CLINICAL AND DIAGNOSTIC STUDIES OF BOVINE VIRAL DIARRHEA IN BUFFALO CALVES AT BASRAH GOVERNORATE ,IRAQ

Hussein A. Abdul Wahid, Kamal M. AL-Saad

Department Of Internal And Preventive Medicine, College of Veterinary Medicine, University Of Basrah,Basrah,Iraq.

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Corresponding Author: kamalsad58@yahoo.com

ABSTRACT

Bovine viral diarrhea BVD has been detected and diagnosed in local buffalo calve breeds of Basrah, Iraq. The study was conducted to examine (980) suspected buffalo calves under one year old and of both sexes. One hundred sixty-eight (168)calves give positive results with PCR test. Twenty-five (25) clinical healthy local buffalo calves are considered as controls. Diseased calves show different clinical manifestations belong to the disease with a significant increase indicated in the body temperature, respiratory and heart rate, as well as the capillary refill time of diseased buffalo calve compared with controls. Results of hematological changes indicated a significant increase in packed cell volume in diseased buffalo calve than in controls, Moreover, A significant leukocytopenia due to a significant lymphocytopenia was also indicated in diseased animals compared with the control group. Results of the clotting factor indices of diseased calves and controls show a significant decrease in total platelet counts, However, the platelet volume and the platelet distribution width, the clotting time, the prothrombin time, the activated partial thromboplastin time was significantly increased in infected animals than in controls. A significant high value was indicated in Aspartate and Alanine aminotransferase, Alkaline phosphatize as well as the blood urea nitrogen, In BVD buffalo animals than in the control animals, On the contrary, a significant decrease was encountered in total protein in diseased calves than in the control group. Results of the acute phase response of the current study revealed a significant increase in haptoglobin in BVD buffalo calves than the

control group, Whereas, a significant decrease in Fibrinogen time has been indicated in BVD calves. The macroscopic examinations of the BVD carcasses revealed severe congestion of the intestinal vessels accompanied by Ecchymotic hemorrhagic enteritis with multiple enlargements of mesenteric lymph nodes along with most parts of the small and large intestine with pasty fecal materials. Furthermore, atrophy of the intestinal villi with sloughing of the epithelial lining of villi of the small intestine, as well to hyperplasia of goblet cells, infiltration of inflammatory cells in the intestinal mucosa, to congestion of blood vessels was also indicated via histopathological examinations. It has been concluded that BVD has very harmful effects on domestic ruminants, which mostly terminated by death, Therefore, applying the control measures is the final and suitable choice to control and eliminate the disease.

INTRODUCTION

Bovine viral diarrhea is a common disease and an important infection of livestock causes many economic losses characterized by depression, fever, diarrhea, and immune suppression (1).

The causative agent, is a BVD virus belong to the genus Pestivirus of the Flaviviridae. Which is considered as a single-stranded RNA genome (2). And have another subspecies named Swine fever and Border disease virus as well as BVDV-1 and BVDV-2, according to genome sequences (3, 4). Cytopathic (Cp) and Non-Cytopathic(Non-Cp) are other classifications according to growth characteristics in cell culture of the two BVDV strain, Since the cytopathic type cause visual cytopathic impact, However, the non-cytopathic could grow in cells without any harm (5, 6).

The disease leads to different forms including subclinical benign diarrhea, the peracute type which mostly highly fatal, Thrombocytopenic, and hemorrhagic form with hemorrhagic diathesis, Reproductive failure, persistently infected (PI) animals of a fatal mucosal disease, Abortions form with malformations (7, 8). Moreover, Animals that are born persistently infected could be considered viral reservoirs and could shed copious amounts of the virus to the environment via aerosols, mucus secretions, and fecal matters (6).

The disease causes high economic loss because of the abortion, congenital defects of newborns, retardation of growth, abnormal reproductive diseases, high mortalities because of mucosal form, and premature isolation, and maybe the culling of PI animals (9, 10, 11).

In general, the disease can occur at all animal ages, However,The consequences of the infection in immune-competent cattle ranges from subclinical or mild to a highly fatal disease, Further, Most domestic animals like sheep, goats, pigs, camels and wild animals, such as deer's and wild boars can be infected with BVD virus and may become an important source of the infection (12, 13).

Bovine viral diarrhea is very scared in local calve buffalo breeds of Basrah Iraq, And little information has been provided, therefore, the present work aims to investigate the clinical and diagnostic criteria including hematological, biochemical, histopathological and evaluation of acute phase response in diseased local buffalo calve breeds of Basrah, Iraq.

MATERIALS AND METHODS

Examined animals and the study areas: This work was design to examine (980) buffalo calve less than one year old and from both sexes from all Basrah province areas, represent fifteen(15) herds with a different management system. The study was started from, January to December. 2020. One hundred and sixty-eight (168) local buffalo calve breeds shows signs of enteritis with bloody diarrhea. Twenty five (25) clinically healthy local buffalo calve breeds of same ages was considered as controls. Complete clinical examinations has been applied to all animals and fecal samples was screened to excluded any infection with gastro-intestinal parasites(employing standard techniques such as direct, floatation and sedimentation, Moreover, Giemsa stained blood smears was also used to rule out the blood parasitic infection (14).

Blood Sampling and hematologic analysis: Ten milliliter of Blood samples collected from each local buffalo calf via jugular vein. Ethylendiaminetetraacetic acid (EDTA-mixed blood(2.5ml) was used to determine Total red blood cell count (RBC), Hemoglobin concentration (HB), Packed cell volume (PCV), Total platelets count, Mean platelet volume, The platelet distribution width, and total leukocyte counts (TLC) on an automatic full digital cell counter from (Beckman, USA). Moreover, Differential leukocyte counts were estimated on blood smears stained with Giemsa, according to (15).

Other, 2.5 ml of blood mixed with Trisodium citrate (with a ratio of 9:1) was used for evaluation of the prothrombin time, activated partial thromboplastin time, and Fibrinogen time using kits from (Biolabo, France). Furthermore, Clotting time

was evaluated according to Bush (16). The remaining blood sample was used for extraction of serum which was stored at -20°C until use. Serum samples extracted from the Blood were tested spectrophotometrically according to the manufactures instructions of (Roche Diagnostics, Indianapolis, GMBH, Germany) to evaluate, Aspartate and Alanine aminotransferase, Alkaline phosphatize as well as the blood urea nitrogen and Total protein. Moreover, the Estimation of Haptoglobin (Sandwich Elisa method), As the color changes of the stop solution was measures at 450 when using a spectrophotometer device).

RNA extraction from serum sample: The RNA of the BVDV extracted from 980 suspected buffalo calves serum samples using the QIAamp® Viral RNA kit (RNA extraction from serum without purification) (ADIAGENE, BioX Dignostic. From France). This procedure was adapted from the usual literature that came with the extraction kit.560 µl of AVL buffer +Carrier RNA was added to each serum sample, homogenized by vortex for fifteen seconds then incubated at room temperature for ten minutes. after that, 100 µl of the supernatant was transferred to a 1.5 ml microtube. For the binding preparations, an amount of 560 µl of ethanol 100% was added, then homogenized by vortex for fifteen Sec..630 µl of the obtained solution was applied to the corresponding column (Identify columns) and centrifuged for 1 minute at 10000 rpm.

