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Immunopathological identification of canine parvoviral enteritis in vaccinated Doberman pup

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Abstract
The present case report gives detailed aspect of the clinical signs, pathology, and molecular diagnosis of canine parvoviral enteritis in 84 days old male Dobermann pup. Examined clinically for vital parameters, complete blood picture, faecal examination for parasites and PCR canine parvovirus antigen. Organs samples were collected at necropsy for confirmatory diagnosis using histopathology, immunohistochemistry and polymerase chain reaction (PCR) for localisation of antigen in tissues. There was a severe anaemia, leukopenia and lymphopenia. The positive antigen faecal test and pathological findings of haemorrhagic enteritis suggested canine parvoviral enteritis disease. Polymerase chain reaction confirmed canine parvovirus as the aetiology of the disease. Vaccination and maternal antibody titre are important to avoid pup from canine parvoviral enteritis.

Keywords: *Canine parvovirus*, pathology, immunohistochemistry, PCR

Introduction
Canine parvoviral enteritis is contagious viral disease of dogs and causes haemorrhagic gastroenteritis in adult and non suppurative myocarditis in young pups. (Chen *et al*. 2019) [4]. Canine parvovirus (CPV) is aetiological agent for canine parvoviral enteritis and produce high mortality rate in young pups due to cardiac form and enteric form in adult dogs. The virus distributed in entire country since forty years (Voorhees *et al*. 2019) [23]. Canine parvovirus is come under the Parvoviridae family and Protoparvovirus genus. Canine parvovirus is single-stranded, icosahedral, non-enveloped and DNA virus. There are two type of viral protein, one is non-structural protein (Two numbers) and another is structural protein (Three numbers). Total viral genome size is five thousand two hundred nucleotides (Miranda and Thompson 2016) [17]. CPV 2a, CPV 2a new variant, CPV 2b and CPV 2c variants are reported in different parts of the world. Canine parvovirus requires the presence of mitotically active cells in order to replicate. Young animals (6 weeks to 6 months, and especially those less than 12 weeks of age) are more likely to develop severe illness; however, disease can also occur in unvaccinated or improperly vaccinated adult dogs. In North America, rottweilers, American pit bull terriers, Doberman pinschers, English springer spaniels, and German shepherd dogs appear to be at increased risk for development of parvoviral enteritis (Houston *et al*. 1996) [14] and Glickman, *et al*. 1985) [11]. Gross lesions of CPV enteritis include thickening and discoloration of the intestinal wall with serosal hemorrhage and enlarged, edematous abdominal lymph nodes. The intestine contains bloody liquid contents, and mucosal haemorrhage. Pale areas of the myocardium of dogs with parvoviral myocarditis. The specific histopathological changes are necrosis of the crypt epithelium in the small intestine, with widespread systemic lymphoid depletion and necrosis. The crypts shows dilated and distended with cellular debris and mucus. The present article report the clinical course, vaccination details, necropsy examination and localisation of antigen in tissue sample affected with canine parvovirus in Doberman pup.

Materials and Methods
Vaccination history
One Doberman young pup was referred to Madras Veterinary College Teaching Hospital, Chennai for regular check-up and vaccination. The male Doberman was born on 01.03.2021. First vaccination was done on 28.03.2021 with commercial vaccine against canine distemper and canine parvoviral enteritis (puppy DP) on 28.03.2021 at breeder place itself with qualified veterinarian (28th day). Second scheduled dose of vaccination was given to pup on 19.04.2021

(50th day) with combined commercial vaccine (contained live canine distemper virus, adenovirus-2, parainfluenza virus, parvovirus vaccine, inactivated *Leptospira canicola*, *Leptospira icterohemorrhagica*, canine adenovirus-1) and same combined commercial vaccine repeated as booster on 10.05.2021 (71st days).

Case history

On 84th day (20.05.2021) male Doberman was referred to Madras Veterinary College Hospital, Chennai for anorexia, fever, dullness and brownish foul smelling diarrhoea. The Doberman was suspected for canine parvoviral enteritis and supportive treatment given to correct fluid loss and broad spectrum antibiotic. Vomiting and bloody diarrhoea continue till 23.05.2021 morning and treatment given morning and evening. On 23.05.2021 (87th day) male Doberman was died and referred to Department of Veterinary Pathology for detailed necropsy examination.

