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Permanent Jejunal Fistula: Promising Method for Obtaining Small Intestinal Chyme without Disturbing Intestinal Function

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Accurate information on changes in small intestinal microflora in dogs is rather limited because of difficulties in obtaining samples of small intestinal chyme. In the study reported here, intussuscepted nipple valves were surgically placed into the jejunum of seven laboratory beagles to obtain intestinal juice samples. The influence of the fistula on intestinal motility was determined by use of barium-impregnated polyethylene spheres (BIPS) and on microflora by use of bacterial culturing. The BIPS were fed two weeks before surgery and again five weeks after surgery. Bacterial samples were collected before (fecal samples), during (small intestinal samples) and 11 weeks after surgery. There were no surgical complications, and the animals tolerated the fistula well. Mean orocolic transit percentage was 93% before and 83% after surgery, and notable changes in gastrointestinal motility were not seen, except in one dog.

The surgery did not markedly alter the bacterial flora in feces. Microflora did change in small intestinal samples; however, methodologic factors may explain most of these differences. In conclusion, the nipple valve is a promising method that creates easy and safe long-term access to the jejunum and appears not to have an influence on intestinal function.

Studies on the microflora of the small intestine of dogs are rather sparse and incomplete (1, 2), compared with the numerous studies done on the large intestine. Especially rare are those studies in which changes in microflora have been monitored over a long period. The main obstacle to studying the ecology of the small intestine is difficulty in obtaining samples. Various techniques have been used to collect intestinal fluid from animals and include laparotomy (3), endoscopy (4) and cannulation (5-9).

Recently, a nipple valve method for long-term access to the small intestine of dogs has been devised (10). This kind of nipple forms a one-way valve between the jejunum and the exit site on the skin surface. It is possible to insert a catheter through the valve into the intestinal lumen. This can be done when the dog is awake and standing, without discomfort to the animal (10). In humans, nipple valves have long been used in operations where there is need to create a reservoir without leakage (10).

The nipple valve technique has been used in dogs mainly for drug administration (10). To the authors' knowledge, studies about the affect of this kind of nipple valve on gastrointestinal motility or intestinal microflora have not been published thus far.

Data on small intestinal motility in dogs is somewhat limited, although abnormal motility, including physical and functional obstructions, is commonly seen in veterinary practice. Small intestinal bacterial overgrowth (SIBO) also is regarded as a consequence of motility disorders (11), and increased bacterial concentrations have been identified after certain gastrointestinal tract surgeries (12).

Motility disorders can be examined using barium contrast studies, breath hydrogen tests, scintigraphy, evaluation of contractions by manometry and electromyography (13) or by use of barium-impregnated polyethylene spheres (14) (BIPS, Chemstock Animal Health Ltd, Christchurch, New Zealand). In the study reported here, BIPS were used because they mimic the normal passthrough in the intestines and are easy to use and particularly helpful in diagnosing functional or partial physical obstructions.

The objective of this study was to evaluate the nipple valve fistula, as a method of obtaining samples of jejunal chyme. The variables examined were intestinal motility and microflora of the small intestine and feces in surgically treated dogs.

Materials and Methods

Surgery. Seven laboratory beagles (one female, 05J, and six males, 057H, 058H, 070H, 086H, 01J, 02J) had a nipple valve surgically inserted into the jejunum approximately 60 cm distal to the pylorus. Age of the dogs ranged between 1 and 2 years, and body weight was between 9 and 14 kg.

All dogs were obtained from Kuopio University's laboratory animal breeding colony in Finland. The dogs were housed in the groups of 2 animals in indoor pens, except the female, which was housed singly. For outdoor activities, all the dogs were allowed to go together in one group. The dogs spend approximately four hours per day outdoors. Pen size was $6m^2$/two beagles and $3.5m^2$/one beagle. Dry dog food (Baron Complete, Raisio Feed Ltd, Raisio, Finland) was fed twice a day at approximately 7:30 a.m. and 3 p.m. The main ingredients were cereal, meat, chicken, animal derivatives, and oils, and the composition was 22% protein, 2.3% fiber, 12% fat, 6.5% ash, 1.1% Ca, and 0.9% P. Water was available ad libitum. Dogs were exposed to natural light at all times, as well as lighting in the daytime from 7 a.m. to 4 p.m.