The collection tube was changed and the rest of the mix was put in the column and centrifuged for one minute at 10000 rpm. The collection tube was changed, and 500 µl of buffer AW1 was added, followed by Centrifuging for one minute at 10000 rpm.The collection tube was changed, 500 µl of buffer AW2 was added and centrifuged for 1 minute at 10000 rpm.The collection tube was changed, followed by centrifuging for 3 minutes at 10000 rpm.The column was transferred to a microtube. Then, 60 µl of buffer AVE was added. This was followed by incubation for 1 minute at room temperature and then centrifuging for 1 minute at 10 000 rpm.The tubes were closed and identified. They were then stored on ice immediately at <-15°C until tested. Viral RNA is stable for up to one year when stored at –30 to –15°C or –70°C.

cDNA synthesis and RT-PCR: For cDNA synthesis, 5 µL of extracted RNA, 1 µL of a specific reverse primer and 14 µL of DEPC treated water were added to the lyophilized master mix contained in Bioneer AccuPowerTM RT PreMix kit (Korea).

The mixture was incubated at 42°C for 60 min. cDNAsynthesis was terminated by incubation at 95°C for 5 min. Amplification of 5′ UTR (288 bp) was carried out on serum samples .RNA using the primers 324 (5′-ATG CCC WTA GTAGGA CTA GCA-3′) and 326 (5′-TCA ACT CCA TGTGCC ATG TAC-3′) to detect pesti-virus infection as described by Vilcek et al. (1994). In individual samples, amplification of cDN As by PCR was performed using the primer pairs 0I 100 (5′-CAT GCC CWY AGT AGG ACT AGC-3′)/1400R (5′-ACC AGT TGC ACC AAC CAT G-3′) as described by Becher et al. (1999) and BD1 (5′-TCT CTG CTG TAC ATG GCA CAT G-3′)/BD2 (5′-TTG TTR TGG TACARR CCG TC-3′) nested PCR) as described by Vilcek et al. (1997) and BD1/BD3 (5′-CCA TCT ATR CAC ACA TAA ATG TGG T-3′) and BD1/BD4 (5′-CCA TCC ACG CAT ACG TAG ATG TG-3′) to detect the strains of BVDV as described by Vilcek et al. (2001).

In the nested PCR, at the first PCR, the outer primers 0I 100 and 1400R were amplified and at the second PCR, 3 µL of the first-round PCR product was amplified with primers BD1/BD2 using the same number of cycles and the same thermal profile to obtain a 738 bp DNA fragment. In addition, to amplify a 428 bp amplicon to detect the BVDV strains NADL and UK the primersBD1/BD3 and BD1/BD4 were employed, respectively. All oligonucleotide primers were got from a commercial origin (Bioneer, INC., Korea).

PCR was carried out in a total volume of 30 µLcontaining 3 µL of 10 x PCR buffer, 0.5 µL of dNTPs(0.16 mM), 1 µL of cDNA, 1 µL of each primer (10pmol), 1.2 µL of MgCl2 (2 mM), 0.3 µL of Taq DNA polymerase (1.5 U) and 22 µL of DNase/RNase free distilled water. Reactions were completed done in an automated thermal cycler (Bio-Rad gradient Thermal Cycle) Cycle parameters for PCR were as follows: initially 95°C for 5 min followed by thirty-five cycles in 3 continuous phases including 94°C for 30 s, 55°C for 100 s, and 72°C for 2 min, and finally terminated by a single cycle of a final extension at 72°C for 10 min. The RTPCR-amplified products were examined by electrophoresis in a 1.5% agarose gel, stained with a 1% solution of ethidium bromide, and visualized using a UV transilluminator.

Gross post-mortem and Histopathology examinations: Animals that died were subjected to post-mortem examinations and laboratory histopathological evaluations. The tissue samples were collected from the intestine. The collected samples were fixed at 10% neutral buffered formalin solution for 72 hrs, then cut to appropriate

sizes and washed, dehydrated, cleared in xylol. Finally, it was embedded in paraffin wax, and sectioned at the 4-5µ thickness, stained with hematoxyline and eosin, and examined under a light microscope (17).

Statistics: The analysis of statistics between diseased and the clinically normal local buffalo calve breeds was done using (Spss) program, The t-test (18). Data will be presented as a mean ± standard error of the mean. $P<0.05$ between diseased and controls.

RESULTS

The present work results, indicates an overall prevalence of (17.14%) in local buffalo calve breeds at Basrah province, Iraq, As, out of (980) suspected cases (168) found positive by PCR test.

Among the most important clinical manifestations that the diseased animals showed was, Anorexia and unable to suck (95.23%),Clear watery diarrhea mixed either with mucous or with blood (93.45%), Dehydration with different degrees (93.45%), Erosive lesions present on the mouth, gums tongue and muzzle (83.92%), Excessive salivation(81.54%), Petechial hemorrhages detected on ocular mucus membranes (79.16%), Lacrimation (52.38%). Weakness and emaciation of diseased animals(38.69%)Table 1.

Table .1: The clinical manifestations of diseased local buffalo calve breeds

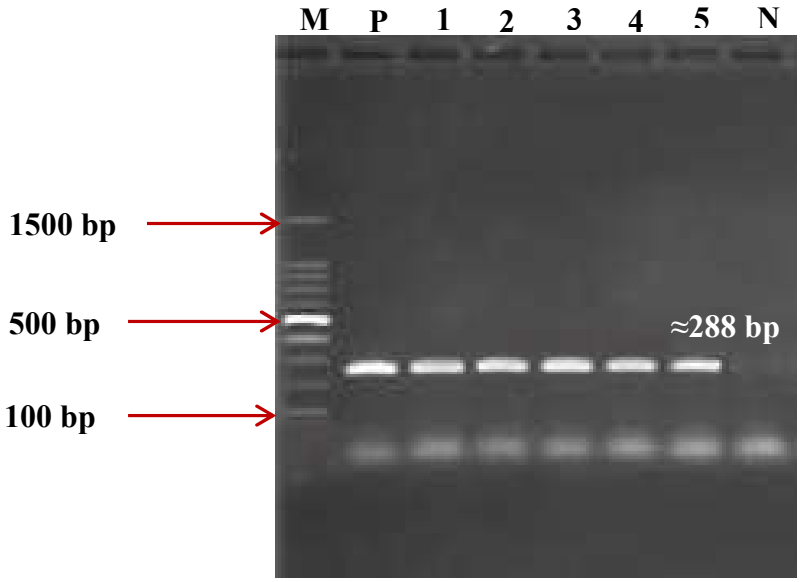
Clinical manifestations	NO. of animals	%
Anorexia and unable to suck	160	95.23
Clear watery diarrhea mixed either with mucous or with blood	157	93.45
Dehydration with different degrees	157	93.45
Erosive lesions present on the mouth, gums tongue and muzzle	141	83.92
Excessive salivation	137	81.54
Petechial hemorrhages detected on ocular mucus membranes	133	79.16
Lacrimation	88	52.38
Weakness and emaciation of diseased animals	65	38.69

Moreover, a significant (P<0.05) increase was indicated in the body temperature, the respiratory and the heart rate, as well as the capillary refill time of diseased local buffalo calve breeds compared with controls Table 2.

