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CLINICO-DIAGNOSTIC STUDY ON BOVINE VIRAL DIARRHEA (BVD) IN SHEEP

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SUMMARY

A private farm at Giza governorate of three hundred sheep was subjected for virological, immuno florescent, immuno cytochemical and histopathological examinations. In this farm three sheep were dead (1% mortality) and forty animals (13% morbidity) showed diarrhea, fever (39°C to 40°C) and muco to muco- purulent nasal discharges associated with mild cough. This study was triggered by isolation of the causative virus from nasal swabs of sick animals and tissues of dead animals on Madin Darby Bovine Kidney (MDBK) cells. The causative virus was identified as Bovine Virus Diarrhea (BVD) by usingindirect immuno fluorescent antibody technique (IFAT) followed by immuno cytochemical identification by using cell culture immuno peroxides test. Dead animals subjected to autopsy and histopathological examination of different organs and lymph nodes. Marked involvement of the lymphoid tissues particularly the mesenteric lymph nodes and peyer's patches of the intestinal ileum were prominent on histopathological examination, in addition to necrosis of the tips of the intestinal villi of the ileum and necrosis of the intestinal gland. This study concluded that infection with BVDV should be considered as one of infectious causes of enteritis.

Key words: BVDV- MDBK cells- IP- IFAT- Pesti virus- Histopatholgical examination.

INTRODUCTION

Border disease virus (BDV) is a Pestivirus of the family Flaviviridae and is closely related to Bovine viral diarrhoea virus (BVDV) and swine fever virus (CSFV) (Van Regenmortel et al, 2000).

BDV spreads naturally among sheep by the oro-nasal route and by vertical transmission. It is principally a cause of congenital disease in sheep and goats, but can also cause acute and persistent infections. Sheep may also be infected following close contact with cattle excreting the closely related bovine viral diarrhoea virus (Carlsson, 1991).

There is serologic evidence of Pestiviral infection in more than 40 species of free-range and captive mammals have been definitively identified as persistently infected with BVDV (Nelson et al., 2008).

The molecular analysis of the ovine pestivirus strains revealed the existence of two distinct groups of sheep derived pestiviruses, namely " true" border disease virus (BDV) strains and bovine viral diarrhea virus (BVDV) - like strains (Becher et al., 1998). The complete genomic sequences of two BD viruses have been determined and compared with those of other pestiviruses (Ridpath & Bolin, 1997and Becher et al, 1998). Phylogenetic analysis shows BD viruses to be more closely related to CSFV than to BVDV (Van Rijn et al, 1997; Vilcek et al, 1997and Avalos-Ramirez et al, 2001).

In Egypt, bovine virus diarrhea-mucosal disease (BVD-MD) was initially detected in sheep as the result of a serological survey of cattle and sheep, using the serum neutralization test. (Baz, 1992). The cell-bound immuno-assay (CBIA) is another serological test established for the detection and titration of antibodies to BVD virus and BD virus (Zaghawa, 1998). Recently Bastawecy et al (2007) also reported the prevalence of BD in 32 out of 161 lambs and adult sheep of various age in Giza governorate using indirect ELISA kit.

The aim of the present investigation was to study an enteric form of suspected pestivirus infection (Border disease) among sheep in a private farm in Giza and to isolate and identify the causative virus from the diseased and dead sheep using immuno-fluorescent techniques and immuno-peroxides test as well as to investigate the histopathological changes associated with the disease .

MATERIAL AND METHODS

Animals:

Forty out of three hundred sheep of native breed belongs to a private farm in Giza governorate, Egypt developed moderate to severe diarrhea, fever (39°C to 40°C body temperature) and muco to muco- purulent

nasal discharges (morbidity rate 13%).Three sheep were dead (mortality rate 1%) .

Samples:

1- Fifteen blood samples on EDTA anticoagulant for separation of Buffy coat were collected from diseased animals for antigen detection.

2- Fifteen nasal swabs were collected from mucoid nasal discharge of diseased animals and immersed in Hank' balanced salt solution for virus isolation on tissue culture cells.

3- Autopsy were performed on the dead sheep and tissues from visceral organs, lymph nodes and brain were collected, parts for virological investigation (freeze or immersed in Hank' balanced salt solution) and parts for histopathological examination (preserved in 10% formalin solution).

Virological examination:

a) Direct Immuno Fluorescent Technique (DFT): Kits of Fluorescein Isothiocynate Conjugated Antibody to Bovine Viral Diarrhea Virus (BVDV) obtained from Central Veterinary Laboratory, New Haw, Weybridge, surrey Kits.

