Bovine Ephemeral Fever: Pathological and Immunohistochemical Studies

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A natural outbreak of Bovine Ephemeral Fever in Egypt during the summer of 2006 had been observed. In Beni Suef province, out of 70 cattle naturally infected with bovine ephemeral fever virus, three fattening calves suffered from subcutaneous emphysema and died and were subjected to post-mortem examination. The findings revealed severe subcutaneous emphysema, interstitial and pulmonary emphysema. The serous membranes were thick, opaque and emphysematous. Microscopically, interstitial and pulmonary emphysema was prominent in most lobes of the examined lungs accompanied with pulmonary oedema and focal leucocytic aggregations in some areas. Angiopathy was demonstrated in all cases. The bronchial and mediastinal lymph nodes showed congestion and hemorrhages. Immunohistochemically, specific reaction for Bovine Ephemeral Fever virus was demonstrated in the lung and lymph nodes of the three cases; the pathogenesis of the disease was discussed.

Bovine ephemeral fever virus is an arthropode-born Rhabdovirus belonging to Rhabdoviridae family (Murphy et al., 1972). Bovine ephemeral fever outbreaks occurred in Egypt during the summer of 2000 and 2002 (Sayed et al., 2001; Abd El-Ghaffar et al., 2002). The disease prevalence was high among dairy cattle with low mortality rate (Yeruham et al., 2000). Pathologically, serofibrinous poly-synovitis, arthritis, cellulitis with patchy oedema in the lung and lymphadenitis were the most common pathological alterations seen in animals infected with bovine ephemeral fever (OIE, 2005). Pneumonia with pulmonary emphysema and subcutaneous emphysema were also noticed in animals infected with ephemeral fever (Theodoridis and Coatzer, 1979; Sayed et al., 2001; Abd El-Ghaffar et al., 2002). The pathological alterations produced by bovine ephemeral fever virus in the musculoskeletal tissue were fully described (Theodoridis and Coatzer, 1979). The virus could be detected in the blood by fluorescence and PCR (Young and Spradbrow, 1977; Stram et al., 2005).

The aim of the present was to study the pathological alterations associated with bovine ephemeral fever in the internal organs, subcutaneous tissue and skeletal muscle, in addition to detection of the virus distribution in different tissues using immunohistochemical technique.

Material and methods

A total of 70 cattle of different ages clinically diagnosed to be suffering from bovine ephemeral fever in an outbreak occurred in Beni-Suef province, Egypt during the summer of 2006. All animals showed manifestations in the form of high temperature 40-41°C, lameness, marked drop in milk production, nasal and ocular discharge with salivation. Three fattened calves had marked subcutaneous emphysema. These cases died and necropsy was performed.

Pathological study. Postmortem examination was carried out for the three dead calves and tissue specimens were collected from the lung, liver, kidneys, spleen, heart, intestine, skeletal muscles, as well as lymph nodes (prescapular, prefemoral, mediastinal, bronchial, and mesenteric lymph nodes).

Tissue specimens were fixed in 10% formalin solution, dehydrated cleared and embedded in paraffin. Tissue sections of 5-7 microns were prepared and then stained with hematoxylin and eosin according to Bancroft and Stevens (1996).

Immunohistochemical study. Indirect avidin-biotin complex technique (ABC) (Dako Envision kit K4011 from Santa Cruz, sc- 565, USA) was applied to formalin fixed tissue embedded materials, according to Dako Envision Kits. Polyclonal serum antibodies were obtained from Institute of Serum and Vaccines Production, Abassia, Egypt. Negative and positive control cases were involved.
Results

Gross pathological findings. Severe subcutaneous emphysema was observed, in the three cases, involving the whole body (Fig.1) in two cases and only localized in the shoulder and neck in the third calf. Subcutaneous fascia was emphysematous, thickened and pale (Fig.2). Diffuse hemorrhage was noticed in the pharyngeal area in one animal. Marked pathological changes were observed in the lungs; some lobes were mosaic in appearance, oedematous and cyanosed. Interstitial and pulmonary emphysema (Fig.3) were predominant in some lobes and appeared as small vesicles especially in the subpleura and interlobular septa. On cut section, there were oedema and emphysema separating the lobules. The bronchial and mediastinal lymph nodes were enlarged and congested. Serous membranes (pleura, peritoneum, and pericardium) were thickened, opaque and emphysematous (Fig.4). The endocardium showed focal congestion. The gall bladder was greatly enlarged. The muscles showed no marked changes. The kidneys showed mild congestion.

Histopathological findings.

