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APPLICATION OF AVIDIN BIOTIN COMPLEX IMMUNOPEROXIDASE AND IMMUNOFLUORESCENT TECHNIQUES FOR DIAGNOSIS OF BOVINE EPHEMERAL FEVER

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SUMMARY

The present study reported field outbreaks of bovine ephemeral fever virus infection in Assiut governorate , Egypt ,during the period of 2000 . A total number of 585 cattle of both sexes belonging to Bani - Morr and Abnoub Holstein Friezian Dairy Stations, Assiut governorate were used in this study .Clinical signs were evident in 267 animals (45.64 %) . The rate of deaths and emergency - slaughtered animals reached 9.36 % .

A total of 25 dead cattle were subjected for thorough postmortem examination .Tissue samples from recently dead cattle were obtained for application of avidin biotin immunoperoxidase and indirect immunofluorescent techniques for the diagnosis of bovine ephemeral fever (BEF) .

These techniques demonstrated that BEF viral antigen was present in the reticuloendothelial cells in the lungs, spleen and lymph nodes as well as in the epithelium lining the alveoli and bronchioles, the hepatocytes and kupffer cells in the liver , the lymphocytic population in the spleen and lymph nodes , the myocardial muscles in the heart and in the endothelial lining the blood vessels. So, all these sites could be considered as sites of BEF viral distribution. The current study also indicated that using of immunoperoxidase and immunofluorescent techniques are rapid and confirmatory methods for the diagnosis of the BEF outbreaks.

INTRODUCTION

Bovine ephemeral fever is caused by an arthropod-borne rhabdovirus, which is the type species of the genus Ephemerovirus. There are number of strains that vary antigenically. Among domestic animals, only cattle are known to be naturally affected. All age groups of cattle are susceptible

but the disease is more common in animals less than 2 years old. Calves less than 6 months of age showing no clinical signs (St.George, 1988 and Uren, 1989).

Bovine ephemeral fever remains an important viral disease for many countries. Ephemeral fever occurs enzootically on the African continent, in most of Asia, the Middle East, the East Indies, and in much of Australia. Clinically affected animal and biological vectors are the source of infection. The disease occurs in the summer months. Outbreaks are clustered and relatively short lived, and depends largely on the insect vector populations and the force and direction of prevailing winds (Radostits et. al., 2000). Davies and walker (1974) isolated ephemeral fever virus from cattle and Culicoides midges in Kenya. Farag et. al. (1998) reported an outbreak of BEF in 1990 in four cattle herds in Saudi Arabia.

During summer 2000, a severe outbreak of BEF was recorded in Egypt. Clinically the disease occurred suddenly in both foreign and native breeds of cattle with severe economic losses (Zaghawa et al., 2000). The disease appeared in Assiut governorate and spreading upward to the rest of Upper Egypt. Foreign and native breeds of cattle as well as buffaloes were variably affected (Sayed et al., 2001). Isolation and identification of the virus was carried out from the affected animals (Khaleel et al., 2001 and Abd El-Rahman, et al., 2002). Gross and histopathological investigations were done by Abd Elghaffar et al. (2002)

The present work was planned to fulfill the followings :

1) Establishment of the avidin biotin immunoperoxidase and indirect immunofluorescent staining techniques for rapid diagnosis of BEF virus by detecting the virus antigens in tissue from naturally infected cattle.
2) Demonstration of the distribution of the viral antigen in the tissues of the diseased cattle and the pathogenesis of the disease.

MATERIALS AND METHODS

Animals:-

A total number of 585 Friesien cattle of both sexes belonging to Bani-Morr and Abnoub Holstein Friesian Stations ,Assiut governorate during the period of 2000 ,were used in this study .The age of these animals varied between 2 months -12 years .Signs of BEF were evident in 267 (45.6%) animals ,25 animals were found dead (9.36%) and 293 animals were apparently healthy (50.09%) .Carful clinical examination of the living animals and postmortem examination of the dead animals were carried out

Samples:-

Specimens from the lymph nodes, spleen, liver, lung, heart and kidneys were collected from 25 recently dead cattle naturally affected with BEF. Thin paraffin sections were prepared and used for application of avidin biotin immunoperoxidase and indirect immunofluorescent techniques.

