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Intestinal Bacterial Overgrowth
and Fat Malabsorption in
Calves with Diarrhea

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INTESTINAL BACTERIAL OVERGROWTH AND FAT MALABSORPTION IN CALVES WITH DIARRHEA

By

Youanes Dawood Youanes

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ABSTRACT

INTESTINAL BACTERIAL OVERGROWTH AND FAT MALABSORPTION IN CALVES WITH DIARRHEA

By

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The concentration of total bacteria, aerobes-facultative anaerobes, coliforms and lactobacilli were measured in the intestine of nine 5 to 21 day old calves with spontaneously occurring diarrhea and four normal controls.

The coliform counts in calves with diarrhea exceeded those of the normal calves throughout the small intestine ($p<0.05$), whereas total bacteria, total aerobes-facultative anaerobes and lactobacilli of calves with diarrhea exceeded the normal calves only in the ileum ($p<0.05$).

The total fecal fat output was positively correlated with fecal quantity ($R=0.67$, $p<0.01$), ileal contents of coliforms and total bacteria ($R=0.72$ and 0.67 respectively, $p<0.01$).

Enteropathogenic agents were isolated only from three of nine calves with diarrhea. One calf had a combined infection with rotavirus and enterotoxigenic Escherichia coli (ETEC), another had ETEC and Cryptosporidium, the third had Cryptosporidium.

It was concluded that bacterial overgrowth of non-pathogenic bacteria in the small intestine may play a role in some cases of neonatal diarrhea.

DEDICATION

To my parents, and my wife, Ferial.

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INTRODUCTION

Neonatal diarrhea in calves is one of the most important causes of morbidity and mortality in dairy and beef herds. In Michigan, diarrhea in young calves is considered as one of the major health problems. Seventy percent of the calf mortality in dairy herds is attributed to diarrhea (Oxender et al., 1973). Eighty percent of diarrhea cases develop before 10 days of age (Acres et al., 1975).

The disease remains largely unchecked because of its complex etiology and the list of microorganisms thought to be responsible is probably incomplete (Acres et al., 1975; Tzipori et al., 1981). In addition, several factors including herd size, colostrum feeding, weather, personnel management, and calving pens, have been considered responsible for neonatal calf mortality in dairy herds (Bakheit and Greene, 1981; Kirk, 1978; Martin et al, 1975 a,b; Oxender et al, 1973; Speicher and Hepp, 1973).

Attempts focusing on controlling the disease have failed. This is because many different etiological agents and many different pathogeneic mechanisms are involved in this disease.

Various pathogenic mechanisms produce diarrhea in man and animals. Thus, recognition of the mechanisms involved in the various types of diarrhea is useful in understanding, diagnosing, and managing enteric diseases. Such mechanisms are hypermotility, increased permeability, hypersecretion and malabsorption (Moon, 1978).

A potential pathogenic mechanism which may play a role in neonatal diarrhea is small intestinal bacterial overgrowth. This has been studied extensively in human medicine but has been given little attention in veterinary medicine. Blind loop syndrome, is a term used in human gastroenterology to define a condition associated with small bowel bacterial overgrowth and characterized by diarrhea, steatorrhea and malabsorption (Donaldson, 1978).

Large numbers of bacteria in the small intestine are capable of deconjugating bile acids. The concentration of bile acid may decline to the point where the critical micellar concentration is not met thus disrupting the process of lipid absorption (Kistler and Giannella, 1980). Deconjugated bile acids are injurious to small intestinal epitheleum, and thereby contribute to fat malabsorption.

Large numbers of bacteria in the small intestine of calves might also compete for important nutrients like vitamin B_{12}, and glucose and make them unavailable for the host.

This research will focus mainly on intestinal bacterial numbers in calves with diarrhea as compared to normal calves and the correlation between intestinal bacterial numbers and fat malabsorption.

The objectives of this study are: 1) to compare the concentration of total bacteria, aerobes-facultative anaerobes, coliforms and lactobacilli in the intestinal tract of normal calves and calves with diarrhea and 2) correlate intestinal bacterial concentration with fat malabsorption.

REVIEW OF LITERATURE

Normal Intestinal Bacteria

The intestinal tract of calves is sterile at birth, but soon after birth it becomes inoculated with fecal organisms from the surrounding environment, particularly of the dam. Different types of organisms are usually present in the intestinal tract of calves and are considered to be normal inhabitants, these include Escherichia coli, Clostridium perfringens, Streptococcus spp., Lactobacillus, and strict anaerobes (Bacteroides) (Mylrea, 1969).

Escherichia coli, Clostridium perfringens and Streptococcus spp. in calves and coliform and Streptococcus spp. in pigs are the first enteric organisms to be found after birth. They achieve high concentration during the first two days of life, this indicates that little destruction is taking place during transit through the upper gastrointestinal tract (Kuarnfor and Mansson, 1972; Smith and Crabb, 1961). The source of these organisms in the intestine of calves is mainly from the forestomachs and abomasum. Considerable numbers of bacteria pass from the

abomasum to the duodenum. The numbers don't vary greatly along the length of the small intestine, and roughly equivalent numbers to that of abomasum pass from the small to large intestine (Mylria, 1969). It seems that the high bacterial content of the large intestine could be due to passage of bacteria from the small intestine (Mylrea, 1969; Smith, 1962; Watase and Takenouchi, 1978 b). This is attributable to the normal peristaltic movement of the small intestine which tends to washout these bacteria (Fossum and Liven, 1974). Then they reside and proliferate in the large intestine which has lower motility. This washout is enhanced by the flow of mucous over the surfaces of villi.

Lactobacilli take a longer time in colonizing the alimentary tract than do E. coli, Clostridium perfringens or Streptococcus spp., but they soon become and remain the most common inhabitants of the stomach (abomasum) and small intestine (Mylrea, 1969; Smith and Crabb, 1961; Smith, 1962).

Bacteroides spp. are not present in calves under two days of age, and after that they are only found in the large intestine where they join the lactobacilli and constitute the largest portion of fecal flora (Mylrea, 1969; Smith and Crabb, 1961; Smith, 1962).

Changes in the numbers of bacteria in the intestine usually occur as the calf advances in age. In calves, lambs and piglets the total viable counts are commonly

about 10^{10} per g of enteric content during the first few weeks of life and slowly decrease with age (Smith and Crabb, 1961; Watase and Takenouchi, 1978 a). Generally, over the first few days of life, there is a reduction in the E. coli concentration from initially high to comparatively low numbers. Some variation occurs in the rate of reduction between species (Mylrea, 1969; Smith and Crabb, 1961). The numbers are found to decrease slowly in calves until the third week and rapidly thereafter (Smith, 1976). The numbers of Clostridium perfringens decline much more rapidly with age and very few are found at 1-3 weeks of age (Smith and Crabb, 1961).

Smith and Crabb (1961) found that streptococci and bacteroides concentrations, like E. coli, decline gradually with age; however, others have found no particular tendency for the streptococci to change with age (Watase and Takenouchi, 1978 a). Lactobacilli also show some decline in numbers as the calves grow older (Smith and Crabb, 1961; Watase and Takenouchi, 1978 a).

Effect of Diet on Intestinal Bacteria

Diet was considered as one of the factors influencing the normal microflora in the intestinal tract of animals. Some workers found that feeding did not affect the concentration of bacteria in the intestine (Mylrea, 1969), while others found a greater decrease in lactobacilli in suckled

calves compared to bottle-fed calves (Watase and Takenouchi, 1978 a). Changes of diet were found to be responsible for the increase in the proportion of "pathogenic E. coli" to total E. coli to about 50% in the feces (Wray and Thomlinson, 1975). Some other researchers found that protein in the diet of pigs may favor the growth of Clostridium perfringens in the intestine (Kuarnfor and Mansson, 1972).

Early weaning of pigs frequently results in a high incidence of diarrhea which starts shortly after separating the pigs from their dams. This may be related to intestinal E. coli concentrations. Barrow et al. (1977) found significantly higher counts of E. coli in stomach, duodenum, and jejunum of the early-weaned pigs but not in samples of ileum. They did not find significantly higher counts of lactobacilli and streptococci.

Feeding high acid milk replacer, in conjunction with hay and concentrates, decreases the population of coliform bacteria but has no effect on lactobacilli (Humphrey et al., 1982). Fermented colostrum has been compared with whole milk as a feed for calves. Ward (1981) found that the intestinal concentrations of all classes of bacteria studied were significantly decreased in calves fed fermented colostrum in comparison to calves fed whole milk. This decrease was especially seen in the E. coli, streptococci and lactobacilli. Ellinger et al. (1980) found that fermented colostrum feeding did not affect

coliform counts of healthy calves. Also, fermented colostrum and other products like Fermatolact[a] and Lactoferment[b] (containing fermenting and lactic acid bacteria) are found to enhance recovery rates and subsequent growth rates in calves suffering from diarrhea (Mulling and Gross, 1980; Otterby et al., 1976). Addition of lactic acid to drinking water or bran to creep feed pellets decreased gastric pH and delayed the multiplication of E. coli in the intestinal tract of pigs with a corresponding decrease in mortality (Thomlinson and Lawrence, 1981). Feeding Lactobacillus acidophilus to calves decreased fecal coliform count with time and also delayed the occurrence of diarrhea (Awad, 1982; Ellinger et al., 1980).

