BACTERIA IN POST-ANTIMICROBIAL ENTERITIS IN WEANED PIGS

by

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A B S T R A C T

This research was undertaken to determine whether or not diarrhoea and enteritis occurred following the withdrawal of antimicrobials used for growth promotion or therapy in the pig.

Cases of post-antimicrobial enteritis were investigated on 6 farms. Post-antimicrobial enteritis could not be confirmed in any instance but was suspected in one unit, Farm 1 on one visit. Inadequate or inappropriate therapy was found to be the main reason for the reappearance of disease following antimicrobial withdrawal. Swine dysentery was responsible on Farms 2 and 3, antimicrobial resistance of Salmonella kedougou to trimethoprim-sulphadiazine used for treatment was responsible on Farm 5 and inappropriate treatment was being given on Farm 1. On two farms (4 and 6) disease had disappeared or was being treated successfully.

Experimental studies were carried out using pigs free from major pathogens and feeding or treating them with antimicrobials reported to cause post-antimicrobial enteritis. The initial study with avoparcin at 80 ppm for 10 days proved inconclusive.Rotavirus and Cryptosporidium spp. (both insensitive to antimicrobials) were identified. Two controlled studies were carried out using lincomycin at 13.3 ppm for 11 days and 20 days (Experiments 2 and 3). Antimicrobial withdrawal was found to affect levels of total coliforms, lactobacilli, campylobacters, Clostridium perfringens type A and Bacteroides spp. in individual pigs but the changes were not statistically significant on a group basis.

A further study was carried out using chlortetracycline in the feed at 300 ppm for 21 days. Weight gains in the treated pigs were higher than those in controls during adaptation to treatment 346g/d compared with 254g/d. A fall in the rate of gain occurred in the treated group following antimicrobial withdrawal. This change provided evidence of a post-antimicrobial effect, but it could not be attributed to any clinical disease, to pathogens in other body systems or to the bacterial species monitored.

It was concluded that a post-antimicrobial effect occurred and that it was not associated with the bacteria being monitored. It was not associated with pathogens such as Clostridium difficile, Serpulina hyodysenteriae, Salmonella spp. or Ileal symbiont intracellularis which have all been implicated in previously reported cases.

AS A RULE, THE SCIENTIST TAKES OFF FROM THE MANIFOLD OBSERVATIONS OF HIS PREDECESSORS, AND SHOWS HIS INTELLIGENCE, IF ANY, BY SELECTING HERE AND THERE THE SIGNIFICANT STEPPING STONES THAT WILL LEAD ACROSS DIFFICULTIES TO NEW UNDERSTANDING. THE ONE WHO PLACES THE LAST STONE AND STEPS ACROSS TO TERRA FIRMA OF ACCOMPLISHED DISCOVERY GETS ALL THE CREDIT. ONLY THE INITIATED KNOW AND HONOUR THOSE WHOSE PATIENT INTEGRITY AND DEVOTION TO EXACT OBSERVATION HAVE MADE THE LAST STEP POSSIBLE.

HANS ZINSSER (1878-1940)

AND IT IS TRULY WONDERFUL THAT A SUBSTANCE, THE VERY ASPECT AND ODOUR OF WHICH ARE SUFFICIENT TO INDUCE AN INEVITABLE NAUSEA, SHOULD BE REGARDED NOT MERELY AS A MATTER OF CURIOSITY AND STUDY, BUT HELD FOR THE HIGHEST REPUTE AS A UNIQUE AND MOST PRECIOUS TREASURE FOR THE PRESERVATION OF HEALTH.

SAMUEL AUGUSTUS FLEMMING, 1738.

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A C K N O W L E D G E M E N T S

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Pedro J.B. de la Salle Fdo. Pradal-Roa

CHAPTER 1

INTRODUCTION AND REVIEW OF THE LITERATURE

1. INTRODUCTION

The subject of this thesis "Bacteria in post-antimicrobial enteritis in weaned pigs", was chosen because there were many reports from the field of diarrhoea having reappeared after the withdrawal of treatment. The reasons for this reappearance of disease were rarely established and it was not even clear whether or not the phenomenon was a real one. For the purposes of this thesis the term "Post-antimicrobial enteritis" is defined as the inflammation of the intestinal mucosa resulting in clinical signs of diarrhoea, occurring after antimicrobial withdrawal.

The literature on post-antimicrobial enteritis is scanty for any species and that on post-antimicrobial enteritis in the weaned pig is no exception. An extensive literature exists on enteritis in the weaned pig and on the effects of antimicrobial treatment on enteritis and the gut flora for many species including man. This general literature is reviewed here, but observations on farms and in experimental studies are necessary to assess the phenomenon of post-antimicrobial enteritis.

Enteric disease in the weaned pig is a complex phenomenon. It may be associated with a number of different infectious agents, with nutrition and with the husbandry conditions prevalent in the affected pig herds. The standard of observation of the clinical signs of enteritis in most farms is poor. The proportion of pigs affected and duration of the disease is rarely recorded accurately. Even well-known diseases like swine dysentery, spirochaetal diarrhoea, clostridial diarrhoeas, salmonellosis and viral diarrhoeas such as rotavirus infections are rarely identified correctly on a farm let alone in individual batches of pigs.

This poor standard of observation and diagnosis by farmers and clinicians is accompanied by the widespread and continuous use of antibiotics to control and prevent common gastroenteric

disorders. In many cases, treatment has been prescribed on presumptive grounds without full identification of the clinical signs and without laboratory confirmation of diagnosis. This poor standard of diagnosis and treatment gives rise to even more confusion about the clinical signs actually present. For this reason it is necessary to study problems of post-antimicrobial diarrhoea on farms to determine whether outbreaks reported as post-antimicrobial enteritis are genuine and then to study the subject experimentally.

2. REVIEW OF THE LITERATURE

A) Enteritis and diarrhoea

The term enteritis literally implies acute or chronic inflammation of the mucous membrane of the small intestine. It is often extended to cover inflammation of other parts or the whole of the gastrointestinal tract as in the term "post-antimicrobial enteritis". Inflammation of the individual portions of the gastroenteric tract is referred to as gastritis when it affects the stomach, caecitis or typhlitis when it affects the caecum, colitis when it affects the colon and proctitis when it affects the rectum. In this review the term enteritis will be used to imply general inflammatory change of the gut unless this is restricted to a single area.

The primary functions of the gastrointestinal tract are the digestion of feed and the absorption of ingested nutrients. There is also absorption of water and reabsorption of endogenous secretions (salivary, gastric, pancreatic, biliary, small intestinal mucosal and colonic secretions) for the maintenance of fluid and electrolyte balance particularly in the colon, and evacuation of the waste products of digestion. The faeces of normal animals are usually firm with relatively little water, electrolyte or residual nutrients.

Changes in the normal mucosa and submucosa occur in enteritis. Specific diseases and conditions produce localised or generalised changes which may be identified as gross lesions in the serosa or mucosa of the gastrointestinal tract. Lesions may consist of loss of the epithelium due to viral, parasitic or bacterial invasion or because of damage from ingested toxins or

bacterial toxins (Binder and Powell, 1970; Gemmell, 1984; Moon, 1978; Savage, 1972; Scotland, 1988). In some cases the damage is not sufficient to cause loss of epithelial cells but may affect their function as in enterotoxic Escherichia coli (E. coli) infections.

When the epithelium is lost, inflammatory changes in the submucosa and lamina propria result. Capillary dilation and exudates of plasma and phagocytes occur. Some diseases may affect the lamina propria alone, others may cause changes which subsequently affect the mucosa. These lesions normally heal and are recolonised by mucosal epithelium. The lamina propria may remain filled with plasma cells and recovery is often accompanied by an immune response. These changes may be seen grossly as mild hyperaemia, oedema, reddening, erosion, villous atrophy, excess mucus production, haemorrhage, adenomatosis and necrosis. Affected submucosa is often thickened and the draining lymph nodes are enlarged. The changes of enteritis can disturb the function of the intestine and the most common result of this is diarrhoea (Moon, 1978).

Enteritis occurs most frequently in the young animal. The neonatal gut is sterile for a few minutes after birth, and in farm animals, is unprotected by antibody. This is provided by maternal colostrum. This antibody protection wanes gradually to disappear at an age depending on the agent concerned and the amount of antibody absorbed. Additional protection is provided by milk antibody, mostly IgA. In neonates which receive no colostrum or where the mother has no active immunity to an agent, the animal will be unprotected. The gut is normally colonized by bacteria, viruses, protozoal and metazoan parasites and, in the absence of passive or active immunity, these can cause diarrhoea. As active immunity develops, the animal becomes increasingly resistant and enteritis and diarrhoea therefore occur most frequently in the young animal. Particular periods of risk include the neonatal period and the post-weaning period. In older animals, the introduction of new agents to a group may result in disease and disease may occur in individuals which have lost immunity or are immunosuppressed. In the case of infectious diseases, the mixing of non-immune and infected animals may give rise to infectious disease in susceptible individuals. Antimicrobial use may stop development of disease

or interrupt colonisation to leave animals naive to a particular agent. The effects of antimicrobial on this process are reviewed below.

Diarrhoea is a condition in which soft, sometimes watery faeces are passed more frequently than normal (Liebler, et al., 1992). Diarrhoea usually results from overstimulation of secretion by crypt epithelium, e.g. by bacterial enterotoxins such as Escherichia coli, interference with absorption e.g. hypertrophied and immature crypts, epithelial proliferation, epithelial loss and villous atrophy (viral and parasitic enteritis and salmonellosis), increased permeability (clostridial enteritis, swine dysentery), and possibly decreased motility or hypermotility. These mechanisms are not mutually exclusive and any could cause fluid faeces alone or together. Where cell loss or tissue destruction has occurred, diarrhoea may be accompanied by an excess of mucus, fibrin, and/or the presence of occasional flecks of blood, necrotic material, melaena or obviously haemorrhagic stools. (Current, 1988; Jubb, et al., 1985; Kent and Moon, 1973; Liebler, et al., 1992; Moon, 1978; Whipp, 1978). Undigested food and unabsorbed nutrients may also be present.

The appearance of the diarrhoeic faeces may be sufficiently distinctive to allow identification of the enteritis concerned.

Diarrhoea results in damage to the host from loss of fluid, nutrients (hypoproteinaemia), and electrolytes leading to dehydration, acid-base imbalance, metabolic acidosis, terminal hyperkalaemia and death from shock. Acute diarrhoea is accompanied by clinical signs of lost appetite, reduced water consumption, abdominal pain and/or distension, fever, vomiting and death.

When disturbances last for long periods, malnutrition, reduced growth rate, weight loss, muscular weakness, anaemia (perhaps with obvious haemorrhages into the gut), and cachexia occur as features of chronic gastrointestinal disorders (Argenzio, et al., 1980; Liebler, et al., 1992; Phillips, 1972).

a) Causal agents in enteritis

A wide variety of agents may cause enteritis. Chief amongst these are infectious agents. Viruses such as coronavirus,

rotavirus and parvoviruses occur in all species but must multiply in the cells of the intestine.

Parasites and bacteria are not restricted to the tissues of the host. Some metazoan and protozoan parasites can exist in the gut lumen apparently dependent only on the gut contents. Examples of this type include Ascaris spp. and some flagellates and ciliates. Others are more closely associated with the mucosal surface and may invade the tissues.

Trichostrongylid worms, coccidia and cryptosporidia may all penetrate to a varying degree while some adult worms such as Oesophagostomum spp. may merely damage the surface. Protozoa such as Balantidium coli and Trichomonas spp. may have a less well defined role. Bacteria also vary in their habitat. Some are considered to be normal inhabitants of the lumen and to be innocuous, others colonise the mucosa only when it is damaged and yet others appear to be pathogens in most animals. Their relationships with the mucosa are complex and are considered below.

Non-infectious agents can also cause enteritis. Physical factors such as the ingestion of irritant food particles or items may abrade the mucosa. Toxic chemicals, bacterial and fungal toxins may be ingested with food and produce enteritis. The diet may be deficient in nutrients such as vitamins essential for the maintenance of the integrity of the epithelium and allergic response may occur to proteins of dietary origin as in coeliac disease in man or to those of microbial origin. All these lesions may be colonized by bacteria present in the affected area of gut or passing it.

B) Normal gastrointestinal microbial flora

a) Normal gastrointestinal flora in animals

Concepts of the gastrointestinal microflora have evolved from Metchnikoff's ideas of noxious products of microbial metabolism in the intestine, through an era of descriptive bacteriology to the current approach of microbial ecology, interrelationships between the various intestinal microorganisms and the symbiotic interactions between the intestinal flora and

the host. Thus, the normal gastrointestinal flora can be considered as the microorganisms which colonize the animal's gut after birth from the mother's skin, faecal and vaginal microflora, from the air and food, and maintain a balance in a saprophytic or commensal relationship with the host animal. This flora is considered by some to prevent the establishment of entero-pathogenic infectious agents (Ducluzeau, 1983; 1985; Hentges, 1970). It is now generally accepted that the most prevalent microorganisms in human and animal faeces are obligate anaerobes e.g. Bacteroides spp. and bifidobacteria (anaerobic lactobacilli), and that the concentration of anaerobes in faeces is approximately 10^{11} per g compared with 10^{3} or less for aerobes (Gorbach, 1971; Hersh, et al., 1970; Moore and Holdeman, 1974; Visek, 1978).

These figures refer largely to the population of the lumen of the large bowel and do not reflect the population of other parts of gastrointestinal tract. The gastrointestinal tract is divided into functional portions. The oral cavity, pharynx, oesophagus, stomach, duodenum, jejunum, ileum, caecum and colon all have characteristic populations of microorganisms (the autochthonous flora) which reflect the environment within that organ. Throughout the gut there are distinct populations of bacteria in the lumen and on the mucosa. Those in the lumen are affected/influenced by pH, peristalsis, food or food residues and oxygen tension while those on the mucosa are also influenced by the epithelial type, the presence of specific antibody and secretions such as mucus. Both populations may be influenced by other elements of the microbial flora and by antimicrobials (Gall, 1970; Haenel, 1970; Hentges, 1970; Kenworthy, 1973).

This stable gastrointestinal flora in the adult is only achieved after colonization by the organisms in the young animal. This complex subject is discussed below in greater detail.

b) The flora of different regions of the gut

The oral cavity and pharynx. The microflora of this region consists largely of bacteria associated with the buccal mucosal cells and the gingival crevice and are considered autochthonous. Any lumenal flora consists of transient or allochthonous

species, only passing through the region or temporarily colonizing a habitat vacated by its autochthonous inhabitants. Streptococci and lactobacilli predominate and a wide variety of anaerobes occurs in the gingival crevice (Russell and Melville, 1978).

The stomach. The adult monogastric stomach and the abomasum of the ruminant are usually at a low pH because of hydrochloric acid secretions. Bacteria and some fungi can persist in the lumen in the food mass. Many of these microorganisms are derived from the food and from the oral cavity, multiplying rapidly until stopped by the fall in pH. Other organisms, principally lactobacilli multiply at this low pH. In addition to this lumenal flora, there is a mucosal flora in the gastric crypts which may be anaerobic or microaerobic and live beneath the protective layer of mucin.

Studies on the gastric bacterial flora of suckling and weaned pigs carried out by Barrow, et al. (1980), Fuller, et al. (1978), McGillivery, et al. (1984) and McGillivery and Cranwell, (1982) have reported that there are between 10^4-10^8 microorganisms/g of gastric homogenised mucosa or contents, and that the dominant bacteria were lactobacilli and Eubacterium spp. Other bacteria isolated were actinomycetes, Bifidobacteria, Peptostreptococcus spp., Streptococci and Veillonella spp. The facultative anaerobes included; Aeromonas spp., Enterobacter spp., E. coli, Klebsiella spp., Micrococcus spp., Serratia spp., Staphylococcus spp. and Streptococci. These bacteria were found adhering to the mucosal epithelium of the pars oesophagea of healthy weaned pigs or isolated from gastric contents by strict anaerobic methods.

In the pig the pars oesophagea consists of keratinized stratified squamous epithelium and may be colonized by lactobacilli and some other indigenous lactic acid bacteria. A similar phenomenon occurs in the oesophagus and stomach of rodents and the crops of chickens in which lactobacilli adhere to the keratinized cells on the surface of the epithelium (Fuller, 1973; 1975; 1977; 1978; 1989; Fuller and Turvey, 1971; Pedersen and Tannock, 1989; Savage, 1970; 1972; 1977; 1984).

The stomach provides an important barrier to microbial overgrowth. Most of the bacteria ingested with food are

destroyed by gastric acid. The bacteria which reach the small intestine are generally acid-resistant and consist of streptococci, fungi, lactobacilli, sporulating bacteria or bacteria protected in ingesta. Bile has inhibitory properties and together with intestinal motility plays an important role in controlling bacterial populations in the small intestine.

The small intestine. In this region there is a lumenal flora of Gram-positive streptococci, aerobic or microaerobic lactobacilli, diphtheroids, fungi and coliforms at concentrations generally less than 10^4 per ml of contents (Gorbach, 1971). The duodenum contains fewest organisms, the jejunum is also relatively sparsely populated but the distal portion of the ileum may contain more Gram-negative bacteria such as aerobic coliforms and anaerobic Bacteroides spp. and clostridia with a total concentration of 10^5 to 10^8 per ml. It represents a transitional zone between the sparse flora of the upper gastrointestinal tract and the high microbial populations of the large intestine. Locally produced antibodies particularly the secretory IgA may be one of the major factors in checking the growth of bacteria in the proximal small intestine where low numbers are recovered in the normal animal (Hersh, et al.,1970).

The mucosal flora comprises aerotolerant species such as enteric streptococci and lactobacilli as well as microaerobic lactobacilli and campylobacters. These are supplemented distally by coliforms and anaerobes.

The large intestine. Just across the ileocaecocolic valve, an important change in the numbers and types of microorganisms can be seen, particularly in the anaerobic populations of the lumen. These comprise/consist of anaerobic lactobacilli, Bacteroides spp., clostridia, Veillonella spp., bifidobacteria, yeasts and fusobacteria, which become the major constituents of the microflora. The anaerobes dominate the flora so completely that they exceed the coliforms and aerobic and facultative flora by a factor of 1,000 to 10,000:1. It appears that the unique environment of the large intestine with its low oxidation-reduction potential and stasis provides anaerobic bacteria with optimal conditions for propagation. It allows the maintenance of a relatively stable bacterial flora by a complex process involving inhibitory substances produced by microbial metabolism

such as short chain fatty acids (butyric, propionic and acetic) and interaction with the physicochemical environment.

The lumenal flora and its interrelationships resembles in some ways that of the rumen in numbers of species and the production of volatile fatty acids.

The mucosal flora of the large intestine includes clostridia, spirochaetes such as Serpulina spp., fusiforms and helicoidal-shaped bacteria, Gram-positive and Gram-negative bacilli, which are known to adhere to substances on or in the mucosal epithelia and are considered to be autochthonous species. In most cases little is known about the adherence mechanisms and the structure(s) to which the organisms adhere on the surface (Savage, 1984).

In all species the volatile fatty acids produced by the flora are absorbed and contribute to some extent to the nutrition of the adult.

Enteric bacteria are ubiquitous in our environment and may contaminate drinking vessels and food for both animals and human beings, leading to colonization of their external and internal surfaces by a wide variety of microorganisms.

c) Colonization

One major phenomenon affecting gastroenteric microbial populations is the colonization of the gut and the development of the adult microflora. This phenomenon has been studied in both monogastric animals and in ruminants. The studies in ruminants provide useful information about the way in which a complex lumenal flora such as that of the large intestine is built up and maintained. These studies have led to the development of many of the techniques used for the study of anaerobes and the identification of key species of gut bacteria. They are reviewed below.

d) Development of gut colonization in animals and man

i) The neonate

The development of the gastroenteric microbial flora of the

healthy full-term neonate begins with a rapid contamination of external and internal surfaces of the foetus when it is exposed to the environment. The gastroenteric tract, sterile during foetal life, becomes colonized within 24 hours of birth. Different bacterial strains and species predominate during the first 2-3 weeks of life and later stabilize, usually by one month of age (Blakey, et al., 1982; Ducluzeau, 1983; 1985; Smith and Crabb, 1961).

Intestinal bacteria colonize the gut in the oral-to-anal direction, and movement in the opposite direction only occurs during pathological conditions such as stasis. The first organisms to colonize the gut are; E. coli, C. perfringens and streptococci within a few hours after birth. By the first 24 hours of life lactobacilli, anaerobic streptococci and flavobacteria can be cultured from all regions of the gastroenteric tract. After reaching a peak, coliform levels drop markedly in the small intestine during the first week of life. After seven days, Bacteroides spp. appear and become the major component of the colonic flora in most mammalian species. One significant change is the increase of anaerobic fusiform organisms accompanied by a decrease in the coliform count at weaning. These fusiforms produce volatile fatty acids (particularly butyric acid) which have an inhibitory effect on the coliforms.

Most reports in colonization have relied on monitoring changes in the faecal flora. The process of colonization in individual regions of the gut has been poorly documented with the exception of the rumen. Here, the extensive knowledge of the flora and the ability to produce fistulated and gnotobiotic animals has provided a fairly comprehensive picture.

The oral cavity is the main portal of entry to the gut for the various microorganisms which later comprise the gastroenteric flora. There are some reports, mainly from man, of the colonization of individual regions of the gut by bacterial species which subsequently affect colonization by other species. An example is that of Staphylococcus epidermidis and E. coli in the human throat, lactobacilli and Salmonella spp. in the avian crop, lactobacilli and E. coli in the pig, Peptostreptococcus elsdenii in the pig caecum (Giesecke, et al., 1970) and Enterococcus faecium and Salmonella typhimurium in germ-free

mice (Barrow, et al., 1980; Fuller, 1975; 1978; 1989; Fuller and Turvey, 1971).

The bacterial colonization in the stomach is mainly by bacteria such as Bacteroides spp. (B. fragilis group) and E. coli in the first 4 days of age. Pseudomonas spp., Enterobacter spp., Clostridium butyricum and Staphylococcus spp. are established after 4 days of life but once gastric hydrochloric acid production is stable, bacteria such as Enterobacter spp. and some streptococci are no longer isolated.

Similar changes are noted in the faecal flora. During the first 4 days of life, Bacteroides spp. were the most common bacteria isolated. By contrast, E. coli and Klebsiella spp. were common after day 4 and persist. During the first 16 days of life Clostridium spp. were isolated with low frequency but after this a steady increase in their numbers was observed. Lactobacilli were seldom isolated and the only viruses identified were rotavirus/enterovirus (Blakey, et al., 1982).

ii) Other animals (Ruminants)

The rumen microflora is the most intensively studied complex gut flora outside the human gingival crevice and has provided information about the enumeration of microorganisms, their identification and the effects of diet and other influences on them. No account of the mammalian intestinal microflora is complete without some consideration of this complex system. It provides some insight into the factors which may govern conditions in the large intestine which has been less well studied.

This organ comprising both, rumen and reticulum, is a large sac-like structure located ahead of the gastric stomach or abomasum and is large in relation with body size (approximately 5 to 10 l in sheep and 100 to 150 l of capacity, in cattle). The temperature in the rumen is near to 39^{o}C because of the heat of fermentation of food and from the normal aerobic tissue metabolism of the animal. The contents are mixed constantly by muscular contractions and relaxations of the rumen wall (Hespell, 1988; Orpin, 1976). The flow of such contents through the rumen is mediated by the water drunk by the animal, but more by the copious production of saliva.

In ruminants the development of colonization by microorganisms begins just after birth but the newborn and young ruminant is, in fact, a non-ruminant, the rumen is small in relation to the abomasum and digestion of milk is by the same process as in any other young mammal, and a mainly lactobacillary-streptococcal, lactose-fermenting type of intestinal flora is present. With the increasing ingestion of solid food as the animal grows, and finally with weaning onto a solid diet, the rumen and its associated complex flora develops. The metabolism of the animal changes to utilize microbial fermentation products rather than sugars in an environment near to neutral pH, buffered by the salts, bicarbonates and phosphates contained in the saliva and the acids produced by bacterial fermentation, giving a final pH of 6.5 which permits the growth of the microorganisms (Orpin, 1976). The development of the rumen and related flora is directly dependent on the diet, but with the different systems and ages of weaning no particular age can be given for the final development of the adult rumen flora.

Under natural conditions the neonatal ruminant is exposed to faecal bacteria and ruminal bacteria from the oral cavity of its mother within a few minutes of birth. The changes associated with colonization have been studied by examining the flora of young ruminants colonized naturally and by examining experimental colonization in gnotobiotic lambs (Lysons, 1975; Lysons, et al., 1971; 1976; Mann and Stewart, 1974).

Gnotobiotic lambs were orally inoculated with a known limited rumen bacterial flora to evaluate the microbial activity and populations in relation to the diet supplemented to the animal. The production of various fermentation products was monitored a different stages according to a bacterial inoculation programme. The process was evaluated using anaerobic bacterial methods of isolation such as selective or differential media (Lysons, 1975; Lysons, et al., 1971; 1976; Mann and Stewart, 1974). The bacterial activity was evaluated by monitoring concentrations of C_2-C_4 volatile fatty acids (VFA), with spectrophotometric determination of formic, succinic and lactic acids, alcohols, and determination of urea and ammonia levels. Hydrogen evolution from the rumen was also measured by the use of mass spectrometer on expired air which contained

gases eructed from the rumen such as methane and carbon dioxide (Hespell, 1988).

As a result of such experiments, the colonization of the rumen by bacteria has been shown to be initiated by Lactobacillus spp. and Streptococcus bovis, which are required for fermentation of sugars in milk and to reduce the rumen fluid's redox potential. Streptococcus bovis comprised 90% of a total viable count of 9 x 10^8/ml of rumen contents at a pH of 6.5, and one day later Lactobacillus spp. increased to about 60%, and the ruminal pH dropped to 5.5 giving at the third day a Lactobacillus spp. count of 8 x 10^8/ml and about 80% of the total in a pH of 4.6. Following this colonization by facultative anaerobes or microaerobic bacteria, Veillonella alcalescens and Megasphaera elsdenii were recovered from lumenal contents during the first 24 hours after inoculation (Lysons, et al., 1976).

At this stage M. elsdenii was also seen in smears and a few days later the pH rose, and the Lactobacillus spp. counts had fallen being just 20% of the total, on day five. The numbers of S. bovis and M. elsdenii increased in a total viable count of 9 x 10^8/ml. In all samples Veillonella alcalescens was observed but the numbers were not established. Seven days later Streptococcus bovis was again the dominant bacterium isolated.

Butyrivibrio fibrisolvens established readily and in more than one study was temporarily the dominant bacteria isolated four days after being orally inoculated. By day 24, observations showed a predominantly Gram-negative flora in total viable counts and Gram stained smears, as in sheep reared conventionally. Bacteroides spp. was from this stage onwards, the principal bacterium isolated with total viable counts of 4 x 10^9/ml. The total viable counts increased from 6.4 x 10^9/ml to an average of 3.3 x 10^{10}/ml, at the end of these studies, and the pH remained relatively constant to an average of 6.5 (Lysons, et al., 1976).

Ruminococcus spp. viable counts were about 10^4/ml of rumen contents, but may have been higher because this bacterium normally colonizes cellulose fibres. The Streptococcus faecium count was 1 x 10^7/ml, Methanobacterium ruminantium varied between 10^4 to 10^6/ml and Anaerovibrio lipolytica was not re-isolated (Lysons, et al., 1976).

These findings are thought to resemble the process of natural colonization.

The adult rumen: The adult rumen shares some bacterial species with the developed monogastric or ruminant caecum. It is highly anaerobic, being bathed in gases which are principally carbon dioxide and methane with only a trace of oxygen, enabling functions such as cellulolysis by bacteria such as Ruminococcus albus and R. flavefaciens and Bacteroides (Fibrobacter) succinogenes to occur.The polysaccharides such as hemicelluloses are hydrolyzed by xylan-fermenting Butyrivibrio fibrisolvens, Bacteroides ruminicola and Eubacterium ruminantium, starch, dextrins and maltose are hydrolyzed by B. amylophilus, and B. ruminicola which are known to be proteolytic and amylolytic, Selenomonas ruminantium is also proteolytic, amylolytic and produces propionate from the extracellular succinate produced by B. ruminicola, Megasphaera elsdenii and Veillonella alcalescens ferment lactic acid produced by lactobacilli and feed lipids are hydrolyzed by Anaerovibrio lipolytica (Hespell, 1988; Hobson and Wallace, 1982a,b).

These bacteria give rise to most of the fermentation products found in the rumen such as carbon dioxide and acetic, propionic, and butyric acids as well as formic, lactic, and succinic acids, ethanol, and hydrogen. All these products are created in a secondary fermentation by bacteria such as Veillonella alcalescens, Peptostreptococcus elsdenii and Selenomonas ruminantium var. lactilytica. They convert lactate or succinate to acetic and propionic acid, carbon dioxide, and hydrogen with Methanobacterium ruminantium which converts hydrogen and carbon dioxide to methane (Giesecke, et al., 1970; Hobson and Wallace, 1982a,b; Mann and Stewart, 1974). In summary, the rumen microorganisms convert feedstuffs into volatile fatty acids which form the animal's energy sources and microbial cells which are its protein source. Also products of microbial metabolism such as B vitamins are available as the microbes and rumen fluid pass through the abomasum and intestines. Similar volatile fatty acids are produced in the large intestines of animals such as the pig.

Bacteria causing proteolysis and deamination are closely related to those mentioned above, and are also extremely sensitive to oxygen. They are preponderantly Gram-negative.

There is also a very close relationship between rumen ciliate protozoa such as Dasytricha, Isotricha and Epidinium spp. a cellulolytic protozoan and the bacteria. They play an important part in the digestion of starch granules and fibres and their population consists of about 10^6/ml (Hespell, 1988).

The actual enumeration of rumen organisms is very important, but is extremely difficult to determine because of the complexity of the system. Bacteria are constantly lost in the ruminal fluid passing to the lower portions of the gut, are attached to particles of feed, and are also attached to the rumen-reticular wall. Methods for the enumeration of the microbial flora in the rumen are and have been used without convincing results because of the difficulties mentioned above (Hobson and Wallace, 1982a,b).

e) Colonization and the normal flora of the pig

i) Normal gastrointestinal flora of the pig

The bacterial flora of the various regions of the gastrointestinal tract of the healthy pig has been studied in both qualitative and quantitative terms by a number of authors mainly during the late 50's and the early 60's. In most cases the papers concerned describe the species and counts of bacteria present in the lumen and contents of the gut and faeces and pay little attention to the mucosal flora. The bacterial species considered to form part of the normal gastrointestinal flora of the pig and the order of their appearance in the tract are reviewed below.

Studies of the faecal microflora of the pig have been conducted by several groups of workers, however, their reports reveal little agreement with regard to numerical relationships between bacterial groups. For example, in studies by Bridges, et al. (1952; 1953), Horvath, et al. (1958) and Larson and Hill, (1955), coliforms, staphylococci, Proteus spp. and Shigella spp. were reported to predominate. Conversely, Briggs, et al. (1954), Fewins, et al. (1957), as well as Fuller, et al. (1960), Kenworthy, (1973), Kenworthy and Crabb, (1963), Pesti, (1962), Quinn, et al. (1953), Smith and Crabb, (1961), Smith and Jones, (1963), Uchida, et al. (1965) and Wilbur, et al. (1960), found

lactobacilli (Lactobacillus acidophilus and L. fermenti) and streptococci (Lancefield's group D, mainly Streptococcus faecium, S. faecalis and S. liquefaciens) to be the most numerous faecal microorganisms. Bifidobacterium spp., Bacteroides spp., Clostridium spp., E. coli, Micrococci, Veillonella spp., bacilli, moulds, yeasts and Pseudomonas spp. were also present in low numbers. Briggs, et al. (1954), Larson and Hill, (1955) and Willingale and Briggs, (1955) studied the flora of the caecum of pigs and concluded that it was essentially the same as that of the faeces, and that counts for both were similar if the caecal counts were increased by $10^{1.9}$ to correct for differences in dry matter.

Studies on the bacterial flora of the alimentary tract as a whole were carried out by Horvath, et al. (1958) in the U.S.A. In Britain, methods for studying the bacterial flora of the alimentary tract were described by Smith and Crabb, (1961) and later applied to investigation of the bacterial flora of pigs by Smith and Jones, (1963). Some other authors who had investigated the gastrointestinal flora of the healthy pig include; Dickinson and Mocquot, (1961), Hill, et al. (1970), Kolacz, et al. (1971) and Uchida, et al. (1965).

ii) Development of gut colonization in pigs

The foetus **in utero** is sterile, but on passage through the vagina during birth it acquires its first microorganisms. The intestinal tract of the newborn pig is initially free from bacteria (Ducluzeau, 1983; 1985; Kenworthy and Crabb, 1963; Muralidhara, et al., 1977; Pesti, 1962; Wilbur, et al., 1960), but within 24 hours of birth, high populations of bacteria reach levels of 10^8 to 10^9/g of faeces. They are derived mainly from the mother's skin and udder, faecal and vaginal microflora, from the air and from the food. A balance becomes established, which in the opinion of Ducluzeau, (1983; 1985) and Hentges, (1970) maintains the gut safe from the establishment of pathogenic infectious agents. By the end of the first day, the whole alimentary tract of the newborn piglet becomes colonized, with large numbers of E. coli and streptococci. Within the first two hours **post partum**, and five to six hours later the populations of these two organisms reach 10^9 to 10^{10}/g of faeces.

Muralidhara, et al. (1977) have studied the development of Lactobacillus spp. and coliforms in healthy newborn pigs and detected Lactobacillus spp. concentrations of 10^4/g of faeces at 4 hours after birth and 10^5 coliforms/g by 8 hours. At 24 hours the two bacteria were present in nearly equal numbers (10^8 to 10^9/g). Clostridium perfringens, lactobacilli, Fusobacterium spp. and Peptostreptococcus spp. are also found in the gut during the first day of life (Ducluzeau, 1983; 1985; Kenworthy and Crabb, 1963; Pesti, 1962; Smith and Crabb, 1961; Smith and Jones, 1963; Wilbur, et al., 1960). After ingestion, these bacteria multiply in the stomach and contents, which contain very little acid in the first 24 hours of life, and also in the proximal intestine.

By the second day of life, the above bacteria are joined by Bacteroides spp. and at the same time the pH of the stomach and contents is sufficiently low to suppress the multiplication of all organisms except the lactobacilli. These then establish themselves as the principal bacterial inhabitants of the stomach, together with the mucosal flora which consists of anaerobic or microaerobic lactic acid bacteria which live beneath the protective layer of mucin in the gastric crypts and in the pars oesophagea (Barrow, et al., 1980; Fuller, et al., 1978).

Studies to estimate the number of viable bacteria/ organisms in the contents of the gastrointestinal tract of pigs were carried out by Horvath, et al. (1958). They showed that facultative anaerobic bacteria are found in numbers which range from 10^3/g to 10^7/g in the proximal gastroenteric tract (stomach, duodenum and jejunum). The numbers increase progressively in the ileum and even more beyond the ileocaecocolic valve, where strictly anaerobic bacteria were found. The site of greatest microbial activity appeared to be the large intestine; while the site of least activity appeared to be, with the exception of lactobacilli, the stomach.

Quantitative and qualitative differences in bacterial populations exist from one region to another. As the pig becomes older, Bacteroides spp., Veillonella spp., Peptostreptococcus spp., Eubacterium spp., yeasts (Torulopsis glabrata and Candida sloofii), and many clostridial species, for example Clostridium butyricum, considered by Baker, et al. (1950) to be the primary

amylolytic agent in the caecum of the pig, also appear in the gut. Some of them are restricted to the large intestine. Alexander, et al. (1976) reported the isolation of Lactobacillus acidophilus, Lactobacillus fermentum, Megasphaera elsdenii, Selenomonas ruminantium and different strains of streptococci from the colon of healthy weaned pigs at concentrations of viable bacteria of about 10^7/g of mucosal scrapings. Streptococcus faecium and Streptococcus faecalis are the two commonly reported species of streptococci in the gut of pigs. Bacteroides spp. were reported to be the predominant organism in the colonic mucosa of healthy pigs, but lower counts than in the contents.

The major change recorded in the bacterial content of the gut at weaning is the tendency of total E. coli counts in the small and large intestinal contents, to increase 3 to 7 days after weaning. Beta haemolytic E. coli predominate at the expense of non-haemolytic strains (Ducluzeau, 1985; Kenworthy and Crabb, 1963; Smith and Jones, 1963). As pigs grow older, there is a tendency for counts of aerobic bacteria to fall and the numbers of organisms voided in the faeces shows a fairly steady decline in aerobic bacteria from 10^{10}/g to 10^8/g (Langlois, et al., 1978b; Smith and Crabb, 1961; Wilbur, et al., 1960).

The median numbers of viable bacteria found in the faeces of adult animals reported by Smith and Crabb, (1961), were $10^{6.5}$/g for Escherichia coli with a range of $10^{5.2}$ to $10^{7.6}$. Clostridium perfringens was found at $10^{3.6}$/g with a range of $10^{2.8}$ to $10^{5.7}$, streptococci were present at $10^{6.4}$/g ranging between $10^{5.7}$-$10^{8.2}$. Bacteroides spp. were present at levels of $10^{5.7}$/g with a range of 0-10^8 and lactobacilli were found at $10^{8.4}$/g with a range of 10^6 to $10^{9.2}$. Fuller, et al. (1960) reported that streptococci were present within the ranges 10^5 to 10^9 organisms/g, lactobacilli, 10^5 to 10^9/g and coliforms 10^4 to 10^7/g wet weight of faeces. Yeasts are commonly found at all levels of the intestine but occur most frequently in the ileum and caecum in a proportion of 0 to 10^7/g as reported by Kenworthy and Crabb, (1963). Veillonella spp. was observed to occur approximately 10^4 to 10^5/g of intestinal contents and Proteus spp., yeasts and moulds were all present at 10^4/g in studies carried out by Horvath, et al. (1958).

Streptococci, which are present within a day or so of birth at concentrations of about 10^8/g, multiply to a lesser extent in the small intestine and to a much greater extent in the large intestine, decreasing rapidly to reach 10^{3-4}/g at 3-4 weeks of age. In the weaned pig, Clostridium perfringens is absent from the stomach and duodenum, but it is usually found in the lower part of the small intestine (distal ileum) and always in the large intestine. Bacteroides spp. are found only in the large intestine of healthy pigs. Veillonella spp. are more common in pigs over 14 days of age, being present in the stomach, small intestine and large intestine. Yeasts are much more common in weaned pigs than in unweaned pigs (Kenworthy and Crabb, 1963; Smith and Crabb, 1961). In all diseased animals the numbers of bacteria in the parts of the alimentary tract other than those directly involved in the pathological process were found to be similar to those in healthy animals (Smith and Jones, 1963).

C) Influences on colonization and the microbial flora

a) Diet

The gastrointestinal flora of healthy and diseased pigs has been shown to be influenced by diet e.g. in pigs fed on an alkaline diet, flooding of the alimentary tract with bacteria was observed (Smith and Jones, 1963). All rectal organisms, with the exception of the total anaerobes and lactobacilli, were found to be lower in numbers when lactose, as compared with starch, was the carbohydrate fed (Wilbur, et al., 1960). In all diseased animals the numbers of bacteria in the parts of the alimentary tract other than those directly involved in the pathological process were found to be similar to those in healthy animals. Since weaning represents the most profound period of dietary change, this is probably the time of greatest susceptibility to these influences in all mammals including man.

i) Dietary antigens

The introduction of a new dietary antigen may promote a number of immunological events specific to the antigen fed, such as antibody response, delayed hypersensitivity and also increased macrophage activity (Miller, et al., 1983; 1984a,b,c;

Stokes, et al., 1983), all of which may affect the type and number of the microbial flora colonizing the gut.

Wilson, et al. (1989) reported that in pigs weaned at three weeks old food protein antigens are absorbed from the intestine. Those antigens produced an antibody response to soya and other proteins such as keyhole limpet haemocyanin to which they had never been exposed. Before the development of tolerance to those antigens, animals pass through a transient gastroenteric immunological hypersensitivity to the dietary antigen often associated with the abrupt introduction of high protein weaner diets, quite frequently in excessive amounts (Bourne and Newby, 1981). This has been implicated as a causal factor in gut damage and postweaning diarrhoea in pigs and also predisposes to gut infection with enteropathogenic E. coli (Miller, et al., 1982; Stokes, et al., 1983).

The high incidence of diarrhoea in the post weaning period has been shown to be associated with dietary factors as well as infectious agents (Hampson, et al., 1988; Miller, et al., 1983; 1984a, b, c; Smith, et al., 1988; Thomson, et al., 1989). This and the similarity between the villous atrophy and the crypt hyperplasia that occurs in the pig after weaning has led to the suggestion that an immunological reaction to dietary antigens may be the initial cause of post weaning malabsorption and diarrhoea (Miller et al., 1983). In marked contrast to the experiments reported by Miller, et al. (1983; 1984a,b,c,), Hampson, et al. (1988) found that their attempts to induce postweaning diarrhoea secondarily to an intestinal hypersensitivity to weaner diets failed, and proved that although postweaning diarrhoea developed it was not related to dietary antigens but to the action of enterotoxigenic E. coli O138 type and to a specific proliferation of rotavirus. They also found that using two different weaner diets the pigs showed no significant differences in food consumption, in bodyweight gain, in ability to absorb xylose, in incidence of diarrhoea, in excretion patterns of enterotoxigenic E. coli and rotavirus or in small intestinal structure.

The withdrawal of the protection afforded by milk antibody is an important factor in the development of diarrhoea after weaning. Miller, et al. (1982) found that the removal of

antibody in sows milk may well be a significant factor in the reappearance of pathogenic E. coli in large numbers in the intestinal tract after weaning, but further experiments suggest that this is not in itself sufficient to provoke diarrhoea due to shifts in microbial populations. Although the direct effects of dietary change last only a few days, the consequences may be prolonged.

b) Immunity

Most work on immunity has been carried out on pathogens. The most detailed has been on Escherichia coli especially in animals such as calves and pigs. It is clear from the studies of Evans, et al. (1980), Klobasa, et al. (1981), McGuire, et al. (1976), Nagy, et al. (1976a,b), Newby and Stokes, (1984), Newby, et al. (1979) and Welliver and Ogra, (1978), that maternal immunoglobulin G (IgG) is absorbed from the colostrum and can prevent disease associated with products of Escherichia coli and prevent adhesion by their fimbriae. Serum immunoglobulin levels reach their peak 12 hours after the beginning of suckling. Serum levels then decline with half lives of 10, 2 and 3 days for IgG, IgA and IgM, respectively, with minimum serum values occurring in weeks 5, 3 and 2 respectively. The effects of maternal antibody and the development of active immunity associated with IgA and IgM in the gut have been documented by Chidlow and Porter, (1979), Hersh, et al. (1970) and Porter, et al. (1974). Although other elements of the gut flora have not been studied in so much detail, it is likely that immunity to them operates in a similar way. This principle is the basis of vaccination of the dam to protect the offspring against enteric disease.

Immunity to certain diseases of the gastrointestinal tract, is not correlated with levels of antibodies in blood serum but is related to the presence of antibody in faeces. This apparently separate immune system operating within the secretions of the gastroenteric tract has been called the secretory or local immune system and over the past two decades has been studied extensively and has been shown to have a number of characteristics that set it apart from that of the circulation (Bourne and Newby, 1981). It is largely mediated by immunoglobulin A (IgA) which is synthesized beneath the

epithelial surface on to which its secreted and is optimally stimulated by immunization at the site of secretion.

It is now recognized that the different local immune systems are interrelated. The most important example of this is the link between the immune system of the gastrointestinal tract and mammary gland. Studies by Bohl, et al. (1972), Chidlow and Porter, (1979) and Evans, et al. (1980) have been shown that immunization of the gastrointestinal tract in pregnant and lactating sows leads to antibody not only in secretions of the gut but also in those of the mammary gland and the antibody in colostrum and milk is directed against organisms inhabiting the gut. Apparently the link is mediated by lymphocytes originating in the gut lamina propria which migrate possibly via lymphatic and blood vessels to lodge and develop in the mammary gland, and use has been made of this link in protecting suckling pigs against neonatal Escherichia coli scours and Transmissible Gastroenteritis (TGE).

Immunity in the gastrointestinal tract is reflected in the variety of ways that it can respond to antigenic stimuli; it does so by antibody production, by reduced responsiveness (tolerance) and by mounting hypersensitivity reactions. Harmless feed antigens provoke a state of tolerance, whilst replicating antigens such as bacteria, viruses and parasites induce an active immunity involving IgA which protects the intestinal tract and maintains the homeostasis of the system (Bourne and Newby, 1981).

c) Other elements of the microbial flora

A variety of microorganisms is known to cause lysis of living bacteria, dead cells or cell wall preparations. Normal intestinal flora reduce the numbers of pathogens by competing for substrates, altering the microenvironment, and producing substances that are bactericidal for pathogens. Among them Bdellovibrios (Starr and Seidler, 1971; Stolp, 1973; 1979) and Microvibrios (Guelin, et al.,1977; 1979) appear to be the more relevant predatory bacteria, but others cause lysis through products of their metabolism. These products are murein-destroying (lytic) enzymes or induce phage-induced lysis,

certain antimicrobials which interfere with cell wall synthesis. Other substances such as gramicidin and tyrocidin from **Bacillus brevis** are highly toxic and lyse pneumococci and streptococci by destroying the cytoplasmic membrane have been also reported by Stolp and Starr, (1963; 1965).

Bacteriophage are viruses that infect bacteria often killing them by lysis. Because of their capacity for lysing bacteria through enzymatic production (lysozymes, virolysins, hyaluronidases and endolysins), phage are an important factor influencing the establishment, maintenance and balance of the flora in the various organs and systems of animals and humans (Calendar, 1970; Stolp and Starr, 1965).

D) Antimicrobials

a) General

Antimicrobials have been used for more than fifty years for the control or suppression of microbial populations involved in human or animal disease and even in plant protection in agriculture. There is a report by Bassett, *et al.* (1980) in which evidence of exposure to tetracycline was found by using fluorescence microscopy in human bones suggesting the use of therapeutic levels of the drug by Sudanese Nubians. The bones came from a cemetery on the west bank of the Nile river, opposite the town of Wadi Halfa in the Sudan during excavations carried out for archaeological studies of an ancient culture (A.D. 350 to 550) in Nubia. This appears to be the first recorded report of the occurrence of antimicrobials in the diet over 1,400 years ago.