Clinical examination, hematologic analysis, and fecal examination for parasites were performed prior to surgery, and the dogs were found to be healthy. The experimental protocol had

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been approved by the local ethics committee for animal experiments in Helsinki, Finland.

The surgical technique has been reported elsewhere in detail (10). Briefly, dogs were premedicated with a combination of medetomidine (20 µg/kg of body weight; Domitor, Lääkefarmos, Turku, Finland) and butorphanol (0.2 mg/kg; Torbugesic, Fort Dodge Animal Health Ltd, Southampton, N.Y.), and anesthesia was induced with propofol (0.5 to 1 mg/kg; Rapinovet, Schering-Plough Animal Health, Farum, Denmark) and maintained with isoflurane in a mixture with oxygen. The surgery was done through midline abdominal incision. An approximately 25-cm segment of the small intestine was separated, 60 cm distal to the pylorus, by dividing the mesentery between the mesenteric vessels. An end-to-end anastomosis was performed on the free ends of the intestine. Two Allis tissue forceps were inserted into the isolated segment, and the intestinal wall was grasped on opposite sides of the lumen to make an intussusception. Because the intestinal lumen diameter in our dogs was quite small, we used method 2 (10) to construct the nipple valve. In this procedure, a chamber to the nipple was created by opening the intestine antimesenterically, then folding back the distal half of the opened segment. Next, the nipple was inserted and sutured in an end-to-side anatomosis to the small intestine.

For flank exteriorisation, a hole was made perpendicular to the skin and abdominal muscle wall layers. The proximal end of the segment was grasped through the hole and pulled out of the abdominal cavity. The end of the nipple was sutured to the parietal peritoneum and abdominal muscles. Finally, the abdominal incision was closed.

Antibiotics were not used prophylactically or postsurgically. Buprenorphine (0.03 mg/kg; Temgesic, Reckitt & Colman, Hull, England) was used for analgesia, and was administered 10 to 15 min prior to completion of surgery and once in the first day after the surgery. There was no need for further analgesia. Recovery time for complete healing of the fistula was four weeks. During that time, the dogs wore Elizabethan collars and were singly housed and walked, to avoid licking of the nipple valve by themselves or by other animals. Postoperative feeding was started the day after surgery, and consisted of small portions of dry dog food provided frequently. At one month after surgery, the feeding routine was returned to normal, with two portions of dry dog food given per day. At that time, the collars were removed and the dogs were allowed to socialize with one another.

Intestinal motility. Two weeks before and five weeks after nipple valve surgery, gastrointestinal motility was examined by use of BIPS. Each dog was fed 10 large and 30 small BIPS markers with a small portion (25% of daily calorie requirement) of dry dog food. The dogs were radiographed 8 and 12 h after feeding. Motility was determined by calculating the orocolic transit percentage, using the following formula:

$$\text{Orocolic transit \% of BIPS} = \frac{\text{max - O. C.}}{\text{max}} * 100\%$$

where max is the maximal number of BIPS fed to the dog (10 large and 30 small BIPS) and O.C. is the number of BIPS in the radiographs that had not reached the colon.

Bacterial culture. Samples from the small intestine and feces of four dogs were analyzed by bacterial culture. Fecal samples were extracted manually per rectum before surgery. Total number of isolated aerobes and anaerobes were determined in fecal samples as were lactic acid bacteria and *Clostridium perfringens*-like bacteria, which were chosen as indicator bacteria. The small intestinal chyme samples were taken during surgery, when the segment of the intestine was separated, by aspirating aseptically, using a catheter and a syringe.

The samples were transported to the laboratory in anaerobic bags (Anaerocult P mini, Merck, Darmstadt, Germany). Because we had some problems with the bag during surgery, wherein the small intestine juice samples were located, and the anaerobic circumstances could not be maintained, analysis of these samples was limited to culture of total aerobes and lactic acid bacteria.