In addition to the type of diet, digestion products also influence the enteric bacterial population. Fatty acids have been shown to have little effect on E. coli in the intestine (Bergeim, 1940; Levison, 1973). In contrast, Ward and Nelson (1982) found that calves fed whole milk (4% fat) had fewer coliforms in the cranial part of the small intestine than calves fed skim milk (fat removed). Stomach contents of the suckling rabbit normally contain antimicrobial substances produced by enzymatic action on rabbit's milk and having an inhibitory effect on the growth of many intestinal bacteria (Smith, 1966). Bile acids also

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[a]CTB Chemotherapia

[b]Selectavet

have been found to inhibit intestinal bacteria *in vitro* (Floch *et al.*, 1970, 1972) and may play a role in controlling *in vivo* intestinal bacterial populations. Oral administration of duodenal fluid to newborn calves lowers the incidence of diarrhea (James *et al.*, 1976).

Etiologic Agents

Different pathogenic organisms have been incriminated as being responsible for causing diarrhea in calves (Tzipori, 1981). The most important ones include enterotoxigenic *E. coli* (ETEC), rotavirus, coronavirus, and *Cryptosporidium*. These organisms are present in eighty percent of calves developing diarrhea before ten days of age (Acres *et al.*, 1975). A combination of two or three etiological agents was also demonstrated by some workers in calves with diarrhea (Moon *et al.*, 1978; Morin *et al.*, 1976). Enterotoxigenic *E. coli* is responsible for the most devastating economic losses in calves, while coronavirus, rotavirus and *Cryptosporidium* cause lesser economic losses (House, 1978). A combination of two pathogens in calves and lambs produces a more severe diarrhea than a single agent alone (Tzipori *et al.*, 1981; Wray *et al.*, 1981). Coccidian parasites of the genus *Cryptosporidium* have been associated infrequently with outbreaks of diarrhea in calves (Pohlenz *et al.*, 1978; Tzipori *et al.*, 1980), lambs (Angus *et al.*, 1982; Berg

et al., 1978), and pigs (Kennedy et al., 1977). The disease in calves occurs between 5 and 21 days old, reflecting the long incubation period of Cryptosporidium compared to other enteropathogens affecting young animals (Tzipori et al., 1980).

Campylobacter spp. is also incriminated as an enteric pathogen of cattle and calves. It has been isolated from the intestinal mucosa of cattle at necropsy (Al-Mashat and Taylor, 1980), and from feces of calves and lambs with diarrhea (Firehammer and Myers, 1981). Enteritis in calves has been experimentally induced by oral inoculation of pure culture of Campylobacter fecalis (Al-Mashat and Taylor, 1981).

Bacteria and Intestinal Epithelum

There is an intimate association between microorganisms and the intestinal epithelium. Some microorganisms are specifically adapted to the intestine and may even be able to use the mucin as a source of carbon and energy (Savage, 1970). The early colonization of the intestine of newborn animals by normal microflora may act as a protective mechanism in preventing establishment of enteric infections. This mechanism is described as interference and inhibits multiplication of pathogens in the animals. Coliform organisms like E. coli and Aerobacter aerogenes are found to be the most antagonistic

for Shigella by inhibiting their multiplication in the intestine (Hentges, 1970).

Enterotoxigenic E. coli are found in close association with the intestinal epithelium, in contrast to non-enterotoxigenic E. coli which are usually confined to the lumen and rarely associated with the brush border (Bertschinger et al., 1972; Hadad and Gyles, 1982 a,b). This intimate epithelial association favors the retention and proliferation of ETEC and results in large numbers of ETEC colonizing the small intestinal epithelium and elaborating toxins leading to diarrhea (Hadad and Gyles, 1982 a; Miniats and Gyles, 1972; Smith, 1976; Suendsen, 1974). Among the ETEC isolated from calves and lambs, the K99 pilus is clearly implicated as a mediator of adhesion to the small intestine (Isaacson et al., 1978; Lariviere et al., 1979; Moon et al., 1976). Other types of enteropathogenic E. coli, which produce neither enterotoxin nor pili are considered invasive (Formal et al., 1978).

Lactobacilli are normally found in the intestinal tract of calves and adhesion of this organism to the intestinal epithelium has been recognized by some workers (Marshall et al., 1982). Some experiments, both in vivo and in vitro, have indicated that lactobacilli inhibit E. coli-induced enterotoxin reactions, perhaps by preventing E. coli from colonizing the jejunum and also by producing substances directed against the enterotoxins (Foster et al., 1980). Other experiments have found no

effect of lactobacilli on the course of enterotoxigenic E. coli diarrhea (Clements et al., 1981).

Disease Mechanism

In order for an enteropathogen to produce a disease, it must first be able to adhere to and colonize the intestinal mucosa. Several factors affect colonization of intestinal mucosa by pathogens. One of these is intestinal motility which helps to washout the microbes from the lumen of the small intestine toward the large intestine, where extensive colonization and proliferation occur. Another is the established flora of the normal gastrointestinal tract which profoundly influences subsequent colonization of the tract by pathogenic or non-pathogenic organisms (Savage, 1977). This may be due to competition for nutrients and space, and occurs mainly in large intestine and is not applicable to the small intestine because of the small numbers of bacteria normally residing there. The small resident population favors the colonization of pathogenic organisms in the small intestine. Other factors involved in colonization of the gut epithelium have also been proposed by some workers. These include adherence to the epithelial surface, colonization of mucin, oxygen, and diet (Savage, 1978). Bacterial pili, produced by some enteropathogenic E. coli, facilitate adhesion and colonization to the small intestial epithelium. Pili

implicated in intestial colonization of swine are K88, K99, and 987P, while K99 is the most important in calves and lambs. In man colonizing factors I and II (CFA I and II) are the most important E. coli pilus antigens. Specific receptors apparently govern the interactions of these pili with epithelial cells. These pili physically hold the pathogens to the small intestinal epithelium and allow the bacteria to resist the mechanical clearance effect of intestinal motility, which in turn enables them to proliferate to high numbers in the small intestine.

Immunity also plays a role in preventing calves from developing enteric disease. This immunity is mostly acquired from feeding colostrum after birth. Colostrum contains specific antibodies for the pathogens to which the dam has been exposed. Therefore, ingestion of colostrum during the first few hours of life is critical in order to supply the calf systemic, as well as local, protection.

Immunoglobulins in the intestinal tract block the target cells for the attachment of the enteropathogens (Rutter et al., 1976). Furthermore, delay in consumption of colostrum will decrease the immunoglobulins absorbed by the calves. In addition, the ability of intestinal epithelium to absorb immunoglobulins will be reduced, mainly due to the early establishment of the microorganisms in the intestinal tract. Staley et al. (1972) found that absorption of the immunoglobulins "whole horse serum" by the intestinal epithelium ceased after

monocontamination with E. coli. James and Polan (1978) also found that orally-administered duodenal fluid, as a source of intestinal microorganisms, influenced absorption of gamma-globulins of colostrum in newborn calves.

So, this local immunity, provided by ingestion of colostrum, will interfere with the stickiness of the enteropathogens to the intestinal epithelium, and these pathogens will be swept distally from the target cells by the action of peristaltic movement.

Pathogenesis of the Disease

Infectious diarrhea in man and other animals is produced by different pathogenic mechanisms, dependent upon the type of pathogen involved. Thus, recognition of the mechanisms involved in the pathogenesis of diarrhea in different diseases is useful in understanding, diagnosing, and managing enteric diseases (Moon, 1978). Besides invasiveness and heat-labile toxin (LT), or heat-stable toxin (ST) production by E. coli, other mechanisms have been described. Escherichia coli O15 (RDEC-1) produce diarrhea in rabbits without evidence of invasion or enterotoxin production (Cantey and Blake, 1977) This infection is characterized by destruction of microvilli and adherence of bacteria to the damaged luminal surface of the intestine in the absence of bacterial invasion or enterotoxin production (Takeuchi et al., 1978). This

mechanism might contribute to the many undiagnosed diarrheal diseases in man and animals. Ulshen and Rollo (1980) have described an infant with chronic diarrhea due to overgrowth of E. coli in the small intestine. This was also characterized by brush-border damage associated with adherent bacteria, remarkably similar in appearance to E. coli 015 (RDEC-1) infection in rabbits (Cantey and Blake, 1977; Takeuhi et al., 1978). Guerrant (1980) suggests at least a fourth mechanism in E. coli diarrhea, one that is associated with close adherence of the organism to small bowel mucosa without recognized invasive or enterotoxigenic properties.

Intestinal Bacteria and Malabsorption

Relationship of intestinal bacteria to malabsorption has been the subject of many investigations in human medicine. However, little in this area has been done in veterinary medicine. Normally, due in part to the mechanical cleansing action, the small intestine contains a very sparse bacterial population (Donaldson, 1970; Fossum and Liven, 1974). Any structural alteration in intestinal mucosa might alter intestinal motility which will create a favorable environment for overgrowth of bacteria in the small intestine. Intestinal bacterial overgrowth has many profound metabolic consequences which may lead to malabsorption, anemia, steatorrhea and vitamin deficiencies,

especially fat soluble vitamins A, D, E, and K (Drude and Hines, 1980; Kistler and Giannella, 1980; Neale et al., 1972). Strombeck et al. (1981) reported a case of chronic diarrhea in a dog which was diagnosed as intestinal bacterial overgrowth. The authors concluded that this case occurred because of a reduced acid concentration in the stomach which resulted in large numbers of bacteria reaching the small intestine. Simpson (1982) reported another case of a dog with intestinal bacterial overgrowth. Coliforms and Pseudomonas aeruginosa were the only organisms recovered. Intestinal malabsorption was present in both of these cases, apparently due to intestinal bacterial overgrowth (Simpson, 1982; Strombeck et al., 1981).