Antimicrobials have been defined as agents which are capable of killing microorganisms (biocidal activity) or suppressing their multiplication or growth (biostatic activity). The effects of such substances is reviewed below as an important factor in the development of gastroenteric upset in mammals, birds and especially in pigs.

The antimicrobial(s) are classified according to the manner in which they affect microorganisms. Those which interfere with

the synthesis of the bacterial cell wall include penicillins, cephalosporins, avoparcin and bacitracin. A second group of antimicrobial agents interfere with the synthesis of nucleic acids such as DNA or RNA. Examples of antimicrobials which exert this kind of bacteriostatic action are nalidixic acid, olaquindox and the rifamycins. A third group of antimicrobial agents changes the permeability of the cell membrane. Their action can be either bacteriostatic or bactericidal, examples include polymyxin B and the ionophore antimicrobials monensin and salinomycin. A fourth group interfere with metabolic processes within the microorganism, most of these agents are bacteriostatic. A major group inhibits bacterial protein synthesis. Streptomycin and the aminoglycosides are bactericidal but macrolides such as tylosin, spiramycin, erythromycin and lincomycin are bacteriostatic. So too are tetracyclines and chloramphenicol. Other antimicrobials inhibit microbial energy production **e.g.** nitrofurans such as nitrofurazone and nitrovin, nitrothiazoles such as dimetridazole, and nitroimidazoles such as metronidazole. Finally, the sulphonamides and trimethoprim inhibit stages in the production of intermediates in the synthesis of folic acid required for nucleotide synthesis.

Antimicrobial agents may be administered topically, orally or injected for the treatment of infectious diseases, used as feed additives to animals as growth promotants, or as preventive measures against unidentified infectious diseases.

b) Antimicrobial resistance and R-factors in bacteria

Antimicrobial agents produce their effect by killing or inhibiting bacteria by the mechanisms listed above. Each antimicrobial has a spectrum of activity comprising the bacteria which it can normally be expected to kill or inhibit. Penicillin G (benzylpenicillin) affects most Gram-positive bacteria such as streptococci, staphylococci and clostridia, and a few Gram-negative bacteria such as certain spirochaetes (*Treponema pallidum* and *Treponema pertenue*). Streptomycin affects Gram-negative bacteria such as *Leptospira spp.* and *Haemophilus spp.* and some Gram-positives. However, it became clear very early in the use of antimicrobials that bacteria not only had inherent resistance to an antimicrobial but that they could acquire it.

Acquired resistance as a result of therapy was reported in staphylococci by Forbes, (1949) who identified the mechanism as a natural ability to produce a penicillinase. The proportion of penicillin-resistant strains found in hospitals had risen to 80% of all Staphylococcus aureus isolated (Lewis, 1983).

The detailed mechanisms by which antimicrobial resistance arises have been reviewed by Lewis,(1983), those associated with transferable drug resistance by Foster, (1983) and Watanabe, (1963) and those in enterobacteria by Anderson, (1968b) and Anderson, et al. (1973) **in vitro** and Lacey, (1975) and Linton, (1977) **in vivo**.

Antimicrobials can affect bacteria at sublethal or sub-minimal inhibitory concentrations (sub-MIC) and these effects have been studied since the discovery of penicillins and during the 1940's. Their effects on bacteria were not systematically investigated until the early 1970's in Europe, America and Japan (Lorian, et al., 1978). The effects included morphological and structural changes and growth inhibition (of at least one logarithm compared to an unexposed control) produced by minute concentrations of antimicrobial agents (MAC). It has been reported that the large staphylococcal cells survived phagocytosis when previously exposed to sub-MIC levels of antimicrobials such as penicillins, cephalosporins and cephamycins (Lorian, et al., 1978).

Similar effects have been demonstrated in E. coli where pathogenic determinants such as F4 (K88) adhesin are suppressed (Deneke, et al., 1985).

The main concern with antimicrobial resistant bacterial populations in man and animals which have arisen by the use of antimicrobials in therapy, prophylaxis or growth promotion is that resistance may interfere with the treatment of infectious diseases (Anderson, 1968c; Guinee, 1972; Lewis, 1983; Linton, et al., 1977; Richmond, 1972; Salzman and Klemm, 1966; Siegel, et al., 1974; 1975; Walton, 1979). The mechanisms of resistance in bacteria are varied but R-plasmid infections are considered to be most important in animals and man (Datta, 1969; Guinee, et al., 1970). Resistant organisms from animals may colonize man and initiate disease as a result of treatments given to animals

(Anderson, 1968c; 1975; Cooke, et al.,1971; Guinee, et al.,1970; Loken et al., 1971). Resistance from animals may result from transfer of resistant organisms directly or by transferable drug resistance (TDR). The classic examples of transfer of resistant organisms from animals to man have occurred in the U.K. with multiply resistant S. typhimurium phage type 29 (1963-69) and phage type 204c (1978-present). These resistant strains arose in calves and spread to man(Anderson, 1965; 1968a,b; Linton, 1977).

Several workers in different countries have demonstrated close relationships between the extent of antimicrobial usage and the incidence of antimicrobial resistant E. coli in the intestinal flora of domestic animals (Anderson, 1974; Braude, 1978; Cooke, et al., 1971; Edwards, 1961; Guinee, 1972; Linton, et al., 1975; Loken, et al., 1971; Mercer, et al., 1971; Smith and Halls, 1966; Wierup, et al., 1975;). For example in pigs and calves 85% of E. coli strains isolated carried resistance to one or more drugs when fed antimicrobials for growth promotion, whereas 16% or fewer E. coli isolated from populations in herds not being fed antimicrobials are resistant (Mercer, et al., 1971). In humans, as in animals, the oral administration of antimicrobials such as tetracycline results in an increase of tetracycline resistant faecal bacteria usually with transmissible R-factors due perhaps to poor absorption of the antimicrobial from the upper gastrointestinal tract (Datta, et al., 1971; Edwards, 1961; Guinee, 1972; Guinee, et al., 1970; Hirsh, et al.,1973; Linton, et al.,1972). Sub-therapeutic levels of antimicrobials also encourage the emergence of resistant E. coli strains (Goldberg, et al., 1961).

In animals such as pigs (Edwards, 1961; Linton et al., 1975), calves (Edwards, 1962; Linton, et al., 1975; Loken, et al., 1971) and poultry where antimicrobials were given orally over short periods, the high initial levels of drug resistance decreased soon after withdrawal. However, on farms where antimicrobials were fed continuously at sub-therapeutic levels the incidence of resistant gastroenteric organisms and the frequency of multiple resistance are greater and persist for a long time after the antimicrobial is removed (Linton, 1977; Linton, et al., 1977; Mercer, et al.,1971; Rollins, et al.,1974; Smith and Crabb, 1957; Smith and Halls, 1966). Their results

show that antimicrobial resistant E. coli strains from the three animal species which in the past have been fed antimicrobials for growth promotion are rich in R-determinants and are of diverse O-serotypes.

Richmond, (1972) reported that the persistence of R-plasmid carrying E. coli strains depends directly on the ability of the bacterium to colonize, and that the best colonizers may persist longer after drug withdrawal. If the R-plasmids are transferred to a sensitive recipient which is also a good colonizer, the resultant strain may also persist for a long time. Such resistant bacteria survive at the expense of sensitive ones under selection pressure from antimicrobials and grow up to persist as the dominant component of the flora (Anderson, 1968b; Edwards, 1961; Lacey, 1975; Lewis, 1983; Linton, et al., 1974; 1977; Richmond, 1972; Solomons, 1978). When this occurs with a pathogen, the consequences can be sometimes fatal as in Clostridium difficile pseudomembranous colitis in man. It is theoretically possible for such resistant bacteria to enter the sensitive gut flora of other animals and man by cross infection (Salzman and Klemm, 1966).

Linton, et al. (1988) reported a 4-year study comprising 12 commercial farms in Suffolk on the use of olaquindox (Bayo-N-Ox; Bayer) fed to pigs as feed additive at a final concentration of 50 ppm of feed and its relation with the emergence of resistance among gut coliforms. From their results a steadily increasing incidence and level of resistance to olaquindox was found on farms using it for growth promotion and also in neighbouring farms that did not.

The use of antimicrobials in animals for both therapy and growth promotion and their potential effect on human pathogens has led to the adoption by the U. K. government and the E. C. of guidelines for antimicrobials used in growth promotion in animals which minimize the possibility that resistance to therapeutic antimicrobials may occur.

c) The use of antimicrobials in animal species

i) Antimicrobials as growth promoters in animals

Early in the 1940's some authors reported that

antimicrobials included in diet were capable of improving the growth rate and also the efficiency of utilization of feed in chicks, turkeys and pigs (Jukes, et al., 1950). The decade of 1950-1960 was the period in which most work on the development and/or discovery of the classic antimicrobials for animal use occurred (Solomons, 1978). Such substances are widely used in agriculture. In animals and in pigs in particular the potential for growth improvement lies somewhere on a scale between the pig growing to its full genetic potential and the pig kept under poor conditions of hygiene and husbandry (Walton, 1979). Those incorporated in feed for both therapy and growth promotion are included in the category of non-nutritive feed additives (Braude, 1978; Mackinnon, 1985). Non-nutritive feed additives comprise pharmaceutical substances and also products intended to affect the quality of stored foods. The use of antimicrobials in feed in many countries is controlled by legislation and some of them also require a prescription by a veterinarian to comply with local law, for example the U. K. Medicines Act (1974). Within the E.C. a restricted list of antimicrobials is in use as growth promoters, E.E.C. Directive 85/249 (July, 1985). This list differs from those available in the U.S.A. and other countries in that therapeutic antimicrobials are not included and can only be used in rations on prescription (Randall, 1969; Report, "Swann Committee", 1969).

The properties which an ideal non-hormonal growth promoter should have have been listed by Walton, (1979) as: effective and economic improvement of performance; it should not cause cross resistance to other antimicrobials; it must not be involved with transferable drug resistance; it must not disturb the balance of the normal gut flora; should not be used therapeutically in human or veterinary medicine; it must not be absorbed from the gut to avoid tissue residues; it should not promote Salmonella spp. shedding; it must not be mutagenic or carcinogenic; it should be non-toxic to the animal and its human handlers and it must not give rise to environmental pollution for example it must be readily biodegradable.

In pigs, several workers have reported the use of various antimicrobials and mixtures of them with the idea of improving daily growth rate, increasing feed efficiency and reducing days

to slaughter (Solomons, 1978). Their work has been reviewed by Mackinnon, (1987) and shows that in the U. K. in 1985, 93% of starter feed and 81% of grower feed contains at least one growth promoter. The results vary but it is clear that under certain conditions antimicrobials have a stimulating effect on growth. Mackinnon, (1985) reports results obtained from 66 field trials in nine European countries conducted between 1976 and 1983 in which average daily gain improved 25 g (42%) per day with a range of 23-27 g/day and feed conversion ratio was with a range of 0.087-0.134 an a mean of 0.113 (3.5%). These results were obtained from a variety of feeding systems, feed types, copper inclusion levels, breeds of pig, location, systems of husbandry and types of antimicrobial of differing chemical nature. Growth promotion occurs mainly in the young fast-growing animal, and as the animal gets older the effect diminishes (Bridges, et al., 1952; Henderickx, et al., 1982). The effects are less obvious in animals with the highest health status, in germ-free chicks and in hysterectomy derived or gnotobiotic pigs they may be non existent (Visek, 1978). In other circumstances the presence of diseases such as pneumonias or enteric disease resistant to the action of the growth promoter used, may mean that their inclusion in the feed may have no effect on growth rate.

There is no single explanation of the way in which they act but it is presumed that they act on intestinal bacteria (Bridges, et al., 1953; Henderickx, et al., 1982; Linton, et al., 1975; Mackinnon, 1985; Visek, 1978; Walton, 1979). The small intestinal villi of animals receiving growth promoters are often longer than those of controls, the mucosa is thinner and the small intestines have less weight, similar to those present in germ-free animals (Braude, et al., 1955). This effect may result from suppression of the autochthonous flora. There is also evidence that the effect of antimicrobials on the gastrointestinal flora is not entirely beneficial as some workers report cases in which such drugs had a negative effect (Parker, 1974). Diarrhoea has occurred after the withdrawal of an antimicrobial (Fuller, 1989; Mackinnon, 1987; Olson, 1986; Olson and Rodabaugh, 1976a; 1977; 1978; 1984; Pradal-Roa and Taylor, 1988). Braude, et al. (1953) reviewed the use of antimicrobials in pig feeding and analyzed the results of trials reported up to the beginning of 1952. The report showed that

chlortetracycline and oxytetracycline gave a better growth response than bacitracin, penicillin or streptomycin, but the number of tests used for such comparisons differed widely, and the concentrations of the antimicrobial(s) added were not always the same. In some trials, removal of the antimicrobial reduced the rate of growth of the treated pigs and their advantage over the non-treated animals was reduced or even abolished (Langlois, et al., 1978b). This finding is one of the important pieces of evidence for the post-antimicrobial effects investigated in this thesis.

ii) Effects of antimicrobial growth promoters on the gut flora of animals

Several workers have carried out bacteriological examinations of the gastrointestinal contents or faeces of animals receiving diets with or without antimicrobials, and have reported that the effect of such drugs on the bacterial flora is very complex (Bridges, et al., 1952; 1953). In some cases a reduction in the total bacterial count is found, in others, there is an increase, in yet others no dramatic or even consistent change was found at all in the composition of the gastrointestinal flora. Even where no spectacular bacteriological change occurs, there is no doubt that the gut population will adapt itself to the presence of the antimicrobial. The development of harmful resistant strains of pathogens or the gradual loss of activity of the antimicrobial through the development of resistance by the gut flora may be the reason (Linton, 1977; Linton, et al., 1975; Threlfall,1984).

The wide range of antimicrobial agents that possess growth-promoting properties when given in the diet has suggested to many workers that they exert this effect through their action on the gut flora. Such action may be by encouraging the growth of organisms that synthesize known or unidentified nutrients, by depressing the growth of organisms that compete with the host for supplies of nutrients, by eliminating known pathogens or by eliminating bacteria which produce toxins that reduce the growth potential of the animal (Linton, et al., 1975).

Some specific examples are known. Toxigenic strains of C. perfringens are generally sensitive to the antimicrobial growth

promoters which usually have an effect on Gram-positive bacteria and form good examples of the type of organism which can be affected. Walton, (1979) describes the effect of zinc bacitracin on Escherichia coli isolated from pigs and poultry in which the bacterial cell wall developed lesions, and there was an increase in the sensitivity to oxytetracycline used at therapeutic levels. This may be due to an increase in the permeability of the cell wall allowing the therapeutic antimicrobial to penetrate the bacterial cell. Francis, et al. (1978) reported reduced Lactobacillus spp. counts and also reduced coliform counts as well as reduced total aerobic counts in the digestive system and in the feed by adding zinc bacitracin to the diet at concentrations of 55 mg/kg of diet to broad breasted white turkey poults.

Tiamulin at concentrations of 10, 20 and 30 ppm has been found to reduce the number of intestinal bacteria which carry R-plasmids in studies carried out in conventional pigs from 15 to 96 kg body weight (Gedek and Hofmann, 1983). Henderickx, et al. (1982), Vervaeke, et al. (1976; 1979) and Visek, (1964), report that virginiamycin and spiramycin used as growth promoters reduce the proteolytic microorganisms responsible for the production of bacterial ammonia in the gut. They also found that virginiamycin added to the feed at 50 ppm increased the number of coliforms and enterococci and that numbers of C. perfringens, lactobacilli and micrococci were somewhat reduced.

iii) Effects of therapeutic levels of antimicrobial on the gut flora of animals

A wide variety of antimicrobials is used in the treatment of animal disease. In some cases the agents are administered in feed and water for convenience and may affect the gut flora directly. In other cases they are given parenterally and may enter the gut in secretions or in the bile. In some cases the antimicrobials are given for diseases of the gastroenteric tract and in others for disease elsewhere. In all cases these therapeutic levels of antimicrobial have a profound effect on the gut flora incidental to their therapeutic effect. In animals these incidental effects have rarely been studied or if studied have not been published with the exception of resistance studies.

The direct effects on intestinal pathogens have been documented in many cases and are described below under the individual disease syndromes of the pig. In some cases antimicrobials can eliminate a bacterial species from the gut completely as in the case of dimetridazole or tiamulin used in the eradication of swine dysentery (Blaha, et al., 1987; Wood and Lysons, 1988). In other cases (Larsen, 1987) tiamulin has been shown to reduce the adhesive ability of F4 (K88) and F5 (K99) enterotoxigenic E. coli **in vitro**. Similar effects have been demonstrated with tetracycline on the attachment of F4 enterotoxigenic E. coli to porcine small-intestinal cells by Deneke, et al. (1985).

iv) Bacterial overgrowth

Another type of effect produced by antimicrobials has been demonstrated by the prolonged use of therapeutic antimicrobials in **humans** which allows the development of pseudomembranous colitis (PMC) which is related to an overgrowth of Clostridium difficile and production of its A and B toxins. This is due to a direct effect on the indigenous gut flora the suppression of which allows the proliferation of the pathogenic clostridia and consequent damage to the epithelium and the development of diarrhoea (Bartlett, 1981; Bartlett, et al., 1978a,c; Borriello, 1990; Borriello and Larson, 1981; George, 1986; George, et al., 1978; Larson, et al., 1978; Lee and Olson, 1986; Mitchell, et al., 1987c; Price, et al., 1979; Young, et al., 1986). Also, Carwardine, (1982) and Berry and Levett, (1986) reported the overgrowth of Clostridium difficile and chronic diarrhoea in **dogs** with prolonged use of antimicrobials such as ampicillin, mimicking the clinical profile present in humans with PMC.

A similar syndrome in which chronic diarrhoea associated with overgrowth of C. difficile followed the prolonged use of various antimicrobials has been reported in **hamsters** by Bartlett (1981), Borriello, et al. (1987), and Price, et al. (1979), in **rabbits** by Guandalini, et al. (1988), LaMont, et al. (1979), Mitchell, et al. (1986) and Rehg and Lu, (1981), in **guinea pigs** by Lowe, et al. (1980) and in **cats** by Taylor, (1994).

Disturbances of the gastrointestinal flora resulting in

illness, diarrhoea, ketosis and death have been reported following the administration of lincomycin to **dairy cattle** (Anon, 1979; 1984b, c; Lang, 1979; Lyne, 1984; Okoh and Ocholi, 1986; Plenderleith, 1988; Rice, et al., 1983), **sheep** (Bulgin, 1988), **rabbits, hamsters** and **guinea pigs** (Bartlett, et al.,1977; 1978b; Katz, et al., 1978; LaMont, et al., 1979; Maiers, et al., 1984; Thilsted, et al.,1981) and **horses** (Raisbeck, et al.,1981). Tetracyclines have been also associated with the development of Colitis "X" in **horses** (Andersson, et al., 1971; Baker and Leyland, 1973; Cook, 1973; MacKellar, et al., 1973; Rooney, et al., 1963; 1966; Sanford, 1976; Schiefer, 1981).

The organisms responsible have not been clearly identified in either the horse or the cattle. In the horse, Finger and Wood (1955) suggested that treatment with oxytetracycline led to the development of salmonellosis. Prescott, et al. (1988) reported the isolation of a Clostridium as a possible agent associated with Colitis "X" in ponies, and Swerczek,(1979), also implicated C. perfringens in the pathogenesis of Colitis "X" in the equine. Schiefer, (1981) suggested that C. perfringens type A was either the principal cause or had an important role in the pathogenesis of the disease. Wierup and DiPietro, (1981) also reported C. perfringens type A as the sole pathogen isolated in horses with Colitis "X" and consequently proposed to change the name of the disease to equine intestinal clostridiosis instead of Colitis "X". Colitis "X" in horses is still, however, known as such and remains of unknown aetiology. Studies of tetracycline administration to horses by Horspool, (1992) have shown that the Colitis "X" may be related to an increase in the total number of viable Clostridium spp. and is unlikely to be due to an increase in the numbers of a single species of clostridia. However, she also reported that problems were encountered in the isolation and identification of C. perfringens, and that C. butyricum was the most commonly identified species of the genus Clostridium.

Similar situations have been documented in animals. In pigs used in experiments for the study of the aetiology of swine dysentery, its treatment and control, the use of different antimicrobials such as tiamulin (22.5 mg/l), tylosin (66 mg/l), ronidazole (0.003%, 0.0015% and 0.00075%/drinking water), virginiamycin (110 mg/kg of feed), cobalt arsanilate (0.05% in

feed), gentamicin (0.0013%/drinking water) and sodium arsanilate (0.01% in feed) showed that the effect on the autochthonous flora is an important factor in the development of post-antimicrobial diarrhoea (Olson, 1980; 1986; Olson and Rodabaugh, 1976a, b; 1977; 1978; 1984).

E) Probiotics

a) General

In 1965, Lilly and Stillwell first used the term probiotic to describe substances produced by one ciliate protozoan which stimulated a consistent 50% increase in growth of another organism. Parker, (1974) used the term to describe "organisms and substances which contribute to intestinal microbial balance", but later such definitions were revised by Fuller, (1989) who defined a probiotic as "A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance". These live supplements have been used as feed additives for many years but recently due to the prospect of bans on antimicrobials used as growth promoters by some countries and actual bans in others, interest in probiotics has received more attention from the scientific community. Attempts have been made to develop them as feasible alternatives to replace the antimicrobials which are widely used in agriculture for the purpose of promoting growth rate and improving feed efficiency in farm animals (Fuller, 1989; Lyons, 1987; Pollmann, et al., 1980).

b) Use of probiotics in animals

The nature of the probiotic supplement resides in the organisms included in it and their composition varies according to the diet, age, the animal species in which such treatment is to be established and the period for which the probiotic is fed. Mordenti, (1986) concluded from his work in pigs that feeding piglets prior to and during weaning, results in the prevention of pathological disorders of the digestive tract.However, many bacteria are host specific, so a probiotic should ideally contain a microorganism isolated from the type of animal which will be treated later on as studies reported by Nielsen, et al.

(1988). The success of probiotic organisms depends on the number of viable bacteria delivered to the gut, their capacity to colonize by attachment to the gut epithelium which can allow slow-growing organisms to colonize and their ability to grow in the gut environment using the available substrate. In addition they must have the capacity to resist the antibacterial mechanisms which operate in the gastroenteric tract such as pH, bile, enzymes, peristalsis, and the competitive exclusion effect from the "normal flora".

Probiotics can be administered to animals by inclusion in the pelleted feed or may be produced in the form of paste, capsules, powder or granules for dosing animals directly or through their food (Fuller, 1989; Lyons, 1987). Almost all the probiotics currently in use contain lactobacilli and/or streptococci and a few contain bifidobacteria. Probiotics may consist of single strains or may contain up to eight strains in which the bacteria most often used are Lactobacillus bulgaricus, L. acidophilus, L. casei, L. helveticus, L. salivarius, L. lactis, Lactobacillus plantarum, Streptococcus thermophilus, Enterococcus faecium, Enterococcus faecalis, Bifidobacterium spp. and E. coli, which are all intestinal strains except for L. bulgaricus and S. thermophilus.

The reason for the use of lactobacilli and streptococci resides in the fact that high populations of these bacteria may be bacteriostatic or bactericidal for pathogenic enterobacteria depending on the number of viable organisms used. Lactobacilli have been shown to have this property both **in vitro** and in the chicken crop **in vivo** (Fuller, 1977; 1978). They may reduce the numbers of E. coli in gnotobiotic chicks, at concentrations of 3.8×10^8/ml of lactobacilli giving a final pH of 4.10, the effect was bactericidal but numbers in excess of 7.0×10^6 only prevented the growth of E. coli. These results are directly related to the low pH produced by the lactobacilli which also inhibited streptococcal growth and were bactericidal to Micrococcus spp. and Bacillus cereus (Fuller, 1977; 1978). Similar effects were reported in conventional weaned pigs by Barrow, et al. (1980), Nielsen, et al. (1988) and Porter and Kenworthy, (1969) and in young piglets by Muralidhara, et al. (1977). Lactobacillus spp. administration decreased the number of coliforms in the faeces of calves (Ellinger, 1978). The inclusion in the diet of L.

acidophilus as a supplement in poultry feeds has been reported by Francis, et al. (1978) to improve weight gain and feed efficiency and also to reduce the total aerobic and coliform counts in the digestive system of turkey poults. Similar effects have been reported by Tortuero, (1973) in broiler and Leghorn chicks and by Fuller, (1977) in chicks.

In the opinion of Fuller, (1989) their effect may be mediated by a direct antagonism against harmful organisms by adhering to the epithelial cells, by their metabolism or by the stimulation of immunity (immunoglobulin levels and phagocytic activity). Lyons, (1987) and Pollmann, et al. (1980) suggested a change in the gastroenteric flora and reduction of Escherichia coli levels, the production of antimicrobial substances such as nicin from streptococci and acidophilin and reuterin (Axelsson, et al., 1989) from lactobacilli, the production of hydrogen peroxide, synthesis of lactate and reduction of pH, and adhesion to epithelial cells of the gastroenteric tract by lactobacilli preventing colonization by pathogenic bacteria as possible modes of action by which probiotics exert their beneficial effects on the host.

From studies carried out with 4 week-old recently weaned pigs (average 7 kg) and with growing-finishing crossbred pigs (from 37 to 57 kg/live weight) by Pollmann, et al. (1980) it appears that the lactobacilli are superior to the streptococci as a feed additive to pig diets and that they also produce a similar effect to that of antimicrobial growth promoters in which a greater response was observed with young than with growing-finishing pigs in average daily weight gain and feed conversion rate. These results were also improved by the addition of lincomycin (Lincomix, Upjohn) at concentrations of 110 mg/Kg suggesting a possible additive effect when L. acidophilus was used.

Underdahl, (1983) used Streptococcus faecium at concentrations of 3.5×10^9 C.F.U./g given twice daily in the milk, for 6 days to gnotobiotic pigs, to study its effect on E. coli diarrhoea. Mild diarrhoea occurred for 47-82 hours after being challenged with 3 different strains of pathogenic E. coli in the treated animals. The other pigs receiving only the pathogenic E. coli, developed persistent severe diarrhoea and in one study 5 out of 8 pigs died and the remaining 3, did not eat

and were in very poor general condition. These results were obtained using gnotobiotic Duroc, Hampshire, Yorkshire, Lacombe, Landrace, Berkshire and some crossbred pigs, and suggest that the feeding of S. faecium limited or slowed colonization with E. coli pathogenic strains, improved weight gain and reduced mortality.

Other bacteria such as "Lactobacillus" sporogenes and Clostridium butyricum ID have been used by Han, et al. (1984a, b) as growth promoters in broiler chicks. Their results show that L. sporogenes fed at levels of 0.04% and 0.06% improved weight gain and feed conversion efficiency, reduced caecal ammonia concentration and also reduced the staphylococci and coliform counts in faeces and in large intestinal contents. Similar results in chicks were obtained when C. butyricum ID at levels of 0.01% and 0.05% was supplemented in the diet. Significant differences were not detected in intestinal pH level among treated and control groups. In the studies using L. sporogenes they report a reduction in intestinal pH in the diverticulum when compared with the control group. Han, et al. (1984c) added C. butyricum to the diet of crossbred growing pigs weighing approximately 14-15 Kg, in order to study its effect on performance and intestinal microbial flora. They found that the supplementation of C. butyricum at levels of 0.01%, 0.02% and 0.03% caused reduction in faecal counts of staphylococci and coliforms, improved the daily weight gain and feed efficiency apparently through the increase of digestibility and nitrogen retention.

F) Disease

Disease affects the microbial flora in a number of ways;

I) Diseases of any system including the gut can reduce feed intake and therefore affect the composition of the gastrointestinal flora.

II) Disease can cause changes in villous height and the digestive capacity of the gut and allow bacterial fermentation of undigested food residues.

III) Pathogenic bacteria can multiply to form the majority of the gut bacteria.

The individual diseases in the weaned pig are described below:

a) Gastroenteric disease of the weaned pig

Enteritis and diarrhoea of weaned pigs continue to be a serious economic problem in piggeries throughout the world (Kyriakis, 1983; 1989a; Leman, et al., 1986; 1992; Nigrelli and Gatti, 1985; Taylor, 1989; Tzipori, 1988; Tzipori, et al.,1980b; Wilson, 1986). Changes in environmental conditions including mixing immune carriers and non-immune piglets at weaning, the loss of maternal (passive) immunity at 6-10 weeks of age, contact with poorly cleaned pens, the introduction to new diets (antigens), the change from a liquid to a dry diet, the effect of feed antimicrobials (growth promoters), the stress of fighting for the establishment of social hierarchies (Kyriakis, 1983; 1989b; Kyriakis and Andersson, 1989; Kyriakis, et al., 1986; Taylor, 1989), have all been shown to contribute to the development of enteric disease (Deprez, 1988; Madec and Josse, 1983). Experimental studies with caesarean-derived, colostrum-deprived pigs, gnotobiotic pigs and naturally raised pigs have provided new information about gastrointestinal disease.

Enteritis in the weaned animal may represent a continuation of earlier infections such as Cryptosporidium, coccidiosis and rotavirus infections, arise at weaning or develop later. The gastroenteric disorders of the weaned pig are reviewed below. Specific diseases may affect the weaned pig and other well defined syndromes such as colitis are currently of unknown aetiology. These will be reviewed last.

G) Bacterial diseases of the weaned pig

The bacterial diseases may respond to treatment, and post-antimicrobial diarrhoea may arise as a result of the mechanisms outlined above. The parasitic and viral diseases may occur without reference to antimicrobials and must be distinguished from them. For this reason the individual diseases are reviewed here.

a) Escherichia coli infections

Infections of the gastroenteric tract associated with pathogenic strains of Escherichia coli have been called "Enteric Colibacillosis" as a generic term covering these clinical

conditions commonly diagnosed in young pigs in all pig-rearing countries (Bertschinger, 1992). Such enteric disturbances associated with diarrhoea are divided accordingly into neonatal diarrhoea,post-weaning enteritis and oedema disease depending on the specific bacterial serogroups and serotypes isolated and the age group of the pigs affected. New isolates of enterotoxigenic **E. coli** (ETEC), enteropathogenic **E. coli** (EPEC), enteroinvasive **E. coli** (EIEC), enterohaemorrhagic **E. coli** (EHEC), enterotoxemic **E. coli** (ETEEC) or attaching and effacing **E. coli** (AEEC) strains are increasingly being identified (Bertschinger, **et al.**, 1992; Hall, **et al.**, 1988; Levine, 1987; Moon, **et al.**, 1988; Thorns, **et al.**, 1989; Wray, **et al.**, 1993). During the past decade other toxins such as verocytotoxin (VT) and cytotoxic necrotising factor (CNF) have been isolated from cases of diarrhoea in pigs and other farm animals (Caprioli, **et al.**, 1983; Chapman, **et al.**, 1989; De Rycke, **et al.**, 1990; Gonzalez and Blanco, 1985; Holland, 1990; Konowalchuk, **et al.**, 1977; Wray, **et al.**, 1993).

i) **Post-weaning enteritis**

Within 3 days of weaning enteritis and diarrhoea are common findings in pig farms all over the world (Kyriakis, 1983). Often such findings are related to the stress of weaning (loss of maternal contact and introduction to strange pens and penmates), change of diet, reduction in ambient temperature and isolation of EPEC, ETEC or ETEEC strains(**e.g.** Abbotstown, O149:K91:H10(B): K88ac (LT^+)(STa^+), O147:K89:H19(B):K88ac, O9:K87:K88ac and O157: K88ac:H43 (LT^+)(STb^+) (Cox, **et al.**, 1986; McAllister, **et al.**,1979; Miller, **et al.**,1984a,b,c; Nagy, **et al.**, 1990; Orskov, **et al.**, 1977; Sellwood, 1979; Smith and Jones, 1963; Soderlind, **et al.**, 1988; Thomlinson, 1963; 1969) are usually isolated from affected pigs at this age (3 to 4 weeks), perhaps from persistence of the bacteria in buildings or from infections during the suckling period. In the immediate post-weaning period **E. coli** counts are abnormally high and composed almost entirely of haemolytic strains (Kenworthy and Crabb, 1963; Wray, **et al.**, 1993). Besides the presence of ETEC/ETEEC in the gut, other factors such as the genetic susceptibility of the host (Rutter, **et al.**, 1975; Sellwood, 1979; 1984; Snodgrass, **et al.**, 1981), nutritional, environmental and immunological factors and the existence of rotaviral infection, can influence the occurrence

and severity of diarrhoea (Bertschinger, et al., 1992; Kyriakis, 1983; Lecce, 1983; Lecce, et al., 1982; 1983; Nabuurs, et al., 1986; Sarmiento and Moon, 1988; Sarmiento, et al., 1988a,b; Tzipori, et al., 1980a,b).

ii) Oedema disease (E. coli enterotoxaemia/Bowel Oedema)

Oedema Disease (OD) was first described by Shanks (1938) in Northern Ireland, nowadays OD occurs worldwide and its also present usually after 1-2 weeks of weaning. It is caused by proliferation of ETEEC strains which produce a toxin(Imberechts, et al., 1992; Timoney, 1986), and is precipitated by the stress of weaning and withdrawal of lactogenic protection (Bertschinger et al., 1988; 1990; Chopra, et al., 1964a, b; Deprez, 1988; Schofield and Davis, 1955; Schofield and Robertson, 1955; Stamm, et al., 1990). The serotypes involved are different to those responsible for neonatal diarrhoea and from those isolated during post-weaning enteritis (Erskine, et al., 1957; Mainil, et al., 1989; Marques, et al., 1987; Nielsen, 1986; Sojka, 1965; Sojka, et al., 1957; 1960; Timoney, 1950; 1957). This disease is characterized by the occurrence of sudden death and the development of oedema and nervous signs. Diarrhoea is not a consistent feature.

b) Campylobacter spp. infections

Campylobacter spp. are bacterial pathogens which have been isolated worldwide from cases of enteric disorders in man and domestic and wild animals (Luechtefeld, et al., 1981; Skirrow, 1982; Skirrow and Benjamin, 1980b). They are also present as part of the normal intestinal flora in domestic animals (Garcia, et al., 1983b; Smibert, 1984; Walton, 1989). Such enteric conditions have the characteristics of diarrhoea with the presence of blood, altered blood and/or mucus. Most of the strains from cattle resembled C. jejuni, whereas those from pigs resembled C. coli; poultry, human and other animals strains are of mixed types (Skirrow and Benjamin, 1980a,b). The pathological changes included a thickening of the mucous membrane of the small, and some times large intestine which histologically appears as a proliferation of the intestinal epithelium leading to a varied clinical signs depending on the nature of the bacterial species involved, age of the animal affected, and from

the nature of the infection (single or mixed).

In pigs C. coli, C. hyointestinalis, C. mucosalis and C. jejuni have been isolated from diarrhoeic animals. In contrast C. jejuni alone appears to be the most common bacteria found affecting human populations(Al-Mashat and Taylor, 1980; Ferreira et al., 1979; Gebhart, et al., 1983; 1985; Hosie, et al., 1979; Lambert, et al., 1984; Olubunmi and Taylor, 1982a, b; Skirrow, 1977; 1982).

c) Porcine Proliferative Enteropathy (P.P.E.)

This disease is widespread (Chang, et al., 1984; Rowland and Lawson, 1986; 1992; McOrist and Lawson, 1993) and may underlie a range of other diseases (Proliferative haemorrhagic enteropathy, Necrotic enteritis, Regional ileitis) in the age group of pigs under consideration here (Jonsson and Martinsson 1976; Lawson, et al., 1977; 1979; Love, et al., 1977; Rowland and Lawson, 1975; Rowland and Hutchings; 1978; Yates, et al., 1979).

Many herds are infected but the exact methods of spread between herds are not yet well recognised, but since rats, rabbits and hamsters all suffer similar enteric conditions cross-species transmission appear possible. On the other hand carrier pigs may be of importance in the introduction of the disease, but up to date the role of vectors on the disease transmission still uncertain (McOrist and Lawson, 1990; Yates, et al., 1979). Mapother, et al. (1987) quote that such a proliferative enteropathies had had an incidence as high as 20% in the U.S.A. with losses of $10 to $20 million annually.

The specific causal agent of these proliferative lesions in pigs resembles a Campylobacter species but is distinct from the other campylobacters found to be at the lesion site (Alderton, et al., 1992; Lawson, et al., 1988a,b; McOrist and Lawson, 1990; McOrist, et al., 1989a). Similar lesions to P.P.E. have been produced in hamsters inoculated with this organism (Gebhart, 1987; McOrist, et al., 1989b) but it has been impossible to cultivate it with the culture media available and in use for this purpose (McOrist, et al., 1989b; Ward and Jones, 1990). The aetiological agent has been visible in tissues for many years as a silver-stained curved bacteria within the cytoplasm of

affected crypt epithelial cells. It has recently been named as Ileal symbiont (IS) intracellularis (Gebhart, et al., 1993). It may be grown in rat enterocytes IEC-18 cell culture in which it multiply slowly (Lawson, et al., 1993; McOrist and Lawson, 1993; McOrist et al.,1989a). Pure cultures have been used to reproduce the disease in conventional minimal disease pigs (Lawson, et al. 1993). The disease has been reproduced in conventional crossbred pigs inoculated orally with organisms isolated in cell free medium, designated RMIT 32A by Alderton, et al. (1992).

d) Swine dysentery

This infectious mucohaemorrhagic colitis with world-wide distribution that primarily affects pigs during the growing-finishing period was first described in 1921 by Whiting, et al. The aetiology of the disease remained unknown for 50 years before it was identified in Britain.

The organism considered to be the primary aetiologic agent of swine dysentery was first identified by Taylor and Alexander (1971) and later named Treponema hyodysenteriae by Glock and Harris, (1972) and Harris, et al. (1972). Initially, both pathogenic and non-pathogenic strains were classified as Treponema hyodysenteriae (Kinyon, et al., 1977). However, recent studies by Stanton, (1992) and Stanton, et al. (1991) resulted on its reclassification in the new genus Serpulina, which also includes the non-pathogenic Serpulina innocens. These studies were based on their genetic, physiological and morphological characteristics which differentiated them from the other members of the genus Treponema. The organism is a Gram-negative, oxygen-tolerant, anaerobic spirochaete. It is 6-8.5um in length, 0.3 to 0.4 um in diameter, loosely coiled, motile and strongly Beta haemolytic (Harris and Glock, 1986; Harris and Lysons, 1992; Taylor, 1972; 1989).

e) Spirochaetal diarrhoea

Since its first description in 1980, this syndrome has been reported from Canada, U.S.A. (Girard, et al., 1989; Jacques, et al., 1989; Spearman, et al., 1988), Poland, the Netherlands, France, and Denmark (Taylor, 1992b). It has been associated with

mucoid diarrhoea in conventional weaned pigs but reports show that in SPF pigs orally inoculated, non-pathogenic effect was found except for soft faeces and transient dysentery (Taylor, et al., 1980).

The causal agent of this syndrome is a thin spirochaete 6-10um in length, 0.25um in diameter and possesses 5-7 fibrils in the axial filament. It forms weakly Beta haemolytic colonies after 3-5 days in horse blood agar, incubated at 37°C under anaerobic conditions. It is distinct from the Gram-negative, indole negative, fructose fermentation positive organism classified as species, Treponema innocens by Kinyon and Harris, (1979). Recently it was renamed as Serpulina innocens by Stanton, (1992) and Stanton, et al. (1991).

f) Clostridial Diarrhoea

The clostridial species most commonly associated with enteric disease in the pig is Clostridium perfringens. The majority of papers and reviews describe a fatal necrotic and haemorrhagic enteritis of pigs less than 1-week-old associated with C. perfringens type C (Bergeland, 1986), but the presence of other types of C. perfringens, especially type A, has been described in some enteric disease outbreaks in recent years (Estrada-Correa, 1986; Estrada-Correa and Taylor, 1986; 1988; Taylor, et al., 1987).

C. perfringens is a Gram-positive, encapsulated, non-motile anaerobic bacilli. The strains consist predominantly of single cells which are stout, large and have truncated ends. Spores are difficult to demonstrate in smears from ordinary media, but when present, in sporulating medium, they are ovoid and central to eccentric. All C. perfringens strains produce exotoxins. Each type is unique in the number, potency, and biological effects of the toxins it produces but all are biochemically similar. Haemolysis patterns on horseblood agar may differ from type to type (Estrada, 1986; Taylor and Bergeland, 1992).

i) Clostridium perfringens type A

C. perfringens type A enterotoxin producing strains have been now identified in the faeces of diarrhoeic piglets and in

the post-weaning period (Nabuurs, et al.,1983). This enterotoxin affects the small intestine mainly in the terminal ileum and also the large intestine of recently weaned pigs (Amtsberg, et al., 1976; Estrada-Correa and Taylor, 1986; 1988; 1989; Moon and Dillman, 1972; Olubunmi, 1982). Older finishing pigs and sows may also develop diarrhoea with loss of condition.

ii) Clostridium difficile

The organisms is a Gram-positive spore-forming (oval and subterminal), anaerobic rod, usually motile in broth cultures, peritrichous and are 0.5-1.9 X 3.0-16.9um. Some strains produce chains of two to six cells aligned end-to-end. Surface colonies on blood agar are 2 to 5 mm, circular, occasionally rhizoid, flat or low convex, opaque, grayish, and have a matt to glossy surface. All strains produce an evanescent pale green fluorescence under long wave length ultraviolet light after 48 h incubation. C. difficile produce two large protein toxins (A and B). Recent studies indicate that sporulation might be closely related to toxin production (Borriello, 1990; Kamiya, et al., 1992). Toxin A (enterotoxin) causes haemorrhage and fluid accumulation in the small intestine and caecum, diarrhoea, and death in hamsters and mice experimentally inoculated (Ketley, et al., 1987; Lyerly, et al., 1985; Mitchell, et al., 1986; 1987c). Toxin B (cytotoxin) is extremely cytopathic for all tissue cultured cells tested (Mayall, et al., 1987; Mitchell, et al., 1987a; 1987b).

The significance of C. difficile as a causal agent of intestinal infections in animals is still not completely understood. It was first described as the cause of human pseudomembranous colitis which had been initiated in the majority of cases by antimicrobial treatment with clindamycin and lincomycin, but cases have been associated with a number of other antimicrobial agents (Bartlett, et al., 1978a,b; Borriello and Larson, 1981; George, et al., 1978; Larson, et al., 1978), and later it was also found in **hamsters** (Bartlett, et al., 1977; Borriello, et al., 1987; Price, et al., 1979), **rabbits** (Rehg and Lu, 1981), **guinea pigs** (Lowe, et al., 1980), in **dogs** suffering from chronic diarrhoea (Berry and Levett, 1986), in household pets was reported by Borriello, et al. (1983); they reported than 23% of dogs were found to excrete C. difficile. Carwardine,

(1982) was the first to report the involvement of this organism in cases of persistent diarrhoea in dogs and cats.

In pigs it has been isolated alone or demonstrated together with its toxins from the small intestine and faeces, but the clinical significance of such findings is still not completely clearly understood. It has been described in swine dysentery (Lysons, et al., 1980), and it has been associated with natural infections accompanied by diarrhoea (Jones and Hunter, 1983), in gnotobiotic pigs dosed orally with C. difficile lesions were seen in the caecum and colon (Lysons and Hall, 1982) and it has also been inoculated in conventional piglets to demonstrate its effects in the colonic mucosa (Sisk, et al., 1982). Although the relationship with antibiotic withdrawal and diarrhoea is clear in human enterocolitis. C. difficile is not yet clearly associated with such disturbances in pigs in spite of the wide use of antimicrobial therapy and antibiotics as growth promoters which itself should affect the internal intestinal balance prevalent in the bacterial flora. However, human cases of C. difficile infections in the absence of antibiotic therapy have also been reported (Rampling et al., 1980).

g) Salmonellosis

This disease is usually a chronic disorder in weaned pigs and occurs with a high morbidity and mortality (some times 100%) is caused by Salmonella choleraesuis or other salmonellas which are not necessarily host specific. It is commonly found in piggeries all over the world and its importance is partly due to its capacity to infect the human population, in food poisoning as well as many other species (Wilcock and Schwartz, 1992).

Early studies by Lawson and Dow, (1966) and Harrington, et al. (1971) placed S. choleraesuis as the main cause of pig salmonellosis in Britain and in the U.S.A. Recently the isolation of S. typhimurium (from rodent-contaminated feed) and exotic salmonellae such as S. saintpaul or S. heidelberg have been reported frequently (Ikeda, et al., 1986; Linton, 1981; Oosterom, 1987; Reed, et al., 1985). S. typhimurium has been implicated as an endemic cause of diarrhoeic disease in some pig farms (Heard and Linton, 1966; Jubb, et al., 1985; Reed, et al.,

1986). It has also been reported as a probable causative agent of rectal strictures (Wilcock, 1974; Wilcock and Olander, 1977a, b). In 1977 Sojka, et al. reported 558 cases of swine salmonellosis in England and Wales between the year 1968 and 1974. Although the number of cases appears to be declining around 100 outbreaks of salmonellosis are recorded annually in pigs in Britain (Linton, 1981; Taylor, 1989).

h) Yersiniosis

Yersinia spp. have been recorded in enteric disease in pigs and in normal pigs faeces (Brewer and Corbel, 1983; De Barcellos and De Castro, 1981; Fantasia, et al.,1993; Hunter, et al.,1983; Neef and Lysons, 1994; Toma and Deidrick, 1975). Until recently the species Y. pseudotuberculosis and Y. enterocolitica had not been properly distinguished and literature published prior to the early 1970's may have referred to Y. pseudotuberculosis when in fact the organism concerned was Y. enterocolitica. In much of the literature an element of uncertainty still remains about the identity of the species isolated, and in this review the species names given by the various authors will be used.

Apart from causing enteric disease in pigs, Yersinia enterocolitica and Yersinia pseudotuberculosis are of significance in pig husbandry because they are pathogenic for humans causing acute gastroenteritis (the most common frequent clinical form of this infection), food poisoning, mesenteric lymphadenitis or terminal ileitis, acute appendicitis, septic polyarthritis, septicaemia, erythema nodosum, abscesses and other varied respiratory symptoms (Bercovier and Mollaret, 1984; Marks, et al., 1980; Toma and Lafleur, 1974; Winblad, 1973; Wooley, et al., 1980). Yersinia enterocolitica and Yersinia pseudotuberculosis have been isolated from the rectal mucosa of pigs with the rectal stricture syndrome (Boyd and Taylor, 1990; Taylor, 1992a). Since it is commonly found in the gastrointestinal tract of pigs, it is a potential significant zoonosis-inducing organism distributed worldwide with most cases being reported from the northern hemisphere, especially Europe. Yersiniosis in other parts of the world is thought to be due to importation of animals from Europe; migration of birds has also probably played a significant role (Obwolo, 1976; Taylor, 1989).

