RESEARCH ARTICLE

Molecular Characterisation *of E. coli* Isolated from Faecal Samples of Pups Affected with Canine Paroviral Enteritis

Shubhangi Warke*, Sunny Magar

Abstract

Canine parvovirus (CPV) is one of the causative agents of enteritis and myocarditis in young puppies. In the present study 100 faecal samples collected from dogs showing clinical sign of gastroenteritis were screened for detection of CPV antigen using Ubioquick VET kit. Among these, 30 (30%) samples were found positive. These samples were further subjected to detailed bacteriological examination, which revealed 22 (73.33%) samples positive for secondary bacterial infection, *E. coli* being predominant. Out of 22 dogs positive for secondary bacterial infection, 14 (63.63%) dogs were infected with CPV + *E. coli*, 5 (22.72%) with CPV + *Salmonella* spp., and 3 (13.63 %) with CPV + *Klebsiella* spp. The remaining 8 dogs had solo-infection with CPV. The predominant bacteria (*E. coli*) were further subjected for detection of *stx*1, *stx*2 and *eae* gene by PCR. The *stx*2 gene amplicon of 584 bp was produced by all the 14 strains, while *stx*1 was not detected in any of the isolates, and 3 (21.42 %) samples were found positive for *eae* gene with the amplicon of 877 bp. *In vitro* antibiogram of the isolates associated with CPV infection revealed a maximum sensitivity towards ceftriaxone-tazobactam and ciprofloxacin (100% each), followed by ceftriaxone and cefotaxime (55.55% each), amikacin (44.44%), furazolidone and amoxicillin-sulbactam (22.22% each), and 100% resistace to metronidazole.

Key words: AST, Canine parvovirus, *E. coli*, PCR, Secondary bacterial infection.

Ind J Vet Sci and Biotech (2024): 10.48165/ijvsbt.20.1.05

Introduction

Canine parvovirus 2 is the causal agent of acute haemorrhagic diarrhoea and myocarditis in dogs. It is extremely contagious and has a high fatality rate (Nandi *et al.*, 2010). Canine parvovirus infection is manifested by two clinical forms: enteritis with emesis (Woods *et al.,* 1980) and myocarditis in dogs below 3 months of age. The incidence of CPV-2 variants in the dog was reported from various states. The incidence of CPV-2a was reported in India in 2001 (Narayanan *et al.,* 2001). The prevalence of CPV-2c was first reported in India in 2010 based on sequence analysis of CPV-2b positive samples (Nandi *et al.*, 2010). The virus spreads by direct contact with infected dogs to susceptible dogs and contact with contaminated stool, environments or people. The virus is sturdy and can last in faeces-contaminated soil for 5 months or more in favourable conditions. The virus can be infected with kennel premises, food and water bowls, neck collars and the hands of person who handle leashes of infected dogs (Reddy *et al.*, 2015). Severe infection is mostly seen in dogs between 6 weeks to 4 months old. Factors that predispose puppies to parvoviral infection include lack of protective immunity, intestinal parasites, over-crowding, unhygienic and stressful environmental conditions.

Canine infectious diarrhoea has been a question for veterinarians because of its pathogenic uncertainty and the concurrent company of viral, parasitic, bacterial and protozoan co-infections (Gizzi *et al,* 2014). Some pathogens last on the mucosal surface and cause potent enterotoxins which can disrupt the fluid flux, others pierce and replicate within intact epithelial cells producing inflammatory damage (Greene and Marks, 2012). Many dogs lodge dormant pathogens without any clinical signs. Intestinal tract damage is seen in Canine parvovirus-2 infection which increases the chance of bacterial alteration leading to coliform septicaemia and the development of systemic inflammatory response syndrome (SIRS) that can progress to septic shock pattern. *In vitro* antibiotic sensitivity test from CPV positive cases is helpful for the physician to initiate the accurate antibiotic for CPV clinical cases associated with secondary bacterial infection. Hence the present study was aimed to determine the bacterial infection in faecal samples of pups affected with canine parvoviral enteritis and carry out detailed

Department of Veterinary Microbiology and Animal Biotechnology, T & R Cell, Nagpur Veterinary College, Maharashtra Animal & Fishery Sciences University, Nagpur-440006, India

Corresponding Author: Dr. Shubhangi Warke, Department of Veterinary Microbiology and Animal Biotechnology, T & R Cell, Nagpur Veterinary College, Maharashtra Animal & Fishery Sciences University, Nagpur-440006, India, E-mail: shubhangi_warke@rediffmail.com

How to cite this article: Warke, S., & Magar, S. (2024). Molecular Characterisation of *E. coli* Isolated from Faecal Samples of Pups Affected with Canine Parvoviral Enteritis. Ind J Vet Sci and Biotech. 20(1), 22-26.