The direct immuno fluorescent technique was used on buffy coat.

b) Virus isolation on tissue culture cells: Madin Darby Bovine Kidney (MDBK) cell line was used for virus isolation. Eagle's essential medium MEM supplemented with 10% fetal calf serum (FCS) and 100 μ g /ml streptomycin sulphate and 100I.U. / ml penicillin G was used for cell growth while 2% FCS was used for cell maintaining. 0.2 of prepared nasal swabs and tissue samples were inoculated for three passages on MDBK cells for virus isolation according to (Clarke et al., 1984).

C) Virus identification (Indirect florescent technique, IFT): The indirect immuno- fluorescent (IF) technique was used on the infected cell cultures with cytopathic effect (CPE) to identify the isolated virus. The technique was carried according to (OIE, 2003).

d) Immuno- Peroxides test (IP): The test was carried out in 96 well plates using MDBK cells according to (Gary et al., 1995).

Pathological examination:

A) Post mortem examination of dead animals was performed and gross lesions were observed in different organs.

B) Tissue specimens from visceral organs, lymph nodes as well as brain of dead sheep were fixed in 10% buffered formalin and processed for paraffin sections which stained with haematoxylin & eosin (H&E) stain for histopatholgical examination according to (Bancroft et al., 1996)

RESULTS

Clinical Findings:

Forty sheep out of the total 300 sheep present in the farm (13 %), showed clinical sickness such as diarrhea, fever, nasal discharges, mild cough, dehydration, recumbence and death of three sheep (1 %).

Virological Findings:

Direct fluorescence antibody technique applied on blood buffy coat smears revealed a positive detection of BVD viral antigen. Isolation of BVDV from swabs and tissues was obtained on MDBK cells and identified by using indirect immuno fluorescence granules (Figure 1).

Fig.1: Infected MDBK cells stained with FITC-conjugated anti-BVDV serum showing specific intra-cytoplasmic fluorescent granules X100.

Positive isolated virus showed clear yellow brownish coloration on immuno peroxides (Figure 2).

