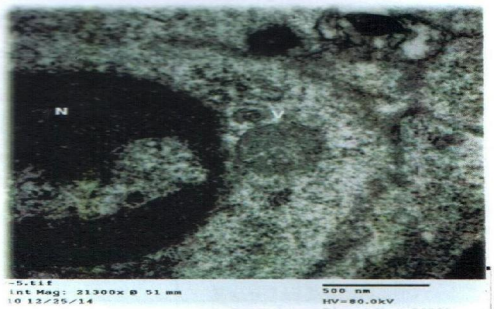


Figure 25:Electron microscopy of lung tissues of buffalo with MCFD showing the characteristic herpes viral particles (white arrow) of Ovine Herpes type 2 (OvH-2) containing the main parts (E: Envelope, T:tegument, C:Core). M:mitochondoria.

Figure(26):Multiple herpes virus particles (ovine herpes virus type 2) in lung of buffalo with MCFD.



Figure(27): Ovine herpes virus type 2 in lymph-nodes of buffalo with MCFD. N: nucleus

Figure(28): Ovine herpes virus type 2 (black arrow) in lymph-nodes of buffalo with MCFD. N:nucleus.

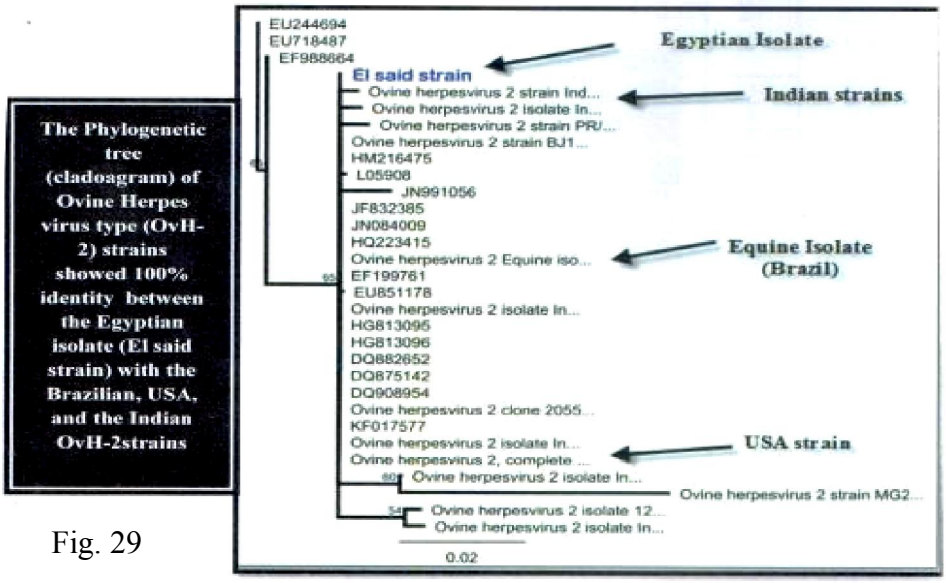


Fig. 29

4. Discussion:

MCF usually occurs as sporadic cases however, several outbreaks have been reported due to both sheep associated type and wildebeest associated type (**Pierson *et al.*, 1973; James *et al.*,1975; Schultheiss *et al.*,2000; Holliman *et al.*,2007** and **Sawi *et al.*,2013**). MCF has been regarded as an emerging threat for the Southern Mediterranean countries as well as the Middle eastern countries specially the sheep associated type. MCF-like signs have been observed in Egypt for many years but the disease was never confirmed and the etiological agent was never detected until 2012 when Bastawecy isolated the causative agent for the first time in Egypt (**Bastawecy and Abd El-Samee, 2012**).

In our study, the observed clinical signs associated with suspected clinical cases of MCF are identical to those reported in veterinary clinical textbooks and in the manual of the OIE (**O'Toole *et al.*,1997; OIE 2013** and **Radostits *et al.*,2000).** Atentative diagnosis of MCF can be based on the typical clinical signs of the disease and a history of contact with sheep, goats or wildebeest.

