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CLINICAL, HAEMATOLOGICAL AND COAGULATION STUDIES OF BOVINE VIRAL DIARRHOEA IN LOCAL IRAQI CALVES

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Summary

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The objective of the present work was to investigate the clinical, haematological and blood chemical changes associated with bovine viral diarrhoea (BVD) in local Iraqi calves. Out of 84 calves with clinical evidence of disease, 66 (55.44%) were ELISA seropositive for BVD. Diseased calves with BVD showed anorexia (89.39%), profuse watery diarrhoea mixed with mucus/or blood (78.78%), dehydration (78.78%), erosive lesions in the oral cavity (65.15%), salivation (60.6%), erosive lesions on the muzzle (48.48%), petechial and ecchymotic haemorrhages of the visible mucosa (40.9%), weakness (37.87%) and lacrimation (31.81%). Statistically significant increase has been detected in body temperature, respiratory and heart rates in diseased calves in comparison with controls ($P<0.05$). The haematological results of control and diseased calves showed no significant difference in erythrocyte count and haemoglobin, however packed cell volume values were significantly higher in diseased calves than in controls. Leukopaenia, lymphopaenia and thrombocytopaenia were encountered in diseased calves. The platelet volume, platelet distribution width, clotting time, prothrombin time and activated partial thromboplastin time values were significantly higher ($P<0.05$) in diseased calves. Blood biochemical changes revealed statistically significant ($P<0.05$) increased fibrinogen and haptoglobin concentrations in BVD calves.

Key words: bovine viral diarrhoea, calves, indirect ELISA, haematology, blood coagulation indices, haptoglobin

INTRODUCTION

Bovine viral diarrhoea virus (BVDV), an enveloped positive–strand RNA virus, is a common pathogen of cattle causing a wide range of clinical syndromes, depending on the age and immune status of the animal at the time of the infection (Edwards, 1990). BVDV is currently classified in the *Pestivirus* genus of the Flaviviridae family, which is also includes the agents of hog cholera virus and border disease (Larson, 1996).

Field isolates of BVDV can be divided into two biotypes according to their ability to induce cytopathogenicity in bovine cell cultures: cytopathic or non-cytopathic. When a seronegative pregnant cow is infected with a non-cytopathic BVDV biotype, the virus can be transferred to the foetus. Infections in foetal life lead to abortion, mummification, teratogenesis and may produce a persistently infected calf (Bock *et al.*, 1986).

Cattle of all ages are susceptible to BVDV infection. The disease causes decreased animal production, pneumonia, abortion and severe acute gastroenteritis and diarrhoea, erosions of the oral mucosa, followed by death. In addition, the virus suppresses the immune system and makes infected animals more susceptible to other diseases (Lindberg, 2003). BVD infections are classified into three clinical syndromes: acute (transient) infection, foetal infection, and persistent infection (PI). Herds become infected by contact with infected animals, especially with so-called carriers, thereby vertical transmission plays an important role in its epidemiology and pathogenesis (Goens, 2002).

The ability of the virus to cross the placenta during the first trimester of pregnancy can result in the birth of immunotolerant and persistently infected (PI) calves which shed the virus during their entire lifespan (Kampa *et al.*, 2007). PI calves are born following exposure to the BVDV during gestation, either via acute infections of the dam or through exposure from dams who are PI themselves (Yapkic *et al.,* 2006). They are responsible for maintaining BVDV infections in cattle populations. Therefore the identification and subsequent removal of PI animals is necessary to rapidly clear infected herds from the virus (Moerman *et al.*, 1993).

Little information is available on the occurrence of BVD in local calves in Mosul, Iraq. Therefore, the objective of the present work was to study the clinical signs, haematological and some blood biochemical changes of infected calves.

MATERIALS AND METHODS

Animals and study design

The study was conducted on 84 local Iraqi calves (male and female), 6–8 months old, from different farms, with indoor feeding system, showing signs of anorexia, diarrhoea, erosive oral lesions, dehydration, rough hair coat and weakness. The study was carried out in Mosul, Iraq. Ten clinically healthy local Iraqi calves served as control. Careful clinical examination had been carried out in all animals. Faecal samples were screened for parasitic load using standard techniques (Coles, 1986).