Lung. The epithelium of bronchioles and bronchi revealed mild degeneration with early necrotic changes. Bronchial epithelium was totally destroyed with minimal inflammatory changes. Cellular debris was found inside some bronchioles which led to obliteration of few bronchial lumens (Fig.5). In some areas, the alveolar epithelium was detached with accumulation of the desquamated cells within the alveolar lumen. Focal leucocytic aggregations comprised of macrophages and few neutrophils degeneration of tunica media could also be seen. were observed in some areas especially those neighbouring to some of the bronchi and bronchioles (Fig.6). The peribronchial lymphoid aggregations were occasionally necrosed.
Apoptosis was frequently observed in some alveolar and bronchial epithelial cells. Many alveoli appeared as if being lined by hyalineized membrane. Angiopathy was demonstrated in all cases in which the blood vessels were dilated and congested, some of which showed necrotic changes in the intima and the endothelial cells float within the lumen. Subintimal oedema and In some areas, severe oedematous changes were observed in the interstitial tissue with minimal inflammatory cells. The alveoli were completely filled with proteinaceous fluids in wide areas (Fig.7). The alveoli were markedly over-distended with air and some were ruptured forming interstitial and alveolar emphysema (Fig.8). The adjacent alveoli underwent atelectasis.

**Lymph nodes.** The lymphoid follicles showed mild hyperplasia with mild degree of necrobiosis in the germinal center of some follicles. Congestion of blood vessels was prevalent (Fig.9). Some areas in the cortex and medulla showed hemorrhage and hemosiderosis. Some of the blood vessels suffered from hyperplasia of intima with thrombus formation. Vasculitis was demonstrated in the medulla. Little of protein-rich odematous fluid was observed in some areas of the stroma. Few neutrophils were found in the paracortex and around medullary cords.

**Kidneys.** The epithelial lining of some renal tubules showed mild degenerative changes in the form of vacuolation with pyknosis in a few nuclei. The blood vessels were dilated and congested.
**Intestine.** Catarrhal enteritis was noticed with hyperplasia of lymphoid follicles.

**Tongue.** The dermal blood vessels were congested and some of which had thrombosis.

**Skeletal muscles.** Some of the muscle fibers were degenerated and necrosed. Oedema was seen in the interstitial area among the muscle bundles.

**Liver.** Vacuolar degeneration was found in the hepatic cells allover the parenchyma. The central veins and portal blood vessels were dilated and congested.

**Heart.** Subendocardial and myocardial blood vessels were congested. The myocardial muscles showed very mild degenerative changes.

**Results of immunohistochemical study.** A positive immunohistochemical reaction (golden brown granules) for bovine ephemeral fever antigen was demonstrated in the lung and lymph nodes. Specific cytoplasmic and perinuclear reaction was mostly detected in macrophages and reticular cells of the peribronchial lymphoid aggregates of the lung (Fig. 10, 11) and also in lymphoid follicles of the lymph nodes (Fig. 12). The negative control cases showed no immunohistochemical reaction (golden brown granules) for bovine ephemeral fever virus.

**Discussion**

Several outbreaks of bovine ephemeral fever (BEF) in cattle have been reported in different localities in Egypt (Sayed et al., 2001; Abd El-Ghaffar et al., 2002). Yeruham et al., (2007) reported that in dairy cattle herd, only 12/50 animals were clinically ill with BEF in the dairy community. The lowest morbidity rate was recorded in young heifers (5.5%) and the highest was in adult cows (75%). Only heifers over the age of three months were clinically affected.

Clinically, variable degrees of subcutaneous emphysema were found at the region of the head, neck and back with mild subcutaneous oedema. The mortality rate was 9.76% in Friesian cattle, 2.19% in native cattle and 0.0% in baffuloes (Sayed et al., 2001). Necropsy findings were in the form of subcutaneous and pulmonary emphysema, pulmonary congestion, petecheal haemorrhages on the serous membranes with swelling and redness of skeletal muscles (Theodridis and Coetzer, 1979; Sayed et al., 2001).

In the present study, focal and diffuse subcutaneous and pulmonary emphysema, with lung congestion were the most predominant lesions in the three dead cases. The emphysematous changes were seen in the interlobular and interstitial areas of the lungs. Also, air bubbles extended into the serous membranes especially pleura and pericardium. Pulmonary and subcutaneous emphysema was the most common pathological lesions experimentally produced in the cases of Bovine ephemeral fever (Burgess and Stradbow, 1977; MacFarlane and Haig, 1955; Theodoridis and Coetzer, 1977).