Intestinal bacteria can deconjugate bile salts. At normal intestinal pH, the bile salts function as detergents to incorporate the products of lipolysis - fatty acids and monoglycerides - into complex molecular particles called micelles. This micellarization helps the dispersion of these poorly water-soluble lipolytic products and allows their nearly complete absorption by intestinal mucosa. The presence of a high number of bacteria in the small intestine might lead to excessive bile salt deconjugation and reduce their concentration below that critical for micelle formation. This would in turn impair micelle formation and finally disrupt the process of lipid absorption (Kistler and Giannella, 1980). In order for

clinically significant malabsorption to occur, a complex flora of strict anaerobes and coliforms, which have the capacity to deconjugate bile salts and to bind vitamin B_{12}, should be present in numbers greater than 10^6 organisms/ml in the proximal small intestine (Kistler and Giannella, 1980; Strombeck et al., 1981). Intestinal lactobacilli also have been recognized to deconjugate bile salts when present at high concentration (Gilliland and Speck, 1977)

Fat malabsorption is one of the most important consequences of intestinal bacterial overgrowth. In addition to steatorrhea it may also lead to deficiencies of fat soluble vitamins. A high concentration of unabsorbed fatty acids in the distal small intestine and colon can contribute to further diarrhea. This mechanism is being described as a conversion of unabsorbed dietary fatty acids to hydroxy fatty acids by enzymes of enteric bacteria (Thomas, 1972). It has been suggested that hydroxy fatty acids might be critically important in the genesis of diarrhea associated with steatorrhea.

Deconjugated bile salts themselves have an injurious effect on intestinal mucosa, which results in disruption of intestinal microvilli and may further contribute to malabsorption (Ament et al., 1972; Shimoda et al., 1974; Teichberg et al., 1981). However, the presence of a high concentration of deconjugated bile acids has an inhibitory effect on intestinal pathogens (Floch et al.,

1970, 1972), thus helping to maintain a favorable balance among species present in the intestinal tract.

MATERIALS AND METHODS

Experimental Animals

Thirteen male Holstein calves, 5-21 days old were subjected to this study. Five calves were from nearby farms experiencing calf diarrhea as a herd problem. An additional four calves were purchased as controls but spontaneously developed diarrhea prior to the experiment. Four healthy, colostrum-fed calves were used as controls.

The calves were housed in metabolism cages and fitted with fecal collection devices consisting of an anal cannula attached to a plastic bag. The cannula was fashioned from the barrel of a 20 ml syringe. The bag was suspended by sutures in the skin over the sacrum. For the first four experimental days the calves were fed whole bovine milk (3.8% butterfat) at approximately 10% of body weight. The diet was divided into two equal feedings per day. On days three and four, quantitative fecal collections were made. On the fifth day, lactose digestion and xylose absorption tests were performed. The results of the latter will not be reported in this thesis. On the sixth day intestinal samples were taken and the calves were euthanatized.

Sample Collection

The calves were sedated with 100 mg of xylazine, the abdominal midline infiltrated with 2% lidocaine, and the abdomen aseptically opened. Three 5 cm segments of intestine were taken from the duodenum, jejunum, ileum and colon. The duodenum segments were taken approximately 100 cm distal to the common bile duct at the site where the mesoduodenum starts to form a long mesentery. The jejunum samples were taken approximately 700 cm distal to the common bile duct and the ileum segments taken 100 cm proximal to the ileocecal valve. The colon samples were taken near the flexure of the spiral colon.

Each intestinal segment was ligated at each end and left *in situ*. After all ligatures had been placed, the calf was euthanatized. Formalin (10%) was injected into one sample from each intestinal segment for histopathological study. Tissue Tek[a] was injected into another sample from each intestinal segment for fluorescent antibody study. The third sample was used for quantitative bacterial counts. Then the segments were quickly removed and placed on ice.

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[a]Lab-Tek Products, Division Miles Laboratories, Inc., Naperville, Illinois 60540.

Quantitative Bacterial Counts

The volume of each intestinal segment was measured by displacement in normal saline. The segments were then homogenized in a sterile Waring blender jar containing a measured volume (20 or 50 ml) of pre-reduced medium 10 (Anaerobe Laboratory Manual, 1972) and an atmosphere of sterile CO_2.

The total bacteria count, which included anaerobes and facultative anaerobes, was determined by dilution in medium 10 (Table 1). Three replicates of 10, 10-fold serial dilutions were made with 1 ml aliquots of homogenate. The medium was prepared in Hungate tubes and inoculated according to Hungate (Anaerobe Laboratory Manual, 1972). The cultures were incubated for four days at 37 C. The highest dilution in which growth occurred was averaged over each of the three replicates and taken to be the reciprocal of the base 10 logarithm of the bacterial concentration of the homogenate, in bacteria per ml. This value was then multiplied by the total volume of the homogenate (in milliliters) to calculate the number of bacteria per 5 cm of intestine.

The number of aerobes and facultative bacteria was determined from the same homogenate by a similar dilution technique; the difference being that the dilutions were made in brain-heart-infusion media under normal atmosphere.

TABLE 1

Media used for determination
of viable counts of various bacteria

Organism	Media	Atmosphere
Coliforms	MacConkey[a]	Aerobic
Lactobacilli	Lactobacillus (LBS)[b] agar	Anaerobic (Anaerobe jars)
Total bacteria	Pre-reduced medium 10[c] (Hungate tubes)	Anaerobic (Hungate tubes)
Aerobes and facultative anaerobes	Brain heart infusion[a]	Aerobic

[a]Difco Laboratories, Detroit, MI
[b]BBL, Division of BioQuest, Cockeysville, MD 21030
[c]Anaerobe Laboratory Manual, p. 21
The Virginia Polytechnic Institute and State University
Anaerobe Laboratory, Blacksburg, Virginia. 1972.

These cultures were incubated at 37 C for 18-24 hrs aerobically.

The numbers of coliforms and lactobacilli were determined by a spread plate technique. A sample (0.1 ml) was taken from first to fourth (for normal calves) and second to sixth (for calves with diarrhea) brain-heart-infusion dilutions and spread with a sterile glass rod over the surface of a MacConkey agar plate and lactobacillus

selective (LBS) plate (Table 1). The cultures were incubated at 37 C for 24 hr; the MacConkey plate was incubated aerobically and the LBS plate was under CO_2 atmosphere. Counts were taken from those plates with between 30 and 300 colonies, except in some cases where the highest dilution resulted in more than 300 colonies. The original concentration was calculated by the following formula:

$$Con = 10 \times 1/10^{-a} \times b$$

where Con is the original concentration in bacteria per 5 cm of intestinal segment, 10^{-a} is the dilution from which the plate inoculum was taken, and b is the volume of the homogenate. The constant factor of 10 accounts for the 0.1 ml of sample which was spread on the plate.

K99 Antigen Detection

K99 Antigen was detected by using the slide agglutination test and the indirect fluorescent antibody technique.

1) Slide Agglutination - This was done by streaking a MacConkey plate from a 18-24 hrs BHI broth culture of the ileal segment. Ileum was chosen because it was considered to be a site frequently colonized by $K99^+$ ETEC (Isaacson et al., 1978). The plate was incubated at 37 C

for 18-24 hrs. Colonies (5-6) chosen at random were inoculated onto Minca Agar Medium plus 1% Iso Vitale X (Guinee et al., 1977), and incubated at 37 C for 18-24 hrs. Samples were taken off the medium from areas of confluent bacterial growth. These were tested for agglutinaion by standard anti K99 serum. An isolate was designated as $K99^+$ if at least one of the three tests was positive. If not, it was considered as $K99^-$.

2) Fluorescent Antibody Technique - Indirect fluorescent antibody technique was used to detect $K99^+$ ETEC. A frozen section of ileum from each calf was treated with a specific immunofluorescent stain (Moon et al., 1977).

Detection of Other Causative Agents

Examination for the presence of rotaviruses and coronaviruses was also made using the fluorescent antibody tissue section technique as described previously (Morin et al., 1974).

The presence of Cryptosporidium spp. was determined by histopathologic examination of small intestinal mucosa.

Fecal Fat Determination

The fat content of each 24 hr fecal collection was determined by the method of Jover and Gordon (1962).

Initially the fat was saponified in strong base. Then the solution was acidified and the fatty acids extracted in toluene. The toluene was evaporated, and fatty acids were dissolved in ethanol. Finally, the solution was titrated to neutrality with NaOH and the fatty acid content calculated to equal the amount of NaOH on a molar equivalent basis. The milligram quantity of neutral fat in the original fecal sample was calculated by multiplying the molar equivalents of fatty acid by 284, the average molecular weight of the fatty acids in milk, and then multiplying by the dilution factor arising in the technique.