Source of support: Nil

Conflict of interest: None

Submitted 26/10/2023 **Accepted** 25/11/2023 **Published** 10/01/2024

© The Author(s). 2023 Open Access This work is licensed under a Creative Commons Attribution-Non Commercial-No Derivatives 4.0 International License.

molecular characterisation of predominant bacteria and their antibiogram.

Materials and Methods

In the present study, a total of 100 faecal samples in duplicate were collected with sterile swabs from pups presented with symptoms suggestive of Canine parvo viral enteritis (vomiting, pyrexia, bloody foul smelling, diarrhoea etc) and subjected to immunochromatography assay (Ubioquick^{vet} Canine parvovirus antigen rapid test kit) as per Soliman *et al.* (2018).

Isolation and Identification of Bacteria

Faecal samples 30 from CPV positive pups were inoculated simultaneously into nutrient broth and selenite broth, and incubated at 37°C for 24 h aerobically, then a loopful of bacterial culture was streaked on blood agar plates. Based on morphology and from Gram's staining, cultures were inoculated into specific or selective media like deoxycholate citrate agar plates, MacConkey agar, Eosin-methylene blue agar. Primary identification of bacteria was done based on colony morphology, type of haemolysis, Gram's staining, biochemical characteristics, and pure cultures were identified up to genus level as per Cowan and Steel (1993) and Cruickshank *et al*. (1975).

Genotypic Characterization

Biochemically confirmed predominant bacterial isolates were subjected to PCR method using published primers described by Cebula *et al*. (1995) and Oswald *et al.* (2000) with suitable modifications. The genomic DNA was extracted as per DNA extraction kit (Promega kit, USA) as per manufacturer instructions. The oligonucleotide primers for detection of the virulence genes *stx1, stx2* and *eae* (Table 1) used in this study were manufactured from IDT. For PCR amplification, each 25 µL reaction mixture for *stx*1 and *stx*2 gene contained 12.5 µL master mix, 1 µL each forward and reverse primer (20 uM), 7.5 µL nuclease free water and 3 µL template. The reaction conditions were: initial denaturation for 5 min at 94 °C, 35 cycles of 1 min at 94 °C (denaturation), 1 min at 54 °C (annealing) and 2 min at 72 °C (extension) and final extension step of 10 min at 72 °C. For *eae* gene PCR amplification, each 25 µL reaction mixture contained 12.5 µL master mix, 2 µL each forward and reverse primer (20 uM), 6.5 µL nuclease free water and 2 µL template. The reaction conditions were: initial denaturation for 5 min at 94 °C, 30 cycles of 1 min at 94 °C (denaturation), 30 sec at 57 °C (annealing) and 1 min at 72 °C (extension) and final extension step of 10 min at 72 °C. The amplified PCR products were run at 1% agarose gel. *E coli* slandered culture was used as positive control.

Antibiotic Sensitivity Test

The antibiotic sensitivity test was performed on the faecal whole culture from nutrient broth by disc diffusion technique (CLSI, 2013) on Mueller Hinton Agar (MHA) plates. The antibiotics used in present study were amikacin, amoxycillin-sulbactam, cefotaxime, ceftriaxone, ceftriaxone-tazobactam, ciprofloxacin, furazolidone and metronidazole. The sensitivity pattern was read by measuring the diameter of Zone of inhibition corresponding to different antibiotics in millimetre as per the interpretation chart provided by the manufacturer.

Results and Discussion

Isolation and Identification of Bacteria

Out of 100 faecal samples of pups screened, thirty samples were found to be positive for CPV infection with lateral flow immunochromatography assay. Earlier in many studies immunochromatoghaphy based test kits were employed for detection of parvovirus in faecal samples of dogs. Out of many available tests, immunochromatography assay (ICA) is on summit in the rapid detection of CPV-2 cases and also give high sensitivity at low cost and can be performed by any veterinarians as well as by pet owners (Esfandiari and Klingeborn, 2000).