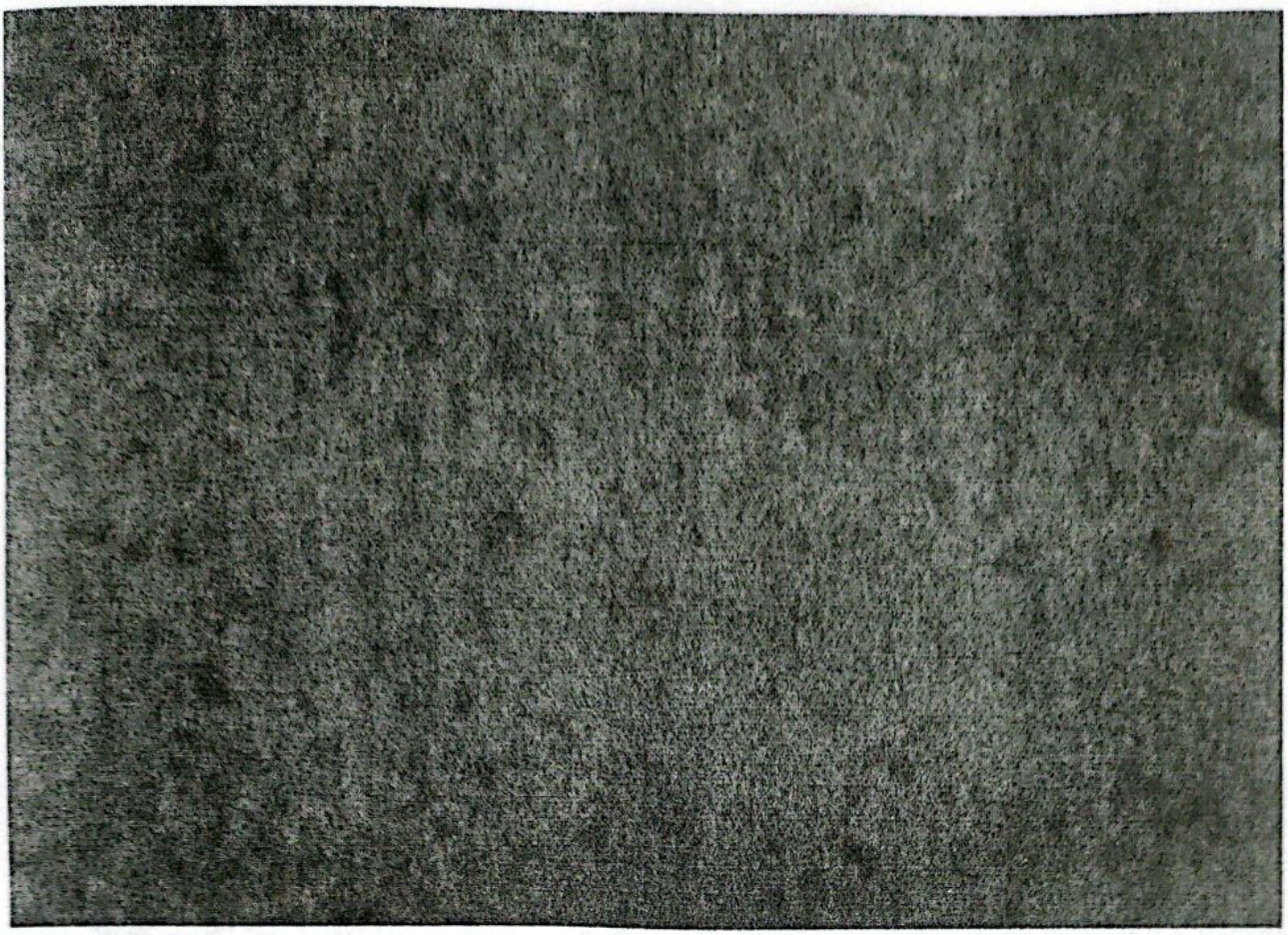

Fig.2: Infected MDBK cells stained showing cytoplasmic brownish coloration granules X100.

Pathological examinations:

Post mortem examination: revealed that the oesophagus and pharynx were edematous and hyperemic. The laryngeal mucosa was hemorrhagic. Congestion of lung with frothy exudates in respiratory passages was present in all three cases. Erosion was observed on the small intestinal mucosa associated with necrosis and hemorrhage of payer's patches. Microscopical examination of the lymph nodes revealed marked involvement of the lymphoid tissues particularly the mesenteric lymph nodes demonstrated as lymphocytic depletion within the lymphoid follicles (Figure 3). Oedema and numerous of macrophages and plasma cells were seen within the medullary sinuses of the mesenteric lymph nodes (Figure 4).

Lymphocytic depletion was marked in payer's patches of the ileum (Figure 5) and in many other lymph nodes as well as the spleen.

Histopatholgical examination of the ileum showed necrosis of the tips of the intestinal villi (Figure 6). Necrosis of

the intestinal gland and infiltration of the sub-epithelial layer occurred with many round cells within the submucosal layer at the site of irritation (Figure7).

The histological examination of the cerebrum showed focal area of gliosis (Figure 8) and area of malacia associated with perivascular and pericellular oedema (Figure 9).

The various organs such as liver and lung showed no significant histopathological changes with exception of vacuolar degeneration in some lobules of the hepatic parenchyma and mild congestion of the lung.