Avidin biotin complex (ABC) immunoperoxidase technique:-

The avidin biotin complex (ABC) immunoperoxidase technique was carried out according to the method of Wilchek and Bayer (1990). Avidin biotin complex (ABC) staining kits and formamid substrate (Sigma Immunochemicals) were used .Refrence hyperimmunesera against BEF virus was kindly supplied by Plum Island USA. Before dehydration in graded ethanol, the prepared paraffin sections were deparaffinized by using xylene 3 times for 5 minutes each. The slides were washed 4 times with distilled water. The endogenous peroxidase was exhausted by 0.3% hydrogen peroxide in methanol for 30 minutes. After washing 3 times with phosphate buffer saline 5 minutes each, the slides were flooded with normal serum for 20 minutes. Before the incubation at the room temperature for 30 minutes, the slides were flooded with rabbit anti-bovine ephemeral fever virus (diluted 1:100). After washing 3 times with PBS, the slides flooded with biotinilated antirabbit immunoglobulins (diluted 1:1000 in PBS) and incubated at the room temperature for 1 hour. After washing 3 times with PBS, the slides were flooded with avidin biotin complex (ABC) reagent for 30 minutes. After washing 3 times with PBS, the slides flooded with formamide. The substrate was diluted in 5 ml of 0.05% M sodium acetate buffer (pH 5.5) and 25 µl of 3% hydrogen peroxide. After incubation for 15 minutes at the room temperature and washing 3 times with PBS and finally with distilled water, the slides were stained with haematoxylin for 10 minutes and washed with distilled water then dehydrated in graded ethanol. The prepared slides were cleaned in xylene and mounted with canada balsam then examined by light microscopy (Zaghawa ,1989).

Indirect immunofluorescent technique:-

Paraffin sections from different organs and tissues were prepared and screened for detection of bovine ephemeral fever virus (BEF) antigen by the indirect fluorescent antibody (IFA) technique according to the method described previously by Goldman (1985). Anti-rabbit immunoglobulin conjugated with fluorescin isothiocynate was supplied by Ames, Low Lab, USA.

RESULTS

Clinical and postmortem findings :-

Most of the affected cattle were characterized by sudden onset of fever (39.5-40.5c) which lasted 12-24 hours accompanied with depression, muscular shivering, stiffness and developing of shifting lameness, associated with respiratory manifestations.

The outstanding features of BEF at postmortem were congestion of the internal organs, consalidation of the lungs, swelling, edema and congestion of the muscles especially of the back and neck,

Fig.(1): Lung showing dense brown granules against BEF virus in the lining bronchial epithelium (arrows) and the wall of some alveoli . Avidin biotin complex immunoperoxidase - counterstained haematoxylin. x 250.

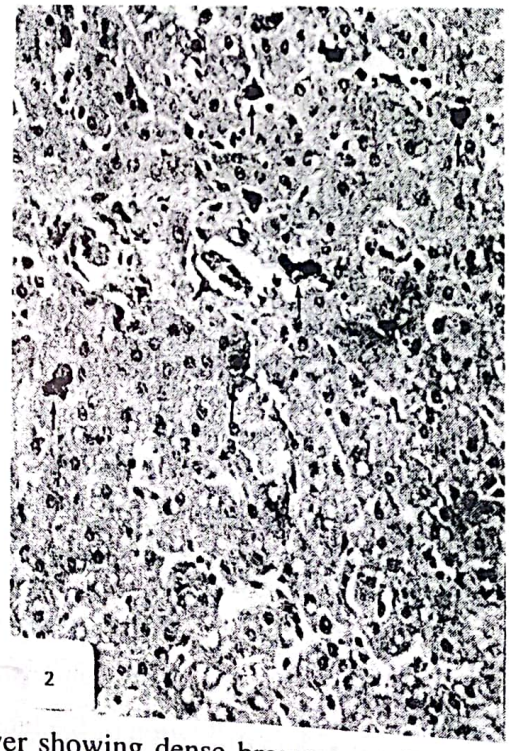

Fig. (2) : Liver showing dense brown granules in the hepatic and kupffer cells (arrows). Avidin biotin complex immunoperoxidase - counter stained haematoxylin. x 200.