Polymerase chain reaction

The faecal sample was collected and tested for gastrointestinal worm eggs. Faecal sample was processed for PCR confirmation.Canine parvovirus DNA from the fecal samples were isolated using QIAamp DNA stool mini kit (Qiagen, Hilton, Germany) according to manufacturer's instruction. The isolated DNA was subjected to PCR assay for detection of parvovirus infection. A reaction volume of 25 µl was prepared, consisting of 0.5 µl of each forward and reverse primer (sense, 5'AAAGAGAGCCAGGAGAGGTA-3'; anti-sense, 5'-TTCTGACAGCAGGTTGACCA-3'), 0.5µl of 2 mM dNTPS (Fermentas), 2.5 µl of Taq DNA buffers A 10X (Tris with15 mM of MgCl2) (Genei, Bengaluru, India), 0.25 µl of Taq DNA polymerase (5U/µl concentration, Genei, Bangalore) and finally 5µl of lysate was added. Then the rest volume was adjusted to 25µl by addition of nuclease-free water (Genei, Bengaluru)

The reaction mixture was prepared in 200 µl PCR tubes. The amplification was performed in a thermocycler (Bio-Rad) with a reaction condition comprised of an initial denaturisation at 95 ºC for 3 min, then 30 cycles of denaturation at 95 ºC for 30 s, primer annealing at 55 ºC for 30 seconds and extension at 72 ºC for 30 seconds, a final extension at 72 ºC for 5 minutes and Hold on 4 ºC for 10 minutes. The amplified PCR product were analysed on 1% agarose gel with the positive control and visualized under UV transilluminator.

Histopathological and immunohistochemical method

The tissues were collected in 10% neutral buffered formalin for histopathological examination. Paraffin embedded tissue section was cut into 4 to 5 micron thickness and stained with Haematoxylin and Eosin (H&E) stain as per procedure described by Bancroft and Gamble (2008) [2].

For immunohistochemical examination, tissue sections were removed the paraffin from tissue section and heat treatment was applied for antigen retrieval. Endogenous peroxidise was blocked and canine parvovirus primary antibody applied over the slide at 1:200 dilution for 12 hours at moist chamber. Tissue sections were washed with PBS for removing excess unbounded CPV primary antibody. The secondary antibody was poured over the tissue sections and incubated for four hours. Tissue section were washed with PBS. Labelled peroxidase-conjugated streptavidin-biotin complex and 3, 3 diaminobenzidine tetrahydrochloride (DAB) were poured over the tissue sections. Tissue section washed with PBS and counter stained with Haematoxylin. Cover glass was applied over tissue sections.

Results

At necropsy, external examination revealed pale oral mucous membrane with dehydration, and dark bloody diarrhoeic content adhered over the anus and perianal region (Fig 1,2 and 3). Internal examination revealed congestion of the serosal surface of the stomach. Stomach contained yellowish green watery content (Fig 4). Serosal surface of the jejunum showed congestion and hemorrhages (Picture 5). The lumen of the jejunum contained brownish watery content. Jejunum mucosa was thickened and congestion. Linear streak of haemorrhage noticed on rectal mucosa (Fig 5 and 6). Heart showed pale streaks on the myocardium with congested blood vessels (Fig 7). Liver and kidney showed enlargement and severe congestion. Lungs revealed patchy area of congestion and edematous (Fig 8 and 9). Mesenteric lymph nodes were enlarged and red.

Histopathological examination of jejunum revealed degeneration and necrosis of the mucosal epithelial cells in the intestinal villi with mononuclear cells infiltration among, shortened and stunted villi (Fig 10 and 11). Eosinophilic intranuclear inclusion observed in the enterocytes in the jejunum portion of villi (Fig 12). Ileum revealed lymphoid cell depletion and fusion of villi (Fig 13). Immunohistochemical examination of jejunum and mesenteric lymph node showed brown positive reaction for canine parvo viral enteritis. The canine parvoviral antigen was detected in the jejunum and ileum and in mesenteric lymph nodes (Fig 14 and 15).

