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Faecal Microbiota Transplantation in Canine Parvoviral Diarrhoea

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ABSTRACT

Background: Faecal microbiota transplantation (FMT) is a novel therapy in the field of gastroenterology which has been studied at current times with greater curiosity.

Methods: FMT was applied as an adjunct to symptomatic treatment in Canine Parvovirus (CPV) affected dogs for a period of 8 days once daily from the day of presentation. The results were compared with a similar group suffering from CPV infection maintained only on symptomatic treatment.

Result: Response to FMT was evaluated on the basis of time taken for resolution of diarrhoea, time of hospitalization, scoring of clinical parameters, recurrence of gastroenteritis within 2 months post therapy of the two groups. Furthermore, adverse events and retention time of the transplant after the procedure were noted. It was observed that the 66.67% (4/6) dogs of FMT treated group had a quicker resolution of diarrhoea (mean 48 hours); FMT treated dogs had better clinical scores compared to symptomatic therapy group (score of 1 or clinically insignificant vs 4 or mildly diseased), lesser recurrence of diarrhoea 2 months post therapy in FMT treated group (16.67% vs 50%). Post FMT one case showed low grade fever (n=1) and epistaxis (n=1) respectively which resolved within 24 hours. The mean retention time of the transplant improved from (20.83±2.39) minutes to (140±12.66) on Day 7th of the trial *(p<0.01)*. Faecal Microbiota Transplantation as an adjunct therapy might aid in early resolution of diarrhoea in acute diarrhoea in dogs.

Key words: Canine, Diarrhoea, FMT, Gastroenterology, IBD, Parvovirus, Veterinary.

INTRODUCTION

The gastrointestinal tract's resident bacteria, viruses and eukaryotic organisms; all of which having symbiotic relationships with their hosts-make up the gut microbiome. The majority of genomic sequences of these bacteria detected in the dog's gastrointestinal (GI) tract using molecular methods belong to the following five phyla: Fusobacteria, Bacteroidetes, Firmicutes, Actinobacteria and Proteobacteria (Mortier *et al.,* 2015). The interactions of somatic cells, the host and gut bacteria regulates several processes. These include the provision of nutrients for the host through metabolic and fermentative processes, the synthesis of vitamins (especially B complex and K), the biotransformation of bile acids, the metabolism of xenobiotics, the proper maturation of gastrointestinal cells, defence against non-resident intestinal pathogens and provision for the development of a healthy thriving epithelium along with the immune system (Mondo *et al.,* 2019); (Schwan *et al.,* 1983) (Blake and Suchodolski, 2016). A dysbiosis of the gut microbiota is associated with changes in the constitution, location and bioactivity of bacteria as well as their interactions with the environment and host genes, among other internal and external factors (Simpson *et al.,* 2002). To restore the state of dysbiosis and restore a healthy microflora, several therapies have been applied which includes antibiotics, probiotics, prebiotics and dietary changes but their efficiency is not clear. In todays' time human and veterinary medicine both extensively practices the use of pre- and probiotics to restore the gut health (Pilla and Suchodolski, 2020). Faecal microbial transplantation (FMT) is a lesser explored procedure which involves introducing a homogenised liquid suspension of faeces from a healthy donor into the patient's gastrointestinal (GI) tract. The first published use of FMT was described in a case series of four patients successfully treated against pseudomembranous enterocolitis followed by another investigating its specific use against Clostridium difficile infection (Eiseman *et al.,* 1958) (Saari *et al.,* 2015). In Dogs, Canine Parvoviral enteritis in dogs have been linked to major disturbances or dysbiosis in the gut microflora that in turn impairs enterocyte nutrition, immunological modulation, gastrointestinal motility and protective barrier function (Kilian *et al.,* 2018). In veterinary practice a study conducted in beagle dogs naturally infected with canine parvovirus (CPV) observed decreased overall diversity indices of gut microflora with significant variations in the microbial community structure and membership signifying that the gut microbiota differs

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between healthy dogs and dogs affected with CPV (Mortier *et al.,* 2015). While it's unclear how FMT eliminates clinical symptoms in dogs suffering from diarrhoea caused by viruses, however, Pereira *et al.,* (2018) hypothesized that the response to the therapy is likely as a result of restoration and engrafting of the intestinal microflora with their corresponding metabolites. The objective of this study was to evaluate the efficacy of FMT as Gastro-intestinal microbiome restorative therapy in conjunction with symptomatic therapy in parvoviral cases.

