IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 11, Issue 3 Ver. II (March 2018), PP 64-70
www.iosrjournals.org

Evaluation of clinical, hematological, blood coagulation and some biochemical parameter changes in clinically infected cattle with bovine viral diarrhea

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Abstract: *The aim of the present study was to evaluate the clinical, hematological, blood coagulation indices as well as some biochemical parameter changes in naturally infected cattle with bovine viral diarrhea disease. Multiplex reverse transcriptase polymerase chain reaction RT-PCR was used for detection of the disease. From January 2017 to August 2017, A total of 494 cattle ear notches (112 cows, 30 young calves and 352 beef lot calves) was sampled. Fifteen milliliter(15) of blood samples was collected from the jugular vein of cattle using for hematological analysis, estimated blood coagulation indices and serum biochemical analysis. The clinical examination card was used to record the clinical finding of all cattle through complete clinical examinations. The overall prevalence of BVDV (BVDV-1 and BVDV-2) in Nineveh province was 13.96%. Infected cattle suffering from an acute form of disease as anorexia, emaciation, erosive and ulcerative lesions in the oral cavity and on muzzle, profuse watery, mucoied or mixed with bloody diarrhea, dehydration, and respiratory disturbance (coughing, nasal discharge). Hematological parameters showed no significant change in the total erythrocyte count and hemoglobin concentration, with significant increase in packed cell volume, However, Lueckocytopenia and lymphocytopenia was indicated. A significant decrease in Total platelet count with a significantly increased of platelet volume and distribution width, clotting time, prothrombin time and activated partial thromboplastin time in diseased cattle in comparison with controls had been found ($P<0.05$). Biochemical analysis of the infected cattle revealed a significant increase in AST, ALT, ALP, BUN, and creatinine, However, no changes have been detected in the protein values (Total protein, albumins and globulins), glucose, and calcium in diseased cattle than in controls ($P<0.05$). It has been concluded that the present study confirms that the circulation of BVDV in Nineveh province and the results of the current study showed significant changes occur in some hematological, blood coagulation indices as well as biochemical parameters during BVDV infection, which might assist in more understanding the pathogenesis of the disease and aid for developing effective treatment strategies and a fit control programs for BVD infection.*

Key words: *BVD, Multiplex RT-PCR, Clinical pathology changes, Nineveh-Iraq.*

Date of Submission: 05-03-2018 Date of acceptance: 23-03-2018

I. Introduction

Bovine viral diarrhea virus BVDV is a significant pathogen associated with gastrointestinal, respiratory, and reproductive diseases of cattle, which is a worldwide distributed. It is a persistent cause of economic losses in cattle industry, mostly due to decreased reproductive performance. The ability of the virus to cross the placenta during early pregnancy can result in the birth of persistently infected (PI) calves (Ahmed and Kawther, 2008., Khodakaram-Tafti, and Farjanikish, 2017).

Bovine viral diarrhea virus is a member of the Pestivirus genus of the family Flaviviridae. It contains a single stranded RNA genome, In this genus there are four species, BVDV-1, BVDV-2, Border disease virus, and classical Swine fever virus (Vilcek *et al*., 2005). Each genotype has been subdivided into sub genotypes and all these mentioned types are endemic in many countries (Ridpath, 2012).

The RNA genome of BVDV consists of a single-stranded, positive-sense, approximately 12.3 kb in length, and single open reading frame (ORF), flanked by 5'- and 3'- untranslated regions (5'-UTR, 3'-UTR) encodes a polyprotein of about 4,000 amino acids. The polyprotein is co- and post-translationally processed into 12 polypeptides in the following order: N-terminal autoprotease (Npro), capsid protein (C),envelope proteins (Erns, E1, and E2), p7, and non-structural (NS) proteins (NS2, NS3,NS4A, NS4B, NS5A, and NS5B) (Collett *et al* ., 1988).