PCR assay was performed for the molecular diagnosis of canine parvovirus. The observation of the 2% agarose gel in the transilluminator allowed the detection of bands or amplified to 535 bp, both in the positive control and in the present dog sample. (Fig 16).

Gross pathology findings

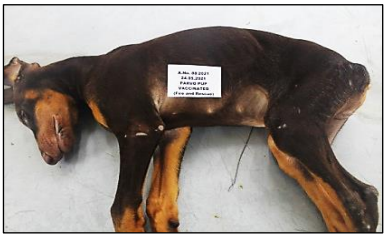

Fig 1: Dehydrated 84 days old Doberman pup

Fig 2: Dark bloody diarrhoic content adhered over the perianal region and anus

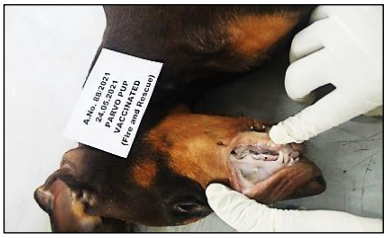

Fig 3: Pale oral mucus membrane

Internal gross lesion in organs

Fig 4: Stomach mucosa stained with yellowish bile with mucus

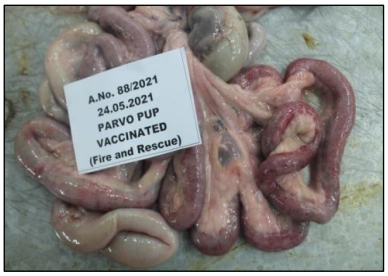

Fig 5: Serosal surface of jejunam showing scattered haemorrhage

Fig 6: Jejunal mucosal showing thickening and diffused haemorrhage

Fig 7: Heart showing vessel congestion and pale streaks

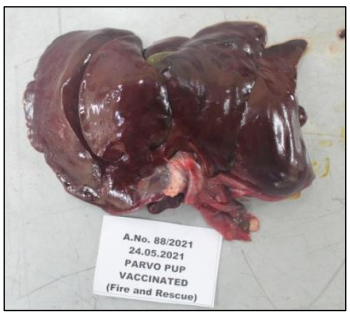

Fig 8: Liver showing slightly enlarged and congestion

Fig 9: Kidneys showing congestion and enlarged

Histopathological changes in organs

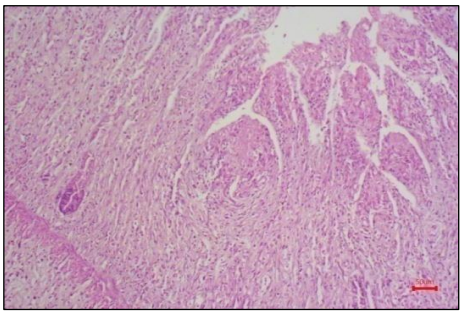

Fig 10: Jejunum-Degeneration and necrosis of the intestinal villi with mononuclear cells infiltration and shortened villi H&E Scale bar x 50µm

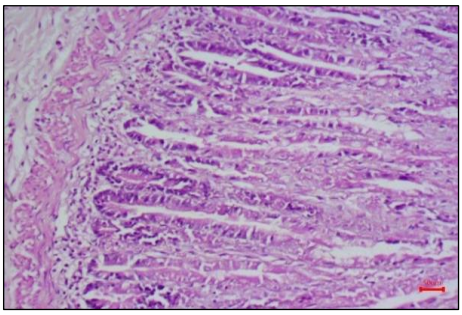

Fig 11: Jejunum showing mononuclear cells infiltration in the lamina propria. H&E Scale bar x 50 µm

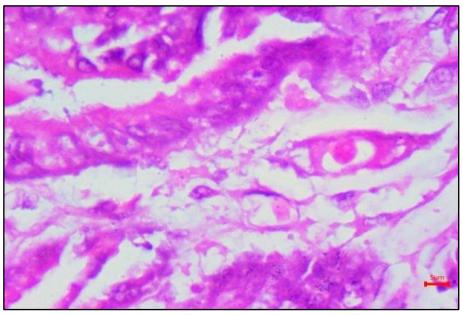

Fig 12: Jejunum villi showing eosinophilic intranuclear inclusion in the enterocytes, H&E Scale bar x 5µm