MATERIALS AND METHODS

Study design

The study conducted was a randomised, longitudinal, prospective study in which a total of 6 CPV infected dogs were treated with FMT as an adjunct therapy during the study period.

Place of study and ethical approval

The study was conducted in the Department of Veterinary Medicine and Dr. I.P. Singh Veterinary Clinical Complex and Trauma Centre, C.V.A.Sc., G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand between October 2021 and March 2022 for a period of 6 months. This study was approved and conducted according to the ethics of Institutional Animal Ethics Committee (IAEC) of College of Veterinary and Animal Sciences, GBPUAT, Pantnagar, Uttarakhand. Owners were detailed about the purpose of the study and all owners consented verbally to participate in the study.

Screening of animals

CPV suspected animals based on clinical signs such as bloody diarrhoea, vomiting, lethargy was subjected to CPV Rapid Antigen Tests (RAT)and Polymerase Chain Reaction (PCR) for confirmatory diagnosis. Out of the positive cases, 12 cases were randomly selected and divided into 2 groups (B and C) for carrying out comparative evaluation of therapeutic efficacy of faecal microbiota transplantation in CPV cases. Accordingly, Group B was treated with symptomatic therapy (S) whereas Group C was treated with Symptomatic therapy along with Faecal Microbiota transplantation (S+FMT) as an adjunct therapy. The procedure was conducted from Day 0 of initiation of treatment to Day 7th of the same. It was hypothesized that administration of a single or more than one transplantation procedure at the proximal colon would result in earlier resolution of diarrhoea and a reduced hospitalization time with improved scores of clinical parameters. Response to FMT therapy was assessed using various criteria. To ensure similarity between animals assigned to the groups B and C; body weight, age, sex and breed data of either group were assessed using a student's t test.

Treatment protocol of faecal microbiota transplantation

A healthy donor is selected based on criteria as mentioned by Pereira *et al.* (2018) for the procedure. The stool donor was a privately owned, 5-year-old naturally born colostrum fed intact male German Shepherd from Pantnagar with no complications or history of gastrointestinal disease and use of antibiotics since past 4 years or more and fed on home-made diet with Chapati, daal, rice, eggs and curd. The animal was properly vaccinated and dewormed. The animal was having a normal body condition score and faecal score. Haemato-biochemical parameters of the donor were analysed to rule out any latent diseases. The donor faces were screened for parasites, protozoans, culturing techniques were carried out for ruling out enteropathogens and a normal GIT microflora. Stool was collected on a daily basis (early morning) into a sterile plastic vial and was immediately processed in the laboratory within 2 hours of collection. The animal was rescreened every 2 months. In addition, rapid antigen tests for canine parvovirus, distemper virus, and tests for haemoprotozoan were carried out. FMT procedure was carried out in the dogs starting from the day of presentation *i.e.*, Day 0 till Day 7 of the same once a day. Within 2 hours of collection of freshly evacuated faeces in the morning, the stool sample is processed and the suspension is stored in -20°C for further use in the same day. About 15 g of collected faeces was mixed with non-bacteriostatic 30 ml 0.9% NaCl; the same is then homogenously mixed with a blender for about 5 minutes; thereafter carefully strained with a strainer to have a uniform mixture free of solids. The suspension is then loaded in 20 ml syringes attached with a red rubber catheter and stored in -20°C. To administer the rectal enema, a minute amount of non-bacteriostatic lubricant was applied on the catheter, and completely introduced into the colon. With the lower abdomen of the animal elevated at about 45° angle, the suspension with a syringe connected to a catheter was introduced per-rectally and the contents were pushed in the proximal section of the rectum. The animal was stabilized for the next 30 minutes thereafter. The procedure was conducted after completion of the symptomatic therapy once a day. Other than routine haemato-biochemical studies, the below mentioned clinical predictors to faecal microbiota transplantation were evaluated:

i. Resolution of diarrhoea.
ii. Effect of FMT on hospitalization time/ recovery.
iii. Clinical scoring of various parameters.
iv. Retention time of faecal slurry after carrying out transplantation.
v. Safety and adverse events.
vi Comparison of recurrence of gastroenteritis episodes till next 2 months post recovery.

