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Suvendu Kumar Behera
Assistant Professor, Department of Veterinary Medicine, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Mizoram, India

Y Damodar Singh
Department of Veterinary Pathology, CVSC and AH, CAU, Selesih, Aizawl, Mizoram, India

Parimal Roychoudhury
Department of Veterinary Microbiology, CVSC and AH, CAU, Selesih, Aizawl, Mizoram, India

Rahul Singh Arya
Department of Veterinary Pathology, CVSC and AH, CAU, Selesih, Aizawl, Mizoram, India

Parthasarathi Behera
Department of Veterinary Physiology & Biochemistry, CVSC and AH, CAU, Selesih, Aizawl, Mizoram, India

M Ayub Ali
Department of Veterinary Physiology & Biochemistry, CVSC and AH, CAU, Selesih, Aizawl, Mizoram, India

H Prasad
Department of Veterinary Medicine, CVSC and All, CAU, Selesih, Aizawl, Mizoram, India

Kalyan Sarma
Department of Veterinary Medicine, CVSC and AH, CAU, Selesih, Aizawl, Mizoram, India

JB Rajesh
Department of Veterinary Medicine, CVSC and AH, CAU, Selesih, Aizawl, Mizoram, India

Chethan GE
Department of Veterinary Medicine, CVSC and All, CAU, Selesih, Aizawl, Mizoram, India

Corresponding Author:
Suvendu Kumar Behera
Assistant Professor, Department of Veterinary Medicine, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Mizoram, India

Clinico-pathological and necropsy findings in a 4-month old mixed-breed pup with canine parvovirus-2 infection and its genetic characterization

Suvendu Kumar Behera, Y Damodar Singh, Parimal Roychoudhury, Rahul Singh Arya, Parthasarathi Behera, M Ayub Ali, H Prasad, Kalyan Sarma, JB Rajesh and Chethan GE

Abstract
A four-month-old male mixed breed dog was presented with a complaint of continuous vomition, bloody diarrhea, anorexia since last five days. Physical examination revealed hypothermia, pale conjunctival mucus membrane, tachycardia, eupnea and poor body condition on the day of presentation. Fecal sample examination using commercial kit confirmed the presence of canine parvovirus-2. Viral DNA was isolated from fecal sample followed by PCR confirmation, cloning, sequencing and phylogenetic analysis which revealed it as CPV-2a variant. Hematology revealed anemia, leukopenia, decreased segmented neutrophil counts with increase in the band neutrophil counts. Plasma biochemistry revealed hypercholesterolemia with increased activities of aspartate aminotransferase, creatinine kinase and lactate dehydrogenase whereas decreased levels of amylase and chloride. In spite of aggressive therapy, the dog succumbed to its injuries on the day of presentation and hence, necropsy was performed with owner's consent. Gross necropsy changes of the small intestine revealed blood-tinged foul hemorrhagic fluid. There were focal round to ovoid ulcerative lesions with punched-out Peyer's patches which were slightly depressed and covered with yellowish thick exudates. The liver was enlarged, soft, friable and showed icteric change with distended gall bladder. The spleen was enlarged and congested. The lung showed consolidation and hemorrhages in diaphragmatic lobes. The kidney was found to be congested. The urinary bladder showed thickened wall and hemorrhagic lesions on the mucosal surface. Histopathology of intestinal tissue revealed complete loss of villi, crypts & columnar cells with severe inflammation of the submucosa.