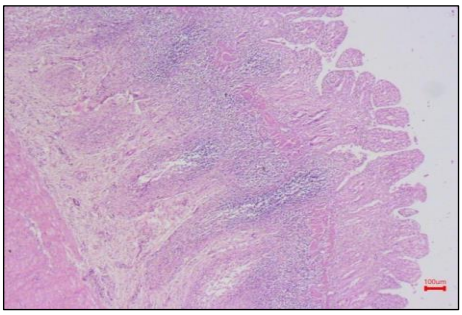

Fig 13: Ileum showing lymphoid cell depletion and fusion of villi, H&E Scale bar x 100 µm

Immunohistochemical localisation of parvoviral antigen in tissues from Doberman with parvoviral infection

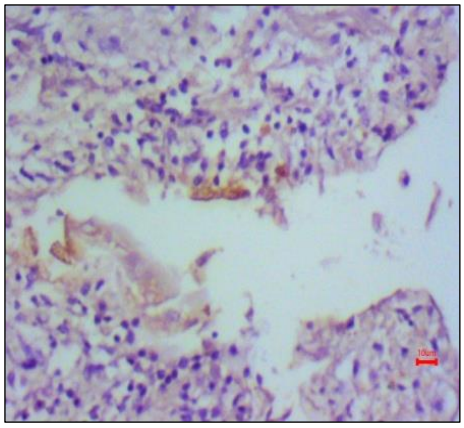

Fig 14: Jejunum showing brown positive immunohistochemical reaction in the villi epithelial cells. Immunohistochemistry method for canine parvovirus Scale barx 10 µm.

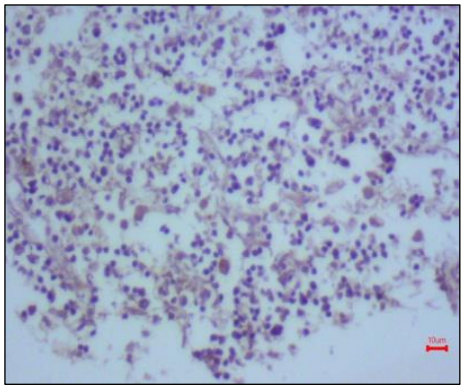

Fig 15: MLN showing brown positive immunohistochemical reaction in the lymphocytes. Immunohistochemistry method for canine parvovirus Scale barx 10 µm.

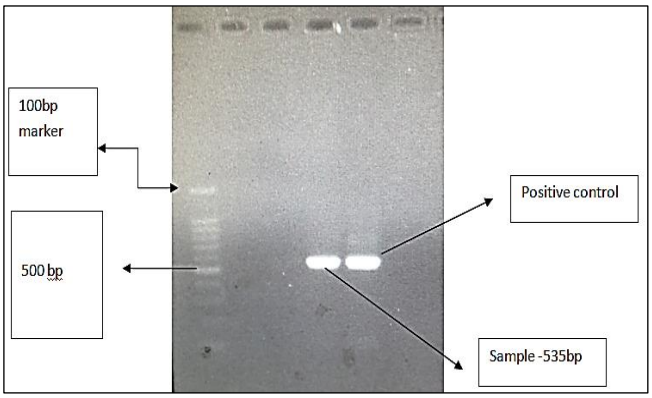

Fig 16: VP2 gene amplicon of CPV isolates from Doberman faecal sample

Discussion

Canine parvovirus causes a highly contagious and fatal disease, developing into acute haemorrhagic enteritis and myocarditis, in dogs (Miranda and Thompson 2016) [17]. In puppies, death occurs due to severe dehydration, hypovolemia from marked gastrointestinal fluid and protein loss and sepsis from bacterial translocation and leukopenia. All these were observed in present case. Recent canine parvovirus showing high virulence in puppies with irrespective of the breed; however, it seems younger puppies succumb to the infection more easily considering the incidence in this report and findings of Ezeokoli *et al.* (1985) [8] and Fagbohun and Omobowale (2018) [9].