Clinical scoring of various parameters of Groups B and C

Once daily, a clinical score adapted from study of Mortier *et al.,* (2015) was analysed for each of the hospitalized dogs suffering from CPV (Table 1).

Follow-up

The pet owners were contacted via telephone weekly to note any adverse events and any recurrence of gastroenteritis, primarily diarrhoea for the next 3 months.

Table 1: Scoring criteria of affected dogs according to various parameters.

Parameter	0	1	2	3
Activity	Active	Mildly lethargic	Moderately lethargic	Lateral recumbency and comatose
Appetite	Normal diet	Able to digest semi-solid food	Able to digest bland liquid diet	Complete anorexia with polydipsia
Vomiting (times/day)	0	1-2	3-5	>5
Faecal consistency	Formed	Semi- Formed	watery	Watery/bloody
Defaecation (times/day)	1	2-3	4-5	>5
Dehydration (%)	<5	5-8	8-10	>12
Total acute diarrhoeal score	0-3	4-5	6-8	9 or more
Clinical significance of the disease	Clinically insignificant	Mild	Moderate	Severe

Relationship between total score and clinical significance: 0-3, insignificant; 4-5, mild; 6-8, moderate; > 8, severe

Statistical analysis

Wherever applicable, analysis of data involved descriptive statistics, application of t tests for normally distributed continuous variables, nonparametric tests *i.e.*, Mann Whitney U tests and Wilcoxin Rank signed sum test for skewed variables, also χ^2 test or analysis of variance (ANOVA) for categorical variables. The data obtained were subjected to statistical analysis by using computer software SPSS. The mean values of different parameters between control and diseased group; and at Day 0, 5 and 7 were compared at 1% and 5 % level of significance using "t" test and "two-way ANOVA".

RESULTS AND DISCUSSION

Resolution of diarrhoea

Out of the dogs undergoing FMT therapy, 66.67% (4/6) cases had resolution of diarrhoea within 48 hours of initiation of therapy, while in 33.33% (2/6) cases, resolution of diarrhoea was seen between 48 and 96 hours of initiation of therapy. In case of dogs undergoing symptomatic treatment 16.67% (1/6) showed resolution within 48 hours, whereas 83.33% (5/6) had resolution between 48-96 hours respectively; the findings had been depicted in Table 2. Similar findings were noted by Pereira *et al.* (2018) in a study where it was hypothesized that the response to the procedure is likely as a result of reconstitution/restoration and engrafting of the intestinal microbiota and its corresponding metabolites.

Hospitalization time

No statistically significant difference was observed was observed on days of hospitalization between the groups (*p>0.05*). Dogs from Group B had mean days (5.17) with median (5) days, range (4-7) days and SD of 1.17 days; while dogs from Group C had mean days (5) with median (5), range (4-7) days and SD of 1.10 days respectively. The data have been depicted in Table 3. As observed by Gal *et al.* (2021) no statistically significant variations (*p=0.41)*

Table 2: Percentage of animals showing resolution of diarrhoea (n=12).

Resolution of diarrhoea	GROUP B (Symptomatic)	GROUP C (Symptomatic+FMT)
Within 48 hours	1/6 (16.67%)	4/6 (66.67%)
48- 96 hours	5/6 (83.33%)	2/6 (33.33%)

between FMT treated dogs and sham-treated control dogs suffering from acute haemorrhagic diarrhoea were seen and both the groups had an average hospitalization time of 3 days. In contrast, Pereira *et al.* (2018) observed that hospitalization time in dogs undergoing FMT+ supportive therapy group was lower *(p<.001)* in comparison to those in the supportive therapy group. It was observed that as a result of reflex vomiting post recovery parenteral therapy was required to be continued for another 2-3 days thus prolonging the hospitalization time. Severe vomiting, severe oesophageal and gastric mucosal erosions might result leading to a delay in initiation of deliberate oral feeding.

Clinical scoring

Dogs of Group C treated did not vary in median (IQR) score from Group B at Day 0 *i.e.* (15.5) (1) *vs.* 16 (1); (p= 0.82[a]) while on Day 7 there were significant differences in scores (1) (1) *vs.* 4 (1); p= 0.02[b]; n= 12). Also, the median (IQR) clinical scores of both Group B and C differed between Day 0 and Day 7 (15.5 (1) *vs.* 1 (1); p= 0.02; n= 6) and (16 (1) vs. 4 (1); p= 0.03; n= 6). The figures have been depicted in Table 4. Improvement in clinical scores of dogs undergoing FMT in comparison to the symptomatic treatment group towards Day 7 indicated a probable useful adjunct in therapy of acute diarrhoea towards faster improvement of the health condition and gut health.