The occurrence of pulmonary emphysema might be attributed to the accumulation of exudates with the presence of cellular debris within the bronchioles which may obstruct the air pathway and induced forced respiration (Theodoridis and Coetzer, 1977). Spencer (1985) attributed the occurrence of pulmonary emphysema to obliteration and destruction of respiratory bronchioles causing air trapping and also due to inflammatory weakening of peribronchiolar alveolar walls.

Cell membrane blebbing and apoptotic bodies in BEFV-infected cells were observed by TUNEL assay and scanning electron microscopy (Chang et al., 2004). Apoptosis of structural cells in the lung might possibly be an important upstream event in the pathogenesis of chronic obstructive pulmonary disease. There was an increase in apoptotic alveolar epithelial and endothelial cells in the lung tissue. So, the net result is destruction of lung tissue and the development of emphysema (Demedts et al., 2006).

It seems possible that the partially blocked air ways combined with necrosis of the mucosa and muscular part of the bronchioles resulting in rupture of bronchioles and alveoli. The air may reach the connective tissue septae and lymphatic of the lung, and then extends subpleurally to reach the mediastinum. From there the air spreads via the thoracic inlet to the subcutaneous tissues (Theodoridis and Coetzer, 1977).

Odiawo, (1989) reported severe respiratory distress and subcutaneous emphysema that observed in 8 cattle with selenium deficiency diagnosed by estimating the glutathione peroxidase (GSHPx) activity which is a selenenzyme. Selenium is an important constituent of the enzyme glutathione peroxidase (Denneke and Fanburg, 1989). Glutathione (GSH) is an antioxidant and plays a vital role in cellular detoxification and enhancement of immune functions and acts to destroy peroxides, thereby protecting cells and membranes against oxidative damage. Glutathione peroxidase is also a constituent of blood platelets and white blood.
polymorphnuclear cells with releasing of their
pericyte hyperplasia, fibrinoid necrosis of the
be also seen, in addition to the oedematous
blood vessels. The latter appeared dilated and
phosphorus and creatinine concentrations, or
packed cell volume, red cell count, haemoglobin
67
pulmonary emphysema in goats (Janof
pathogenesis of pulmonary emphysema
by intravenous injection of virulent virus
m
(www.springboard4health.com).
immune system and blood clotting mechanism
with a concurrent decline in the number of
In our study, the pathological lesions in most
organs were accompanied with angiopathy of
blood vessels. The latter appeared dilated and
congested, some of which showed necrotic
changes in the intima and the endothelial cells
were desquamated within the lumen. Subintimal
oedema and degeneration of tunica media could
be also seen, in addition to the oedematous
changes that were predominated in the lung
tissue. Young and Spradbrow, (1990-b) reported
increase in permeability of blood vessels
associated with serosal surfaces especially
synovial, pericardial, thoracic and abdominal
serosae in cattle infected with bovine ephemeral
fever. Yellowish turbid fluids were found in the
abdominal, thoracic, and pericardial cavities.
Swelling and hyperplasia of the endothelial cells,
pericyte hyperplasia, fibrinoid necrosis of the
small arteries and perivascular hyperplasia with
occasional thrombosis of vessels in muscles
were detected. Endocarditic and pleuritis were
also seen. BEF virus may be directly chemotactic
to neutrophils. Phagocytosis of the virus may
result in neutrophil degranulation and release of
vasoactive amines which induce vascular
permeability
In the present study, immunohisto-
chemically, the virus could be demonstrated
within leucocytes in the lungs and lymphoid
tissues of the dead animals. Using a direct
fluorescent antibody technique, (Young and
Spradbrow, 1985) found that BEF virus antigen
was identified in synovial, pericardial, thoracic
and abdominal fluids, in synovial membranes
and epicardium. In synovial membranes and
epicardium, specific fluorescence was observed
in two cell types, mesothelial cells and
neutrophils. In the fluids, fluorescence was
restricted to neutrophils. Elimination of
circulating neutrophils in cattle infected with
BEF virus resulted in vireamia without
development of clinical disease (Young and
Spradbrow, 1980). Specific fluorescence,
indicating the presence of BEF viral antigen,
could be detected at the time of peak clinical
response in individual cells in the lungs, spleen
and lymph nodes as well as neutrophils. Before
and after the peak fever some fluorescence was
seen in cells which appeared to be reticular cells
in the lymph nodes. Viral isolation in mice could
be made from blood, lungs, spleen and lymph
nodes over a period of no more than 3 days. It is
postulated that viral growth takes place mainly in
the reticuloendothelial cells in the lungs, spleen
and lymph nodes and not in vascular
endothelium or lymphoid cells (Young and
Spradbrow, 1977). Using real-time PCR assay,
the bovine ephemeral fever virus was detected in
the cow blood (Stram et al., 2005).

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