Table. 2 : Body temperature, respiratory, heart rate and capillary refill time of diseased local buffalo calve breeds and controls

Clinical parameters	Control buffalo calves **n=25**	Diseased buffalo calves **n=168**
Body temperature / ºC	38.4±0.28	40.6±1.32*
Respiratory rate / min	22.0±3.45	68.3±10.78*
Heart rate / min	88.4.3±6.85	133.4±12.67*
Capillary refill time/ min	1.42 ± 0.63	5.23 ± 0.72*

In the current study diagnosis of BVDV was confirmed by using PCR technique (Fig:1), As, all selected blood samples give positive results



Figure(1): The Gel electrophoresis image showing: lane M) Exact Mark 100-1500bp DNA ladder; Lane P) cDNA extracted from the diseased local buffalo calve breeds used as positive control for the BVDV; Lane 1, 3, 4, 5) the Conventional PCR test detected only BVDV in (approximately band size) ≈288bp; Lane N) cDNA extracted from the BVDV-free animal used as a negative control.

Results of hematological changes indicated a significant increase (P<0.05) in (PCV) in diseased buffalo calve breeds than in controls, Moreover, A significant

decrease($P<0.05$) in leukocyte count due to a significant lymphocytopenia was also indicated in diseased animals compared with control group. Table. 3.

Table.3. Alterations in hematological parameters in diseased buffalo calve breeds and controls

Parameters	Control buffalo calves **n=25**	Diseased buffalo calves **n=168**
RBC $x10^6$	7.41±0.46	7.33±1.51
Hb g/dl	12.88±1.74	12.78±1.89
PCV %	34.55±4.73	44.21±6.73*
TLC $x10^3$	11.63±1.53	9.65± 0.81*
Lymphocytes /Absolutes	5990.82±300.43	4100.05± 100.62*
Nutrophiles /Absolutes	4870.33± 300.12	4800.86±2.32
Monocytes /Absolutes	320.87±10.21	318.87±30.12
Eosinophiles /Absolutes	300.34±10.23	300.62±10.9

Results concerning the indices of clotting factors of diseased buffalo local calve breeds and control show a significant ($P<0.05$) decrease in total number of platelet counts. However, the values of platelet volume and platelet distribution width, the clotting time, the prothrombin time, as well as the values of activated partial thromboplastin time was significantly increased ($P<0.05$) in infected buffalo calves than in controls. Table 4.

Table.4: Clotting factor indices of diseased buffalo local calve breeds and controls

Parameters	Control buffalo calves **n=25**	Diseased buffalo calves **n=168**
Total platelet counts × 10^3	443.12± 28.12	255.43± 76.32*
Platelet volume /fl	11.14± 1.21	16.15± 1.84*
Platelet distribution width %	17.66± 1.28	25.72± 3.12*
Clotting time / mint	3.21± 0.71	4.91± 1.65*
Prothrombin time /sec	14.55± 1.15	18.54± 2.32*
Activated partial thromboplastin time /sec	52.75± 3.17	68.16± 4.11*

A significant difference has been encountered in biochemical analysis of diseased buffalo local calves breeds than the control animals. As significance (P<0.05) increase was indicated in AST, ALT, ALP and BUN, In diseased buffalo calve breeds than in controls, However, a significant (P<0.05) decrease were encountered in total protein in infected animals with BVD than in the control group.Table.5.

Table.5: Changes of biochemical analysis in diseased buffalo local calve breeds and controls

Parameters	Control buffalo calves **n=25**	Diseased buffalo calves **n=168**
AST U/L	58.15 ± 11.76	87.51 ± 13.04*
ALT U/L	38.53 ± 4.15	68.22 ± 8.78 *
ALP U/L	61.35 ± 7.93	79.28 ± 12.71*
BUN mg/dL	15.42 ±3.42	32.72 ± 9.15 *
Total protein g/dL	7.37 ± 0.26	6.11 ± 1.23*

Results of the acute phase response of the current study reveled a significant increase(P<0.05) in Haptoglobin values in diseased buffalo local calve breeds than controls, however, a significant (P<0.05) decrease in Fibrinogen time has been indicated in BVD calves compared with controls .Table. 6.

Table. 6: Acute phase response of diseased buffalo local calve breeds and controls

Parameters	Control buffalo calves **n=25**	Diseased buffalo calves **n=168**
Haptoglobin g/dl	0.0018±0.011	0.042±0.008*
Fibrinogen time/sec	22.24± 1.37	12.66 ± 4.41*

Ten of severely diseased local calve breeds was dying and macroscopic examination of the carcasses revealed a severe congestion of the intestinal vessels accompanied with Ecchymotic hemorrhagic enteritis with a multiple enlargement of mesenteric lymph nodes along most parts of the small and large intestine with pasty fecal materials (Fig 2, 3and 4).

Figure 2: Severe congestion of intestinal blood vessels (black arrows)

Figure 3: Ecchymotic hemorrhagic enteritis with pasty fecal materials (black arrows)

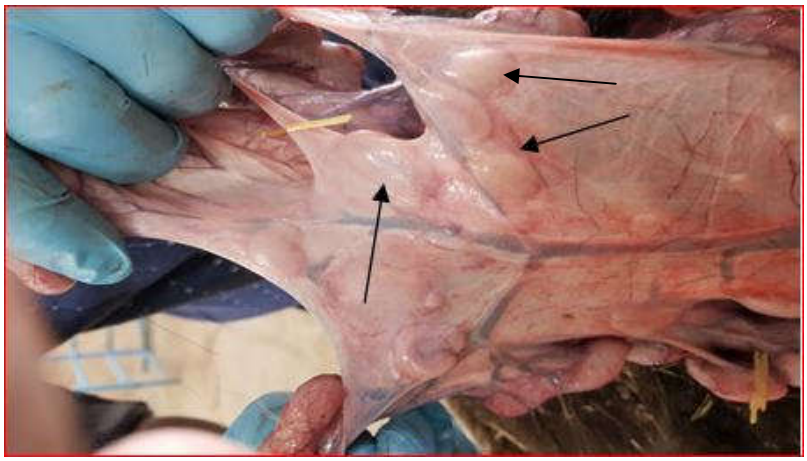


Figure 4: Multiple enlargement of mesenteric lymph nodes(arrows)

Furthermore, the results of histopathological analysis of those dead animals show atrophy of the intestinal villi with sloughing of the epithelial lining of villi of the small intestine, as well to hyperplasia of goblet cells. Moreover, infiltration of inflammatory cells in the intestinal mucosa, and congestion of blood vessels was also indicated .Fig. 5,6 and 7.