Retention time

It was seen that 100 % of dogs had clinical signs consistent with severe category of clinical score at presentation. Retention time of the faecal transplant was noted from the first day of the therapy till the 7th day and was observed that

Table 3: Effect of FMT on hospitalization time/ recovery (n=12).

Groups	Mean (days)	Median (days)	Range (days)	Standard deviation (days)	*p* value
Group B	5.17	5	4-7	1.17	≥0.05
Group C	5	5	4-7	1.10	

Table 4: Table depicting median (IQR) scores, *p* values and clinical significance of groups b and c from day 0 to day 7.

Groups	Median score	IQR	*p* value	Clinical significance of the disease
Group B				
Day 0	16	1	0.82a	100% (severe)
Day 7	4	1	0.02b	Mild
Group C				
Day 0	15.5	1	0.82a	100% (severe)
Day 7	1	1	0.02b	Clinically insignificant

*Statistically significant at p≤0.05.

Table 5: Retention time of the faecal slurry after carrying out transplantation (n=6).

Days	Retention time (Mean±SE)	*p* value
Day 0 (minutes)	20.83±2.39	<0.01
Day 7(minutes)	140±12.66	

Table 6: Recurrence of gastroenteritis/ subsequent episodes of diarrhoea within next 2 months of therapy.

Treated cases	Recurring diarrhoea	Percentage (%)
Group B (n=6)	3	50%
Group C (n=6)	1	16.67%

the mean retention time during the first day of the therapy was found to be (20.83±2.39) minutes whereas there was significant improvement in retention time on the 7th day of the therapy (140±12.66) minutes respectively (p<0.01). The figures have been depicted in Table 5. Lesser retention time in the initial phase might be due to increased frequency of peristalsis during the earlier stage of the disease and mild discomfort may be felt by the animal as it is not habituated to the procedure. In the later phase, resolution of diarrhoea and familiarization of the procedure resulted in a significant increase in retention time. Sood *et al.* (2020) in a study in human patients suffering from Ulcerative colitis also noted similar increase in retention time ((3.27±1.06 h) for the first session vs (5.12±0.5 h) for the seventh one) and stated that adequate retention of fecal slurry is significant for engraftment of microbiota even though fecal transplant retention time did not modify results with FMT, this however larger structured trials are required for its establishment.

Safety and adverse events

No serious adverse events were noted, however one case showed low grade fever (n=1; 16.67%) and epistaxis (n=1; 16.67%) respectively which resolved within 24 hours. The figures have been depicted in Table 6. This is concurrent to findings of Simpson *et al.* (2002) who noted low grade fever in 7/140(5%) human patients who underwent FMT in acute ulcerative colitis. In contrast, Pereira *et al.* (2018) did not observe any clinical abnormalities during in their study.

Recurrence of diarrhoea

Recurrence of non-hemorrhagic diarrhoea within 2 months was seen in 1/6 (16.67%) cases of Group C had while 3/6 (50%) dogs of Group B had recurrence within the same time period (Table 6). The development of fever and epistaxis could not be directly linked to FMT; fever and epistaxis as a consequence of DIC could be linked to sepsis like conditions, however, however other criteria of sepsis like condition were not fulfilled thus eliminating the possibility. FMT could be considered safe because group did not develop any clinical abnormalities that were attributable to the procedure. A small number of FMT research in people and canines have evaluated the microbiome, but this was not done in our study. The determination of changes in microbial diversity was outside the scope of this study and requires more investigation.

CONCLUSION

It can be concluded in this study that Faecal Microbiota Transplantation in parvovirus-infected puppies was associated with quicker resolution of diarrhoea and lower incidence of recurrent gastroenteritis post recovery. FMT is one of the comparatively inexpensive and hassle-free method of non-conventional therapies which could be further explored both in gastroenterology as well as in other branches of medical and veterinary sciences. Further studies with larger study groups need to be conducted to analyse and bring the procedure to a routine practice in the future.

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Conflict of interest

The authors declared no conflict of interest.

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