Enteric bacteria: Friend or foe?

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INTRODUCTION

The normal gastrointestinal tract contains an enormous number of aerobic and anaerobic bacteria which represent a complex ecosystem that has important implications for the health of the host. This flora normally enjoys a symbiotic relationship with the host, but it can have adverse effects with local and systemic consequences.

REGULATION AND EFFECTS OF NORMAL FLORA

Normal gastrointestinal microflora

The small intestine constitutes a zone of transition between the sparsely populated stomach and the luxuriant bacterial flora of the colon. The number of organisms increases from approximately $10^2$ to $10^6$ colony forming units (cfu)/ml in the proximal small intestine to between $10^6$ and $10^9$ cfu/ml in the distal small intestine of dogs and then increases dramatically in the large intestine to approximately $10^{10}$ to $10^{11}$ cfu/ml in the colon (Burrows and others 1995). There is a wide variation in the bacterial flora between normal individuals, and concentrations may be affected by a variety of circumstances including environment, diet, scavenging and coprophagy. This is evident in the proximal small intestine where, under some circumstances, the number of bacteria found in apparently clinically healthy dogs can reach more than $10^8$ cfu/ml (Batt and others 1992, Willard and others 1994).

These findings contribute to a debate on the upper limit of normality, and it is therefore helpful to consider studies in kennelled beagles which emphasise that these higher numbers of bacteria are likely to be associated with mucosal damage although this can be clinically silent (Batt and others 1992, Morris and others 1994). There is no doubt that increased numbers of direct damage to the intestinal mucosa, and bacterial metabolism of intraluminal constituents, for example forming deconjugated bile acids and hydroxylated fatty acids which stimulate fluid secretion. Additional problems arise if there is interference with the mucosal barrier since this can result in increased passage of bacteria and bacterial products stimulating mucosal inflammation, while bacterial translocation can result in bacteraemia and septicaemia. Problems associated with bacterial pathogens are illustrated by the properties of the spectrum of pathogenic Escherichia coli, some of which facilitate long-term colonisation by adherence to the surface or invasion of enterocytes.
bacteria in the small intestine carry an increased risk of a harmful outcome, and the reasons for this are apparent in the subsequent sections.

Whether this effect is manifest clinically will depend on individual circumstances including the composition of the diet, which bacteria can convert to potentially harmful metabolites. The nature of a host response to the increased load is also likely to be particularly important, and this may involve stimulation of immunoglobulin and mucus production, suppression of potentially damaging cell-mediated immune responses, a compensatory production of structural and functional epithelial cell proteins, and increased epithelial cell turnover.

The threshold between apparent normality and clinical disease may therefore differ between individuals and be influenced by a delicate balance between the microflora and the host. Interestingly, relatively high numbers of bacteria (10^5 to 10^8 cm/ml), frequently including obligate anaerobes, have been found in the proximal small intestine of normal cats, but these do not appear to affect the mucosa and may reflect adaptation to a strictly carnivorous diet (Johnston and others 1993).

The quantitative proximal-distal gradient in the gastrointestinal tract is accompanied by qualitative changes in the microflora. Aerobic and Gram-positive species, including streptococci, staphylococci and lactobacilli, are typically predominant in the proximal small intestine of dogs, while coliforms are found in relatively low concentrations and anaerobes only intermittently. In the distal ileum of dogs, Gram-negative bacteria begin to outnumber Gram-positive organisms, while coliforms are consistently present and anaerobic bacteria such as Bacteroides, Fusobacterium and Clostridium species are found in substantial concentrations. The bacterial population in the colon is also very diverse; in the dog, bacteria of 84 different species have been identified, representing 27 different genera, as well as five genera of fungi. Most colonic bacteria are anaerobic with Bacteroides, Bifidobacterium, Peptostreptococcus, Eubacterium, Clostridium and Peptococcus species predominating, while various species of Enterobacteriaceae are also common.