Conventional PCR is presented as a good alternative of choice because of its high sensitivity and high specificity, compared to traditional methods used clinically. PCR result in the present case is in accordance with Cavalli *et al.* (2001) [3].

Steinel *et al.* (2001) [22] reviewed infections of the different antigenic types of CPV-2a and-2b. The tropism of the virus often determines the pathological manifestation. CPV- 2a is capable of causing myocarditis depending upon timing of infection. Infection in utero or in first few weeks of life allows the virus to attack most rapidly dividing cells in the body including cardiac myocytes (Prittie and Barton 2004 [20]; Sime *et al.* 2015) [21]. The virus also infects the intestinal epithelium, resulting in crypt necrosis, crypt dilatation and villous atrophy, which is diagnostic of the disease (Cooper *et al.* 1979) [5]. The most common enteric form of CPV targets the lymphoid tissue of the oropharynx, the mesenteric lymph nodes and/or the thymus as its initial site of replication. The virus then disseminates through the body hematogenously and ultimately destroys intestinal villi (Goddard *et al.* 2010) [12]. All the finding are in accordance with present case and observed in Doberman pup.

The presence of the viral antigens revealed by immunohistochemistry in the small intestines (jejunum and ileum) due to the destruction of cells in which viral agents were heavily present before the development of clinical signs. Jejunal villi revealed intranuclear inclusion and confirmed the presence of viral antigen. The immunohistochemistry result correlate with Macartney and McCartney (1986) [16]. Vaccination is considered to have a significant impact on CPV. Vaccinated animals were probably less infected, as reported in a previous study (Hasan *et al.*, 2017) [13]. However, a considerable number of cases were found where dogs were infected by CPV, despite a history of vaccination(Glickman *et al.* 1985) [11] and El-Neshwy *et al.* 2019) [7]. As the vaccine variants do not cover all field variants, it may act as a determining factor. Furthermore, different veterinary practitioners use distinct vaccination protocols. Several causes of vaccination failure are known, such as not maintaining cold chain, maternal antibody, worm load, poor nutritional status, lack of protective antibody titers against heterologous CPV antigenic types, and faulty vaccination (Glickman *et al.* 1985) [11] (Freisl *et al.* 2017) [10] (Altman *et al.* 2017) [1] (Kapiya *et al.* 2019) [15]. Some researchers suggest that the CPV2b vaccine provides cross-protection against CPV2a and CPV2c, but a more intensive study is needed to confirm this possibility (Odueko *et al.* 2020) [17] (Altman *et al.* 2017) [1] (Wilson *et al.* 2014) [24] (Opriessnig *et al.* 2020) [19]. In the present case, the Doberman pup was vaccinated against canine parvo viral enteritis, but pup was infected with virulent canine parvovirus and died. Above finding correlated with present case.

Conclusions

The results of the present study revealed severity of the canine parvoviral enteritis in Dobermann pup even with proper vaccination protocol. Histopathological and immunohistochemical result were confirmed canine parvoviral enteritis in Dobermann pup. Vaccination and maternal antibody level are very important to prevent canine parvoviral infection in pups. Identification of present variant of parvovirus and accordingly vaccination protocol should be

followed to control parvoviral infection.