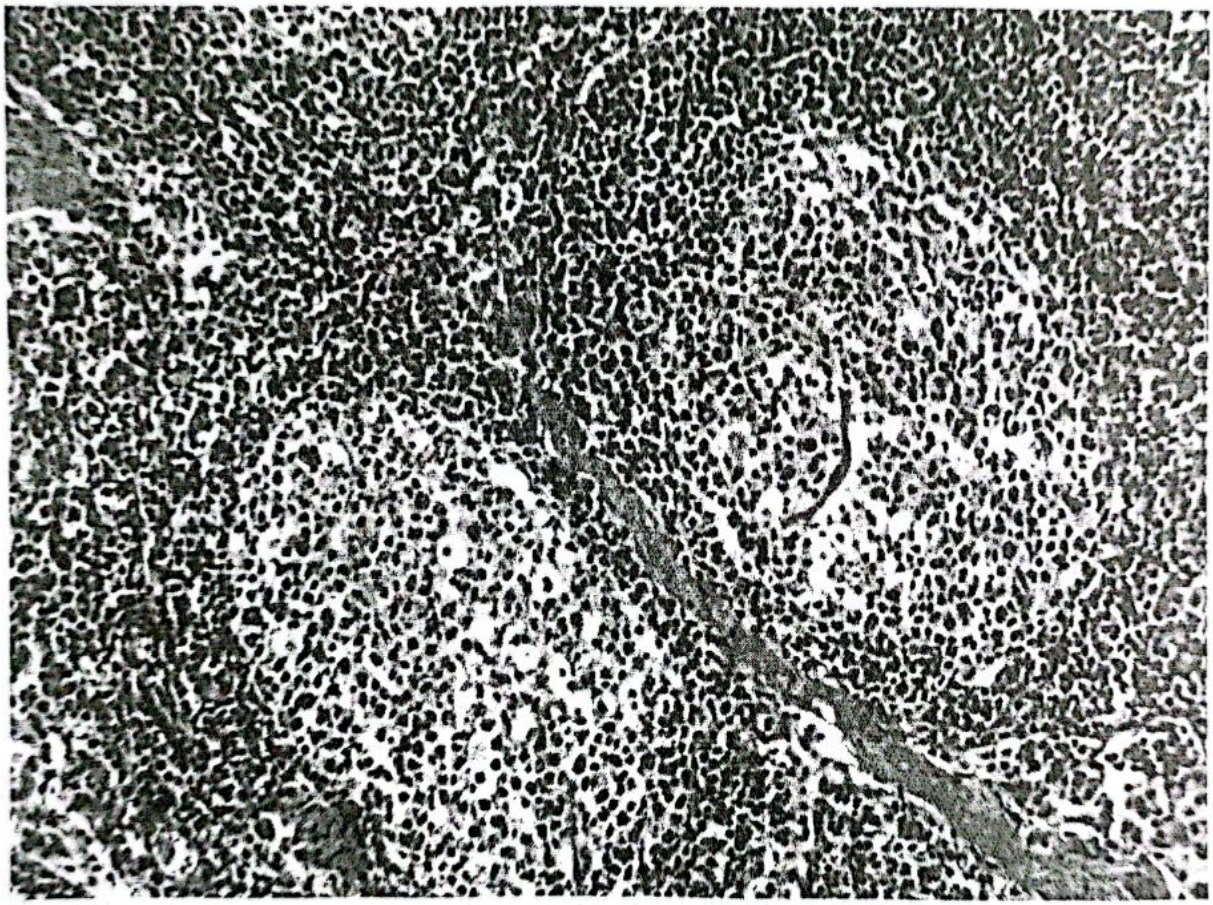

Fig.3: Lymph node showing lymphocytic depletion within lymphoid follicles. H&E tain, X 400.