Bovine viral diarrhea BVD causes different forms of diseases, including subclinical benign diarrhea, peracute highly fatal diarrhea, hemorrhagic and thrombocytopenic disease, reproductive failure, fatal mucosal disease of PI animals, abortions and malformations (Hilbe *et al* ., 2007). In addition, BVDV causes a general immunosuppression. Animals that are born might persistently be infected (PI) with BVDV and are considered the direct viral reservoirs that shed copious amounts of virus into the environment through aerosols, mucus secretions and fecal matter (Kaiser et al ., 2017).

The clinical manifestation of a BVDV infection depends on viral strain and the animal's immune status. Severe cases are characterized by fever (40-41°C), anorexia, depression, erosions and hemorrhages of the gastrointestinal tract, diarrhea and dehydration. In mild cases, diarrhea may not be prominent. Most BVD infections are subclinical, and the course of the disease varies from 2-3 days up to 4 weeks (Rodninga et al ., 2012). In cattle, BVDV has been associated with a pathologic effect in several organs, including the respiratory, hematologic, immunologic, neurologic, and reproductive systems. Acute infection of an immunocompetent animal, most commonly leads to subclinical infection, although clinical signs of lethargy, decreased milk production, lymphopenia, thrombocytopenia, diarrhea, and death because of fulminating disease also may occur in some cases (Brock, 2004). Viral infections may be a cause of haemostatic dysfunctions in cattle (Radwińska, 2010). Moreover, DeMaula et al. (2002) noted increased levels of blood coagulation in cattle affected by the bluetongue disease. Cattle attacked by the BVD virus may show haemostatic imbalance, including primary as well as secondary haemostasis manifested by extravasations or hemorrhge at mucous membranes which could deteriorate the functioning of internal organs as DIC progresses. One of the most potential outcomes of acute BVD infection is hemorrhagic disease, which is characterized by thrombocytopenia and an increased susceptibility to bleeding (Walz *et al* ., 1999). Studies on serum biochemical changes in cattle naturally infected with BVD disease are scanty, Since, The measurement and evaluation of some biochemical profile might helpful in elucidating the pathogenesis and prognosis of the disease. Significant changes were indicated in the blood biochemical parameters of animals exposed to viral diseases (Akalın *et al* ., 2015).

Several diagnostic methods are available for the detection of antigen, antibody and viral components (antigen and nucleic acids) of BVDV. These include virus isolation from blood or tissues, immunohistochemical staining of skin samples, reverse transcriptase-polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) for detection of antigen or antibody (Hilbe *et al* ., 2007). The PCR method is highly sensitive and suitable for a large variety of samples, including blood, milk, saliva, follicular fluid and tissue as well as for genotyping for detected and isolated BVDV (Lanyon *et al* ., 2014). The aim of this study was to evaluate the clinical, hematological and some serum biochemical parameter changes in clinically infected cattle with bovine viral diarrhea at Nineveh province.

II. Materials and methods

Animals and sample collection

From Jan 2017 to Aug 2017, 494 ear notch samples (1 centimeter square) were collected from the external ear pinna by using a sterile disposable surgical blades, the samples were placed in a sterile test tube and transported to the laboratory by using ice bag were stored at -20 °C until analysis. Samples comprising adult cows (n=112), young calves (n=30) and beef lot calves (n=352) of both sexes and of different breeds. Blood samples were collected from the jugular vein of cattle using 18G needle into three sterile vacutainers® tubes (5ml each), two with anticoagulant one contain ethylene diamine tetraacetic acid (EDTA) for hematological analysis, The second was contain trisodium citrate (9:1 ratio) for estimated blood coagulation indices and third tube without any anti-coagulant for serum which was separated by centrifuging at 2500 rpm (280 G-force) for 15 min and stored at -20°C for serum biochemical analysis. Clinical examination was conducted to all cattle and clinical findings were recorded in a clinical examination card.