H) Parasitic infections of the weaned pig

Both the protozoa and metazoa are known to be involved in enteric diseases of pigs. In Britain, enteric parasitic infection is generally subclinical and therefore difficult to diagnose except by laboratory and necropsy procedures. The relative rarity of nematode infection in Britain is probably due to the modern system of husbandry, where the majority of animals are kept on concrete for much of their lives. Where they occur, internal parasites continue to be an economic factor in pork production. Subclinical infections inhibit weight gain and decrease feed conversion, therefore the time required to reach market weight is longer. Lesions of the alimentary tract and other organs are responsible for these setbacks and may result in condemnation of carcasses at slaughter. In many cases the lesions associated with nematode infections are poorly described. Infections with parasites will be largely unaffected by antimicrobial treatment and they are therefore included here because of their potential involvement in enteritis.

a) Coccidiosis

Coccidia are a group of sporozoa in the family **Eimeriidae** commonly parasitic in epithelial cells of the intestinal tract, but also found in the liver and other organs, it includes three genera **Eimeria**, **Isospora** and **Cystoisospora**. Coccidiosis occurs universally but is of most importance where animals are confined or housed in small areas (Lindsay, **et al.**, 1992). Until recently coccidiosis was thought to be very rare in Britain, but Roberts, (1980) showed that it is a widespread subclinical condition. The presence of coccidia in the faeces of piglets with diarrhoea has been reported by Roberts, **et al.** (1980), Stuart, **et al.** (1982) and in pigs with postweaning diarrhoea by Nilsson, (1988).

b) Cryptosporidiosis

Cryptosporidium spp. is commonly found in the microvillous border of epithelial cells lining the digestive and respiratory tracts.These coccidian parasites of the family **Cryptosporidiidae** were first observed by J.J. Clarke in 1895 and later described by E.E. Tyzzer in 1907. They infect mainly the cells at the tips of the villi in the jejunum and ileum of young animals (Current, **et al.**, 1983; Kennedy, **et al.**, 1977; Snyder, **et al.**, 1978) but there are also reports of the identification of **Cryptosporidium spp.** in gallbladder, bile ducts, pancreatic tubules (rhesus

monkey), respiratory tract (turkey) and gastric glands (mouse). In domestic animals such as calves it has been often associated with diarrhoea (Morin, et al., 1976; Pearson and Logan, 1978; Pohlenz, et al., 1978a, b; Snodgrass, et al., 1980; Tzipori, et al., 1980b). Pigs orally inoculated with calf cryptosporidia by Moon and Bemrick, (1981) and Tzipori, et al. (1980a; 1981b; 1982b) develop infection and diarrhoea. Experimental reproduction in lambs (Tzipori, et al., (1981a; 1982a) and in calves (Tzipori, et al., 1982b) has also been reported.

In recent years Cryptosporidium spp. have been recognized as an important, widespread cause of diarrhoeal disorders in several animal species (Angus, 1983; Kennedy, et al., 1977; Schultz, 1983; Tzipori, 1983) including humans (Angus, 1987; Anon, 1984a; Current, et al., 1983; Meisel, et al., 1976; Nime, et al., 1976). The disease has occurred in a veterinary student in contact with diarrhoeic calves affected with cryptosporidiosis (Anderson, et al., 1982). In humans epidemiological surveys based on the examination of stool specimens show that Cryptosporidium is distributed worldwide, including east and west Africa, north, central and south America, Australia, Europe and Asia (Ungar, et al., 1986).

c) Balantidium coli

Balantidium coli, a ciliated protozoan, is found mainly in the caecum and anterior colon of pigs and is known to be a secondary invader of lesions already present on the intestinal mucosa. On its own, it cannot penetrate an intact intestinal mucosa (Corwin and Stewart, 1992; Corwin, et al., 1986). Its ability to invade secondary lesions is said to be due to the production of hyaluronidase, which breaks down the ground substance and enlarge the lesion. The effect of this secondary invasion is seen clinically as anaemia, enteritis and reduced growth in affected young pigs. B. coli is often seen in necrotic debris of chronic salmonellosis (Wilcock and Schwartz, 1992). The organism is unaffected by antimicrobials (Taylor, 1993).

d) Other parasites

i) Oesophagostomum

Oesophagostomum infections have been reported to cause diarrhoea in pigs, but infections are more frequently associated with poor weight gain (Hale, et al., 1986). Oesophagostomum is

the most common helminth in British pigs. Eighty-five per cent of sows and 45% of weaners are known to be infected (Pattison, et al., 1980). The parasitic burden in many of the infections does not appear to be heavy enough to cause clinical disease characterized by diarrhoea and weight loss. In heavy infections, the clinical disease is produced.

O. dentatum and O. quadrispinulatum are the two major species found in pigs in Britain (Taffs, et al., 1969). Experimental reproduction of diarrhoea and anorexia with heavy doses of larvae of Oesophagostomum has been reported (Poelvoorde and Berghen, 1981). The mechanical damage to the mucosa of the large intestine in very heavy infection may be sufficient to provide an environment conducive to the proliferation of other pathogens.

ii) Trichuris suis

T. suis infection is common in porkers and baconers in Britain, but the numbers present are usually low. In other countries it is sometimes responsible for a severe ulcerative typhilitis (Corwin and Stewart, 1992; Corwin, et al., 1986).

Infection follows the ingestion of infective eggs. T. suis is known to reside in the caecum and colon, with migration limited to the walls of the gut. All subsequent development occurs in the caecal and colonic mucosa. In heavy infection, irritation of the intestinal mucosa may result in diarrhoea, sometimes accompanied by mucus and blood, anorexia, reduced performance and dehydration in growing pigs (Hale, et al.,1986). Experimental reproduction of the disease with heavy doses of eggs has been reported (Beer and Lean, 1973; Hass and Collins, 1973). Bacterial infection of the lesions has been reported.

The pathology of the affected gut may range from virtually no host tissue reaction (Beer, 1973), to a catarrhal enteritis with oedema and nodule formation (Powers, et al., 1960; Batte, et al., 1977).

Pigs are most susceptible to clinical diarrhoea or bloody scours due to T. suis in the 2 to 6 months of age. Immunity appears to keep infections low thereafter (Powers, et al., 1959). Normal bacterial flora of the gut is known to affect the severity of the disease and infectivity of larvae, as eggs fail to hatch in gnotobiotic pigs (Burden, et al., 1987; Rutter and Beer, 1975).

Necropsy is the best method of diagnosis of Trichuris

infection. Detection of eggs in the faeces may be less helpful in diagnosis as Trichuris spp. do not lay eggs continuously (Powers, et al., 1960). Treatment with flubendazole at 30 ppm for five days has been reported by Thienpont, et al. (1982) to control the natural infection in weaned pigs.

Other small intestinal nematode parasites e.g. Strongyloides ransomi, have been reviewed by Corwin, et al. (1986) and Corwin and Stewart, (1992).

I) Viral diseases of the weaned pig

Viruses are a common cause of diarrhoea in pigs. Although many different viruses have been isolated from the intestine or from diarrhoeic faeces, only three species are considered as major enteric pathogens: Transmissible Gastroenteritis virus (T.G.E.), Porcine Epidemic Diarrhoea virus (P.E.D.) and Rotavirus. Pathogenically the course of these infections is comparable; malabsorption is predominant, due to a loss of membrane-bound digestive enzymes and to destruction of absorptive enterocytes, causing excessive intraluminal accumulation of osmotically active material in the intestine. Secretion of crypt epithelial cells and immature villous enterocytes may increase further the amount of intestinal fluid. The severity of clinical signs depends on the extent of enterocyte damage and resulting villous atrophy. Although classical T.G.E. is regarded as the most severe diarrhoeal disease known, P.E.D. is moderately severe and rotavirus as rather mild, great variations occur in clinical signs in practice. An aetiological diagnosis has always to be based on the detection of virus antigen.

a) Transmissible Gastroenteritis (T.G.E.)

Transmissible Gastroenteritis is a highly contagious viral enteric infection of pigs resulting in a very high mortality in pigs under two weeks of age. The mortality rate in pigs over 5 weeks of age is very low. T.G.E.is caused by a coronavirus which is relatively host-specific for pigs (Cartwright, 1969; Garwes, 1988; Giles, 1977; Pritchard, 1982; 1983; Saif and Bohl, 1986; Saif and Wesley, 1992; Wood, 1979).

b) Porcine rotaviral infection

Rotavirus infections are commonly diagnosed in young pigs

suffering of profuse diarrhoea, all over the world (Bridger, 1988; Pospischil and Guscetti, 1989). The first reports of rotaviruses isolated from pigs in various countries were by Bohl, et al. (1978), Lecce, et al. (1976), Woode and Bridger, (1974) and Woode, et al. (1976). Such rotaviral infections are associated generally with pathogenic strains of E. coli which worsen the clinical picture of the disease, and increases considerably the mortality which may rise until 100% in fully susceptible animals. The incidence of infection has been reported by Woode, (1986) to be as high as 90% in piglets. When infection is prevalent into a herd 33% of the affected young pigs may die. In weaned pigs mortality by rotavirus is low but diarrhoea or soft faeces are always present and represents the economically most important effect of the disease due to loss of growth in recovered pigs (Bohl, 1979; Pritchard, 1983).

c) Adenoviral infection

The first association of porcine adenovirus with enteric disease of pigs was recorded by Haig, et al. (1964) who isolated the virus from a rectal swab from a 12-day-old piglet with diarrhoea. Enteric adenoviral infection is largely associated with mild disease in piglets although infection may persist after weaning. Four serotypes of porcine adenovirus are known, and serological surveys indicate that infection is widespread in pig populations. Antibodies to the virus are found in 70-80% of slaughtered pigs in Britain. The virus is excreted in the faeces most frequently in the postweaning period (Coussement, et al., 1981; Derbyshire, et al., 1966), while adult pigs rarely excrete the virus, even though they have high serum antibody levels.

Diarrhoea has consistently been reproduced in experimental infection of pigs with Type 4 porcine adenovirus (Shadduck, et al., 1967), and has also been observed as one of the clinical signs in experimental infection of pigs with other serotypes of the virus (Derbyshire, et al., 1975; Harkness, et al., 1971). Adenovirus infection is diagnosed by viral isolation and serology (Derbyshire, 1992a).

d) Porcine Epidemic Diarrhoea (P.E.D.)

P.E.D. of pigs has been described as a highly contagious disease characterised by vomiting, diarrhoea and inappetence of pigs at all ages. This diarrhoea syndrome was first described in England by Wood, (1977) and in Belgium by De Bouck and Pensaert

(1980) and Pensaert and De Bouck (1978). The disease has since been reported in other parts of the world. The syndrome appears to be due to a coronavirus distinct from T.G.E. and Haemagglutinating Encephalomyelitis Virus (H.E.V.) and material in which it occurs can reproduce a syndrome resembling the classic disease (Tyrrell, et al., 1978).

Type I disease was first recognised in Yorkshire in 1971 by Oldham, (1972). Since then it has been seen in Lancashire, West Midlands, East Anglia and Aberdeen. Type II disease was first described in 1977 by Wood. The pathogenesis of P.E.D. has been reviewed by Pensaert, (1986; 1992) and is similar to that of T.G.E.. In partially immune piglets only a localised area of the gut may show villous atrophy.

e) Other viral diseases

i) Enteroviruses

Enteroviruses have been isolated from and are known to be present in enteric lesions in the pig. They are frequently isolated from the faeces of piglets with diarrhoea. Enteric strains of porcine enteroviruses appear to be ubiquitous. Transmission of infection occurs by the faecal-oral route. Endemic infection with several serogroups of these viruses can be demonstrated in conventional herds and is probably maintained in groups of weaned pigs (Bohl, et al., 1972). Infection is normally acquired by piglets shortly after weaning when maternal antibody protection is lost, and pigs from several litters are mixed together, and it persists for at least several weeks. Adult pigs rarely excrete enteroviruses, but they are known to have high antibody levels. Pigs of any age are, however, fully susceptible to infection with a sero-group to which they have not previously been exposed.

Natural infection follows an ingestion of the virus and it is well established that initial replication of the virus occurs in the tonsils and intestinal tract (Baba, et al., 1966; Long, 1985). The ileum and the large intestine are most frequently infected and contain higher titres of virus than the upper small intestine. Serological identification of the virus isolate by

immunofluorescence and immunoperoxidase staining has been suggested by Derbyshire, (1992b) and Watanabe, (1971).

In natural field infection, transient diarrhoea has been described as the major clinical sign seen in infection with enteroviruses. Diarrhoea has also been produced experimentally by enteroviruses in piglets believed to be free of other pathogens. The diarrhoea is mild and relatively transient (Derbyshire, 1992b).

ii) Caliciviruses in Diarrhoea

Caliciviruses are R.N.A.-containing viruses 37 nm in diameter with a petal-like arrangement of capsomeres and have been identified in cases of post-weaning diarrhoea in pigs in Britain, U.S.A. and Japan (Flynn, et al., 1988). Porcine isolates have been shown to be antigenically distinct from the calicivirus of vesicular exanthema. Calici-like viruses have been grown in pig kidney cells in the presence of gnotobiotic intestinal content and eventually cause rounding and detachment of cells (Flynn and Saif, 1988). Infection of gnotobiotic piglets caused diarrhoea lasting 3-7 days. Virus was present in the villous epithelial cells of the duodenum and jejunum and caused significant villous atrophy. It could be demonstrated in intestinal contents (Bridger, 1980).

iii) Toroviruses in Diarrhoea

Recently, Scott, et al. (1987) reported the finding of torovirus-like particles in a 3-week-old piglet suffering from severe enteritis by direct electron microscopy, from contents of the small intestine. Such viruses have been named Torovirus due to their physical appearance under electron microscope. Two types have been identified, one was a kidney-shaped form and the other was spherical or oval, both may be found forming clumps or alone and they had a clearly defined fringe of short peplomers different to that seen on coronavirus. Torovirus-like particles have been reported in humans (Beards, et al., 1986) and other animal species such as goats, sheep and pigs (Weiss, et al., 1984). Horzinek and Weiss, (1984) first proposed this new family of viruses, the Toroviridae. Their clinical significance

still to be determined.

J) Colitis as a novel syndrome

In recent years a "new" clinico-pathological enteric feature was reported to affect weaned to fattening pigs in Britain, and later reports from other countries in Europe and America followed. Since 1985 there appears to be an increase, veterinarians now believe 5 to 10% of herds in the U.K. have had colitis (Lysons, et al., 1988; Taylor, 1989). Colitis or "non-specific colitis" as had been called by some, is a mixture of clinical signs which itself shows similarities to known enteric diseases, but with the peculiarity of inflammation of the large intestinal mucosa distinct from that of swine dysentery. In many cases described in the literature (Duncan and Lysons, 1987; Smith and Nelson, 1987; Wilkinson and Wood, 1987) agents known to cause disease in this age group have not been sought (Taylor, et al., 1987).

The aetiology and pathogenesis is unknown, but it has been related with an infectious component and nutrition. Observations from the field suggest that the syndrome occurs on a particular feed and that when that feed is changed the disease disappears (Smith, et al., 1988; Spearman, et al., 1988). In other studies in which the diet was fed as pellets, meal and as ground pellets the disease occurred on the pelletised ration and not on the meal (Thomson, et al., 1989). It has been also associated with spirochaetal diarrhoea and S. innocens infection by Spearman, et al. (1988) and recently with Yersinia pseudotuberculosis infection by Neef and Lysons, (1994). Typically, growing affected animals develop softening of the faeces and some times pass mucus. The syndrome is most common in the 8-10 week age group but can occur later. Between 10 to 20% of pigs in a herd may be affected. The epidemiology of colitis is quite different from swine dysentery. It is present in high health status herds where swine dysentery has never been seen and methods for eradication of swine dysentery by medication and cleaning have not eliminated colitis (Lysons, 1986; Taylor, 1989). Growth rates may be depressed and feed conversion may be affected. There may be little effect on body condition or, on some farms, a number of animals may become thin, despite of treatment.Deaths from the disease are very rare.

This review indicates that direct descriptions of post-antimicrobial enteritis are rare. The association between antimicrobial use and the occurrence of antibiotic associated disease is well documented especially with regard to **Clostridium difficile** enteritis in man and other species (Bartlett, **et al.**, 1978a,b,c; Borriello and Larson, 1981; George **et al.**, 1978; Larson, **et al.**,1978). It is also clear that inadequate treatment or inappropriate treatment are commonly associated with the continuation or reappearance of enteric disease. This failure of treatment or recrudescence have been recognised in the pig and described by Olson, (1980;1986) and Olson and Rodabaugh, (1976a, b; 1977; 1978; 1984) for swine dysentery. Such recrudescence is typically associated with appearance of the disease within the normal incubation period following withdrawal of treatment. Antimicrobial resistance and its development also represent another common reason for antimicrobial associated enteritis.

The literature reviewed suggests that colonisation depends upon access of organisms to the gut and on factors preventing their colonisation. The inhibition of colonisation followed by explosive colonisation and enteritis is well known from post-weaning enteritis where diet exposure to agents and waning immunity all combine to affect colonisation. This study hypothesises that the prolonged administration of antimicrobial particularly over weaning, should produce similar syndromes and explain and reproduce the scarce reports from the field of post-antimicrobial enteritis not associated with recrudescence of disease.

In doing so, two approaches were adopted, one was to examine the possibility that the phenomenon could be identified in the field and the other was to reproduce the disease by withdrawing treatment given to relatively healthy pigs free from many enteric diseases. The review of enteric disease of the weaned pig indicates the potential complexity of diagnosis of disease and the fact that viral and parasitic diseases in particular occur and may not respond to antimicrobial therefore complicating any assessment.

CHAPTER 2

GENERAL MATERIALS AND METHODS

The materials and methods described below were used throughout this study.

1. DIAGNOSTIC MATERIALS

These consisted of rectal/faecal samples, samples of gastrointestinal tissue and contents obtained from pigs on the experimental farm belonging to the Glasgow University Veterinary School (Cochno farm), from farms in the west of Scotland and elsewhere in the United Kingdom.

Rectal faecal samples were examined and post mortem material was also examined from pigs which had died or been submitted live to Glasgow University Veterinary School for the diagnosis of gastroenteric disorders.

The amount of information given with these samples varied but they were mostly from cases or outbreaks in which treatment had been unsuccessful or might have been associated with bacterial overgrowth.

The date and origin was recorded for each sample and details such as pig number, sex, the nature of the sample, treatment history, etc., were recorded if available.

A) Faecal samples and intestinal contents

Faecal samples and intestinal contents submitted for diagnosis were examined by methods used throughout this study. In every case the colour, consistency, smell, where relevant, and the presence or absence of blood, mucus, necrotic material and undigested food was recorded. Both quantitative and qualitative bacteriological examinations were carried out as described below and limited virological and parasitological examinations were also carried out.

B) Necropsy procedure

All pigs in these studies were treated in the same general way regardless of whether they were presented for diagnosis or were experimental. In some cases where animals had died some time before submission, only a limited series of examinations were performed.

a) Ante mortem clinical inspection

Live pigs submitted for diagnosis were examined by the methods described below for clinical examination (Section 9) whenever possible and the results recorded.

b) Blood sampling

Blood samples were taken from the external jugular vein (Douglas, 1977) prior to euthanasia and were allowed to clot. The clotted samples were left at room temperature for about 5 hours and later the serum was removed with a pasteur pipette, placed into glass tubes and centrifuged at 1, 500 g for 10 minutes in a GPR centrifuge at 25°C (Model Cat. No. 349703, Beckman Instruments, Ltd.). The serum was removed and stored at -20°C in 5 ml amounts in sealed plastic sterile bijou bottles until required for further serological diagnostic procedures.

c) Euthanasia

All live animals were killed by the injection of Pentobarbitone Sodium B.P. (Vet.), ('Euthatal' May and Baker, Ltd., Dagenham) into the anterior vena cava followed by exsanguination.

d) Gross post mortem examination

Any external abnormalities were recorded. Particular attention was paid to dehydration, the presence of scalding of the perineum and evidence of diarrhoea.

This procedure was carried out as soon after death as possible (Thorpe and Thomlinson, 1967) using the general

procedures described by Wells, (1977), Straw and Meuten, (1986; 1992) and Taylor, (1989). Particular attention was paid to the gastrointestinal tract but the other organs of the abdominal and thoracic cavity were also examined for the presence of gross lesions (Rowland, 1989). The gross changes noted were recorded.

The examination was carried out with the animal on its back. Disarticulation of the hip joints and section of the muscles attaching the scapula to the body wall was carried out. The posterior abdominal wall was pinched so that it was clear of the abdominal contents and a cut was made cranially to remove a strip of the wall. The cut was continued cranially through the costochondral junctions, up the neck and to the mandibular symphysis. The colour of carcase muscles was recorded as well as the presence or absence of dehydration.

The abdominal cavity was examined for any superficial pathological changes of the viscera (colour, changes in size and shape, surface texture, fluid exudates or adhesions within the cavity itself) and the anatomical location. After initial inspection, the ribs were broken to expose the thoracic cavity and its contents. After superficial examination the thoracic viscera were freed from the carcase and examined in more detail. Any samples required for histopathology and microbiology were taken at this time.

The appearance of the liver was examined and the state of the gallbladder noted. The gastrointestinal tract was examined more thoroughly and serosal abnormalities, congestion, adhesions and abnormalities in position recorded. The tract was then removed by cutting through the oesophagus at the cardia and cutting the mesentery at the root. The rectum was cut at the pelvic brim and the gastrointestinal tract removed. The caecum and ileo-caeco-colic junction were located and the tract orientated for detailed examination. The stomach was opened along its greater curvature and the presence or absence of oedema in its wall was recorded. The contents were examined and samples were taken for bacteriological examination. The mucosa was examined and then rinsed for further examination. Samples were taken for histological examination. The duodenum, jejunum, ileum, and the mesenteric lymph nodes were then examined. The bowel was opened and the appearance of the contents noted, and

samples taken for bacteriology. The mucosa was examined and then rinsed for further study. Samples of bowel wall were taken for examination for the presence or absence of villi and for histology and bacteriology. This process was repeated in the caecum and colon respectively.

Freshly taken portions of small and large intestinal mucosa were placed in sterile physiological saline and examined using a dissecting microscope for the presence or absence of villi, the villous architecture and inflammatory change.

Samples of lymph node and other abdominal organs were taken for bacteriological and histological examination when relevant.

e) Histopathological techniques

Sections of stomach, duodenum, jejunum, ileum, caecum and colon were fixed in 10 per cent neutral buffered formol saline for at least 72 hours, before trimming and embedding. The samples were embedded in paraffin wax and sections were cut at 3-4 um mounted on glass slides and stained routinely with Haematoxylin and Eosin (H & E). All the stained sections were examined under the light microscope and the results recorded.

C) Sampling methods for bacteriological examination

a) Faecal samples

Rectal faecal samples were collected daily directly from the rectums of the pigs in the experimental studies using either a thermometer or a gloved finger to stimulate the production of faeces. Each whole rectal faecal sample obtained was placed in a sterile plastic or glass universal container which was identified beforehand with; pig tag number-colour and date of collection, and returned to the laboratory for the bacteriological, virological and parasitological examinations described below.

b) Gastrointestinal contents

Samples from stomach, duodenum, jejunum, ileum, caecum and colon were obtained after the gross post mortem examination was

performed, the external abnormalities recorded and the tract removed from the carcase. Each part of the gastroenteric tract was opened with a separate pair of scissors, the contents examined for colour, consistency, smell and the presence or absence of blood, mucus, necrotic material and undigested food. The findings were recorded, and then approximately 20 g of the contents of each section were placed in a sterile plastic or glass universal container which was already identified with; pig tag number-colour, date of collection and site from which the sample had been taken. The samples were then returned to the laboratory for the bacteriological, parasitological and virological examinations described below.

Both rectal faecal samples and gut content samples reached the laboratory within one hour of collection.

2. BACTERIOLOGICAL EXAMINATION

This was similar for samples of diagnostic and experimental origin and for both gut contents and rectal faecal samples. Samples from organs other than the gastrointestinal tract were examined on media and in conditions appropriate to the pathogens thought to be present.

Upon arrival at the laboratory direct smears were prepared from each sample on clean glass slides, air dried and heat fixed, stained by Gram's method (Soltys, 1963) and examined by light microscopy using an oil immersion lens for the presence of bacteria. The presence of morphologically distinct bacteria such as spirochaetes, campylobacters, clostridia and their spores was recorded. Smears from each region of the gut and from faeces were also prepared, air dried and fixed with methylated spirits for examination of cryptosporidial oocysts. These smears were stained by a modified Ziehl-Neelsen method (Garcia, et al., 1983a; Henriksen and Pohlenz, 1981) using cold strong carbol fuchsin for 10 minutes, decolourized with 3% acid alcohol for 5 minutes and counterstained with Loeffler's methylene blue, washed with tap water and air dried. Cryptosporidial oocysts appeared as red circular bodies 4 um in diameter when examined under the microscope using the oil immersion lens. The results were recorded.

A) Qualitative bacteriological examination of enteric samples

The qualitative examination of gastrointestinal contents and rectal faecal samples was carried out by streaking onto different selective and non-selective solid media prepared according to the manufacturer's instructions (Oxoid Manual, 1982) using a stiff bacteriological wire loop (Medical Wire & Equipment Co., Ltd.) and incubated under the appropriate atmospheric conditions as described below.

a) Aerobic enteric bacteria

Rectal faecal samples and contents from stomach, duodenum, jejunum, ileum, caecum and colon were cultured in aerobic conditions after being inoculated onto sheep blood agar plates (Blood Agar Base No. 2, Oxoid, Ltd. CM 271) with added 7% defibrinated sheep blood (GIBCO, Ltd. Cat. No. 101-01 E), and MacConkey agar plates (MacConkey Agar, Oxoid, Ltd.CM 7 with added 0.075 g neutral red). Beta haemolytic *E. coli* and non-lactose fermenting colonies were isolated on these media. The identity of the bacteria isolated was confirmed from the colonial morphology and the effect on the media, smears were prepared and Gram stained as described above after a period of incubation at 37°C for 24 hours. When required, further biochemical tests were performed using the API 20 E strip System. (Appendix A).

i) Faecal Streptococci

The presence or absence of faecal streptococci was achieved by inoculation onto Slanetz and Bartley agar plates (Slanetz & Bartley Medium, Oxoid, Ltd. CM 377). To confirm the identity of the bacteria isolated after 72 hours aerobic incubation at 37°C, the API 20 STREP System (Appendix B) was used on characteristic colonies on a limited number of occasions.

ii) Salmonellae

A single sample from the gut of each pig and each faecal sample used in these studies was examined for the presence or absence of *Salmonella spp.* by the inoculation of a loopful of

the faecal material into Tetrathionate broth (Tetrathionate Broth Base, Oxoid, Ltd. CM 29) to which 0.2 ml of Iodine solution (Iodine 6 g, Potassium iodide 5 g in 20 ml of distilled water) had been added. The inoculated broth was incubated for 24 hours at 37°C in aerobic conditions and subcultured by plating onto Salmonella-Shigella Agar plates (SS Agar [Modified] Oxoid, Ltd. CM 533) and Desoxycholate Citrate Agar plates (Desoxycholate-Citrate Agar [Hynes modification] Oxoid, Ltd. CM 227). After 24 hours incubation at 37°C two non-lactose fermenting colonies from each plate were examined further by the inoculation of Urea broth (Urea Broth Base, Oxoid, Ltd. CM 71) with 5 ml of added 40% urea solution (Oxoid, Ltd. SR 20). The inoculated urea broth was incubated at 37°C for 18-24 hours. Colonies that failed to hydrolyse urea were further tested by stabbing and streaking onto Triple Sugar Iron (TSI) Agar (Triple Sugar Iron Agar, Oxoid, Ltd. CM 277) slants and incubated for 24 hours at 37°C to determine hydrogen sulphide production, gas production and sugar(s) fermentation.

Slide agglutination tests were carried out on presumptive Salmonella isolates with Salmonella polyvalent H specific and non-specific, and Salmonella polyvalent O antisera (Wellcome Diagnostics Reagents, Ltd.). In order to confirm their identity as salmonellae further biochemical examination was carried out using the API 20 E strip System (Appendix A). The positive identification of any culture was recorded and reported to the Divisional Veterinary Officer and then the culture was sent to the Scottish Salmonella Reference Laboratory, Department of Bacteriology, Stobhill General Hospital, Glasgow, for confirmatory testing and serogrouping.

b) Microaerobic organisms

i) Campylobacters

The samples under study were inoculated onto specific culture media for the isolation of Campylobacter spp. These bacteria were isolated by inoculation onto Blood Agar plates (Blood Agar Base No. 2, Oxoid, Ltd. CM 271) with added 7% defibrinated horse blood (Oxoid, Ltd. SR 50) and Campylobacter selective supplement (Skirrow) (Oxoid, Ltd. SR 69).

ii) Lactobacilli

Lactobacillus spp. was isolated by the inoculation of de Man, Rogosa and Sharpe Agar plates (M.R.S. Agar, Oxoid, Ltd. CM 361). Incubation for the isolation of both campylobacters and lactobacilli was carried out in anaerobic jars (Don Whitley Scientific, Ltd.) as described below but without (the inclusion of) catalysts. The final oxygen concentration was 4%. Further examination of colonies found to be morphologically similar to the ones under study was carried out by preparation of smears stained by Gram's method and examination under the light microscope using the oil immersion lens.

c) Anaerobic enteric bacteria

The anaerobic bacteria considered in these studies as potentially important enteric pathogens were:

i) Enteric spirochaetes

Large Gram-negative spirochaetes (Serpulina hyodysenteriae and Serpulina innocens), were isolated from the gastroenteric contents and rectal faecal samples by streaking onto Spectinomycin Agar plates (Blood Agar Base No. 2, Oxoid, Ltd. CM 271) with added 7 % defibrinated horse blood (Oxoid, Ltd. SR 50) and supplemented with 400 ug/ml spectinomycin of SPECTAM injectable (CEVA, Ltd. 5488) for serpulinas.

ii) Clostridium perfringens

Large Gram-positive anaerobic rods (Clostridia-like organisms) were isolated from rectal faecal samples and also from gastroenteric contents by streaking onto Perfringens Agar plates (Perfringens Agar Base [T.S.C. & S.F.P.], Oxoid, Ltd. CM 587) with added selective antibiotic supplement T.S.C. (Oxoid, Ltd. SR 88). For Clostridium perfringens type A, Egg yolk agar plates (Blood Agar Base No. 2, Oxoid, Ltd. CM 271) with 10 ml/200 ml added Egg yolk emulsion (Egg Yolk Emulsion, Oxoid, Ltd. SR 47) were also prepared, inoculated and incubated anaerobically at 37^{o}C as described below.

iii) Clostridium difficile

Clostridium difficile agar plates (Clostridium difficile Agar Base, Oxoid, Ltd. CM 601) with added 7% defibrinated horse blood (Oxoid, Ltd. SR 50) and C. difficile selective supplement (Oxoid, Ltd. SR 96) were prepared, inoculated and incubated in anaerobic jars (Don Whitley Scientific, Ltd.) at 37°C with the inclusion of cold catalyst(s) as described below.

Both vegetative and spore-forming strains of clostridia were sought by the methods described below. When required, further biochemical and serological tests were carried out using reversed passive slide latex agglutination and the API 20 A strip System. (Appendix B).

iv) Bacteroides spp.

Neomycin agar plates were prepared for Bacteroides spp. isolation using Blood Agar Base No. 2 (Oxoid, Ltd. CM 271), with 7% defibrinated horse blood added (Oxoid, Ltd. SR 50) and supplemented with 126 mg/200 ml of Neomycin Sulfate (SIGMA No. N-1876), the plates were inoculated and incubated in anaerobic jars (Don Whitley Scientific, Ltd.) at 24, 48, 72 or more hours with the inclusion of cold catalyst(s) as described below.

v) Other anaerobic bacteria

Other important enteric anaerobic bacteria found to be present in gastroenteric disorders such as Fusobacterium spp., neomycin susceptible Bacteroides spp. and other Clostridium species, were isolated by inoculation onto antimicrobial free supplemented Horse Blood Agar plates (Blood Agar Base No. 2, Oxoid, Ltd. CM 271) with added 7% defibrinated horse blood (Oxoid, Ltd. SR 50). Inoculated plates were incubated at 37°C in anaerobic conditions using anaerobic jars (Don Whitley Scientific, Ltd.) with the inclusion of cold catalyst(s).

B) Quantitative bacterial examination of enteric samples

a) Dilutions procedure for bacterial cultivation

As soon as examination of the initial direct smears had been carried out, the porcine faecal specimens and/or gut contents were examined quantitatively for vegetative bacteria and spores as follows: 1g of the sample was placed into weighed (Balance, Oertling HB 63) sterile universal bottles and these aliquots were diluted in 9 ml of sterile phosphate buffered saline (PBS) (Phosphate Buffered Saline [Dulbecco 'A'] Oxoid BR 14a) to give a final dilution of 1:10 and homogenised thoroughly using a rotamixer (Rotamixer de Luxe, Hook & Tucker Instruments, Ltd.). The universals were subject to centrifugation at 3000 g for 15 minutes in a GPR Centrifuge at 4^{o}C (Model Cat. No. 349703, Beckman Instruments, Ltd.) to spin down faecal debris.

Ten-fold serial dilutions were carried out with the supernatant using an 8 channel 5 - 50 ul pipette (Titertek Microtitration Equipment, EFLAB, Labsystems and Flow Laboratories). 20 ul of each sample were placed either into a sterile tissue culture multi-well plate, comprising 96 flat bottom wells (Cat. No. 1-63320, Nunclon Delta SI, Nunc, Inter Med, Denmark) with lid or a sterile irradiated tissue culture multi-well plates with 96 flat bottom wells 1.0 x 0.6 cm approximately. (Cat.No. 76-008-05, Titertek, Flow Laboratories, Ltd.) with lid or a sterile irradiated tissue culture multi-well plates, 96 U shaped wells 1.0 x 0.7 cm approximately with lid (Cat. No. 76-013-05, Titertek, Flow Laboratories, Ltd.) in 180 ul of sterile PBS. 20 ul amounts of the dilutions were taken with a P 20 Gilson pipetman and were inoculated onto the surface of the eight selective and non-selective solid culture media which were prepared according to the manufacturer's instructions and listed below;

The plastic pipette tips (PR 800-50, Ref. PT 84, 5-100 ul non-sterile disposable pipette tips, A + J Beveridge, Ltd.) used in the dilution procedures were all sterilized beforehand with wet steam under 2.0 bar of pressure and 120^{o}C of temperature for 30 minutes, in an autoclave (Model No. 704-8000-DSE, National Appliance, Co., a Heinicke Company [Sterilin Instruments]) and disposed of after single use.

TABLE 2.1 Quantitative bacterial cultivation

Medium (Agar)	Organisms	Atmosphere of incubation	Time (Hours)
Sheep blood	Escherichia coli	aerobic	24
MacConkey	Non lactose fermenters	aerobic	24
Slanetz & Bartley	Streptococcus faecalis	aerobic	72
Campylobacter de Man, Rogosa & Sharpe	Campylobacter spp.	microaerobic	48-72
	Lactobacillus spp.	microaerobic	48-72
C. perfringens	Clostridium perfringens	anaerobic	48-72
C. difficile	Clostridium difficile	anaerobic	48-72
Neomycin blood	Bacteroides spp.	anaerobic	48-72
Spectinomycin	Serpulina hyodysenteriae	anaerobic	48-72
Horse Blood	Others	anaerobic	48-72

b) Aerobic enteric bacteria

MacConkey agar plates (MacConkey Agar, Oxoid, Ltd. CM 7 with added 0.075 g neutral red), were used for coliform enumeration. Sheep blood agar plates (Blood Agar Base No. 2, Oxoid, Ltd. CM 271) with added 7% defibrinated sheep blood (GIBCO, Ltd. Cat. No. 101-01 E) were used for the enumeration of Beta haemolytic E. coli and Slanetz and Bartley agar plates (Slanetz & Bartley Medium, Oxoid, Ltd. CM 377) were used for faecal streptococci enumeration.

c) Microaerobic enteric bacteria

Campylobacter agar plates (Blood Agar Base No. 2, Oxoid, Ltd. CM 271) with added 7% defibrinated horse blood (Oxoid, Ltd. SR 50) and added Campylobacter supplement [Skirrow], (Oxoid, Ltd. SR 69) were used for the enumeration of Campylobacter spp. and de Man, Rogosa and Sharpe agar plates (M.R.S. Agar, Oxoid, Ltd. CM 361) were also inoculated and incubated at 37^{o}C for Lactobacillus spp.

d) Anaerobic enteric bacteria

Spectinomycin blood agar (Blood Agar Base No. 2, Oxoid, Ltd. CM 271) with added 7 % defibrinated horse blood (Oxoid,

Ltd. SR 50) plus 400 ug/ml spectinomycin SPECTAM injectable (CEVA, Ltd. 5488). C. difficile agar plates (C. difficile Agar Base, Oxoid, Ltd. CM 601) with added 7 % defibrinated horse blood (Oxoid, Ltd. SR 50) supplemented with C. difficile selective supplement (Oxoid, Ltd. SR 96). Perfringens agar plates (Perfringens Agar Base [T.S.C. and S.F.P.], Oxoid, Ltd. CM 587) with added selective antibiotic T.S.C. (Oxoid, Ltd. SR 88). Neomycin Agar plates using Blood Agar Base No. 2 (Oxoid, Ltd. CM 271) with added 7% defibrinated horse blood (Oxoid, Ltd. SR 50) supplemented with 126 mg/200 ml of Neomycin Sulfate SIGMA No. N-1876), were prepared, inoculated and incubated for the enumeration of S. hyodysenteriae, C. difficile, C. perfringens type A, Bacteroides spp. and Fusobacterium spp. respectively.

C) Quantitative procedures for spore-forming clostridia

Counts of spore-producing clostridia strains were carried out using the same 1:10 final dilution of faeces, heated in a water bath (Grant Instruments [Cambridge], Ltd. Type JB 2) at 80°C for 12 minutes. Ten-fold serial dilutions were performed as above and then inoculated onto Perfringens Agar plates (Perfringens Agar Base [T.S.C. & S.F.P.], Oxoid, Ltd. CM 587) with added selective antibiotic T.S.C. (Oxoid, Ltd. SR 88) and C. difficile agar plates (C. difficile Agar Base, Oxoid, Ltd, CM 601) with added 7% defibrinated horse blood (Oxoid, Ltd. SR 50) supplemented with C. difficile selective supplement (Oxoid, Ltd. SR 96) was carried out.

For the isolation and enumeration of C. difficile spore-forming strains, 0.5 ml of unheated faeces diluted 1:10 was added to an equal volume of absolute ethanol (Borriello and Honour, 1981; Borriello, et al., 1983; Koransky, et al., 1978) and mixed on a rotamixer (Rotamixer de luxe, Hook & Tucker Instruments, Ltd.). After being allowed to stand at room temperature (25°C) for one hour, serial ten-fold dilutions were prepared and samples of 20 ul of the serial dilutions were plated onto C. difficile Agar plates and incubated in anaerobic jars as described below.

All inoculated plates were left to dry at room temperature and then incubated at 37°C in aerobic, microaerobic and anaerobic conditions depending on the bacteria sought and the plates incubated for 24, 48 and 72 hours.

Although this method is not the best for high survival rate of strict anaerobes there was no other alternative available, e.g. anaerobic cabinet, which may be the best system currently in use for more accurate bacteriological studies.

3. CONDITIONS OF CULTIVATION

A) Atmospheric conditions

Cultures were incubated at 37°C in aerobic, microaerobic and anaerobic conditions according to the cultural requirements of the organisms (Bergey's Manual of Systematic Bacteriology, 1984/1986).

Aerobic conditions were obtained by placing the inoculated plates directly into the incubator (LTE Qualitemp 80).

Microaerobic conditions were produced by evacuating anaerobic jars (Don Whitley Scientific, Ltd.) containing the culture plates without a cold catalyst, to a pressure of 500 millimetres of mercury, using a vacuum pump (Model ECB 1, Edwards High Vacuum, Ltd.). The jars were then flooded with 5% carbon dioxide and 95% hydrogen mixture (British Oxygen Co., Ltd., Special Gases Division).

Anaerobic conditions were produced by evacuating loaded anaerobic jars (Don Whitley Scientific, Ltd.) containing the culture plates and fitted with cold catalyst(s) to a pressure of 600 millimetres of mercury using a vacuum pump (Model ECB 1, Edwards High Vacuum, Ltd.). The evacuated jars were then filled with the carbon dioxide/hydrogen mixture. Gassed jars were evacuated once more and gassed again in order to flush out any remaining oxygen.

B) Incubation temperature

All cultures were incubated at 37°C under the atmospheric conditions required for each specific bacteria.

C) Period of incubation

All primary aerobic cultures on routine, selective and non-

selective culture media were incubated for 24 hours, examined and reincubated. Plates for anaerobic bacteria were incubated for 48 hours examined, and reincubated if necessary. Slanetz and Bartley plates and microaerobic plates were incubated for 72 hours, examined and, if further incubation was not required, the plates were placed on the bench and discarded one week after the last incubation.

4. BACTERIAL IDENTIFICATION CRITERIA

Following incubation, the plates were examined and bacterial colonies identified presumptively by colonial morphology, effect on the media, and Gram stain reaction. Smears were prepared as previously described to identify the bacteria present and a record of each finding was made. The bacterial counts were considered to give the number of colony forming units (Miles, Misra and Irwin, 1938).

A) Examination of plates

This was carried out on a presumptive basis in most cases (Vervaeke and Van Nevel, 1972). Examination of colonies were made by the naked eye and by using a dissecting microscope, the colonies seen were described in terms of their morphological characteristics such as size, elevation, outline, colour, consistency, odour and their effect on the medium, such as haemolysis. Colonies were presumptively identified by these characters. Thin smears were prepared from colonies on clean dry glass slides, air dried and heat fixed, they were stained by Gram's method. The stained smears were examined under the oil immersion lens of a light microscope to determine cellular morphology, reaction to Gram's stain and the results recorded. Further identification of bacteria was carried out in more detail using the criteria of Carter, (1979), Cowan and Steel, (1981) and Bergey's Manual Systematic Bacteriology,(1984/1986).

For C. difficile identification the MicroScreen latex agglutination test (Mercia Diagnostics, Ltd. M 41) was carried out using a 1:10 faecal dilution as described above, to compare the sensitivity of the serological test with the culture results and to confirm the presence or absence of the bacteria. RPLA

tests (PET-RPLA, Oxoid, Ltd.) were also carried out routinely for the demonstration of C. perfringens type A enterotoxin.

When a known pathogenic bacterium was isolated from samples submitted for diagnosis, subculture was carried out to achieve culture purity. This procedure enabled antimicrobial susceptibility tests to be performed. The antimicrobial sensitivity of each isolate could then be recorded.

B) Antimicrobial susceptibility tests

Once a pure culture of the bacteria was obtained, antimicrobial susceptibility tests were carried out using an antimicrobial 'Multodisk' code 7695 E (Oxoid, Ltd.) and individual (Bauer, et al., 1966) antibiotic discs (Oxoid, Ltd.) listed below. Such antimicrobial discs were placed with a flamed pincers onto the surface of two selective or non-selective plates, and then incubated for the period of time and atmospheric conditions required for the bacteria isolated. After incubation the plates were examined and the results were recorded.

Antimicrobial 'Multodisk' (Oxoid, Ltd. Code No. 7695 E):

AMP_{25} - Ampicillin
C_{10} - Chloramphenicol
FR_{50} - Furazolidone
N_{10} - Neomycin
OT_{30} - Oxytetracycline
S_{10} - Streptomycin
SF_{100} - Sulphafurazole
SXT_{25} - Sulfamethoxazole-Trimethoprim

Individual discs (Oxoid, Ltd.):

AML_{25} - Amoxycillin
APR_{15} - Apramycin
MY_{10} - Lincomycin
PN_{10} - Ampicillin
SH_{10} - Spectinomycin, and
AUG_{30} - Augmentin (Mast Laboratories, Ltd.)

C) Immunological techniques

a) Counterimmunoelectrophoresis

Agarose (L.K.B. Instruments Ltd.) at one per cent concentration was dissolved by heat in 0.025 ionic-strength barbital-acetate buffer pH 8.6. Slides were layered with melted agarose and allowed to harden for at least 20 minutes and stored in a moist chamber until use.

Barbital-acetate buffer pH 8.6 was prepared in distilled water. The composition of stock buffer was 3.09 per cent sodium barbital, 0.552 per cent barbital and 1.23 per cent sodium acetate. The buffer solution was heated to approximately 90°C for 15 minutes to dissolve the buffer salts and was then diluted to a 0.025M ionic-strength buffer with distilled water. Parallel rows of wells 0.3 cm in diameter were cut 0.5 cm apart in the agar. Ten ul of antigen were placed in wells near the cathode. The samples to be tested were placed in the wells near the anode in similar volumes. The electrode vessel contained barbital-acetate buffer of the same ionic strength (0.025M), as was used for the gel preparation. Electrophoresis was carried out in an electrophoresis chamber (Gelman Instrument Co.) at room temperature with a constant current of 6 mA per slide for 60 minutes. Filter paper was used as an electrode wick. After electrophoresis, the slides were removed to a moist chamber and allowed to develop for 30 minutes. For a permanent record the slides were washed for 24 hours in phosphate buffered saline and for 6 hours in distilled water before staining with Coomassie brilliant blue at 0.025 per cent.