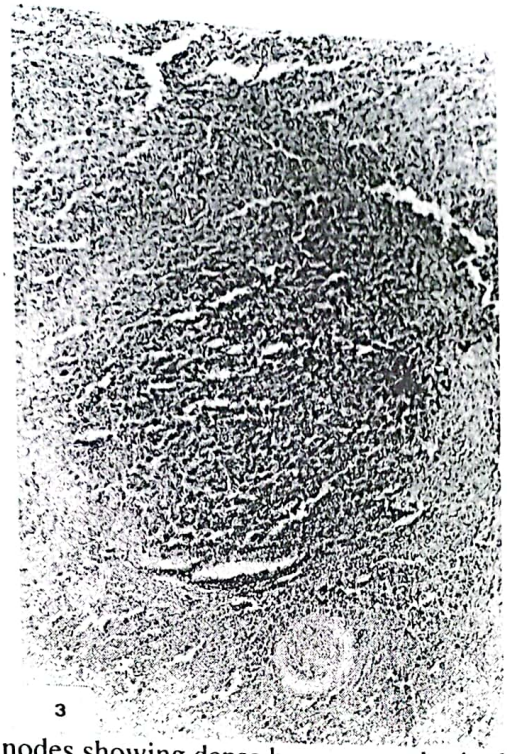

Fig. (3) : Lymph nodes showing dense brown granules in the macrophage and lymphocytes of the lymphoid follicles (arrows) . Avidin biotin complex immunoperoxidase - counter stained haematoxylin. x 165.

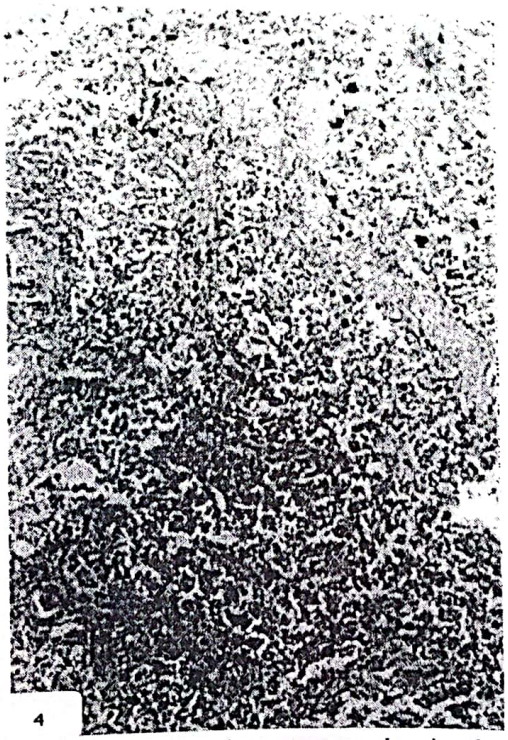

Fig. (4) : Spleen showing dense brown granules in the macrophage and lymphocytes of the red and white pulp (arrows). Avidin biotin complex immunoperoxidase - counter stained haematoxylin. x 250.