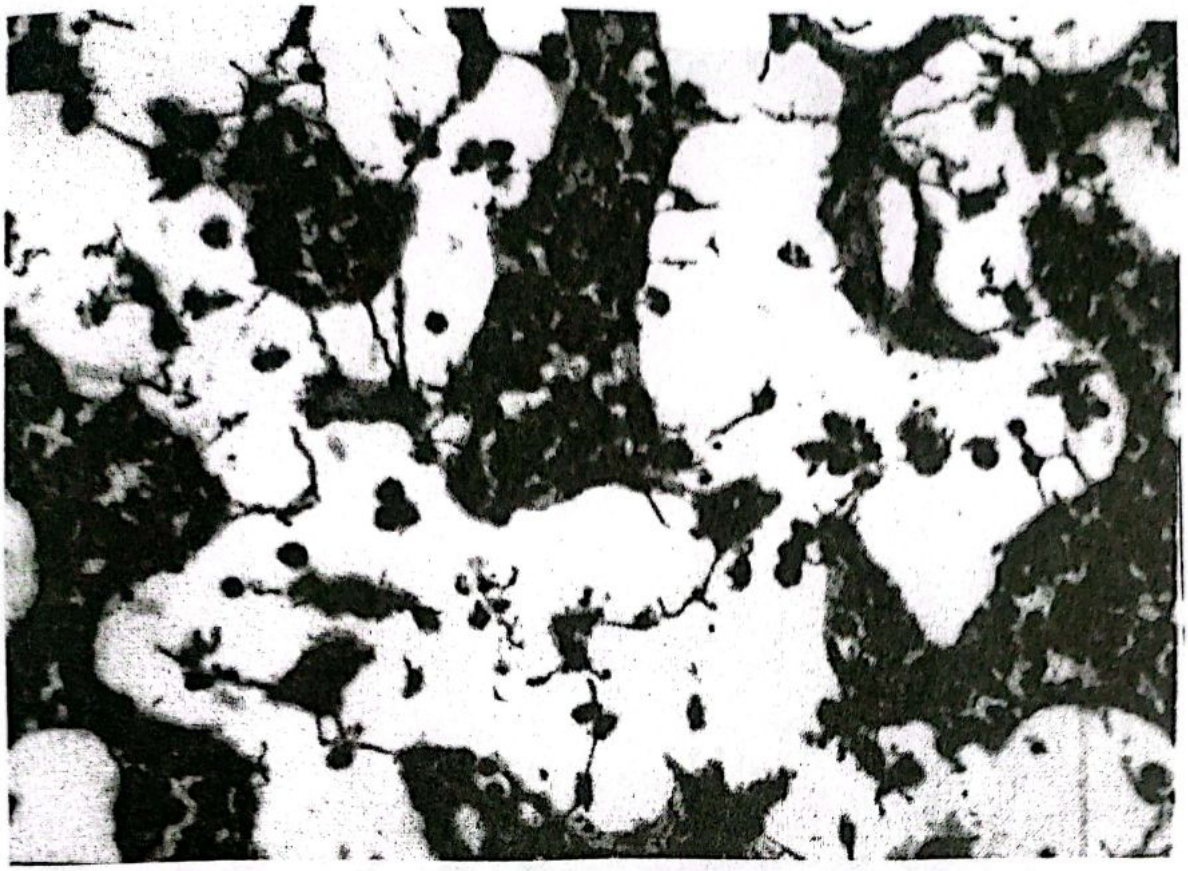

Fig.4: Lymph node showing numerous numbers of macrophages and plasma cells within the medullary sinuses. H&E stain, X 400

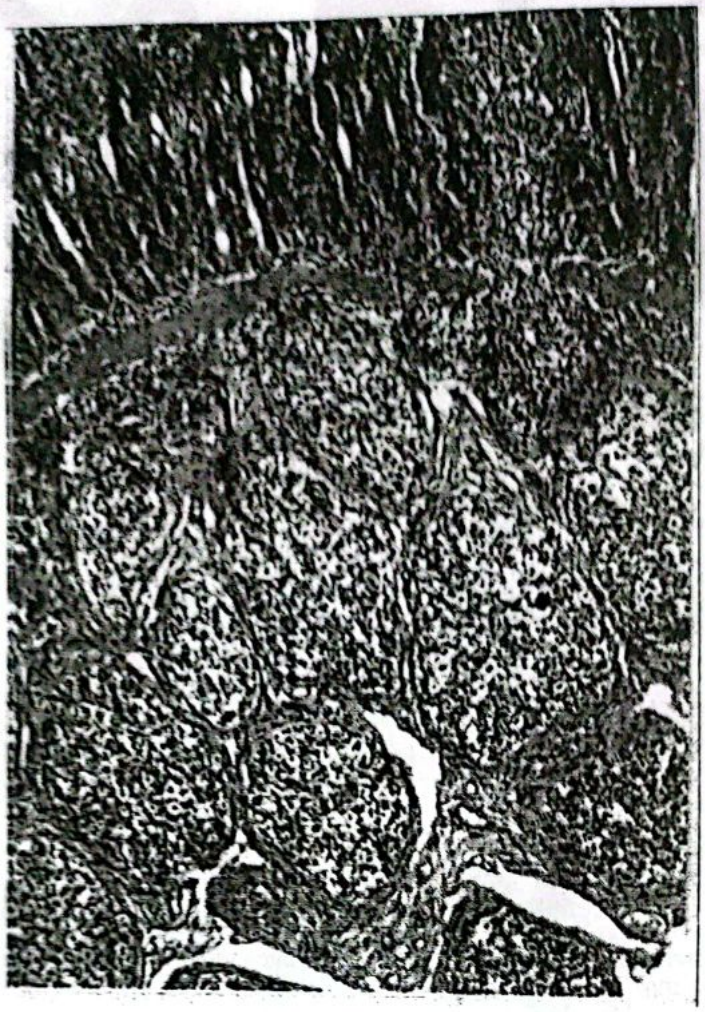

Fig.5: Ileum showed marked lymphocytic depletion within the payer's patches. H&E stain, X100. showe