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Reference

1. Altman KD, Kelman M, Ward MP. Are vaccine strain, type or administration protocol risk factors for canine parvovirus vaccine failure? Vet. Microbiol 2017;210:8-16.
2. Bancroft JD, Gamble M. Theory and Practice of Histological Techniques. 6th 283 ed. Churchill Livingstone, Elsevier Health Sciences, 2008, 1-725
3. Cavalli A, Bozzo G, Decaro N, Tinelli A, Aliberti A, *et al*. Characterization of a canine parvovirus strain isolated from an adult dog. New Microbiol 2001;24(3):239-242.
4. Chen C, Guo X, Liang H, Ning B, Li J, Zhong S *et al*. Determination of parvovirus antibodies in canine serum using magnetic bead-based chemiluminescence immunoassay. Biotechnol Appl Biochem 2019;66:586-590.
5. Cooper BJ, Carmichael LE, Appel MJ, Greisen H. Canine viral enteritis. II. Morphologic lesions in naturally occurring parvovirus infection. Cornell Vet 1979;69:134-144.
6. Cotmore SF, Agbandje-McKenna M, Chiorini JA, Mukha DV, Pintel DJ, Qiu J, *et al*. The family Parvoviridae. Arch Virol 2014;159(5):1239-1247.
7. El-Neshwy WM, El-Zahar H, Morsi AM, Shety T. Molecular and phylogenetic analysis of Canine parvovirus variants (2a-2b-2c) in Egypt. Res J Vet. Pract 2019;7(3):74-82.
8. Ezeokoli CD, Umoh J, Adeyanju JB, Abdullahi SU. Parvovirus enteritis in Nigerian dogs. J Small Anim. Pract 1985;26(11):669-673.
9. Fagbohun OA, Omobowale TO. Sequence and phylogenetic analysis of canine parvovirus-2 isolates in dogs revealed circulation of three subtypes in Nigeria. Virus disease 2018;29(3):411-415.
10. Freisl M, Speck S, Truyen U, Reese S, Proksch AL, Hartmann K. Faecal shedding of Canine parvovirus after modified-live vaccination in healthy adult dogs. Vet. J 2017;219:15-21.
11. Glickman LT, Domanski LM, Patronek GJ *et al*. Breed-related risk factors for canine parvovirus enteritis. J Am Vet Med Assoc 1985;187:589-594.
12. Goddard A, Leisewitz AL. Canine parvovirus. Vet. Clin. North Am. Small Anim. Pract 2010;40:1041-1053.
13. Hasan MM, Jalal MS, Bayzid M, Sharif MAM, Masuduzzaman M. A comparative study on Canine parvovirus infection of dog in Bangladesh and India. Bangladesh J Vet. Med 2017;14(2):237-241.
14. Houston DM, Ribble CS, Head LL. Risk factors associated with parvovirus enteritis in dogs: 283 cases (1982-1991). J Am Vet Med Assoc, 1996, 542-546.
15. Kapiya J, Nalubamba KS, Kaimoyo E, Changula K, Chidumayo N, Saasa N, *et al*. First genetic detection and characterization of Canine parvovirus from diarrheic dogs in Zambia. Arch. Virol 2019;164(1):303-307.
16. Macartney L, Macartney CM. Canine parvovirus: Development of immunofluorescence and immunoperoxidase techniques. Res. Vet. Sci 1986;40:201-208.
17. Miranda C, Thompson G. Canine parvovirus: The worldwide occurrence of antigenic variants. J Gen Virol 2016;97(9):2043-2057.
18. Odueko FD. Literature review on canine parvoviral enteritis variants in Nigeria. J Dairy Vet. Anim. Res 2020;9(1):26-32.
19. Opriessnig T, Karuppannan AK, Halbur PG, Calvert JG, Nitzel GP, Matzinger SR *et al*. Porcine circovirus type 2a or 2b based experimental vaccines provide protection against PCV2d/porcine parvovirus 2 co-challenge. Vaccine 2020;38(8):1975-1981.
20. Prittie J, Barton. Canine parvoviral enteritis: A review of diagnosis, management and prevention. J Vet. Emerg. Crit. Care 2004;14:167-176.
21. Sime TA, Powell LL, Schildt JC, Olson EJ. Parvoviral myocarditis in a 5-week-old Dachshund. J Vet. Emerg. Crit. Care 2015;25:765-769.
22. Steinel A, Parrish CR, Bloom ME, Truyen T. Parvoviurs infection in wild carnivores. J Wild life Disease 2001;37(3):594-607.
23. Voorhees IEH, Lee H, Allison AB, Lopez-Astacio R, Goodman LB, Oyesola OO, *et al*. Limited intra-host diversity and background evolution accompany 40 years of canine parvovirus host adaptation and spread. J Virol, 2019, 2.
24. Wilson S, Illambas J, Siedek E, Stirling C, Thomas A, Plevová E, *et al*. Vaccination of dogs with Canine parvovirus type 2b (CPV-2b) induces neutralizing antibody responses to CPV-2a and CPV-2c. Vaccine 2014;32(42):5420-5424.