In the present study, culturing of faecal samples from 30 CPV affected dogs revealed the presence of Gram-negative bacteria such as *E coli, Salmonella* spp., *Klebsiella* spp. in different combinations along with CPV infection (Table 2). Cultural and biochemical characteristics of different species of bacteria isolated from faecal samples are depicted in Fig. 1. Invasion of secondary bacteria in the present study might be due to leukopenia and compromise of immune system. CPV causes necrosis of gut associated lymphoid tissue resulting diminished local immunity to infectious agents and predisposing to bacterial infection, as suggested by Turk *et al.* (1992). Similarly secondary bacterial infection with *E coli* (Isogai *et al*., 1989, Reddy, 2013), and *Salmonella* spp., *Klebsiella* spp. (Reddy, 2013) was reported from isolation studies on faecal samples of dogs with parvoviral enteritis. These results were

Table 1: Details of primers specific for *E. coli* used in PCR

Gene	Primer name	Sequence	Product size (bp)	Reference
stx1	LP30	CAGTTAATGTGGTGGCGAAGG	348 bp	Cebula *et al*. (1995)
	LP31	CACCAGACAATGTAACCGCTG		
stx2	LP43	ATCCTATTCCCGGGAGTTTACG	584 bp	Cebula *et al*. (1995)
	LP44	GCGTCATCGTATACACACAGGAGE		
eae	Intimin	CCCGAATTCGGCACAAGCATAAGC	877 bp	Oswald *et al.* (2000)
		GCTTATGACCGCTCTGCCTAGGCCC		

also found to be closely related to observations made by Bhargavi *et al.* (2017), who concluded that commonest bacteria involved along with CPV infection were of Gram negative species (*E. coli*, *Salmonella* spp. and *Klebsiella* spp.). *E coli, Salmonella* spp. *Campylobacter* spp. and *Clostridium perfringens* can also be associated with diarrhea in canines, and they vary between studies (Marks *et al.*, 2011). *E. coli* is a normal resident of the intestinal tract of humans and animals with a capacity to result from mild to severe pathological conditions and it is considering as major cause of diarrhea in dogs. *Kebsiella pnemoniae*, *E. coli* and *Enterobacter* spp. were also isolated from bacteriological culture of swabs obtained from intravenous catheters placed to parvoviral infected dogs (Lobetti *et al.*, 2002; Greene and Decaro, 2012). Nivy *et al.* (2011) isolated an unusually resistant *E coli* strain from the cerebrospinal fluid of a CPV affected dogs and stated that dogs with CPV infection have an increased risk of secondary bacterial infections due to neutropenia and immunosuppression.

Table 2: Mixed infection of CPV with other bacterial species

Types of isolates	No. positive	%
CPV + *E. coli*	14	63.63
CPV + *Salmonella* spp.	05	22.72
CPV + *Klebsiella* spp.	03	13.63

Extraction of DNA and Molecular Characterization of *E. coli* Isolates

The DNA was extracted from *E coli* isolates (14) by using DNA extraction kit (Promega kit, USA) as per manufacturer's instruction and screened for the presence of *stx*1, *stx*2 and *eae* genes. The *stx*2 gene with amplicon of 584 bp was produced by all strains (Fig. 2), while *stx*1 was not detected in any of the isolates. 3 (21.42 %) samples were found positive for *eae* gene with the amplicon size of 877 bp (Fig. 3). The results of the present study were somewhat in contradiction with that conducted by Hasan *et al.* (2016), who got highest percentage of *stx*1 in CPV associated cases.