**Regulation of the gastrointestinal microflora**

Regulation of the gastrointestinal microflora depends on complex interactions between many host and microbial factors, the most important of which are summarised in Table 1 (Frederickson and Stephanopoulos 1981, Mackowiak 1982, Strombeck and Guilford 1990). These factors maintain the numbers and balance of indigenous microorganisms, and also help to prevent colonisation by potential pathogens.

<table>
<thead>
<tr>
<th><strong>Table 1. Regulation of the gastrointestinal microflora</strong></th>
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<tr>
<td><strong>Host factors</strong></td>
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<tr>
<td>Gastrointestinal motility (normal peristaltic activity)</td>
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<tr>
<td>Gastric acid</td>
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<td>Antibiocidal nature of biliary and pancreatic secretions</td>
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<td>Intestinal immunity (secretory IgA in biliary and intestinal secretions)</td>
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<td>Mucus layer</td>
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<td><strong>Microbial factors</strong></td>
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<tr>
<td>Alterations in redox potential</td>
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<tr>
<td>Substrate depletion</td>
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<tr>
<td>Production of growth inhibitors, eg, short chain fatty acids and ‘bacteriocins’</td>
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<td>Suppression of bacterial adherence</td>
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Peristaltic activity is a major host factor that inhibits colonisation of the upper small intestine, supported by the antibacterial activities of gastric acid, bile and pancreatic juice. Mucous provides additional protection at the mucosal surface because the viscous high molecular weight glycoproteins provide a physicochemical barrier which acts in concert with secreted immunoglobulins to entrap bacteria (Forstner 1978). The carbohydrate component of mucus can also compete, and therefore interfere, with receptor-specific binding of microbes and microbial toxins.

The role that microbial interactions play in the regulation of the microflora is most evident in the densely populated colon (Frederickson and Stephanopoulos 1981, Mackowiak 1982). Facultative bacteria which grow both aerobically and anaerobically help to maintain a low redox potential allowing oxygen-sensitive organisms to survive in the colon and providing an unfavourable environment for many pathogenic aerobes. Potentially this could also occur elsewhere in the gastrointestinal tract. Competition between bacteria may also occur due to the depletion of nutrients that are essential for the growth of some microorganisms, and the production of bacterial metabolites that help to create an intraluminal environment that restricts microbial growth. For example, the production of the short-chain fatty acids - acetic, propionic and butyric acid - can inhibit bacterial proliferation, and a decrease in butyrate concentration is thought to permit proliferation of salmonella in some species of animal. Bacteria can also produce a variety of antibiotic-like substances called bacteriocins that inhibit the growth of other species of bacteria or even control their growth in an autoregulatory manner (Simon and Gorbach 1987).

**Bacterial metabolism of exogenous and endogenous substances**

In addition to involvement in microbial interactions, the metabolic activity of the indigenous microflora can have a major impact on the host. Action on exogenous substances (Table 2)
typically involves reduction and hydrolysis of dietary constituents by a variety of enzymes (Scheline 1973). Beneficial consequences include the production of short-chain fatty acids from fermentable carbohydrate, because these provide a valuable source of nutrient for the colonic mucosa. Bacterial synthesis of vitamins, including biotin, folate and vitamin K, can also be beneficial and can supplement the dietary supply.

Deleterious effects of bacterial metabolism include a compromised nutritional value of the diet, particularly when there are large numbers of bacteria in the proximal small intestine where nutrient absorption is maximal. Gas production resulting in distension of the intestine and flatus can be an unpleasant consequence of nutrient metabolism. In addition to competition for calories, enteric bacteria may compete for and deplete essential dietary nutrients; examples include cobalamin which can be bound and internalised by enteric bacteria, and taurine for which there is good indirect evidence of degradation by enteric bacteria in the cat (Anantharaman-Barr and others 1994, Backus and others 1994). Bacteria in the proximal small intestine can also convert dietary long-chain fatty acids to polar hydroxy fatty acids which then cannot be absorbed by passive diffusion and remain in the lumen stimulating colonic secretion of fluid (King and Toskes 1979, Donaldson and Toskes 1989). This also occurs when fatty acids reach the luxurient microflora in the distal intestine in clinical conditions that result in fat malabsorption.