OvHV-2 was suspected to be the etiology of MCF in Egypt. Cattle and buffalos developed clinical symptoms characteristic for MCF, ranged from mild to severe signs which ended with death.

Pathological examination revealed typical gross pathology and histopathology demonstrated characteristic pansystemic vasculitis and lymphocytic proliferation in lymphaid and non-lymphoid organs of clinically affected animal as stated by **O'Toole *et al.* (1995,1997).**

In our study, the causative agent was identified as herpesvirus using positive staining EM. EM has the advantage of differential diagnosis **(Bastawecy and Abd El-Samee,2012**) so, it must be the front line of diagnosis in emergent situation (**Hazelton and Gelderbolm,2003**). EM excludes infection with foot and mouth disease (FMD) and bovine viral diarrhea-mucosal disease (BVD-MD) which can be also suspected due to presence of oral and hoof lesions. Typical herpesvirus particles have been detected in lung tissue which is the site for OvHV-2 replication and in lymph nodes where lymphoid tissues are the preferred predilection seats for gamma herpesviruses.

Further confirmatory identification was performed with semi-nested PCR assay (**Baxter *et al.*,1993**) which still considered by many researches to be the best molecular assay for detection of SA-MCF in clinical samples (**Li *et al.*,1995** and **Muller-Doblies *et al.*,1998**). Primers used to detect a fragment within the open reading frame 75 (ORF75) of OvHV-2 (coded by tegument protein gene). These primers do not react with AIHV-1 (**Li *et al.*,1994**). We found that PCR assay could be improved when the test performed on the whole blood samples rather than the peripheral blood leukocytes (Pb1) only. This may be due to the presence of free virus in the blood plasma of the infected animal.

The most common clinical form of MCF encountered in our study was the head and eye form (64.3%). This is in accordance with the report of **Brenner *et al.* (2002).**

Skin lesions (35.7%) in association with other typical sings clearly confirm MCF infection (**O'toole *et al.*,2014**).

Typical corneal opacity of MCF has been observed only in 50% of infected animals, this may imply to that opacity may not be a consistent clinical feature of MCF (**Pardon *et al.*,2009**).

Corneal opacity of MCF usually begins to develop along the limbus as a thin gray margin and them spreads to the center to cover the entire cornea (**Whiteley *et al.*,1985** and **Mirangi and Rossiter, 1991**).

The combination of persistent fever, corneal opacity and mucosal lesions are not commonly encountered in any disease other than MCF (**Murray and Blood,1961**).

Sheep (age ranged from more than 6 months up to 4 years) showing no clinical signs were positive PCR results where most of the previous studies stated that sheep were carrier without showing clinical symptoms and molecular approach was the method of choice (**Taus *et al.*,2006 and Taus *et al.*,2007).**

The geographical distribution of the PCR confirmed cases is nearly equal in Upper and Lower Egypt and this can be explained by the spread of sheep associated-MCF (OvHV-2) all over the Egyptian governorates.

The highest incidence of MCF cases (56%) was recorded mainly during winter as mentioned by blood et al.(1983) but the other clinical cases (44%) tend to occur in Egypt at any time during the year.

The phylogenetic analysis of the PCR products of the Egyptian strains of OvHV-2 revealed 100% identity with OvHV-2 strains of India, USA and Brazil. It was interesting to find that our local OvHV-2 strain (El-Said strain, Accession number: KT725443) showed 100% homology to OvHV-2 detected in clinical case of equine in brazil. Therefore, the future epidemiological SA-MCF studies in Egypt should involve the equine species.

In our study, the most of MCF cases (Cattle and buffaloes) were associated with lambing ewes and recently weaned lamb and these results in agreement with those obtained with **Li *et al.* (2004).**

On conclusion, rearing sheep and goats in the same grazing area of the susceptible species can lead to increased chances of contracting the infection. Therefore, our recommendation is the separation of grazing areas for cattle and buffalos from those of sheep and goats. Also, studies on the different ages of sheep and goats must be done on large scale to know the most proper age for transmitting infection with MCF for the susceptible species.

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