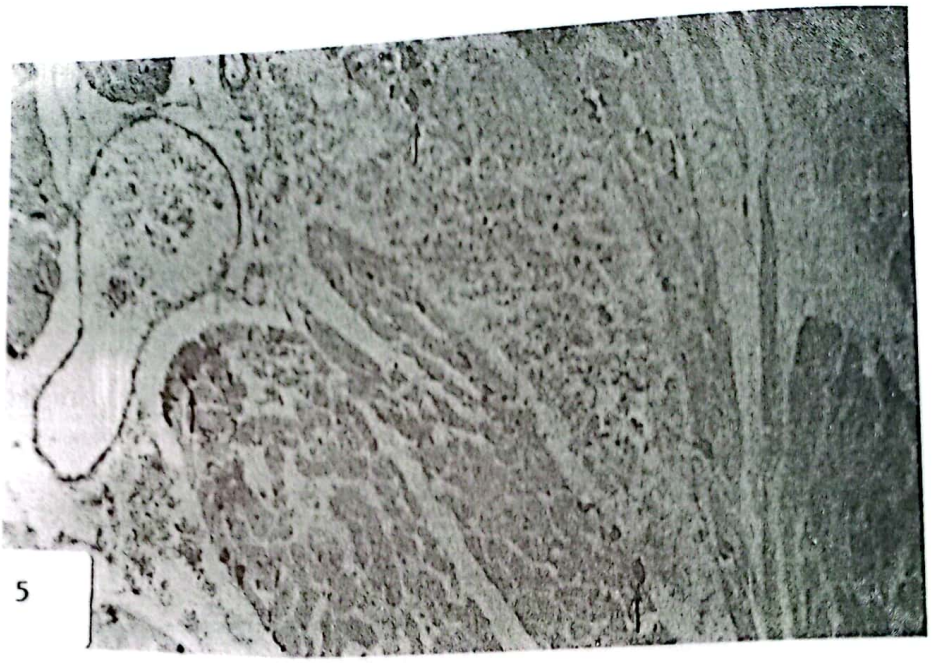

Fig. (5) : Heart showing dense brown granules inbetween the cardiac muscle fibers (arrows). Avidinbiotin complex immunoperoxidase - counter stained haematoxylin. x 200.

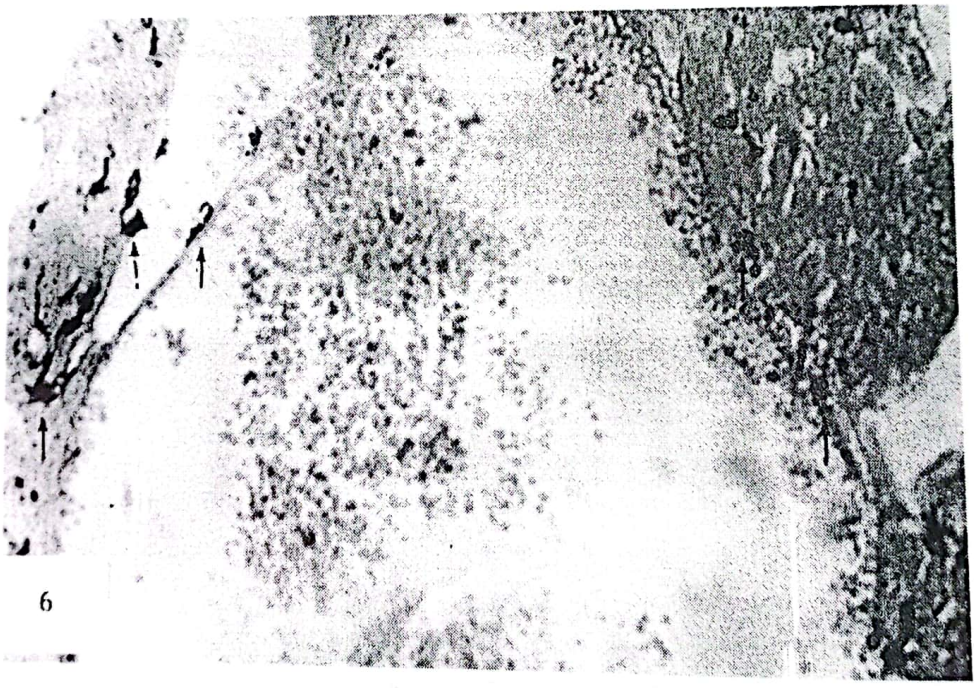

Fig.(6) : Dense brown granules in the lining endothelium of the blood vessels (arrows). Avidin biotin complex immunoperoxidase - counter stained haematoxylin. x 400.

