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Clinical, Biochemical and Pathological Changes Associating Bovine Respiratory Syncytial Virus Pneumonia in Calves

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Abstract: The current study was undertaken to evaluate the clinical, histopathological, hematological and biochemical changes associating BRSV in calves. A total number of 19 calves were subjected to study at private farm (Mosha village, Assiut Governorate, Egypt). Out of them, 13 calves (4 males and 9 females) showed sudden onset of fever and respiratory distress. Sudden death occurred in 4 calves. The remained 6 animals were clinically healthy and represented the control group. Animals were examined clinically and then whole blood and serum samples were collected from all calves under the study. In addition, specimens from lungs, liver and kidneys from dead calves were taken for histopathological and ultrastructural examinations. The present study revealed that bovine respiratory syncitial virus infected both type I and II pneumocyte. The virus induced necrosis in type I pneumocyte and induced proliferation, hypertrophy and necrosis in type II pneumocyte. Infection with BRSV cause hepatic and renal damage and may led to bone marrow depression.

Key words: BRSV, calves, clinical, histopathological, anaemia

INTRODUCTION

Bovine Respiratory Syncytial Virus (BRSV), a pneumovirus in the family Paramyxoviridae is an important cause of acute respiratory disease, especially in young calves (Elvander, 1996). The infection causes huge economic losses for cattle worldwide (Snowder *et al*., 2006). Bovine respiratory syncytial virus is broadly distributed in many countries (Baker *et al*., 1986; Van der Poel *et al*., 1993; Paton *et al*., 1998; Uttenthal *et al*., 2000; Arns *et al*., 2003; Snowder *et al*., 2006). In Sudan, BRSV is one of the major causes of respiratory infection in camel (Dioli and Stimmelmayr, 1992). In Egypt, the virus was detected in cattle (Ghoniem, 1995, 2002; Saber *et al*., 1996; Sahar, 1998; El-Mokamer, 2002; El-Hakim, 2003).

In outbreak situations, morbidity is high but the fatality rate has reached up to 20% and may be attributable to bacterial pneumonia (Murphy *et al*., 1999). Bovine respiratory syncytial virus infection in cattle is characterized by sudden onset of fever, rhinitis, coughing, respiratory distress, increased bronchial sounds, abdominal breathing and reduced appetite (Gillette and Smith, 1985). Pathological lesions with severe disease is characterized by bronchiolitis, multifocal and interstitial edema, emphysema and some cases progressing to sever bronchopneumonia may end with death (Ellis *et al*., 2001). The virus develops numerous syncytial cells and intracytoplasmic eosinophilic inclusion bodies (Rosenquist, 1974; Murphy *et al*., 1999).

Although, many researches were done on BRSV in Egypt (Tawifik, 1992; Saber *et al*., 1996; Ashraf, 2001) but it still threatens livestock production and causes great economic losses. On the other hand, studying the effect of BRSV on blood constituents is lacking. The current study was undertaken to evaluate the clinical, histopathological, hematological and biochemical changes associating BRSV in calves.

MATERIALS AND METHODS

Animals: A total number of 19 calves were subjected to study at a private farm (Mosha village, Assiut governorate, Egypt). Out of them, 13 calves (4 males and 9 females) showed sudden onset of fever and respiratory distress. Sudden death occurred in 4 calves. The remained 6 animals were clinically healthy and represented the control group. The age of calves was 2-4 months and weighted about 45-65 kg. A thorough clinical examination was conducted in which mucous membranes were examined, heart rate and respiratory rate were also determined, rectal temperature was measured and a description of respiratory sounds and the appearance and amount of nasal discharge were recorded. Any coughing was noted during the clinical examination and calves were also examined for presence of diarrhea.

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Samples: Whole blood and serum samples were collected from all animals under study. Whole blood samples were collected from the Jugular vein in Vacutainer tubes containing EDTA and used for hematological analysis using Veterinary Hematology Analyzer (Medonic Vet. 620 CA, Sweden). Samples for blood serum were collected from the jugular vein in plain Vacutainer tubes and processed for separation of serum according to Coles.

Serum samples were used for biochemical analysis using test kits supplied by Spinreact (Spinreact, GIRONA, Spain) and by means of Digital UV Spectrophotometer (Optizen 3220 UV, Mecasys Co., Ltd. Korea). Total Antioxidant Capacity (TAC) was measured in serum using commercial test kits supplied by Bio-Diagnostics (Cairo, Egypt).

Postmortem examinations: Postmortem examination was carried out in all animals.

Histopathology: Specimens from lungs, liver and kidneys were fixed in 10% buffered formalin and embedded in paraffin by routine methods. Sections, 4 μm thick were stained with Hematoxylin and Eosin (HE) (Bancroft *et al*., 1996).