5. VIROLOGICAL AND PARASITOLOGICAL PROCEDURES

A) Virological examinations (Porcine Rotavirus)

These tests were carried out on caecal and colonic contents and faecal samples from all the pigs under study. Each sample was suspended in sterile physiological saline (0.85 per cent of Sodium Chloride in deionised water) and centrifuged at 11.000 g for 15 minutes in a GPR centrifuge at 4°C (Model Cat. No. 349703, Beckman Instruments, Ltd.) for clarification. The supernatant was taken and subject to filtration through a 0.22

ug blue sterile disposable syringe filter holder (Minisart NML, SM 165 34K, Prod. Code No. FC 800-15. Sartorius-Instruments, Ltd.) as described by Tzipori, et al. (1980c). After filtration the RPLA test was performed using either the Wellcome Rotavirus Latex test, ZL 40 (Wellcome Diagnostics, a Division of The Wellcome Foundation, Ltd.) or the RotaScreen M 80 a rapid slide latex test (Mercia Diagnostics, Ltd.) (Scott, et al., 1988), for the qualitative detection of rotavirus or rotavirus antigen and the results recorded. The remaining filtrates were then stored at -20°C for further examination if required.

B) Parasitological examinations

Scrapings of ileal mucosa, rectal faecal samples and intestinal contents were examined for cryptosporidial oocysts using light microscopy. Smears of faeces, intestinal scrapings and contents stained by modified Ziehl-Neelsen (Henriksen and Pohlenz, 1981; Garcia, et al., 1983a) were prepared and examined for the presence of Cryptosporidium spp. The presence or absence of which was recorded.

Histological sections of the small intestinal mucosa stained by Haematoxylin and Eosin (H & E) were examined under the light microscope for the presence of Isospora suis and Balantidium coli and the results recorded.

6. PIGS USED IN THE EXPERIMENTAL STUDIES

The animals used in the experimental studies were of minimal disease origin obtained from a high health status farm (National Pig Development) and maintained at Cochno farm which belongs to the Glasgow University Veterinary School and is situated at Duntocher, 6 miles from the Veterinary School at the Garscube in Bearsden, Glasgow.

A) Farm description

a) Cochno Farm, Animal Husbandry unit (Piggery)

Situated at Duntocher, 6 miles northwest from the Glasgow Veterinary School this farm has 40 sows and 3 boars from the same genetic origin and of high health status (National Pig

Development). The gilts are supplied from a multiplier farm and the boars from the nucleus farm. The productive cycle ends in sale at 65-70 kg liveweight (Pork weight) pigs to the local meat market in Glasgow. The routine management of the farm begins with the pregnant sows/gilts which are kept in cement floored sow stalls in the dry sow house (Figure I) with a diet containing 20% crude protein (C.P.) (DALGETY Agriculture Ltd., 320 Ultragrade and 320 Ultrabreed pellets), and rationed to 2 kg/day, with free access to water. One week before parturition they are washed prior to moving into the farrowing house (Fig. I) where they are placed into the farrowing crates with temperatures in the range of 25-30^{o}C allowing the sow/gilt and their litter to be as comfortable as is possible under intensive conditions of production.

Recently born piglets have their tails clipped at two days of age and are injected intramuscularly in the hind limb at three days with 200 mg in 1 ml of Iron ("Gleptosil", FISONS, Plc., Animal Health, 337-14-121). For three weeks the piglets suck their mother's milk when they are weaned. At ten days of age solid food, containing 25% C.P. (B.O.C.M., Silcock, Ltd., Basingstoke, Hampshire, 401, Weanercare Pellets) with 100 mg/kg of Olaquindox and 175 mg/kg of copper sulphate added as growth promoters, "Intagen" 10 g/kg (E. coli polysaccharide antigen) is available in small quantities until weaning. On reaching 4 weeks of age the pigs are relocated in the weaning area (Figure I) which is composed of four flatdecks with 15-20 spaces each, this area has a temperature varying between 23-27^{o}C, regulated by an individual lamp positioned above each pen and the air-conditioning extractor fan, the floor is of metallic wired mesh. They are fed **ad libitum** with feed containing 22% C.P., 40 mg/kg of tylosin phosphate (Tylamix) and 175 mg/kg of copper sulphate were added as growth promoters (B.O.C.M., Silcock, Ltd., Basingstoke, Hampshire, 483, Growercare Pellets) and water is available **ad libitum** from a nipple drinker. At the same time the mother is returned to the dry sow's house (service area) to recover from the lactation period and to be served for further pregnancy. The boars are also penned in the service area on cement floored, fencing pens (Fig. I), and fed on a diet containing 20% C.P. and rationed at 2 kg/day; water is freely available.

The pigs are kept in the flatdecks until they reach 20-25

kg of weight, or 8-9 weeks of age, and are then rehoused in cement floored and partially slatted pens in the fattening house (Fig. I). The pens have the capacity for an average of 25 recently weaned pigs which are regrouped according to size and weight when necessary. The pigs are housed at a temperature of between 21-24°C and are fed **ad libitum** from 8-9 weeks of age on a diet containing 22% C.P. and 175 mg/kg of copper sulphate as growth promoter (DALGETY Agriculture, Ltd., 320 Ultragrade Pellets), until they reach slaughter weight. Water is available **ad libitum** from a nipple drinker and there are two extractor fans to avoid excessive gas concentrations and help with the regulation of the temperature.

All pigs used were conventionally weaned and for the purpose of the different experiments they were aged between 3 to 6 weeks at the beginning of the studies.

B) Identification method

All pigs from either treated or control groups were individually identified using numbered and coloured ear tags before any experimental procedures were instituted. The pigs were selected at random and allocated to groups in either flatdecks or cement floored pens.

C) Accommodation

According to the requirements of the particular Experiment and the availability of space in the weaning area inside the fattening house (Cochno farm), two different type of pens were used. Either elevated flatdecks (15-20 spaces) with a heat lamp positioned above each one and one extractor fan which combined to keep the room temperature between 23-27°C, or cement floored pens (25 spaces) (Figure I) with straw bedding and one slatted section at the back in the cement floored ones (Figure I). Each pen was equipped with an automatic feeder and water was freely available from a nipple drinker.

D) Food

a) Standard diet

All the pigs were fed at the beginning of each of the studies with the existing diet which was available **ad-libitum** and provided by British Oil and Cake Mills, (B.O.C.M.) Silcock, Ltd. Basingstoke, Hampshire. The ingredients are listed below;

BOCM 401-Weanercare Pellets; (Suckling-weaned piglets)		BOCM 483-Growercare Pellets; (Weaned pigs)	
Crude protein	25.00%	Crude Protein	22.00%
Oil	10.00%	Oil	6.00%
Fibre	1.75%	Fibre	3.00%
Ash	7.00%	Ash	5.00%
Vitamin 'A'	15 000 IU/Kg	Vitamin 'A'	10 000 IU/Kg
Vitamin 'D_3'	2 000 IU/Kg	Vitamin 'D_3'	2 000 IU/Kg
Vitamin 'E'	150 IU/Kg	Vitamin 'E'	60 IU/Kg
Selenium	0.3 mg/Kg	Selenium	0.3 mg/Kg
Olaquindox	100.0 mg/Kg	Copper Sulphate	175.0 mg/Kg
Copper Sulphate	175.0 mg/Kg	Tylosin phosphate	40.0 mg/Kg
"Intagen"	10.0 g/Kg		

b) Experimental diets

In Experiment 1 the diet was prepared specially for the Experiment in the B.O.C.M. company mills with AVOTAN 50 (80 g/ Tonne) added to the regular commercial formulation used for the company on their 483, B.O.C.M. Feedstuffs (see above for ingredients).

As in Experiment 1, in Experiment 4 the diet used was prepared specially for the Experiment by the staff at the experimental unit (Cochno farm) under the directions and formulation provided for the Department of Animal Husbandry-Veterinary School. The formulation of this diet contained;

Ingredients	% in ration	Protein content	Kilograms
Barley	73.0	10% = 2550	255
Soya	11.4	40% = 1600	40
Fish meal	14.3	50% = 2500	50
Mins. & Vitamins	1.4	0% = 0000	5
TOTAL	100.00%	= 6650 +	350=**19% C.P.**

c) Recording of feed use

Measured amounts of feed were added to the feeder when empty. The amount of feed used per day could therefore be recorded while still allowing free access by all pigs.

7. ANTIMICROBIALS USED IN THE EXPERIMENTAL STUDIES

A) Antimicrobials

There were three different antimicrobials used which were either added to the basic diet or mixed in the drinking water during the experimental studies;

a) Experiment 1: In Experiment 1, AVOTAN 50 was added in proportions of 80 g/tonne. The antimicrobial was included into a B.O.C.M. 401-2697 Weanercare Pellets basic ration at the mill. The diet was available one week before and one week after the rectal faecal sampling began and was then withdrawn, the rectal faecal sampling was continued one week afterwards.

b) Experiment 2: In Experiment 2, Lincocin* Soluble Powder (*Lincomycin hydrochloride, 400 mg/g) (Lot. No. 17006, Upjohn, Ltd., Animal Health Division) was added to the drinking water in a proportion of 166 mg/5 litres (giving a concentration of 13.3 ppm) in a clean bucket, mixed and then put into the automatic drinker into the pen occupied by the treated group for a period of one week and then withdrawn. The control was supplied with plain water in a similar drinker. Water consumption was recorded daily from both control and experimental groups by refilling the header tank when empty and recording the amount required. This procedure was also applied in Experiment 3.

c) Experiment 3: In Experiment 3, Lincocin* Soluble Powder was given as above during a period of two weeks to compare differences between treatments as described in Chapter 5.

d) Experiment 4: 300 ppm of Chlortetracycline hydrochloride ('AUROFAC* 100', Cyanamid Laboratories) (*Aureomycin, Lot. No. 1515-62) was included at a rate of 3 Kg/tonne of the special diet prepared at the experimental unit (Cochno farm). The

medicated diet was available **ad libitum** for a period of two weeks before the faecal sampling routine started and during two weeks afterwards, making a period of in-food medication of 4 weeks. The faecal sampling was continued for a further two weeks to evaluate the effect of withdrawal on the gut flora.

8. GENERAL EXPERIMENTAL OBSERVATIONS AND PROCEDURES

In each experiment daily rectal faecal samples were obtained for bacteriological, parasitological and virological examination as described above. Rectal temperatures were obtained daily or every third day and recorded. All the animals were weighed either daily or weekly depending on the Experiment. Daily feed consumption was recorded and the conversion rate calculated. Daily water consumption was recorded in Experiments 2 and 3. Pigs were sacrificed before medication began, after one week and two weeks and at the end of each Experiment for evaluation of internal changes and lesions.

9. CLINICAL EXAMINATION

The pigs used in the experimental studies were examined individually and/or as a group before any treatment or change in management was carried out (Straw and Meuten, 1986; 1992). The clinical findings were evaluated to determine their overall level of health and productivity of each animal. Those signs were:

A) General physical appearance

Skin: Integrity, colour, cleanliness, presence or absence of dermatitis, wounds (rubbed elbows), obvious/visible haemorrhages, presence or absence of lice and hair condition.

Extremities: Deformities, wounds, lesions (cuts, bruising, cracks or infection), lameness, arthritis, swelling and abscessation of feet and joints.

Head: Skin condition, eyes, nasal morphology (crumpling and/or distortion of the face), ears and presence or absence of mange.

Clinical signs: Presence or absence of any nervous signs

such as convulsions or tremor.

B) Physiological standards

Rectal temperature, ocular and rectal/vaginal mucosa colour and respiratory rate.

C) Social behaviour

Individually and with pen-mates, fights, vices (such as tail, ear and flank biting, penis sucking) and appetite. Scratching or rubbing and attitude to the examiner.

D) Clinical observations

General bodily condition, consistency of the faeces, presence or absence of nasal discharge, sneezing, coughing, and any apparent locomotor disorders (e.g. swollen joints). During the experimental period in which daily movement and sample collection was done, the clinical signs recorded were;

Physical appearance, rectal temperature, faecal consistency, mucosa colour, bodily condition and weight. All were recorded from individuals in experimental and control groups and on the various pigs in the different farms from which clinical material was obtained.

10. FARM STUDY PROTOCOL

Where farms differed from the one belonging to the Glasgow University Veterinary School (Cochno farm), a general basic scheme was established to obtain the history of the operation from which clinical specimens were obtained and used. The herd profile of health and productivity was based on;

Number of animals, breed (s), source of breeding stock, diet, and additives currently in use and used before, clinical signs observed for the Veterinarian and/or staff, treatments given, type of housing, floor type and condition, pig flow through buildings, age at weaning, immunization programme(s), purpose of the operation (e.g. weaner production, fattening or

breeding-fattening), previously diagnosed diseases, mortality rates, any programme of medication, control or disease eradication currently in force or previously used before and any other information available such as source of feed, is it regularly from the same company, or home made, ingredients used in the ration, sanitary precautions, personnel employed, etc.

The conditions of husbandry inside the various piggeries visited were evaluated to determine the proposal of solutions to the farmer of basic problems such as ventilation, humidity concentration, level of contamination with dust and gases (Ammonia), temperature and draughts which, together with the state of the buildings maybe partially responsible for the development of some clinical and subclinical diseases in the piggery.

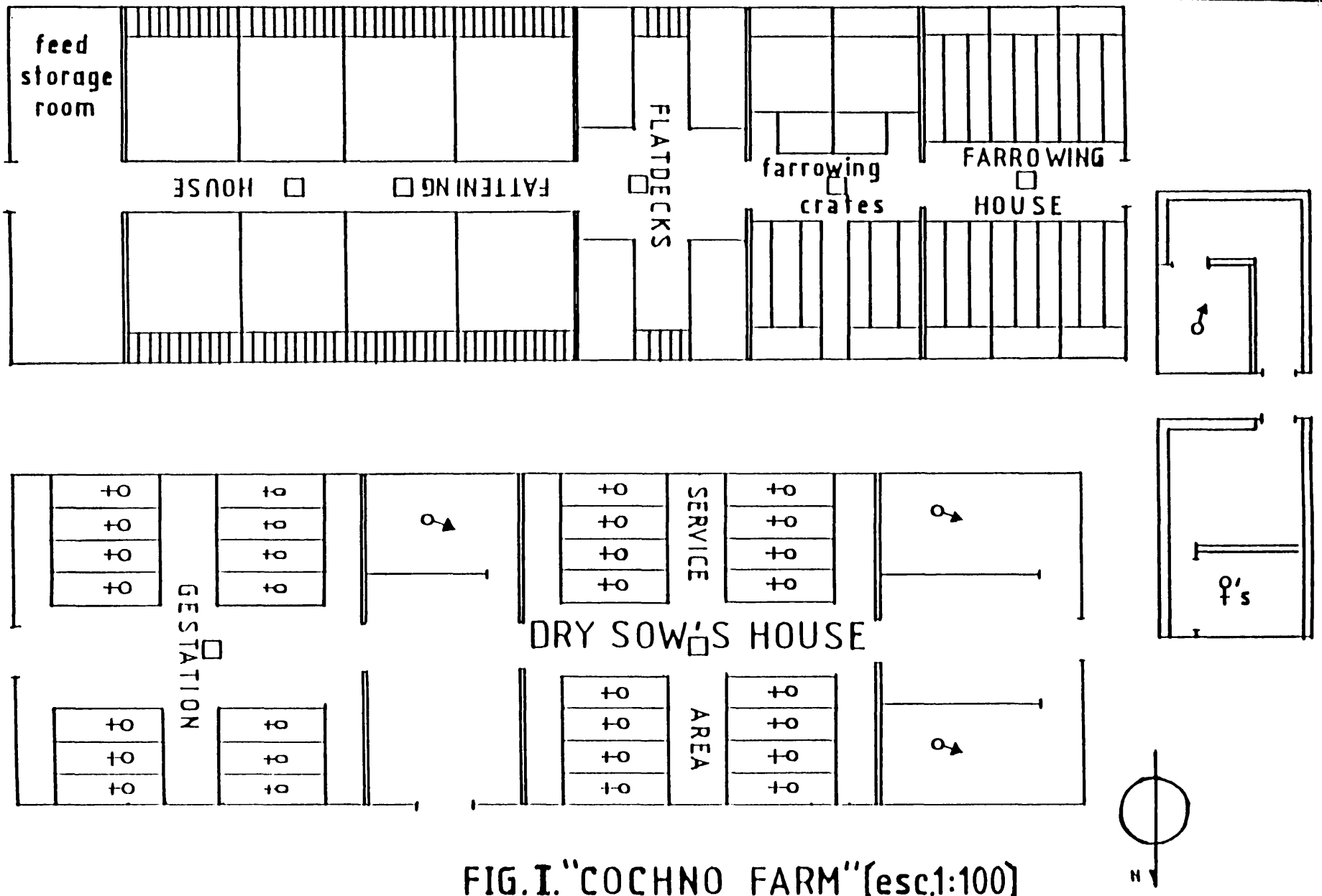


FIG. I. "COCHNO FARM" (esc. 1:100)

CHAPTER 3

SURVEY OF FIELD MATERIALS

1. INTRODUCTION

The faecal samples and pigs used in this study came from a small number of farms in Scotland and England and were mainly from recently weaned young animals. Only a limited number of field cases of potential post antimicrobial enteric disease were reported during this study. In most cases, the disease reported concerned recently weaned animals. Initial notification was usually accompanied by samples and in the majority of cases described here it was possible to visit the unit to verify the occurrence of post antimicrobial enteritis. The visits were considered necessary as it was difficult to form a judgement about the nature of the disease from samples and histories alone. In many cases, the scanty history of the cases and the state of preservation of the source material limited the information which could be obtained. The results of this chapter are presented below in the form of reports on each unit and the evidence they provide for post antimicrobial enteritis is discussed.

2. MATERIALS AND METHODS

The majority of the materials and methods used have been described in Chapter 2. The faecal samples and few pigs concerned came from 6 farms, details of which are given below and together with the results.

A) Brief description of the farms visited

Farm 1 (Southwest Scotland)

This farm of 200 sows finishing all progeny at 90 kg was part of a mixed farm of approximately 100 ha. It was close to the Veterinary School (40 km) and was visited on 4 occasions in this study.

The housing varied in quality. Dry sows were housed in a purpose-built fully-slatted stalled sow house and farrowing took

place in a fully slatted environmentally-controlled converted building or in solid floored, cold farrowing crates in another building. Sows and litters were moved from this building to solid floored draughty rearing pens. All pigs were weaned at 3 weeks and went to either purpose built temperature controlled flat decks or to conventional solid floored strawed pens with poor temperature control and outside runs. One half of the house had pens with a continuous dunging passage. 25 kg pigs from both types of accommodation were mixed at the next stage and entered the grower house, a continuously-stocked, fully-slatted temperature controlled unit. At 60 kg they went to the slatted finishing house for finishing to 90 kg. Creep feed was purchased (Norvite), but all other feeds were home-mixed and presented as a meal at this time.

The majority of breeding stock were home bred with boars being purchased from the nucleus herds of major breeding companies or from other high health herds.

A number of enteric diseases had been demonstrated on this farm in the past and its productivity was also low. The enteric diseases identified included neonatal diarrhoea not known to be associated with the presence of Beta-haemolytic Escherichia coli or rotavirus, post-weaning E. coli diarrhoea, PPE, spirochaetal diarrhoea but not swine dysentery and no salmonellosis. Other diseases such as pleuropneumonia caused by Actinobacillus pleuropneumoniae, Bordetella bronchiseptica infection, pasteurellosis and Haemophilus parasuis had all been identified previously and all remained endemic in the farm at the time of sampling.

Post-antimicrobial enteritis was suspected in weaned pigs of 5-8 weeks of age. Post-weaning diarrhoea was being treated with neomycin sulphate (11 mg/kg) in the drinking water for 5 days or lincomycin (10 mg/kg) for 5 days. Diarrhoea was said to respond to both treatments but recurred following withdrawal. Olaquindox (Fedan) was included in the creep feed (Norvite Feeds) at 100 ppm and this was fed until 1 week post weaning. Diarrhoea was also present in pigs aged from 8-13 weeks and was normally treated with a course of neomycin or lincomycin-medicated drinking water. Later rations contained no antimicrobial growth promoters.

Farm 2 (Bristol, England)

This unit with a finishing herd buying in pigs and finishing 30,000 pigs **per annum** on a continuous throughput system was located in the vicinity of Nailsea, Bristol. The reports were forwarded by the veterinarian in charge of the operation.

It appears from the original report that weaned pigs were suffering from an non-specific form of 'colitis' thought to be caused by *S. hyodysenteriae*. Only faecal samples were available and the history supplied was poor. It also appeared that the 'colitis' had failed to respond to conventional antimicrobial therapy (unspecified) and some cases of the 'colitis' had occurred after treatment.

Farm 3 (Lanarkshire, Scotland)

This small unit of 60 sows finishing all progeny as light pork at 65-70 kg had been established two years prior to the visit using purchasing breeding stock following depopulation without cleaning. Sows were housed in an open yard and farrowed in a large house with poor temperature control also containing flat deck/bungalow units for recently weaned pigs. Pigs were transferred at 25 kg to outside pens with slatted floors and poor temperature control. Feed was purchased and fed **ad libitum**. The herd had been the subject of previous visits to design a control programme for swine dysentery. A programme of partial depopulation, cleaning and tiamulin treatment had been devised in August 1985. Six months before the beginning of this study a number of enteric diseases had been demonstrated on this farm. These diseases included swine dysentery, infection with *Campylobacter spp.*, salmonellosis (*S. panama*), neonatal and post-weaning *E. coli* diarrhoea and Oedema disease. In May 1987 further problems with diarrhoea were being experienced and swine dysentery had been confirmed at the Veterinary Investigation Centre one month previously. It was reported that tiamulin soluble in water was now ineffective after $2^1/2$ days treatment, that injectable tiamulin was not working and that only feed medication for one week was having an effect. It appeared from the original description by the owner that post-antibiotic diarrhoea might be occurring.

Farm 4 (Cumbria, England)

Small herd of 30-40 sows finishing all progeny to light pork at 60-70 kg. Weaning at 3-4 weeks into flat decks and subsequently into Thomas houses where they were carried on until slaughter. The pigs were fed on a high protein rations, Ultra gain and Ultra link (Dalgety) containing 10 ppm tylosin and copper only. Diarrhoea was said to occur at weaning and again at movement into the Thomas houses and was then treated with neomycin in drinking water. Diarrhoea recurred after this and led to reduced productivity.

Farm 5 (Yorkshire, England)

The visit to this farm of 3,000 sows divided into five indoor units was undertaken to confirm the presence of a post-antimicrobial enteritis reported to be affecting the weaned pigs between 6-8 weeks of age. The worst problem was said to be in a multiplier herd of 300 Landrace sows.

Only two units were inspected. The first was a 300 sow unit finishing pigs at 90 kg. The piglets were weaned at 3 weeks of age, mixed and moved when they reach 5 weeks of age to flat decks for a further 2-4 weeks (average 3 weeks). From these flat decks (at 8 to 9 weeks of age) they were put in the 2nd flat decks for 1-2 weeks and later moved to the Verandah house for 12 weeks. Finally they were accommodated in the pens of the finisher house (growing house) from which they went to slaughter. The mortality rate between weaning and slaughter was 4.5% and the FCR had fallen from the previous 2.4 to 2.6.

Five months earlier the pigs had been on ration 482 and later ration 483 Growercare pellets (TC), both from B.O.C.M. and containing oil 6%, crude protein 22%, copper 175 mg/Kg and 40 mg/kg of tylosin phosphate as growth promoters. They were moved to Thompson's feeds, first with finisher (451: 4.5% oil, 20% crude protein + probiotic). An improvement was noted which then declined giving a poorer growth from weaning to 90 kg (FCR of 2.5:1). Pigs were medicated with 1.5 kg of tiamulin and 3 kg of chlortetracycline/tonne which provided a definite improvement but 10 days following this treatment a deterioration was

observed. All the feed was provided ad libitum. It was reported that at growing stage 2% of the pigs were being removed from the pens to hospital pens and put on water medication. The sows in this unit were vaccinated against neonatal E. coli diarrhoea (Gletvax 5 and later with Nobivac). It was also claimed that the stock was free from Enzootic Pneumonia, pleuropneumonia, Atrophic Rhinitis, lice, mange, worms, Glasser's Disease, salmonellosis (S. typhimurium) and Streptococcus suis.

The second unit with 450 sows producing 24 pigs/sow/year sold had a FCR of 2.3 and the pigs were slaughtered at 150 days weighing 95 kg. The piglets were weaned at 3 weeks of age, mixed and moved when they reach 5 weeks to flat decks for further 2-4 weeks (average 3 weeks). From these flat decks (at 8 to 9 weeks of age) they were put in the 2nd flat decks for 1-2 weeks and later moved to the Verandah house for 12 weeks. Finally they were accommodated in the pens of the finisher house (growing house) from which they went to slaughter.

The first feed provided to the piglets was Cuckoo One (B.P. Nutrition [UK] Ltd.) with 9% oil and 24% crude protein. 175 mg/kg of copper and 40 mg/kg of tylosin were included in the creep feed for growth promotion. The second ration available in the flat deck accommodation was Flat Deck 1 with oil 9%, crude protein 23% and medicated with 175 mg/kg of copper and 40 mg/kg of tylosin. The third ration Flat Deck 2 varied from the previous in contents of oil (8%) and crude protein (22%). These two rations were obtained from A-One Feed Supplements Ltd., York. The final feed for this stage was Multiwean 6/20 (S. C. Associates, Melmerby, Ripon) contained Oil 6%, crude protein 22% and 175 mg/kg of copper as a growth promoter. It also contained 40 mg/kg of tylosin, 50 mg/kg of Trimethoprim B.P. and 250 mg/kg of Sulphadiazine B.P. (Uniprim 150) for therapy.

It was reported that this unit had been kept free of diseases such as TGE, Oedema disease, worms, swine dysentery, spirochaetal diarrhoea, coccidia, Cryptosporidium spp., C. perfringens and rotavirus. Louse infection, mange, Atrophic Rhinitis, Enzootic Pneumonia, pleuropneumonia, greasy pig disease, Streptococcus suis and Glasser's Disease had not been seen but P.P.E. had been present in the past. The antimicrobials

used for treatment of diarrhoea were Amphipen injection (piglet diarrhoea, 10 to 15 days old), Noridyne (crystopen, streptomycin and cortisone) and oxytetracycline.

Farm 6 (East Scotland)

This unit of 400 sows was visited to establish the aetiology of enteritis which prevailed in weaned pigs of 4-5 weeks of age in spite of treatment with antimicrobials.

The sows were kept outdoors in arks. Farrowing was outdoors and piglets are weaned at 3 weeks and brought into kennel accommodation in a large barn. They were bedded on straw and heat lamps were used to maintain temperature. At six weeks the pigs were moved to a further kennel/bungalow within the same barn with solid floors. Weaners are then graded for sale as weaners at 27 kg. Some animals were being kept on for finishing in covered yards/large pens on solid floors with deep straw in an adjacent unit.

An outbreak of diarrhoea had begun at the end of July 1989 with the loose faeces and by the 11^{th} August diarrhoea was clearly a problem in recently weaned pigs.On the 14^{th}-16^{th} of August Salocin (Hoechst) was added to the ration without effect. Zinc oxide was added to the ration at high level >1,000 ppm for two weeks and after $3^{1}/2$ days the diarrhoea dried up.

Examination of the pigs at the Edinburgh V.I.C. failed to demonstrate S. hyodysenteriae on FAT, no clostridial enterotoxins were demonstrated and no salmonellae were found. Rotavirus, Cryptosporidium spp and parasites were not checked. E. coli scour had been identified in the first kennels and a $K88^{+}$ve (F4) Escherichia coli had been isolated. No specific treatment for this had been given.

3. R E S U L T S

A) Farm 1 (Southwest Scotland)

a) Visit 1

Clinical inspection: Diarrhoea was present in pigs aged 7

days (samples 10 and 11), nine days (samples 9 and 12) and 8 weeks of age (samples 1 to 8). Those of 8 weeks of age had received treatment with lincomycin, but diarrhoea had recurred following the withdrawal of treatment.

Upon inspection no diarrhoea was found in this age group of pigs. All rectal samples taken were firm or pasty. No diarrhoea was seen on the pen floor.

The two nine day old pigs were diarrhoeic, one presented yellow to creamy stools and the other showed bloody diarrhoea. Similar diarrhoea was found in the pigs aged 7 days. One of them had soft, yellow faeces, the other had yellowish diarrhoea containing blood.

All the rectal faecal samples taken from the pigs in the supposedly affected group were examined for the presence of enteric pathogens.

i) Bacteriological findings

The bacteria isolated from these samples after culture under aerobic, microaerobic and anaerobic conditions included the following potential pathogens: Beta haemolytic *E. coli*, *Campylobacter mucosalis*, *C. coli*, *Bacteroides spp.*, *Bacteroides melaninogenicus*, *B. fragilis*, *F. necrophorum*, *C. perfringens* type A, some of which were enterotoxin producing. No salmonellae, serpulinas, cryptosporidia or rotaviruses were identified. The organisms isolated are given in Table 3.1.

In addition faecal streptococci, coliforms, *Proteus spp.*, non-haemolytic *E. coli*, and alpha haemolytic streptococci were isolated from the faeces of younger diarrhoeic pigs. Beta haemolytic *E. coli* from these younger pigs were sensitive in vitro to antimicrobials as shown below in Table 3.2.

ii) Virological and parasitological findings

Rotaviral particles and parasites were not present according to the tests carried out on the samples either by Ziehl-Neelsen and Gram's stained smears and/or by RPLA tests.

TABLE 3.1 Organisms isolated from weaned pigs with possible post-antimicrobial enteritis on Farm 1 (Visit 1).

ORGANISM	PIG NUMBER							
	1	2	3	4	5	6	7	8
Beta haemolytic								
E. coli	+	-	-	-	-	-	-	-
C. mucosalis	-	-	-	-	-	-	-	+
C. coli	-	-	-	-	-	+	-	-
Bacteroides spp.	+	+	-	-	-	-	-	-
B. melaninogenicus	-	-	+	-	-	+	-	-
B. fragilis	-	-	-	+	-	-	+	+
B. vulgatus	-	-	-	-	+	-	-	-
F. necrophorum	-	-	-	+	-	-	-	-
Clostridium spp.	-	-	+	-	-	-	+	-
C. perfringens type A	+	+[a]	+[a]	+	+	+[a]	+	+[a]
C. ramosum	-	+	-	-	-	+	-	+
Bacillus spp.	+	+	-	+	+	+	-	+

+ = Positive, - = Negative

[a] = Enterotoxin positive by counterimmunoelectrophoresis

TABLE 3.2 Antimicrobial sensitivity of Beta haemolytic E. coli.

PIG NUMBER	ANTIMICROBIAL SENSITIVITY								
	AMP_{25}	C_{10}	FR_{50}	N_{10}	OT_{30}	S_{10}	SF_{100}	SXT_{25}	AML_{25}
9	R	S	S	R	R	R	R	R	R
10	S	S	S	S	R	R	R	R	S
11	S	S	S	S	R	R	R	R	S
12	R	S	S	R	R	R	R	R	R

S = Sensitive, R = Resistant

TABLE 3.2 (Cont.) Antimicrobial sensitivity of Beta haemolytic E. coli

PIG NUMBER	ANTIMICROBIAL SENSITIVITY				
	APR_{15}	MY_{10}	PN_{10}	SH_{10}	AUG_{30}
9	S	S	R	S	S
10	S	R	S	S	S
11	S	R	S	S	S
12	S	S	R	S	S

S = Sensitive, R = Resistant

b) Visit 2

Clinical inspection: On the second visit diarrhoea was also reported to be present in the 8 weeks old pigs which had recently been given lincomycin. Inspection of the pens showed that diarrhoea was present in the outside runs. 4 pigs from this age group had died with varying degrees of faecal staining of the perineum. No faecal samples were taken on this occasion but samples from the colonic contents were taken from the dead animals for bacterial examination only during post mortem examination on the farm and the results are given below.

Pig I. Gross findings suggested that changes were restricted to the colon which contained blood. No excess mucus was noted. Coliforms, alpha haemolytic streptococci, haemolytic and non-haemolytic Bacillus spp., Clostridium spp. and fungi were isolated from the colonic sample. No salmonellae were found. No obvious bacterial pathogens were identified.

Pig II. Gross findings of intestinal haemorrhage and "bloody gut" were found. The colonic contents were taken for examination. Coliforms including Proteus spp., Bacillus spp., fungi, Campylobacter-like organisms and low numbers of clostridial spores (2.5×10^2/g of contents) were present. No other pathogens, and no evidence for the presence of C. perfringens types A and C or clostridial enterotoxin were found by counterimmunoelectrophoresis.

Pig III. A 6-8 week old pig from a group suffering from diarrhoea following lincomycin treatment. There was reddening of the ileal mucosa and the colon contents were soft. Colonic contents were taken for examination. Beta haemolytic E. coli, non-haemolytic E. coli, coliforms, Campylobacter spp., Proteus spp., non-haemolytic and haemolytic Bacillus spp., and Clostridium spp. were all isolated. Low numbers of clostridial spores (8.0×10^2/g) were present. The isolate of Beta haemolytic Escherichia coli was sensitive to AML_{25}, AUG_{30}, SH_{10}, APR_{15}, FR_{50}, N_{10} and C_{10}. The culture was resistant to SXT_{25}, AMP_{25}, OT_{30}, MY_{10}, PN_{10}, S_{10} AND SF_{100}.

Pig IV. A further animal from this lincomycin treated group was also available and ileal inflammation was the most obvious finding. Blood was present in the ileal lumen in this animal. Ileal contents were examined further. Coliforms, Beta haemolytic colonies of E. coli, Campylobacter spp., C. perfringens type A,

Bacillus spp. and Proteus spp. were isolated. The haemolytic E. coli strains were sensitive in vitro to N_{10}, FR_{50}, C_{10}, APR_{15}, AML_{25}, AUG_{30} and SH_{10} and resistant to SXT_{25}, AMP_{25}, OT_{30}, MY_{10}, PN_{10}, S_{10} and SF_{100}.

Colonic samples from pigs II and III were further examined for the presence of clostridial antibodies and/or enterotoxin by counterimmunoelectrophoresis but the results were negative.

c) Samples obtained from necropsied pigs

Five pigs of approximately 5 weeks of age supplied by Farm 1 were sacrificed and necropsied at the post-mortem room of the Veterinary School as necropsy technique practice for final year undergraduate students. They were also examined for the existence of known pathogenic bacteria from their gastroenteric tract, since all of them were presenting clinical signs of enteric disorders. All these animals were sneezing and coughing, and nasal discharge was also seen. The ear numbers of the pigs were: 6363, 6380, 6383, 6392 and 6393.

i) Clinical signs, gross post mortem lesions, and bacteriological findings

Prior to euthanasia the pigs passed liquid diarrhoeic faeces which was grey in colour but without blood or excessive mucus. Their hindquarters and perineums were stained with faeces and they were in poor condition showing clear signs of dehydration. Rectal temperatures are given below.

At necropsy, fluid contents were observed in the large intestine. The last portion of the ileum was slightly hyperaemic, the caecum was enlarged, the colonic mucosa was mildly inflamed and patchily congested, but the rest of the gut was apparently normal. Direct smears stained with Gram and Ziehl-Neelsen showed an excess of Gram positive rods but were negative for cryptosporidial oocysts.

Pig 6363. The rectal temperature was 40.1°C. The lungs showed few bloody spots and also presented a grade 3 atrophy of the turbinate bones of the snout. Ileal contents were taken and examined further. Alpha haemolytic streptococci, Proteus spp., coliforms, Campylobacter spp. and Bacillus spp. were all isolated. No spirochaetes were present.

Pig 6380. This animal presented respiratory distress, loose faeces and rectal temperature of 40.1°C. At the necropsy some lung lesions were apparent including an small area of enzootic pneumonia. Caecal contents were pasty in consistency and yellowish in colour. Samples from this section were taken and examined for the presence of potential enteric pathogens. Coliforms, alpha haemolytic streptococci, Staphylococcus spp., Campylobacter spp., Bacillus spp., Bacteroides spp. and peptostreptococcus were all isolated.

Pig 6383. No clinical signs apart from diarrhoea were found in this pig. Its rectal temperature was 39.7°C. Gross post mortem examination showed no apparent respiratory lesions. The digestive tract was also normal in appearance but the contents in the large intestine were soft in consistency and were taken for further examinations. Streptococci, Staphylococcus spp., profuse growth of coliforms, Campylobacter spp., non-haemolytic colonies of Bacillus spp., Bacteroides spp., fungi and Proteus spp. were all found from this sample.

Pig 6392. The rectal temperature of this pig was 40.0°C and at the necropsy rhinitis grade 1 and small scattered individual lobules in the lungs were found. No changes were observed in the gastrointestinal tract.However, ileal contents were examined for potential enteropathogenic organisms. Streptococci, haemolytic and non-haemolytic Bacillus spp. and coliforms were the only enterobacteria isolated.

Pig 6393. Gross findings showed the lungs and trachea to be normal in appearance. Rhinitis grade 3 was present. The rectal temperature was 38.6°C. Streptococci, Staphylococcus spp., coliforms, Proteus spp., Campylobacter spp., Bacteroides spp. and non-haemolytic Bacillus spp. were all isolated.

ii) Histological examination

Pig 6963. The ileal mucosa was grossly normal, except for the slightly short villi fused locally. There were some immature cells and cryptosporidial oocysts present. The colonic mucosa was normal but bacteria were seen adhering to the luminal epithelium.

Pig 6380. Some adhering bacteria, cryptosporidia and few

coccidia were seen in the ileal epithelium. There was no evidence of inflammation. The colonic mucosa was slightly low but crypts were normal. Mild colitis and numerous bacteria adhering to the epithelial surface were also observed.

Pig 6383. The ileal epithelium was grossly normal, except for the presence of neutrophils in some crypts and eosinophils in the lamina propria. Signs of mild colitis were observed. Bacteria were seen adhering to the epithelial surface, some crypts were dilated and a few of them contained neutrophils. Locally there was some epithelial loss. Inflammatory cells and plasma cells were present in the lamina propria.

Pig 6392. In the ileum the mucosa was normal. Some crypts were seen with debris inside. No changes were observed in the colonic section.

Pig 6393. Numerous cryptosporidial oocysts were observed in the ileal epithelium together with bacteria adjacent to the mucosal surface. Villous atrophy, some dilated crypts and capillary dilatation in the lamina propria were also seen. In the colon there was some epithelial loss with areas of attaching and effacing organisms (E. coli) with pedestal formation. Balantidium coli was also seen adjacent to the mucosal colonic epithelium.

d) Faecal samples obtained at the department

The results given below correspond to four samples of diarrhoeic faeces submitted to the Bacteriology section of the Department of Veterinary Pathology for the isolation and identification of suspected salmonellosis, campylobacteriosis and spirochaetosis (swine dysentery). The samples were identified as 1 (liquid brownish coloured faeces), 2 (liquid faeces containing blood), 3 (from a weaned pig-the faeces were liquid in consistency and brown in colour) and 4 (the faeces were blood-stained and brown to black in colour).

i) Bacteriological findings

The bacteria isolated from these samples is presented below in Table 3.3. There were no other potential pathogens such as

salmonellae or serpulinas present in the samples examined.

TABLE 3.3 Organisms isolated from faecal samples (Farm 1) obtained at the Veterinary Pathology Department

ORGANISM	SAMPLE NUMBER			
	1	2	3	4
Coliforms	+	+	+	+
Staphylococcus albus	+	+	-	-
Beta haemolytic staphylococci	+	+	+	+
Alpha haemolytic streptococci	+	+	-	+
Non-haemolytic E. coli	-	+	+	-
Beta haemolytic E. coli	-	-	+	-
Campylobacter spp.	+	+	+	+
Pseudomonas spp.	+	-	-	-
Proteus spp.	+	-	-	+
Bacillus spp.	+	+	-	-
Bacteroides spp.	+	-	-	-
Clostridium spp.	-	+	+	-
C. ramosum	-	-	-	+
Yeasts	-	+	-	-

+ = Positive, - = Negative

e) Visit 3

i) Clinical inspection and bacteriological findings

On the third visit to the farm diarrhoea was present in pigs from different age groups. They were receiving treatment with lincomycin in the drinking water. The feed conversion ratio was said to be 4.6:1, and the main problem at the time of inspection was reported to be related with respiratory disease. 10 faecal samples were obtained to be examined for enteric pathogens present in the pigs with diarrhoea or soft faeces. The samples were identified with progressive numbers from 1 to 10. Samples 1 and 10 were obtained from the Farrowing House from pigs between 4 to 5 weeks of age. Samples 2, 3, 4, 5, 7, 8, and 9 were obtained from the Growing House (General House) in which the enteric disease was apparent and the diarrhoea observed was fluid or mucoid in consistency. Sample 6 was obtained from the Fattening House. The untreated animals with diarrhoea had not

received any treatment for the previous three weeks. A table (Table 3.4)showing the identity, characteristics of the samples, and the results of bacteriological findings are presented below.

During the visit, one pig from pen 14 in the Growing House identified with the ear-tag number C8461 died showing prolapse of the rectum. At the necropsy the macroscopic findings were bilateral pneumonia and chronic peritonitis with rupture of rectum. Gut contents and numerous adhesions were present all over the abdominal cavity. Liver, spleen and kidneys were normal in appearance. The cause of death was considered to be due to peritonitis and rectal prolapse.

TABLE 3.4 Identity, characteristics of the samples, and results of bacteriological examinations (Farm 1, Visit 3)

Sample Number	1	2	3	4	5	6	7	8	9	10
Age of pig (Weeks)	4-5	8	8	8	16	16	16	16	15	4-5
Diarrhoea present	-	+	-	++	-	-	+	-	++	-
Mucus present	-	+	-	-	-	-	-	-	-	-
Soft faeces	+	-	+	-	-	+	-	-	-	-
Lincomycin treatment	+	-	-	-	-	-	-	-	-	+
Rectal temperature ^{o}C	-	-	-	41.6	-	-	-	-	-	-
Organisms isolated:										
Coliforms	+	+	+	+	+	+	+	+	+	+
Escherichia coli	-	+	-	-	-	-	+	+	-	+
Beta haemolytic E.coli	-	-	-	-	-	-	+	-	-	+
Fungi	+	+	+	-	-	+	+	++	-	-
Alpha haemolytic streptococci	-	+	+	+	+	+	+	+	+	-
Beta haemolytic staphylococci	-	+	+	-	-	-	-	-	-	-
Staphylococcus albus	-	+	+	-	-	+	-	-	-	-
Campylobacter spp.	+	+	+	+	+	+	+	-	+	+
Proteus spp.	+	-	-	-	-	-	-	-	+	+
Bacillus spp.	+	+	+	+	-	+	+	+	+	+
Bacteroides spp.	-	+	+	+	+	+	+	+	+	+
Serpulina spp.	-	-	-	+[a]	+	-	+[a]	-	+[b]	-
Clostridium spp.	-	+	-	+	+	-	-	-	-	-

+ = Positive, - = Negative,
[a] = S. innocens, [b] = S. hyodysenteriae

Beta haemolytic E. coli were isolated only from pigs 7 and 10. Their sensitivity to antimicrobials in vitro showed that the strains isolated from pig 10 were sensitive to FR_{50}, AMP_{25}, C_{10},

N_{10}, OT_{30}, AML_{25}, AUG_{30}, PN_{10} and APR_{15}, and resistant to SXT_{25}, SF_{100}, S_{10}, MY_{10} and SH_{10}. The Beta haemolytic E. coli isolated from pig 7 presented a different pattern to the effect of antimicrobials, being sensitive to FR_{50}, C_{10}, AMP_{25}, N_{10}, OT_{30}, SXT_{25}, S_{10}, AML_{25}, AUG_{30}, PN_{10}, APR_{15} and SH_{10} and resistant to SF_{100} and MY_{10}.

f) Visit 4

i) Clinical inspection and bacteriological findings

In order to maintain a continuous assessment of the scale of the enteric disease, a fourth visit to the farm was carried out one week later. In this occasion the health of the piggery showed a remarkable clinical recovery. However, to continue the evaluation of enteric pathogens present in the farm seven faecal samples were obtained, 6 from the Growing (General) House, and 1 from the Fattening House. The samples were identified with Roman numerals to avoid confusion with the samples previously examined and are presented in Table 3.5.

TABLE 3.5 Identity, characteristics of the samples, and results of bacteriological examinations (Farm 1, Visit 4)

Sample Number	I	II	III	IV	V	VI	VII
Age of pig (Weeks)	12	15	16	16	12	12	14
Diarrhoea present	+	-	-	-	-	-	-
Soft faeces	-	+	+	+	+	+	+
Rectal temperature °C	39.7	39.6	39.8	38.8	39.7	39.9	-
Organisms isolated:							
Coliforms	+	+	+	+	+	+	+
Escherichia coli	+	+	-	-	+	+	+
Fungi	+	+	+	+	+	+	+
Alpha haemolytic streptococci	-	-	-	-	-	-	+
Staphylococcus albus	-	-	-	-	+	-	-
Campylobacter spp.	+	+	-	+	+	+	+
Proteus spp.	-	-	+	+	-	-	-
Bacillus spp.	+	+	+	-	-	+	-
Bacteroides spp.	+	-	+	-	-	-	-
Clostridium spp.	-	+	-	-	-	-	-
Peptostreptococcus spp.	-	+	-	-	-	-	-
Eubacterium spp.	-	+	-	-	-	-	-

+ = Positive, - = Negative

No potential pathogens were isolated with the exception of

Campylobacter spp. which were isolated from 7/8 animals, Bacteroides spp. and clostridia.

B) Farm 2 (Bristol, England)

Weaned pigs from this farm were suffering from a non-specific form of colitis thought to be caused by Serpulina hyodysenteriae. Only faecal samples were available and the history supplied was poor. It appeared that the colitis had failed to respond to conventional antimicrobial therapy (unspecified) and some cases of the colitis had occurred after treatment. Two sets of faecal samples were sent for examination for possible enteric pathogens. Further investigation of the possibility that this represented post-antibiotic diarrhoea was to depend upon the outcome of this examination.

a) First samples examined

Three faecal samples were received from this farm to be examined for bacterial pathogens thought to be the cause of the enteric disease prevalent in the piggery. The samples received were dark-green in colour and soft in consistency. They were identified as 1, 2 and 3. Direct smears of the faeces stained with Gram and Ziehl-Neelsen were negative for the presence of spirochaetes and Cryptosporidium spp.

i) Bacteriological findings

The organisms isolated were coliforms, non-haemolytic E. coli, Streptococcus spp. and alpha haemolytic Staphylococcus spp. Beta haemolytic E. coli in sample 3 and contamination by Proteus spp. in the three samples were also present.