The main actions of enteric bacteria on endogenous substances in the lumen are the conversion of urea to ammonia, formation of urobilinogen from bilirubin, and the formation of deconjugated and secondary bile acids (Table 3) (Drasar siid Hill 1974). The effects on bile acids appear to be of little consequence in the colon where all oile acids are deconjugated and secondary bile acids predominate. However, these metabolites can have a damaging effect on membranes in the small intestine, where a reduction in the concentration of conjugated bile acids can interfere with micelle formation and hence fat absorption (King and Toskes 1979, Donaldson and Toskes 1989). Release of taurine from tauro-conjugated bile acids in the small intestine and subsequent metabolism by enteric bacteria could interfere with the availability of this essential amino acid in the cat. Degradation of mucus and glycocalyx at the surface of intestinal epithelial cells by the action of bacterial glycosidases can potentially interfere with the integrity of the mucosal barrier if compensatory rates of synthesis are inadequate. Consequential exposure of specific carbohydrates may also facilitate binding of bacteria and dietary lectins which can then cause mucosal damage.

The beneficial effects of the normal enteric flora can, therefore, include the competitive exclusion of potentially pathogenic organisms, the production of short-chain fatty acids, which represent an important energy source for the colonic mucosa, and synthesis of essential nutrients such as vitamins (Table 4). These advantages are counterbalanced by the detrimental effects of the enteric flora which can include competition for calories and essential nutrients, especially small bowel flora, eg, vitamin B₉, taurine. Production of metabolites with adverse effects such as short-chain fatty acids, urobilinogen, and secondary bile acids can cause or exacerbate inflammatory bowel disease.

**OVERGROWTH OF RESIDENT MICROFLORA**

The beneficial effects of the normal enteric flora can, therefore, include the competitive exclusion of potentially pathogenic organisms, the production of short-chain fatty acids, which represent an important energy source for the colonic mucosa, and synthesis of essential nutrients such as vitamins (Table 4). These advantages are counterbalanced by the detrimental effects of the enteric flora which can include competition for calories and essential nutrients, especially small bowel flora, eg, vitamin B₉, taurine. Production of metabolites with adverse effects such as short-chain fatty acids, urobilinogen, and secondary bile acids can cause or exacerbate inflammatory bowel disease.
for calories and essential nutrients, particularly by bacteria located in the small intestine, production of metabolites with adverse effects, and a capacity to damage the mucosa, in some circumstances causing or contributing to inflammatory bowel disease (Table 4). These detrimental effects can be accentuated by factors that interfere with the physiological regulation of the bacterial microflora and allow colonisation of the proximal small intestine with increased numbers of normal residents or bacteria not normally present at that site.

Causes of bacterial overgrowth

Host factors that predispose to proximal overgrowth include defective gastric acid secretion, interference with normal motility or stasis, and defective local immunity (Table 5) (King and Toskes 1979, Toskes and Donaldson 1989, Kirsch 1990). Other factors, such as a potentially stressful or contaminated environment, diet and coprophagy, may also play a role and could contribute to the relatively high numbers of bacteria reported in laboratory beagles (Batt and others 1992, Morris and others 1994). The pathophysiological consequences may involve direct damage to the intestinal mucosa, and bacterial metabolism of intraluminal constituents, for example forming deconjugated bile acids and hydroxylated fatty acids which interfere with fat absorption and stimulate fluid secretion. Additional problems can arise if there is interference with the mucosal barrier since this can result in increased passage of bacteria and bacterial products stimulating mucosal inflammation, while bacterial translocation can potentially result in bacteraemia and septicaemia.