For culture of anaerobic bacteria, the samples were processed in an anaerobic chamber and plated on the following media: *Brucella* agar supplemented with sheep blood and vitamin K1 to determine total anaerobes and tryptose sulfite cycloserine agar (TSCA, Merck) for *Clostridium perfringens*-like bacteria. The plates were incubated at 37°C for 5 to 7 days in anaerobic jars filled with mixed gas (85% N_2, 10% CO_2, 5% H_2) by use of the evacuation-replacement method (Anoxomat, Mart, Lichtenvoorde,

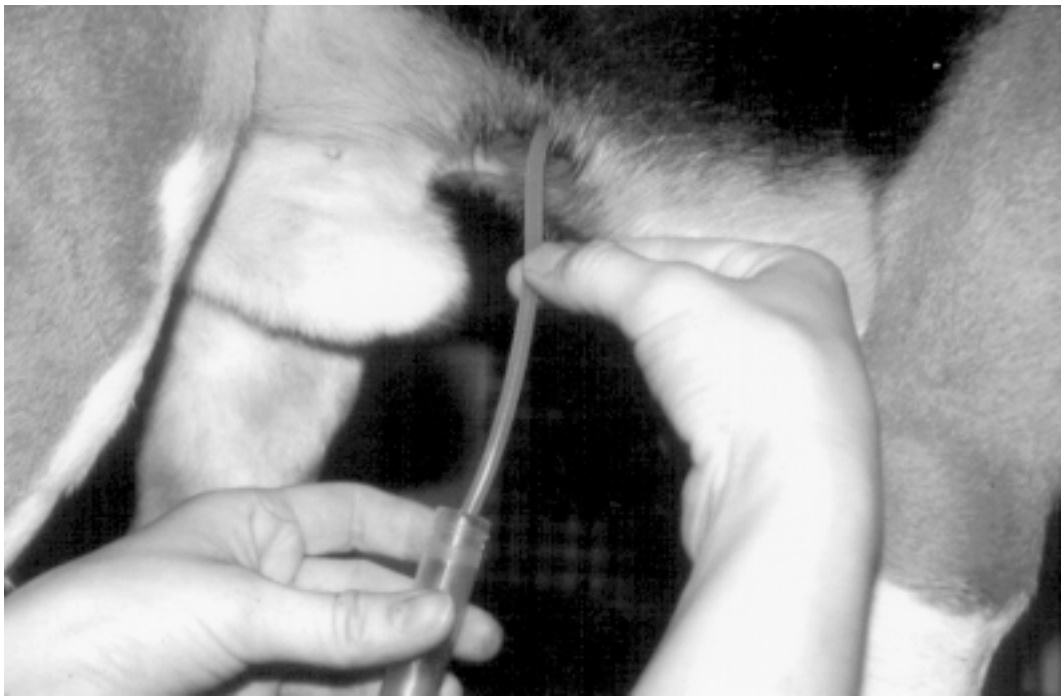

Figure 1. Obtaining small intestinal chyme sample by inserting a silicon tube through the nipple valve in a standing dog.

Table 1. Orocolic transit percentages before and after surgery*

	Before (%)		After (%)	
Dog	8 h	12 h	8 h	12 h
1	75.0	97.5	75.0	75
2	85.0	97.5	87.5	-
3	62.5	100	-	97.5
4	80.0	80.0	77.5	77.5
5	87.5	87.5	77.5	77.5
6	67.5	100	67.5	87.5
7	75.0	100	17.5	22.5

*Radiographs were taken eight and 12 h after feeding barium-impregnated polyethylene spheres (BIPS).
- = Not radiographed.

The Netherlands). For culture of lactic acid-producing bacteria (LAB) and total aerobes, samples were plated on Rogosa agar (Oxoid, Hampshire, England) and nutrient agar (NA), respectively, under aerobic conditions. Rogosa agar plates were incubated at 37°C for 3 to 4 days in jars containing anaerobic bags (Anaerocult A, Merck). Nutrient agar plates were incubated in ambient air at 37°C for 2 days. All colonies from *Brucella* agar, NA, and Rogosa agar plates were enumerated. Black-pigmented colonies from TSCA plates were counted as *C. perfringens*-like bacteria.