Campylobacters were isolated from all the samples examined. All the samples gave negative results for enteric spirochaetes.

b) Second samples examined

Seven faecal samples from pigs suffering from diarrhoea were received with the specific request of examination for the presence of S. hyodysenteriae. The farm owner now suspected more firmly that swine dysentery was present in the piggery. Direct smears of the samples stained with Gram's were examined under the microscopy and showed the presence of organisms with the morphology of campylobacters, streptococci, clostridia, and few large serpulina-like bacteria. For the bacteriological report and examination procedures, the samples were identified with progressive arabic numerals according to the list below:

1.- Faecal sample from House 1 - Pen 3 (H-1 P-3).
2.- Faecal sample from House 2 - Pen 88 (H-2 P-88).
3.- Faecal sample from House 3 - Pen 40 (H-3 P-40).
4.- Faecal sample from House 3 - Pen 87 (H-3 P-87).
5.- Faecal sample from House 2 - Pen 31 (H-2 P-31).
6.- Faecal sample from House 1 - Pen 18 (H-1 P-18).
7.- Faecal sample from House 2 - Pen 75 (H-2 P-75).

TABLE 3.6 Organisms isolated from the samples examined (**Farm 2**)

ORGANISM	SAMPLE NUMBER						
	1	2	3	4	5	6	7
Coliforms	+	+	+	+	+	+	+
Escherichia coli	+	+	+	+	+	+	+
Beta haemolytic E. coli	-	+	-	-	+	-	-
Fungi	+	+	+	+	+	+	+
Staphylococcus spp.	-	+	+	-	-	+[a]	-
Alpha haemolytic streptococci	+	+	-	+	+	+	+
Proteus vulgaris	-	-	+	-	-	-	-
Campylobacter spp.	+	+	+	-	+	-	+
Proteus spp.	+	+	+	++	+	++	+
Bacillus spp.	+[a]	+[a]	+	+[a]	+[a]	+[a]	+[a]
Bacteroides spp.	-	-	-	+	-	-	-
Clostridium spp.	-	-	+	-	-	-	-
C. perfringens type A	-	-	-	-	-	+	-
C. septicum	-	-	-	-	-	+	+

+ = Positive, - = Negative, [a] = Haemolytic colonies

Serpulina hyodysenteriae was isolated from these samples only from sample number 1 which was obtained from the farm's House I-Pen 3. The identity of the bacterium was confirmed by Gram stained smears and slide agglutination test.

C) Farm 3 (Lanarkshire, Scotland)

a) Farm visit

Clinical inspection: Examination of the farm showed that hygiene was poor, temperature control was poor and that geese and dogs had free access to pig pens and feed.

Swine dysentery was present in a number of pens. Upon enquiry it appeared that the treatments given were inadequate. Tiamulin water soluble was given in the drinking troughs but not as the only source of water. Even this degree of treatment continued for only $2^{1}/2$ days and was not associated with disinfection or cleaning. The parenteral treatment with tiamulin consisted of the injection of individual very sick animals with little or no supportive treatment other than access to medicated water. Top dressing of feed was carried out for periods of one week, but affected animals in these treated pens were not separated or always treated parenterally. All feed (Super Meat Maker pellets) contained 20 g/tonne of tylosin as a growth permitter. It appeared from the farm inspection that the problem was not one of post-antimicrobial diarrhoea, but rather one of totally inadequate management of the swine dysentery. Faecal samples were taken to confirm this.

Twenty samples were taken from animals on treatment and from untreated pens in 3 different age groups. They were examined for enteric pathogens such as bacteria, rotavirus and Cryptosporidium spp. The samples obtained were identified with Roman numerals and their nature, origin, and characteristics of the pigs sampled are presented below in Table 3.7.

NOTE: All those animals 8 weeks or over had been treated previously with tylosin.

TABLE 3.7 Identity, characteristics of the samples and results of bacteriological examinations (Farm 3, Farm Visit)

Sample Number	I	II	III	IV	V	VI	VII	VIII	IX	X
Age of pig (Weeks)	4	4-5	4-5	4-5	4-5	8	8	8	8	8
Diarrhoea present	-	++	++	++	++	-	-	-	-	++
Soft faeces	+	-	-	-	-	+	+	+[a]	+	-
Farrowing house	+	+	+	+	+	-	-	-	-	-
Open yard	-	-	-	-	-	+	+	+	+	+
Tylosin in feed	+	+	+	+	+	+	+	+	+	+
Organisms isolated:										
Coliforms	+	+	+	+	-	+	-	-	+	+
Escherichia coli	+	+	+	+	+	+	+	+	+	+
Beta haemolytic										
Escherichia coli	+	+	+	-	+	-	-	-	+	-
Fungi	+	-	-	-	+	+	-	-	+	+
Alpha haemolytic										
streptococci	+	+	+	+	+	+	+	+	+	+
Staphylococcus spp.	-	-	-	+	-	+	+	-	-	-
Campylobacter spp.	+	+	+	+	+	+	+	+	+	+
Proteus spp.	-	+	+	+	+	-	-	+	+	-
Bacillus spp.	+	+	-	+	+	+	+	+	-	+
Bacteroides spp.	-	-	+	+	-	+	-	-	-	+
Clostridium spp.	-	+	-	-	-	-	-	-	-	-
C. perfringens type A	-	-	-	+	-	-	-	-	-	-
Peptostreptococcus spp.	-	-	-	-	+	-	-	-	+	-

+ = Positive, - = Negative, [a] = Mucus

TABLE 3.7 (Cont.) Identity, characteristics of the samples, and results of bacteriological examinations (Farm 3, Visit)

Sample Number	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX
Age of pig (Weeks)	8	8	12	12	12	12	12	14	14	52
Diarrhoea present	++	-	++	++	-	-	++	+++	-	-
Soft faeces	-	+	-	-	+	+	-	-	+	-
Open yard	+	+	-	-	-	-	-	-	-	-
Fattening house	-	-	+	+	+	+	+	+	+	-
Tylosin in feed	+	+	+	+	+	+	+	+	+	+
Tiamulin in water	-	-	+	+	+	+	-	-	+	-
Tiamulin water+injectio	-	-	-	-	-	-	-	-	+	-
Organisms isolated:										
Coliforms	-	-	+	-	+	-	+	+	-	+
Escherichia coli	+	+	+	+	+	+	+	+	+	+
Beta haemolytic E. coli	+	-	-	-	-	-	-	-	-	-
Fungi	+	+	-	-	-	+	-	-	+	+
Alpha haemolytic streptococci	+	+	+	+	+	+	+	+	+	+
Staphylococcus spp.	-	+	+	+	+	+	+	+	+	+
Campylobacter spp.	+	+	+	+	+	+	-	+	+	+
Proteus spp.	-	+	-	+	-	-	-	-	-	-
Bacillus spp.	+	+	+	+	+	+	+	+	+	+
Bacteroides spp.	+	-	+	-	-	+	-	+	+	+
Clostridium spp.	+	-	-	-	-	+	-	-	-	+
Peptostreptococcus spp.	-	-	-	+	-	-	-	-	-	+

+ = Positive, - = Negative

i) Bacteriological findings

The major pathogens identified were Beta haemolytic E. coli in 4/5 pigs from the farrowing house and 2/7, 8 weeks pigs from the outside pens. All the samples yielded Campylobacter spp. except the sample from pig XVII. C. coli was identified from pigs, XV and XVI and C. hyointestinalis was found in sample from pig VIII. These isolates were tested for their sensitivity to metronidazole 5, 10 and 50 ug (Flagyl), tylosin 25, 30 and 100 ug (Tylan) and nalidixic acid (Negram). The results showed that both isolates were sensitive only to nalidixic acid and resistant to tylosin and metronidazole.

The Beta haemolytic E. coli isolated were tested in pure cultures for their sensitivity to antimicrobials. The results of these tests showed that strains of pigs II and III were sensitive to, AML_{25}, PN_{10}, APR_{15}, SH_{10}, C_{10}, FR_{50}, AMP_{25}, N_{10}, S_{10} and AUG_{30}, and resistant to MY_{10}, OT_{30}, SF_{100}, and SXT_{25}. Pig V gave other results being sensitive to AML_{25}, PN_{10}, APR_{15}, SH_{10}, C_{10}, FR_{50}, N_{10} and S_{10}. The resistance was identified against MY_{10}, OT_{30}, SF_{100}, SXT_{25} and AMP_{25}.

Characteristic colonies of S. hyodysenteriae were isolated from pig VIII. The morphology was confirmed by the examination of Gram stained smears and their biochemical reactions were tested by the methods established in the laboratory and used for routine diagnosis of swine dysentery. MIC determinations for S. hyodysenteriae were carried out and the organism was found to be sensitive in vitro to tiamulin (MIC 0.5 ug/ml).

D) Farm 4 (Cumbria, England)

a) Farm visit

Clinical inspection: Farm inspection failed to reveal any evidence of the post-antimicrobial enteritis. All weaned pigs were in good condition with firm faeces. Although diarrhoea had occurred 14 days previously in 6-8 weeks old pigs, it had been treated successfully and of the 70 pigs in the appropriate age group, only two with soft faeces were seen. These animals were in excellent condition and were not dehydrated and were afebrile. Their faeces were shiny but contained no excess mucus or blood. It was concluded form the inspection that no post-antimicrobial enteritis was present, but faecal samples were taken to confirm the absence of enteric pathogens. After bacteriological examination of the samples no enteric pathogens were isolated.

E) Farm 5 (Yorkshire, England)

a) Farm visit

Clinical inspection: The examination of the farm indicated that a problem was present in the weaned pigs of the flat decks. Animals with diarrhoea and high rectal temperatures were seen

here. In the Verandah house (solid floored pens) the pigs (30 to 40 kg body weight) were dirty, the environment was hot but the pigs were afebrile and in good condition. Coughing was noticed but was being treated, tail-biting was also observed and some animals presented signs of rectal stricture and swollen ears. Infection by Haemophilus parasuis was apparently present. In these pens brown/dark soft to fluid diarrhoea with a little mucus was apparent. The pigs were pale possibly due to the presence of PHE or PPE. In the growing-finishing house the pigs were between 60 to 80 kg in weight and diarrhoea was clearly observed. In some animals it was reddish and fluid and in other pigs with soft reddish stools excess of mucus was observed. Diarrhoea was present both in pigs receiving treatment and in untreated animals. It was decided that until this diarrhoea could be identified, post-antimicrobial enteritis could not be said to be present.

Fifteen faecal samples were taken from the pigs of three different age groups and located in the flat deck house (samples 1 and 2), the Verandah house (3 to 11) and in the finishing house (growing house) (12 to 15), for examination of potential pathogens responsibles of the diarrhoea observed. The list below presents the identification number of each sample, its origin, nature of the samples and characteristics of the pigs from which each sample was obtained.

Identity and characteristics of the samples:

1.- Pig 2 weeks old; Soft faeces; Flat deck; 40.1°C.
2.- Pig 2 weeks old; Diarrhoea; Flat deck; 39.9°C.
3.- Pig 5-6 weeks old; Fluid faeces; Verandah; 39.6°C; Pen 2.
4.- Pig 5-6 weeks old; Soft faeces; Verandah; Room 1; Pen 2.
5.- Pig 5-6 weeks; Soft faeces; Verandah; 39.5°C;Room 1;40 kg.
6.- Pig 5-6 weeks old; Soft faeces; Verandah; PPE; Pen 6.
7.- Pig 5-6 weeks;Firm faeces;Verandah;Room 1;Rectal stricture.
8.- Pig 5-6 weeks old; Dark soft faeces;Verandah;Room 1;Pen 6.
9.- Pig 5-6 weeks old; Dark soft faeces;Verandah;Room 1;Pen 6.
10.-Pig 5-6 weeks old; Diarrhoea dark; Verandah; Room 1;Pen 7.
11.-Pig 5-6 weeks old; Soft faeces; Verandah; Room 1; Pen 9.
12.-Pig 4-5 months old; Soft faeces; Growing house 2.
13.-Pig 4-5 months old; Soft faeces; Growing house 3.
14.-Pig 4-5 months; Fluid reddish diarrhoea; Growing house 3.
15.-Pig 4-5 months; Mucoid reddish diarrhoea; Growing house 3.

TABLE 3.8 Organisms isolated from the samples examined (Farm 5)

ORGANISM	SAMPLE NUMBER							
	1	2	3	4	5	6	7	8
Escherichia coli	+	+	-	-	+	+	+	+
Beta haemolytic E.coli	-	-	++	++	-	-	-	-
Streptococcus spp.	-	-	+	+	+	+	-	+
Alpha haemolytic streptococci	+	++	-	-	-	-	+	-
Campylobacter spp.	-	-	+	-	+	-	+	+
Lactobacillus spp.	-	-	+	+	-	+	-	+
Bacteroides fragilis	+	+	+	-	-	-	-	-
B. melaninogenicus	-	-	-	-	-	-	+	-
B. vulgatus	-	-	-	-	-	-	+	+
B. distasonis	-	-	-	-	+	-	-	-
F. necrophorum	-	-	-	-	-	+	+	-
Clostridium spp.	-	-	+	+	+	+	+	+

+ = Positive, - = Negative

Beta haemolytic E. coli strains isolated from pigs 3 and 4 from the Verandah house were sensitive in vitro to the following antimicrobials: AMP_{25}, C_{10}, FR_{50}, N_{10}, AUG_{30}, tylosin (TY_{30}), gentamicin (CN_{10}), erythromycin (E_{10}) and spectinomycin (SH_{25}). Resistance was present against OT_{30}, S_{10}, SF_{100} and SXT_{25}.

TABLE 3.8 (Cont.) Organisms isolated from the samples examined

ORGANISM	SAMPLE NUMBER						
	9	10	11	12	13	14	15
Coliforms	+	+	+	+	+	+	+
Escherichia coli	+	+	+	+	+	+	+
Staphylococcus albus	-	+	-	+	-	-	-
Streptococcus spp.	-	-	-	-	-	-	+
Alpha haemolytic streptococci	++	-	+	-	+	-	-
Salmonella kedougou	-	-	+	-	+	+	-
Campylobacter spp.	-	+	+	-	+	-	+
Lactobacillus spp.	+	+	+	-	+	-	+
Yeasts (Small intestine)	-	-	+	-	-	-	-
Bacteroides vulgatus	-	-	+	-	-	-	+
Clostridium spp.	+	+	+	-	-	-	-

+ = Positive, - = Negative

i) Bacteriological findings

Samples from pigs 11, 13 and 14 which were positive by slide agglutination tests (agglutinated with polyvalent H and O antiserum) and API 20 E strip biochemical tests (99.5% and 99.9% +ve) were considered to belong to the genus Salmonella. Subcultures sent to the Scottish Salmonella Reference Laboratory, were confirmed as belonging to the serovar Salmonella kedougou. All three isolates were tested for their sensitivity to antibiotics and were sensitive in vitro to AUG_{30}, AMP_{25}, C_{10}, N_{10} and FR_{50}, and resistant to SXT_{25}, SF_{100}, S_{10}, and OT_{30}.

Campylobacter spp. were present in most of the samples examined (see Tables above). The colonial and microscopical morphology of the organisms isolated suggested that both C. coli and C. hyointestinalis were present. Many clostridial spores were observed at microscopic examination from the anaerobic HBA and clostridia plates.

None of the cultured samples presented any growth which suggested the presence of spirochaetes. However, serological tests (i.e. slide agglutination) gave positive results for the presence of S. hyodysenteriae antigens in the samples examined.

ii) Virological findings

Samples from pigs 4, 8, 9, 10 and 14 were examined further to determine if rotavirus was present in the farm. RPLA tests were carried out on the faecal filtrates of the samples, but the results were all negative for porcine rotavirus in these units.

iii) Parasitological findings

Few selected samples were examined for the presence of the protozoan Cryptosporidium spp. Ziehl-Neelsen stained smears showed that this coccidial parasite were not present.

F) Farm 6 (East Scotland)

a) Farm visit

i) Clinical inspection and bacteriological findings

When the unit was inspected, diarrhoea was seen in pigs aged 4-4^{1}/2 weeks associated with poorly cleaned kennels. Most animals had firm faeces but some animals aged 6-7 weeks had grey-brown soft faeces with some mucus after 13 days in the pen. Some coughing and an early case of mange were also noted.

Upon enquiry it appeared that regular vaccination against E. coli infection was only carried out in incoming gilts using Duphar's Suvaxyn E. coli.

It was concluded from the clinical inspection of the farm that post-antibiotic enteritis was not present. However, ten faecal samples were obtained to confirm the clinical findings. Samples 1 to 4 were taken from diarrhoeic pigs of 4^{1}/2 weeks of age and samples 5 to 10 from older pigs (> than 4^{1}/2 weeks) with soft to firm faecal consistency.

TABLE 3.9 Organisms isolated from the samples examined (Farm 6)

ORGANISM	SAMPLE NUMBER									
	1	2	3	4	5	6	7	8	9	10
Coliforms	+	+	+	+	+	+	-	+	+	+
Escherichia coli	+	-	-	-	+	-	+	-	+	-
Beta haemolytic E.coli	-	++	+	+	-	-	-	+	-	+
Fungi	+	+	+	+	+	+	+	+	+	+
Alpha haemolytic streptococci	+	-	+	+	+	++	+	+	+	+
Staphylococcus spp.	-	-	-	-	+	-	++	+	+	+
Campylobacter spp.	+	+	+	+	+	+	+	+	+	+
Proteus spp.	-	-	-	-	-	-	-	+	+	-
Bacillus spp.	-	-	-	-	-	-	-	-	+	-
Bacteroides spp.	+	+	+	-	-	-	-	-	+	-
C. perfringens type A	+	+	+	+	+	+	+	+	+	+

+ = Positive, - = Negative

C. perfringens type A were isolated from all samples inoculated, and a large number of sporulated organisms was observed microscopically in Gram's stained smears.

TABLE 3.10 Antimicrobial sensitivity of 5 Beta haemolytic *E. coli* isolates from Farm 6 (Farm Visit)

PIG NUMBER	ANTIMICROBIAL SENSITIVITY								
	AMP_{25}	C_{10}	FR_{50}	N_{10}	SXT_{25}	OT_{30}	S_{10}	SF_{100}	MTZ_5
2	S	S	S	S	S	R	R	R	R
3	S	S	S	S	S	R	R	R	R
4	S	S	S	S	S	R	S	R	R
8	S	S	S	S	S	S	S	R	R
10	S	S	S	S	R	R	S	R	R

S = Sensitive, R = Resistant

The results obtained from the antimicrobial sensitivity of Beta haemolytic *E. coli* colonies showed differences in their sensitivity pattern which indicates the presence of three different strains. Samples from all pigs yielded colonies suggestive of campylobacters but only two (5 and 7) were confirmed as *C. hyointestinalis* which were sensitive to the antimicrobials presented below in Table 3.11.

TABLE 3.11 Antimicrobial sensitivity of *Campylobacter spp.*

PIG NUMBER	ANTIMICROBIAL SENSITIVITY								
	AMP_{25}	C_{10}	FR_{50}	N_{10}	SXT_{25}	OT_{30}	S_{10}	SF_{100}	MTZ_5
5	S	S	S	S	R	S	R	R	S
7	S	S	S	S	R	S	R	R	S

S = Sensitive, R = Resistant

b) Samples obtained from necropsied pig

Two weeks after visiting the farm a 6 week-old diarrhoeic pig from the kennel/bungalow accommodation was brought to the Veterinary School for a detailed post mortem examination. The pig was killed and examined for gross pathological lesions. Particular attention was paid to the gastrointestinal and

respiratory tracts.

i) Post mortem findings

The skin was covered with a waxy deposit and there was mange in the ears. Mites were seen in large numbers. The lungs had small haemorrhages, possibly Ascaris spp. migration tracks but no liver lesions were identified. The small intestine was grossly normal but the large intestine was dilated with old serositis on the colon wall. The colonic mucosa was pale and the contents were soft, light in colour and contained shiny mucus. Black gritty material was present in the contents. There was inflammation of the rectum, and massive iron staining in the hind-limb muscles was also present.

ii) Bacteriological findings

Direct smears from the ileal, caecal and colonic contents were made,stained with Gram and examined under the microscope. From the ileum Gram +ve cocci, Gram +ve clostridia-like rods and few large Gram -ve rods were observed.The caecum contained many Gram -ve organisms with the morphology of campylobacters, some Bacteroides-like bacteria, few clostridia-like rods, some yeasts, some bacilli and few Gram +ve cocci. The colonic smears contained many large Gram -ve spirochaetes, some campylobacters, a few large Gram +ve clostridia-like cells and some other bacilli. Smears from the faeces stained with the modified Ziehl-Neelsen technique for the presence of cryptosporidia were also made and the results were negative. Mucosal smears from the jejunum, ileum, caecum and colon stained with Gram's yielded mainly Gram -ve bacteria which included spirochaetes, campylobacters-like, Bacteroides-like, Fusobacterium-like and few Gram +ve clostridia-like organisms. The bacteria isolated and identified from these samples cultured on solid media are presented below in Table 3.12.

TABLE 3.12 Organisms isolated from the samples examined (Farm 6)

ORGANISM	ILEUM	CAECUM	COLON
Coliforms	-	+	+
Escherichia coli	-	+	+
Beta haemolytic E. coli	+	+*	+
Non-haemolytic streptococci	-	+	+
Alpha haemolytic streptococci	-	-	+
Fungi	++	++	+
Campylobacter spp.	+	+	+
Proteus spp.	+	-	-
Bacillus spp.	-	+	+
Bacteroides spp.	+	+	+
C. perfringens type A	+	++	+

+ = Positive, - = Negative

* Only one colony of haemolytic E. coli was seen

Although campylobacters and Bacteroides spp. were isolated and identified from all the samples examined, their colonies were found in reduced numbers due to heavy fungal contamination present in the original plates. A small number of clostridia were isolated from the ileum and colon but the caecal sample gave high counts of 10^6/g. The identity of these organisms was confirmed as C.perfringens type A by the methods used previously.

iii) Histological examination

The sections processed for this examination were obtained from the stomach, jejunum, ileum, caecum, colon and lung.

Examination of the gastric mucosa showed that post mortem changes were present (i.e accumulated cell shedding on the epithelial surface). Local accumulation of macrophages and plasma cells, capillary dilatation and an area of regenerative mucosa, perhaps a healing ulcer were observed in the tissue. In the jejunum the villi were shortened and fused locally but few active inflammatory lesions were present. Some bacteria were adjacent to the mucosal surface.No coccidia were present. The ileal mucosa was grossly normal, except for relative shortening of the villi, the presence of Cryptosporidium spp., some

eosinophils in lamina propria and some intraepithelial lymphocytes.

The findings observed in the caecum included local accumulation of plasma cells in the lamina propria, lowered mucosal epithelium, interruption of the mucosal epithelium with clumps of Balantidium coli, local capillary dilatation in lamina propria and bacterial organisms in some crypts. Signs of chronic inflammatory colitis were present in the colonic section. This section was clearly inflamed, high numbers of neutrophils were present in the lamina propria, bacteria were adherent to the mucosal surface and B. coli were also present adjacent to the colonic epithelium.

In the section from the lung mild bronchitis and interstitial pneumonia were present. Some evidence of mild fibrous pleurisy and local areas of peribronchiolar lymphocytes indicating early enzootic pneumonia were observed.

4. D I S C U S S I O N

The studies described in this Chapter were intended to identify field examples of post-antimicrobial enteritis and to study the bacteria and other pathogens involved. A small series of suitable farms was identified over the three years of the project and each was examined to see if post-antimicrobial enteritis was occurring or had occurred.

The histories all suggested that post-antimicrobial enteritis was possible. The history of enteric disease on Farm 1 was typical. Diarrhoea recurred following the withdrawal of treatment with neomycin or lincomycin for 5 days in weaned pigs 5-8 weeks of age. This history suggested that postweaning diarrhoea was being controlled and was then developing once more as the cause had not be eliminated. Examination of affected pigs during visit 1 failed to find evidence of diarrhoea. Diarrhoea was identified during visits 2 and 3 but no clear evidence for the cause was determined although individual animals yielded potentially pathogenic bacteria such as Beta haemolytic E. coli, Campylobacter spp., Bacteroides spp. and C. perfringens type A (enterotoxin producing strains). Serpulina spp. but not S. hyodysenteriae were also presumptively identified on visit 3. Enteric lesions were present but could not be ascribed to any

single syndrome.

In subsequent visits or with subsequent material it was difficult to relate the appearance of enteritis to the medication used.

Although the history of the **Farm 2** suggested that post-antimicrobial enteritis was present the faecal samples sent failed to reveal any clear cause of the enteritis. *Salmonella spp.* were absent but *S. hyodysenteriae* was present suggesting that the cause of the problem reported was linked to underlying poorly controlled swine dysentery. Further information about the outbreak was not forthcoming and there appeared to be little point in continuing investigation. The problems involved in monitoring this case by samples alone led to the adoption of visits as the appropriate method of investigating the problem.

Farm 3 appeared to be a case of swine dysentery occurring as a post-antimicrobial enteritis. Inspection showed that treatment was being given in an incorrect manner and, often, at inadequate levels. It was not, therefore, possible to identify this farm as one with post-antimicrobial enteritis, but rather one of totally inadequate management of the swine dysentery. Clinical signs suggestive of swine dysentery were in fact present in some animals on clinical inspection and laboratory findings confirmed that the problems were associated primarily with swine dysentery.

Farm 4 represented a further case of the problem associated with visit 1, **Farm 1**; disease was absent when inspection was carried out.

Farm 5 was a more complex case but inspection of the affected animals showed that disease was actually occurring in animals receiving treatment as well as post-treatment. The isolation of a *Salmonella spp.* from this enteritis which was resistant to the antimicrobial used suggested the cause of the problem. Post-antimicrobial enteritis could not be confirmed on this unit either.

Farm 6 appeared to have a post-antimicrobial enteritis but treatment was changed prior to the visit and when the animals were inspected treatment (with zinc oxide) was fully effective.

These results had all shown in different ways that post-antimicrobial enteritis was difficult to identify in the field.

Only Farm 1 on visit 2 was there any suggestion that post-antimicrobial enteritis was actually present. In investigating this phenomenon in the field it was clearly necessary to have a good history and to be able to identify all the possible pathogens. Farm inspection was necessary as the history was often unreliable and gave no details of inadequate treatment (Farm 3) which could only be determined by inspection.

The clinical inspection of the farms included in this survey showed that the presence of post-antimicrobial enteritis was not a certain finding as the reports from the farms owners suggested. The lack of accurate information about the treatments used, the history of the diarrhoeic syndrome and poor observation of the epidemiological factors from the different pig units made difficult to determine the presence of a real post-antimicrobial enteritis. In some cases it was found that poor management of the treatment was the key to the solution of the problems.

The results from the bacteriological examinations proved that different enteropathogenic bacteria were present and that they were in some cases closely related to the clinical signs of diarrhoea present in the different piggeries under study. The presence of strains of Beta haemolytic E. coli, C. perfringens type A, Campylobacter spp. and Bacteroides spp. was clearly related to the clinical disease found in some farms. The result of the studies on antimicrobial sensitivity carried out mainly on strains of E. coli showed that resistance to various drugs was present and that when the recommended therapy was followed diarrhoea cases were controlled successfully.

Detailed examination of the one possible case of post-antimicrobial enteritis found (Farm 1, visit 2) had failed to reveal any clear association with any single causal agent. The presence of Beta haemolytic E. coli and cryptosporidia on the farm suggested that the recurrence of diarrhoea might have been due to the inability of the treatment given (lincomycin) to affect either of these agents or to represent a true post-antimicrobial enteritis associated with as yet unidentified pathogens. It was decided, therefore to examine the effects of antimicrobial withdrawal on the gastrointestinal flora in experimental studies.

CHAPTER 4

THE EFFECT OF IN-FEED AVOPARCIN ON GASTROINTESTINAL BACTERIAL FLORA AND ITS RELATIONSHIP WITH ENTERITIS FOLLOWING ITS WITHDRAWAL IN WEANED PIGS

1. INTRODUCTION

This chapter describes the first of a series of experiments planned to assess the effect of withdrawal of antimicrobials on the "normal" gastrointestinal flora present in the gut of recently weaned piglets. The antimicrobial chosen was avoparcin, a commonly used growth promoter, fed to pigs for long periods. The experiment was carried out to assess the effects of withdrawing treatment with the growth promoter avoparcin (Avotan 50*, Cyanamid) given at 80 ppm for 10 days over a period when adjustment to the normal flora would be taking place in the gut. The animals were monitored for 7 days during treatment and for 7 days thereafter in order to detect short-term changes following withdrawal Pigs from a high health status herd were used in order to minimize the effects of diseases such as swine dysentery, P.P.E. and salmonellosis. The effects of withdrawal on the pigs was monitored by examining clinical signs and on their gastroenteric flora by examining faecal samples over the 14 day period of observation. Sample animals were euthanased and examined post mortem. The time required for sampling was so great that only 5 pigs would be monitored continuously. It was decided to sacrifice 5 others at the intervals shown below (Table 4.1) in order to monitor pathological processes in the group.

The relationship between withdrawal of avoparcin and clinical disease, macroscopic and microscopic lesions as well as the effect on growth-productive parameters was evaluated.

2. MATERIALS AND METHODS

The majority of the materials and methods used have been described in detail in Chapter 2.

A) Experimental animals

Source of the experimental animals: The ten eight-week-old minimal disease pigs weighing between 10.5 and 15.3 kg used in

this study were obtained from the Veterinary School Animal Husbandry Department's Farm at Cochno described in Chapter 2 and shown in Figure I.

a) Identification system

Individual identification of the pigs (Table 4.1) was achieved by the application of plastic, numbered, coloured tags in the right ear. They were assigned at random to one of two groups. Tags of colours (white and orange) were used to differentiate between pigs monitored daily (white tags) and those to be killed (orange tags);

TABLE 4.1 Individual identification of pigs in Experiment 1

Tag Number and colour	Sex	Initial Weight(Kg)	Study Group	Killed on Study day
66 white	M	15.3	S	ND
67 white	M	11.5	S	ND
68 white	M	13.7	S	ND
69 white	F	11.8	S	ND
70 white	M	10.5	S	ND
59 orange	M	ND	K	0
93 orange	F	ND	K	7
91 orange	M	ND	K	8
94 orange	F	ND	K	9
40 orange	M	ND	K	14

M = Male, F = Female, S = Sampled pigs
K = Sacrificed pigs, ND = Not Determined (Done)

b) Maintenance of experimental animals

The pigs were housed in two elevated flatdecks in a temperature controlled room (23-27^{o}C). Each pen was equipped with an automatic feeder and water was freely available from a nipple drinker. They were reared under commercial conditions in the piggery of the Glasgow University Veterinary School at Cochno.

c) Food

The pigs had been fed at the beginning of the study on the

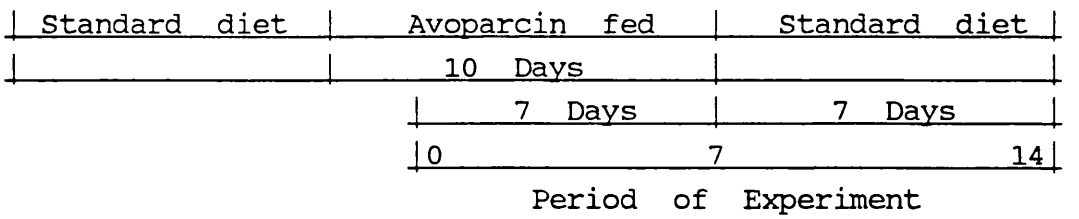
existing diet (B.O.C.M. 401 Weanercare pellets + Intagen) which was available **ad libitum** and provided by B.O.C.M. Silcock, Ltd., Basingstoke, Hampshire.

For the purpose of this study an experimental ration (401-2697) was prepared specially at the B.O.C.M. mill with 80 g/tonne of avoparcin (Avotan 50*) added to the regular commercial formulation of their 401 BOCM feedstuff (Chapter 2). It was not possible to formulate a ration with avoparcin as the only antimicrobial due to the limited availability of the product. An existing ration had to be supplemented.

B) Experimental procedures

General: Pigs were maintained on the standard diet B.O.C.M. 401 weanercare pellets + Intagen and then changed to the experimental diet B.O.C.M. 401-2697 for 10 days to allow the animals to become accustomed to it. The ration was then changed back to B.O.C.M. 401 (weanercare pellets + Intagen).

The five pigs of the sampled group were monitored daily during seven days prior to the second ration change and for seven days afterwards. The experiment therefore took place as shown below:



C) Clinical observations

The general bodily condition of the experimental pigs was noted daily.

a) Rectal temperature, faecal consistency and samples and daily live weight gain

i) Rectal temperatures were taken and recorded daily from the sampled group. The measurements were all carried out at a similar time of the day.

ii) Appetite and faecal consistency were noted daily. Any deviation from this general pattern of clinical examination was recorded.

iii) Faecal samples were taken daily for laboratory examinations.

iv) Weight gains in the sampled group were measured daily and recorded.

b) Serum samples

Blood samples were collected from the external jugular vein before treatment began and from all pigs from the sacrificed group prior to euthanasia. The sera were stored at -20°C in 5 ml amounts until required.

c) Post mortem examination

The pigs of the sacrificed group were killed during the period of avoparcin treatment (Pig 59-orange, Day 0) and after 1, 2 and 3 days after withdrawal of avoparcin, (pigs 93, 91 and 94-orange, Days 7, 8 and 9). The last pig (Pig 40-orange) was killed on day 14 of the study with an intravenous overdose of pentobarbitone, followed by exsanguination.

Post mortem examination was carried out by the methods described in Chapter 2, as soon as possible after death. All five pigs were examined for gross changes or lesions in the organs of the abdominal and thoracic cavities. Particular attention was paid to the gastroenteric tract which was investigated in detail.

D) Laboratory examinations

a) Microbiological and parasitological examinations

Rectal faecal samples were examined qualitatively and quantitatively for bacteria, and qualitatively for rotavirus and protozoal parasites by the methods described in Chapter 2. Care was taken to examine the samples immediately (within 1-2 hours). Contents from stomach, jejunum, ileum, caecum and colon obtained from the sacrificed pigs after post mortem examination were also subject to a similar examinations.

Air-dried heat fixed smears of faeces, intestinal scrapings and contents from the following five portions of the

gastrointestinal tract: stomach, jejunum, ileum, caecum and colon were made and stained by Gram's method and modified Ziehl-Neelsen's method for bacteria and the presence of cryptosporidial oocysts respectively.

b) Histological examinations

Sections of stomach, jejunum, ileum, caecum and colon were taken after the gross post mortem examination was completed. After processing, histological sections stained by H & E were examined by light microscopy to detect any abnormalities present in the gastrointestinal epithelium and for the presence of Cryptosporidium spp. and Balantidium coli.

c) Examination for enteric agents and their products

The following tests were also carried out on faeces and contents. The test used throughout the study for the demonstration of rotaviral particles was the rapid reversed passive latex agglutination (RPLA) test. Subculture of isolates onto egg yolk and sheep blood agar plates was carried out to confirm the presumptive identity of Clostridium perfringens type A by its lecithinase production (Nagler reaction) and its double zones of haemolysis respectively. Counterimmunoelectrophoresis was performed in two occasions for the demonstration of C. perfringens type A enterotoxin but, RPLA was used routinely throughout for the same purpose. C. difficile antigens were also screened using commercial RPLA kits available for agglutination from faecal dilutions by the methods outlined in detail in Chapter 2.

d) Bacteriological examinations

The bacteria considered in these studies as potentially important enteric pathogens were selected from the agents more often isolated in the studies carried out in Chapter 3 and included; a) Aerobic organisms, b) Microaerobic organisms and c) Anaerobic organisms.

Bacterial identification was presumptive in most cases, incubated plates were examined for colonial morphology and

effect on the media. Cellular morphology was confirmed by Gram's stain. Further identification of bacteria was carried out in more detail where the identity of isolates was in doubt using the methods described in Chapter 2. The bacterial counts provided the number of Colony Forming Units (CFU).

E) Statistical analysis

The daily individual bacterial counts of each organism recorded from the pigs of the sampled (treated) group were compared between themselves and between the periods which represented the pigs under treatment and after its withdrawal. The bacteriological results were analyzed for significance by using the "StatWorks^{TM} Data (Kruskal-Wallis Test) statistical package (1991). The results of clinical observations (rectal temperature) and production parameters (DLWG) were analysed by using a two sample paired student's t-Test for means (Microsoft Excel, Version 4.0 [(c)1992]). The latter test was used to make statistical comparisons between the periods of treatment and withdrawal. All the data were analysed using the 5 per cent level of significance ($P<0.05$).

3. R E S U L T S

The results are presented here in the order in which routine examinations established beforehand were carried out. The bacterial counts presented were obtained from the daily record sheet, an example of which is shown on page 155.

A) Clinical signs

The general appearance of the sampled and sacrificed pig groups used in this study were of animals in good condition, healthy and active. None of them were suffering from external conditions such as wounds, haemorrhages or ectoparasites, or any apparent locomotor disorders at the beginning of the Experiment. They remained clinically normal during the period prior to treatment with the exception of pigs 66 and 67 which were diarrhoeic from day zero. After the withdrawal of avoparcin, pigs which were clinically diarrhoeic showed loss of condition and hairy coats as well as dehydration, general weakness, raised temperature, low DLWG and their appearance was dirty. The individual daily record of clinical signs identified from the sampled group are presented below in Tables 4.2-4.5 and Fig. II.

DAILY BACTERIOLOGY RECORD SHEET

BACTERIAL COUNTS Date:15.05.88

Medium (Bacteria)	Pig No.	Highest Dilution	Colonies found	C.F.U. per/g	Isolation Comments
MacConkey	66	N G	N G	N G	None
(Coliforms)	67	10^2	3	1.5×10^4	NH/*E. coli*
24 hours	68	10^4	6.5	3.25×10^6	None
Aerobic	69	10^2	3	1.5×10^4	BH/*E. coli*
	70	10^2	1	5.0×10^3	None
S. and B.	66	10^2	3.5	1.75×10^4	
(Streptococci)	67	10^2	3	1.5×10^4	
48 hours	68	10^2	1	5.0×10^3	
Aerobic	69	10^1	2	1.0×10^3	
	70	10^1	2	1.0×10^3	
Campylobacter	66	10^3	3	1.5×10^5	
(Campylo. spp.)	67	10^2	4	2.0×10^4	
24-48 hours	68	10^4	1	5.0×10^5	
Microaerobic	69	10^4	1	5.0×10^5	
	70	10^2	3.5	1.75×10^4	
M.R.S.	66	10^2	2	1.0×10^4	
(Lactobacilli)	67	10^1	4	2.0×10^3	
48-72 hours	68	10^3	1	5.0×10^4	
Microaerobic	69	10^1	1	5.0×10^2	
	70	10^2	1	5.0×10^3	
Neomycin Agar	66	N G	N G	N G	
(*Bacteroides*)	67	10^6	45	2.5×10^8	
48 hours	68	10^5	2	$n \times 10^7$	
Anaerobic	69	10^6	3	$n \times 10^8$	
	70	10^6	1	$n \times 10^7$	
C. difficile	66	10^4	1.5	7.5×10^5	
(vegetative)	67	10^4	4	2.0×10^6	
48 hours	68	10^6	2.5	1.25×10^8	
Anaerobic	69	10^5	1	5.0×10^6	
	70	10^5	2	1.0×10^7	
C. difficile	66	N G	N G	N G	
(spores)	67	10^2	1	5.0×10^3	
48 hours	68	10^1	1	5.0×10^2	
Anaerobic	69	10^2	1	5.0×10^3	
	70	N G	N G	N G	
C. perfringens	66	N G	N G	N G	
(vegetative)	67	10^2	6	3.0×10^4	
48-72 hours	68	10^6	2	1.0×10^8	
Anaerobic	69	10^4	8	4.0×10^6	
	70	10^4	7.5	3.75×10^6	

N G = No Growth, NH = Non-Haemolytic, BH = Beta Haemolytic

Rectal temperature: The temperature recorded from individual pigs varied and did not always relate to the other clinical signs such as diarrhoea.When the rectal temperature was elevated the presence of any disease was recorded.

The detailed figures are given in Table 4.2. No change in rectal temperature occurred upon withdrawal of medication.

Faecal consistency: Diarrhoea was common in the pigs of the sampled group (Table 4.3). Only two pigs were showing diarrhoeic faeces at the start, but during treatment there were 14 diarrhoea days out of 35 pig/days and 21/35 after the withdrawal of the antimicrobial from the diet.

Changes in faecal consistency from soft and diarrhoeic to normal were recorded from pigs 67 and 69, during the period of treatment. After its withdrawal, Pig 67 became diarrhoeic and Pig 69 remained normal. The addition of avoparcin to their diet and its withdrawal affected the faecal consistency of the sampled group as may be observed in Figure II below.

The diarrhoeic faeces were in most cases fluid in consistency and fairly dark in colour. Pig 66 which maintained such fluid-watery stools throughout the period of study passed an excess of mucus on day one. After withdrawal, the faeces of Pig 68 contained streaks of blood on days 8 and 9, and the colour was pale. On day 10 the faeces of Pig 67 also contained blood in its pasty-soft faeces. There was a general change noticed in colour, from dark-brown to pale-yellowish in the faeces of the sampled group after the withdrawal of avoparcin.

Daily live weight gain: The weights obtained are given in Table 4.4. Daily live weight gains for the periods 0 to 6 and 7 to 14 days are given in Table 4.5 with mean daily liveweight gain for each period. The mean daily liveweight gains were almost identical for the two periods 510 g/day on avoparcin and 511 g/day after withdrawal, but the individual weights varied.

TABLE 4.2 Daily individual rectal temperatures recorded from the the sampled pigs in Experiment 1

Pig	Day of Experiment										
Number	1	2	3	4	5	6	7*	8	9	10	11
66	40.0	39.4	39.7	39.3	39.6	39.3	39.5	39.8	39.4	39.8	39.5
67	39.7	39.6	39.2	39.4	39.1	39.1	39.2	39.0	39.3	39.4	39.3
68	39.0	39.7	39.8	39.7	39.4	39.8	39.7	39.8	39.6	39.8	39.4
69	42.0	39.8	39.6	40.0	39.7	39.6	39.6	39.5	39.9	39.3	39.9
70	39.7	39.7	39.7	39.8	39.9	39.4	39.5	39.6	39.7	39.4	39.5
X	40.1	39.6	39.6	39.6	39.5	39.4	39.5	39.5	39.6	39.5	39.5

* = Withdrawal of treatment

NOTE: Temperatures are expressed in degrees centigrade.