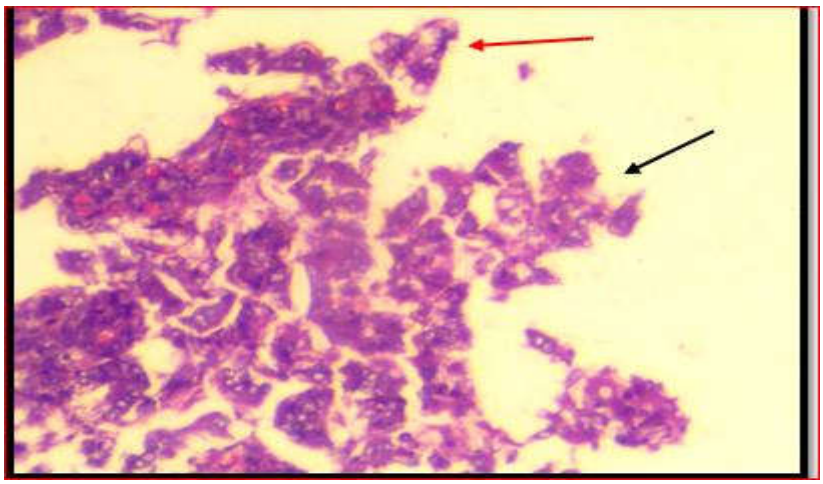


Figure 5.: Histopathological section of small intestine showed sloughed epithelial lining of villi (black arrow), as well to hyperplasia of goblet cells (red arrow). H&E stain. 10X.

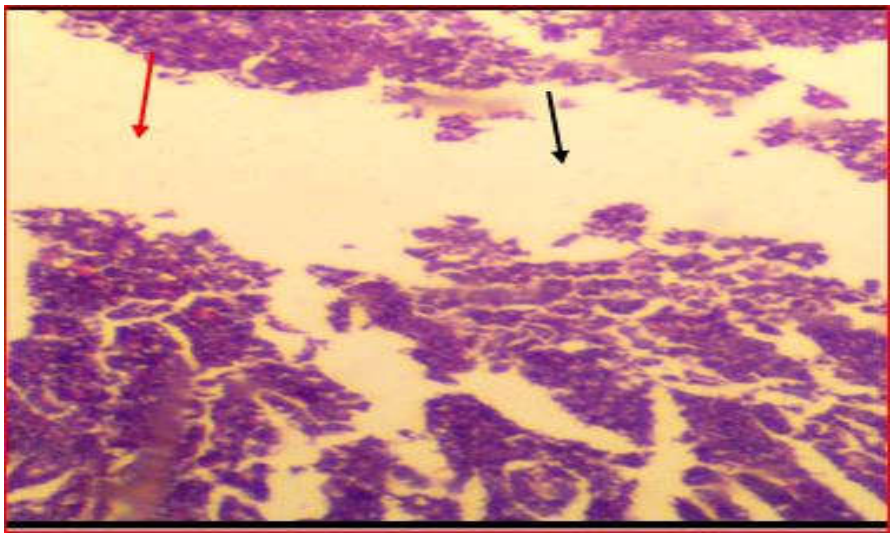


Figure 6: Histopathological section of small intestine showed severe sloughing of epithelial lining of villi (black arrow), as well to atrophy of intestinal villi (red arrow). H&E stain. 10X.

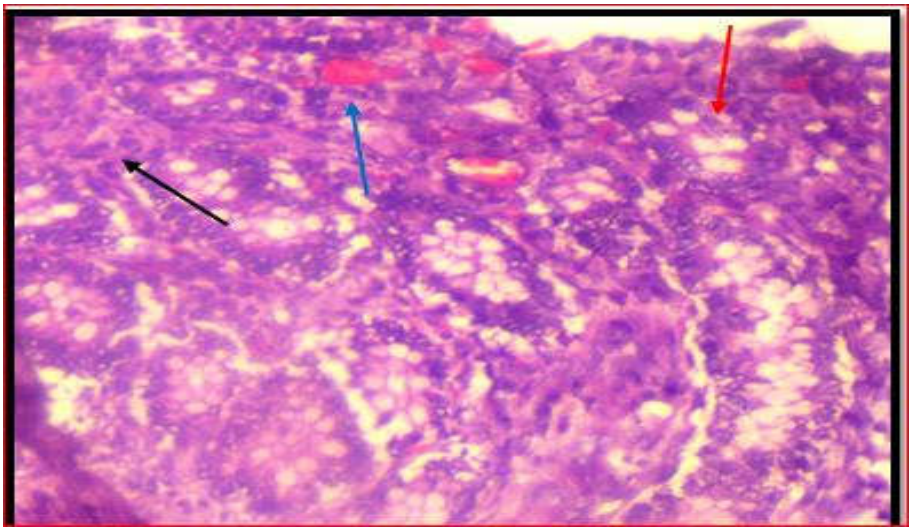


Fig.7: Histopathological section of small intestine showed infiltration of inflammatory cells in the intestinal mucosa (black arrow), as well to hyperplasia of goblet cells (red arrow), and congestion of blood vessels (blue arrow). H&E stain. 40X.

Discussion

The infection with bovine viral diarrhoea is considered one of the most important diseases of cattle distributed globally (1,10). However, the treacherous feature of the causative infectious virus will contribute to substantial economic losses in both the beef as well as the dairy animals worlds wide (19). The common sequelae of BVDV infection in cattle are digestive disorders, respiratory, as well as reproductive complications. Therefore, the most important losses due to the disease's reproductive disorders might be the most economically important consequences (20). The results of the present study indicate that an overall percent prevalence rate of (17.14%) in local buffalo calves breeds at Basrah province, Iraq was indicated for the first time, using PCR techniques. Results of (6%) prevalence rate were obtained by (21) at Al-Anbar province (the western region of Iraq). Moreover, a prevalence rate of (13.96%) was indicated by Hasan and Alsaad (22) in Nineveh in cattle. On the other hands, it was found a different prevalence rate among different countries around the worlds, In Iran, a prevalence rate of 18.23% (23), in Turkey 11.45% (24), In Egypt 17.2% (25), In Tunisia,2.65% (26), Moreover, in Poland 3.9% (27), and in Canada 10.44% (28).

It was documented that, Prevalence, refers to the amount of the disease in a known population at the same time without differences between old and new cases (29). Different biological measurements can be used for determining the occurrence of disease infection, including, the clinical, clinical-pathological, the virus virulence, serological methods, and production measures. However, the importance of each measure could depend on the purpose of the investigation (1). Therefore, The prevalence of this disease differed from country to another and from place to another even within the same country because of the different diverse management systems, type and number of samples, the sensitivity of the diagnostic technique which used, availability and the efficacy of the control programs, the variations in climate, control activity of animal movement, The variation in the animal numbers size, and presence and persistence of the disease (30,31).

For more understanding of the disease feature which might be considered as a continual challenge for the owners as well as the veterinarians, Researchers described different types of Bovine viral diarrhoea, The Acute BVD form, The Severe Acute form, The Thrombocytopenic hemorrhagic form, The Acute BVD–Bovine Respiratory Disease Form, and The Acute–immunosuppression form of BVD.

Although in most cases a significant percentage of the disease could result in subclinical type infections. Nevertheless, whether the disease is one of the acute types or is a subclinical form in nature, there is a specific period of the virus for shedding (5,32).

The infection of local buffalo calves breeds with BVD reflected different clinical signs, Although they are varied and might depend on the genetic mutation tendency in BVDV strains and the virulence(33). However, The clinical response to infection could be more complex and depends on different criteria including agent factors, virulent factors, host factors, etc. which might affect the result of the clinical disease include immune-tolerance, immune-competence, the general immune status whether its passive or active immunity (34). Local buffalo calve breeds of the current study show different clinical manifestations that were mentioned by others (1,12, 35, 36, 37).