Blood collection and analysis

Blood samples (10 mL) were obtained from each calf by jugular veni-puncture. EDTA-mixed blood (2.5 mL) used to determine erythrocyte count (ER), haemoglobin (HB), packed cell volume (PCV), platelets (PLT), mean platelet volume (MPV), platelet distribution width (PDW) and total leukocyte counts (TLC) on an automatic full digital cell counter (Beckman, USA). Differential leukocyte counts were estimated on Giemsa-stained blood smears (Coles, 1986). Another 2.5 mL of blood mixed with trisodium citrate (9:1 ratio) was used to determine prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen using commercial kits (Biolabo, France). Clotting time (CT) was estimated according to Bush (1975).

Serum haptoglobin concentrations (Bovine Haptoglobin ELISA) were assayed according to Hiss *et al.* (2004). Commercial indirect ELISA kits (Svanova Biotech AB Uppsala/ Sweden) were used for detection of BVDV antibodies in serum samples according to manufacturer's instruction.

Statistical analysis

The significance of variations between diseased and healthy calves were statistically analyzed using one way analysis of variance (SPSS) (Leech *et al.*, 2007).

RESULTS

Results showed that out of 84 diseased calves 66 (55.44%) were seropostive for BVDV antibodies. Most of diseased calves with BVD showed anorexia (89.39%), profuse watery diarrhoea mixed with mucus/or blood (78.78%), dehydration (78.78%), erosive lesions in the oral cavity (65.15%), salivation (60.6%). Other clinical signs included erosive lesions on the muzzle (48.48%), petechial and ecchymotic haemorrhages of the visible mucosas (40.9%), weakness (37.87%) and lacrimation (31.81%) (Table 1).

The body temperature, respiratory and heart rates were statistically significantly increased ($P<0.05$) in diseased calves in comparison with controls (Table 2).

The haematological results in control and diseased calves are presented in Table 3. Packed cell volume values were significantly higher in diseased calves. Moreover, a substantial decrease ($P<0.05$) was established in total leukocyte and lymphocyte counts in BVD calves.

Blood coagulation indices and acute phase protein concentrations are presented in Table 4. The platelet count was lower ($P<0.05$) in diseased calves. The platelet volume, platelet distribution width, clotting time, prothrombin time and activated partial thromboplastin time were significantly higher ($p<0.05$) than the respective values in healthy control animals. Serum fibrinogen and haptoglobin concentrations were also significantly ($P<0.05$) elevated in infected calves as compared to controls (Table 4).

DISCUSSION

Infection of cattle with BVDV can result in a wide spectrum of clinical manifestations. The clinical response to infection is complex and depends on several host

Table 1. Clinical signs of calves infected with bovine viral diarrhoea (n=66)

Clinical signs	Number of cases	%
Anorexia	59	89.39
Profuse watery diarrhea mixed with mucous/or blood	52	78.78
Dehydration	52	78.78
Erosive lesions in oral cavity	43	65.15
Drooling saliva	40	60.60
Erosive lesions on muzzle	32	48.48
Petechial and ecchymotic hemorrhages of the visible mucosa	27	40.90
Weakness	25	37.87
Lacrimation	21	31.81

Table 2. Clinical parameters of calves infected with bovine viral diarrhoea (BVD) and healthy calves. Data are presented as mean ± standard error of mean

Parameters	Control (n=10)	BVD (n=66)
Body temperature, ºC	38.7±0.72	40.8±1.83*
Respiratory rate, min^{-1}	21.0±2.35	66.0±8.53*
Heart rate, min^{-1}	63.0±4.52	128.0±11.23*

* $P<0.05$ between BVD and healthy calves.

Table 3. Blood parameters of calves infected with bovine viral diarrhoea (BVD) and healthy calves. Data are presented as mean ± standard error of mean

Parameters	Control (n=10)	BVD (n=66)
Erythrocytes, T/L	6.92±1.34	6.71±1.76
Haemoglobin, g/dL	12.37±1.54	12.21 ±1.22
Packed cell volume, %	33.32±2.41	47.00±4.25*
Total leukocyte counts, G/L	12.36±1.35	10.41±1.52*
Neutrophils, %	44.25±2.43	48.62±4.32
Lymphocytes, %	47.52±3.61	40.84±2.54*
Monocytes, %	4.52±1.11	4.83±2.23
Eosinophils, %	4.28±1.25	4.71±1.34
Basophils, %	1.30±0.25	1.40±0.12

* P<0.05 between BVD and healthy calves

Table 4. Blood coagulation parameters and acute phase protein concentrations in calves infected with bovine viral diarrhoea (BVD) and healthy calves. Data are presented as mean ± standard error of mean