Electron microscopy: Samples of lungs (0.1 mm^3) were fixed in glutaraldehyde 5% in cacodylate buffer (0.1 M, pH 7.2) for 3 times, 20 min each. After dehydration, the samples were embedded in Epon. Semithin sections were stained with toluidine blue. Ultrathin sections were contrasted with uranyl acetate and lead nitrate and the slides were examined using a transmission electron microscope (Jeol, CX11) at 80 kV (Electron Microscope Unit, Assiut University).

Statistical analysis: Data are presented as mean and standard deviation. Data from calves suffered from BVSV were compared with the control group. Statistical significance was determined using Statistical Package for the Social Sciences for Windows (SPSS, Version 10.0, Chicago, IL, USA). Statistically significant differences were determined at $p \leq 0.05$. Data were expressed as mean±SD.

RESULTS AND DISCUSSION

Clinical findings: The noticed abnormal signs were firstly abdominal breathing, anorexia, paleness of all mucous membranes, tachycardia (95-110 beat min^{-1}), hurried respiration (55-80 respiratory cycle/min), rectal temperature (39.5-41°C), paroxysmal dry coughing and nasal discharge was varied from serous, mucopurulent). Researchers also noticed abnormal sounds on auscultation of the lungs, increased bronchial sounds and the animal's demeanor was varied from bright, mildly or severely depressed. Four calves were dead from a total of 13 diseased calves after blood sampling.

Hematological findings: There were significant decreases ($p<0.01$) in RBCs count, Hb concentration and PCV% in BRSV group when compared with the control group. In addition, there were no significant changes in mean corpuscular volume, mean corpuscular hemoglobin and also in total and differential WBCs count (Table 1).

Biochemical findings: Comparing serum biochemical data from BRSV with those from the control group revealed significant increases in serum γ-Glutamyl Transferase (GGT), Alkaline Phosphatase (ALP), iron and Blood Urea Nitrogen (BUN) levels. On the other hand, there were significant decreases in serum phosphorus, magnesium, calcium, glucose and Total Antioxidants Capacity (TAC) in the BRSV group.

Gross lesion: Large areas of consolidation were present in the cranioventral parts of the lungs of infected calves (Fig. 1).

Table 1: Hematological findings in calves infected with BRSV

Parameters	Control group (No. = 13)	BRSV (No. = 6)
RBCs count ($\times 10^{12}$/L)	6.32±0.68	2.75±0.49**
Hb. (g L^{-1})	73.60±6.80	33.90±5.30**
PCV (%)	20.26±2.04	8.91±1.47**
MCV (fl)	32.08±1.88	32.70±3.23
MCH (pg)	11.68±0.56	12.62±1.48
MCHC (%)	36.58±2.84	38.64±3.11
WBCs count ($\times 10^9$/L)	9.71±1.99	10.99±5.32
Neutrophils ($\times 10^9$/L)	3.64±0.91	4.52±2.41
Band cells ($\times 10^9$/L)	0.23±0.14	0.18±0.16
Eosinophils ($\times 10^9$/L)	0.32±0.14	0.24±0.26
Basophils ($\times 10^9$/L)	0.00±0.00	0.00±0.00
Lymphocytes ($\times 10^9$/L)	5.33±0.86	5.52±2.81
Monocytes ($\times 10^9$/L)	0.32±0.18	0.44±0.38

Data were presented as mean±SD

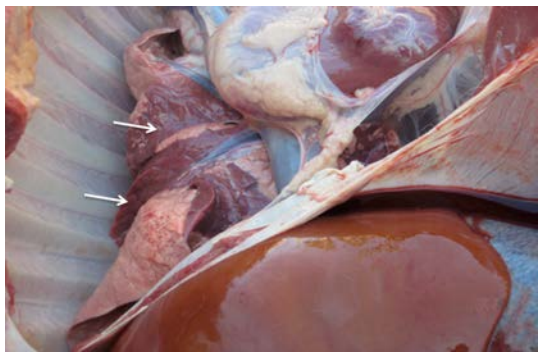

Fig. 1: Consolidation in the cranioventral parts of the lungs (arrows)

Histopathology: Lungs of infected animals showed interstitial pneumonia in which there was severe hyperplasia of type II pneumocytes (Fig. 2a). The proliferation of type II pneumocytes caused narrowing of the alveolar lumen (Fig. 2b). Evidence of giant cell formation can be seen (Fig. 2c). Eosinophilic inclusions were observed in type II pneumocyte (Fig. 2d). There was also hyperplasia of the bronchiolar epithelium. Thrombosis and hemorrhage were constant findings in the liver, lungs and kidneys. In the kidneys, there was a proliferation of the mesangial cells and infiltration of mononuclear cells in the interstitium (Fig. 3a). In the liver, there was vacuolation of the hepatocytes and severe infiltration of mononuclear cells (Fig. 3b).

