Assiut Vet. Med. J. Vol. 43 No. 85, April 2000

Dept. of Pathology & Clinical Pathology,
Dept. of Biochemistry,
Dept. of Pathology,
Animal Health Research Institute Dokki, Giza ARE (Zagazig Branch)

DIAGNOSTIC STUDIES OF BOVINE VIRAL DIARRHEA -MUCOSAL DISEASE IN SHARKIA GOVERNORATE

(With 2 Tables and 10 Figures)

By

IBTISAM M.G.EL.DIN; MOHGA SHOWKAT; MOUCHIRA M.M. EL DIN and A. ALI*

*: Dept. of Pathology, Faculty of Vet. Med., Zagazig University

(Received at 29/3/2000)

دراسات تشخيصية على الإسهال الفيروسي المعدي في محافظة الشرقية

ابتسام محمد جمال الدين ، مهجة شوكت ، مشيرة محمد محيى الدين ، عبد المنعم على

تمت هذه الدراسة على أحد مزارع تسمين الأبقار بمدينة منيا القمح – محافظة الشرقية. حيث وجدت العجول تعانى من إسهال بدرجات متفاوتة و ارتفاع في درجة الحرارة بين ٣٩,٥ الى ٤١ درجة مئوية بالإضافة إلى بعض المشاكل بالجهاز التنفسي والأجهزة الأخرى بالجسم . وقد دفعنا هذا الى عمل هذه الدراسة لبحث الأعراض الإكلينيكية للمرض و ما يصحبه من تغيرات إكلينكية – معملية وباثولوجية – لقد أجرى هذا العمل على عشرين عجل مصاب من هذه المزرعة و عشر حالات أخرى كمجموعة ضابطة من مزرعة سليمة اكينكيا. و قد تم تشخيص المرض عن طريق أخذ عينتين من دم الحيوان بينهما أسبوعين في كل حالة من الحالات السابقة لقياس مستوى الأجسام المناعية في المصل ضد مرض الأسهال الفيروسي حيث أوضحت النتائج ارتفاع مستوى الأجسام المناعية للفيروس بين(٤/١ : ٢٥٦/١) في العجول المصابة. أما في المجموعة الضابطة فلم يوجد أجسام مناعية ضد هذا المرض في مصل الحيوان. و أوضحت النتائج وجود فقر دم بالعجول المصابة حيث قلت معنويا عدد كرات الدم الحمراء و نسبة الهيموجلوبين بالدم كذلك انخفض العدد الكلى لكرات الدم البيضاء و الصفائح الدموية و خلايا النيتروفيل واللمفوسيت انخفاضا معنويا في المجموعات المصابة مقارنة بالمجموعة الضابطة .ودلت نتائج المجموعة المصابة على ارتفاع مستويات اليوريا والبوتاسيم ارتفاعاً معنويا بينما انخفض مستوى البروتين الكلى – الألبومين – الجلوبلين – الصوديوم – الكلور يد انخفاضاً معنوياً مقارنة بالمجموعة الضابطة . و كانت التغيرات

الباثولوجية في العجول النافقة نتيجة للإصابة بهذا المرض. عبارة عن وجود قرح – تنكـرز بالجهاز الهضمي، مع نقص في عدد الخلايا الليمفاوية الموجودة بالغدداللليمفاويـــة بالجـــهاز الهضمي مع نقص وتتنكرز الخلايا الليمفاوية بالطحال بالإضافة الي وجود بقع نزيفيـــة فـــي الأمعاء ، الكلى والقلب.

SUMMARY

This study was conducted in a private fattening cattle farm in Minet El Kamh City, Sharkia Governorate, A.R.E. The animals were not previously vaccinated with BVD vaccines in this farm. The calves were suffering from diarrhea of varying degrees, fever ranged from 39.5 to 41^0c. Anorexia and erosions in mouth & nose were present. The calves suffered from respiratory problems and high calf losses. Twenty affected calves from this farm constituted the material of our study and from another farm ten healthy ones were taken as control. For serodiagnosis paired blood samples were collected with two weeks interval in-between. The titer of antibodies against BVDV, was determined by (B-procedure of serum neutralization test). Antibody titer was rising and varied from 1:4 to 1:256 in affected calves indicating positive bovine viral diarrhea virus infection .The control healthy animals were seronegative for BVD virus. For clinico-pathological study, two blood samples were obtained from each of affected calves and healthy ones. The first sample was used for hematological studies, it revealed hypoplastic anaemia where there were significant decrease in RBCS count, Hb concentration, PCV, thrombocytic & total leukocytic count. Neutrophils and lymphocytic percentage were also significantly decreased. Serum obtained from the second sample was used for biochemical examination, the results revealed significant increase in BUN& significant decrease in total protein, albumin and globulin. There was significant decrease in serum sodium and chloride but serum potassium showed significant increase ($p<0.05$) in severely affected calves. The pathological changes of dead calves showed depletion and necrosis of the lymphocytes in spleen & lymph nodes, focal hemorrhage and hemosiderosis were evident among lymphoid follicles of mesenteric lymph node. Renal medulla and epicardium showed focal or diffuse hemorrhages. GIT revealed ulceration, coagulative necrosis and leukocytic infiltration in the lamina propria.