RNA extraction and amplification from cattle ear notches

The RNA of BVDV was extracted from 494 ear notches employing the QIAamp® Viral RNA kit (RNA extraction from ear notches without purification) (ADIAGENE, BioX Dignostic. France). The procedure was adapted from the literature that came with the extraction kit. Amplify the highly conserved region 5' UTR gene of BVDV from ear notch samples (n=494), as a target in multiplex PCR technique using QIAGEN OneStep RT-PCR Kit (Qiagen, Germany) for BVDV detection and determination of genotypes. The oligonucleotides of specific primers were designed by (Gilbert *et al.*, 1999) which were provided by First BASE Laboratories Sdn. Bhd. Malaysia (Table 1). In this PCR process, two positive cDNA derived from persistently infected calve was used as positive control. The GenBank accession numbers for cDNA positive controls were (MF347399, MF491394) for BVDV1 and BVDV2 respectively. Further, cDNA extracted from non-infected calve was used as negative control for each PCR amplification. In this study, all the animals tested positive were re-sampled 30 days after the first round of testing to differentiate persistently infected animals. Multiplex PCR reactions were conducted in a total volume of 50 µl, composed of 10µl 5XQaigen RT PCR buffer, 3µl of each

primer, 2µl of dNTP mix, 2µl of RT-PCR enzyme, 10µl of template (RNA sample) and 17µl dH2o. The mixture was briefly centrifuged and reverse transcription was done at 550C for 30 min in the thermo-cycler machine. This was followed initial denaturation at 95 ºC for 15 min, followed by 40 cycles of denaturation at 94 ºC for 20 s, annealing at 50 ºC for 30 s, extension at 72 ºC for 30 s and final Extension-72 ºC for 15 min, The samples were removed at the end of the program when the temperature on the screen showed 4ºC

Table 1: The Oligonucleotide primers used to amplify the 5'UTR gene.

primer	**Sequences 5'-3'**	**Amount of oligo (nMoles)**	**size(bp)**
BVD_F	TGG AGA TCT TTC ACA CAA TAG C	30.7	
V1-R	GGG AAC CTA AGA ACT AAA TC	33.7	360
V2-R	GCT GTT TCA CCC AGTT (A/G)TA CAT	27.8	604

Estimation of hematological, serum biochemical parameters and blood coagulation indices

The blood in tubes with anticoagulant was utilized to verify the total erythrocyte counts (TECs), hemoglobin concentration (Hb), packed cell volume (PCV), and total leukocyte counts (TLC) by using a Hematology analyzer (Mythic 18VET/France). Differential leukocyte counts (DLC) were estimated on Giemsa-stained blood smears as mentioned by (Weis and Wardrop, 2010). 2.5 mL of blood in tubes with EDTA was used for platelet counts, mean platelet volume, and platelet distribution width, by using a Hematology analyzer (Mythic 18VET/France). Another 2.5 mL of blood mixed with trisodium citrate was used to determine prothrombin time (PT) and activated partial thromboplastin time (APTT) using commercial kits (Biolabo, France).Moreover, serum was used for biochemical analysis, including aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), creatinine, blood urea nitrogen (BUN), total protein, albumins, globulins, glucose and calcium, using special cassettes for each in a Chemistry analyzer (IDEXX - Vet Test, Arachem/USA).

Statistical analysis

The significance of variations between diseased and healthy cattle in hematology, coagulation indices and serum biochemical parameters was statistically analyzed using independent sample t-tests and one way analysis of variance in IBM-SPSS statistics version19 program. All the significant differences were determined at ($P<0.05$).

III. Results

Diagnosis of Bovine viral diarrhea virus was confirmed by using Multiplex reverse transcriptase polymerase chain reaction RT-PCR (Fig.1).