The coefficient of fat digestion was calculated by dividing the 24 hr fecal fat content by 24 hrs fat consumption and multiplying by 100.

Data Analysis

Intestinal bacterial contents in normal calves and calves with diarrhea were compared by the Wilcoxon Rank Sum Test (Rimm et al., 1980).

Differences in bacterial counts among segments were analyzed by the Kruskal Wallis Test (Sokal and Rohlf, 1969).

Spearman's Rank Correlation Coefficient (Steel and Torrie, 1960) was used to evaluate the correlation between fecal quantity and fecal fat excretion. The same test was

used to find the correlation between total bacterial counts and fecal fat excretion.

RESULTS

Bacterial Numbers

Calves with diarrhea had significantly more viable coliforms ($p<0.01$) and lactobacilli ($p<0.04$) than normal calves in duodenum segment (Figs. 1,2 and Tables 2,3), while total viable bacteria and total viable aerobes-facultative anaerobes were approximately the same in these two groups of calves.

The mean counts of all types of viable bacteria in jejunal segments were higher in calves with diarrhea than in normal calves; however, the differences were significant ($p<0.05$) only in regard to coliforms (Fig. 1 and Table 2).

The numbers of all types of bacteria studied in ileal segments were higher in calves with diarrhea than in normal calves. The variation was most marked in total aerobes-facultative anaerobes, coliforms and total bacteria with 100,000, 10,000 and 1,000-fold differences, respectively ($p<0.001$) (Figs. 4,1,3 and Tables 5,2,4), whereas lactobacilli were 10 to 100 fold higher in calves with diarrhea than normal calves ($p<0.01$) (Fig. 2 and Table 3).

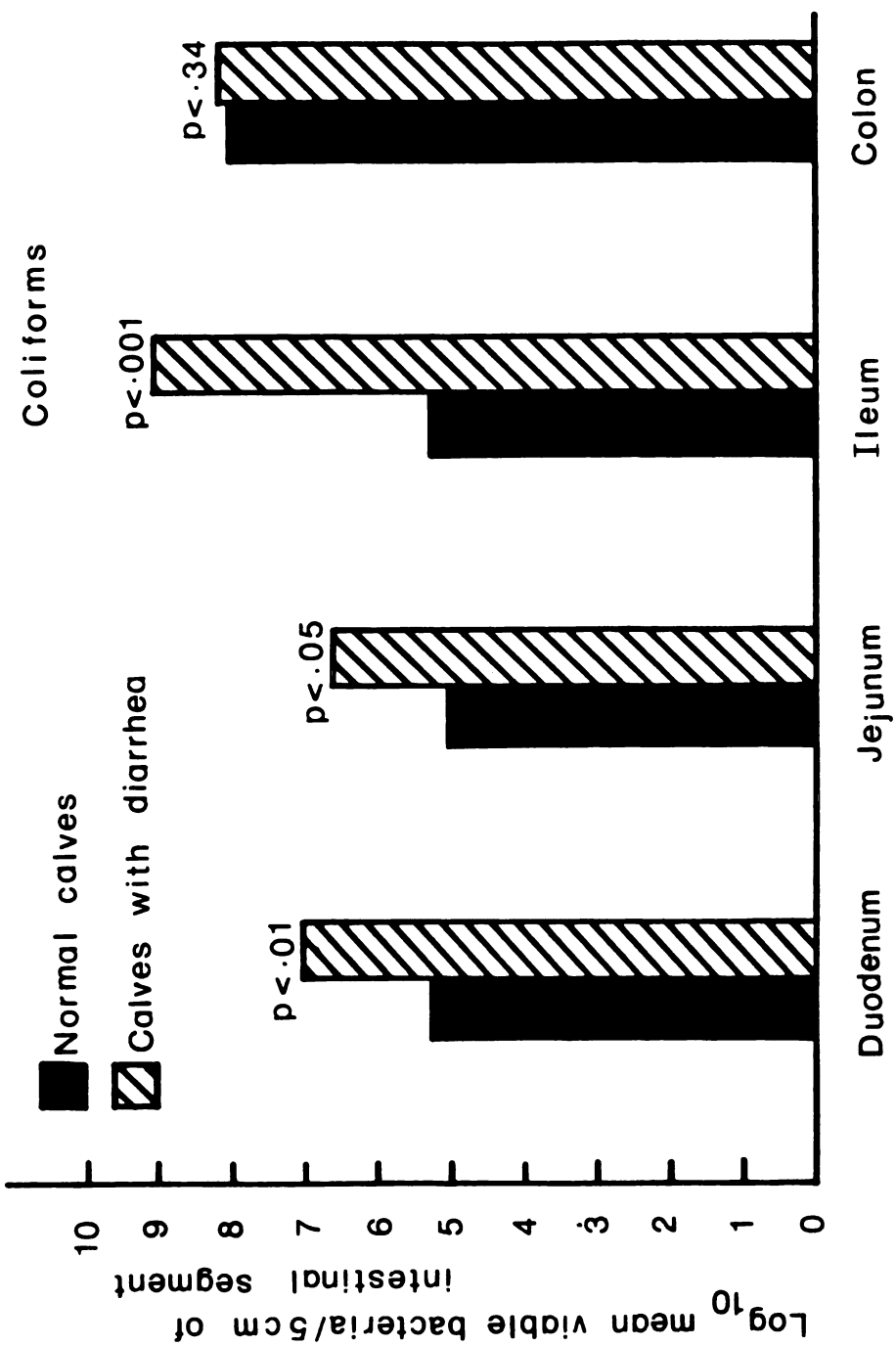

Fig 1 – Log_{10} mean viable coliforms count / 5cm of intestinal segment

TABLE 2

Coliform counts in intestinal segments
of normal calves and calves with diarrhea

Intestinal segments	Classification of calves						P value
	Normal			Diarrhea			
	N	Range	$\bar{x}$	N	Range	$\bar{x}$	
Duodenum	4	(4-6)	5.25[a]	9	(5-9)	7.1	<0.01
Jejunum	4	(4-6)	5.0	8	(4-10)	6.6	<0.05
Ileum	4	(4-6)	5.25	9	(8-10)	9.0	<0.001
Colon	4	(6-10)	8.0	8	(6-10)	8.1	<0.34

[a] Log_{10}/5 cm of intestinal segment

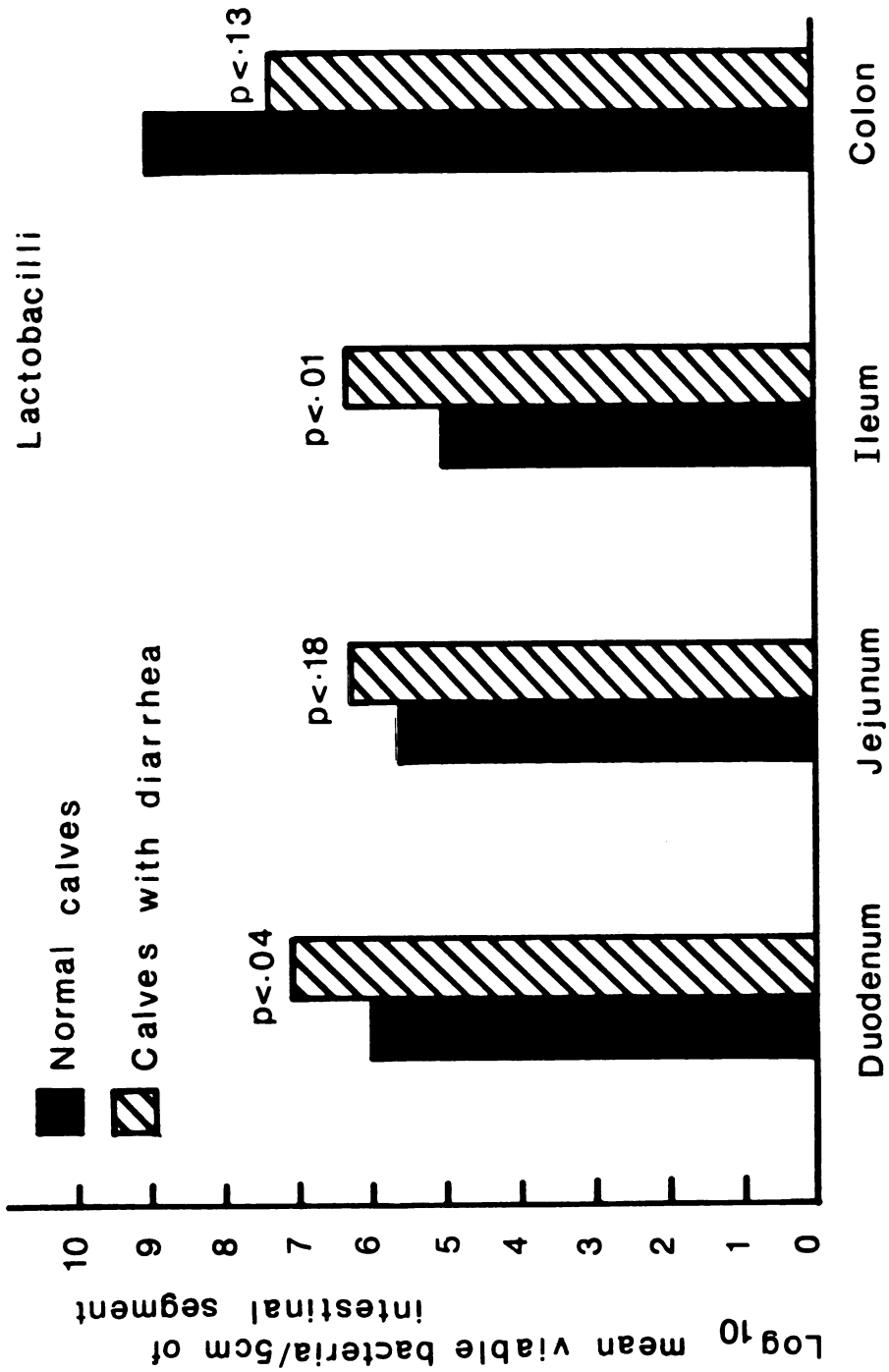

Fig 2 — Log_{10} mean viable lactobacilli count / 5 cm of intestinal segment

TABLE 3

Lactobacilli counts in intestinal segments of normal calves and calves with diarrhea