Electron microscopy: There was a great evidence of proliferation and hypertrophy of type II pneumocyts (Fig. 4a). Hypertrophied type II pneumocyte forming epithelial giant cells. Few typical type I pneumocyte was observed. Some type II pneumocytes showed swelling of mitochondria which revealed that the cells undergo necrosis (Fig. 4b). Inclusions of bovine respiratory syncitial virus were noticed in hypertrophied type II pneumocyte (Fig. 4c). Exudate in the alveolar lumen consisted of neutrophils and necrotic debris (Fig. 4d).

Bovine respiratory syncytial virus is one of the most important viruses affecting the respiratory tract in cattle, especially in young calves. Samples used in the present study, proved to be the best one in the process of the virus isolation. This fact was explained by Viuff *et al.* (1996).

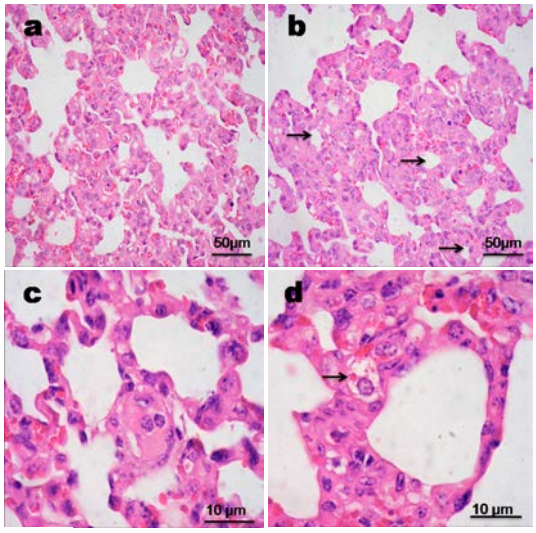

Fig. 2: a) Interstitial pneumonia. Hyperplasia of type II pneumocyte; b) narrowing of the alveolar lumen (arrow); c) giant cell and d) eosinophilic intracytoplasmic inclusions (arrow). Lung, H&E

Grossly, lungs of calves infected with BRSV showed large areas of consolidation in the cranioventral parts. This is a consistent finding observed in field cases and following experimental infection with BRSV (Sacco *et al.*, 2013). The area of consolidation can be also present throughout the cranial, middle and accessory lobes (Gershwin *et al.*, 1998).

Microscopically, lungs of infected animals showed interstitial pneumonia. A variety of infectious agents may induce interstitial pneumonia in cattle including BRSV (Bryson, 1993), larvae of Dictyocaulus viviparous Ascaris suum (Ellis *et al.*, 1996). BRSV induced bronchointerstitial pneumonia, necrotizing bronchitis, hyperplasia of type II pneumocyte (Caswell and Williams, 2008) (Table 2).

From the results, BRSV induced proliferation and hypertrophy of type II pneumocytes. This proliferative response may be contributing to the damage to the alveolar epithelium of type II pneumocytes and migration of leukocytes into the lung (Shami *et al.*, 1986).

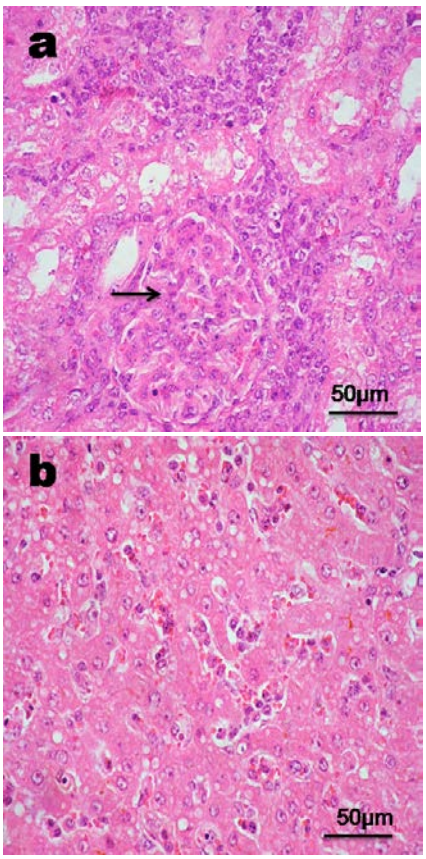

Fig. 3: a) Proliferation of the mesangial cells (arrow). Infiltration of mononuclear cells in the interstitium. Kidney, H&E and b) vacuolation of the hepatocytes, infiltration of mononuclear cells and obstructive jaundice. Liver, H&E