Clinical presentation and mucosal changes

Small intestinal bacterial overgrowth (SIBO) in the proximal small intestine is emerging as an important condition in many breeds of dog. Typically, this presents in young animals as chronic intermittent small bowel diarrhoea, which may be accompanied by loss of bodyweight or failure to gain weight (Batt and McLean 1987, Rutgers and others 1993, 1995a). An association with partial villous atrophy and inflammatory bowel disease has been reported, but in most cases there are relatively minor or no obvious histological changes (Rutgers and others 1995a). However, studies in German shepherd dogs have provided evidence that SIBO can have a direct effect on enterocytes and that high numbers of anaerobes typically cause more severe damage than aerobes (Batt and others 1984, 1988, Batt and McLean 1987).

For reasons considered above, the clinical outcome of SIBO may depend not only on the numbers and types of bacteria, but also on factors such as composition of the diet and host responses to the flora. The latter include local non-specific and immunological protection, compensatory stimulation of epithelial cell production and the capacity for mucosal repair. There is some evidence that mucosal immunity may be particularly important.

Relatively low concentrations of immunoglobulin A (IgA) have been found in the serum and duodenal juice of German shepherd dogs with SIBO, whereas the overgrowth flora and consequential mucosal damage would have been expected to stimulate mucosal IgA production (Batt and others 1991, Whitbread and others 1984, Willard and others 1994). In contrast, high rather than low concentrations of IgA have been found in serum from a series of clinically healthy laboratory beagles with SIBO (Batt and others 1992 and unpublished observations), suggesting that local immunity may have better protected the mucosa against the potentially damaging effects of SIBO in these dogs.

Diagnosis of bacterial overgrowth

Diagnosis of SIBO should follow baseline investigations in order to eliminate enteric pathogens and parasites, partial obstruction, systemic disorders and exocrine pancreatic insufficiency (Burrows and others 1995). At present, there is no single, simple, sensitive and specific test for the detection of bacterial overgrowth. An increased serum folate or reduced cobalamin concentration can provide indirect evidence for overgrowth in dogs, once other possible causes of these changes such as pancreatic insufficiency have been eliminated. Analysis of data from a series of 80 clinical cases has shown that these vitamin estimations have a fair specificity for the detection of bacterial overgrowth, but a low sensitivity (Table 6) (Rutgers and others 1995a). This emphasises that normal serum concentrations of these vitamins do not exclude the possibility of

Table 5. Causes of bacterial overgrowth

<table>
<thead>
<tr>
<th>Defective gastric acid output</th>
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<td>Gastritis, gastric atrophy</td>
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<th>Defective motility or stasis</th>
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<tr>
<td>Idiopathic, neuropathy, inflammatory bowel disease</td>
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<tr>
<td>Intussusception, foreign body, tumour</td>
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<td>Defective or absent ileocaecal valve</td>
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<th>Defective local immunity</th>
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<td>Secretory IgA deficiency</td>
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<td>Defective secretory piece</td>
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<td>Malnutrition</td>
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<th>Exocrine pancreatic insufficiency</th>
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<th>Defective mucus production</th>
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| Unabsorbed nutrients            |

0
Table 6. Serum folate and cobalamin concentrations for the detection of bacterial overgrowth in dogs

| Index • Sensitivity Specificity Positive-predictive value Negative-predictive value |
|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
| Serum folate                        | Serum cobalamin                     | Serum folate                        | Serum cobalamin                     |
| >8.8 μg/litre                       | <200 ng/litre                        | >8.8 μg/litre                       | <200 ng/litre                        |
| 51%                                | 24%                                 | 5%                                 | 100%                                |
| 79%                                | 87%                                 | 100%                                | 100%                                |
| 72%                                | 67%                                 | 100%                                | 50%                                 |
| 61%                                | 52%                                 | 61%                                 |                                     |

Sensitivity, specificity, positive and negative predictive values of high folate and low cobalamin concentrations in serum for detection of small intestinal bacterial overgrowth. Based on data from 39 culture-negative dogs and 41 dogs with overgrowth diagnosed by concurrent culture of duodenal juice (From Rutgers and others 1995a)

overgrowth, because these alterations depend on factors such as the type and numbers of organisms present, and also on the severity of any secondary mucosal damage which may interfere with folate absorption despite a high intraluminal concentration. Furthermore, although a high folate concentration can be associated with SIBO, this may also be a consequence of high folate intake perhaps due to vitamin supplementation, a high folate diet and coprophagy.