Intestinal segments	Classification of calves						P value
	Normal			Diarrhea			
	N	Range	$\bar{x}$	N	Range	$\bar{x}$	
Duodenum	3	(6)	6.0[a]	8	(5-9)	7.1	<0.04
Jejunum	3	(4-7)	5.6	8	(5-8)	6.25	<0.18
Ileum	3	(4-6)	5.0	8	(5-8)	6.37	<0.01
Colon	3	(9)	9.0	8	(5-9)	7.37	<0.13

[a] Log_{10}/5 cm of intestinal segment

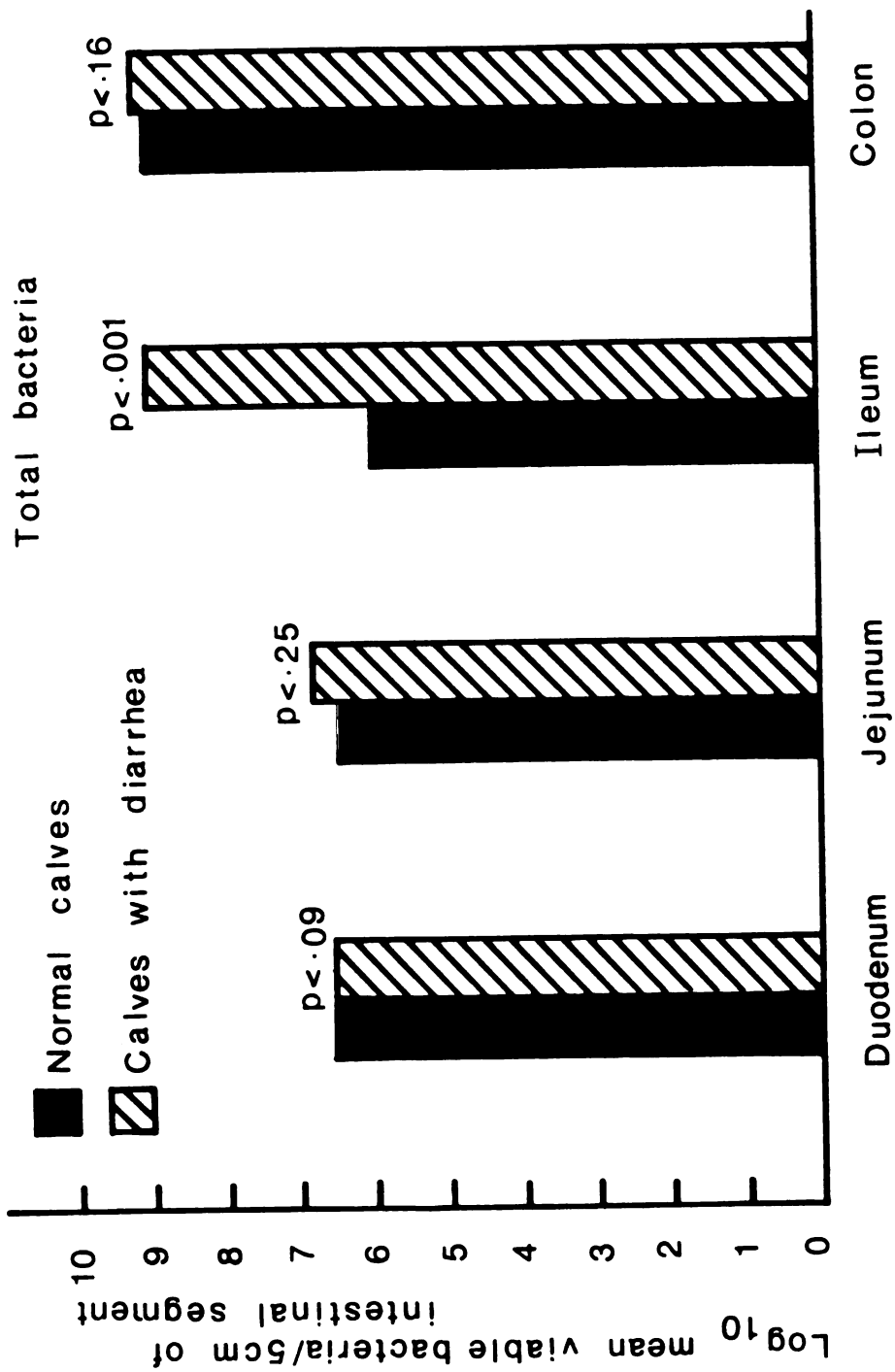

Fig 3 – Log_{10} mean viable total bacteria count/5cm of intestinal segment

TABLE 4

Total bacteria counts in intestinal segments of normal calves and calves with diarrhea

Intestinal segments	Classification of calves						P value
	Normal			Diarrhea			
	N	Range	$\bar{x}$	N	Range	$\bar{x}$	
Duodenum	4	(5-7)	6.5[a]	9	(5-11)	6.5	<0.09
Jejunum	4	(5-8)	6.5	9	(3-11)	6.8	<0.25
Ileum	4	(5-7)	6.0	9	(8-10)	9.0	<0.001
Colon	4	(5-11)	9.0	9	(5-11)	9.2	<0.16

[a] Log_{10}/5 cm of intestinal segment

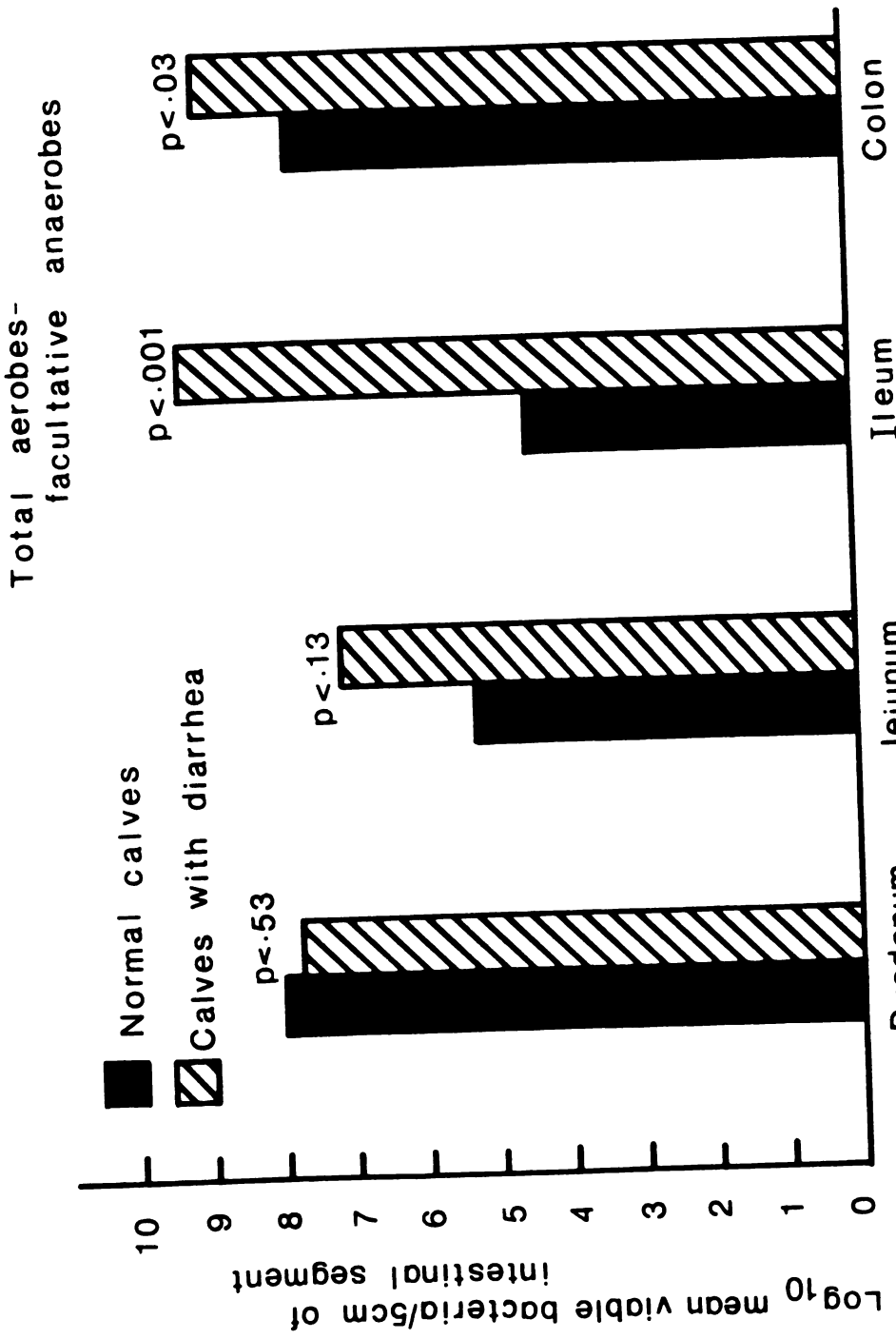

Fig 4 – Log_{10} mean viable total aerobes-facultative anaerobes count / 5cm of intestinal segments