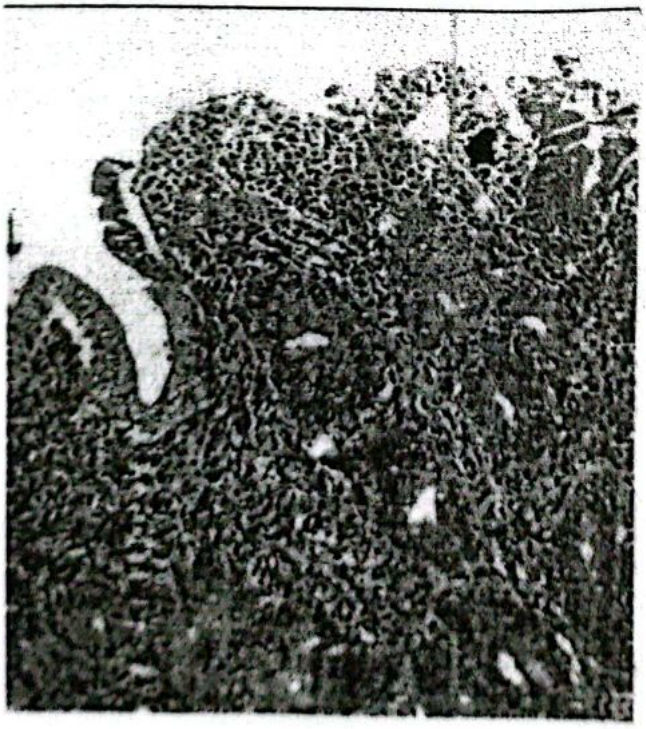

Fig.6: Ileum showing variable degrees of necrosis of the tips of intestinal velli, its infiltration with round cells. H&E stain, X 200.

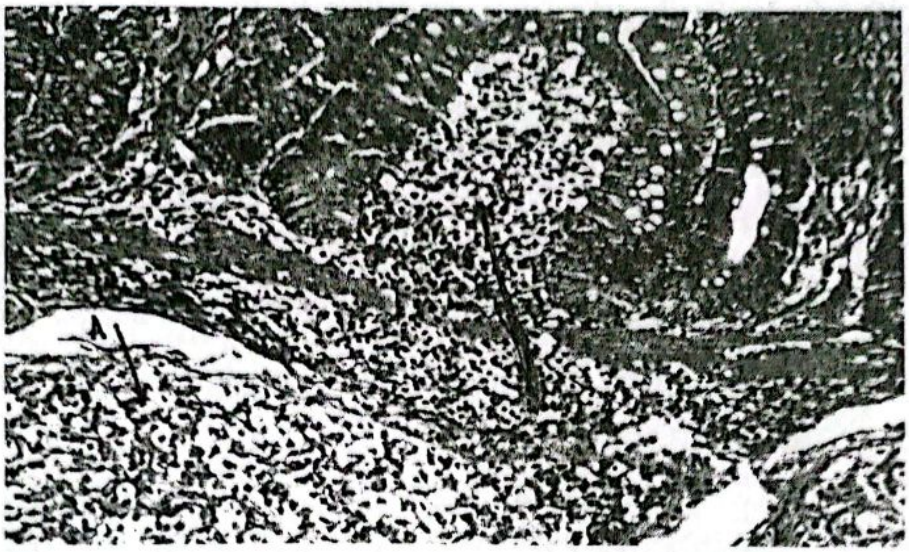

Fig.7: Ileum showing lymphatic depletion (arrow A) associated migration of round cells from the submucosal layer to the site of irritation (arrow B). H&E stain, X 400.

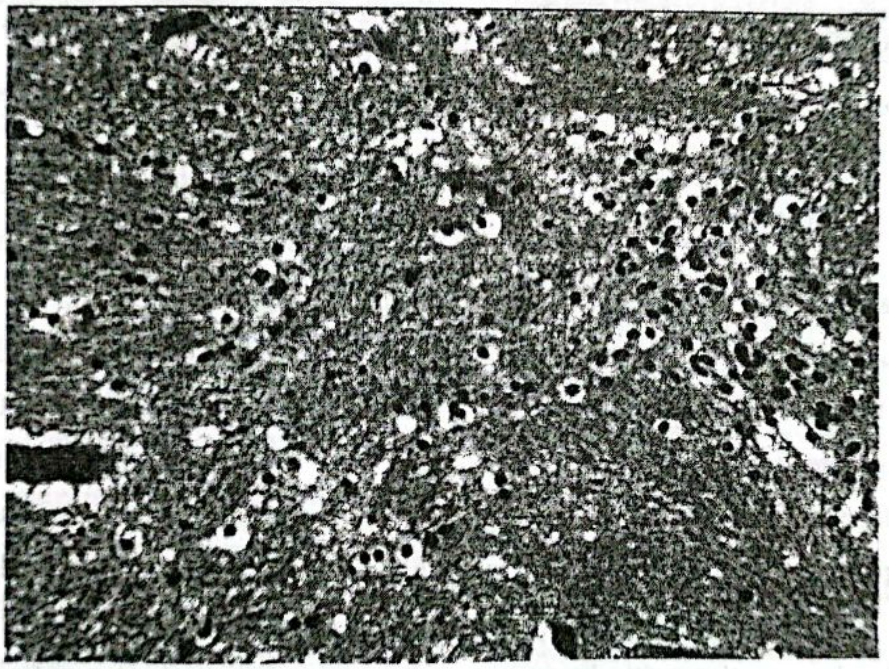

Fig.8: Cerebrum showing focal area of gliosis. H&E stain, X100.