Increased intestinal permeability has been demonstrated in laboratory beagles (Batt and others 1992, Morris and others 1994) and in approximately 50 per cent of a series of 63 clinical cases with high numbers of intraluminal bacteria (Rutgers and others 1995c), but was normal in three dogs with SIBO which did not have obvious histological damage (Quigg and others 1994). Permeability tests may, therefore, help in detecting and assessing the severity of mucosal damage in overgrowth, but an abnormal result needs to be repeated following antibiotic therapy to assess whether such changes are due to bacterial overgrowth.

The potential value of hydrogen breath testing following a meal or oral administration of individual sugars for the investigation of gastrointestinal disease in small animals is becoming apparent (Muir and others 1991, Rutgers and others 1995b, Washabau and others 1986a,b) and this offers a relatively simple procedure for the presumptive diagnosis of SIBO in dogs. This merits further evaluation, although its application is likely to be restricted to specialist centres.

Microbiological culture of duodenal juice obtained endoscopically or at laparotomy is needed to confirm the diagnosis of SIBO, and should demonstrate greater than $10^5$ total or greater than $10^3$ obligate anaerobic cfu/ml in dogs (Burrows and others 1995, Rutgers and others 1995b). A definite association between these numbers of bacteria, clinical disease and mucosal damage has been established in dogs (Batt and McLean 1987, Batt and others 1988, 1992, Rutgers and others 1995a) although higher numbers may be found in apparently clinically healthy dogs for reasons discussed above (Willard and others 1994). The most frequent isolates typically include enterococci and E coli in dogs with aerobic overgrowth, and clostridia in dogs with anaerobic overgrowth.

**Treatment of bacterial overgrowth**

The first objective is to consider treatment of any identified underlying abnormality that could be responsible for the overgrowth, such as partial obstruction due to intussusception, tumours or foreign bodies (Burrows and others 1995). In most cases, however, the underlying cause will not be apparent and management is directed towards the overgrowth flora.

Oral broad-spectrum antibiotic therapy with oxytetracycline (10 to 20 mg/kg bodyweight, repeated every eight hours, for 28 days) has been particularly successful, but may need to be continued for extended periods if clinical signs recur on withdrawal of medication (Rutgers and others 1995a). Metronidazole (10 mg/kg every 12 hours) and tylosin (10 mg/kg every eight hours) have proved to be effective alternatives. Dietary management with a low fat diet may also be valuable, because this can minimise the secretory diarrhoea which is a consequence of bacterial metabolism of fatty acids and bile salts. Deficiencies of trace elements, vitamins and, perhaps, essential amino acids which may be metabolised by bacteria may also make a significant contribution to the clinical consequences of SIBO, and the potential for appropriate supplementation should not be neglected.

**COLONISATION WITH ENTERIC PATHOGENS**

Colonisation by pathogenic bacteria can have detrimental effects on the intestinal tract, and long-term colonisation may be favoured by similar circumstances that interfere with the physiological regulation of the the normal flora resulting in bacterial overgrowth. Bacterial factors that can also contribute to colonisation, and hence pathogenicity of these organisms, include the presence of flagellae, enzymes (mucinases, glycosidases, proteases), adherence determinants (pili, fimbriae, close attachment) and factors that result in impairment of bowel motility (Giannella 1981).