It has been documented that anorexia and unable to suck occur because of the erosive lesions that existed on the mouth, gums, and tongue due to localization of the virus in the oral mucus membranes causing severe inflammation of these tissues (stomatitis), which makes the animal refrain from eating or feeding because of the severe pain resulting from the severity of inflammation with the descent of large amounts of saliva (5,20). Moreover, different types of diarrhoea were affected diseased buffalo calve breeds was resulting from, damage to epithelial surfaces of the gastrointestinal system, However, more virulent strain could lead to more severe inflammation which results in severe damage and could reach to the deep intestinal layers causing severe damage of the intestinal blood vessels resulting in hemorrhagic enteritis along the intestine (38, 39,40).

On the other hand (41,42) added that Acute type of the disease in newborn animals might reflect severe enteritis and or / pneumonia. Those clinical manifestations are related to calves suffering from a failure of passive transfer, as passively acquired maternal antibodies are thought to be a protector. Since the clinical disease in the face of adequate passive transfer may be related to antigenic diversity among infecting viral strains and viral strains against which passive immunity was developed. Lastly, inadequate passive immunity combined with immunosuppressive effects of BVD infection may result in secondary diseases affecting various organ systems. Also (25,27), describe a thrombocytopenic hemorrhagic type as a severe acute form of the disease that appears to be related to a non-cytopathic form of the

disease, As diseased calves were suffering significant thrombocytopenia, petechial and ecchymotic haemorrhages of mucosal surfaces, epistaxis from the nose, clear bloody diarrhoea, fever, leukopenia, and death, same signs were indicated in buffalo diseased calves of the current study.

Local buffalo calves of the present study show signs of excessive salivation. This could be a usual reflection of inflammation of the oral cavity, as BVD oral lesions characterized by the erosive type of lesions causing pain, loss of appetite, unable to suck teats, and might terminate with grinding sound(43). Furthermore, increase the representation of the vital sign with an increase in body temperature, respiratory and heart rate as well as increase the capillary refill time which show the acute form of the disease will also be mentioned by (1,37). It has been documented that the capillary refilling time is a fast technique apply to check some clinical sign troubles such as systemic dehydration, peripheral vascular disease, and shock as well as hypothermia, therefore, the prolonged time of the refill time of the blood vessels might indicate the low amount of blood flow which will reach to tissues (19).

The results of the current study show no significant changes in values of total erythrocyte count and hemoglobin concentration, However, PCV was found high, the same results were also demonstrated by (33,44, 45,46) which might be referred to the excessive loss of body fluid and dehydration and the haemoconcentration mechanism of the blood, which lead to decrease plasma volume in diarrheic buffalo calve breeds.
It has been documented that, The primary result of BVD infections is a decrease in immune system ability and strength due to leukocytopenia (immune-suppression) due to suppression of the defence cells like macrophages, nutrophiles and lymphocytes, which are the first to respond to infection (47,48). The results of the current study show a significant leukopaenia and Lymphopenia in BVD of buffalo local calve breeds which agreed with the same data reported by (37) who refers to the low number of both B- and T-lymphocytes in peripheral blood as a consistent finding in acute BVD infection. Furthermore, (49) added that, previous studies indicate that the causative agent was localized in the lymph nodes, Peyer's patches, enterocytes, spleen, thymus, tonsils and liver causing destruction of lymphocytes in those tissues.
The Infection due to BVD could result in mild, less or severe decrease Lymphopenia, which always has a good associated with the infection and the lesions of the lymphoid tissues. Further, it was proven that, during BVD infection, Cytotoxic T-lymphocytes will be more influenced than the helper one with less or with no any

harm on circulating γ/δ T-cells. The CD4+ depletion increases the period of virus shedding (50). Therefore, BVD infection could increases the susceptibility to another secondary microbial infections because the lymphocytes from diseased animals will has impaired memory responses to BVDV and other antigens (42,51).

A significance difference has been indicated in clotting factor indices of diseased buffalo calve breeds and controls in the current study, Since, it was shown that, thrombocytopenia might occurs in a regular pattern when BVD was acute, although, the low values of thrombocytes will not always reflected a severe hemorrhages (52,53), It was also indicated by the results of the present study. The exact reason of decreased platelets count might unclear completely, however, the damage of megakaryocytes and the reduction of thrombocytes production by megakaryocytes as well as the increased consumption of the platelet cells in the periphery, and functional defects of the platelets have all been indicated as such a contributing factors (54). Moreover, (55), added that the development of thrombocytopenia is directly related to the infection of bone marrow with BVD and that in the bone marrow, BVD can be detected in all cellular elements including megakaryocytes. On the other hand, Bleeding tendency or hemorrhagic diathesis might only occur when thrombocytes have reached a very low number (56,57), Which was also detected in the current study, reflecting the intense of the clinical signs in BVD diseased buffalo calve breeds.

In specific situations , some virulent strains of BVDV might result in high mortalities at the beginning of the disease. As, the bone marrow tissue could be affected in late stages than other lymphohematopoietic tissues, and thrombocytopenia develops after infection of the bone marrow, Therefore, the animal might die before hemorrhagic diathesis will confirm, this could clarify the difference in frequency of bleeding indicated in the fields and the experimental cases of severe acute disease (58). The present study indicates a significantly high value of clotting factor indices in diseased buffalo calve breeds than in controls, which also agreed by(45,57). BVD in buffalo calves alters the coagulation system which might enhance the development of disseminated intravascular coagulation(DIC). The most common coagulation abnormality in diseased animals is a status of Hyper-coagulation associated with the (DIC) with a severity counting on the duration and virulence of the infectious agent (59).

Different significant changes have been detected in biochemical analysis of diseased buffalo calve breeds compared with controls. As, the results show significant high values of the Aspartate and Alanine aminotransferase, Alkaline phosphatize as well as the blood urea nitrogen which, could reflect the harmful effects of the skeletal and cardiac muscles, hepatic as well as renal tissues. High values of the Aspartate and Alanine aminotransferase was estimated in the diseased buffalo calves with acute BVD. Both the aminotransferase could be an indicator of hepatic tissue injuries, However, it could also originate from other tissues such as skeletal and cardiac muscles. Both of those enzymes could be liberated and found during the pathological conditions. These findings agree with the same evidence of the literature (33, 60). Moreover, ALP was also used as an index of a hepatic injury, However, It could also be useful in Skelton diseases. Since, The enzyme was found in the intestinal, hepatic, and renal tissues beside bones and will increase with its level when those tissues were already damaged (61). An increased level of BUN may indicate indirect damage of renal tissue, and the presence of globins catabolites, however, Heart failure and dehydration will also elevate the BUN level, which is indicated in the deceased buffalo calves of the current study (62).

Significant slight hypoproteinemia was indicated in diseased buffalo calve breeds compared with controls. Same results were also obtained by (44, 46), who concluded that the relative difference and reduction in total protein level might be due to, starvation, malabsorption, and diarrhoea, (digestive disturbances) destruction of proteins due to fever, However, it could be attributed to stress which might affect the hepatic parenchyma causing hepatic depletion resulting in less protein synthesis.