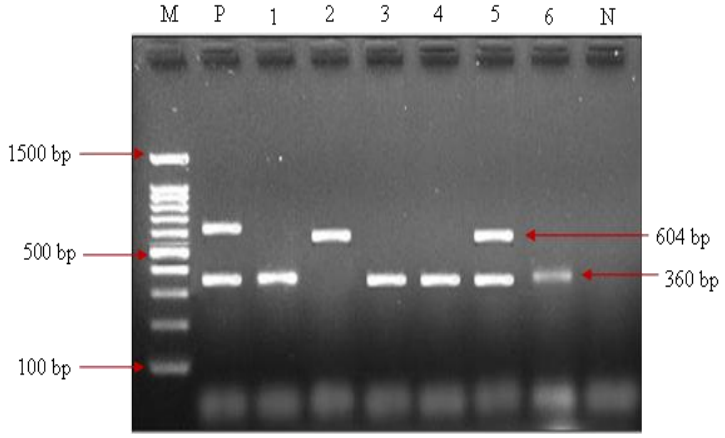

Figure 1*: Gel electrophoresis image showing: lane M) Exact Mark 100-1500bp DNA ladder; Lane P) cDNA extracted from PI calf used as positive control for BVDV1 and BVDV1; Lane 1, 3, 4, 6) Multiplex PCR technique detected only BVDV1 in approximately band size 360bp; Lane 2) Multiplex PCR technique detected only BVDV2 in approximately band size 604bp; Lane 5) Multiplex PCR technique detected both BVDV1 and BVDV2 in approximately band size 306 and 604bp respectively; Lane N) cDNA extracted from BVDV-free calf used as negative control.*

The overall prevalence of BVDV (BVDV-1 and BVDV-2) in Nineveh province was 13.96% (69 out of 494) (Table 1). Infected cattle with acute BVD disease were suffering from fever, anorexia and emaciation, profuse watery and bloody diarrhea mixed with mucus, dehydration, respiratory disturbance (coughing, nasal discharge) and erosive lesions in the oral cavity, muzzle and interdigital space, drooling saliva, lacrimation,

petechial and ecchymotic hemorrhages of the visible mucosa, epistaxis, poor growth, decrease of milk production, with different frequency percentage (Figure 2 and 3).

In the present study the haemogram of cattle infected with BVD was found to be no significant changes in TECs, Hb. Whereas, a significant increase ($P<0.05$) in PCV and a significant decrease ($P<0.05$) in TLC due to lymphocytopenia in BVD cattle compared to the healthy group was indicated (Table 3). The results of the blood coagulation indices in cattle infected with BVD showed a significant decrease in platelet volume. However, platelet distribution width, clotting time, prothrombin time and activated partial thromboplastin time was significantly increased ($P<0.05$) than the respective values in healthy group (Table 4).

Serum biochemical analysis of the infected cattle revealed a significant increase ($P<0.05$) in aspartate aminotransferase AST, alanine aminotransferase ALT, alkaline phosphatase ALP, blood urea nitrogen BUN, and creatinine, while no significant changes ($P<0.05$) in the total protein (albumins and globulins), glucose and calcium values compared to the healthy group .(Table 5).

Table 2: Prevalence of bovine viral diarrhea virus in cattle at Nineveh province using multiplex PCR.

Type of test	No. of samples	No. of positive samples	Overall prevalence of BVDV
Multiplex PCR	494	69	13.96%

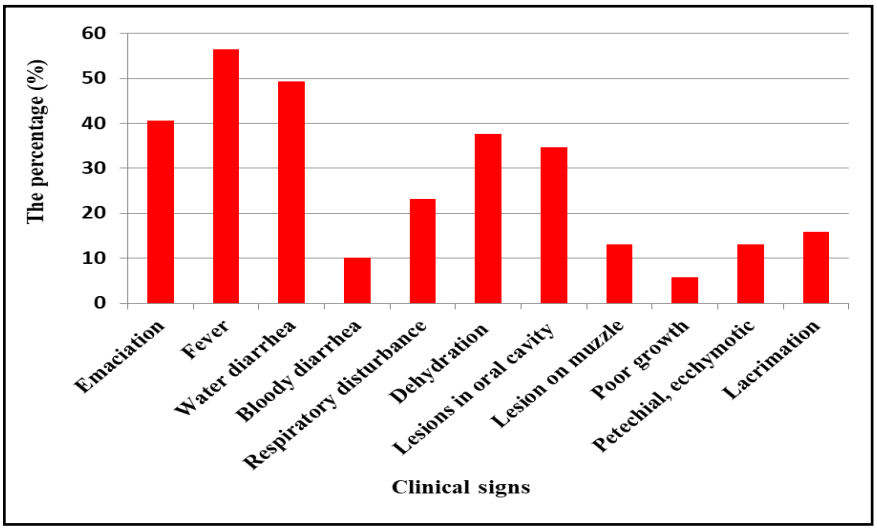

Fig. 2 Percentage of clinical signs in cattle infected with the BVD disease (n=69).