Eleven weeks after surgery, after food had been withheld for 12 h, samples of feces and small intestinal juice were again collected. Stool samples were taken manually per rectum and small intestinal chyme was collected by inserting a silicon tube, approximately 15 cm long and 5 mm in diameter, through the nipple valve into the jejunum. The samples were cultured as described previously.

Statistical analysis. The Wilcoxon signed rank test was used to test the differences in bacterial counts before and after surgery. Before statistical analysis, the bacterial counts were logarithmically transformed. In statistical analysis for dog 4, the value $< 10^3$ was extrapolated as 10^3 for small intestinal lactic acid bacteria count before surgery.

Results

Recovery after surgery. A nipple valve was surgically inserted into seven dogs for permanent intestinal access. After the surgery, the dogs were carefully monitored (including wound inspection and clinical examination, abdominal palpation, and measurements of body temperature, defecation, and appetite) twice daily. Except for transient anorexia in three dogs in the first two days after surgery, complications were not seen during recovery. There was no leakage from the valve and no signs of infection. Approximately four months after the surgery, dog 7 had to be euthanized because of leakage of small intestinal contents from the nipple valve that could not be fixed. At necropsy, adhesions between the nipple, small intestine, and peritoneum were observed and the intussusception had straightened itself.

In the remaining six dogs, which still being studied, the function of the nipple has been excellent. No leakage of intestinal content has been discovered from the fistulas. By inserting a silicon tube through the nipple valve, small intestinal chyme has been collected hundreds of times without difficulty from these dogs. When the tube is inserted (Fig.1), the jejunal juice runs out passively, at least six hours after feeding, obviating the need for aspiration, which might damage the intestinal mucosa.

Intestinal motility. Results of motility analyses indicated that, in all dogs, most of the BIPS had passed through the stomach and small intestine and reached the colon, on average, in 12 h, and in six dogs, the transit percentages remained practically the same before and after the surgery (Table 1). Mean transit percentage (12 h after BIPS) before surgery was 93% (range, 80 to 100%, six dogs), and after surgery was 83% (range, 67.5 to 87.5%, five dogs). In the seventh dog, the orocolic transit percentage changed markedly following the operation (before, 100%; after, 22.5%). This was the dog which subsequently had to be euthanized because of leakage of the fistula and adhesions between the nipple, small intestine, and peritoneum.

Bacterial counts before and 11 weeks after fistulation. Bacterial counts in fecal samples are shown in Table 2. No change was observed in fecal counts of total anaerobes and lactic acid-producing bacteria before and after fistulation in any of the four dogs. A decrease was observed in *C. perfringens*-like bacteria and total aerobes in fecal samples from three dogs.

Samples from the small intestine. Bacterial counts in small intestinal samples are shown in Table 3. In small intestinal samples after fistulation from three of four dogs, counts of lactic acid-producing bacteria increased. In contrast, counts of total aerobes decreased (two dogs) or remained unchanged (two dogs).

Statistical results. The statistical *P* values between the bacterial counts in feces before and after fistulation were as follows: total aerobes, $P = 0.13$; total anaerobes, $P = 0.88$; lactic acid bacteria, $P = 0.86$; and *Clostridium perfringens*-like bacteria, $P = 0.38$. For small intestinal bacterial counts, the *P* values were: 0.37 for total aerobes and 0.25 for lactic acid bacteria. The statistical results are expressed as means, medians, and first and third quartiles in Tables 2 and 3.

Discussion

Numerous species of bacteria are harbored in the gastrointestinal ecosystem; numbers and types of bacteria vary according to location within the tract (15). A healthy gastrointestinal microflora forms a barrier against invading organisms, and changes in normal gut flora may result in development of disorders (16). Information on the small intestinal flora and the environment within the small intestine of the dog is rather limited (1, 2), the main obstacle being gaining regular access to small intestinal chyme.