Fig. (7) : Lung showing fluorescent reaction distributed throughout the alveolar and bronchial wall against BEF virus. Paraffin section x 200.

Fig. (8) : Liver showing fluorescent reaction against B E F virus distributed throughout the hepatic parechyma. Paraffin section x 200.

Fig. (9) : Lymph node showing fluorescent reaction within the lymphoid follicles . paraffin section x 400.

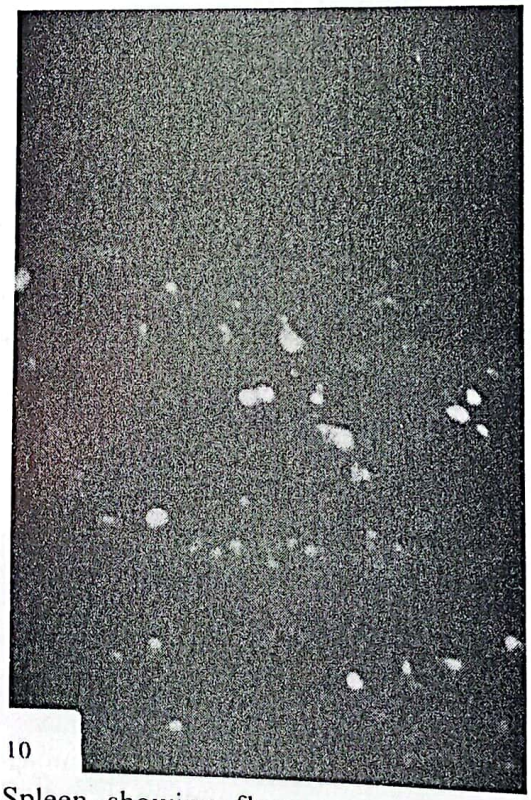

Fig.(10): Spleen showing fluorescent reaction within the white and red pulp. paraffin section . x 200.

enlargement of the liver and lymph nodes as well as serous exudates in the pericardium and thoracic cavities .

Immunohistochemical findings :-

Application of avidin biotin complex (ABC) immunoperoxidase and indirect fluorescent antibody (IFA) techniques on paraffin sections prepared from bovine organs and tissues collected from different localities in Assiut Province indicated the infection of these animals with bovine ephemeral fever virus.

Avidin biotin complex (ABC) immunoperoxidase technique:-

The avidin biotin complex (ABC) immunoperoxidase technique revealed intense brown stained-granules in the lining bronchial and alveolar epithelium in the lung (Fig. 1), in the cytoplasm of most of the hepatic and Kupffer cells in the liver (Fig. 2), in the macrophages and lymphocytes of lymphoid follicles in the superficial lymph nodes (Fig. 3) and in the white and red pulps of the spleen (Fig. 4). Also, dense brown granules against BEF virus were clearly observed in between the cardiac muscle fibers (Fig. 5) and in the endothelial cells lining most of the blood vessels (Fig. 6). Meanwhile, weak positive reaction was seen in the kidneys.

Indirect immunofluorescent technique

The results of the indirect fluorescent antibody (IFA) technique indicated positive fluorescence reaction in the alveolar and bronchial wall of the lung (Fig. 7), inside the cytoplasm of the hepatic cells (Fig. 8), the lymphoid follicles of the lymph nodes (Fig. 9) in addition to white and red pulps of the spleen (Fig. 10). Weak positive reaction was noticed in the cardiac muscles and no reaction could be seen in the kidneys.

DISCUSSION

Bovine ephemeral fever remains as a viral disease of considerable importance to many countries including Egypt .The disease was firstly recorded in 1909 in Nile Vally .Then in 1991 in lower Egypt (Hassan et al.,1991) .During summer 2000, severe outbreaks of BEF have been recorded in Egypt mainly in Assiut and Fayoum governorates (Nawal et al., 2001).

Nandi and Negi (1999) found that ephemeral fever is a disease of economic importance and its rapid diagnosis is the first step to plan a suitable control program. Diagnosis of BEF depends on detection of virus antigen by immunofluorescence and virus isolation and identification as well as detection of specific antibodies in paired serum samples by neutralization test (Tziporie,1975).