Keywords: Canine parvovirus-2, commercial kit, antigenic variant, hemato-biochemistry, necropsy

Introduction
Canine parvovirus-2 (CPV-2), a member of the genus Parvovirus and family Parvoviridae is one of the most significant viral causes of acute haemorrhagic gastroenteritis and myocarditis in puppies, causing heavy morbidity and mortality [15]. The virus contains a single strand DNA genome of about 5200 nucleotides that is packaged in an icosahedral capsid [7]. Phylogenetic analysis revealed that all CPV-2 variants were descended from single ancestor who emerged during the mid-1970s and was closely related to feline panleukopaenia virus (FPV) which infects cats, minks, and raccoons but not dogs. Because of continuous mutation, the virus subsequently gave rise to many variants such as CPV-2a, CPV-2b, CPV- 2c, New CPV-2a, New CPV-2b and Asp 300 (2a/2b). The virus has three capsid proteins: VP1, VP2 and VP3. VP2 is the highly antigenic major capsid protein, and it plays an important role in determining viral host range and tissue tropism [11]. Amino acids substitutions in VP2 gene have been responsible for genetic and antigenic properties [17]. All the newer antigenic variants differ from the original type CPV-2 for a few amino acids in the VP2 protein. Currently, the original virus, CPV-2 has been completely replaced by these new variants [15]. The identification of the subtypes of CPV-2 that are currently circulating in the canine population is essential for the understanding of viral evolution and the development of measures to control its spread [16]. As scientific literatures remain silent about the clinico-pathobiology of CPV-2 in Mizoram, so, the aim of the present communication was to discuss in detail the clinical signs, diagnosis, antigenic variant of the virus and changes with respect to hemato-biochemistry and necropsy(gross and histopathologic) in a mixed-breed pup with CPV-2.

Materials and Methods

Case history

A four-month-old male mixed breed dog weighing 11 kg was presented to Teaching Veterinary Clinical Complex of the college with a history of continuous vomition (6-8 times/day), bloody fetid diarrhea (6-8 times/day), anorexia, marked depression since last five days. The animal had a dietary history of home cooked food. Animal had improper immunization and deworming history.

Clinical observations and laboratory evaluations

Clinical examination revealed hypothermia (rectal temperature of 98º F), pale conjunctival mucus membrane, tachycardia (260 bpm), eupnea (25 bpm), increase capillary refill time (4 sec), skin turgor test (> 8sec) and poor body condition score (3 on 9 scale) on the day of presentation. Fecal sample examination of the pup using commercial kit (SNAP PARVO, IDEXX Laboratories) confirmed the presence of canine parvovirus-2 (Fig. 1). 2ml Blood sample was collected from the cephalic vein in EDTA vial for haematological examination with the help of an automated blood cell counter (MS4e, France) followed by centrifugation of the blood @ 3000 rpm for 5 minutes to harvest plasma for plasma biochemistry with the help of automated dry chemistry analyzer (Fujifilm DRI-CHEM 4000i, Japan). Viral DNA was extracted from the fecal sample and the published primer pairs such as H*for* (5'-CAGGTGATGAATTTGCTACA-3' with nucleotide position 3556–3575 of VP2) and H*rev* (5'-CATTTGGATAAACTGGTGGT-3'with nucleotide position 4185–4166 of VP2) yielded 630bp [4] product (Fig. 2). The PCR product was then cloned followed by sequencing, phylogenetic analysis and submitted to GenBank.

Results and Discussion

Hematology revealed marked decrease in the levels of hemoglobin, hematocrit, total erythrocyte count, total leukocyte count and segmented neutrophils count with marked increase in the band neutrophils count compared to reference range (table 1). Plasma biochemistry revealed increase in the levels of total cholesterol (TCHO), aspartate aminotransferase (AST), creatinine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) whereas decreased levels of amylase and chloride (Cl) compared to reference range (table 1). Phylogenetic analysis of the sequence revealed similarity ranged between 99.2-99.8% with other CPV-2a sequences from the database.