The present study shows a significant difference in the acute phase response. Since high values of haptoglobin were indicated in diseased buffalo calves than controls. This might reflect the unspecific and complex innate reaction that occurs quickly after tissue injuries, As, the pro-inflammatory cytokines could be released initially at the site of an insult and will be responsible for the starting of local and systemic defences (63). It has been shown that the major mediators of acute-phase protein synthesis in the liver, are, Tumor necrosis factor-alpha (TNF-α), inflammatory cytokines, the interleukin-6, and interleukin-1-beta. (64). On the other hand, where is knows that The main and important functions of acute-phase protein and response are an enhancement of phagocytosis, activation of the immune system, clearance of the product of inflammations? Nevertheless, It has been thought that the acute phase

protein response is more active and sensitive than leukocyte count as a marker of inflammations, and are more stable than cellular components, Moreover, they also confirmed that the acute phase response has a faster response rather than alterations in leukocyte count in situations where new leukocytes must be generated by the bone marrow(65).

Hypo-fibrinogenemia and prolonged clotting time which indicated in the diseased buffalo calve breeds of the present study might suggest the prevalence of petechial and ecchymotic hemorrhages which detected on the ocular mucous membranes, However, the thrombocytopenia might also play a good role because of the depression of the activities, of the bone marrow, enlargement of spleen and sequestration of the thrombocytes, which reflected due to disturbed homeostasis and could terminate with infarction due to micro-thrombosis of an important tissue such as the pulmonary, the brain a well as the intestine (53,55).

Results of the present study show different macro and micro pathological changes of the dead carcasses which were also mentioned by (5,66,67), who confirmed that BVD could affect all body tissues and caused harmful changes when occurring in different ages, however, in young ages, different degrees of enteritis including the hemorrhagic type was predominant some time. Which is associated with sloughing of the epithelial lining of the villi of the small intestine, as well as the hyperplasia of goblet cells, Furthermore, with infiltration of different inflammatory cells in the intestinal mucosa, and congestion of blood vessels which indicated in the buffalo calve breeds of the current study.

دراسة سريرية وتشخيصية للإسهال البقري الحموي في عجول الجاموس لمحافظة البصرة ، العراق

كمال الدين مهلهل السعد , حسين عبد الكريم عبد الواحد

فرع الطب الباطني والوقائي ، كلية الطب البيطري ، جامعة البصرة

الخلاصة

تم الاشتباه بإصابة عجول جاموس محلية في محافظة البصرة بمرض الاسهال البقري الحموي. أذ تم فحص (٩٨٠) عجل جاموس بعمر اقل من سنه واحدة ومن كلا الجنسين ،تاكد اصابة(١٦٨) منهم بفحص تفاعل البلمرة المتسلسل كما استخدم خمس وعشرون من عجول الجاموس المحلية السليمة سريرياً كمجموعة سيطرة .اظهرت العجول المريضة علامات سريرية مختلفة تعود لمرض الاسهال البقري الحموي مع حدوث ارتفاع معنوي في معدلات درجات حرارة الجسم ، معدلات التنفس ، ضربات القلب وسرعة رجوع الدم في

الاوعية الدموية في العجول المريضة بالمقارنة مع مجموعة السيطرة .كما اظهرت النتائج حدوث زيادة معنوية في معدل حجم خلايا الدم المرصوصة في عجول الجاموس المصابة بالمقارنة مع الصحيحة في حين انخفضت معنويا معدلات العدد الكلي لخلايا الدم البيض بسبب انخفاض معدلات الخلايا اللمفية في العجول المريضة بالمقارنة مع العجول السليمة . وقد اوضحت نتائج معاملات تخثر الدم حدوث انخفاض معنوي في العدد الكلي للصفيحات الدموية في حين ارتفعت معاملات حجم الصفيحات وسرعة انتشارها ، معدل تخثر الدم ، معدل الخثرين وحرك الخثرين الجزيئي في العجول المريضة بالمقارنة مع مجموعة السيطرة . كما تبين ايضا حدوث ارتفاع معنوي في خمائرالاسبارتيت ناقلة الامين و الالنين ناقلة الامنين والفوسفاتاز القاعدي ويوريا نتروجين الدم في العجول المريضة بالمقارنة مع السليمة وعل العكس من ذلك انخفضت معنويا معدلات البروتين الكلي في العجول المصابة بالمرض بالمقارنة مع مجموعة السيطرة . بينت نتائج الدراسة وجود ارتفاع معنوي ملحوظ في معدلات بروتينات الطور الحاد حيث ارتفعت معنويا معدلات الهابتوكلوبين في العجول المصابة بالإسهال البقري الحموي بالمقارنة مع مجموعة السيطرة بينما انخفضت معدلات منشيء الليفين في العجول المريضة بالمقارنة مع العجول السليمة . تبين من الفحص العياني للجثث النافقة بسبب المرض وجود احتقان الاوعية الدموية المعوي الشديد المترافق مع التهاب الامعاء النزفي الكدمي فضلا عن تضخم متعدد وملحوظ للغدد اللمفية المساريقية على طول اجزاء الامعاء الدقيقة والغليظة مع وجود مود برازية عجينية فضلا عن ضمور واضح للزغابات المعوية وتقشر البطانة الظهارية لزغابات الامعاء الدقيقة وكذلك تضخم الخلايا الكأسية وانسلال الخلايا الالتهابية مع احتقان واضح للاوعية الادموية . استنتج من خلال هذة الدراسة ان لمرض الاسهال البقري الحموي تأثير ضار وسلبي على المجترات المصابة والتي في الغالب ينتهي بهلاكها لذلك فأن اتخاذ تدابير الوقاية اللازمة والصحيحة هو الخيار النهائي والمناسب للسيطرة والقضاء على المرض بشكل نهائي .

REFERENCES

1-Constable, P.D., Hinchcliffe, K.W., Done, SH., Grunberg, W.(2017). Veterinary medicine: A textbook of the diseases of cattle, sheep, goats and horses. 11th ed. Philadelphia: WB Saunders Co.

2-Liu, L.H., Xia, H.Y., Wahlberg, N., Belak, S. and Baule, C.(2009). Phylogeny, classification and evolutionary insights into pestiviruses. Virol.385:351-357.

3-Yazici, Z., Serdar, M.S., Gumusova, S.O. and Albayrak, H. (2012). Molecular diagnosis and seroepidemiology of pestiviruses in sheep. Vet. Arhiv. 82: 35-45.

4-Ridpath, J.F. (2015). Emerging pestiviruses infecting domestic and wildlife hosts. Anim. Health Res. Rev.16:55–59.

5-Bachofen, C ., Braun, U ., Hilbe, M ., Ehrensperger, F ., Stalder, H., Peterhans, E.(2010). Clinical appearance and pathology of cattle persistently infected with

bovine viral diarrhea virus of different genetic subgroups. Vet Microbiol. 141(3): 258–267.

6-Lanyon, S.R., Hill, F.I., Reichel, M.P., Brownlie, J. (2014). Bovine viral diarrhoea: Pathogenesis and diagnosis. Vet. J. 199:201-209.

7-Firat, I.,Bozkurt, H.H., Turan, N. and Bagcigil, F. (2002). Distribution of bovine viral diarrhoea virus (BVDV) in the genital system tissues of cattle. Vet. Arhiv. 72:35−48.