Virus isolation and neutralization have many disadvantages as they are time consuming, bacterial contamination and toxic effect of samples on tissue cultures (Burleson et al., 1992).

During the last 2 reported outbreaks of BEF in Saudi Arabia in 1990 and 1996, trials of Farag et al. (1998) and Abu ElZein et al. (1999) to isolate the causative virus in cell culture and baby mice were unsuccessful.

Fluorescent antibody technique was used by Theodoridis (1969) and Hassan et el. (1991) for diagnosis of BEF in tissue samples and blood films, respectively.

Immunoperoxidase staining was proved to be a simple, rapid and accurate method for diagnosis of different viral infections. Monoclonal antibody based immunohistochemical was used by Haines et al. (1992) for detection of bovine viral diarrhea (BVD) virus in formalin-fixed paraffin-embedded tisuues. Also, Gehan et al. (1996) used the same procedure for diagnosis of mixed infection of BVD and foot and mouth disease. El-Manakhly et al. (1997) and Gehan et al. (2001) used this technique for diagnosis of infectious bovine rhinotracheitis. Khaleel et al. (2001) used this technique for detection of BEF virus in buffy coats of infected cattle.

The avidin biotin complex immunoperoxidase technique is a system used to label antibodies by using a strong binding affinity of avidin and biotin. This technique was applied by Nawal et. al. (2002) for detection of bovine virus diarrhea virus (BVDV), bovine herpes virus-1 (BHV-1) and parainfluenza type-3 virus (PI-3) antigens in paraffin sections of different tissues and organs of naturally affected animals from three cattle herds in Lower Egypt.

In the present study, avidin biotin complex (ABC) immunoperoxidase is applied for the first time in Egypt for detection of BEF viral antigen in paraffin sections of different organs and tissues of naturally infected cattle. Detection of the BEF viral antigen within the paraffin sections of infected animals tissues (lung, liver, spleen, lymph nodes and heart) using ABC immunoperoxidase and immunofluorescent techniques, as indicated in the current study, is rapid and confirmatory method for the diagnosis of the BEF outbreaks. The test is simple, rapid, sensitive and can be considered as a field test in the presence of diagnostic reagents.

Burgess and spradbrow (1977) postulated that BEF viral replication takes place mainly in the reticuloendothelial cells in the lungs, spleen and lymph nodes and not in the vascular endothelial or lymphoid cells. However Jubb, et al., 1993 reported that the pathogenesis BEF is poorly understood and the site of viral replication remains unknown. In the present study, avidin biotin complex (ABC) immunoperoxidase and immunofluorescent techniques demonstrated that, BEF viral antigen was present in the reticuloendothelial cells in the lungs, spleen and lymph nodes as well as in the epithelial lining the alveoli and bronchi-

oles in the lung, the hepatocytes and kupffer cells in the liver , the lymphocytic population in the spleen and lymph nodes , the myocardial muscles in the heart and in the endothelial lining the blood vessels. So all thess sites could be considered as sites of BEF viral replication. The pulmonary lesions, the appearance of intracytoplasmic acidophilic inclusion bodies in the hepatic cells and myositis, in addition to the necrosis of the lymphocytes in the lymphoid organs and the angiopathic lesions which were described in cattle naturally infected with BEF by Abd Elghaffar et al. (2002) would confirm this conclusion.

Conclusion

The current events led us to conclude that avidin biotin complex immunoperoxidase is a rapid, specific and sensitive method for detection of BEF virus antigen in paraffin section of infected cattle. Avidin biotin complex immunoperxidase technique was more accurate method than the immunoflourescent as the non-specific reaction is less common.

The reticuloendothelial cells in the lungs, spleen and lymph nodes as well as the epithelial lining the alveoli and bronchioles in the lung, the hepatocytes and kupffer cells in the liver, the lymphocytic population in the spleen and lymph nodes, the myocardial muscles in the heart and the endothelial lining the blood vessels could be considered as sites of BEF viral replication.

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