TABLE 4.2. (Cont.) Daily individual rectal temperatures recorded from the sampled pigs in Experiment 1

Pig	Day of Experiment			Group
Number	12	13	14	X
66	39.3	39.2	39.2	39.5
67	39.4	39.4	39.4	39.3
68	39.5	39.4	39.5	39.6
69	39.4	39.7	39.5	39.7
70	39.3	39.6	39.6	39.6
X	39.4	39.5	39.4	39.5

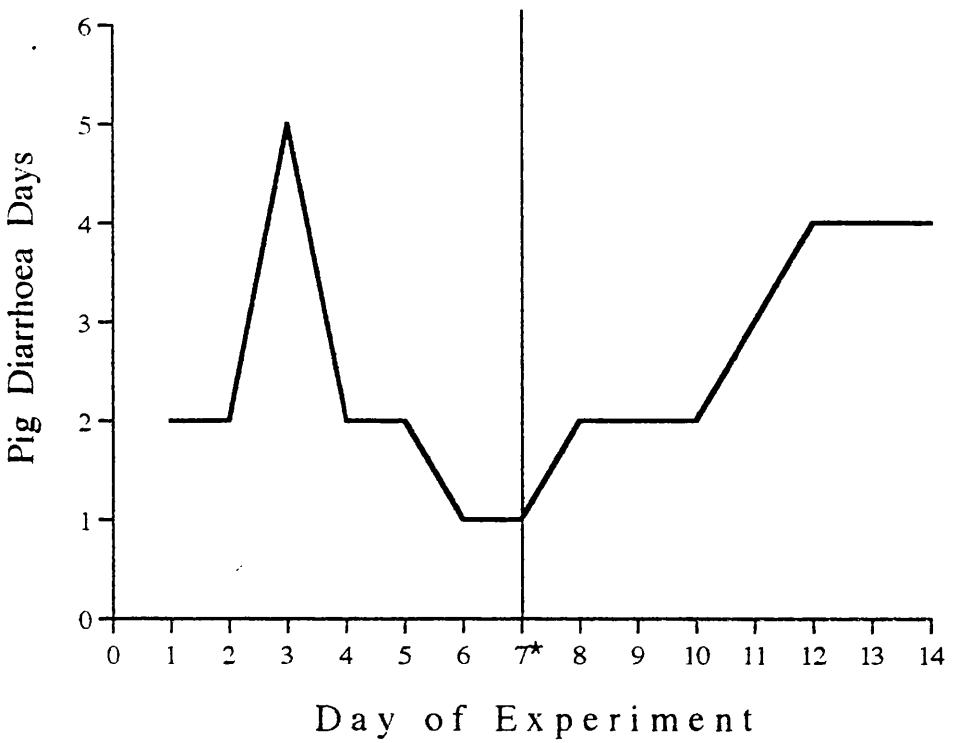
TABLE 4.3 Daily individual faecal consistency changes recorded from the sampled pigs in Experiment 1

Pig	Day of Experiment														Total
Number	1	2	3	4	5	6	7*	8	9	10	11	12	13	14	D/Days
66	D	D	D	D	D	D	D	D	D	D	D	D	D	D	14/14
67	D	D	D	N	S	N	S	S	S	S	S	D	D	D	6/14
68	S	S	D	S	S	N	S	D	S	D	D	D	D	D	7/14
69	S	S	D	N	N	N	N	N	N	N	N	N	N	N	1/14
70	S	S	D	D	D	N	S	S	D	S	D	D	D	D	8/14

N = Normal, S = Soft, D = Diarrhoea

* = Withdrawal of treatment

FIGURE II. Changes in daily faecal consistency from the sampled pigs in Experiment 1



* Withdrawal of treatment

TABLE 4.4 Daily individual live weights of the sampled pigs in Experiment 1, expressed in kilograms

Pig	Day of Experiment										
Number	1	2	3	4	5	6	7*	8	9	10	11
66	15.3	15.0	15.2	15.4	16.2	17.3	17.8	18.4	19.2	19.4	19.2
67	11.5	11.8	12.4	12.8	13.2	14.1	14.6	15.2	15.8	16.4	16.8
68	13.7	13.6	14.3	15.2	16.2	16.4	17.0	17.4	18.2	18.3	18.8
69	11.8	11.2	11.9	12.1	13.1	13.4	14.1	14.8	14.7	15.3	15.8
70	10.5	11.0	11.5	12.4	13.8	13.8	14.6	15.7	16.1	16.7	17.1
Group	62.8	62.6	65.3	67.9	72.5	75.0	78.1	81.5	84.0	86.1	87.7

* Withdrawal of treatment

TABLE 4.4 (Cont.) Daily individual live weights of the sampled pigs in Experiment 1, expressed in kilograms

Pig	Day of Experiment			Total
Number	12	13	14	Gain
66	19.8	19.9	20.6	5.3
67	17.2	17.5	18.5	7.0
68	19.3	19.2	19.8	6.1
69	16.3	17.1	17.7	5.9
70	17.8	18.5	19.4	8.9
Group	90.4	92.2	96.0	33.2

TABLE 4.5 Individual daily live weight gains (Kg) of the sampled pigs for days 0-6 and 7-14, in Experiment 1

Pig	Day of Experiment	
Number	0-6	7-14
66	0.416	0.400
67	0.516	0.557
68	0.550	0.400
69	0.383	0.514
70	0.683	0.685
X	0.510	0.511

B) Bacteriological examination of faeces

The results in respect of quantitative bacteriological examinations performed on solid media from faeces are presented in Tables 4.6 to 4.12 and Figures III to VII.

a) Aerobic organisms

i) Total coliforms: Table 4.6 below gives the counts obtained on MacConkey agar plates before, over the period of treatment and after the withdrawal of avoparcin from faecal samples. It shows that coliforms as a group represented mainly by E. coli were present in numbers varying between 10^2 to 10^7 bacteria/g of faeces. The average numbers were slightly higher (10^5/g) at the end of the treatment but returned to normal (10^4/g) by day 9. Pig 68 showed an increase from 10^4 to 10^7/g after withdrawal, and also diarrhoea and raised temperature but the mean levels of E. coli remained uniform throughout the course of the study(Fig. III). On day 10 there was contamination by Proteus spp.

ii) Faecal streptococci: Slanetz and Bartley selective medium gave reliable selective conditions for the isolation of faecal streptococci, and the results are given in Table 4.7. The identity of 3 isolates from the first counts was confirmed using the API 20 Strep System as S. faecalis (2) and S. faecium (1). The numbers ranged between 10^2 to 10^5/g but on some days no growth was found on the plates (Fig. IV). Pigs 66 and 68 showed an increase in their counts after 24 hours and pigs 66 and 67 after 72 hours. The mean counts were higher (10^5/g) at the beginning of the study but during the use of avoparcin they were maintained at an average of 10^3 organisms/g. As in coliforms, there was a rise after withdrawal which returned to normal levels remaining at 10^3/g for the rest of the period of observation.

iii) Salmonella species were not isolated from any of the animals of either group. Similar techniques in routine use in the same laboratory frequently yield the organism when present.

b) Microaerobic organisms

i) Campylobacter spp. were isolated in numbers which varied

TABLE 4.6 Daily individual bacterial counts of total coliforms/g faeces from the sampled pigs in Experiment 1

Study							
Date	Days	Pig No. 66	Pig No. 67	Pig No. 68	Pig No. 69	Pig No. 70	$\bar{X}$
13.10.87	1	2.25×10^4	2.25×10^4	3.25×10^5	1.5×10^4	1.5×10^4	8.0×10^4
14.10.87	2	5.0×10^4	5.0×10^2	5.0×10^3	N G	5.0×10^2	1.4×10^4
15.10.87	3	N G	1.5×10^4	3.25×10^6	1.5×10^4	5.0×10^3	8.2×10^5
16.10.87	4	5.0×10^3	5.0×10^2	5.0×10^5	N G	7.5×10^2	1.4×10^4
17.10.87	5	2.0×10^3	N G	5.0×10^3	N G	2.0×10^3	3.0×10^3
18.10.87	6	3.0×10^4	5.0×10^4	2.5×10^4	1.0×10^4	1.75×10^4	2.6×10^4
19.10.87	7*	1.5×10^5	2.0×10^4	1.0×10^4	5.0×10^5	3.5×10^4	1.4×10^5
20.10.87	8	2.0×10^5	1.5×10^4	1.0×10^7	5.0×10^3	3.0×10^4	2.5×10^5
21.10.87	9	3.0×10^4	3.5×10^5	5.5×10^4	1.5×10^4	2.5×10^5	9.5×10^4
22.10.87	10	N G	N G	N G	N G	N G	Contamination
23.10.87	11	6.5×10^3	9.0×10^4	5.0×10^5	11.0×10^3	4.5×10^3	1.2×10^5
24.10.87	12	1.5×10^4	5.0×10^3	5.0×10^3	N G	N G	8.3×10^3
25.10.87	13	5.0×10^2	3.5×10^4	8.0×10^3	1.5×10^3	5.0×10^3	1.0×10^4
26.10.87	14	2.5×10^5	3.0×10^4	5.0×10^3	2.0×10^4	5.0×10^4	7.1×10^4

* Withdrawal of treatment, N G = No Growth

TABLE 4.7 Daily individual bacterial counts of faecal streptococci/g faeces from the sampled pigs in Experiment 1

Study

Date	Days	Pig No. 66	Pig No. 67	Pig No. 68	Pig No. 69	Pig No. 70	$\bar{X}$
13.10.87	1	1.0×10^3	5.0×10^4	6.25×10^5	N G	2.0×10^4	1.7×10^5
14.10.87	2	5.0×10^2	N G	3.25×10^3	N G	N G	1.8×10^3
15.10.87	3	1.75×10^4	1.5×10^4	5.0×10^3	1.0×10^3	1.0×10^3	7.9×10^3
16.10.87	4	5.0×10^3	1.0×10^3	5.0×10^2	N G	N G	2.1×10^3
17.10.87	5	N G	5.0×10^2	1.0×10^3	N G	1.5×10^3	1.0×10^3
18.10.87	6	N G	N G	7.5×10^2	N G	3.0×10^3	1.8×10^3
19.10.87	7*	1.0×10^3	N G	7.5×10^3	N G	2.0×10^3	3.5×10^3
20.10.87	8	1.0×10^4	N G	1.0×10^4	N G	1.5×10^3	7.1×10^3
21.10.87	9	1.0×10^3	1.5×10^4	3.0×10^3	5.0×10^2	N G	4.8×10^3
22.10.87	10	2.0×10^4	1.5×10^5	6.75×10^3	5.0×10^2	N G	4.4×10^4
23.10.87	11	N G	2.0×10^3	N G	5.0×10^2	1.25×10^3	1.2×10^3
24.10.87	12	N G	N G	N G	N G	1.0×10^4	1.0×10^4
25.10.87	13	N G	7.5×10^2	5.0×10^2	7.5×10^2	5.0×10^2	6.2×10^2
26.10.87	14	N G	5.0×10^3	N G	5.0×10^2	5.0×10^2	2.0×10^3

* Withdrawal of treatment, N G = No Growth

FIGURE III. Daily individual bacterial counts of total coliforms in Experiment one

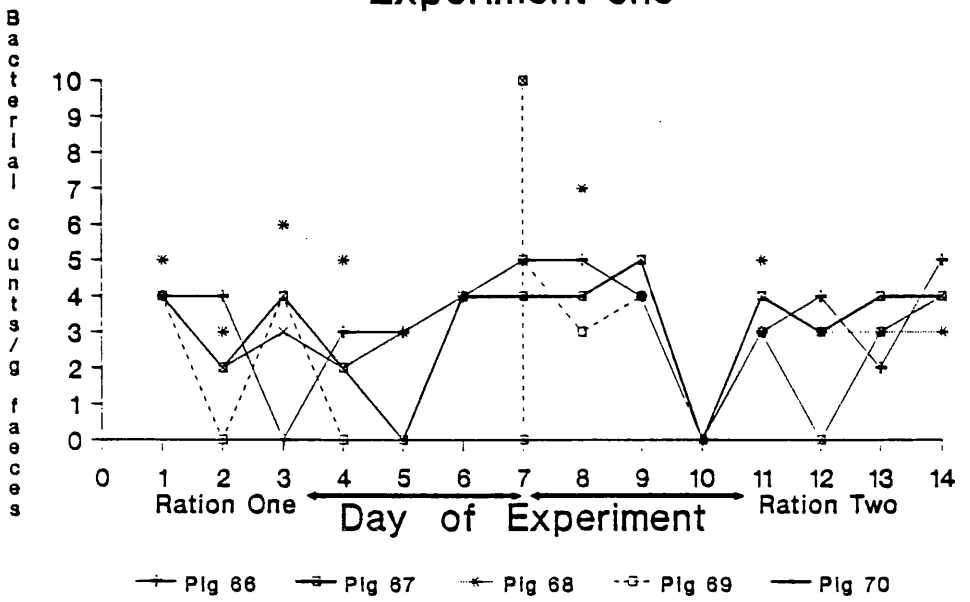
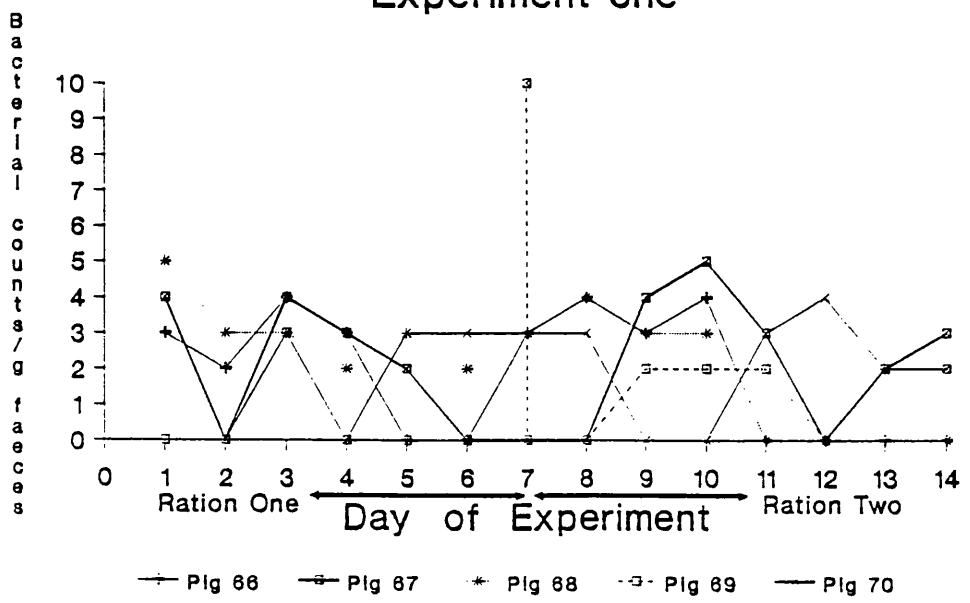


FIGURE IV. Daily individual bacterial counts of faecal streptococci in Experiment one



from 10^2 to 10^7/g as reported in Table 4.8. In this study, only one pig (Pig 68) showed an increase in Campylobacter numbers after withdrawal of the drug, the remaining animals maintained an average of 10^4 C.F.U./g (Fig. V).

ii) Lactobacilli were not isolated on every occasion, but, when present occurred in numbers which mean varied from 10^2 to 10^3 per gram of faeces. No significant changes were observed in their counts individually or per group during the period of study as may be observed in Table 4.9.

c) Anaerobic organisms

i) Enteric spirochaetes: No Serpulina hyodysenteriae or other enteric spirochaetes were isolated. Beta haemolytic areas developed on spectinomycin agar plates to levels of 10^2 to 10^3/g. Presumptive identification as spirochaete colonies on their macroscopic appearance was not confirmed in Gram's stained smears carried out from the cultures. No organisms were identified and the presence of spirochaetes could not be confirmed.

ii) Bacteroides spp. were not isolated from every sample. They were found in numbers varying from 10^2 to 10^8/g faeces on neomycin blood agar plates (Table 4.10 and Figure VI). The identity of sample isolates was confirmed as B. fragilis (4) and B. vulgatus (2) and 7 remained unidentified. B. vulgatus could be identified presumptively as a Beta haemolytic colony but totals were not recorded. Black colonies presumptively identified as B. melaninogenicus were also seen present throughout. Changes in numbers of Bacteroides spp. were in more than one case closely related to the development of disease and variation in clinical signs from pigs with diarrhoea.

iii) Clostridium difficile (vegetative cells.- Colonies developed on Clostridium difficile selective medium in numbers of 10^2 to 10^{10}/g which was considered at the time to indicate the presence of vegetative cells of C. difficile, because of the selectivity of the cultural medium used. However, the organisms isolated proved not to be C.difficile after further confirmatory testing using API 20 A (anaerobe) strips and were an unidentifiable Clostridium spp.

TABLE 4.8 Daily individual bacterial counts of Campylobacter spp./g faeces from the sampled pigs in Experiment 1

Study						
Date	Days	Pig No. 66	Pig No. 67	Pig No. 68	Pig No. 69	Pig No. 70	$\bar{X}$
13.10.87	1	3.5×10^6	7.0×10^6	3.25×10^7	5.0×10^6	5.0×10^6	1.0×10^7
14.10.87	2	1.75×10^3	12.5×10^4	4.0×10^4	2.0×10^3	1.0×10^3	3.3×10^4
15.10.87	3	1.5×10^5	2.0×10^4	5.0×10^5	5.0×10^5	1.75×10^4	2.3×10^5
16.10.87	4	5.0×10^5	2.5×10^4	5.0×10^5	3.75×10^3	7.5×10^3	2.0×10^5
17.10.87	5	5.0×10^4	N G	1.0×10^3	5.0×10^3	N G	1.8×10^4
18.10.87	6	N G	1.0×10^3	N G	N G	4.75×10^3	2.8×10^3
19.10.87	7*	N G	1.0×10^3	5.0×10^3	1.5×10^3	5.0×10^2	2.0×10^3
20.10.87	8	N G	N G	1.0×10^4	5.0×10^2	N G	5.2×10^3
21.10.87	9	1.0×10^3	3.0×10^3	7.5×10^3	1.5×10^3	1.0×10^3	2.8×10^3
22.10.87	10	5.0×10^4	1.5×10^4	5.0×10^4	5.0×10^3	3.5×10^3	2.4×10^4
23.10.87	11	N G	11.0×10^4	5.0×10^4	1.0×10^4	N G	5.6×10^4
24.10.87	12	N G	N G	N G	1.0×10^3	N G	1.0×10^3
25.10.87	13	5.0×10^4	N G	3.0×10^3	N G	N G	2.6×10^4
26.10.87	14	5.5×10^3	1.0×10^3	2.0×10^4	1.5×10^3	N G	7.0×10^3

* Withdrawal of treatment, N G = No Growth

TABLE 4.9 Daily individual bacterial counts of lactobacilli/g faeces from the sampled pigs in Experiment 1

Study							
Date	Days	Pig No. 66	Pig No. 67	Pig No. 68	Pig No. 69	Pig No. 70	$\bar{X}$
13.10.87	1	5.0×10^3	1.5×10^3	1.0×10^4	5.0×10^2	1.0×10^3	3.6×10^3
14.10.87	2	2.0×10^3	1.0×10^4	5.0×10^3	N G	5.0×10^2	4.3×10^3
15.10.87	3	1.0×10^4	2.0×10^3	5.0×10^4	5.0×10^2	5.0×10^3	1.3×10^4
16.10.87	4	5.0×10^3	1.0×10^3	1.0×10^3	N G	5.0×10^2	1.8×10^3
17.10.87	5	1.0×10^3	N G	7.5×10^2	5.0×10^2	2.5×10^3	1.2×10^3
18.10.87	6	5.0×10^2	N G	1.5×10^3	N G	3.25×10^3	1.7×10^3
19.10.87	7*	N G	N G	1.0×10^3	N G	5.0×10^2	7.5×10^2
20.10.87	8	N G	N G	5.0×10^2	N G	N G	5.0×10^2
21.10.87	9	N G	N G	1.5×10^3	N G	1.0×10^3	1.2×10^3
22.10.87	10	N G	N G	3.0×10^3	1.0×10^3	5.0×10^3	3.0×10^3
23.10.87	11	N G	5.0×10^2	N G	N G	5.0×10^2	5.0×10^2
24.10.87	12	N G	N G	N G	N G	N G	N G
25.10.87	13	N G	N G	N G	1.0×10^3	N G	1.0×10^3
26.10.87	14	1.0×10^3	5.0×10^2	5.0×10^2	5.0×10^2	5.0×10^2	6.0×10^2

* Withdrawal of treatment, N G = No Growth

FIGURE V. Daily individual bacterial counts of Campylobacter spp. in Experiment one

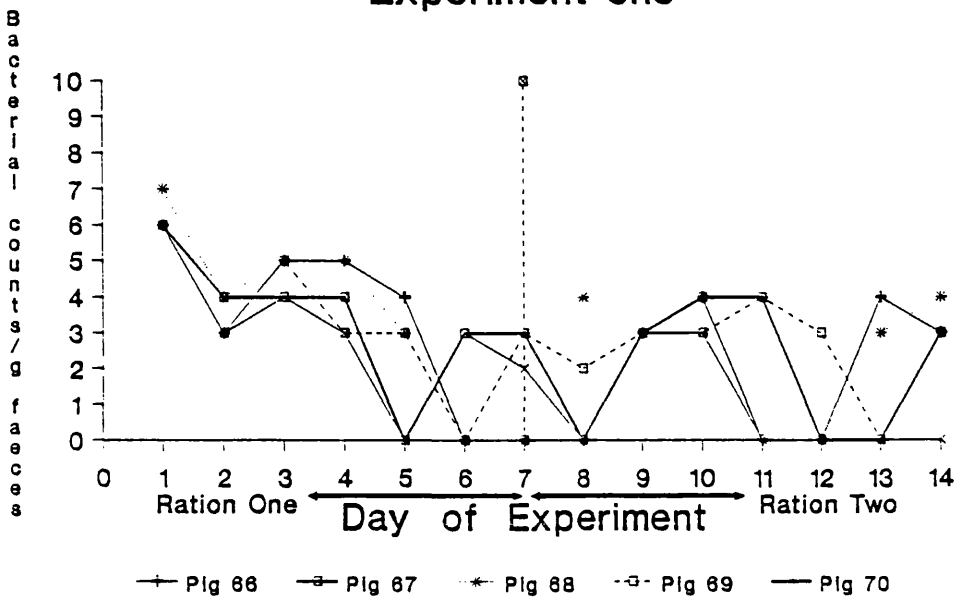


FIGURE VI. Daily individual bacterial counts of Bacteroides spp. in Experiment one

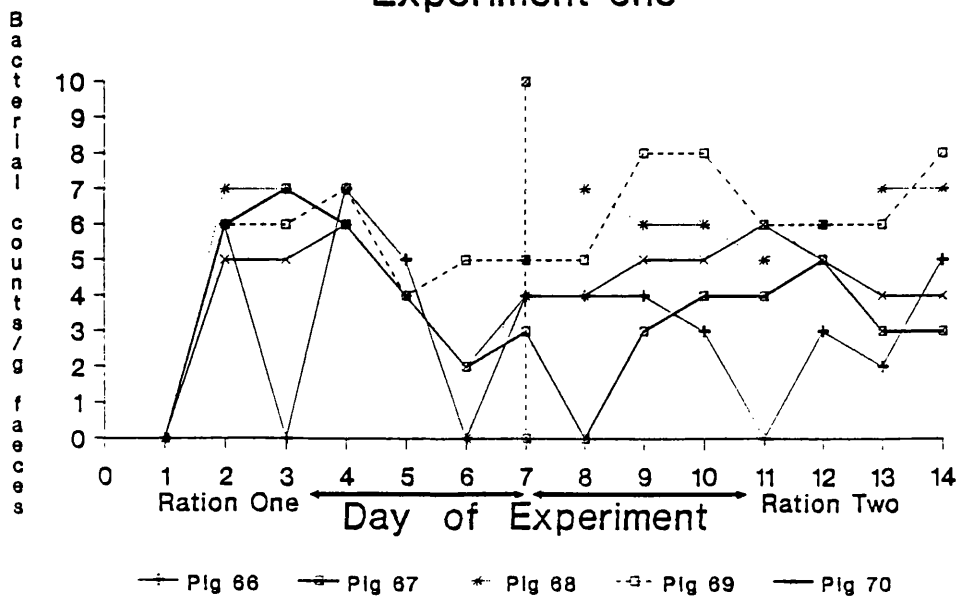


TABLE 4.10 Daily individual bacterial counts of Bacteroides spp./g faeces from the sampled pigs in Experiment 1

Study							
Date	Days	Pig No. 66	Pig No. 67	Pig No. 68	Pig No. 69	Pig No. 70	$\bar{X}$
13.10.87	1	N G	N G	N G	N G	N G	N G
14.10.87	2	1.0×10^6	4.25×10^6	2.0×10^7	7.5×10^6	3.0×10^5	6.6×10^6
15.10.87	3	N G	22.5×10^7	1.0×10^7	1.5×10^6	5.0×10^5	8.6×10^6
16.10.87	4	5.0×10^7	1.0×10^6	5.0×10^6	3.5×10^7	1.5×10^6	1.8×10^7
17.10.87	5	2.0×10^5	7.5×10^4	5.0×10^4	3.75×10^4	2.5×10^4	7.7×10^4
18.10.87	6	N G	5.0×10^2	N G	5.0×10^5	5.0×10^2	1.7×10^5
19.10.87	**7***	$\mathbf{1.0 \times 10^4}$	$\mathbf{1.0 \times 10^3}$	$\mathbf{2.0 \times 10^5}$	$\mathbf{1.5 \times 10^5}$	$\mathbf{2.5 \times 10^4}$	$\mathbf{7.7 \times 10^4}$
20.10.87	8	5.0×10^4	N G	1.0×10^7	5.0×10^5	1.0×10^4	2.6×10^6
21.10.87	9	2.0×10^4	1.0×10^3	2.5×10^6	1.0×10^8	2.5×10^5	2.0×10^7
22.10.87	10	1.5×10^3	5.0×10^4	3.5×10^6	5.0×10^8	4.0×10^5	1.0×10^8
23.10.87	11	N G	1.0×10^4	1.0×10^5	1.5×10^6	5.0×10^6	1.6×10^6
24.10.87	12	5.0×10^3	1.0×10^5	5.0×10^6	2.0×10^6	1.5×10^5	1.4×10^6
25.10.87	13	5.0×10^2	2.5×10^3	5.0×10^7	3.0×10^6	9.5×10^4	1.1×10^7
26.10.87	14	2.0×10^5	5.0×10^3	1.0×10^7	1.5×10^8	5.0×10^4	3.2×10^7

* Withdrawal of treatment, N G = No Growth

Spore-forming strains.- Colonies only developed after heating on one day, after heat-shock treatment of the ten fold diluted faecal sample but, like the vegetative cells were not confirmed as C. difficile. There was therefore no confirmed evidence for the presence of C. difficile.

iv) Clostridium perfringens type A: C.perfringens vegetative cells were not isolated in every occasion. High numbers were isolated on day 1 (mean 10^{11}/g). Numbers fell during the period of observation. The vegetative cell counts were from 10^2 to 10^{11}/g but an increase in numbers was noted 48-72 hours (days 9 and 10) after withdrawal of treatment (Table 4.11 and Fig. VII). The increase was from 10^3 to 10^6-10^9 and from 10^2 to 10^7-10^{10} organisms/g faeces after which a drop in numbers occurred. Spore-forming strains of C. perfringens type A were not found on every occasion but were mainly found during the use of avoparcin and after it was withdrawn they almost disappeared (Table 4.12). Isolates were confirmed as C. perfringens type A by cultural characters including Nagler's reaction.

C) Pathological findings in the sacrificed group

a) Gross post mortem examinations

The time of the day for the slaughter of the animals depended on factors such as transport facilities (vehicle) from the farm to the Veterinary School post mortem room and on the availability of space and personnel in the place, after the pigs arrived from the farm, to carry out the procedures of killing, gross examination and sampling of the animals. However, they were all euthanased during the morning of the day previously planned.

No gross changes were seen in pigs 93 and 94 killed on days 7 and 9 of the study. In most cases the villi in the sacrificed pigs were only slightly reduced in height and the large intestinal mucosa was normal in appearance regardless of the consistency of its contents. Often the stomach was either empty or half-filled with feed, the duodenum was always partially empty and contained a bubbly-mucoid yellowish material, the jejunum and ileum were generally half filled and the caecum and colon were always filled with contents which varied in

TABLE 4.11 Daily individual bacterial counts of Clostridium perfringens type A (vegetative cells)/g faeces from the sampled pigs in Experiment 1

Study

Date	Days	Pig No. 66	Pig No. 67	Pig No. 68	Pig No. 69	Pig No. 70	$\bar{X}$
13.10.87	1	5.25×10^5	2.0×10^7	5.0×10^{11}	5.0×10^7	1.0×10^7	1.0×10^{11}
14.10.87	2	5.0×10^4	5.0×10^5	1.25×10^6	8.25×10^9	1.25×10^5	1.6×10^9
15.10.87	3	N G	3.0×10^4	1.0×10^8	4.0×10^6	3.75×10^6	2.7×10^7
16.10.87	4	5.0×10^5	1.75×10^6	7.5×10^7	1.5×10^7	1.0×10^7	2.0×10^7
17.10.87	5	2.0×10^4	1.0×10^3	5.0×10^3	5.0×10^2	1.0×10^4	7.3×10^3
18.10.87	6	2.5×10^4	5.0×10^4	4.0×10^4	1.5×10^3	2.5×10^4	2.8×10^4
19.10.87	7*	1.0×10^4	1.5×10^3	1.0×10^4	5.0×10^3	1.0×10^3	5.5×10^3
20.10.87	8	N G	N G	2.0×10^4	5.0×10^3	5.0×10^2	8.5×10^3
21.10.87	9	5.0×10^3	1.0×10^3	1.5×10^6	2.5×10^6	1.5×10^7	3.8×10^6
22.10.87	10	5.0×10^7	5.0×10^7	5.0×10^6	3.0×10^9	1.0×10^{10}	2.6×10^9
23.10.87	11	N G	N G	N G	2.0×10^3	1.5×10^3	1.7×10^3
24.10.87	12	N G	N G	2.5×10^3	N G	1.0×10^4	6.2×10^3
25.10.87	13	5.0×10^5	2.5×10^5	1.0×10^7	5.0×10^6	2.5×10^6	3.6×10^6
26.10.87	14	1.0×10^6	1.0×10^4	1.0×10^4	5.5×10^4	5.0×10^4	2.2×10^5

* Withdrawal of treatment, N G = No Growth

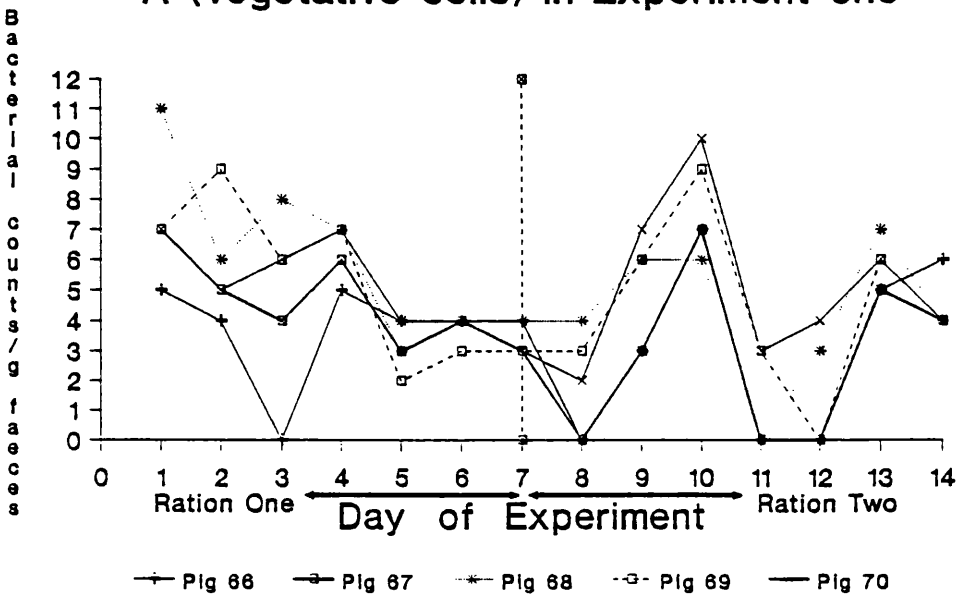
TABLE 4.12 Daily individual bacterial counts of *Clostridium perfringens* type A (spores)/g faeces from the sampled pigs in Experiment 1

Study

Date	Days	Pig No. 66	Pig No. 67	Pig No. 68	Pig No. 69	Pig No. 70	$\bar{X}$
13.10.87	1	N G	N G	N G	N G	5.0×10^2	5.0×10^2
14.10.87	2	N G	5.0×10^2	5.0×10^2	N G	1.0×10^4	3.6×10^3
15.10.87	3	N G	N G	N G	N G	N G	N G
16.10.87	4	N G	N G	N G	N G	N G	N G
17.10.87	5	N G	N G	N G	N G	5.0×10^2	5.0×10^2
18.10.87	6	1.0×10^4	N G	1.0×10^3	N G	5.0×10^2	3.8×10^3
19.10.87	7*	1.0×10^3	N G	N G	N G	N G	1.0×10^3
20.10.87	8	N G	N G	N G	N G	N G	N G
21.10.87	9	N G	N G	N G	N G	1.0×10^3	1.0×10^3
22.10.87	10	N G	N G	N G	N G	N G	N G
23.10.87	11	N G	N G	N G	N G	N G	N G
24.10.87	12	N G	N G	N G	N G	N G	N G
25.10.87	13	N G	N G	N G	N G	N G	N G
26.10.87	14	5.0×10^2	N G	N G	N G	5.0×10^2	5.0×10^2

* Withdrawal of treatment, N G = No Growth

FIGURE VII. Daily individual bacterial counts of Clostridium perfringens type A (vegetative cells) in Experiment one



consistency.

Pig 59 was killed on day 0 when in-feed treatment started. This animal had diarrhoea at the time of death but it was in good bodily condition showing no apparent external abnormalities or lesions. When the carcase was opened, the serosal surfaces and organs contained in both thoracic and abdominal cavities appeared grossly normal. Its intestinal tract appeared grossly normal except for the presence of local hyperaemia on the mucosal surface of the caecum. The contents of the caecum and colon varied from fluid to pasty and contained clear mucus which could be seen on the mucosal surface locally hyperaemic. In contrast Pig 91 killed on day 8 showed areas of collapse of the ventral portion of the left lung. The lesions were characteristic of a Bordetella bronchiseptica pneumonia. The gastrointestinal tract, mucosa and contents were all normal in appearance.

There were no gross pathological changes in the viscera of both thoracic and abdominal cavities of Pig 40 killed at the end of the study (day 14). At the detailed examination of the gastroenteric tract the stomach was normal except for the presence of excess mucus on the mucosal surface. The jejunum and ileum were normal and the caecum and colon had pasty contents adhered locally to the mucosal surface. After washing, the mucosa appeared grossly normal.

b) Histological findings

Mild histological changes were noted in the stomach, small and large intestines of all the pigs killed, in sections stained by H & E and examined by light microscopy. These changes included the presence of lymphoid accumulations and crypt abscesses, local congestion, occasional haemorrhages, local oedema and capillary dilatation. Eosinophils were seen in the lamina propria and lymphocytes were present in the crypts. Bacteria, yeasts and neutrophils were present, adjacent to or attached to the lumenal epithelium. There was loss of the epithelial cells and the presence of shed epithelial cells, fibrin and debris on the lumenal surface of the epithelium in some areas. Some of the lumenal debris and bacteria (cocci and rods) could be seen in dilated crypts. Villous anastomosis and fusion were seen occasionally in the small intestine.

Cryptosporidium spp. and other coccidia-like organisms were seen in the small intestinal mucosa but the identity of the latter could not be confirmed. The detailed findings seen in each section of the digestive tract of the sacrificed pigs are presented below.

Those changes mentioned above were restricted in Pig 59 to the presence of congestion in the lamina propria, neutrophils, yeast cells, cocci and a bacterial microcolony in the lumen of the gastric mucosa. In the jejunum the changes seen were villous anastomosis and cell desquamation. The villi were slightly stunted and oedema and eosinophils were present in the lamina propria. Neutrophils were seen on the mucosa of the ileum and lymphocytes were also present as an exudate. There was capillary dilatation in the lamina propria. In the caecum the lumenal epithelium was low but largely normal, but there were large numbers of cocci and rod-shaped bacteria in the crypts. The colon was largely normal except for the presence of bacteria (mainly cocci) attached to the epithelium. There was some mucosal damage which was considered to have occurred after death or during the processing of the sections.

In Pig 93 killed on day 7 of the study, the findings in the stomach comprised congestion and mild oedema in the lamina propria. In the jejunum Cryptosporidium-like organisms were seen on the mucosal epithelium. The ileum was normal. The caecal crypts were dilated and contained large numbers of bacterial organisms. There was cell desquamation and few haemorrhages in the lamina propria. The only changes in the colonic mucosa was some congestion of the submucosa and the muscular layers.

Pig 91 was killed on day 8 and histological sections from the stomach were normal in appearance. Mild capillary dilatation and fusion of the villi was present in the jejunum. Some cellular debris and bacteria were seen in the crypts and shedding of lymphocytes and congestion of lamina propria in the ileum. Bacteria were present in dilatated crypts of the caecum and colon.

There was submucosal congestion in the stomach of Pig 94 and the capillaries in lamina propria were dilated. A patch of yeast-like cells were seen on the epithelium. There were bacterial organisms inside the villi of the jejunum and in the

lumen. Slight congestion of the lamina propria was seen in the ileum as well as some cellular debris in the crypts. Structures resembling parasitic oocysts were present on the epithelium of the intercrypt spaces of the caecum and some **post mortem** changes were also present. Only slight capillary dilatation in the lamina propria was seen in the colon.

At the end of the study another pig (Pig 40) was killed and its histological features were; in the stomach epithelial cell shedding into the mucus on the mucosal surface. In the jejunum excess mononuclear cells and polymorphs were present in the lamina propria as well as increased number of intraepithelial lymphocytes, but the villi were normal. The ileum was normal except for the presence of a crypt abscess. Bacteria could be seen in clumps adjacent to the intercrypt areas of the caecal mucosa together with some cellular debris on the mucosa. In the dilated crypts there were a large number of bacteria-like organisms. The mucosa was folded and there were local areas of plasma cells and lymphocytic infiltration. The colon showed infiltration of the lamina propria with polymorphonuclear leucocytes and eosinophils. Bacteria were also present in the lumen.

D) Bacteriological examination of gastrointestinal contents

The results of quantitative bacteriological examinations carried out from gastrointestinal contents of stomach, jejunum, ileum, caecum and colon obtained from the pigs of the sacrificed group are given below in Tables 4.13 to 4.19.

a) Aerobic organisms

i) Total coliforms: The results on coliform counts obtained from the sections of the digestive tract are presented on Table 4.13.

Stomach.- Prior to treatment with avoparcin coliforms were isolated from gastric contents in numbers of 10^5/g. During treatment their numbers dropped to 10^2/g but after withdrawal an increase in populations was found on days 8 (10^3/g), 9 (10^6/g) and at the end of the study (10^7/g).

Jejunum.- No counts were made from the jejunum at the

beginning but, during treatment their numbers were 10^4/g and after avoparcin withdrawal they increased to 10^5/g on day 8 and 10^9/g on day 9, falling back to 10^5/g at the end of the study on day 14.

Ileum.- Coliforms were isolated in numbers of 10^5/g before treatment, during in-feed avoparcin they were 10^6/g and after treatment they declined to 10^5-10^4/g on days 8 and 9 respectively. Counts of 10^4/g were found on day 14.

Caecum.- The presence of coliforms from this section of the intestine showed no marked changes in their populations being 10^4/g before avoparcin, 10^5/g during treatment and after withdrawal their number were found to be 10^4/g which did not change until the end of the study on day 14.

Colon.- Important variations in coliform bacteria were found in this section. They were 10^5/g at the start, 10^4/g during treatment and raised markedly up to 10^{12}/g by day 9, returning to 10^6/g by day 14.

TABLE 4.13 Coliform counts from the gastrointestinal contents of sacrificed pigs of Experiment 1

Necropsy Date	Study Day	Pig No.	Stomach	Jejunum	Ileum	Caecum	Colon
12.10.87	0	59	$5.0x10^5$	N D	$1.5x10^5$	$5.0x10^4$	$2.75x10^5$
19.10.87	7*	93	$5.0x10^2$	$5.0x10^4$	$3.0x10^6$	$5.0x10^5$	$5.25x10^4$
20.10.87	8	91	$5.0x10^3$	$1.0x10^5$	$5.0x10^5$	$1.0x10^5$	$1.5 x10^4$
21.10.87	9	94	$1.5x10^6$	$1.0x10^9$	$1.5x10^4$	$2.0x10^4$	$1.0x10^{12}$
26.10.87	14	40	$1.5x10^7$	$3.0x10^5$	$2.5x10^4$	$2.0x10^4$	$1.0 x10^6$

N D = Not Done, * = Withdrawal of treatment

ii) Faecal streptococci: Streptococcal counts are shown in Table 4.14. They were only isolated in numbers of 10^4/g from the stomach at the start of the study but did not appear either during or after treatment with avoparcin. It was isolated from the jejunum only after withdrawal of the drug on day 8 at levels of 10^3/g. At the end of the study on day 14 they were isolated from the ileum at 10^3/g. Streptococci were more often isolated in the caecum being 10^3/g before treatment and following

treatment counts remained at 10^3/g on day 8 and 10^2/g on days 9 and 14. From the colon as from the stomach they were isolated only at the beginning (10^4/g) but not during treatment or after it was withdrawn.

iii) Salmonella spp.: As in faecal samples these bacteria were not isolated from the contents of either gastric or intestinal regions throughout the whole experimental period.

b) Microaerobic organisms

i) Campylobacter spp.: The results given in Table 4.15 show that these bacteria were isolated from the stomach at levels of 10^4/g only before the treatment was established. From the jejunum it was found in numbers varying from 10^2/g (day 7) to 10^4/g (day 8) but no further isolation was recorded at the end of the study. Numbers of 10^3 C.F.U./g were found before, during and after avoparcin treatment in the ileum. Campylobacters were present in the caecum on day 0 at 10^6/g, during treatment their numbers dropped to 10^3/g and counts of 10^4/g were finally recorded on day 14. No isolations were made immediately after withdrawal of the drug. These organisms were isolated from the colon at 10^5/g before avoparcin treatment which reduced their numbers to 10^3/g, remaining at this level throughout the rest of the study.

ii) Lactobacilli: These organisms were isolated from the contents of the gastrointestinal tract in numbers which varied from 10^2 to 10^5 C.F.U./g (Table 4.16). The highest counts were found in the stomach on day 0 and no organisms were identified during or after the treatment. They were isolated from the jejunum on day 7 at 10^3/g and on day 14 at 10^4/g. Lower counts (10^2/g) were found from the ileum on day 0, during and after treatment they were isolated at 10^3/g. No isolations were recorded from caecal contents except on day 0 (10^3/g), and from the colon they were only found at 10^3/g on day 0 and 10^2/g on day 7. Afterwards no apparent growth was found.

TABLE 4.14 Faecal streptococcal counts from the gastrointestinal contents of sacrificed pigs of Experiment 1

Necropsy Date	Study Day	Pig No.	Stomach	Jejunum	Ileum	Caecum	Colon
12.10.87	0	59	$1.25x10^4$	N D	N G	$1.5x10^3$	$1.5x10^4$
19.10.87	7*	93	N G	N G	N G	N G	N G
20.10.87	8	91	N G	$1.0x10^3$	N G	$1.0x10^3$	N G
21.10.87	9	94	N G	N G	N G	$5.0x10^2$	N G
26.10.87	14	40	N G	N G	$1.5x10^3$	$5.0x10^2$	N G

N D = Not Done, N G = No Growth, * = Withdrawal of treatment

TABLE 4.15 *Campylobacter spp.* counts from the gastrointestinal contents of sacrificed pigs of Experiment 1

Necropsy Date	Study Day	Pig No.	Stomach	Jejunum	Ileum	Caecum	Colon
12.10.87	0	59	$5.0x10^4$	N D	$5.0x10^3$	$5.0 x10^6$	$5.0x10^5$
19.10.87	7*	93	N G	$5.0x10^2$	$2.0x10^3$	$3.25x10^3$	$1.0x10^3$
20.10.87	8	91	N G	$1.5x10^4$	N G	N G	$4.0x10^3$
21.10.87	9	94	N G	N G	$3.5x10^3$	N G	N G
26.10.87	14	40	N G	N G	N G	$1.0 x10^4$	$2.5x10^3$

N D = Not Done, N G = No Growth, * = Withdrawal of treatment

TABLE 4.16 *Lactobacillus spp.* counts from the gastrointestinal contents of sacrificed pigs of Experiment 1

Necropsy Date	Study Day	Pig No.	Stomach	Jejunum	Ileum	Caecum	Colon
12.10.87	0	59	$5.5x10^5$	N D	$5.0x10^2$	$1.5x10^3$	$5.0x10^3$
19.10.87	7*	93	N G	$5.0x10^3$	$2.5x10^3$	N G	$5.0x10^2$
20.10.87	8	91	N G	N G	N G	N G	N G
21.10.87	9	94	N G	N G	N G	N G	N G
26.10.87	14	40	N G	$1.5x10^4$	$1.0x10^3$	N G	N G

N D = Not Done, N G = No Growth, * = Withdrawal of treatment

c) Anaerobic organisms

No enteric spirochaetes were demonstrated and no C. difficile colonies were isolated.

i) Bacteroides spp.: Colonies similar to those isolated and identified as B. fragilis, B. vulgatus and B. melaninogenicus from faeces were isolated from contents in numbers varying from 10^2 to 10^7/g, and are reported in Table 4.17. Because the media for these organisms were unavailable on day 0 no cultures were made. On day 7, these organisms were present in the stomach in numbers of 10^2/g, 10^3/g in the jejunum and ileum, 10^6/g in caecum and 10^7/g in the colon. No growth was found in the ileum on day 8 but high counts of 10^5/g were found in the caecum and 10^6/g in the colon. On days 8, 9 and 14 there were no further isolations either from stomach or jejunum. From the ileum low numbers (10^2/g) were isolated on day 9 and on day 14 (10^3/g). High isolation rates of Bacteroides spp. from the caecal and colonic contents were maintained. On day 9 and 14 10^6 C.F.U./g were found in the caecum. The colonic counts of 10^7/g were also maintained.

ii) Clostridium perfringens type A: The counts shown below in Table 4.18 demonstrate that these organisms were present during the whole period of study in the contents obtained from most sections of the gastroenteric tracts of the sacrificed pigs. C. perfringens type A was isolated from the stomach (10^6/g) only on day 0. During treatment (day 7) its numbers in the jejunum were 10^2/g, by day 8 they increased to 10^3/g, and 10^2 C.F.U./g were found on day 14. They were found at 10^3/g on day 0, 10^5/g on day 7, 10^2/g on day 9 and 10^3/g on day 14 from the ileal contents. The caecum showed counts of 10^5/g on days 0 and 7, 10^2/g on day 8 and by day 14 their numbers returned to 10^4 C.F.U./g. Some days no growth was found on the inoculated plates. This organism was regularly found in the colonic contents at counts of 10^6/g at the beginning of the study and 10^5, 10^3, 10^5 and 10^4/g respectively on days 7, 8, 9 and 14 of the study.

TABLE 4.17 Bacteroides spp. counts from the gastrointestinal contents of sacrificed pigs of Experiment 1

Necropsy Date	Study Day	Pig No.	Stomach	Jejunum	Ileum	Caecum	Colon
12.10.87	0	59	N D	N D	N D	N D	N D
19.10.87	7*	93	$5.0x10^2$	$5.0x10^3$	$1.5x10^3$	$1.5x10^6$	$2.0x10^7$
20.10.87	8	91	N G	N G	N G	$5.0x10^5$	$1.0x10^6$
21.10.87	9	94	N G	N G	$5.0x10^2$	$2.0x10^6$	$1.5x10^7$
26.10.87	14	40	N G	N G	$5.0x10^3$	$1.5x10^6$	$2.0x10^7$

N D = Not Done, N G = No Growth, * = Withdrawal of treatment

TABLE 4.18 Clostridium perfringens type A (vegetative cells) counts from the gastrointestinal contents of sacrificed pigs of Experiment 1

Necropsy Date	Study Day	Pig No.	Stomach	Jejunum	Ileum	Caecum	Colon
12.10.87	0	59	$5.0x10^6$	N D	$2.25x10^3$	$5.0x10^5$	$1.0x10^6$
19.10.87	7*	93	N G	$5.0x10^2$	$1.0 x10^4$	$2.5x10^5$	$1.0x10^5$
20.10.87	8	91	N G	$5.0x10^3$	N G	$5.0x10^2$	$1.5x10^3$
21.10.87	9	94	N G	N G	$5.0 x10^2$	N G	$1.0x10^5$
26.10.87	14	40	N G	$5.0x10^2$	$5.0 x10^3$	$1.0x10^4$	$2.5x10^4$

N D = Not Done, N G = No Growth, * = Withdrawal of treatment

TABLE 4.19 Clostridium perfringens type A (spores) counts from the gastrointestinal contents of sacrificed pigs of Experiment 1

Necropsy Date	Study Day	Pig No.	Stomach	Jejunum	Ileum	Caecum	Colon
12.10.87	0	59	N G	N D	$5.0x10^2$	$7.5x10^2$	N G
19.10.87	7*	93	N G	N G	N G	N G	$5.0x10^2$
20.10.87	8	91	N G	N G	N G	N G	N G
21.10.87	9	94	N G	N G	N G	N G	N G
26.10.87	14	40	N G	N G	N G	N G	$5.0x10^2$

N D = Not Done, N G = No Growth, * = Withdrawal of treatment

Spores of C. perfringens type A were only isolated in numbers of 10^2/g from ileum and caecum on day 0 and from colon on days 7 and 14 (Table 4.19).