Figure 3: A) Weakness and emaciation of persistently calf ; B) Bloody diarrhea in acutely BVD infected calf ; C) Ulcerative lesions on muzzle in acutely BVD infected calf ; D) Erosive lesions in oral cavity in acutely BVD infected calf .

Table 3: Blood parameters of calves infected with bovine viral diarrhoea (BVD) and healthy group.

Parameters	Healthy group (n=15)	Infected cattle (n=69)
Total Erythrocytes count $\times 10^6$ µl	6.71 • ± 0.85	6.69 • ±0.72
Hemoglobin, g/ dL	11.97 • ± 1.74	11.93 ±1.64
Packed cell volume, %	32.27 • ± 3.68	45.22 ± 2.23*
Total leukocyte counts, $x10^3$µl	11.96 • ±1.53	9.54 • ± 0.9*
Neutrophils, %	47.33 • ± 3.12	46.62 • ±2.32
Lymphocytes, %	48.82 • ±3.93	41.04 • ± 2.62*
Monocytes, %	3.97 • ±1.02	3.83 • ±3.33
Eosinophils, %	3.18 • ±1.10	3.22 • ±1.9
Basophils, %	1.20 • ±0.15	1.23 • ±0.13

Data are presented as mean • ± standard error of mean.P<0.05 between infected cattle and healthy group

Table 4: Blood coagulation indices in cattle infected with bovine viral diarrhoea (BVD) and healthy cattle.

Parameters	Healthy group (n=15)	Infected cattle (n=69)
Platelet counts, $x10^3$ µl	465.32 • ± 38.22	311.53 • ± 53.44*
Mean platelet volume, (fl)	10.15 • ± 2.31	14.18 • ± 1.93*
Platelet distribution width (%)	18.72 • ± 1.26	24.22 • ± 4.16*
Clotting time (min)	3.38 • ± 0.92	4.96 • ± 1.83*
Prothrombin time/ Sec	13.85 • ± 1.35	17.44 • ± 2.52*
Activated partial thromboplastin time/ Sec	53.45 • ± 4.77	65.56 • ± 5.21*

Data are presented as mean ± standard error of mean. P<0.05 between infected cattle and healthy group

Table 5: Serum biochemical parameters of cattle infected with bovine viral diarrhea (BVD) and healthy cattle.

Parameters	Healthy group (n=15)	Infected cattle (n=69)
AST U/L	55.45 ± 18.76	97.61 ± 23.03*
ALT U/L	36.63 ± 10.75	62.17 ± 11.88 *
ALP U/L	64.65 ± 21.13	76.78 ± 26.11*
BUN mg/dL	17.80 ±6.22	28.44 ± 6.45 *
Creatinine mg/dL	0.87 ± 0.19	1.85 ± 0.24 *
Total protein g /dl	7.57 ± 0.66	7.22 ± 0.48
Albumin g/dl	3.66 ± 0.65	3.47 ± 0.50
Globulin g/dl	3.83 ± 0.21	3.67 ± 0.32
Glucose mg/dL	59.46 ± 11.86	59.21 ± 8.97
Calcium mg/dl	9.12 ±1.69	9.9 ±1.82

Data are presented as mean ± standard error of mean.P<0.05 between infected cattle and healthy group