Anemia, leukopenia and neutropenia were in agreement with previous reports reported by various authors [5, 14]. Anemia might be attributed to the enteric blood loss. Leukopenia and neutropenia develop secondary to marrow infection or sepsis and generally parallel the severity of clinical infection [10]. Some authors suggested that neutropenia was primarily the result of net consumptions of neutrophils at the injured intestinal mucosa rather than a primary failure of granulopoiesis [6]. Leukopenia was a constant finding, with white blood cell counts dropping below 2000–3000 cells/µL of blood [9]. Marked leukopenia and neutropenia (decrease in segmented neutrophils and increase in band neutrophils) in the present case were suggestive of grave prognosis [2].

Hypercholesterolemia in the present study was in contradiction with earlier report where serum hypocholesterolemia and hypertriglyceridemia were reported in dogs with severe septic form of parvoviral enteritis [18]. Hyperlipidemia (hypercholesterolemia and hypertriglyceridemia) has been reported in infectious and inflammatory diseases which was attributed to both increase in lipoprotein production and decrease in lipoprotein clearance as a host defense mechanism [1].Increased activity of plasma aspartate aminotransferase (AST) was in agreement with other reports [2, 12] which may be due to their increased synthesis and secretion or due to reduced catabolism and/or by increase extracellular leakage resulting from increased cell permeability. Large quantities of AST are present in red blood cells, liver, heart, muscle tissue, pancreas, and kidneys. Destruction of any of these tissues results in release of large quantities of this enzyme in the blood [13]. Increase in the activities of CK-MB and LDH were in agreement with previous report [3, 18]. CK-MB, an isoenzyme of CK, is predominantly found in heart muscle and is a sensitive marker for myocardial cell damage in humans and animals [19]. LDH is found in muscle, heart, red blood cells, liver and kidneys. Destruction of any of these tissues results in liberation of large quantities of this enzyme in the blood. In this case, elevated level of LDH may be taken as a complementary test to CK-MB test to help determine that the origin of LDH is from muscle and most probably, myocardium. Hypochloremia in the present case might be secondary to vomiting and diarrhea [14] and was in agreement with earlier report [12].

Fluid and electrolytes have remained the cornerstones of therapy against CPV-2 enteritis. In spite of intensive therapy the animal collapsed on the day of presentation which might be due to advanced stage of the disease and hence necropsy was performed with owner's consent.

Gross changes of the small intestine particularly the duodenum and jejunum showed moderate to severe degree of congestion and hemorrhage with blood-tinged hemorrhagic fluid (Fig. 3). There were focal round to ovoid ulcerative lesions with punched-out Peyer's patches which were slightly depressed and covered with yellowish thick exudates (Fig. 4). The stomach was filled with blood-tinged fluid and some pieces of stones with hyperemic mucosae. The mesenteric lymph nodes were enlarged and edematous with hemorrhages in the cortex. The liver was enlarged, soft, friable and showed icteric changes with distended gall bladder (Fig. 5). The spleen was also enlarged and congested (Fig. 6). Kidney was found to be congested (Fig. 7). The urinary bladder showed thickened wall and hemorrhagic lesions on the mucosal surface (Fig. 8). The lung showed consolidation and hemorrhages in diaphragmatic lobes (Fig. 9). All the above gross changes were in agreement with earlier report [10].

Histopathology of intestinal tissue revealed complete loss of villi, crypts and columnar cells (Figs. 10 and 11) which was in agreement with earlier report [10]. As the virus multiplies in the rapidly dividing cells of intestinal crypts, causing epithelial destruction and villous collapse and combined with neutropenia due to bone marrow aplasia favours bacterial invasion and septicemia, sequentially, and may lead to dehydration, endotoxic and/or hypovolemic shock and death [8, 9].

Table 1: Hemato-biochemical profile of the dog with CPV-2a infection on day 0.