TABLE 5

Total aerobes-facultative anaerobes counts
in intestinal segments of normal calves and calves with diarrhea

Intestinal segments	Classification of calves						P value
	Normal			Diarrhea			
	N	Range	$\bar{x}$	N	Range	$\bar{x}$	
Duodenum	4	(7-11)	8.0[a]	9	(5-11)	7.7	<0.53
Jejunum	4	(4-6)	5.25	9	(4-11)	7.1	<0.13
Ileum	4	(3-7)	4.5	9	(8-11)	9.3	<0.001
Colon	4	(5-10)	7.75	9	(8-11)	9.0	<0.03

[a] Log_{10}/5 cm of intestinal segment

In the colonic segment, total coliforms and total bacteria were the same in both diarrhea and normal calves (Fig. 1,3 and Tables 2,4). Total aerobes-facultative anaerobes were significantly ($p<0.03$) higher in calves with diarrhea than normal calves (Fig. 4 and Table 5). Lactobacilli counts were 100 to 1000 fold higher in normal than calves with diarrhea (n.s) (Fig. 2, Table 3).

Differences in viable counts of bacteria among the four intestinal segments in calves with diarrhea were also investigated. The total coliform and total bacteria numbers varied significantly among the intestinal segments ($p<0.05$ and $p<0.025$, respectively) (Table 6). Differences in counts of aerobes-facultative anaerobes and lactobacilli were not significant.

Enteropathogenic Agents

Enterotoxigenic *E. coli* was also investigated by demonstrating the K99 pilus antigen by slide agglutination and FA. The results revealed two diarrhea calves (#8 and #10) which were positive in both slide agglutination and FA (Table 7). No K99 antigen was detected in any of the normal calves.

Rotavirus and coronavirus were also investigated by using the FA technique. The results revealed that only one calf with diarrhea (#10) was positive for rotavirus

TABLE 6

Differences in viable bacterial counts
among the intestinal segments in calves with diarrhea

Bacteria	Intestinal segments				
	Deudenum	Jejunum	Ileum	Colon	p[a]
Coliforms	7.0[b] (5-9)[c]	6.6 (4-10)	9.0 (8-10)	8.1 (6-10)	<0.05
Total Bacteria	6.5 (5-11)	6.8 (3-11)	9.0 (8-10)	9.2 (5-11)	<0.025
Aerobes-facultative anaerobes	7.7 (5-11)	7.1 (4-11)	9.3 (8-11)	9.0 (8-11)	NS
Lactobacilli	7.1 (5-9)	6.25 (5-8)	6.37 (5-8)	7.37 (5-9)	NS

[a]Probability of no difference between means

[b]Data expressed as mean Log_{10}/5 cm of intestinal segment

[c]Values in parenthesis are ranges

NS - $p>0.05$

TABLE 7

Distribution of enteropathogenic agents
in normal calves and calves with diarrhea

	Calf #	K99		Rotavirus	Coronavirus	Cryptosporidium
		Slide Aggl.	FA	FA	FA	Histopathology
Normal	26	-	-	-	-	-
	2	-	-	-	-	-
	13	-	-	-	-	-
	14	-	-	-	-	-
Diarrhea	1	-	-	-	-	-
	6	-	-	-	-	+
	8	+	+	-	-	+
	9	-	-	-	-	-
	10	+	+	+	-	-
	11	-	-	-	-	-
	12	-	-	-	-	-
	25	-	-	-	-	-
	47	-	-	-	-	-

FA - Fluorescent antibody technique

(Table 7). This calf was also positive for K99 E. coli, as previously stated.

Cryptosporidium, a protozoan parasite was also found histopathologically in two calves with diarrhea (#6 and #8) (Table 7). Cryptosporidium was the only enteropathogen isolated from calf #6, while calf #8 had a dual infection with Cryptosporidium and $K99^+$ E. coli.

The total numbers of viable coliforms in the ileal segment of K99 positive E. coli calves (#8 and #10) were 10^8/5cm segment for both calves, while these numbers were 10^{10}/5cm segment in Cryptosporidium infected calf (#6). These numbers were not greater than those in the other calves with diarrhea in which known enteropathogens were not identified.

The total bacteria and total aerobes-facultative anaerobes in ileum segment of calves #6,#8, and #10, were between 10^8-10^{10}/5cm segment. These, again, were not higher than in the other calves with diarrhea without known enteropathogens.

Fat Digestion

Fecal fat appearing in the feces of calves during diarrhea was also investigated in this experiment. Percentage of daily fat intake appearing in the feces of normal calves was between 0.7% and 3.3% (Table 8), whereas in calves with diarrhea it was between 15.6% and 102%

(Table 9). The total fecal fat output (expressed as the percentage of daily fat intake) was positively correlated with fecal quantity ($R=.67$, $p<0.01$).

The regression of fecal quantity on fat content is:

$Y = 44.3 \times +403.9$
where,
Y = gram feces/day
X = fecal fat
as presented in Figure 5.

The correlation coefficients between each class of intestinal bacteria studied and fecal fat were also determined (Table 10). The strongest positive correlation between fecal fat and bacterial numbers occurred in the ileum. The correlation coefficients for coliforms, total bacteria and total aerobes-facultative anaerobes were $R=.72$, $.67$, ($p<0.01$) and $.59$ ($p<0.025$), respectively (Table 10). Significant correlations were also found between fecal fat and total aerobes-facultative anaerobes in the colon ($R=.55$, $p<0.05$), lactobacilli in the duodenum and ileum ($R=0.55$ with $p<0.05$), and coliforms in duodenum ($R=.47$ with $p<0.05$) (Table 10).

TABLE 8

Fecal quantity and fat content of normal calves

Calf #	Fecal Wt (gm)	Fat %[a]
2626	176	3.3
2	95	1.1
13	63	1.3
14	135	0.7

TABLE 9

Fecal quantity and fat content of calves with diarrhea

Calf #	Fecal Wt (gm)	Fat %[a]
1	1,385	25.0
6	2,204	45.0
8	10,254	44.0
9	1,988	22.0
10	1,199	15.6
11	3,741	83.0
12	1,065	60.0
25	5,672	102.0
47	1,152	57.0