There have been many inherent difficulties with the methods previously used to sample small intestinal content. Laparotomy and endoscopy require general anesthesia. Food must be withheld from the dogs before surgery; thus, volume of the samples has been minimal (17). Both methods also are time consuming and do not allow frequent sampling over time, nor is it possible to follow up the changes in intestinal chyme. With use of the instrumented models, complications due to the presence of the foreign material of the fistula in the intestine have been numerous (e.g., leakage and/or tissue rejection, abscess, cannula extrusion, and ulceration of the skin [9]). Because plugs are needed to prevent leakage from the fistula, collars are required to protect the implants and dogs must be housed singly.

The fistula method described here allows for an easy and repeatable way to obtain representative small intestinal chyme samples from the jejunum. Simply by inserting a catheter through the valve into the intestinal lumen, it is possible to extract samples several times a day. All procedures have been performed on a conscious dog, without discomfort to the animal.

In following the dogs several months after surgery now, complications, infections, or leakage were not observed, except in dog 7, which had adhesions between the nipple valve, small intestine, and peritoneum; the intussusception has straightened itself, which likely caused the failure.

Table 2. Mean, median, and first and third quartile (Q1 and Q3) bacterial counts* in fecal samples from four dogs before and 11 wks after installation of permanent fistulas

	Total anaerobes		*C. perfringens* -like		LAB		Total aerobes	
Dog	Before	After	Before	After	Before	After	Before	After
1	1.1×10^{10}	9.4×10^{9}	1.7×10^{9}	2.5×10^{6}	1.0×10^{9}	2.5×10^{8}	2.2×10^{9}	1.8×10^{7}
2	2.1×10^{9}	5.2×10^{9}	2.0×10^{7}	7.0×10^{5}	5.9×10^{8}	1.6×10^{9}	1.8×10^{9}	6.6×10^{8}
3	1.0×10^{10}	7.3×10^{9}	1.0×10^{7}	4.0×10^{7}	1.8×10^{9}	2.0×10^{9}	3.0×10^{9}	8.7×10^{7}
4	2.4×10^{9}	3.6×10^{9}	5.0×10^{7}	5.0×10^{5}	3.1×10^{8}	8.0×10^{7}	2.2×10^{9}	1.0×10^{8}
Mean	6.4×10^{9}	6.4×10^{9}	4.5×10^{8}	1.1×10^{7}	9.3×10^{8}	9.8×10^{8}	2.3×10^{9}	2.2×10^{8}
Median	6.2×10^{9}	6.3×10^{9}	3.5×10^{7}	1.6×10^{6}	8.0×10^{8}	9.3×10^{8}	2.2×10^{9}	9.4×10^{7}
Q1	2.3×10^{9}	4.8×10^{9}	1.8×10^{7}	6.5×10^{5}	5.2×10^{8}	2.1×10^{8}	2.1×10^{9}	7.0×10^{7}
Q3	1.0×10^{10}	7.8×10^{9}	4.6×10^{8}	1.2×10^{7}	1.2×10^{9}	1.7×10^{9}	2.4×10^{9}	2.4×10^{8}

*Bacterial counts are expressed as colony-forming units (cfu/g wet weight, as determined by bacterial culture).
1–4 = dog ID numbers.
LAB = Lactic acid-producing bacteria, *C. perfringens*-like = *Clostridium perfringens*-like bacteria.
Q1 = First quartile, Q3 = Third quartile.

Table 3. Mean, median, and Q1 and Q3 bacterial counts in small intestinal samples from four dogs before and 11 wks after installation of permanent fistulas

	LAB		Total aerobes	
Dog	Before	After	Before	After
1	6.0×10^{4}	4.5×10^{7}	1.8×10^{6}	1.1×10^{7}
2	5.0×10^{3}	3.0×10^{3}	1.8×10^{8}	5.0×10^{3}
3	5.0×10^{5}	8.0×10^{7}	1.0×10^{7}	5.4×10^{4}
4	$<10^{3}$	1.9×10^{5}	7.5×10^{6}	1.0×10^{6}
Mean	1.4×10^{5}	3.1×10^{7}	5.0×10^{7}	3.0×10^{6}
Median	3.3×10^{4}	2.3×10^{7}	8.8×10^{6}	5.3×10^{5}
Q1	3.8×10^{3}	1.4×10^{5}	6.1×10^{6}	4.2×10^{4}
Q3	1.7×10^{5}	5.4×10^{7}	5.3×10^{7}	3.5×10^{6}

See Table 2 for key.