E) Virological examinations of faeces and gastrointestinal contents

All faecal samples and caecal contents were subject to screening for rotaviral particles using the RPLA test. Neither rotavirus or rotaviral antigen were detected before the beginning of the treatment from faeces and colonic contents of Pig 59 which was killed on day zero of the study. During the period of treatment only Pig 68 was positive to the test on day 5. Rotavirus and/or its antigen was also detected from the caecum and colon of Pig 94 on day 9 of the Experiment. No further positive tests were recorded either from faeces or from contents for the remaining days post-treatment. However, three faecal samples gave a weak agglutination on days 6 (Pig 68), 8 (Pig 70) and 14 (Pig 69) of the study, but according to the recommendations of the manufacturer those results must be and were considered as negative.

F) Parasitological examinations of faeces and gastrointestinal contents

Smears of faecal samples from the sampled group and contents collected at the post mortem examination from standard sites in the intestinal tract, and ileal scrapings from the sacrificed group were all found to be negative for cryptosporidial oocysts. However, other coccidia-like organisms and protozoa were present, but they could not be identified.

4. D I S C U S S I O N

A) Experimental design

The experimental design was limited by the availability of the ration and the fact that the avoparcin had to be incorporated at the mill and could not be incorporated on farm. In addition the work load involved in culturing samples meant that it was not possible to monitor the animals in the group which were to be sacrificed. The history of these animals in terms of daily liveweight gain, faecal consistency, rectal temperature and bacterial counts was therefore not available. The small number of animals used made statistical analysis of the results difficult as did the absence of controls.

Transport of the samples may also have contributed to the low numbers of some anaerobes isolated from faecal samples, since they were transported without the addition of any transport medium from either the farm or the post mortem room.

Although all samples obtained from gastrointestinal contents and faeces were processed as soon as it was possible the average time for such procedures was between 1-2 hours.

All factors affecting these studies will be discussed below with the results of the individual examinations.

B) Clinical findings

No change in rectal temperature was seen upon withdrawal of medication. There was, however, an apparent effect on faecal consistency. Diarrhoea was more common in the period following withdrawal of treatment, but statistically ($P>0.05$) these changes were not significant (Table 4.3, Figure II). The reasons for this apparent increase are discussed below.

C) Production parameters

The mean daily liveweight gain of the sampled group was almost identical during and after treatment, but the daily individual weigh gains of pigs varied. Pigs 66, 68 and 69 lost weight after introduction of treatment but not after withdrawal. Although the daily liveweight gain of pigs 67 and 69 improved after drug withdrawal, no change was recorded on the liveweight gain of Pig 70 either during or after the treatment. These changes could not be linked consistently with diarrhoea as Pig 69 had diarrhoea for one day only had a similar weight gain to that of Pig 66 which had diarrhoea throughout the study. These findings maybe due to another infectious condition affecting a non-enteric system.

D) Faecal and gastrointestinal bacteriology

The examinations carried out from pigs in the sampled and sacrificed groups showed that a wide variety of bacteria could be isolated from the faecal sample and gastroenteric contents. More than one species of bacterium occurred in high numbers after the withdrawal of avoparcin in some individuals. Although there were individual differences clearly noted on the bacterial counts reported from the sampled group, after the statistical

analysis of such data no significant difference ($P>0.05$) was found either between groups or between the periods of the study, before, during or after the antimicrobial was used.

Some of the bacteria isolated are known to cause enteric disease and lesions in pigs **e.g.** C. perfringens type A and Campylobacter spp., but others had been considered to be normal members of the alimentary tract flora **e.g.** faecal streptococci and lactobacilli. However, some of the bacteria isolated **e.g.** Bacteroides spp. are described in the literature as causes of lesions other than those of the enteric tract and may be important in pig gastroenteric disorders.

Isolation of important pathogens such as Salmonella spp. and S. hyodysenteriae was attempted throughout the whole period of study but they were not identified by any of the methods used. It was surprising that C. difficile could not be isolated using the selective medium. The colonies isolated were tested exhaustively and shown not to be C. difficile. This is of considerable interest since this bacterium and its toxins are commonly reported in high numbers from human cases of pseudomembranous colitis and enterocolitis in hamsters, following the recent use of orally administered antimicrobials (Bartlett, 1981; Bartlett, et al., 1978a,b,c,; Borriello, 1990; Borriello and Larson, 1981; George, et al., 1978). Although C. difficile has been isolated from cases of experimentally induced swine dysentery in pigs (Lysons and Hall, 1982) and from colonic necrotic lesions of naturally occurring enterocolitis (Jones and Hunter, 1983), not associated with swine dysentery they were not isolated in this study.

Bacteria which appeared to rise in numbers following withdrawal of avoparcin included coliforms (Table **4.6**, Figure III), faecal streptococci (Table **4.7**, Figure IV), campylobacters (Table **4.8**, Figure V), Bacteroides spp. (Table **4.10**, Fig.VI) and vegetative cells of C. perfringens type A (Table **4.11**, Figure **VII**). In individual animals this increase was associated with diarrhoea, in Pig **67** (C. perfringens type A) and in Pig **68** (coliforms, streptococci, campylobacters and Bacteroides spp).

Populations rose after withdrawal of the drug and these variations were closely related to changes in faecal consistency. Bacteroides spp. have been isolated from enteric lesions of swine dysentery (Alexander, et al., 1976; Lysons, et al., 1980; Robinson, et al., 1982; 1984; Whipp, et al., 1979; 1980), and may have contributed to the development of diarrhoea in Pig **68**. The findings reported in Table **4.10** and Figure **VI** show that Bacteroides spp. must be considered in the routine screening for the isolation of aetiological agents from enteric diseases involving diarrhoea as a clinical feature. A marked

rise in numbers in Pig 69 was not associated with any clinical abnormality.

All of the bacterial results could have been affected by the time consuming processes and methods such as the serial dilutions and plating, which may have altered the rate of isolation and survival of the strictly anaerobic bacteria. Aerobic organisms were easy to grow but their accurate identification by genera and species was difficult in the sense of time expended on each isolate. The cultures of microaerobic bacteria were found in many cases to be contaminated by fungi. In some cases reading of the bacterial colonies, their numbers and their morphology was impossible. In this study the conditions of cultivation might have been improved if more sophisticated selective culture media had been used. However, bacteria such as Bacteroides spp. which are highly sensitive to exposure to air suffered during the process of counting and almost certainly produced lower numbers than those originally present inside the gastroenteric tract.

The use of anaerobic jars for both microaerobic and anaerobic organisms produced satisfactory results except when fungi contamination was found in the jars used. This late complication could be avoided if an anaerobic cabinet were used but at the time this equipment was not available.

E) Parasitological and virological examinations

The identification of Cryptosporidium spp. and other coccidia-like organisms in the pigs of the sacrificed group by histological examination suggested that the clinical signs, and the macroscopic and histological findings reported, might be related to organisms other than bacteria. The fact that oocysts of Cryptosporidium spp. were not seen from the smears made from faeces and contents stained with the modified Ziehl-Neelsen technique was surprising, since they were seen in the histological sections examined.

In this study the presence of rotavirus and/or its particles confirmed by RPLA tests could not be directly related to lesions on the epithelial surface of the digestive tract.

Agents such as rotavirus or cryptosporidia were not demonstrated in every pig at the time of examination, but it cannot be assumed that they were not present as they might have been either in reduced numbers or present at a time prior to the time of sampling and/or examination. The presence of two types of agent which are incapable of response to antimicrobials makes interpretation of the results of this study more difficult.

F) Pathological examinations

Gross pathological changes were slight. Where fluid intestinal contents were present, the mucosa was mildly inflamed or macroscopically normal. Similar changes were seen in animals with normal intestinal contents. There was not clear association between the gross changes present and the withdrawal of treatment.

Histological examination of the intestinal mucosa showed changes which were not macroscopically apparent. They were classified as mild and could not be related to any specific infectious process or to the withdrawal of treatment. They did however reveal the presence of cryptosporidia in the experimental pigs. It is possible that some changes seen histologically may reflect minor differences in treatment prior to slaughter and may have affected the gastroenteric tracts.

G) Concluding remarks

In this study there were some technical problems which might have an effect on the results obtained and in the way of controlling the two groups of sacrificed and monitored pigs. However, from the results obtained it could be said that:

a) There was some association between the appearance of clinical signs (diarrhoea) and withdrawal of in-feed avoparcin, but were not significant statistically.

b) There was no clear association between the withdrawal of treatment and the production parameters.

c) There was no evidence for the presence of Clostridium difficile, a noted post-antimicrobial pathogen of man.

d) Expected rises in individual bacteria such as coliforms, faecal streptococci, Campylobacter spp., Bacteroides spp. and notably C. perfringens type A, occurred after the withdrawal of treatment.

e) Two agents not likely to be susceptible to antimicrobials (cryptosporidia and rotavirus) were demonstrated in the pigs used in this study.

The absence of enteropathogenic bacteria i.e. beta haemolytic Escherichia coli from the faecal samples and gastrointestinal contents examined was in contrast to the findings of previous studies (Chapter 3) in which their presence was a common feature. This result suggests that they may have been suppressed by Intagen and other antimicrobials especially the olaquindox in the rations used.

CHAPTER 5

THE EFFECT OF WATER MEDICATION WITH LINCOMYCIN ON THE GASTROINTESTINAL BACTERIAL FLORA AND ITS RELATIONSHIP WITH ENTERITIS FOLLOWING ITS WITHDRAWAL IN WEANED PIGS

1. INTRODUCTION

The Experiments described in this Chapter follow from the results obtained in the previous study (Chapter 4) which were inconclusive. It was decided to chose a different antimicrobial for the studies described here. In the previous study the drug used was avoparcin, a common growth promoter but in this Chapter the therapeutic product chosen was lincomycin. This has a wider spectrum of activity and is related to clindamycin, a known cause of antibiotic associated colitis in other animal species and man.

It was also decided that lincomycin should be given for a longer period than recommended to exaggerate the effects of withdrawal. In these studies the antimicrobial would be administered by a different route, being incorporated in the drinking water because of the reluctance of any commercial mill to incorporate this product in-feed. The use of water medication allowed any diet to be used, thus reducing the problems experienced in the previous study with avoparcin.

In order to assess the effect of lincomycin on the "normal" gastrointestinal flora of recently weaned pigs, Experiments 2 and 3, both incorporating controls, were carried out to assess the effects of withdrawal of the product. Medication was given at a concentration of 13.3 ppm in water for 11 days in Experiment 2 and for 20 days in Experiment 3. Clinical signs, productive parameters and faecal bacteriological studies were recorded in Experiment 2 with particular emphasis on the period following withdrawal. Post mortem examinations and bacteriology of the contents from six portions of the digestive tract were also carried out in Experiment 3.

The studies were intended to evaluate the relationship of lincomycin use and withdrawal with; clinical disease, the presence of gross and microscopical lesions, variation of bacterial populations (qualitative and quantitatively) in faeces and in gastrointestinal contents, and the effect on productive parameters and water consumption. The presence or absence of rotavirus and/or its particles and some intestinal parasites was also studied.

2. MATERIALS AND METHODS

The majority of the materials and methods used in both Experiments have been described in detail in Chapter Two and briefly mentioned above in Chapter 4.

A) Experimental animals

Source of the experimental animals: In Experiment 2, ten four-week-old minimal disease weaned pigs weighing between 5.2 and 10.6 kg were used. In Experiment 3, twenty, three-week-old minimal disease recently-weaned pigs weighing between 4.3 and 6.8 kg were used. The animals in both Experiments were obtained from the Veterinary School Animal Husbandry Department's farm at Cochno described in Chapter 2 and shown in Figure I. They were from a high health status herd and were free from salmonellosis, swine dysentery or P.P.E.

a) Identification system

As in Chapter 4 all the animals used in these two Experiments were individually identified by the application of plastic, numbered, coloured tags in the right ear. For the purpose of each Experiment they were assigned at random to one of two groups. Orange tags were used for pigs under treatment and white-coloured tags for pigs used as controls. The individual identification of the pigs in both Experiments are shown below in Tables 5.1 and 5.2.

TABLE 5.1 Individual identification of pigs in Experiment 2

Tag Number and colour	Sex	Initial Weight(Kg)	Study Group
68 orange	M	8.1	T
69 orange	F	6.4	T
97 orange	M	7.6	T
98 orange	F	6.6	T
99 orange	M	7.7	T
50 white	M	10.6	C
95 white	F	7.3	C
97 white	F	9.4	C
99 white	M	5.2	C
100 white	F	10.3	C

M = Male, **F** = Female, **T** = Treated, **C** = Control

TABLE 5.2 Individual identification of pigs in Experiment 3

Tag Number and colour	Sex	Initial Weight(Kg)	Study Group	Killed on Study Day
61 orange	F	5.3	T	22
63 orange	F	5.4	T	ND
64 orange	F	5.7	T	34
65 orange	F	4.6	T	24
67 orange	M	4.6	T	7
84 orange	F	5.2	T	ND
86(63) orange	F	6.8	T	20
87 orange	M	5.8	T	ND
88 orange	M	6.8	T	ND
90 orange	F	6.5	T	ND
27 white	M	5.5	C	24
57 white	F	3.8	C	7
61 white	M	4.7	C	22
88 white	M	4.5	C	ND
89 white	F	4.8	C	34
90 white (L)	F	5.7	C	ND
90 white (R)	F	4.3	C	20
92 white	M	5.9	C	ND
93 white	F	5.6	C	ND
94 white	F	6.3	C	ND

M = Male, **F** = Female, **T** = Treated, **C** = Control,
ND = Not Done, **L** = Left ear, **R** = Right ear

b) Maintenance of experimental animals

Pigs used in both Experiments were housed in two separated cement floored pens with slats at the back. The nipple drinker in each pen was turned off. The pens were temporarily equipped with an automatic drinker with a capacity of five litres so that water was freely available. The temperature of the room in which the pens were located varied from 23-27^{o}C, additionally there was an individual source of heat from a lamp (150w bulb) in each pen which was positioned over the solid floored front creep area in which an automatic feeder was also available providing feed **ad libitum**. Except for the individual drinkers the pigs were reared under the commercial conditions prevailing in the piggery of the Glasgow University Veterinary School Cochno Farm.

c) Food

The pigs in both Experiments were fed on Growercare 483 diet (B.O.C.M. Silcock, Ltd.). The ration contained the ingredients listed below:

Declaration B.O.C.M. 483 Growercare pellets;

Crude protein	22.00%
Oil	6.00%
Fibre	3.00%
Ash	5.00%
Vitamin 'A'	10,000 IU/kg
Vitamin 'D_3'	2,000 IU/kg
Vitamin 'E'	60 IU/kg
Selenium	0.3 mg/kg
Copper Sulphate	175 mg/kg
Tylosin Phosphate	40 mg/kg

B) Antimicrobial administration

For the purpose of both studies 166 mg of 40% lincomycin powder (as Lincocin* Soluble Powder, Upjohn, Ltd.) was added to 5 litres of drinking water in a clean bucket, mixed and then put into the automatic drinker fixed into the pen occupied by the treated group of pigs to give 13.3 ppm lincomycin hydrochloride.

Plain water in the same quantity was supplied to the control group of pigs in a similar drinker. Both medicated and non-medicated water was freely available and its consumption was recorded daily from either groups.

C) Experimental procedures

The effects of the drug and its withdrawal on the pigs was monitored by examining clinical signs and on their gastroenteric flora by examining faecal samples (Experiment 2), and in Experiment 3, gastroenteric contents were also examined. Treated and control groups in either Experiment were studied over the periods of 18 and 34 days of observation. In Experiment 3 animals from each group were euthanased at specific times and examined **post mortem** for macroscopic pathological lesions and microscopically for histological changes and the presence or absence of intestinal parasites.

In Experiment 2 the ten pigs from either treated and control groups were monitored once before treatment began and daily for six days while being medicated with lincomycin and for five days after its withdrawal.

In Experiment 3 the twenty pigs were monitored for one week before treatment. One week was allowed for adaptation to the treatment and once this was achieved they were monitored every third day for two weeks (seven days) and for two weeks more (seven days) after the withdrawal of the treatment.

D) Clinical observations

The general bodily condition of the experimental pigs from both groups in both Experiments before, during and after treatment was noted daily or every third day.

a) Faecal consistency, rectal temperature, feed and water consumption and daily liveweight gain

i) Appetite and individual faecal consistency were recorded every day from Experiment 2 (Tables 5.3 and 5.4) and every third day from Experiment 3 (Tables 5.24 and 5.25).

ii) Rectal temperatures were taken and recorded every day or every third day (Tables 5.5, 5.6, 5.26 and 5.27) from the experimental animals of the treated and control groups in the two Experiments. The measurements were all carried out at a similar time of the day.

iii) Feed consumption and water intake were measured daily or every third day in both groups in both Experiments and recorded. Feed use was recorded and the pigs were weighed on the days when animals were removed for slaughter.

iv) Weight gains of the pigs in the two Experiments were measured daily or every third day respectively Tables 5.10, 5.11, 5.31 and 5.32).

Any deviation from this general pattern of clinical examination was recorded.

b) Serum samples

Blood samples were collected from the external jugular vein of all pigs before treatment began in both Experiments. Samples were also collected from all pigs sacrificed from Experiment 3 prior to euthanasia. The sera obtained were stored at -20^{o}C in 5 ml amounts until required.

c) Post mortem examination

The pigs sacrificed from the treated and control groups in Experiment 3 were killed on the 7th, 20th, 22nd, 24th and 34th day of the study (Table 5.2).

Post mortem examination was carried out by the methods described in Chapter 2, as soon as possible after death. All ten pigs were examined for gross changes or lesions in the organs of the abdominal and thoracic cavities. Particular attention was paid to the gastroenteric tract which was investigated in detail.

E) Laboratory examinations

a) Microbiological and parasitological examinations

In Experiments 2 and 3 rectal faecal sampling was carried out daily or every third day and samples were examined qualitatively and quantitatively for bacteria, and qualitatively for rotavirus and protozoal parasites by the methods described

in Chapter 2. Care was taken to examine the samples immediately as soon as possible (within 1-2 hours). Similar examination was carried out using the contents from the following six portions of the gastrointestinal tract: stomach, duodenum, jejunum, ileum, caecum and colon, obtained after the **post mortem** examination of pigs killed in Experiment 3.

Air-dried heat fixed smears of faeces (Experiments 2 and 3), intestinal mucosal scrapings and contents from the intestinal portions mentioned above (Experiment 3) were made and stained by Gram's method and modified Ziehl-Neelsen's method for bacteria and the presence of cryptosporidial oocysts respectively.

b) Histological examinations

Sections of stomach, duodenum, jejunum, ileum, caecum and colon were taken at the gross **post mortem** examination of the sacrificed pigs in Experiment 3. After processing, histological sections stained by H & E were examined by light microscopy to detect any abnormalities present in the gastrointestinal epithelium and for the presence of *Cryptosporidium spp.*, *Isospora suis* and *Balantidium coli*.

c) Examination for enteric agents and their products

The following tests were also carried out on filtrates of faeces and contents. The test used throughout the studies for the demonstration of rotavirus or its particles was the Reversed Passive Latex Agglutination test (RPLA). RPLA tests (PET-RPLA, Oxoid, Ltd.) were also used routinely throughout for the demonstration of *C. perfringens* type A enterotoxin. The presumptive identity of *Clostridium perfringens* type A was confirmed by its lecithinase production (Nagler reaction) and its double zones of haemolysis (Alpha and Beta). Subculture of isolates was carried out onto egg yolk and sheep blood agar plates anaerobically incubated at 37^{o}C. *C. difficile* antigens were also screened using commercial RPLA kits (MicroScreen LA test, Mercia Diagnostics, Ltd.) on faecal filtrates by the methods outlined in detail in Chapter 2.

d) Bacteriological examinations

Faecal and gastroenteric samples were examined for aerobic, microaerobic and anaerobic bacteria by the methods described in Chapter 2.

Bacterial identification was presumptive in most cases, incubated plates were examined for colonial morphology and effect on the media. Cellular morphology was confirmed by Gram's stain. Further identification of bacteria was carried out in more detail where the identity of isolates was in doubt using the methods described in Chapter 2. The bacterial counts provided the number of Colony Forming Units (CFU).

3. RESULTS

The results obtained from the two studies are presented here in the order in which routine examinations established were carried out. The daily bacterial counts presented were obtained from the daily results sheet and summarised in Tables (5.12 to 5.23, Experiment 2 and 5.33 to 5.48, Experiment 3) and represented in Figures (XII to XXIII, Experiment 2 and XXX to XLV, Experiment 3).

3.1 EXPERIMENT 2

A) Clinical signs

The weaned minimal disease pigs used in experiment 2 were in good condition, healthy and active. Diarrhoea was the major clinical abnormality noted and occurred in both treated and untreated groups of pigs both during the period of treatment and following its withdrawal. Pigs which were clinically diarrhoeic showed hairy coats, dehydration, loss of condition, general weakness, raised temperature or fever, low feed consumption and DLWG, their appearance was dirty and the consumption of water increased. Rectal temperatures remained within the normal range in the treated group receiving treatment, but in the controls and following withdrawal of treatment raised temperatures were observed.

The daily individual record of clinical signs identified from either group in this Experiment are presented below in Tables 5.3 to 5.11, and Figures VIII to XI.

Faecal consistency: The consistency of the faeces recorded from the pigs on Experiment 2 varied consistently from soft to diarrhoeic, however some pigs showed diarrhoea within a day of passing faeces of normal consistency. In the treated group (Table 5.3) there was an important change recorded in faecal consistency after the withdrawal of lincomycin. During treatment there were only 4 diarrhoea days but after withdrawal of the antimicrobial from the drinking water this figure increased to 8 diarrhoea days out of 25 pig/days (Figure VIII). On day 17 of the study the faeces of Pig 68 contained streaks of blood, and Pig 98 on day 18 passed an excess of mucus on its soft faeces.

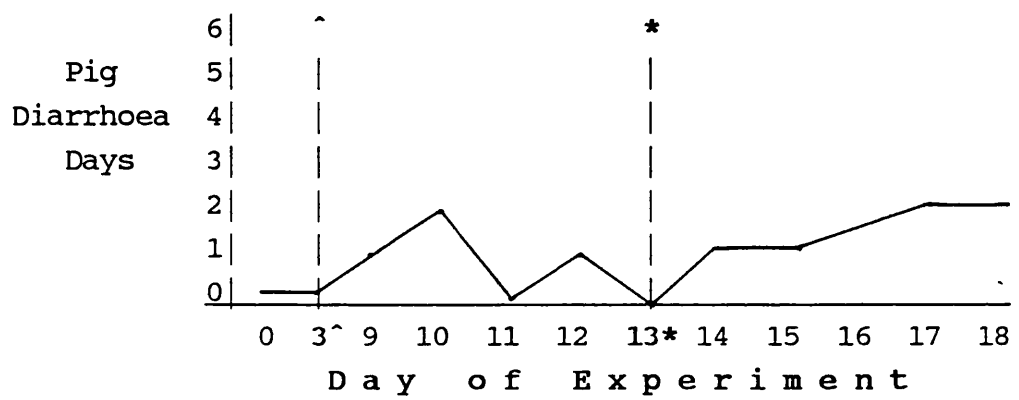
TABLE 5.3 Changes in faecal consistency recorded from the treated pigs in Experiment 2

Pig	Day of Experiment												Total
Number	0	3^	9	10	11	12	13*	14	15	16	17	18	D/Days
68	**N**	N	S	D	S	S	**N**	S	S	N	S	**S**	1/12
69	**N**	S	S	D	S	S	**S**	D	S	S	S	**D**	3/12
97	**S**	S	S	S	N	D	**S**	S	S	S	D	**D**	3/12
98	**N**	N	D	S	S	S	**S**	S	D	D	D	**S**	4/12
99	**N**	S	N	S	S	S	**S**	S	N	D	S	**S**	1/12

N = Normal, **S** = Soft, **D** = Diarrhoea

^ Beginning of treatment, * Withdrawal of treatment

FIGURE VIII. Changes in faecal consistency of the treated pigs in Experiment 2



^Beginning of treatment, *Withdrawal of treatment

The control group showed a higher number of diarrhoea days recorded over the periods in which its replica group was

treated, and after treatment withdrawal. Similar distribution of diarrhoea days occurred in the control group as may be seen on Table 5.4 and Figure IX, below;

TABLE 5.4 Changes in faecal consistency recorded from the control pigs in Experiment 2

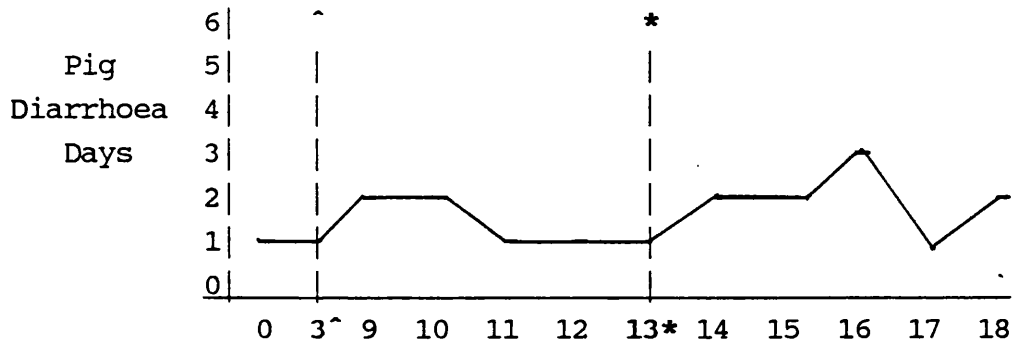
Pig	Day of Experiment												Total
Number	0	3^	9	10	11	12	13*	14	15	16	17	18	D/days
50	S	S	N	D	N	N	S	S	S	S	S	S	1/12
95	S	S	N	N	S	S	S	S	S	D	S	S	1/12
97	N	S	D	S	S	S	S	D	D	D	S	D	5/12
99	D	N	S	N	N	N	S	S	S	N	S	S	1/12
100	S	D	D	D	D	D	D	D	D	D	D	D	11/12

N = Normal, S = Soft, D = Diarrhoea

^ Beginning of treatment, * Withdrawal of treatment

Diarrhoea occurred in Pig 100 from this group for 11/12 days starting at the beginning of the treatment, and on days 15, 16 and 17 of the study its diarrhoea was clearly fluid in consistency with some gas bubbles.

FIGURE IX. Changes in faecal consistency of the control pigs in Experiment 2



^Beginning of treatment, *Withdrawal of treatment

Rectal temperature: The temperatures recorded from individual pigs varied and did not always relate to the other clinical signs such as changes in faecal consistency and water or feed consumption. When this parameter changed drastically the presence of disease if any, was recorded. The detailed figures are given in Tables 5.5 and 5.6.

TABLE 5.5 Individual rectal temperatures of the treated pigs (orange tags) in Experiment 2

Pig	Day of Experiment								
Number	0	3^	9	10	11	12	13*	14	15
68	39.8	39.4	39.2	39.1	39.1	39.3	39.2	39.6	39.5
69	39.4	39.5	39.6	39.5	39.5	39.3	39.4	39.9	39.5
97	39.4	39.5	39.6	39.6	39.2	39.5	39.2	39.6	39.6
98	39.5	39.4	39.1	39.5	39.5	39.5	39.2	39.7	39.8
99	39.5	39.4	39.2	39.5	39.3	39.2	39.3	39.5	39.3
X	39.5	39.4	39.3	39.4	39.3	39.4	39.3	39.7	39.5

^ Beginning of treatment, * Withdrawal of treatment

NOTE: Temperatures are expressed in degrees Centigrade

TABLE 5.5 (Cont.) Individual rectal temperatures of the treated pigs (orange tags) in Experiment 2

Pig	Day of Experiment			Group
Number	16	17	18	X
68	39.5	39.6	39.7	39.4
69	40.0	39.8	39.6	39.6
97	39.8	39.4	39.5	39.5
98	39.6	39.7	39.8	39.5
99	40.1	39.6	39.8	39.5
X	39.8	39.6	39.7	39.5

NOTE: Temperatures are expressed in degrees Centigrade

The rectal temperatures recorded from the pigs in the treated group of Experiment 2 averaged 39.5^{o}C with a range of 40.1^{o}C and 39.1^{o}C. The average temperature in the control group was 39.7^{o}C with a range of 40.4^{o}C and 39.3^{o}C. The maximum temperature was recorded in Pig 99 (white) on days 3 and 10 of treatment. The differences between the groups were statistically significant (t=-11.22), during the period of treatment, by using a student's t-Test for means (Microsoft Excel, Version 4.0).

TABLE 5.6 Individual rectal temperatures of the control pigs (white tags) in Experiment 2

Pig	Day of Experiment								
Number	0	3^	9	10	11	12	13*	14	15
50	39.5	40.0	39.8	40.0	39.8	39.8	ND	39.8	39.8
95	39.4	39.6	39.0	39.7	39.6	39.6	ND	39.7	39.9
97	39.5	39.9	40.1	39.7	39.6	39.6	ND	40.1	39.9
99	39.7	40.4	40.0	40.4	40.0	39.8	ND	39.7	40.3
100	39.4	39.8	39.7	39.7	39.6	39.5	ND	39.3	39.5
$\bar{X}$	39.5	39.9	39.7	39.9	39.7	39.7	ND	39.3	39.9

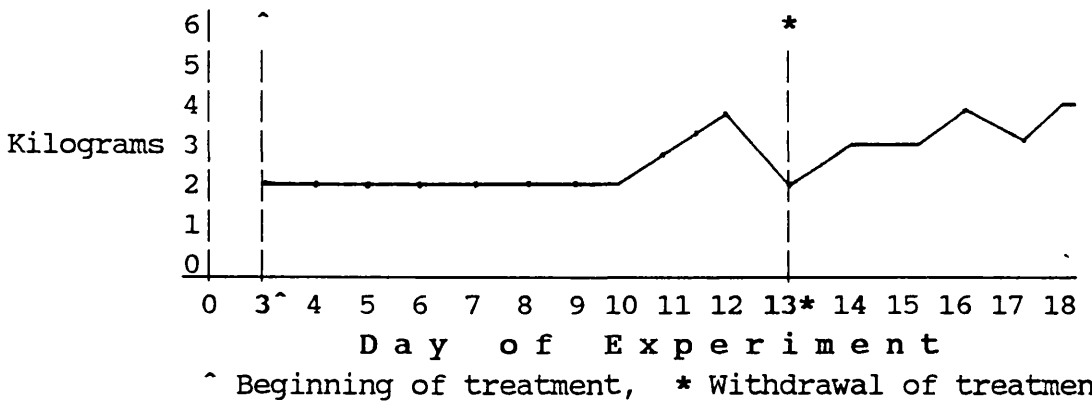
^ Beginning of treatment, * Withdrawal of treatment
ND = Not Determined (broken thermometer)

TABLE 5.6 (Cont.) Individual rectal temperatures of the control pigs (white tags) in Experiment 2

Pig	Day of Experiment			Group
Number	16	17	18	$\bar{X}$
50	39.8	39.9	39.8	39.8
95	39.6	39.3	39.3	39.3
97	40.1	40.0	39.8	39.8
99	39.8	39.5	40.0	40.0
100	39.8	39.8	39.7	39.6
$\bar{X}$	39.8	39.7	39.7	39.7

Feed and water consumption: The feed consumption records from the treated group in Experiment 2 are presented in Figure X, and the water consumption of the treated group for the periods before, during treatment and after withdrawal are presented below in Table 5.7 and Figure XI. Feed and water consumption of the controls was not recorded.

FIGURE X. Feed consumption of the treated pigs in Experiment 2

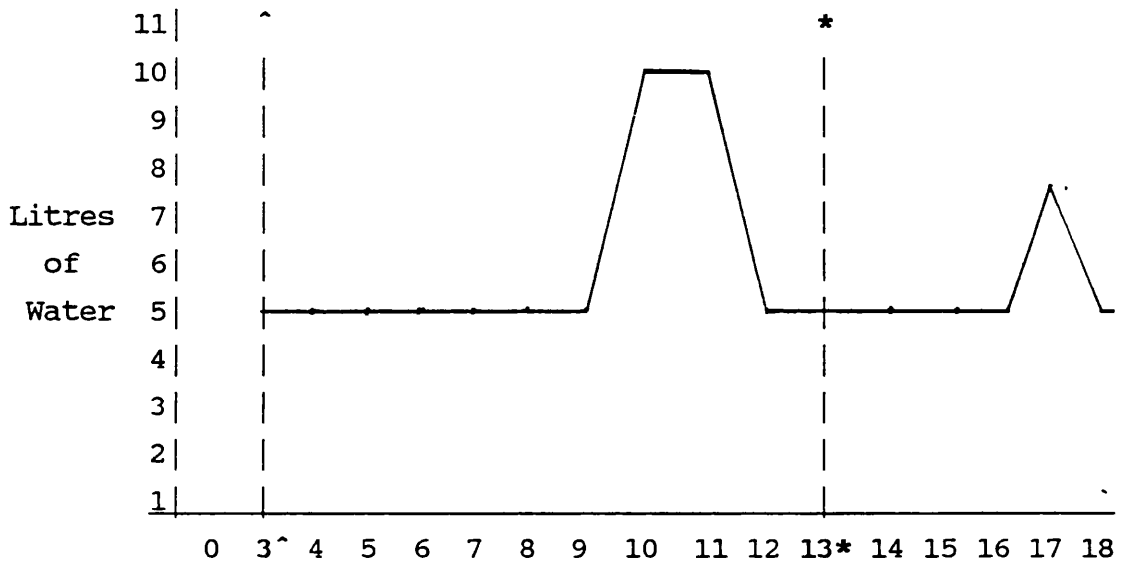


^ Beginning of treatment, * Withdrawal of treatment

TABLE 5.7 Total water consumption of the treated group in Experiment 2

Days before treatment (1)	Days during treatment (11)	Days after treatment (6)
5 litres	65 litres	36.5 litres

FIGURE XI. Water consumption of the treated pigs in Experiment 2



^ Beginning of treatment, * Withdrawal of treatment

Daily liveweight gain: The individual live weights recorded from the pigs in Experiment 2 in both treated and control groups are given in Tables 5.8 and 5.9. Daily liveweight gains for the periods 0 to 3, 3 to 13 and 13 to 18 days (Experiment 2) are presented in Tables 5.10 and 5.11, below.

TABLE 5.8 Individual live weights of the treated pigs in Experiment 2, expressed in kilograms

Pig	Day of Experiment												Total
No.	0	3^	9	10	11	12	13*	14	15	16	17	18	Gain
68	8.1	9.7	10.9	10.9	12.5	12.2	12.7	13.3	14.2	14.8	14.6	14.9	6.8
69	6.4	7.8	9.5	9.9	10.3	10.5	10.9	10.8	12.6	13.5	13.6	14.6	8.2
97	7.6	9.0	11.9	12.7	13.4	13.8	14.1	14.9	15.6	15.8	15.8	16.7	9.1
98	6.6	8.1	9.4	9.8	10.4	10.6	11.2	11.9	13.1	12.9	13.3	13.7	7.1
99	7.7	9.0	11.3	12.0	12.5	13.1	13.8	14.7	15.8	16.1	16.3	17.2	9.5
Total	36.4	43.6	53.0	55.3	59.1	60.2	62.7	65.6	71.3	73.1	73.6	77.1	40.7

^ Beginning of treatment, * Withdrawal of treatment

TABLE 5.9 Individual live weights of the control pigs in Experiment 2, expressed in kilograms

Pig	Day of Experiment												Total
No.	0	3^	9	10	11	12	13*	14	15	16	17	18	Gain
50	10.6	11.1	13.3	13.9	15.3	16.1	16.5	17.4	18.1	18.8	19.1	19.9	9.3
95	7.3	8.2	8.3	9.3	9.8	10.2	10.6	11.8	12.1	12.3	12.7	13.3	6.0
97	9.4	9.9	11.8	12.1	12.7	13.3	13.9	15.2	14.9	15.7	16.2	16.6	7.2
99	5.2	5.4	5.6	5.8	6.2	6.5	6.8	7.7	7.9	8.4	8.5	8.8	3.6
100	10.3	11.3	12.8	13.1	13.3	13.9	14.1	14.6	14.9	15.1	15.5	15.9	5.6
Total	42.8	45.9	51.8	54.2	57.3	60.0	61.9	66.7	67.9	70.3	72.0	74.5	31.7

^ Beginning of treatment, * Withdrawal of treatment

TABLE 5.10 Individual daily live weight gains of the treated pigs for days 0-3 (before treatment), 3-13 (treatment) and 13 to 18 (withdrawal), in Experiment 2

Pig	Day of Experiment		
Number	0-3	3-13	13-18
68	0.533	0.300	0.440
69	0.466	0.310	0.740
97	0.466	0.510	0.520
98	0.500	0.310	0.500
99	0.433	0.480	0.680
X	0.480	0.382	0.576

NOTE: Weight gains are expressed in kilograms

TABLE 5.11 Individual daily live weight gains of the control pigs for days 0-3, 3-13 and 13-18, in Experiment 2

Pig Number	Day of Experiment 0-3	3-13	13-18
50	0.166	0.540	0.680
95	0.300	0.240	0.540
97	0.166	0.400	0.540
99	0.066	0.140	0.400
100	0.333	0.280	0.360
X	0.206	0.320	0.504

NOTE: Weight gains are expressed in kilograms

B) Bacteriological examinations of faeces

Over the periods before, during and after lincomycin treatment quantitative and qualitative bacteriological examinations were performed on faeces obtained individually from the pigs from both treated and control groups. The results of these examinations are presented in Tables 5.12 to 5.23 as arithmetic means, and graphically in Figures XII to XXIII.

a) Aerobic organisms

i) Total coliforms: The counts obtained on MacConkey agar plates from both treated and control groups in Experiment 2 are presented in Tables 5.12, 5.13 and Figures XII and XIII. In the treated group the population of coliforms was present in numbers varying between 10^4 to 10^9 bacteria/g of faeces. The average number of coliforms found before treatment was 10^8/g, during treatment the numbers decreased to 10^5/g but returned to 10^8/g at the end of the study by day 18. The results were different in the control group showing that an average of 10^5 coliforms/g was present before treatment. During the period of treatment of the other group, the bacterial counts in this group were 10^8/g and finally, when the study finished, their numbers were slightly higher (10^9/g) by day 18 of the Experiment. The population in this group varied from 10^4 to 10^{11} bacteria/g.

ii) Faecal streptococci: Few colonies of faecal streptococci developed on Slanetz and Bartley agar plates from the faecal

TABLE 5.12 Daily individual bacterial counts of total coliforms/g faeces from the treated group in Experiment 2

Study

Date	Days	Pig No. 68	Pig No. 69	Pig No. 97	Pig No. 98	Pig No. 99	X
05.03.88	0	N D	N D	N D	N D	N D	N D
08.03.88	3^	1.0×10^9	1.0×10^4	2.0×10^5	2.5×10^4	2.5×10^4	2.0×10^8
14.03.88	9	5.0×10^5	5.0×10^4	3.5×10^5	3.0×10^5	5.0×10^4	2.5×10^5
15.03.88	10	5.0×10^5	5.0×10^4	4.5×10^5	1.0×10^5	3.0×10^5	2.8×10^5
16.03.88	11	4.5×10^4	4.5×10^4	5.0×10^5	11.0×10^4	5.0×10^5	2.2×10^5
17.03.88	12	1.0×10^5	4.0×10^4	1.5×10^6	5.0×10^5	2.0×10^6	8.2×10^5
18.03.88	13*	5.0×10^6	1.0×10^5	5.0×10^6	2.5×10^6	1.5×10^6	2.8×10^6
19.03.88	14	2.5×10^5	1.0×10^5	1.0×10^7	5.0×10^6	2.0×10^7	7.0×10^6
20.03.88	15	1.0×10^6	5.0×10^4	5.0×10^4	1.0×10^5	3.5×10^5	3.1×10^5
21.03.88	16	1.0×10^7	12.0×10^4	4.0×10^5	2.0×10^5	5.0×10^4	2.1×10^6
22.03.88	17	1.0×10^5	2.0×10^5	5.0×10^9	1.0×10^7	5.0×10^8	1.1×10^9
23.03.88	18	5.0×10^6	5.5×10^4	5.0×10^7	5.0×10^4	1.5×10^5	1.1×10^7

N D = Not Done (Determined)

^ Beginning of treatment, * Withdrawal of treatment

TABLE 5.13 Daily individual bacterial counts of total coliforms/g faeces from the control group in Experiment 2

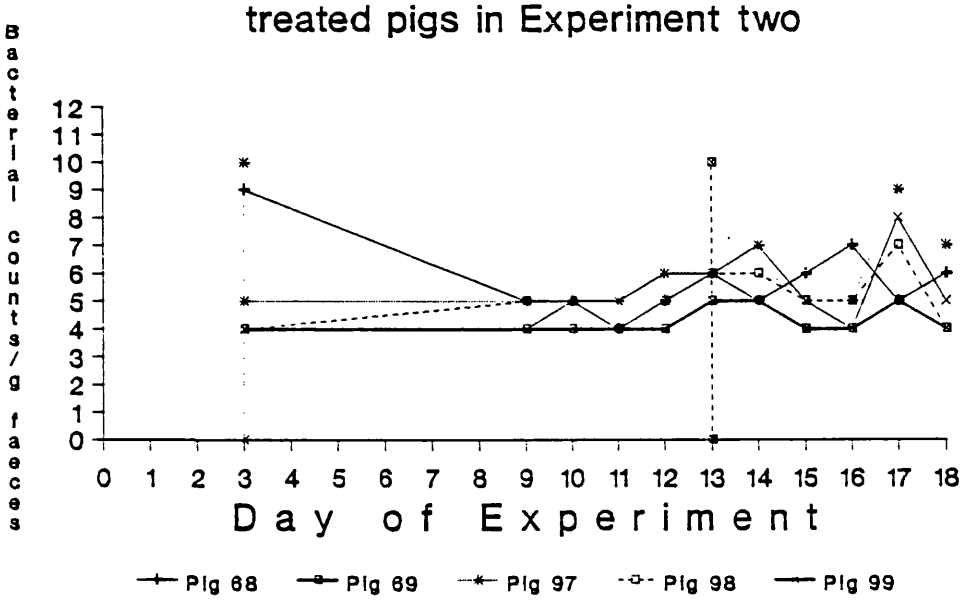
Study

Date	Days	Pig No. 50	Pig No. 95	Pig No. 97	Pig No. 99	Pig No. 100	X
05.03.88	0	N D	N D	N D	N D	N D	N D
08.03.88	3^	4.5×10^5	1.0×10^5	3.5×10^4	1.5×10^4	2.0×10^5	1.6×10^5
14.03.88	9	1.5×10^5	1.0×10^7	2.5×10^5	1.5×10^5	5.0×10^5	2.2×10^6
15.03.88	10	1.5×10^6	4.5×10^5	1.5×10^6	5.0×10^4	1.5×10^5	7.3×10^5
16.03.88	11	3.0×10^6	5.0×10^5	5.0×10^5	5.0×10^5	1.5×10^5	9.3×10^5
17.03.88	12	1.5×10^6	5.0×10^5	3.0×10^5	1.5×10^5	5.0×10^8	1.0×10^8
18.03.88	13*	5.0×10^5	2.5×10^5	5.0×10^9	5.0×10^9	1.0×10^{10}	4.0×10^9
19.03.88	14	1.0×10^6	5.5×10^5	3.5×10^5	5.0×10^5	1.5×10^5	5.1×10^5
20.03.88	15	2.0×10^6	5.0×10^5	5.0×10^6	5.0×10^7	5.0×10^6	1.2×10^7
21.03.88	16	1.5×10^8	9.5×10^4	1.5×10^5	1.0×10^9	2.0×10^{11}	4.0×10^{10}
22.03.88	17	1.0×10^{10}	5.0×10^5	5.0×10^7	3.5×10^6	5.0×10^5	2.0×10^9
23.03.88	18	5.0×10^7	2.0×10^5	2.0×10^5	2.5×10^5	6.0×10^5	1.0×10^7

N D = Not done (Determined)

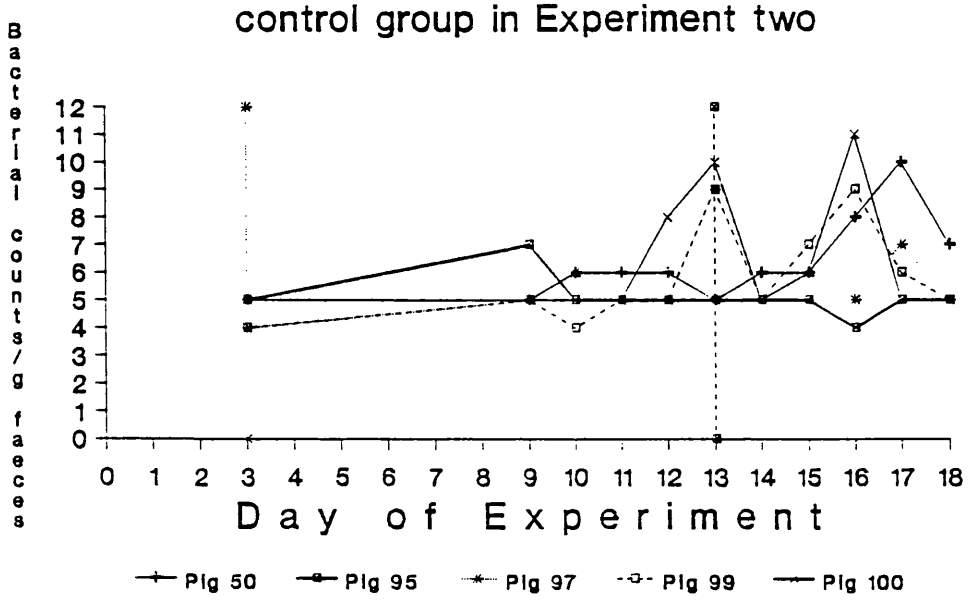
^ Beginning of treatment, * Withdrawal of treatment

FIGURE XII. Daily individual bacterial counts of total coliforms from the treated pigs in Experiment two



. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE XIII. Daily individual bacterial counts of total coliforms from the control group in Experiment two



. . . . Beginning of treatment
---- Withdrawal of treatment

samples of pigs in Experiment 2. The maximum number isolated in any pig of the treated group was 10^2 bacteria/g before treatment, 10^3/g during treatment and after its withdrawal 10^2/g (Figure XIV). Streptococci were present in the control group only before treatment at 10^3/g, and during lincomycin treatment in the treated group maximum numbers were 10^2/g. No streptococci were isolated after withdrawal (Figure XV).

iii) Salmonella spp.: No Salmonella species were isolated from any of the pigs of either group in this Experiment.

b) Microaerobic organisms

i) Campylobacter spp. were isolated in the treated group in numbers which varied from 10^2 to 10^7/g (Figure XVI), detail numbers are given in Table 5.14 (Appendix C). The populations remained at 10^4/g before and during treatment, but after withdrawal they increased to an average of 10^6/g. A similar pattern was observed in the control group(Figure XVII). Detailed figures are given in Table 5.15 (Appendix C). Mean numbers of organisms in the controls were 10^4/g, followed by a dramatic increase to 10^{10}/g between days 14 and 18 of the study.