[a] % of daily fat intake

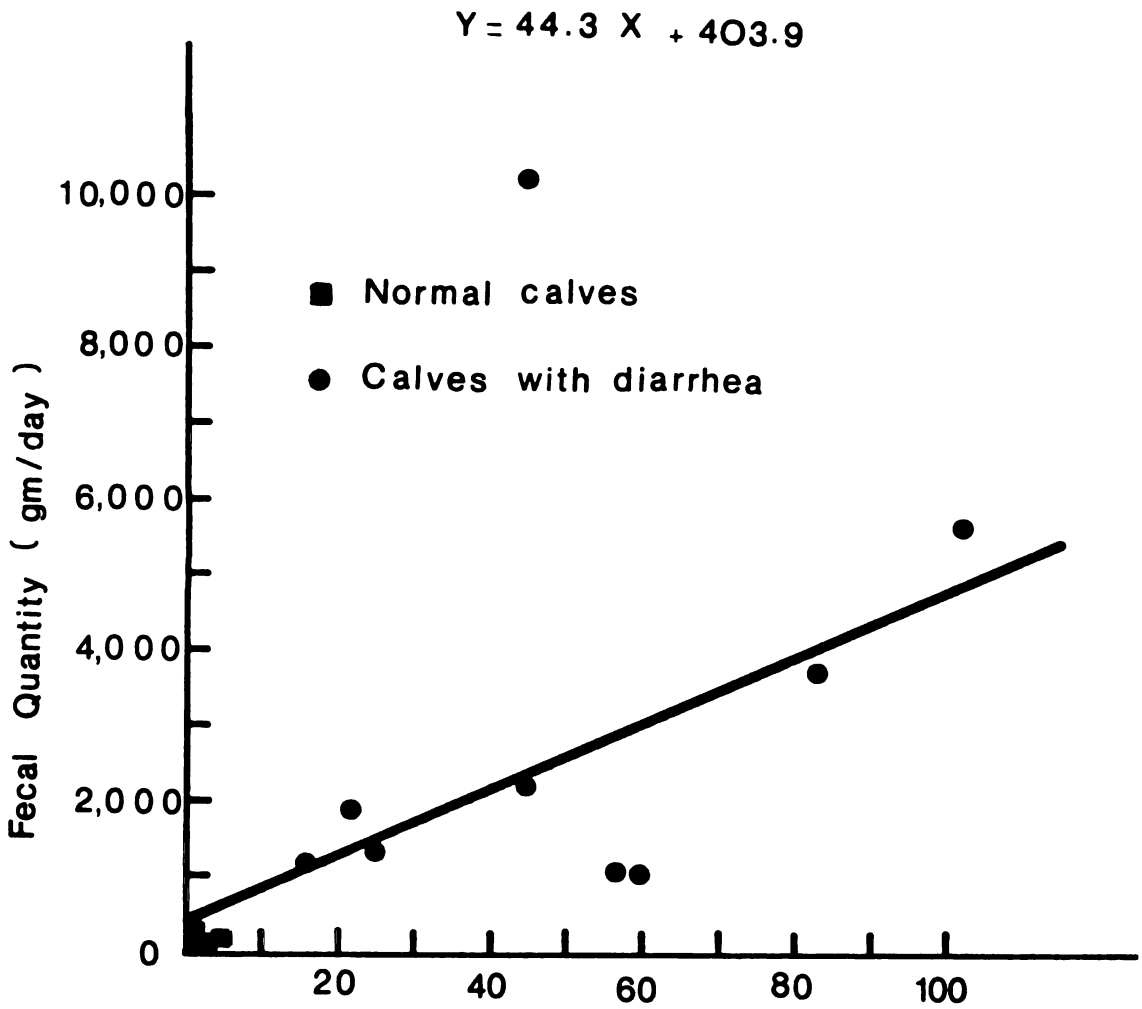

Fig 5 – Regression of Fecal Quantity on Fecal Fat

TABLE 10

Correlation coefficients
between bacterial concentration and fecal fat content

Intestinal segments	Coliforms		Total bacteria		Aerobes		Lactobacilli	
	RHO*	P	RHO	P	RHO	P	RHO	P
Duodenum	0.47	<0.05	0.13	NS	0.01	NS	0.55	<0.05
Jejunum	0.005	NS	-0.14	NS	0.11	NS	-0.32	NS
Ileum	0.72	<0.01	0.67	<0.01	0.59	<0.025	0.55	<0.05
Colon	0.02	NS	0.31	NS	0.55	<0.05	-0.32	NS

NS - p>0.05
* Correlation coefficient

The regression of ileal coliform counts on fecal fat content is:

$$Y = .0377\ X + 6.5$$

where:

$Y = \text{Log}_{10}$ viable coliforms

X = Fecal fat

as presented in Figure 6.

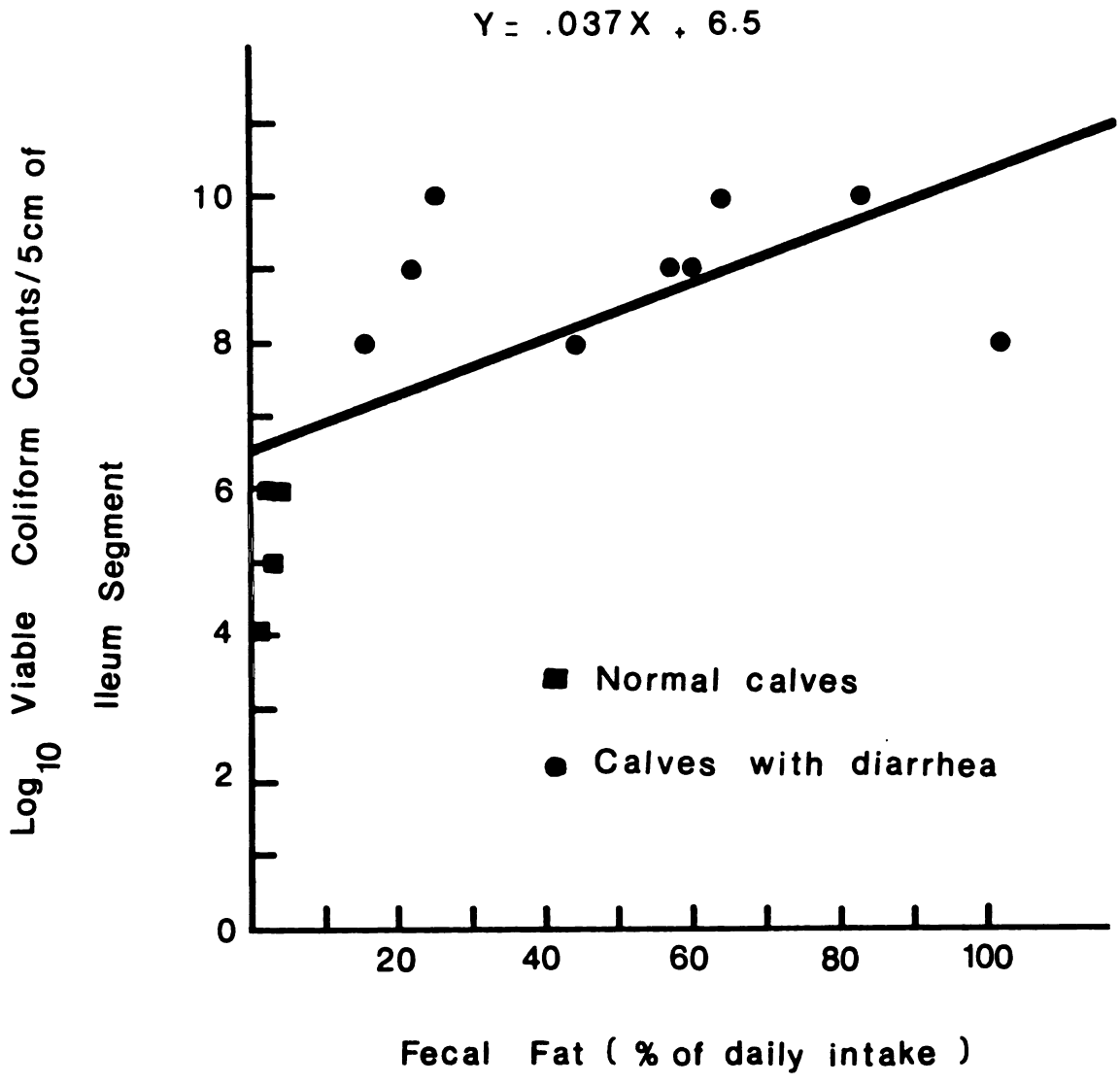

Fig 6 _ Regression of Coliform Counts on Fecal Fat

DISCUSSION

The results of this experiment indicated that total viable bacterial counts of all types of organisms studied were higher in the small intestine of calves with diarrhea than in normal calves.

In normal calves, a mixed population of bacteria existed over the length of the intestine. The duodenal segment showed that the total aerobes-facultative anerobes were 10-fold higher than the total bacteria (Figs. 3 and 4). The difference was not due to coliform numbers, because the latter were 1000-fold lower than the total aerobes-facultative anaerobes (Fig. 1). The discrepancy might have been caused by some gram positive cocci, like streptococci which passed from the oral cavity, resisted abomasal acidity, passed through the abomasum, and remained at high concentration in the duodenum.

In the jejunum and ileum, the total aerobes-facultative anaerobes dropped 10-fold below the total bacteria, while the numbers of coliforms remained approximately equal to total aerobe-facultative anaerobes numbers. This would indicate that almost all the total aerobes-facultative anaerobes in the jejunum and ileum were

coliform organisms. Therefore, the 10-fold difference between the total aerobes-facultative anaerobes and total bacteria could be due to some anaerobic organisms which grow well in prereduced medium 10. These anaerobes probably were Clostridium perfringens and Lactobacilli organisms. Bacteriodes spp. are not usually found in the normal small intestine, but are only found in the large intestine with the lactobacilli, they constitute the largest proportion of the fecal flora (Mylrea, 1969; Smith and Crabb, 1961; Smith, 1962).

Total bacteria increased markedly in the colon and were 10-fold higher than the total aerobes-facultative anaerobes and coliforms (Figs. 3, 4 and 1). The difference was accounted for by lactobacilli, which existed in very high numbers in the colon (Fig. 2).

In calves with diarrhea, the total aerobe-facultative anaerobes numbers were similar to the total bacteria except in the duodenum, in which the total aerobes-facultative anaerobes were 10-fold higher than the total bacteria. Again, as in the normal calves, this discrepancy may be due to oral flora not adapted to growth in medium 10. Throughout the small intestine coliform numbers were approximately equal to total bacteria and total aerobes-facultative anaeroes.

In the colon, coliforms were 10-fold lower than total bacteria. This difference did not appear to be made up by

lactobacilli (Fig. 3), which were about 100-fold lower than the total bacteria (Fig. 2).

Various factors may have accounted for the differences in bacterial numbers in the small intestine between calves with diarrhea and normal calves. One probable factor is altered intestinal motility. Normal intestinal motility is considered to be the single most important factor in maintaining the low bacterial population in the small intestine (Drasar et al., 1969). Gastrointestinal motility is reduced in calves with diarrhea. Bueno et al. (1980) found a low level of spiking activity in the abomasum, small intestine and colon during infectious diarrhea of calves, indicating reduced motility. Thus reduced intestinal motility may have led to bacterial overgrowth in calves with diarrhea in this study.