Key words: Bovin viral diarrheamucousal disease.

INTRODUCTION

Bovine viral diarrhea (BVD) is a disease caused by the bovine viral diarrhea virus (BVDV), it was first recognized in the United States (Olafson et al., 1946). BVDV is an RNA virus of the family flaviviridae, genus pestivirus It causes important economical losses associated mainly with reproductive failure (Kobrak and Weber 1997).Mucosal disease is sporadically occurring and cause highly fetal-enteric form of BVDV infection and usually involves cattle ranging from 6 months to 2 years of age or all ages. The case fatality rate approaches 100%(Lee et al., 1994).

Bovine viral diarrhea is a serious disease that cause electrolyte imbalances, hematological abnormalities and alimentary tracts affections which severely affect the animals (Fisher, 1971and Lewis & Phillips, 1978, Blood et al., 1983,Bolin et al., 1985, Argenzio 1992 & Taniyama et al., 1995, Braun et al.., 1996)

Johnson and Muscoplat (1973) reported that the BVDV has an affinity for lymphocytes and rapidly dividing cells. Thus it causes leukopenia and lymphoid depletion in lymph nodes and payer's patches. The authors suggested that BVDV infection have an immunosuppressive effect.

This work was planned after a visit to a fattening cattle farm in Minet El Kamh City where calves suffered from diarrhea, respiratory problems and other symptoms suggesting the possibility of BVDV/MD infection. Our aim was to study the effect of the disease on clinical, hematological and biochemical parameters in calves together with the associated histopathological changes in different organs.

MATERIALS and METHODS

Animals:

This study was conducted on 20 animals in a private fattening cattle farm at Minet El Kamh city, Sharkia Governorate, A.R.E. The animals in this farm were not previously vaccinated with BVD vaccine. They suffered from diarrhea of varying severity ranging from watery to hemorrhagic diarrhea. Fever ranged from 39.5 to 41^{0}c anorexia, erosion &ulceration of the oral mucosa. Salivation and areas of hemorrhage in the mouth & tongue were evident in affected calves. Calves suffered from varying degrees of respiratory symptoms ranging from nasal discharge to severe cough. High calf losses occurred between birth and weaning time. According to the severity of diarrhea, calves were divided

into two groups: one group composed of ten mildly affected animals where there was watery diarrhea and mild symptoms and another group comprised ten severely affected animals showing hemorrhagic diarrhea accompanied by severe general and respiratory symptoms. Ten healthy calves from another far away farm were used as a control group.

Virus used in serum neutralization test (SNT):

Local Imanstrain of Bovine viral diarrhea virus (BVDV) was supplied by the Virology Laboratory (BVD-Unit) of the VET. Serum and Vaccine Research Institute, Abbasia. It was adapted on Madin Darby Bovine Kidney (MDBK) cells. It gave a titer of 10^{5} $TCID_{50}$/ml, the virus was preserved at --196c°

Serum sample:

For serodiagnosis of the disease: Paired blood samples with two weeks apart were collected from diseased and healthy animals in a clean, dry centrifuge tube, left to clot and serum was separated for screening of BVDV antibodies. Serum neutralization test (B-procedure of SNT) was applied according to Frey and Liess (1971) for screening and titration of BVDV neutralizing antibodies Examination of paired serum samples collected from actually infected &convalescent individual animals were employed to give a presumptive retrospective diagnosis .any significant rise in the titer of neutralizing anti bodies could be considered as an indication of recent infection with the virus(Frey and Liess,1971).

Clinico pathological study:

Two blood samples were obtained from each of twenty affected calves and ten healthy ones. The first sample was taken on EDTA for hematological studies; according to standard techniques described by Jain (1986). The second sample was taken in dry clean tube, left to clot then centrifuged, serum obtained was used for biochemical examination: Serum sample was used for estimation of blood urea nitrogen (Patton and Crouch 1977), total protein and albumin (Peters, 1968 and Drupt, 1974) chloride (Felidkamb, 1974). Sodium and potassium were estimated by Flame Photometer (Hawk, 1965).

The mortality rate in the study farm was 10%. Dead calves were necropsied for Post -Mortem examination and the lesions were recorded. Tissue specimens were collected from lung, intestine, spleen, lymph node liver, heart and kidneys, fixed in 10%formalin, processed and stained with (H&E) for microscopic examination (Lillie and Fulmen, 1976)

The data obtained were statistically analyzed (Armitage and Berry, 1990).

RESULTS

Neutralizing antibodies to BVDV were detected in bovine sera collected from clinically affected animals. The estimated neutralizing titers varied from 1:4 to 1:256 against BVDV. The 2nd sample taken after two weeks showed rising titer. The control animals were seronegative.

The clinical symptom of the affected calf showed diarrhea of varying severity ranging from watery to bloody diarrhea. Fever ranged from 39.5 to 41 c, anorexia, erosion & ulceration of oral mucosa. Salivation and areas of hemorrhage in the mouth and tongue were present. The calves suffered from respiratory problem ranging from serous to seromucoid nasal discharge, dyspnea and frequent coughing.

Post mortem examination revealed hemorrhage from the mouth, ulceration in nose, alimentary tract and mouth. The intestinal contents were watery in mildly affected animals with enlarged mesenteric lymph nodes. In the severely affected animals the intestinal contents were bloody and the mesenteric lymph nodes were also enlarged and hemorrhagic. Congestion and petechial hemorrhage on the surface of liver, kidneys and heart were present. Lungs were congested and firm in consistency.

The homeroom of the affected calves in both mild and severe diarrhea, showed significant decrease in RBCS count, Hb concentration, packed cell volume, thrombocytic count, white blood cells count, neutrophils and lymphocytes percentage when compared with the control group. The decrease was more significant ($p<0.001$) in severely affected animals compared to mildly affected animals (Table 1).

The biochemical parameters of the affected calves revealed a significant increase in BUN in both groups compared to control group. Total protein, albumin and globulin showed significant decrease compared to the control group. Serum Sodium and chloride showed significant decrease whereas serum potassium revealed significant increase compared to the control group. The changes were more significant ($p<0.001$) in severely affected animals and less significant ($p<0.01$) in mildly affected animals (Table 2)

The mucosa of GIT revealed coagulative necrosis particularly upper portions of the intestinal villi., beside numerous leukocytic infiltrations in the lamina propria (Fig. 1). Moreover, numerous

extravasated erythrocytes and siderocytes could be seen inside the intestinal lumen mixed with desquamated epithelial cells and mucus indicated intestinal ulceration (Fig. 2). The glands of GIT. revealed either metaplasia of goblet cells or focal necrosis of their lining epithelium (Fig. 3). Lymphoid tissue (spleen & lymph node), showed necrotic changes, they revealed depletion of lymphocytes (Fig. 4) Focal hemorrhage and hemosiderosis were evident among lymphoid follicles of the mesenteric lymph nodes beside edema in the capsule and septa (Fig. 5&6). The main blood vessels in the lungs particularly arterioles showed hyperplasia of their tunica media and partial hyalinization with damage of their endothelial lining beside perivascular edema (Fig .7). Some bronchial lumen showed inflammatory exudate and destruction of their lining epithelium. The hepatic parenchyma showed either focal degenerative changes or coagulative necrosis with polymorph cell infiltration (Fig. 8). The renal tissue showed focal or diffuse hemorrhage and hemosiderosis particularly in the renal medulla (Fig. 9). The surrounding tubules suffered from nephrotic changes. The epicardium appeared thickened by extensive hemorrhage, hemosiderosis and edema (Fig. 10).

DISCUSSION

Bovine viral diarrhea virus (BVDV) is a primary pathogen responsible for bovine enteric, respiratory and reproductive failure (Reddy et al., 1999). The disease has various clinical mainfastions, including diarrhea, fever, weight loss, lymph node enlargement, dehydration, and respiratory symptom. In study farm these clinical manifestations were recorded which are nearly similar to those mentioned by Hashiguchi et al. (1978) Doll and Gerbermann (1989), Braun et al., (1996) and Taylor et al. (1997).

A serum neutralizing titer of 1:4 or higher is considered to be an indication of pervious infection with BVDV or of passive immunization (Malmquist, 1968 and Campen et al., 1998). In the present study, neutralization titers were varying from 1:4 to 1:256 for antibodies to BVDV. The absence of any vaccination programs against this disease suggests that the neutralizing antibodies to BVD virus in the farm animals had arisen as a consequence of natural infection with BVD viruses.

Post mortem examination of the dead calves in this farm showed ulceration of alimentary tract from mouth till rectum, The severe cases

showed hemorrhagic contents of intestine. Lungs were congested and firm. Congestion and petechial hemorrhage on the surface of liver, kidney and heart were evident. Similar results were mentioned by Corapi et al. (1990), Jewett et al. (1990), Taniyama et al. (1995) and Taylor et al. (1997).

The hematological examination of the affected calves revealed significant decrease ($P<0.001$) in RBCS count Hb concentration and PCV in severely affected animals compared to control group. Our results accord with those mentioned by Blood et al. (1983) Elsobaie etal; (1984), Bolin et al., (1985), Ahmed (1987), Argenzio(1992) and Pernthaner et al; (1997). Anemia may be the result of hypoplasia and necrosis of cells of bone marrow due to the virus (Marshall et al., 1996 and Ellis et al.; 1998).

Thrombocytopenia, leukopenia, neutropenia and lymphopenia were observed in study groups. Similar findings were reported by Blood et al. (1983) Bolin et al. (1985) Rebhun et al. (1989), Corapi et al. (1990), Bolin and Ridpath (1992) Pernthaner et al. (1997) and Ellis et al. (1998). Corapi et al., (1990) believed that thrombocytopenia is attributed to direct viral effect on platelets, rather than to lack of production of platelets in the bone marrow.

The disease was associated with hemorrhages and petchae on the surfaces of different organs as liver, kidney, heart, spleen and intestine which accords with Rebhun et al., (1989) and Corapi et al., (1990), they reported hemorrhagic syndrome in BVDV affection with bloody diarrhea accompanied with multiple petchea and echymosis on all mucosal surfaces and on all various internal organs.

Johnson and Muscoplat (1973); Heuschele (1978); Lamontagne et al. (1989) and Pernthaner et al; (1997) discussed the mechanism of leukopenia -due neutropenia- and lymphopenia in cases of BVDV/MD infection., as the virus has the affinity to lymphoid tissues (lymphotropic virus), that induces immunosuppression, leading to secondary bacterial or viral infection. The spleen and lymph nodes in dead cases revealed, depletion and necrotic changes of lymphocytes which accords with Marshall et al. (1996), and Brodersen and kelling (1998) where they found degeneration and necrosis with severe lymphoid depletion.

There was significant increase ($p<0.01$, $p<0.001$) in BUN in the mild and severely affected calves respectively. This result is similar to that obtained by Argenzio, (1992) and Braun et al;(1996). Azotemia may be due to hypovolemia and renal failure (Argenzio, 1992). In this study

kidney showed nephrotic changes in the renal tubules with diffuse hemorrhage and hemosiderosis especially in renal medulla which accords with the results of Piero et al. (1997).

Significant decrease ($p<0.01$) in the values of serum sodium and chloride were observed in calves affected with BVD/MD in this study. These results agree with Tennant et al. (1972) and Argenzio (1992). Tennant et al. (1972) stated that the decrease in serum sodium may be due to loss of sodium with intestinal secretion associated with diarrhea. Tasker (1971), reported that serum chloride level usually follows that of sodium because chloride is usually found in the form of sodium chloride, this explains the concomitant reduction of chloride level associated with decrease in the level of sodium. Microscopic examination of the intestine revealed desquamation of the mucosa with extravasation of erythrocytes and hemosidren into the lumen, the mesenteric lymph nodes showed focal hemorrhage and hemosiderosis which accords with Marshall et al. (1996), Brodersen&kelling (1998)and Odeon et al. (1999).

Significant increase ($p<0.05$) in serum potassium of affected calves in this study agrees with Argenzio (1992) who stated that the elevation of plasma potassium is the result of hypovolemia and acidosis due to diarrhea. Hyperkalemia may be due to exchange of extra cellular H for intracellular K in skeletal muscles (Lewis and Phillips, 1973). Thus, the intracellular muscle compartment acts as buffer for the extracellular fluid, at the expense of hyperkalemia. Cardiotoxic effects occur when hyperkalemia is severe and might, in fact be the ultimate cause of death in diarrhea induced acidosis.

Significant reduction in total protein, albumin and globulin values ($p<0.001$) &($p<0.01$) in severely and mildly affected animals were evident, respective results were obtained by Mottelib (1972) and Hassaan et al. (1984). The significant decrease may be attributed to loss of serum proteins via the intestinal tract in diarrheatic calves (Cornell et al., 1969) or due to mal-absorption of dietary constituent from the intestinal tract (Coles, 1980). The significant decrease in globulin of calves infected with BVD/MD may be due to immunosuppressive action of the virus as mentioned by El-Sebaie and Hassaan (1985).

It is concluded that BVD/MD has a deleterious effect on blood cytology, blood chemistry of affected calf so the vaccination, programs in late stage of pregnancy is mandatory to avoid high calf losses.

ACKNOWLEDGEMENT

Special thanks to prof.Dr.Gaber Fakery El-Bagory Prof. and Head of Department of Virology Faculty of Vet. Medicine Zagazig University Banha branch for his help in the virology part

REFERENCES

Ahmed, A.H.S. (1987): Clinical and some biochemical blood changes accompanying alimentary and respiratory mainfistations among fattening buffaloe calves.Thesis M.V.Sc Fac.Vet.Med. Assiut Usniversity.

Argenzio, R.A. (1992): Pathophysiology of diarrhea in Gastroentrology 2nd sEd. Edited by Anderson Philadelphia,Lea &Febiger London.

Armitage, P. and Berry, G. (1990): Statistical methods in medical research .2nd Ed Black Well .Scientific Puplication .Oxford ,London , Edinburgh, Boston&Melbowne.

Braun, U, Thur, B., Weiss, M. and Giger , T. (1996): Bovine virus diarrhea /mucosal disease in cattle –Clinical findings in 130 calves and cattles. Schweiz-Arch-Tierheilkd.;138(10):465-75.

Blood, D.C.; Radostitis, O.M and Henderson, J..A (1983): Vet. Medicine. 6th Ed.Bailliere, Tindall

Bolin, S.R.; McClurkin, AW.; Cutlip, R.C. and Coria, M.F. (1985): Severe clinical disease induced in cattle persistently infected with non cytopathic BVD by super infection with non cytopathic BVD. A.J.V.R. 46.3.572-578.

Bolin, S.R. and Ridpath, J.F. (1992): Differences in virulence between two non-cytopathic bovine viral diarrhea viruses in calves .Am .j .Vet. Res. Nov.,53:2157-63.

Brodersen, B.W. and kelling C.L. (1998): Effect of concurrent experimentally induced bovine respiratory syncytial virus and bovine viral diarrhea virus infection on respiratory tract and enteric disease in calves. Am.J. Vet. Res. Nov; 59(11): 1423-30.

Campen, H.Van; Huzurbazar, S.; Edwards, J.; Cavender, J..L (1998): Distribution of antibody titers to bovine viral diarrhea virus in infected ,exposed, and uninfected beef cattle..J.of Vet. Diag. Invest. 10 (2): 183-186.

Corapi, W.V; Elliott, R.D, French, T.W., Arthur, D.G., Bezek, D.M. and Dubovi, E.J. (1990): Thrombocytopenia and hemorrhages in veal calves infected with bovine viral diarrhea virus. J.A.V.M.A ,196(4) Feb 15.

Coles, E. H. (1980): Vet. Clinical Pathology W.B. Saunders Company, Philadelphia and London.

Cornell,L. M., Charloes, A. M. and Norman R. U. (1969): Loss of serum proteins via intestinal tract in calves with infectious diarrhea.A.J.V.R. 30 163-166.

Doll,K and Gerbermann, H. (1989): Clinical observation in cattle with persistent bovine diarrhea virus infection.Praktische Tierarzt(1989) 70 (Sondernummer) 38-40.

Drupt, F. (1974): Colorimetric method for determination of albumin. Pharm.Bio; 9,777

Ellis, J.A, West, K.H, Cortese, V.S., Myers, S.L., Carman, S. Martin , K.M and Haines, D.M. (1998): Lesion and distribution of of viral antigen following an experimental infection of young sero negative calves with virulent bovine virus diarrhea virus-type 11.an.J.Vet.Res.Jul.,62:161-9.

EL-Sebaie, A.H., Ibrahim, A.A.; Hofmann,W. and Amer, A.A. (1984): Investigation on an outbreak of enzootic broncho-pneumonia in fattening buffaloe calves in Assiut A.R.E. 1. Clinical, microbiological and hematological studies.Assiut Vet.Med.J. 11, 22: 166-167.

EL-Sebaie, A.H. and Hassaan, N.K. (1985): Reports on infectious bovine rhinotrachtitis and virus diarrhea infection in fattening calves. Assiut Vet.Med.J. vol. 14. 14 no 27,247.

Felidkamb, C.S, (1974): Calorimetric determination of chloride. Z.Klin. Chim. Klin. Biochem. 12:146 –150.

Fisher, E.W. (1971): Hydrogen ion and electrolytes disturbances in neonatal calf diarrhea. Ann Ny. Acad. Sci. 17 223-230.

Frey, H.R. and Liess, B. (1971): Growth curve studies and applicability of a highly cytopathogenic BVD-MD virus strain for diagnostic purposes using the micro-titer method.Zbl.Vet.Med.18, 61:71.

Hashiguchi, Y. Inui, S.; Nanba, K and Kumagai, T. (1978): Bovine viral diarrhea – Mucosal disease. II.Isolation and characterization of cytopathogenic virus and experimental production of the disease. Natl. Inst Anim. Health Q Tokyo. Winter; 18(3-4): 118-27.

Hawk, P.B. (1965): Determination of serum sodium by using flamephotometer. Hawk's physiological chemistry. 14th ed .B.L.M.C.Graw-Hill ,Inc NEW YORK.

Hassaan, N.K.; EL-Sebaie, A.H. and Hassan, MS. (1984): Investigation on an outbreak of enzootic broncho-pneumonia in buffaloe calves in Assiut A.R.E. 11 Clinical, significance of blood serum protein level and electrophoretic serum protein fractionation in infected calves. .Assiut Vet.Med.J. 11,22:177.

Heuschele, W.P. (1978): New Perspectives of the epidemiology of bovine virus diarrhea-mucosal disease (BVD) Bovine Pract. 12:51-53.

Jain N.C. (1986): Schalm's Vet Hematology 4th Ed.Lea and Fibiger Philadelphia,USA.

Jewett, C.J.; Kelling, C.L.; Frey, M. L. and Doster, A.R. (1990): Comparative pathogenicity of selected bovine viral diarrhea virus isolates in genotobiotic lambs. Am.J. Vet. Res. Oct.; 51(10): 1640-4.

Johnson, D.W. and Mucoplat, C.C. (1973): immunologic abnormalities in calves with chronic bovine viral diarrhea. Am J Vet Res 34:1139-1141

Kobrak, A. and Weber, E.L (1997): (Bovine viral diarrhea virus An up date) Rev-Argent-Microbiol. Jan –Mar, 29:47-61.

Lamontagen, Lafortune, P. and Fournell, M. (1989): Modulation of the cellular responses to –cell- dependent antigens in lambs with induced bovine viral diarrhea virus infection. AM. J.of Vet. Res. 50(9) 1604-1608.

Lee, S.C.; Borgmann, L. E. and Gobin, N.A. (1994): A severe outbreak of mucosal disease in central Alberta. Can. Vet. J. 35:641-642.

Lewis, L.D. and Phillips, R.W. (1973): Diarrheic induced changes in intracellular and extracellular ion concentration in neonatal calves. .Ann. Res.Vet.,4:99-105.

Lewis L.D and Phillips R.W. (1978): Pathophysiology changes due to corona virus –induced diarrhea in calf. J. Am. Vet. Med. Assoc. 173:636-642.

Lilile, R.D. and Fulmen, H.M.(1976): Histopathiological Technique and Practical Histopathology. The Blauiston Division, New York, Acad. Sic.,111,789-792

Marshall, D.J., Moxley., R.A. and Kelling, C.L (1996): Distribution of virus and virus antigen in specific pathogen-free calves following inoculation with noncytopathic bovine viral diarrhea virus. Vet-Pathol.33(3):311-8.

Malmquist, W.A. (1968): Bovine viral diarrhea – Mucosal disease etiology, pathogenesis and applied immunity. JAVMA. Vol. 152, No 6,763:768.

Mottelib, A.A. (1972): A study on changes of blood in buffaloe calves suffering from enteritis due to different causitive agents. Thesis Fac. Vet. Med. Assiut Univ.

Odeon, A.C.; Kelling, C.L.; Marshall, D.G.; Estela, E.S. Dubovi, E.J. and Donis, R.O. (1999): May;11(3):221-8. Experimental infection of calves with bovine viral diarrhea virus genotype II (NY-93). J.Vet.Diagn.Invest. May 11(3):221-8.

Olafson, P.; MacCullum, A.D. and Fox, F. H. (1946): An apparently new transmissible disease of cattle. Cornell Vet. 36:205-213.

Patton, C.J. and Crouch, J.E. (1977): Enzymatic determination of urea.Anal. Chem., 49,464-469.

Peters, T. (1968): Colorimeteric method for determination of total serum proteins. Clin.Chem., 14, 1147.

Pernthaner, A., Schilcher, F. and Baumgartner, W. (1997): Acute bovine viral diarrhea virus infection in Australian cattle. Israel journal of Vet Med .52(2/3) 104 –107

Piero, F, DEL. Wilkins, P.A. and Dubovi, E.J. (1997): BVD virus antigen in purkinje fibres in a calf. Vet. Rec. (1997) 140 (15) 407-408.

Reddy, J.R, Kwang, J., Varthakavi, V, Lechtenbergk. F. and Minocha, H.C. (1999): Semiliki forest virus vector carrying the bovine viral diarrhea virus NS3(P80) CDNA induced immune responses in mice and expressed BVDV protein in mammalian cells. Comp-Immunol-Microbiol infect- Dis.Oct;22(4)231-46.

Rebhun, W.C.; French, T.W., Perdrizer, J.A., Dill, S.G. and Karcher, L.F. (1989): J. of Vet. Internal Medicine 3(1): 42-46.

Tasker, J.B. (1971): Fluid, electrolytes and acid- base balance. In:Clinical biochemistry of domestic animals vol. 2,edited by Kaneko,J.J.:Cornellus,C.B.2nd Ed. Acad. Press,NewYork.

Taniyama, H Ushiki, T., Tajima, M., Kurosawa, T., Kitamura, N., Takahashi,, K., Matsukawa, K., and Itakura, C. (1995): Spontaneous diabetes mellitus associated with persistent viral diarrhea (BVD) virus infection in young cattle .Vet. Path .May, 32: 221-9.

Taylor L.F.; Janzen, E.D.; Ellis, J.A. Hurk, J.V. Van Den; Ward, P. (1997): Performance, Survival,necropsy, and virological findings from calves presistently infected with bovine viral diarrhea virus originating from a single Saskatchewan beef herd. Candian Vet. J. 38(1): 29-37.

Tennant, B.; Harrold, D. and Reina-Cuerra, M. (1972): Physiological and Metabolic factors in the pathogenesis of neonatal enteric infection in calves.J.A.V.M.A., 161: :993-1007.

Table 1: Mean values (+SD) of some hematological parameters in both clinically healthy and affected calves.

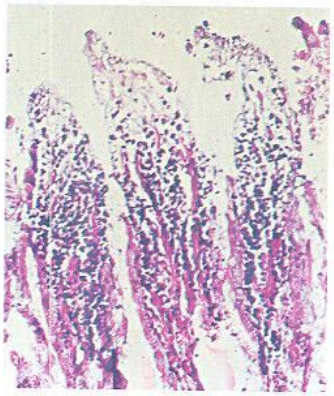
Group	RBCS X 10^6/mm	Hb Gm/dl	PCV %	Thrombocytes $X10^3$ /mm	WBCs $X10^3$/ mm	Neutrophils %	Lymphocytes %
Healthy Calves (n=10)	7.92+0,51	10.12+1.65	34.0+4.06	390.0+43.24	7.18+0.61	28.2+0.61	60.0+7.91
Mild Diarrhea (n=10)	6.34+0.63***	8.32+0.63*	28.6+3.51**	300.0+25.49***	6.5+0.79*	20.6+3.36**	42.4+3.36**
Severe Diarrhea (n=10)	5.04+0.79***	6.5+1.11***	22.6+3.86***	208.0+27.7***	5.48+0.95**	16.0+2.23***	37.0+5.15***

* Significant P<0.05 ** Highly significant P<0.01 *** Very highly significant P<0.001

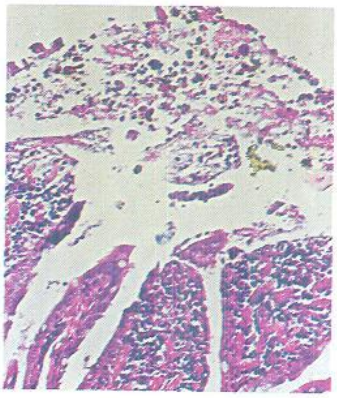
Table 2: Mean values (+SD) of some biochemical parameters in both clinically healthy and affected calves.

Group	BUN mg/dl	Total Protein gm/dl	Albumin gm/dl	Globulin gm/dl	Sodium mEq/l	Potassium mEq/l	Chloride mEq/l
Healthy calves (n=10)	33.76+4.88	7.5+0.61	3.58+0.44	3.92+0.42	140.0+12.74	5.68+0.89	95.0+11.18
Mild Diarrhea (n=10)	52.3+10.36**	6.0+0.38**	3.0+0.25**	3.0+0.25***	120.0+14.5**	6.1+0.15	82.0+9.3**
Severe Diarrhea (n=10)	68.0+7.83***	5.5+0.68***	2.86+0.23**	2.64+0.45***	110.0+15.8***	6.74+0.96*	70.0+7.9***