Parameters	Day 0	Reference range
Hb (g/dl)	8.22	12-19
Hematocrit (%)	25.1	35-57
TEC (x 10^6/µl)	4.4	5.0-7.9
TLC (x 10^3/µl)	4.1	5.0-14.1
Platelet (x 10^5/µl)	2.52	2.11-6.21
Segmented Neutrophil (%)	48	60-75
Band N (%)	13	3-6
L (%)	27	8-21
M(%)	10	2-10
E (%)	2	0-9
Plasma biochemistry		
TCHO (mg/dl)	290	135-278
AST (U/L)	39	13–15
CK-MB (U/L)	290	124-173.0
AMYLASE (U/L)	104	226-1063
LDH (U/L)	296	0–236
Cl (mEq/L)	104	110–124

Fig 1: Commercial Dot ELISA based CPV-2 Ag Test (IDEXX Lab.) showing positive result

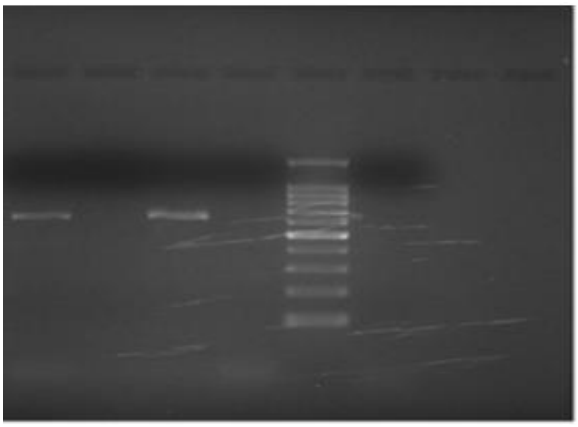

Fig 2: Gel picture showing the PCR amplified. 630bp fragment of VP2 gene of CPV-2 isolates. From left: lane 1 showing positive amplification, 2 showing negative amplification, 3 positive control, 4 negative control and M 100 bp plus DNA ladder

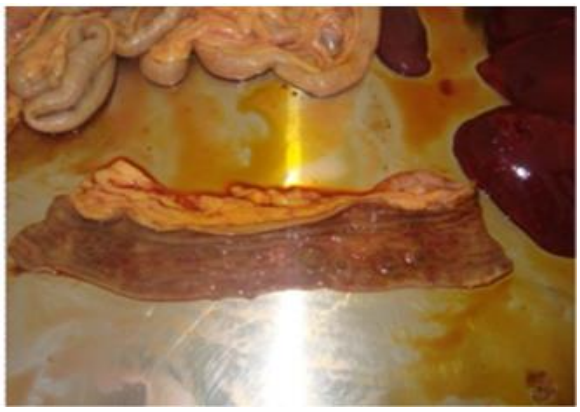

Fig 3: Gross necropsy examination of small intestine containing blood- tinged. hemorrhagic fluid, linear congestion and haemorrhages

Fig 4: Reddish ulcerated and ovoid punched-out Payer's patches

Fig 5: Enlarged. soft, friable liver with icteric changes and distended gall bladder