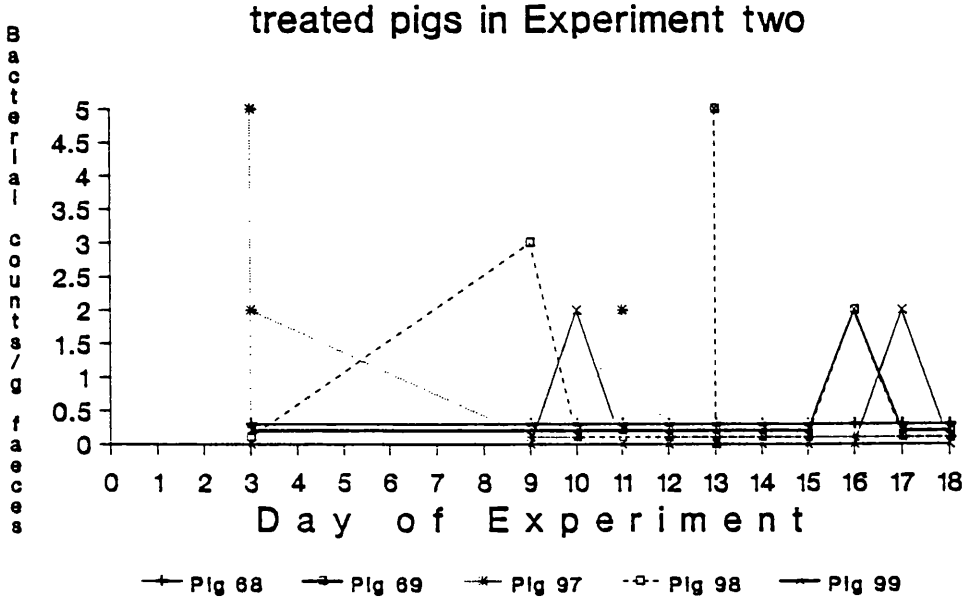
ii) Lactobacilli: Tables 5.16 and 5.17 give the counts of lactobacilli isolated, and they are also shown in Figures XVIII and XIX. The populations obtained from the treated group averaged 10^6/g before treatment, remained at 10^6/g during treatment and increased slightly to 10^7/g after withdrawal. From the control group in the same Experiment an average of 10^5/g was recovered at the beginning, increasing to 10^9/g during treatment and decreasing to 10^8/g at the end of the study on day 18.

c) Anaerobic organisms

i) Enteric spirochaetes: Spirochaetes such as Serpulina hyodysenteriae or S. innocens were not isolated from any of the animals of either group.

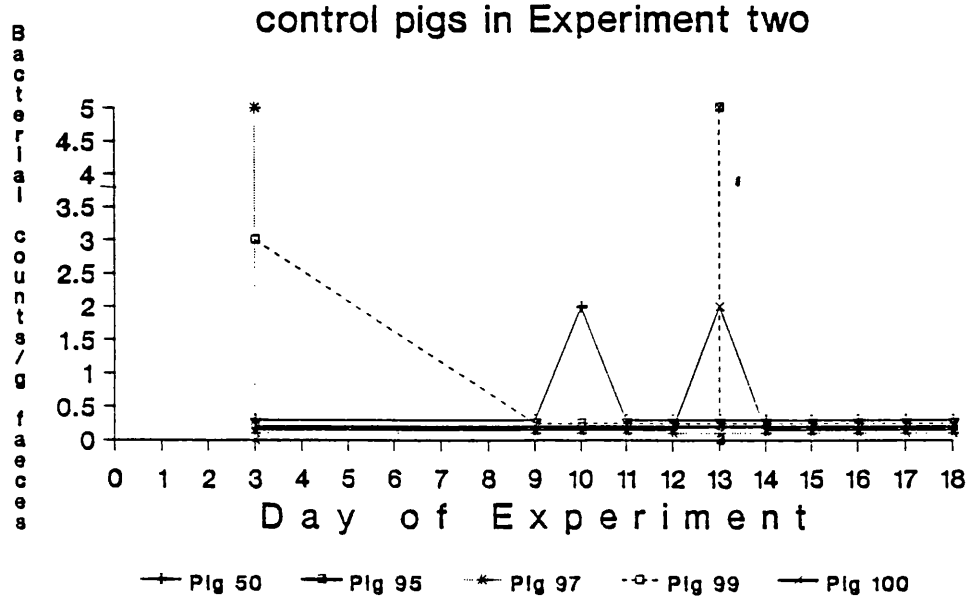
ii) Bacteroides spp.: Faecal bacterial counts of Bacteroides spp. are presented in Tables 5.18 and 5.19 from the treated and control groups of this Experiment. Counts were low

FIGURE XIV. Daily individual bacterial counts of faecal streptococci from the treated pigs in Experiment two



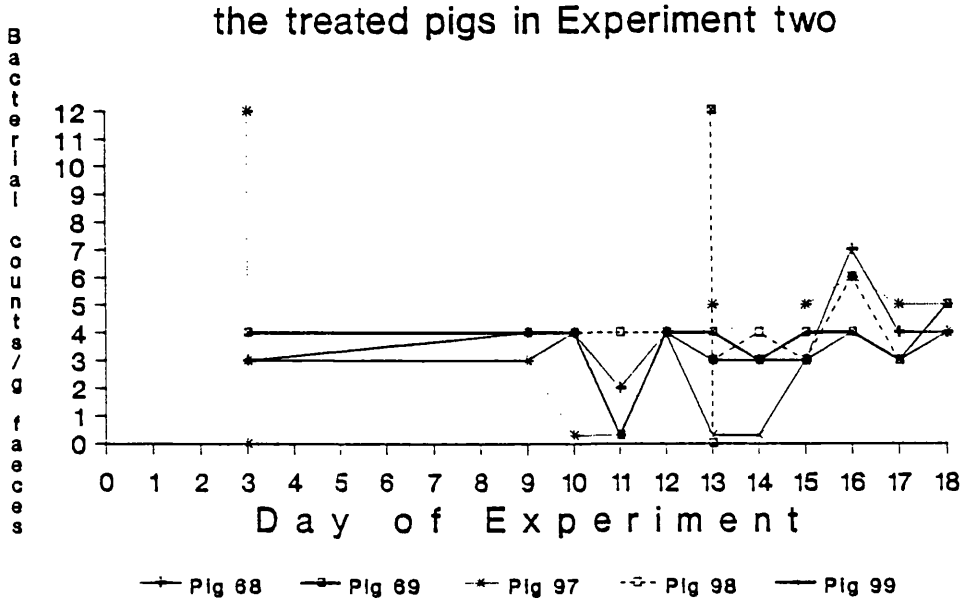
. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE XV. Daily individual bacterial counts of faecal streptococci from the control pigs in Experiment two



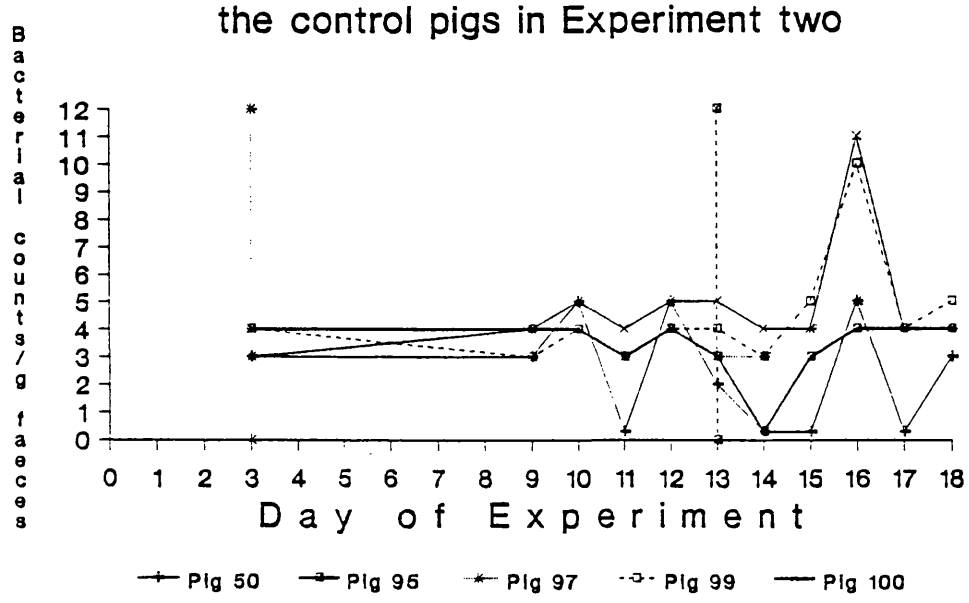
. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE XVI. Daily individual bacterial counts of Campylobacter spp. from the treated pigs in Experiment two



. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE XVII. Daily individual bacterial counts of Campylobacter spp. from the control pigs in Experiment two



. . . . Beginning of treatment
---- Withdrawal of treatment

TABLE 5.16 Daily individual bacterial counts of lactobacilli/g faeces from the treated group in Experiment 2

Study

Date	Days	Pig No. 68	Pig No. 69	Pig No. 97	Pig No. 98	Pig No. 99	$\bar{X}$
05.03.88	0	N D	N D	N D	N D	N D	N D
08.03.88	3^	2.0×10^7	1.0×10^5	2.5×10^5	6.0×10^4	5.0×10^4	4.0×10^6
14.03.88	9	1.5×10^5	5.0×10^3	5.0×10^4	2.0×10^5	5.0×10^5	1.8×10^5
15.03.88	10	5.0×10^4	1.5×10^5	5.0×10^5	2.5×10^5	5.0×10^6	1.1×10^6
16.03.88	11	2.5×10^5	2.5×10^4	3.5×10^5	2.0×10^5	1.5×10^5	1.9×10^5
17.03.88	12	3.0×10^5	3.0×10^4	2.0×10^5	3.0×10^5	5.0×10^5	2.6×10^5
18.03.88	13*	1.0×10^7	5.0×10^5	2.0×10^6	1.5×10^6	5.0×10^6	3.8×10^6
19.03.88	14	1.5×10^8	5.5×10^4	1.0×10^7	5.0×10^6	5.0×10^5	3.3×10^7
20.03.88	15	5.0×10^4	1.5×10^5	1.5×10^5	5.0×10^4	5.0×10^6	1.0×10^6
21.03.88	16	4.0×10^4	2.0×10^5	5.0×10^4	3.0×10^4	2.0×10^4	6.8×10^4
22.03.88	17	1.0×10^5	1.5×10^5	5.0×10^8	5.0×10^6	5.0×10^7	1.1×10^8
23.03.88	18	1.0×10^6	2.0×10^4	2.0×10^6	5.0×10^6	5.0×10^5	1.7×10^6

N D = Not Done (Determined)

^ Beginning of treatment, * Withdrawal of treatment

TABLE 5.17 Daily individual bacterial counts of lactobacilli/g faeces from the control group in Experiment 2

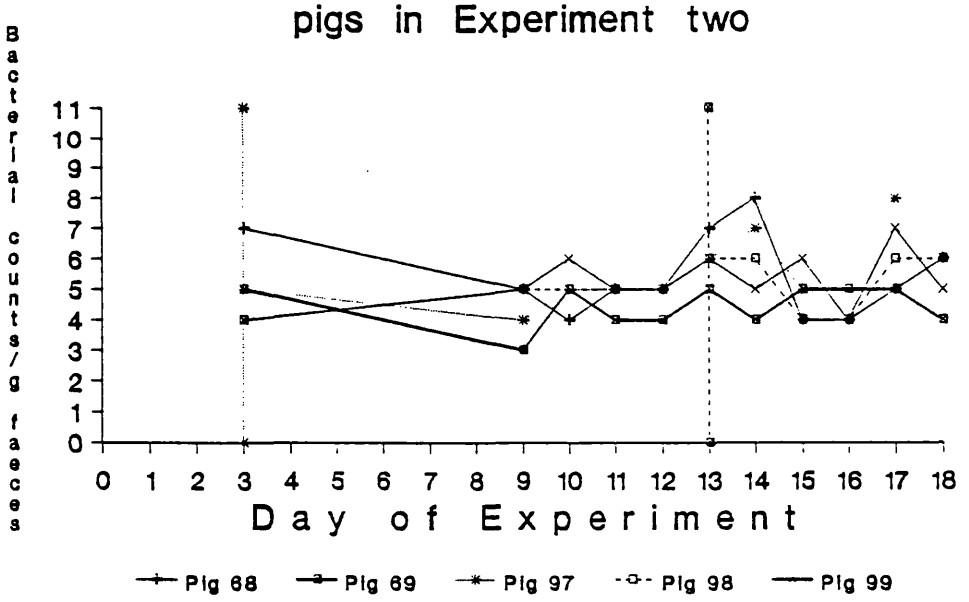
Study

Date	Days	Pig No. 50	Pig No. 95	Pig No. 97	Pig No. 99	Pig No. 100	$\bar{X}$
05.03.88	0	N D	N D	N D	N D	N D	N D
08.03.88	3^	9.5×10^5	1.5×10^5	5.0×10^4	5.0×10^3	1.5×10^5	2.6×10^5
14.03.88	9	2.0×10^5	1.0×10^7	5.0×10^5	1.5×10^5	1.0×10^7	4.1×10^6
15.03.88	10	5.0×10^5	4.0×10^5	5.0×10^5	6.5×10^4	5.0×10^5	3.9×10^5
16.03.88	11	1.5×10^6	1.0×10^7	1.0×10^6	7.5×10^4	2.0×10^5	2.5×10^6
17.03.88	12	2.0×10^6	5.0×10^6	5.0×10^5	2.5×10^6	1.5×10^6	2.3×10^6
18.03.88	13*	5.0×10^7	2.0×10^5	1.5×10^9	1.0×10^{10}	1.5×10^{10}	5.3×10^9
19.03.88	14	5.0×10^7	1.0×10^{10}	1.0×10^8	4.5×10^5	5.0×10^4	2.0×10^9
20.03.88	15	5.0×10^7	1.0×10^7	5.0×10^6	5.0×10^7	1.0×10^6	2.3×10^7
21.03.88	16	5.0×10^4	3.5×10^4	5.0×10^3	1.5×10^5	1.0×10^5	6.8×10^4
22.03.88	17	1.0×10^7	5.0×10^5	1.0×10^8	5.0×10^8	1.5×10^6	1.2×10^8
23.03.88	18	3.0×10^6	5.0×10^5	5.0×10^5	5.0×10^6	1.0×10^8	2.1×10^7

N D = Not Done (Determined)

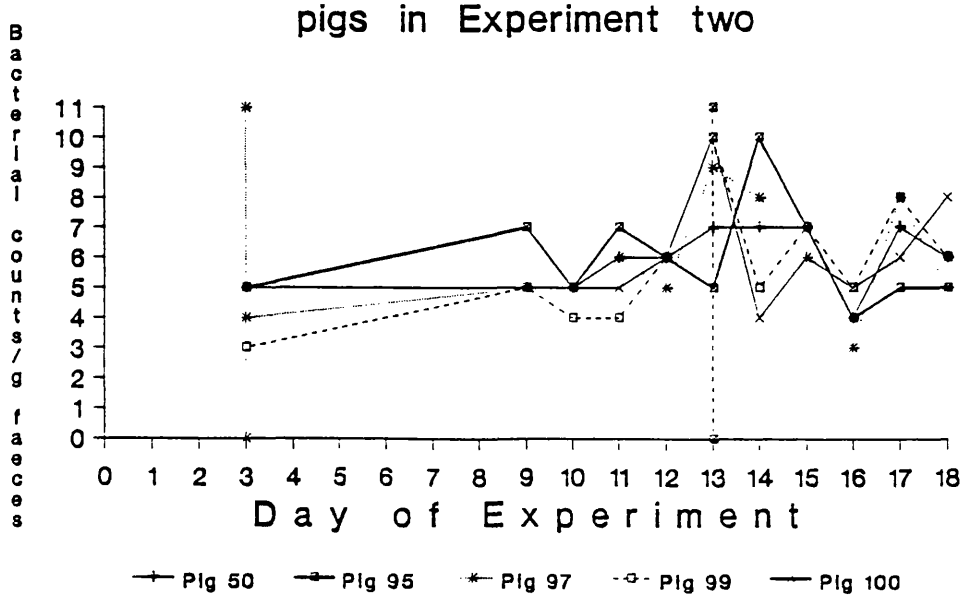
^ Beginning of treatment, * Withdrawal of treatment

FIGURE XVIII. Daily individual bacterial counts of lactobacilli from the treated pigs in Experiment two



. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE XIX. Daily individual bacterial counts of lactobacilli from the control pigs in Experiment two



. . . . Beginning of treatment
---- Withdrawal of treatment

and varied from 10^2 to 10^6/g in the treated group. On some days no bacteria resembling Bacteroides spp. were isolated (Figure XX). The average numbers before treatment were 10^4/g, during treatment 10^3/g, and after withdrawal an increase to 10^5/g was found. Days without Bacteroides spp. also occurred in the control group (Figure XXI) but when present their numbers were on average 10^4/g before, 10^3/g during treatment and 10^4/g at the end of the Experiment. Numbers varied from 10^2 to 10^5/g (Fig. XXI).

iii) Clostridium difficile was not isolated from any of the faecal samples examined from the pigs in this Experiment. In some cases colonies developed on the selective media used. However, after confirmatory testing the organisms proved not to be C. difficile in either vegetative or spore form.

iv) Clostridium perfringens type A (Vegetative cells): Colonies in the numbers shown in Tables 5.20 and 5.21 developed on the selective media used for the isolation of these organisms from faecal samples of pigs in both treated and control groups. Numbers in the treated group varied between 10^2 and 10^6 bacteria/g of faeces(Figure XXII). At the beginning of the study the average population was 10^4/g and it remained at this level during treatment and after withdrawal 10^4 organisms/g were still being isolated. No growth was detected in the treated group on some days(Fig. XXII). Populations between 10^2 to 10^5/g of faeces were detected in control pigs (Figure XXIII) and their average numbers were uniform throughout the Experiment, being 10^4/g.

Spore-forming strains.- Colonies which developed after heat-shock treatment were uncommon (Tables 5.22 and 5.23, Appendix C). Faecal samples of only three pigs of the treated group yielded the organisms, one at the beginning of the Experiment for three days, another (10^2/g) at the beginning and after withdrawal of the drug and the last only after the withdrawal of treatment at 10^2/g. All pigs in the control group yielded spore-forming C. perfringens type A but only for one or two days, and in low numbers (10^2-10^3/g).

3.2 E X P E R I M E N T 3

The results of this Experiment are presented here in the

TABLE 5.18 Daily individual bacterial counts of *Bacteroides spp.*/g faeces from the treated group in Experiment 2

Study

Date	Days	Pig No. 68	Pig No. 69	Pig No. 97	Pig No. 98	Pig No. 99	X
05.03.88	0	N D	N D	N D	N D	N D	N D
08.03.88	3^	5.0×10^4	2.5×10^4	1.5×10^5	1.5×10^4	1.0×10^5	6.8×10^4
14.03.88	9	5.0×10^3	N G	5.0×10^2	N G	N G	2.7×10^3
15.03.88	10	5.0×10^2	5.0×10^2	N G	N G	5.0×10^2	5.0×10^2
16.03.88	11	1.0×10^3	5.0×10^4	2.0×10^3	5.0×10^4	5.0×10^3	2.1×10^4
17.03.88	12	5.0×10^2	2.5×10^3	N G	N G	N G	1.5×10^3
18.03.88	13*	5.0×10^2	N G	N G	N G	N G	5.0×10^2
19.03.88	14	1.0×10^3	5.0×10^4	1.5×10^4	1.0×10^4	N G	1.9×10^4
20.03.88	15	5.0×10^4	2.0×10^5	1.5×10^5	5.0×10^4	5.0×10^4	1.0×10^5
21.03.88	16	5.0×10^3	2.0×10^4	1.0×10^4	N G	1.0×10^3	9.0×10^3
22.03.88	17	5.0×10^3	5.0×10^4	1.5×10^5	2.5×10^4	1.0×10^6	2.4×10^5
23.03.88	18	5.0×10^6	5.0×10^5	5.0×10^4	5.0×10^4	1.5×10^5	1.1×10^6

N D = Not Done (Determined) N G = No Growth

^ Beginning of treatment, * Withdrawal of treatment

TABLE 5.19 Daily individual bacterial counts of Bacteroides spp./g faeces from the control group in Experiment 2

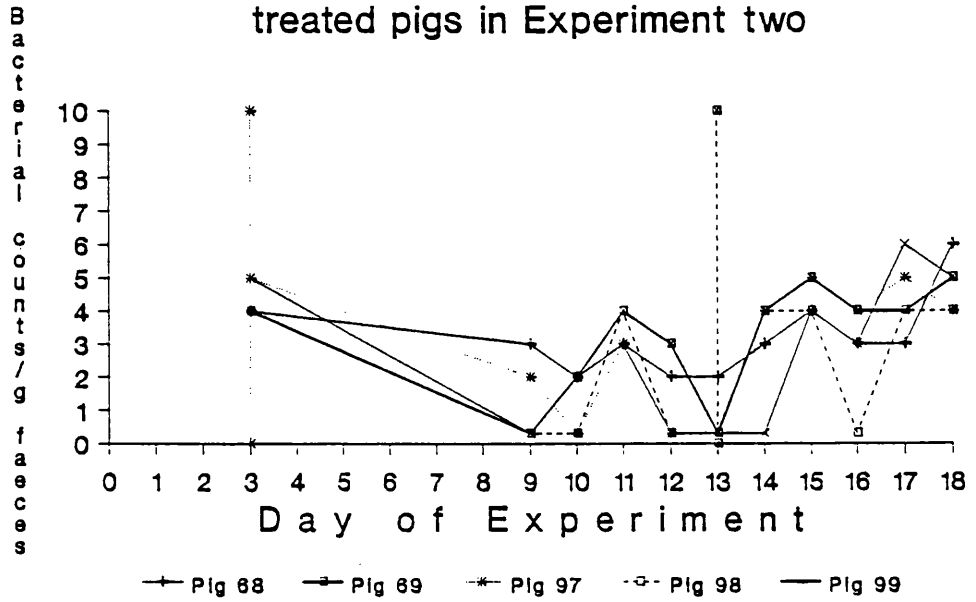
Study

Date	Days	Pig No. 50	Pig No. 95	Pig No. 97	Pig No. 99	Pig No. 100	X
05.03.88	0	N D	N D	N D	N D	N D	N D
08.03.88	3^	5.0×10^4	1.0×10^3	5.0×10^3	1.0×10^5	1.5×10^3	3.1×10^4
14.03.88	9	1.0×10^3	5.0×10^2	5.0×10^2	N G	1.0×10^3	7.5×10^2
15.03.88	10	5.0×10^2	N G	1.5×10^4	N G	5.0×10^2	5.3×10^3
16.03.88	11	2.0×10^3	5.0×10^3	1.0×10^3	N G	N G	2.6×10^3
17.03.88	12	N G	5.0×10^2	5.0×10^2	1.0×10^3	N G	6.6×10^2
18.03.88	13*	1.5×10^4	1.5×10^3	6.0×10^3	3.0×10^3	2.0×10^3	5.5×10^3
19.03.88	14	5.0×10^4	1.0×10^4	2.0×10^3	1.0×10^3	1.5×10^3	1.2×10^4
20.03.88	15	5.0×10^4	1.0×10^5	4.5×10^4	1.5×10^4	5.0×10^4	5.2×10^4
21.03.88	16	5.0×10^3	1.0×10^3	5.0×10^3	2.0×10^4	1.0×10^4	8.2×10^3
22.03.88	17	5.0×10^3	1.0×10^3	1.5×10^3	2.5×10^4	5.0×10^3	7.5×10^3
23.03.88	18	5.0×10^4	2.5×10^4	5.0×10^4	3.5×10^3	5.0×10^3	2.6×10^4

N D = Not Done (Determined) N G = No Growth

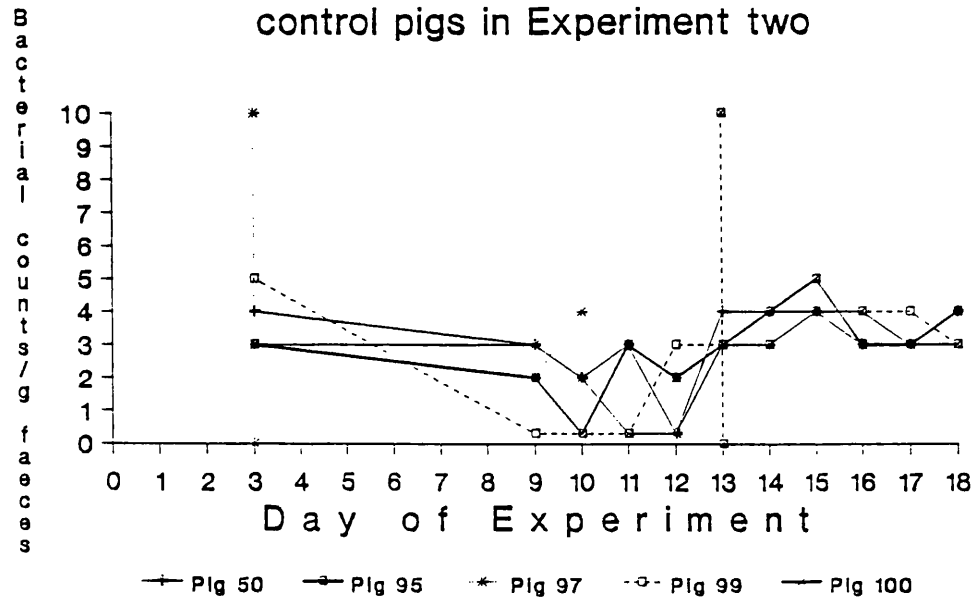
^ Beginning of treatment, * Withdrawal of treatment

FIGURE XX. Daily individual bacterial counts of Bacteroides spp. from the treated pigs in Experiment two



. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE XXI. Daily individual bacterial counts of Bacteroides spp. from the control pigs in Experiment two



. . . . Beginning of treatment
---- Withdrawal of treatment

TABLE 5.20 Daily individual bacterial counts of *Clostridium perfringens* type A (vegetative cells)/g faeces from the treated group in Experiment 2

Study

Date	Days	Pig No. 68	Pig No. 69	Pig No. 97	Pig No. 98	Pig No. 99	$\bar{X}$
05.03.88	0	N D	N D	N D	N D	N D	N D
08.03.88	3^	5.0×10^4	5.0×10^3	5.0×10^4	2.5×10^4	1.0×10^5	4.6×10^4
14.03.88	9	2.0×10^3	1.0×10^3	1.0×10^4	1.0×10^3	2.0×10^3	3.2×10^3
15.03.88	10	N G	N G	5.0×10^2	N G	5.0×10^2	5.0×10^2
16.03.88	11	1.0×10^4	1.5×10^3	1.5×10^5	5.0×10^3	1.5×10^5	6.3×10^4
17.03.88	12	5.0×10^2	1.0×10^3	1.0×10^3	1.5×10^3	2.5×10^3	1.3×10^3
18.03.88	13*	5.0×10^3	1.5×10^3	N G	3.0×10^3	5.0×10^2	2.5×10^3
19.03.88	14	1.0×10^4	1.0×10^5	2.0×10^3	5.0×10^3	5.0×10^3	2.4×10^4
20.03.88	15	2.0×10^4	5.0×10^4	5.0×10^3	5.0×10^3	1.0×10^5	3.6×10^4
21.03.88	16	5.0×10^3	5.0×10^4	1.0×10^5	1.0×10^5	5.0×10^3	5.2×10^4
22.03.88	17	1.5×10^4	3.0×10^4	5.0×10^3	2.5×10^4	1.5×10^6	3.1×10^5
23.03.88	18	5.0×10^2	5.0×10^2	5.0×10^4	N G	1.0×10^4	1.5×10^4

N D = Not Done (Determined) N G = No Growth

^ Beginning of treatment, * Withdrawal of treatment

TABLE 5.21 Daily individual bacterial counts of Clostridium perfringens type A (vegetative cells)/g faeces from the control group in Experiment 2

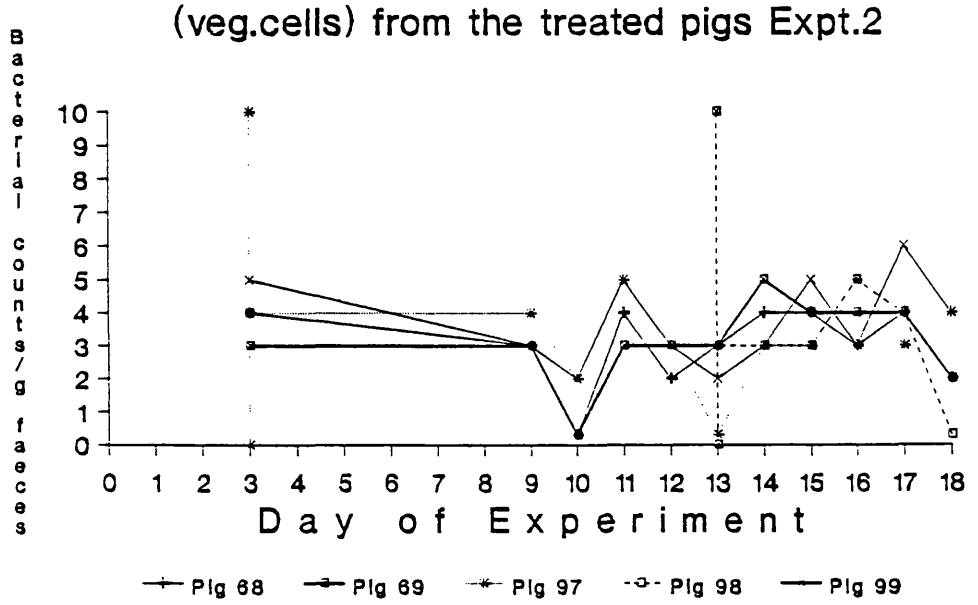
Study

Date	Days	Pig No. 50	Pig No. 95	Pig No. 97	Pig No. 99	Pig No. 100	$\overline{X}$
05.03.88	0	N D	N D	N D	N D	N D	N D
08.03.88	3^	5.0×10^4	1.0×10^5	5.0×10^4	5.0×10^4	1.0×10^4	5.2×10^4
14.03.88	9	1.5×10^5	5.0×10^4	2.0×10^3	4.0×10^3	5.0×10^3	4.2×10^4
15.03.88	10	5.0×10^2	2.0×10^3	1.5×10^3	N G	2.0×10^3	1.5×10^3
16.03.88	11	7.0×10^4	1.5×10^5	5.0×10^4	5.0×10^4	2.0×10^4	6.8×10^4
17.03.88	12	1.0×10^3	4.5×10^3	3.5×10^3	N G	2.5×10^3	2.8×10^3
18.03.88	13*	5.0×10^3	5.0×10^3	5.0×10^3	5.0×10^4	1.0×10^4	1.5×10^4
19.03.88	14	3.5×10^3	1.5×10^4	3.0×10^3	5.0×10^2	N G	5.5×10^3
20.03.88	15	2.0×10^3	5.0×10^3	5.0×10^4	5.0×10^3	1.5×10^3	1.2×10^4
21.03.88	16	1.0×10^4	2.0×10^3	5.0×10^3	5.0×10^5	5.0×10^3	1.0×10^5
22.03.88	17	5.0×10^4	2.0×10^4	1.0×10^4	5.0×10^3	5.0×10^4	2.7×10^4
23.03.88	18	N G	5.0×10^3	N G	2.0×10^4	N G	1.2×10^4

N D = Not Done (Determined) N G = No Growth

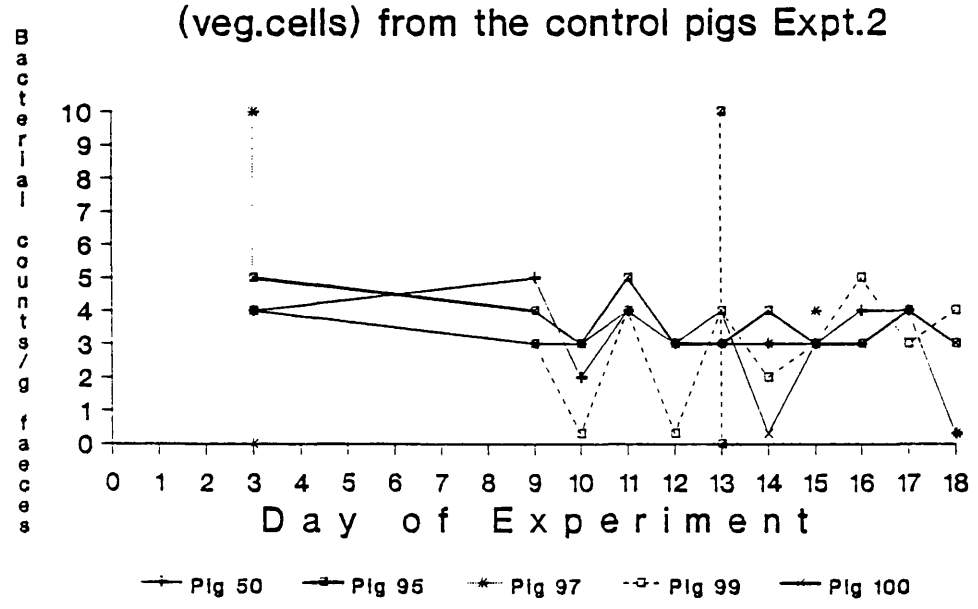
^ Beginning of treatment, * Withdrawal of treatment

FIGURE XXII. Daily individual bacterial counts of Clostridium perfringens type A (veg.cells) from the treated pigs Expt.2



. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE XXIII. Daily individual bacterial counts of Clostridium perfringens type A (veg.cells) from the control pigs Expt.2



. . . . Beginning of treatment
---- Withdrawal of treatment

order in which routine examinations were carried out. The bacterial counts presented were obtained from the results and summarised on Tables and represented in Figures. Farm staff sent pig 86 for slaughter in error on day 20. From day 20 of the Experiment pig 63 was monitored to provide individual data for the remaining period.

A) Clinical signs

The majority of the weaned minimal disease pigs used in Experiment 3 were in good condition, healthy and active. Pigs 67 and 88 from the treated group and pigs 27, 88, 89 and 93 from the control group were diarrhoeic on day zero but they recovered completely on day one when treatment started. During and after treatment the clinical signs from both groups were recorded and are presented below in Tables 5.24 and 5.32 and Figures XXIV and XXIX.

Diarrhoea was the major clinical abnormality noted and occurred in both treated and untreated groups of pigs both during the period of treatment and following its withdrawal. Pigs which were clinically diarrhoeic showed dehydration and general loss of condition, their appearance was dirty and the consumption of water increased. Rectal temperatures varied considerably but individuals in both treated and control groups had temperatures of 40^{o}C or more both during the initial period when treatment was being given and after it had been withdrawn. Pig 93 white developed a swelling over the hip joint on day 29 but remained in good condition and ate well until day 34 when observations ceased. No treatment was given.

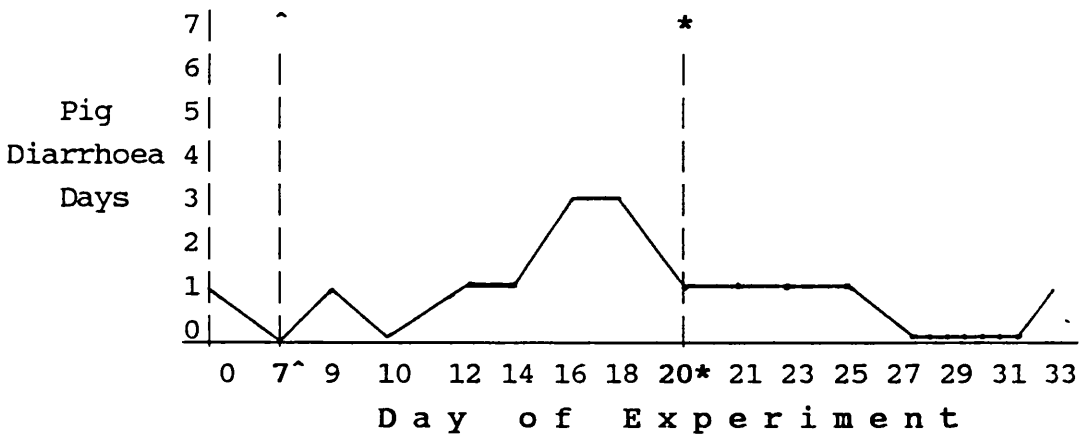
Faecal consistency: In this Experiment an increase in days with soft faeces occurred in the treated group after withdrawal of the antimicrobial particularly in pigs 86(63) and 88 (Table 5.24). However, the diarrhoea days decreased from 9 to 5 in the periods of treatment and withdrawal respectively (Figure XXIV). In this group pig 84 remained diarrhoeic for 9 days starting 2 days after treatment began and continuing over the periods of treatment (5) and withdrawal (4). At the end of the study its faeces remained soft in consistency.

TABLE 5.24 Individual faecal consistency changes recorded from pigs of the treated group in Experiment 3

Pig	Day of Experiment																Total
Number	0	7^	9	10	12	14	16	18	20*	21	23	25	27	29	31	33	D/Days
61	N	ND	ND	ND	ND	ND	ND	ND	ND	K							0/10
63	N	ND	ND	ND	ND	ND	ND	ND	N	N	S	S	N	S	N	S	0/8
64	N	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	K	0/16
65	N	ND	ND	ND	ND	ND	ND	ND	ND	ND	K						0/11
67	D	K															1/2
84	N	N	D	N	D	D	D	D	D	D	D	D	S	S	N	S	9/16
86(63)	N	S	N	N	N	N	D	S	K								1/8
87	N	N	S	N	N	N	S	S	N	N	S	N	S	S	N	S	0/16
88	D	N	N	N	N	N	D	D	N	S	S	S	S	N	N	D	4/16
90	N	N	N	N	N	N	N	D	N	N	S	N	N	N	N	N	1/16

N = Normal, S = Soft, D = Diarrhoea, ND = Not Determined, K = Killed, ^Beginning of treatment, *Withdrawal of treatment

FIGURE XXIV. Changes in faecal consistency recorded from pigs of the treated group in Experiment 3



^Beginning of treatment, *Withdrawal of treatment

In the control group 4/10 pigs were diarrhoeic before treatment, but on day 7 of the Experiment all those monitored were completely recovered. On day 12 Pig 93 was diarrhoeic and two days later pigs 92, 93 and 94 all developed diarrhoea which remained for 4 further days during and after treatment. After withdrawal pigs 92 and 93 continued with diarrhoea for 4 more days but Pig 94 showed a partial recovery passing soft faeces

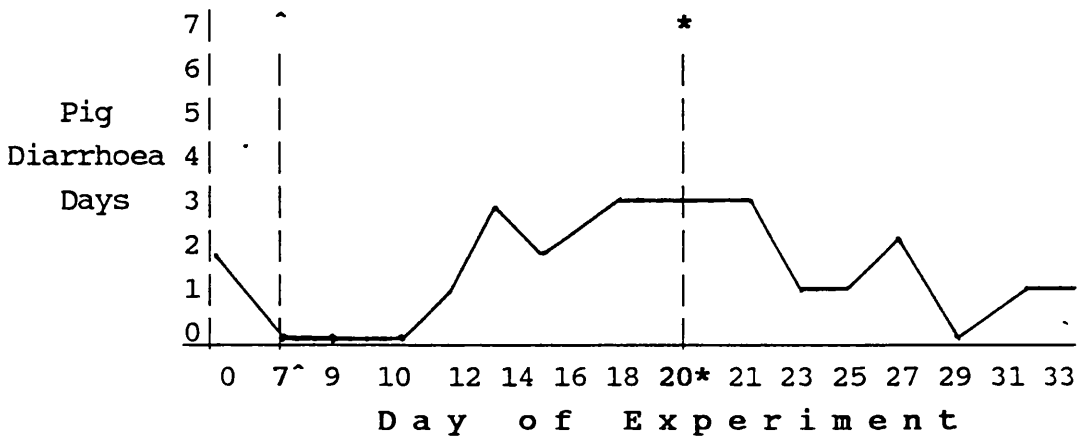
until the end of the study. Soft faeces increased after withdrawal of lincomycin (8 to 14), and diarrhoea days (9 to 12) also increased (Table 5.25 and Figure XXV).

TABLE 5.25 Individual faecal consistency changes recorded from pigs of the control group in Experiment 3

Pig	Day of Experiment																Total
Number	0	7^	9	10	12	14	16	18	20*	21	23	25	27	29	31	33	D/Days
27	D	ND	ND	ND	ND	ND	ND	ND	ND	ND	K						1/11
57	N	K															0/2
61	N	ND	ND	ND	ND	ND	ND	ND	ND	K							0/10
88	D	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	1/16
89	D	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	K	1/16
90(L)	N	N	S	S	S	N	N	N	N	N	S	S	S	S	S	N	0/16
90(R)	N	ND	ND	ND	ND	ND	ND	ND	K								0/9
92	N	N	N	N	N	D	D	D	D	D	D	D	D	S	N	N	8/16
93	D	S	S	N	D	D	S	D	D	D	S	S	D	S	D	D	9/16
94	S	N	S	N	S	D	D	D	D	D	S	S	S	S	N	S	5/16

N = Normal, S = Soft, D = Diarrhoea, ND = Not Determined,
K = Killed, (L) = Left ear, (R) = Right ear
^ Beginning of treatment, * Withdrawal of treatment

FIGURE XXV. Changes in faecal consistency recorded from pigs of the control group in Experiment 3



^Beginning of treatment, *Withdrawal of treatment

The diarrhoea in the treated group of Experiment 3 was fluid in pigs 67 and 88 (orange) on day 0, the latter also

showed fresh blood in its diarrhoeic stools. Neither blood nor excessive mucus were found in the remaining diarrhoeic samples. The diarrhoea in Pig 27 (white) of the control group was fluid, in Pig 89 (white) it was watery and in pigs 88 and 93 (white) it was scored as diarrhoea on day zero. On day 16 Pig 88 (white) showed normal stools except for an excess of mucus and dark blood. As in the treated group the remaining diarrhoeic samples obtained appeared to be free from blood or excess mucus.

The colour of these faecal samples varied from brown to dark brown except for the diarrhoeic stools which were usually of a yellowish appearance.

Rectal temperature: The temperatures recorded from individual pigs varied and did not always relate to the other clinical signs such as changes in faecal consistency and water or feed consumption. When this parameter changed drastically the presence of disease if any, was recorded. The detailed figures are given in Tables 5.26 and 5.27.

TABLE 5.26 Individual rectal temperatures of the treated pigs (orange tags) in Experiment 3

Pig	Day of Experiment										
Number	0	7^	9	10	12	14	16	18	20*	21	23
61	39.7	ND	ND	ND	ND	ND	ND	ND	ND	K	
63	39.8	ND	ND	ND	ND	ND	ND	ND	40.4	39.9	40.1
64	39.9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
65	40.6	ND	ND	ND	ND	ND	ND	ND	ND	ND	K
67	39.5	K									
84	39.9	39.8	40.0	39.7	39.9	39.8	39.5	39.7	39.8	39.3	39.5
86(63)	39.3	40.0	39.9	39.7	39.7	39.9	39.8	39.9	K		
87	39.7	39.7	40.0	39.4	39.7	39.5	40.4	39.9	40.7	39.5	40.4
88	40.3	39.8	39.6	39.5	39.6	39.5	39.7	40.6	40.4	40.2	39.6
90	39.7	40.0	39.5	39.5	39.8	39.9	39.7	40.0	40.5	39.8	40.1
X	39.7	39.9	39.8	39.6	39.7	39.7	39.8	40.0	40.4	39.7	39.9

K = Killed, N D = Not Determined

^ Beginning of treatment, * Withdrawal of treatment

NOTE: Temperatures are expressed in degrees Centigrade

TABLE 5.26 (Cont.) Individual rectal temperatures of the treated pigs (orange tags) in Experiment 3

Pig Number	Day of Experiment 25	27	29	31	33	Group X
61						39.7
63	40.1	39.9	40.0	39.4	40.2	40.0
64	ND	ND	ND	ND	K	39.9
65						40.6
67						38.5
84	40.1	40.0	39.5	39.9	40.8	39.8
86(63)						39.8
87	39.8	39.5	39.6	39.6	39.7	39.8
88	39.7	39.6	39.4	39.6	40.4	39.8
90	40.1	39.7	39.5	39.5	39.6	39.8
X	40.0	39.7	39.6	39.6	40.1	39.8

ND = Not Determined (Not Done), K = Killed

NOTE: Temperatures are expressed in degrees Centigrade

TABLE 5.27 Individual rectal temperatures of the control pigs (white tags) in Experiment 3

Pig Number	Day of Experiment 0	7^	9	10	12	14	16	18	20*	21	23
27	40.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	K
57	39.4	K									
61	40.0	ND	ND	ND	ND	ND	ND	ND	ND	K	
88	40.2	39.6	39.4	39.6	39.6	39.6	39.8	39.7	39.6	39.6	39.7
89	39.9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
90(L)	39.6	40.3	40.2	39.9	40.2	39.5	39.9	39.9	40.5	40.3	40.2
90(R)	39.6	ND	ND	ND	ND	ND	ND	ND	K		
92	39.6	39.7	39.7	39.9	39.8	39.7	39.7	39.6	39.5	39.6	39.8
93	40.2	40.0	40.0	39.6	39.9	40.1	39.7	39.8	40.2	40.0	40.2
94	39.9	39.5	39.9	39.5	39.7	40.5	40.8	40.7	40.3	39.9	39.9
X	39.8	39.8	39.8	39.7	39.8	39.9	40.0	39.9	40.0	39.9	40.0

ND = Not Determined, K = Killed, (L)= Left ear, (R)= