THE ART AND SCIENCE OF ENTERIC BACTERIOLOGY IN COMPANION ANIMALS

BACTERIOLOGY is as much an art as a science, and while some diagnoses, for example the isolation of Staphylococcus aureus from an abscess, present no difficulties, in contrast the determination of the aetiology of diarrhoea is often fraught with difficulty because of the vast number of micro-organisms that colonise the gut.

The term "normal gut flora" is confusing and a misnomer which is sometimes used by laboratories when the bacteria isolated appear unremarkable.

The gut, which usually means the intestine, varies in different animal species and the term suggests that all microbial types found on, or in, faeces or cavities are normal residents of habitats in those sites. This is not the case and many microbial types that can be isolated from the gastro-intestinal tract cannot be regarded as indigenous to the system and must be regarded as transients.

Transients can be transported to a habitat by food and other materials, or even by passing down from habitats above the one being sampled.

Certain transients, some of which may be pathogens, may temporarily colonise niches in habitats in perturbed eco-systems, for example due to the use of antimicrobial drugs, starvation or other forms of malnutrition and certain environmental conditions.

The gastro-intestinal tracts of mammals and birds have five major sections: oral cavity/ oesophagus; stomach; small intestine; caecum; CLIFFORD WRAY BVMS, PhD, FRCPath, MRCVS EDWARD WRAY BSc, ARCS and PETER F. NAYLOR MIBiol, CBiol, MRSH

of Greendale Veterinary Diagnostics provide an overview of the variety of micro-organisms that colonise the gut of companion animals, and suggest the best way forward in the diagnosis and treatment of gastro-intestinal disorders

and large intestine. Depending on the species of animal, any or all of these areas may contain habitats for indigenous micro organisms.

Such habitats may include the contents of the lumen, the epthelial surface and the Crypts of Lieberkuhn.

The large intestines of mammals contain enormous populations of micro organisms: > 10^{11} microbes/gram dry weight of content, and over 400 species have been detected in the microflora.

Ninety per cent of these populations are strict anaerobes, for example Bacteriodes, Fuso bacterium, *Clostridium and Peptostreptococcus*.

Major differences exist between the intestinal microflora of each animal species, for example in rabbits E.coli are present only in low numbers, usually less than $10^3/g$ faeces, whereas the intestine of dogs and cats contains more that $10^7/g$ faeces.

Even in these two species the number of micro-organisms differs with age and puppies and kittens usually have a different microflora from that of the adult, S. aureus being a common inhabitant of the intestine of puppies (Smith, 1965).

The delicate relation and ecological balance between the bacterial species of the intestinal microflora are poorly understood and it is likely that the different diets used nowadays to feed cats and dogs will also affect the numbers of different bacterial species in the intestine.

If either carcases or intestinal contents are submitted to the laboratory it must be remembered that rapid bacterial multiplication can occur within a short period after death and this will also affect the structure and appearance of the intestinal mucosa.

To maximise the chance of determining the cause of bacterial enteritis it is better to submit faeces rather than a swab and to dispatch them to the laboratory by the quickest means possible.

If a swab is used it is important that the correct transport medium is used.

The commonest causes of bacteria



Figure 1. E. coli with fimbriae



Figure 2. Cup and pedestal lesions in the intestine

gastro-enteritis in companion animals are Campylobacters, pathogenic Escherichia coli, and Salmonellae, which are also of public health significance.

Campylobacter have been detected in 32 per cent of faeces samples from dogs suffering from diarrhoea and an association was found between Campylobacter infection in dogs and diarrhoea in human households (Food Standards Agency, 2002).

All samples should be examined for these bacteria using a range of appropriate selective and non selective culture media, and a Gram stain of a faecal smear can also provide useful information.

Provided the samples for diagnosis are taken at the onset of symptoms and before therapy, one sample should suffice because of the large numbers of pathogens present in the faeces.

We have always regarded E. coli as important and it is now receiving more attention as a possible cause of diarrhoea in companion animals and not just a commensal organism.