8-Hilbe, M., Stalder, H., Peterhans, E., Haessig, M., Nussbaumer, M. and Egli, C. (2007). Comparison of five diagnostic methods for detecting bovine viral diarrhea virus infection in calves. J. Vet. Diag.Invest.19:28−34.

9-Ezanno, P., Fourichon, C. and Seegers, H.(2008). Influence of herd structure and type of virus introduction on the spread of bovine viral diarrhoea virus (BVDV) within a dairy herd. Vet. Res., 39(5):39.

10-Garoussi, M.T., Haghparast, A.R. and Rafati, M.S. (2011). The prevalence of bovine viral diarrhea virus in persistently infected cows in industrial dairy herds in suburb of Mashhad-Iran. Iran. J. Vet. Med.5: 198-203.

11-Hessman, B.E., Fulton, R.W., Sjeklocha, D.B., Murphy, T.A., Ridpath, J.F. and Payton, M.E. (2009). Evaluation of economic effects and the health and performance of the general cattle population after exposure to cattle persistently infected with bovine viral diarrhoea virus in a starter feedlot. Am. J. Vet. Res.70:73−85.

12-Everman, J.F. and Ridpath, J.E . (2002). Clinical and epidemiologic observations of bovine viral diarrhea virus in the northwestern United States. Vet. Microbiol., 89: 129-139.

13-Kaiser, V., Nebel, L., Schüpbach-Regula, G. R., Zanoni, G. and Schweizer, M. (2017). Influence of border disease virus (BDV) on serological surveillance within the bovine virus diarrhea (BVD) eradication program in Switzerland. Vet. Res.13:21.

14-Zajac, A.M., and Conboy, G.A. (2012). Vet. Clin. Parasite., 8th ed, (p 3-39). UK: Wiley-Blackwell.

15-Weiss, DJ., Wardrop, KJ. (2010). Schalm's Veterinary Hematology,6th ed(Ames, Wiley-182 Blackwell).

16-Bush, B. M. (1975). Veterinary Laboratory Manual, The Gresham Press, London.

17-Maxie .M.G .(2016). Pathology of domestic animals Sixth Ed (Vol. 2,3). Academic press. Elsever.

18-Leech,N.,Barrett,K., Morgan,G.A. (2013).SPSS for intermediate statistics :Use and interpretation. Routledge.

19-Smith, BP.(2004). Large animal internal medicine, 4th ed., New York, Mosby.

20-Almeida, L.L., Miranda, I.C.S., Hein, H.E., Neto, W.S., Costa, E.F., Marks, F.S., Rodenbusch, C.R., Canal, C.W. and Corbellini, L.G. (2013). Herd-level risk factors for bovine viral diarrhea virus infection in dairy herds from Southern Brazil. Res. Vet. Sci.95: 901-907.

21-Al-Ajeeli, K. S. A. and Hasan, A. S. h.(2011). Detection of Bovine Viral Diarrhea Virus by Conventional RT-PCR: A comparative Study. Al-Anbar J. Vet. Sci. 4 (2):121-128.

22-Hasan, S. D. and Alsaad, K.M. (2018a). 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) Volume 11, Issue 3 Ver. II .64-70

22-Hasan, S. D and Alsaad, K.M.(2018b). Bovine Viral Diarrhea And Persistently Infection Of cattle At Nineveh Province, Iraq. Bas.J.Vet.Res. 17(2).14-32.

23-Safarpoor, D. F. and Haghighi, N. (2012). Detection of bovine viral diarrhea virus in bovine and buffalo milk thorough conventional and real-time reverse transcriptase polymerase chain reaction. Res. Opin. Anim. Vet. Sci. 2: 263-267.

24-Yilmaz, V.(2016).Prevalence of antibodies to Bovine Viral Diarrhea Virus (BVDV) in blood and milk serum in dairy cattle in Kars district of Turkey., Indian J. Anim. Res. 50 (5) : 811-815.

25-Soltan, M. A., Wilkes, R. P., Elsheery, M. N., Elhaig, M. M., Riley, M. C. and Kennedy, M. A .(2015). Circulation of bovine viral diarrhea virus – 1 (BVDV-1) in dairy cattle and buffalo farms in Ismailia Province, Egypt. J . Inf. Dev. Ctri., 9(12):1331-1337.

26-Thabti, F., Kassimi, L. B., M'zah, A., Ben Romdane, S., Russo BEN Said, P. M.S., Hammami, S. and Pepin, M. (2005). First detection and genetic characterization of bovine viral diarrhea viruses (BVDV) types 1and 2 in Tunisia. Rev. Med. Vet., 156(8-9):419-422.

27-Wernicki, A. R., Urban-Chmiel, D.,Stęgierska1, Ł., Adaszek, M.,Kalinowski, A. and Puchalski, M.(2015). Detection of the bovine viral diarrhoea virus (BVDV)

in young beef cattle in eastern and southeastern regions of Poland. Polish J. Vet. Sci.,18(1):141–146.

28-Deregt, D., Carman, P.S., Clark, R.M. Burton, K.M.,Olson, W.O. and Gilbert, S.A. (2002). A comparison of PCR with and without RNA extraction and virus isolation for detection of BVD virus in young calves. J .Vet. Diagn. Invest.14(5):433-437.

29-Stevenson, Mark .(2005). An Introduction to Veterinary Epidemiology. EpiCentre, IVABS. Massey University. New Zealand

30-Farhad, S. D.(2011). Prevalence study of Bovine viral diarrhea virus by evaluation of antigen capture ELISA and RT-PCR assay inBovine, Ovine, Caprine, Buffalo and Camel aborted fetuses in Iran. AMB Express.,1(32):1-6.

31-Graham, D. A.,Clegg, T. A., Lynch, M. and More, S. J. (2013). Herd-level factors associated with the presence of bovine viral diarrhea virus in herds participating in the voluntary phase of the Irish national eradication program. Prev. Vet. Med. 112:99–108.

32-Grooms, D., Baker, J.C., Ames, T.R. (2002). Diseases caused by bovine virus diarrhea virus. In: LargeAnimal Internal Medicine, 3rd Ed. Ed. Smith BP,pp: 707–714. Mosby, St. Louis, MO.

33-Hasan, S. D and Alsaad, K.M.(2018b). Evaluation of clinical, hematological, blood coagulation and some biochemical parameter changes in clinically infected cattle with bovine viral diarrhea. IOSR-JAVS. 11(3)Ver. II .64-70.

34-Zhong, F., Li, N., Huang, X., Guo, Y., Chen, H., Wang, X., Shi, C. and Zhang, X. (2011).Genetic typing and epidemiologic observation of bovine viral diarrhea virus in Western China. Virus Gen. 42: 204-207.

35-Van Vuuren, M. (2006).A review of bovine viral diarrhea virus infection in livestock in Southern Africa. Perspectives in Agriculture. Vet. Sci. Nutrit. Natural Resou. 1:9.

36-Alsaad, K. M., Al-Obaidi, Q. T. and Hassan ,S. D.(2012a). Detection of bovine viral diarrhea virus antibodies in cows and buffaloes milk in Mosul, Iraq. Res. Opin. Anim. Vet. Sci.,2(3):158-160.