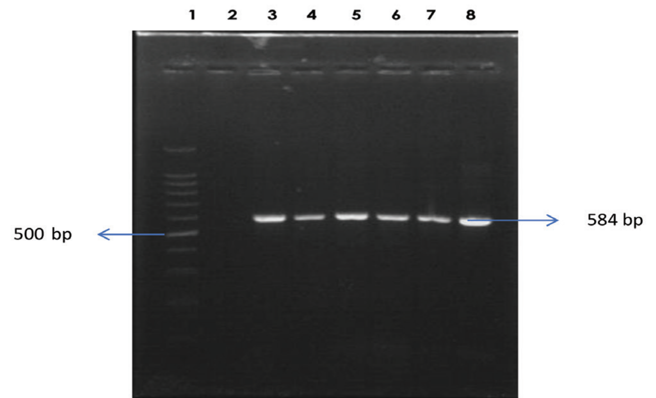
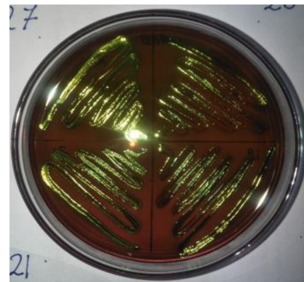


Fig. 2: Amplification of *stx*2 gene of *E.coli* by PC: Lane M: Ladder 100 bp. Lane 1: Blank, Lane 2: *E. coli* reference strain *stx*2 (584 bp), Lane 3: Sample no.2, Lane 4: Sample no.13, Lane 5: Sample no.26, Lane 60: Sample no.39, Lane 7: Sample no.42

In vitro Antibiogram

In vitro antibiogram of the isolates associated with CPV infection (Table 3) revealed a maximum sensitivity towards

MacConkey agar



EMB agar

DCA agar

Fig. 1: Cultural characteristics of different spp. of bacteria isolated from faecal samples of affected dogs

Table 3: Details of the antibiogram pattern of bacterial isolates associated with CPV positive cases (n=22)

Sr. No.	Name of Antibiotic	Symbol of Antibiotic	No. of isolates sensitive	Percentage sensitivity
1	Amikacin	AK	4	44.44%
2	Amoxycillin-Sulbactam	AMS	2	22.22%
3	Cefotaxime	CTX	5	55.55%
4	Ceftriaxone	CTR	5	55.55%
5	Ceftriaxone- Tazobactam	CIT	22	100%
6	Ciprofloxacin	CIP	22	100%
7	Furazolidone	FR	2	22.22%
8	Metronidazole	MT	0	0%

ceftriaxone-tazobactam and ciprofloxacin (100% each), followed by ceftriaxone and cefotaxime (55.55% each), amikacin (44.44%), furazolidone and amoxicillin-sulbactam (22.22% each), and 100% resistance to metronidazole.

In the present study, amikacin, an aminoglycoside showed 44.44 % sensitivity. Most of the Gram-positive aerobes are less sensitive (Maddison *et al.,* 2008). Earlier, Dutta *et al.* (2008) reported 72.22 % of sensitivity to amikacin for *Salmonella* spp. isolated from diarrhoeic dogs. The *in vitro* drug sensitivity of both ceftriaxone and cefotaxime was found to be 55.55 % in the present study. Both included under third generation cephalosporin group had decreased Gram positive, but increased Gram-negative activity (Susceptible Gram-negative bacteria include *Escherichia coli, Klebsiella* spp., *Salmonella* spp. as stated by Maddison *et al.,* 2008). But the less sensitivity in the present study could be due to the involvement of Gram-negative spp. against which the sensitivity is variable (Maddison *et al*., 2008). Earlier Dutta *et al.* (2007) reported 55.56 % sensitivity of Salmonella isolates from diarrhoeic dogs to cefotaxime, while in another study cefotaxime exhibited 85.03 % sensitivity to bacteria isolated from diarrhoeic dogs (Singh, 2008). Emergence of many resistant strains of Gram-negative bacteria and increasing prevalence of *Klebsiella pneumoniae* and *Enterobacter* spp. as pathogens might be responsible for decreased spectrum of amoxicillin as they are intrinsically non-susceptible to aminopenicillins (Maddison *et al.,* 2008).

Metronidazole exhibited 100 % resistance, which might be attributed to the frequent and indiscriminate use of this antimicrobial agent. Previously, Sharma (2003) and Dutta *et al.* (2007) reported 100 % resistance of bacteria isolated from diarrhoeic dogs to metronidazole.