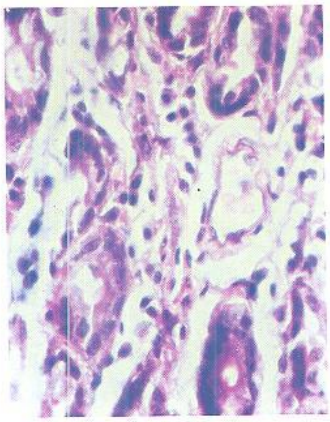
* Significant P<0.05 ** Highly significant P<0.01 *** Very highly significant P<0.001



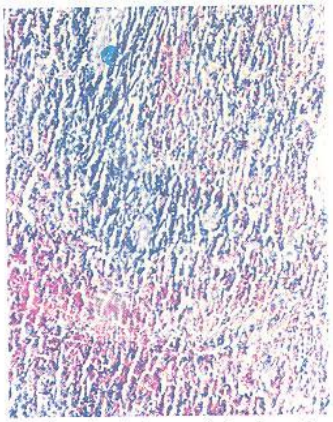
(Fig.1) Small intestine of dead calf (BVD) showing focal necrosis of the upper portions of the intestinal villi and numerous leukocytic infiltrations (H&EX600)



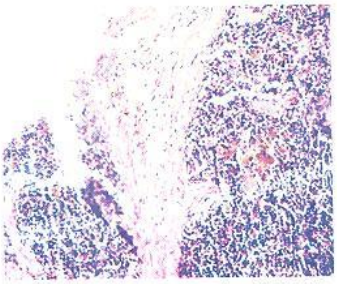
(Fig.2) Instestine of dead calf (BVD) showing destructed mucosa with presence of exudate inside intestinal lumen (H&E X600)



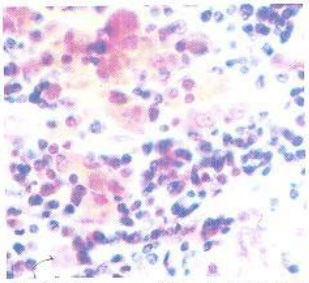
(Fig3) Glandular stomach of dead calf (BVD) showing focal necrosis of their lining epithelium (H&E X1200)



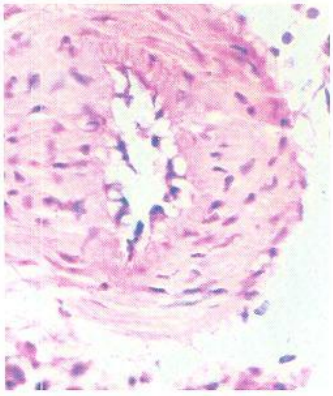
(Fig. 4) Spleen of dead calf (BVD) showing depleted white pulp (H&E X 150)



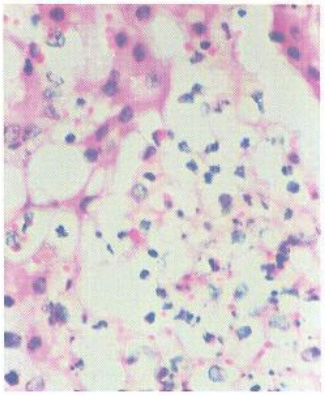
(Fig5) Lymph node of calf (BVD) showing edematous capsule and subcapsular hemosiderosis (II&E 150)



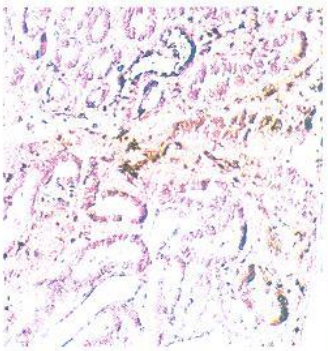
(Fig 6) High power of the pervious figure to show extensive hemosiderosis and necrosis of some lymphoid cells (H&E X1200)



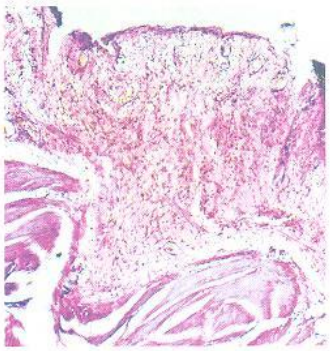
(Fig.7) Lung of dead calf (BVD) showing arteritis and perivascular edema (H&E 1200)



(Fig.8) Liver of dead calf showing focal coagulative necrosis of the hepatic parenchyma (H&E 1200)



(Fig.9) Kidney of dead calf (BVD) showing interstitial hemorrhage in the renal medulla (H&E 600)



(Fig.10) Heart of dead calf showing thickened and hemorrhage on the epicardium (H& E X150)