The range of normal gastrointestinal transit time is difficult to determine (13) because motility varies widely in different animals and even within the same animal if diet is changed. Motility disorders are sometimes seen after intestinal surgery (11); intestinal transit time can be delayed, which can result in SIBO. Excess amount of bacteria is considered to be responsible for this pathologic condition, manifested by chronic diarrhea and maldigestion. It is, therefore, important to determine the possible alterations to intestinal motility caused by fistula surgery before beginning any trial with fistula-operated dogs. Dogs with abnormal transit time are at risk of developing SIBO, and should, thus, be excluded from the trials.

Our results indicate that, barring complications, the anastomosis in nipple valve surgery does not disturb intestinal transit time. On the other hand, dog 7 illustrated quite clearly that intestinal surgery can change intestinal motility if complications, such as adhesions, develop. This dog must have already had the adhesions at the time the motility test was performed, although clinical signs of such did not appear until four months after surgery.

Regulation of gastrointestinal microflora depends on many factors (16), including normal gastrointestinal peristaltic activity, which prevents abnormal bacterial colonization. However, it is also known that there is a considerably large variation in gastrointestinal microflora (1, 2, 18-23).

In the study reported here, all bacterial counts in fecal samples, before and after the nipple valve operation, were almost identical, and the subtle differences can be considered as normal variation. Hence, the fistula does not change the colonic microflora.

The results from the small intestinal bacterial cultures were not as constant. This could be explained by the natural variation between individual samples. In addition, some problems in the handling of the samples were encountered during surgery. These problems do not, however, explain why there is a bigger disparity in the number of aerobic bacteria in small intestinal, compared with fecal samples. One reason might be that the sampling techniques during and after surgery were not identical, which could have influenced the results. Another explanation is that it is difficult to get representative samples from the small intestine in a dog from which food has been withheld. In a recent study, using the fistulated dogs, we observed that the number of bacteria in small intestine changes remarkably in consecutive samples from dogs from which food was withheld (24).

The culture results concerning small intestinal bacteria must be considered to have only limited value, further devalued by the fact that traditional culturing methods are cumbersome and include many risk factors. It is also known, that only a small minority of intestinal bacteria can be isolated by culturing, and bacterial counts depend on the culturability of bacterial species (25). Furthermore, it is not clear whether the general anesthesia will alter the small intestinal microflora. Novel, more reliable methods should be developed for examining the changes in intestinal microflora. The nipple valve method described in this study allows easy sampling of small intestinal contents, facilitating more studies to be performed on intestinal microecology. Our results indicated that the nipple valve does not cause SIBO, because the total aerobic bacterial count had not increased in samples taken after surgery. Clinical signs of SIBO (diarrhea, flatulence, borborygmus) or changes in the dogs' physical condition were not evident, and the functioning of the nipple was judged to be excellent. This indirectly supports the view that fistulation either has no or minimal effect on normal functioning of the small intestines.

In conclusion, nipple valve surgery was easy to perform, the recovery time was only four weeks, and except for transient loss of appetite, typical postoperative complications did not develop. This kind of fistula makes it possible to collect samples many times per day over a long period compromising the quality of life of the animals; dogs can be housed together and allowed access to outdoor activities. The nipple valve enables small intestinal chyme content to be examined more frequently and more accurately than does use of techniques requiring endoscopy, laparotomy, or the current fistula models. Larger amounts of chyme can be extracted as often as needed, because there is no need to anaesthetize the animal, making it possible to take post- feeding samples as well. The nipple valve enables further investigations where, for instance, the effect of feeding on small intestinal microflora can be determined and the diurnal alterations in bacterial contents can be examined. The fistula also makes it possible to investigate the effects of various medications on small intestinal microflora, mucosal immunology, and bacterial metabolites.

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