Fig 6: Enlarged and congested spleen

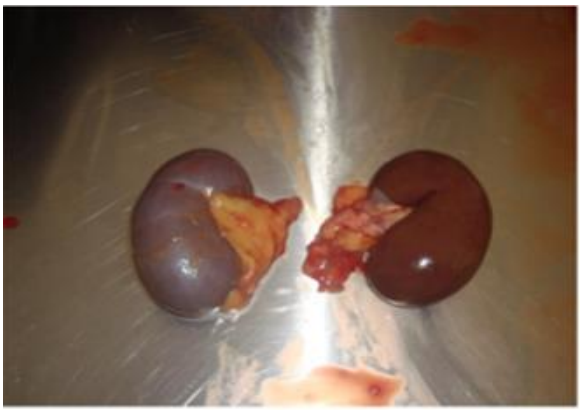

Fig 7: Congested left kidney compared to right kidney

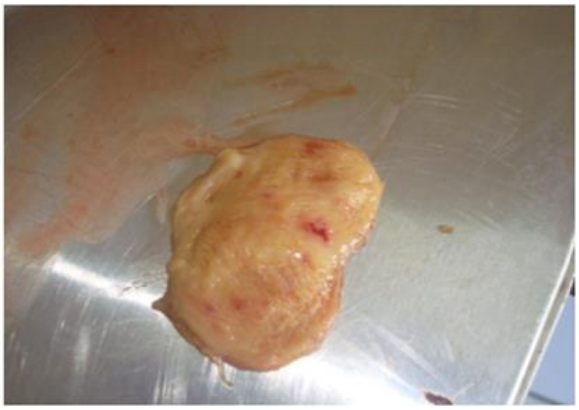

Fig 8: Thickened urinary bladder and hemorrhagic lesions on the mucosal surface

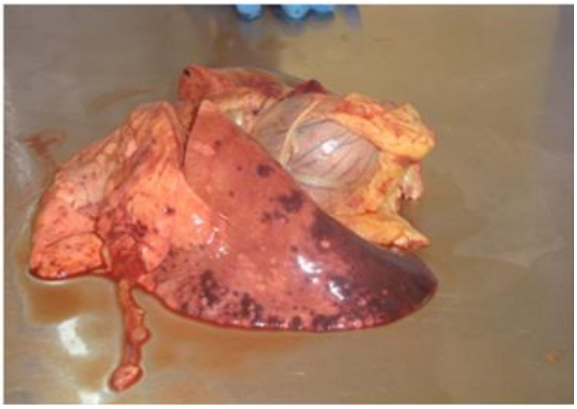

Fig 9: Lungs showing consolidation and hemorrhages in diaphragmatic lobes

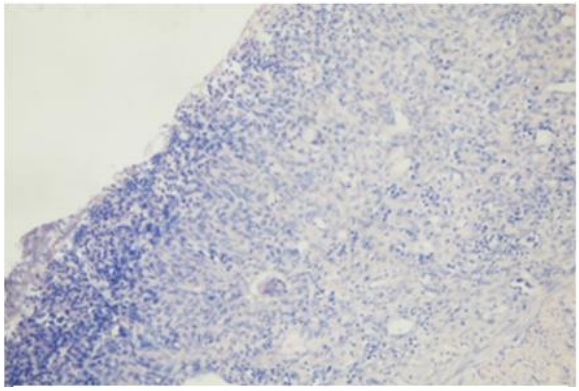

Fig 10: Histopathology of intestinal tissue showing absence of columnar cells, clubbed villi and completely merged crypts (H and E stain, 100x)

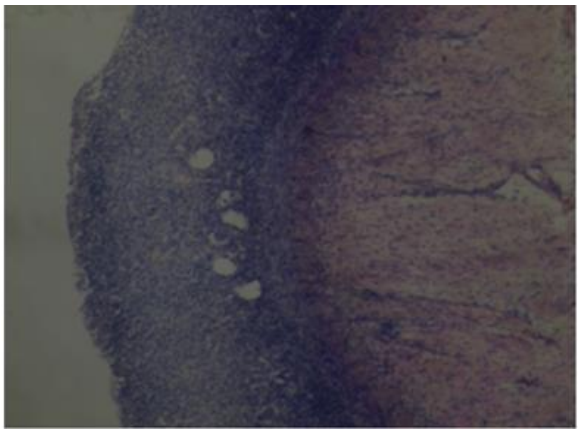

Fig 11: Histopathology of intestine revealing sever inflammation of the submucosa with infiltration of inflammatory cells (H & E stain, 100x)

Conclusion

Confirmatory diagnosis of CPV-2 was made by using both commercial kit and PCR assay. Phylogenetic analysis revealed the variant to be CPV-2a which is widespread among dog populations, including India and other Asian countries. This report is the first of its kind from the state of Mizoram where a detailed description of hemato-biochemical, pathological and virological aspects of naturally occurring CPV-2a infected dog has been described and will help furthering our knowledge with respect to the disease's pathogenesis.

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