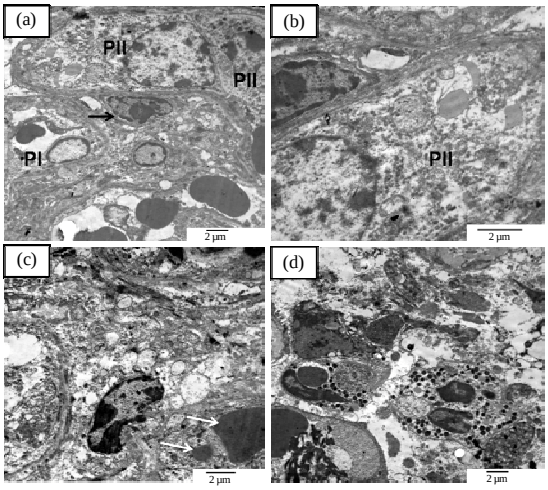

Fig. 4: Electron micrograph of lung: a) increased numbers of Pneumocyte type II (PII). The cells are hypertrophied, X3600. Flattened cells (arrow) can be observed between type II and I Pneumocyte (PI); b) mitochondrial swelling in type II pneumocyte, X7200; c) an aggregate of the viral nucleocapsids are present within the cytoplasm of type II pneumocyte (arrows), X4800 and d) necrotic debris and neutrophils is present within the alveolar lumen, X3600

Table 2: Biochemical findings in calves infected with BRSV

Parameters	Control group (No. = 13)	BRSV (No. = 6)
Total protein (g L^{-1})	69.78±8.0100	62.71±7.7200
Albumin (g L^{-1})	31.83±3.6000	31.55±2.7700
Globulins (g L^{-1})	37.93±9.5800	31.18±7.5600
A/G ratio	0.90±0.3200	1.08±0.3500
AST (U L^{-1})	30.45±4.6800	48.94±27.390
ALT (U L^{-1})	12.63±3.3600	13.04±5.0400
LDH (U L^{-1})	599.48±181.66	791.42±270.37
ALP (U L^{-1})	19.91±10.120	178.21±65.990**
GGT (U L^{-1})	8.69±1.8400	26.17±8.3600**
Iron (µg dL^{-1})	212.06±45.870	269.54±114.80*
BUN (mg dL^{-1})	34.33±11.320	87.10±49.830*
Phosphorus (mg dL^{-1})	7.76±1.9700	4.30±1.1100**
Magnesium (mg dL^{-1})	2.42±0.9300	0.69±0.2300**
Calcium (mg dL^{-1})	10.29±1.1800	4.59±1.5200**
Glucose (mg dL^{-1})	77.28±8.7500	52.01±20.870**
TAC	0.20±0.0400	0.12±0.0300**

Data were presented as mean±SD

The proliferation of type II pneumocytes caused narrowing of the alveolar lumen which causes complications in gas exchange at the alveolar level in calves with respiratory syncytial virus pneumonia (Bryson *et al*., 1991). Evidence of multinucleated giant cell formation can be seen. This result is also described by Bryson *et al*. (1991) and Viuff *et al*. (2002). Mitotic figures occasionally observed within type II pneumocyte cytoplasm and syncytia may have resulted from the fusion of daughter cells following cell division (Bryson *et al*., 1991). Exudate in the alveolar lumen consisted of neutrophils and necrotic debris was seen. Viuff *et al*. (2002) revealed that the lumen of the alveoli was obstructed by neutrophils, cellular debris, macrophages and occasionally eosinophils.

In the present study, the significant increase in serum GGT and ALP activities may be attributed to the vacuolation of the hepatocytes, severe infiltration of mononuclear cells and obstruction of bile ducts. Serum GGT activity is specific to liver disorders of cattle (Mullen, 1976; Pearson and Maas, 1990) and it is a sensitive indicator for cholestasis (Braun *et al*., 1995), biliary hyperplasia and hepatocellular damage (Craig *et al*., 1991). Anaemia in the investigated calves was normocytic normochromic anaemia and may be attributed to depression of the bone marrow.

Measuring TAC gives a complete picture of the oxidative stress state in the body by looking at the levels of markers for the ongoing oxidative damage in serum (Arguelles *et al*., 2004). In the present study, the significant dencrease in TAC, demonstrated a state of increased oxidative stress in the blood of calves with BRSV.

CONCLUSION

Bovine respiratory syncitial virus infected both type I and II pneumocyte. The virus induced necrosis in type I pneumocyte and proliferation, hypertrophy and necrosis in type II pneumocyte. Infection with BRSV cause hepatic and renal damage and may led to bone marrow depression and also contribute to increased oxidative stress.

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