Pathogenicity may also be due to virulence factors which can include the secretion of
enterotoxins, the ability to invade the epithelium and resistance to phagocytosis. Bacteria that are to be considered to be enteric pathogens in small animals include *Salmonella*, *Clostridium*, *Campylobacter* and *Yersinia* species and pathogenic *E coli* (Burrows and others 1995). Their deleterious effects can be illustrated by considering the properties of the spectrum of pathogenic *E coli* that have now been identified, some of which are capable of long-term colonisation by adherence to the surface or invasion of enterocytes. This group of pathogenic bacteria is particularly interesting and may be more prevalent in small animals than suspected because they would be overlooked in routine microbiological culture of faeces. The problem is that they cannot be readily distinguished from non-pathogenic *E coli* which represent a major component of the normal flora, and need to be identified using either DNA probes to pathogenic factors or appropriate in vitro assays (Hart and others 1993).

Considerable effort has been focused on human infections, and five main categories of pathogenic *E coli* are now recognised based on virulence properties (Hart and others 1993).

1. **Enterotoxigenic E coli (ETEC)** possess protein spikes called pili or fimbriae that allow attachment to receptors on the mucosal surface and hence promote colonisation. They produce toxins that, although not causing intestinal damage, have a biochemical effect on epithelial cells typically resulting in a short-lived secretory diarrhoea.

2. **Enteropathogenic E coli (EPEC)** produce a non-inflammatory osmotic diarrhoea. This is a consequence of a loss of microvilli following intimate attachment of the bacteria to the surface of enterocytes in the small intestine resulting in malabsorption of small osmotically active molecules such as disaccharides and monosaccharides.

The other three categories of pathogenic *E coli* typically produce a more aggressive clinical picture associated with an inflammatory diarrhoea.

3. **Enteroinvasive E coli (EIEC)** attach to and invade colonocytes where multiplication leads to cell death, necrosis and ulceration of the large bowel.

4. **Enterohaemorrhagic E coli (EHEC)** cause attaching-effacement of microvilli, in common with EPEC. However, differences are that EHEC infection is typically limited to the terminal ileum and colon, and that the release of one or both verotoxins (VT1, VT2) results in a haemorrhagic colitis due to death of epithelial cells. If EHEC enter the circulation they can also damage endothelial cells and precipitate the haemolytic-uraemic syndrome. This presents as acute renal failure, thrombocytopenia and coagulopathy with evidence of microangiopathic haemolytic uraemia.

5. **Enteroaggregative E coli (EAggEC)** also damage colonic villi by haemorrhagic necrosis and can result in chronic diarrhoea, but the precise mechanism is unclear.

Pathogenic *E coli* have been studied extensively in food animal species, but their potential importance as primary enteropathogens in small animals is not clear. However, ETEC and EPEC have been isolated from dogs with diarrhoea (Olson and others 1985, Drolet and others 1994) and there is good evidence for the presence of attaching-effacing (eae) and verotoxin-producing *E coli* (VT) in cats (Pospischl 1987, Abaas and others 1989). Furthermore, susceptibility of canine small intestine to EPEC has been confirmed by demonstrating classic attaching-effacing lesions following infection with EPEC in organ culture (Fig 1) (Hart and others 1990).

Our own studies have used DNA probes to examine *E coli* in faecal samples from dogs with diarrhoea and clinically healthy dogs, all of which were maintained in a household environment. Preliminary results indicate that significantly more affected dogs were excreting pathogenic *E coli* that hybridised with probes for EPEC (eae: acute 17-5 per cent, n=57; chronic 15 per cent, n=40: P<0.05) and verotoxin (VT1: acute 24-5 per cent; chronic 35 per cent: P<0.01) compared with the control dogs (n=30) in which no eae or VT1 positive *E coli* were found (Sancak and others 1995). These findings support the possibility that pathogenic *E coli* could play a role in the pathogenesis of acute and chronic diarrhoea in dogs.

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REFERENCES


SCHELINE, R. R. (1973) Metabolism of foreign compounds by gastrointestinal microorganisms. Pharmacology Reviews 25, 451-432