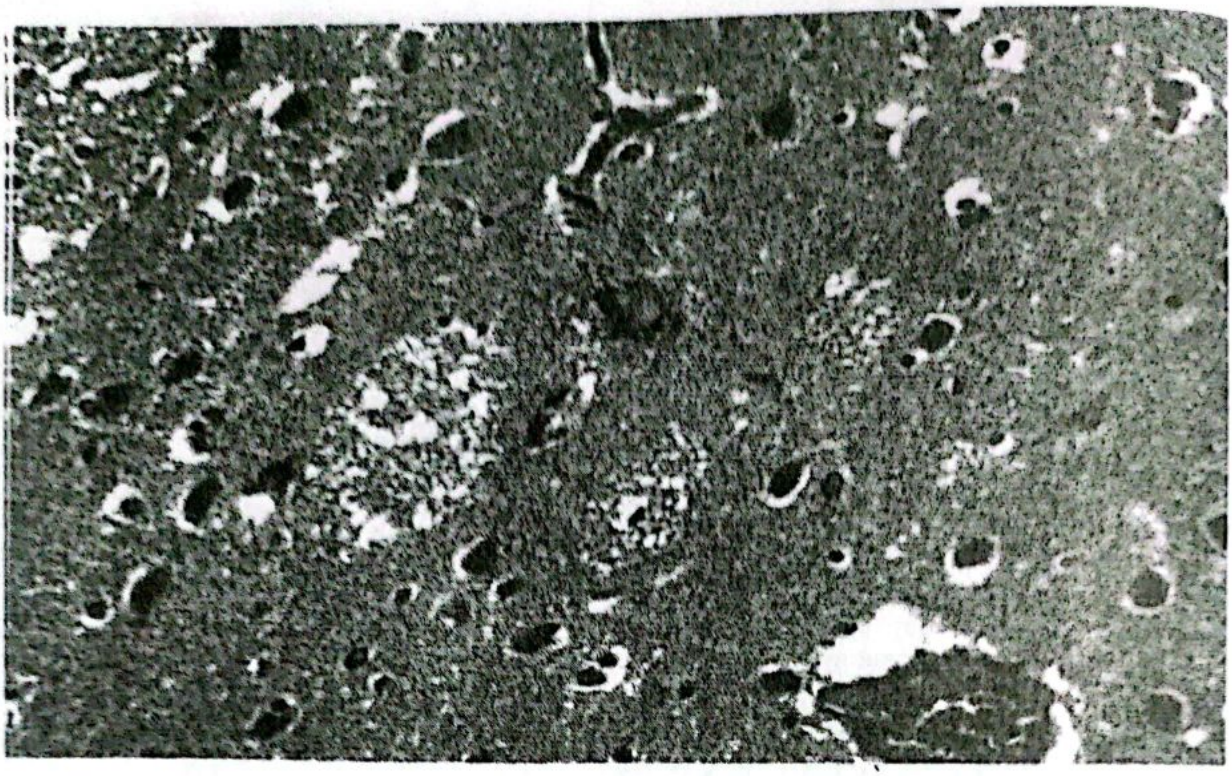

Fig.9: Cerebrum showing area of malacia associated with perivascular and pericellular oedema. H&E stain, X100.

DISCUSSION

The BVDV and BDV infections have been reported throughout the world among ruminants, and the wide spectrum of clinical syndromes associated with this single stranded enveloped RNA virus makes it one of the most important viral pathogen (Corapi et al., 1989).

Similar to the situation with bovine viral diarrhea/ mucosal disease (BVD/MD) the existence of persistently infected sheep with BDV is proven (Wolf and Buttner 1994).

Occasionally BDV isolates have been shown to produce an acute form of enteric disease with high fever, profound and prolonged leucopenia, anorexia, conjunctivitis, nasal discharge, dyspnoea and diarrhea and 50% mortality in young lambs.One such isolate was recovered from a severe epidemic of BD among dairy sheep in 1984 (Chappuis et al, 1986).