Gastric acid also contributes to maintaining the relative sterility of the proximal bowel (Drasar et al., 1969). So, altered gastric acid secretion may also influence intestinal bacterial numbers (Strombeck et al., 1981). Although the effect of infectious diarrhea on gastric acid secretion in the calf does not appear to have been investigated. In general, factors such as nervous and endocrine stimulation, which promote gastric motility, also promote gastric acid secretion. Calves with diarrhea may have reduced gastric motility, resulting in reduced gastric acid secretion. Thus fewer bacteria are killed in the

abomasum. These factors may contribute to small intesinal bacterial overgrowth.

One other factor that might have led to high numbers in the small intestine of calves with diarrhea is the interference with the normal mucosal integrity. Mucins found in the mucous coat lining intestinal epithelial surfaces have molecular structure similar to components of the epithelium that appear to act as receptors for microorganisms or their toxins (Springer, 1970; Strombeck and Harrold, 1974). Thus, bacterial attachment sites may bind to mucin rather than enterocytes. This would allow them to be washed out of the gut with the normal flow of mucus. Rotavirus or cryptosporidia, which were identified in some cases of diarrhea in our experiment, or other unknown pathogens, might have altered or destroyed the intestinal mucous coat, thus allowing bacterial proliferation.

The intestinal immune system, as well as the mucosa may have been altered in the calves with diarrhea. Specific infectious organisms, such as viruses and cryptosporidia, may have compromised the intestinal immune system and thus allowed for proliferation of normally present nonpathogens. Small intestinal bacterial overgrowth might also have resulted from deficiency of immunoglobulins supplied to calves during their life. Immunodeficient patients have increased numbers of intestinal bacteria as compared to normal patients

(Ament et al., 1973; Brown et al., 1972; Hersh et al., 1970).

High intestinal bacterial concentrations may also have resulted from malnutrition. Mata et al. (1972) found proliferation of bacteria in the small intestine associated with protein-calorie malnutrition.

Bacterial adherence factors also might have played a role in bacterial proliferation in the small intestine. Calves #8 and #10 had K99 positive ETEC and they had coliform counts of 10^8/5 cm of ileum. These results are similar to those of Isaacson et al. (1978) with respect to the numbers of K99 positive E. coli/5 cm segment found in calves with diarrhea. However, my results are in contrast with those of Isaacson with respect to the numbers of coliforms found per 5 cm segment in calves with diarrhea without K99 positive E. coli. Coliform counts in those cases were as high or higher than 10^8/5 cm segment. This may be due to other adherence factors. Infection with strain RDEC-1 E. coli as described by Cantey and Blake (1977), and Takeuchi et al. (1978) is characterized by destruction of microvilli and adherence of bacteria to the luminal surface without bacterial invasion or enterotoxin production. The existence of this type of bacteria as a pathogen for calves has not been proven, but it may occur and might have contributed to bacterial proliferation in some of the diarrheal cases. Other unknown adherence factors may also exist and could account

for some cases of bacterial overgrowth. It could also be that in the presence of reduced motility, adherence factors are not necessary for bacterial proliferation in the small intestine.

The presence of high numbers of bacteria in the small intestine may have many profound metabolic consequences. These include maldigestion and malabsorption of essential nutrients. One probable factor which might have led to maldigestion in the calves with diarrhea, is bile acid deconjugation. Bile acids are deconjugated in the presence of large numbers of coliforms, such as occurred in this experiment. Excessive bile acid deconjugation leads to fat malabsorption and steatorrhea (Drude and Hines, 1980; Kistler and Giannella, 1980; Neale et al., 1972). Fat malabsorption was strongly correlated with bacterial deconjugation in experiments of others (Kim et al., 1966; Rosenberg et al., 1967; Tabaqchali et al., 1968).

In addition to inducing steatorrhea, deconjugation of bile salts has a direct injurious effect on intestinal mucosa which may have resulted in disruption of intestinal microvilli and may have further contributed to malabsorption (Ament et al., 1972; Shimoda et al., 1974; Teichberg et al., 1981).

Maldigestion and malasorption might also have resulted from infectious agents which were present at the time of the absorption tests but were not identified at

post mortem. In most cases the calves developed diarrhea on the farm and were affected for at least 12 hours before being brought into the clinic. After arrival at the clinic, a minimum of five days was required to complete the digestive studies. So, it was six to seven days, and in some cases more, after the onset of diarrhea that the calves were necropsied. This period of time may have been sufficient for some of the calves to have eliminated their primary infectious agents. Thus, unidentified agents may have occurred and resulted in mucosal damage and further contributed to malabsorption. Halpin and Caple (1976) concluded that calves with diarrhea associated with reovirus-like agent have a reduced ability to utilize dietary lactose. This was due to reduced mucosal beta-galactosidase activity which was associated with abnormal mucosal morphology, as indicated by a decreased villus/crypt length ratio. Such lesions cannot be assessed from data presented in this experiment, but may have occurred and contributed to malabsorption.

Unabsorbed fat and lactose may contribute further to diarrhea. Unabsorbed fat in the jejunum and ileum are modified by bacteria to hydroxylated fatty acids, which will stimulate jejunal and ileal secretion of water and electrolytes (Ammon and Phillips, 1974; Ammon et al., 1974). Hydroxylated fatty acids in the colon also stimulate colonic secretion of water and electrolytes.

Unabsorbed lactose will further be metabolized by bacteria to a variety of osmotically active molecules, creating an osmotic diarrhea similar to that seen in lactase deficiency. In addition, conjugated and deconjugated bile acids reaching the colon also stimulate colonic secretion of water and electrolytes (Hofmann, 1969).

Known enteropathogens may have initiated many of the cases of diarrhea investigated in this thesis. However, these organisms appeared not to be responsible for the genesis of persistent diarrhea. Proliferation of intestinal bacteria, in spite of the cause, might be responsible in some cases of diarrhea in calves. Cohen et al. (1967) and Gorbach et al. (1971) concluded that any type of diarrhea may lead to increased intestinal bacterial concentrations. Intestinal bacterial overgrowth leads to malabsorption of essential nutrients which further contribute to diarrhea which might be responsible for some chronic cases of diarrhea.

CONCLUSION

From the results of this experiment, it was concluded that bacterial overgrowth of non-pathogenic bacteria in the small intestine may play a role in some cases of neonatal calf diarrhea. Small intestinal bacterial overgrowth is known to cause fat malabsorption in some species. Fat malabsorption may, in turn, lead to diarrhea due to the effects of unabsorbed fats in the distal ileum and colon. Thus, fat malabsorption may be a cause of some cases of diarrhea occurring in calves with small intestinal bacterial overgrowth, as suggested by the results of this experiment.

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APPENDIX

*Data of total coliform counts in normal calves

Calf #	Intestinal Segments			
	Duodenum	Jejunum	Ileum	Colon
26	4	5	5	8
2	7	6	6	6
13	4	4	6	8
14	6	5	4	10

*Data of total coliform counts in calves with diarrhea

Calf #	Intestinal Segments			
	Duodenum	Jejunum	Ileum	Colon
1	7	4	10	9
6	8	10	10	10
8	9	5	8	9
9	7	9	9	6
10	5	6	8	9
11	8	9	10	No Data
12	6	4	9	6
25	7	No Data	8	7
47	6	6	9	9

*Data expressed as Log_{10}/5 cm segment

*Data of total Lactobacillus counts in normal calves

Calf #	Intestinal Segments			
	Duodenum	Jejunum	Ileum	Colon
26	6	4	5	9
**2	-	-	-	-
13	6	7	6	9
14	6	6	4	9

*Data of total Lactobacillus counts in calves with diarrhea

Calf #	Intestinal Segment			
	Duodenum	Jejunum	Ileum	Colon
**1	-	-	-	-
6	6	7	7	8
8	8	6	6	7
9	7	7	6	5
10	8	8	7	8
11	9	7	8	9
12	7	5	6	7
25	7	5	6	6
47	5	5	5	9

*Data expressed as Log_{10}/5 cm segment

** Lactobacillus counts were not done for these calves

*Data of total bacteria counts in normal calves

Calf #	Intestinal Segments			
	Duodenum	Jejunum	Ileum	Colon
26	7	5	7	9
2	5	6	5	5
13	7	8	5	11
14	7	7	7	11

*Data of total bacteria counts in calves with diarrhea

Calf #	Intestinal Segments			
	Duodenum	Jejunum	Ileum	Colon
1	8	11	10	11
6	5	7	10	11
8	9	6	8	11
9	8	8	9	5
10	7	8	9	9
11	11	9	10	11
12	6	3	9	6
25	7	5	8	8
47	6	5	8	11

*Data expressed as Log_{10}/5 cm segment

*Data of total aerobe-facultative anaerobe counts in normal calves

Calf #	Intestinal Segments			
	Duodenum	Jejunum	Ileum	Colon
26	8	5	4	8
2	11	6	7	5
13	6	4	3	8
14	7	6	4	10

*Data of total aerobe-facultative anaerobe counts in calves with diarrhea

Calf #	Intestinal Segments			
	Duodenum	Jejunum	Ileum	Colon
1	11	11	11	11
6	9	10	11	10
8	10	5	9	10
9	7	9	10	6
10	5	6	9	10
11	10	10	9	10
12	5	4	8	6
25	7	4	9	8
47	6	5	8	10

*Data expressed as Log_{10}/5 cm segment