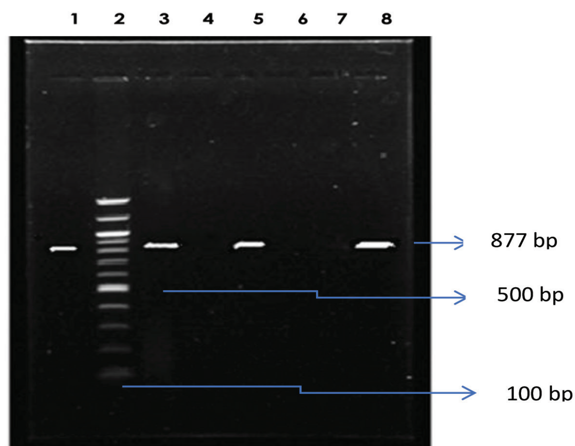


Fig. 3: Amplification of *eae* gene of *E. coli* by PCR: Lane 1: *E. coli* reference strain *eae* (877 bp), Lane M: Ladder 100bp, Lane 3: Sample no. 17, Lane 5: Sample no. 25, Lane 8: Sample no. 29, Lane 4,6 & 7: Blank

Conclusion

From the findings of present study, it was concluded that the most common bacteria involved in Canine parvoviral infection were of Gram-negative species (*E. coli*, *Salmonella* spp., and *Klebsiella* spp). Antibiogram studies of the isolates associated with CPV infection revealed a maximum sensitivity towards ceftriaxone-tazobactam and ciprofloxacin (100% each), followed by ceftriaxone and cefotaxime (55.55%), amikacin (44.44%), furazolidone and amoxicillin-sulbactam (22.22%). and 100% resistance to metronidazole.

Acknowledgments

Authors are thankful to the Associate Dean, Nagpur Veterinary College, Nagpur for the facilities and cooperation extended for this work.

References

Bhargavi, M., Shobhamani, B., Kumari, N. K., & Srilatha, C.H. (2017). Antibiotic sensitivity pattern of different bacterial species isolated from faecal samples of pups affected with canine parvoviral enteritis. *International Journal of Current Microbiology and Applied Sciences, 6*(10), 2875-2882.

Cebula, T.A., Payne, W.L., & Feng, P. (1995). Simultaneous identification of strains of *Escherichia coli* serotype O157: H7 and their Shiga-like toxin type by mismatch amplification mutation assay- multiplex PCR. *Journal of Clinical Microbiology, 33,* 248-250.

CLSI, Clinical and Laboratory Standards Institute (2013). Approved Standards: Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved standard. 11th edn., Clinical and Laboratory Standards Institute, Wayne, PA.

Cowan, S.T., & Steel, K.J. (1993). *Manual for the identification of medical bacteria*. The syndics of the Cambridge University Press. Bentlay House, 200 Euston Road, London, UK.

Cruickshank, R., Duguid, J.P., Marmoin, B.P., & Swain, R.H.A. (1975). *Medical Microbiology.* Vol. 2, 12th edn., Churchill Livingstone, Edinburgh, London and New York.

Dutta, P.R., Phukan, A., Borah, P. & Hazarika, G.C. (2007). *In vitro* drug sensitivity pattern of salmonella isolated from diarrhoeic dogs. *Indian Journal of Veterinary Medicine, 27*(2), 147-148.

Dutta, P.R., Phukan, A., Borah, P., & Hazarika, G.C. (2008). Pathogenicity and *In vitro* drug sensitivity pattern of salmonella isolated from diarrhoeic dogs. *Indian Veterinary Journal, 85*(11), 1223-1224.

Esfandiari, J., & Klingeborn, B. (2000). A comparative study of new rapid and one-step test for the detection of parvovirus in feaces from dogs, cats, and mink. *Zoonosis and Public Health, 47*(2), 145-153.

Gizzi Aline Baumann da Rocha, Simone Tostes Oliveira, Christian M Leutenegger, Marko Estrada, Denise Adamczyk Kozemjakin, Rafael Stedile, Mary Marcondes, & Alexander Welker Biondo (2014). Presence of infectious agents and co-infections in diarrheic dogs determined with a real-time polymerase chain reaction-based panel. *BMC Veterinary Research, 10,* 23

Greene, C.E., & Marks, S.L. (2012). Gastrointestinal and intra-abdominal infection of dogs and cats. *Infectious Diseases, 4,* 950-981.