IV. Discussion

The results illustrated that the overall prevalence of BVDV in Nineveh was 13.96%, which was higher compared to previous studies in Iraq and other countries that used the PCR technique to diagnose this disease. The prevalence was 6% in cattle by using conventional RT-PCR in regions around Baghdad (Al-Ajeeli and Hasan, 2011). It had been found that the prevalence of BDV in Bsarah and Nassirya cities in Iraq, was 10 % in cattle (Jarullah *et al* ., 2012). In Tunisia, 2.65% (Thabti *et al* ., 2005), in Poland 3.9% (- Wernicki *et al* ., 2015), in Canada 10.44% (Deregt et al ., 2002), in Iran 18.23% (Safarpoor and Haghighi, 2012), In Egypt 17.2% (Soltan et al ., 2015) and in Turkey 11.45%(Yilmaz, 2016). The prevalence of BVD disease differs from country to another and evern aqt the regions within the same country and that might due to the different in management practices, number of animals samples, types of sample, how sensitive the diagnostics methods used, incidence of competent vectors, cattle activity, presence and efficacy of control programs, the climatic variations, extensive cattle trade and uncontrolled animal movement, The variation in the cattle population size, biosecurity, and persistence of BVDV (Farhad, 2011., Graham *et al* ., 2013).

The current study showed that the clinical signs of cattle ranged from mild to moderately severe signs of BVDV, and these signs were the same as those reported by (Alsaad *et al* ., 2012 ., Brodersen, 2014). The clinical signs of BVD disease are variable due to genetic mutation tendency in BVDV strains. Most of the clinical signs are uncharacteristic of BVD (Everman and Ridpath, 2002).

In the current study the haemogram of cattle infected with BVD was found to be no significant changes in TECc, Hb. These findings were consistent with (30). The results showed a significant increase in PCV of cattle infected with BVD. This was in agreement with (Fernández-Sirera *et al* ., 2011 ., Oguzhan *et al* ., 2014). This change may be attributed to haemoconcentration, excessive loss of body fluid and dehydration, which lead to decrease plasma volume in diarrheic calves (Galbat *et al* ., 2015).

This study showed significant decrease in total leukocytes count TLC. This was due to lymphopenia observed in infected cattle. This finding could be indicative of the lesions in lymphoid tissue, which result in destruction and decrease of the lymphocytes and it is usually seen in the acute stage of infection. Studies previously done showed that the virus localize in enterocytes, Peyer's patches, thymus, spleen, lymph nodes, tonsils and liver (Wayne et al ., 1989., Pedrera *et al* ., 2012 ., Lokhandwala *et al* ., 2017).

The present study showed that the results of the blood coagulation indices in cattle infected with BVD disease were significant decrease in platelet volume. While, platelet distribution width, clotting time, prothrombin time and activated partial thromboplastin time were significantly increased in infected cattle. Our results agree with the former researchers (Radwińska , 2010). Thrombocytopenia was seen in severely affected cattle with the significant decrease platelet count. These changes in the haemogram finding suggest that BVD infections could lead to the development of disseminated intravascular coagulopathy DIC and also may shorten the platelet life span due to large platelet consumption during hemorrhage. In contrast (Paul *et al* ., 1999 ., Fiore *et al* ., 2006) did not observe any changes in the plasma coagulation system of six cows infected with the BVD virus.

This current study showed that the serum biochemical analysis of the cattle infected with BVD revealed a significant increase in AST, ALT, ALP, BUN, and creatinine. This may be indicative of the damage to the skeletal and heart muscles, hepatocytes and kidneys. The elevation in both ALT and AST was detected in the clinically infected cattle with acute BVD. Increased ALT and AST is relatively specific for liver damage, but also may originate from other tissues such as heart and skeletal muscles These enzymes may be released and detected during the pathological situation. These findings concurred with the evidence in the literature of (Galbat *et al* ., 2015). Although the present data revealed no significant changes in the total protein (albumins and globulins) in infected cattle, However, the relative difference may be due to starvation, malabsorption, and hepatic depletion. Also, it could be attributed to stress of diarrhea which may affect the hepatic parenchyma, which lead to decrease of protein synthesis, this result consistent with (Fernández-Sirera, 2011 **.,**). On the other hand, there is little information about the changes of some electrolytes such as calcium, glucose, magnesium and other trace elements, and their relationships with clinical signs in cattle infected with BVD disease.

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Sadam, D.Hasan "Evaluation of clinical, hematological, blood coagulation and some biochemical parameter changes in clinically infected cattle with bovine viral diarrhea." IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) 11.3 (2018): 64-70.