In farm animals the classification of disease producing E coli has been the subject of intensive investigations and a wide range of toxins and adherence factors have been identified. Fimbrae (Figure I) allow the attachment of the organism to the mucosa. enterotoxogenic E. coli produce toxins which cause fluid loss and diarrhoea and enteropathogenic E. coli possess the attaching and effacing gene which produces the characteristic cup and pedestal lesions in the intestine (Figure 2). Less research has been carried out in companion animals but during a recent survey of acute and chronic diarrhoea by the University of Liverpool (Sancak et al. 2004), pathogenic E. coli were detected in 40 per cent of the cases, which included dogs of all ages. Enteropathogenic E. coli were usually the only abnormality in dogs with acute diarrhoea, whereas in chronic diarrhoea additional diagnoses were present.

These included small intestine bacterial overgrowth (SIBO) and the presence of enteropathogenic E. coli in duodenal juice. Enterotoxic E. coli have also been shown to be a cause of diarrhoea in young dogs (O son et al, 1985). Thus, the isolation of E. coli in pure culture should be treated as possibly significant and related to the clinical findings. The identification of pathogenic E. coli in companion animals presents problems because specialised laboratory facilities are necessary for the identification of toxins and adherence factors. If further investigation is required, full serotyping and toxin production can be arranged by your laboratory.

SIBO is a recently identified condition in dogs and emphasises the potentially damaging effect of the intestinal microflora when the balance is disturbed. This presents typically as chronic intermittent diarrhoea in young animals, which may be accompanied by weight loss (Rutgers et al, 1995).

In most cases the cause cannot be identified, but host factors that are known to predispose to bacterial overgrowth include interference with normal motility or stasis, defective gastric acid secretion and impaired local immunity (Burrows et al, 1995).

Diagnosis of SIBO is difficult and microbiological culture of duodenal juice should demonstrate $a > 10^5$ cfus of a mixed culture of Enterococci and E. coli.

Anaerobic bacteria, especially Clostridia, have also been shown to cause diarrhoea in companion animals. However, we do not routinely culture for anaerobes because staining techniques are simpler and quicker for the identification of Clostridia, although if required culture and toxin tests can be arranged.

Clostndium perfringens Type A is a frequent isolate from normal faeces, and its presence, and the detection of toxin, should be related to clinical signs.

A predominantly Gram-positive faecal flora in dogs and cats suggests a dietary imbalance, rather than the presence of pathogens (though in other species predominately Gram-negative flora can be an indicator of infection).

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Antibacterial sensitivity testing and treatment

Susceptibility tests provide guidance to the practitioner as to the most appropriate antibiotic to use in the prevailing circumstances.

If susceptibility tests for Campylobacters are performed correctly, the results are valid and helpful because of the limited range of antibiotics to which this organism is susceptible. This is especially important in the case of the fluoroquinolones because of the possibility that isolates may be resistant to this class of antibacterials.

Salmonella gastro-enteritis in mature animals is usually self-limiting and in general fatalities are uncommon. This may not be the case in young or older animals, particularly if the latter have some underlying disease and in these cases treatment is necessary.

Although Salmonella are associated with about 10 per cent of the cases of diarrhoea in companion animals (Carter and Quinn, 2000), Salmonella typhimurium DT 104, at present one of the commonest isolates from cases of diarrhoea, is resistant to a wide range of antibiotics and susceptibility testing is advisable.

In human medicine, the use of antibiotics for treatment of uncomplicated gastro-enteritis has generally been contra-indicated because of lack of response and possible prolongation of *Salmonella* shedding.

However, following the introduction of fluoroquinolones, a number of clinicians have advocated their use for the treatment of Salmonella because of their efficacy in reducing the severity of symptoms, duration of illness and *Salmonella* shedding.

Similar considerations may apply to companion animals, especially when there are young children, elderly or immuno-compromised people at risk because of the large number of *Salmonella* shed during episodes of diarrhoea.

The use of antibiotics, especially fluoroquinolones, needs to be considered carefully given the possibility that resistant strains may develop. Whether to treat Salmonellosis must depend on the veterinary surgeon's judgement, based on the clinical and laboratory findings.

Submitting faecal samples from animals with enteric disease is useful as it can allow the types of bacteria present to be determined, their significance assessed and sensitivity testing can be performed to determine likely drug efficacy.

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