Greene, C.E., & Decaro, N. (2012). Canine viral enteritis. In: Greene C.E, *Infectious Diseases of the Dogs and Cats.* 4th edn., W.B. Saunders, Elsevier. 67-74.

Hasan, M.S., Yousif, A.A., & Alwan, M.J. (2016). Detection of virulent genes in *E. coli* O157:H7 isolated from puppies and adult dogs by polymerase chain reaction. *Research Journal of Veterinary Practitioners, 4*(1), 1-6.

Isogai, E., Isogal, H., Onuma, M., Mizukoshi, N., Hayashi, M., & Namioka, S. (1989). *Escherichia coli* associated endotoxemia in dogs with parvovirus infection. *Nihon Juigaku Zasshi, 51(*3), 597-606.

Lobetti, R.G., Joubert, K.E., Picard, J., Castens, J., & Pretorius, E. (2002). Bacterial colonisation of intravenous catheters in young dogs suspected to have parvo viral enteritis. *Journal of American Veterinary Medical Association, 220*(9), 1321-1324.

Maddison, L.E., David, A., Watson, J., & Elliott, J. (2008). In: Maddison J.E, Page S.W. and Church D.B. 2008. *Small Animal Clinical Pharmacology*, Second edition, W.B. Saunders, Philadelphia, p. 148-185.

Marks, S.L., Rankin, S.C., Byrne, B.A., & Weese, J.S. (2011). Enteropathogenic bacteria in dogs and cats: Diagnosis, epidemiology, treatment and control. *Journal of Veterinary Internal Medicine, 25*, 1195-1208.

Nandi, S., Chidri, S., Kumar, M., & Chauhan, R.S. (2010). Occurrence of canine parvovirus type-2c in the dogs with haemorrhagic enteritis in India. *Research in Veterinary Science, 88,* 169-171.

Narayanan, S.A., Kumar, P.K., Chug, S., Yadav, R., & Sharma, M.L (2001). Strain characterization of Indian isolates of Canine parvo virus by PCR-RFLP. *Remount Veterinary Corp, 40,* 67-74.

Nivy, R., Hahn, S., Perl, S., Karnieli, A., Karnieli, O., & Aroch, I. (2011). A fatal outbreak of parvovirus infection. *Israel Journal of Veterinary Medicine, 40,* 459-467.

Oswald, E., Schmidt, H., Morabito, S., Karch, H., Marches, O., & Caprioli, A. (2000). Typing of intimin genes in human and animal enterohemorrhagic and enteropathogenic *Escherichia coli*: Characterization of a new intimin variant. *Infectious Immunology, 68,* 64-71.

Reddy, B.K. (2013). Clinico-epidemiological studies on canine parvo viral infection in and around Tirupati. *M.V.Sc. thesis.* Sri Venkateswara Veterinary University, Tirupati. India.

Reddy, B.K., Shobhamani, B., Sreedevi, B., Prameela, D.R., & Reddy, B.R. (2015). Prevalence of *Canine parvo* viral infection in dogs in and around Tirupathi of India. *International Journal of Livestock Research, 5*(3), 93-98.

Sharma, A. (2003), Clinical and therapeutic studies on canine gastroenteritis. *M.V.Sc, Thesis*.. CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, India.

Singh (2008). Studies of etiological agents of canine diarrhoea with special reference to Canine parvovirus. *M.V.Sc*. *Thesis*. CSK Himachal Pradesh krishi Vishvavidyalaya, Palampur, India.

Soliman, R.M., Baker, N.M., Nasr, M.Y., & Khodeir, M.H. (2018). Clinical, virological and molecular characterization of *canine parvo virus* in dogs. *Journal of Applied Veterinary Science, 5*(4), 525-535.

Turk, J., Fales, W., Miller, M., Pace, L., Fischer, J., Johnson, G., Kreeger, J., Turnquist, S., Pittman, L., Rottinghaus, A., & Gosser, H. (1992). Enteric *Clostridium perfringens* infection associated with parvoviral enteritis in dogs: 74 cases (1987-1990). *Journal of the American Veterinary Medical Association, 200*(7), 991-994.

Woods, C.B., Pollock, R.V.H., & Carmichael, L.E. (1980). Canine parvo viral enteritis. *Journal of the American Animal Hospital Association, 16,* 171-179.