Parameters	Control (n=10)	BVD (n=66)
Blood coagulation indices		
Platelet counts, G/L	453.64±27.32	312.26±62.46*
Mean platelet volume (fL)	9.63±3.43	14.13±3.72*
Platelet distribution width (%)	19.51±3.25	23.45±5.18*
Clotting time (min)	3.35±1.13	4.92±2.72*
Prothrombin time, s	14.54±1.72	18.37±3.48*
Activated partial thromboplastin time, s	51.34±3.71	63.53±6.24*
Acute phase proteins		
Fibrinogen, g/L	4.122±0.313	5.875±0.168*
Haptoglobin, g/L	0.0023±0.0011	0.0076±0.0021*

* P<0.05 between BVD and healthy calves

and agent factors (Ames, 1986). Host factors that influence the outcome of clinical disease include immunocompetence, immunotolerance, pregnancy status, gestational age of the foetus, the immune status (passive or active immunity from previous infection or vaccination), and the level of environmental stress (Baker, 1995).

The primary result of BVD infections is a decrease in immune system capability due to reduction in the white blood cells (immunosuppression) and much of this is due to suppression of the macrophages, neutrophils and lymphocytes, which are the first to respond to infection (Roth *et al.*, 1981). Our results showed a significant leukopaenia and lymphopenia in BVD calves in support of data reported by Bolin *et al.,* (1985) who observed decreased number of both B- and T-lymphocytes in peripheral blood as a consistent finding in acute BVDV infection. Infection with BVDV results in mild (10–20% decrease) or severe lymphopaenia, correlating well with the infection and lesions in lymphoid tissue

(Bruschke *et al.*, 1998; Brodersen & Kelling, 1999). Cytotoxic T-lymphocytes (CD8+) are affected more than helper T-lymphocytes (CD4+) with little or no effect on circulating γ/δ T-cells. The CD4+ depletion increases the period of virus shedding (Ellis *et al.*, 1988). Thereby BVDV increases the susceptibility to secondary infections because lymphocytes from BVDV-infected animals have impaired memory responses to BVDV and other antigens (Lamontagne *et al.*, 1989).

Thrombocytopaenia occurs regularly in cases of severe acute BVD, although the reduction of platelets does not always result in marked haemorrhages (Rebhun *et al.*, 1989, Corapi *et al.*, 1990). It was also confirmed by our results. The cause of thrombocytopenia is not completely understood, however necrosis of megakaryocytes, reduced production of thrombocytes by megakaryocytes, increased consumption of thrombocytes in the periphery, and functional defects of thrombocytes have all been suggested as contributing factors (Walz *et al.*, 1999a). Moreover, Spagnuolo *et al.* (1997) added that the development of thrombocytopaenia is directly related to the infection of bone marrow with BVDV and that in the bone marrow, BVDV can be detected in all cellular elements including megakaryocytes. Bleeding (haemorrhagic diathesis) occurs only when thrombocytes have reached very low numbers (Walz *et al.*, 2001). It was also observed in the present study, indicating the severity of clinical signs in BVD-infected calves. In some cases, highly virulent BVDV may cause mortality early in infection. Since the bone marrow is infected later than other lymphohaematopoietic tissues, and thrombocytopaenia develops after infection of the bone marrow, the death of the animal may occur before haemorrhagic diathesis becomes established. This might explain the variation in frequency of bleeding observed in field and experimental cases of severe acute BVDV infections (Walz *et al.*, 1999b).

In the current study the results showed increased values of clotting time, prothrombin time and activated partial thromboplastin time in infected calves with BVD, as also mentioned by Corapi *et al.* (1989). BVD in calves very often induces changes in the coagulation system which may lead to the development of disseminated intravascular coagulation. The most common coagulopathy in BVD calves is a hypercoagulable state associated with disseminated intravascular coagulation with intensity depending on the severity and duration of the disease (Walz *et al.*, 1999a).

Our results also indicated significant increase in blood haptoglobin concentrations in calves infected with BVD, similarly to data reported by Ganheim *et al.* (2003). The main function of haptoblobin is to bind free haemoglobin. This property has a bacteriostatic effect, as it limits free iron available for bacteria, moreover haptoglobin has numerous other functions related to the host defense response in infection and inflammation, for example stimulation of angiogenesis and modulation of granulocyte activity (Wassell, 2000). The inhibitory effect of haptoglobin on granulocyte activity has been suggested to be beneficial in acute inflammation by reducing the late inflammatory response, which can be harmful to the host (Rossbacher *et al.*, 1999).

In conclusion, bovine viral diarrhoea infection in local Iraqi calves was exhibited through different clinical signs, with significant changes between diseased animals and healthy controls concerning haematological and blood biochemical values.

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