37-Brodersen, B. W. (2014). Bovine Viral Diarrhea Virus Infections: Manifestations of Infection and Recent Advances in Understanding Pathogenesis and Control. Vet.Pathol.51(2): 453-464.

38-Bazargani, T. T., Hemmatzadeh, F., Nadjafi, J. and Sadeghi, N. A. (2008). BVDV induced gastro-neuropathy outbreak in a feedlot calves around Tehran (Iran). Iran. J. Vet. Res.9(3):271-276.

39-Talafha, A,; Hirche, S.; Ababneh, M.; Al-Majali, A. and Ababneh, M. .(2009). Prevalence and risk factors associated with bovine viral diarrhea virus infection in dairy herds in Jordan. Trop. Anim. Health Prod., 41: 499-506.

40-Tao, J., Liao, J.H., Wang, Y., Zhang, X.J., Wang, J.Y. and Zhu, G.Q.(2013). Bovine viral diarrhea virus (BVDV) infections in pigs. Vet. Microbiol. 165:185-189.

41-Liebler-Tenorio, E.M., Ridpath, J.F,, Neill, J.D (2003a). Distribution of viral antigen and development of lesions after experimental infection of calves with aBVDV 2 strain of low virulence. J. Vet. Diagn. Invest. 15:221–232.

42-Liebler-Tenorio, E.M., Ridpath, J.F., Neill, J.D (2003b). Lesions and tissue distribution of viral antigen in severe acute versus subclinical acute infection with BVDV2. Biologicals 31:119–122.

43-Brock, K.V. (2004). The many faces of bovine viral diarrhea virus. Vet. Clin. North Am. Food Anim. Prac. 20:1-3.

44-Fernández-Sirera, L., Mentaberre, G., López-Olvera, J. R., Cuenca, R., Lavín, S. and Marco, I.(2011). Hematology and serum chemistry of Pyrenean chamois (Rupicapra pyrenaica) naturally infected with a border disease virus. Res. Vet. Sci. 90(3): 463-467.

45-Oguzhan, A., Sibel, Y. and Oya, B.(2014). Changes in Hematological Parameters in Cattle Infected with Bovine Viral Diarrhea Virus. Acta Sci. Vet. 42: 1173.

46-Galbat, S. A., El-Shemy, A. and Keshta. H. G.(2015). Clinical, Hematological and some Biochemical alterations in calves during diarrhea. Int. J. Adv. Res., 3(12): 191 – 196.

47-Bruschke, C., Weerdmeester, K., van Oirschot, J. and van Rijn, P. (1998).Distribution of bovine virus diarrhoea virus in tissues and white blood cells of cattle during acute infection.Vet. Microbiol. 64:23–32.

48-Goens, S.D.(2002). The evolution of bovine viral diarrhea: A review. Can. Vet. J.43:946–954.

49-Pedrera, M., Gómez-Villamandos, J.C., Risalde, M.A., Molina, V. and Sanchez-Cordon, P.J.(2012b). Characterization of apoptosis pathways (intrinsic and

extrinsic) in lymphoid tissues of calves inoculated with non-cytopathic bovine viral diarrhoea virus genotype 1. J. Comp. Patho.,146: 30–39.

50-Ellis, J. A., W. C. Davis, E. L. Belden & D. L. Pratt. (1988). Flow cytofluorimetric analysis of lymphocyte subset alterations in cattle infected with bovine viral diarrhea virus.Vet. Pathol. 25, 231–236.

51-Ridpath, J.F., Bendfeldt, S., Neill, J.D., and Liebler-Tenorio, E.(2006). Lymphocytopathogenic activity in vitro correlates with high virulence in vivo for BVDV type 2 strains: Criteria for a third biotype of BVDV. Virus Res.,118(1-2):62-69.

52-Corapi, W., French, T. and Dubovi, E.(1989). Severe thrombocytopenia in young calves experimentally infected with noncytopathic bovine viral diarrhea virus. J. Virol.,63:3934–3943.

53-Radwińska, j. (2010). Effect of the BVD-MD virus on coagulation And fibrinolytic systems in dairy cows. Bull .Vet. Isnt. Pulawy. 54:293-298.

54-Walz, P., Steficek, B. and Baker, J.(1999a). Effect of experimentally induced type II bovine viral diarrhea virus infection on platelet function in calves. Am. J. Vet. Res.60:1396–1401.

55-Walz, P. H., Bell, T. G., Grooms, D. L., Kaiser, L., Maes, R. K. & Baker, J. C. (2001).Platelet aggregation responses and virus isolation from platelets in calves experimentally infected with type 1 or type II bovine viral diarrhea virus. Can. J. Ve. Res. 65: 241–247.

56-Rebar, A.H., Mas Williams, P.S., Feldman, B.F., Metzger, F.L., Pollock, R.V.,Roch,J.(2005). Platlets: Overview ,Morphology,Quantity ,Platelets function disorders. Int. Vet. Inf.21:805-825.

57-Alsaad, KM., Al-Obaidi, QT. And Hassan, SD. (2012b). Cinical, haematological and coagulation studies of bovine viral diarrhoea in local Iraqi calves ,Bulg. J.Vet.Med.15: (1) 44-50.

58-Walz, P. H., Bell, T. G. , Steficek, B. A. , Kaiser, L. , Maes, R. and Baker, J. (1999b). Experimental model of type II bovine viral diarrhea virus-induced thrombocytopenia in neonatal calves. J. Vet. Diag. Invest.11:505–514.

59-Bick, RL.(2003). Disseminated intravascular coagulation: Current concepts of etiology, pathophysiology, diagnosis and treatment. Hematol. Oncol. Clin. North. Am.17:149.

60-Fiore, F., Cubeddu, G.M., Lai, M.G. and Pintori, G. (2006). Clinical considerations of bovine viral diarrhea (BVD). XIV Cong. Inte.Fe. Me. S. P. Rum, Lugo (Spain).

61-Kaneko,J.J., Harvey, J.W., Bruss.M.L.(2008). Clinical biochemistry of domestic animals.6th ed. Elsevier

62-Stockham, S.L., Scott, M.A.(2008). Fundamentals of Veterinary Clinical Pathology, 2nd ed. Ames, IA: Blackwell.

63-Cary, C., Zaias, J. and Altman, N.H. (2009). Acute Phase Response in Animals:A Review. Cattle. Vet. Rec. 162, 514-517.

64-Jain, S., Gautam, V., and Naseem, S. (2011). Acute-phase proteins: As diagnostic tool. J. Pharm. Bioallied.Sci. 3(1): 118–127.

65-Tothova, C., Nagy, O., Kovac, G.(2014). Acute phase proteins and their use inthe diagnosis of diseases in ruminants: a review. Veterinarni Medicina,59.(4): 163–180.

66-Acland, S. T. (2001), Tissue distribution of bovine viral diarrhea virus antigens in persistently infected cattle. J. Vet. Sci. 2:81–84.

.67-Duncan, C. G ., Ridpath, J. F ., Palmer, M ., Spraker, T.(2008). Histopathologic and Immunohistochemical Findings in Two White-Tailed Deer Fawns Persistently Infected with Bovine Viral Diarrhea Virus. Vet. Diag. Investigate.