All pestiviruses are antigenically cross-reactive. Although antigenic differences do not exist between pestvirus species, they are not extensive enough for pestivirus species to be recognized as serotypes. (Ridpath, 2003). However, animal –to- animal variation and diveregence among viruses from the same pestivirus species can make it difficult to reproducibly and reliably differentiate pestivirus species based on serology alone (Muller et al, 1997; Bolin and Ridpath, 1998).

The present study recorded the presence of the enteric mucosal disease -like pestivirus infection in sheep with high morbidity (13%) and low mortality (1%). Clinically, fever, anorexia, depression, diarrhea with

dehydration and respiratory manifestations, muco purulent nasal discharge and dyspnia were the most observable signs in the affected herd. These findings are similar to those reported in sheep infected with acute form of pestivirus infection (Chappuis et al, 1986). Also similar clinical signs of BVDV/MD infection in calves were observed by (Baker 1995), Marshall 1996, Stoffregen et al 2000and Al-Afaleq et al., 2006).

In the present work we used fluorescein isothiocynate conjugated antibody to BVDV for the detection of pestiviruses (Plant et al. 1973). Accurate diagnosis of BVDV depends upon isolation of the virus from nasal swabs or blood or tissue samples from affected animals in a diagnostic laboratory (Donis 1988, Haines et al., 1992 and Abd EL Rahim and Grunder 1996). Laboratory investigations revealed isolation and identification of BVDV from nasal swabs and tissue samples collected from infected sheep using the MDBK cells and the immuno fluorescent technique.

The immuno peroxides method was used as a rapid way of confirming the demonstration of BVDV in the infected MDBK cells. The results were similar to the observations reported by several authors (Garey et al., 1995, Hosney et al., 1996, and Aly et al., 2003).

Antigen of BVD virus is regularly found in lymphoid tissues, mucose of the upper and lower digestive tract, the respiratory tract and parenchymal cells of other organs (Marshall et al, 1996, Ellis et al., 1998, Odeon et al, 1999 and Stoffregen et al, 2000).

The pathological changes which accompanied severe clinical syndrome characterized by diarrhea and/ or respiratory distress in sheep associated with inflammatory lympho- proliferative lesions in several organs, notably the CNS and intestinal tract (Barlow et al., 1983).

Microscopically examination of the lymph nodes revealed marked involvement of the lymphoid tissues particularly the mesenteric lymph nodes, peyer's patches and spleen demonstrated as lymphocytic depletion within the lymphoid follicles. These findings are in accordance with the descriptions of several authors in cattle and sheep (Bolin et al, 1985, Wihelmsen et al, 1991, Marshall et al, 1996, Ellis et al., 1998, Odeon et al, 1999 and Stoffregen et al, 2000). Lesions of necrosis of epithelium in the small intestinal tract and necrosis of the tips of the intestinal villi of the ileum were consistently observed histopathologically in our study. These results are in agreement with (Baker, 1995, Marshall et al, 1996, Stoffregen et al, 2000 and Al-Afaleq et al, 2006).

Examination of the brain cerebrum showed mild histological changes in the form of focal area of gliosis and area of malacia associated with perivascular and pericellular oedema. It is reasonable because there was no any nervous

manifestation (Physick-sheard et al 1980 and Monies et al 2004).

The present study concluded that, infection with pestivirus is an important cause of enteritis. Consequently, preventive and control measures should be applied. The immuno peroxides technique is one of the most rapid and accurate laboratory tests for the BVDV diagnosis.

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دراسه اكلينكية تشخيصية على مرض الاسهال الفيروسى البقري فى الاغنام.

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تم فحص مزرعة خاصة بها 300 راس اغنام بمحافظة الجيزه لكل من الفيرولوجى والهستوباثولوجى نتيجة وجود حالات وفيات لثلاث من الحيوانات (1% وفيات) واصابة 40 حالة (13% اصابات) باسهال وحمى مع وجود سعال وافرازات انفية.
هذه الدراسة الغرض منها التعرف على المسبب المرضى وعزله مع تصنيفة من خلال المسحات الانفية على الخلايا المناسبة وقدتم تحديد المسبب وهو فيروس الاسهال البقرى باستخدام اختبار الفلورسنت الغير مباشر واختبار البيروكسيديز فى الخلايا.
تم فحص الانسجة باثولوجيا وتسجيل التغيرات المرضية فى الاحشاء والغدد الليمفاوية, ولوحظ ضمور فى الامعاء والغدد المعوية.
هذه الدراسة تاكد ان مرض الاسهال البقرى الفيروسى هو احد اسباب الاصابات المعوية فى الاغنام والتى ينتج عنة اصابات ووفيات للاغنام فى مصر.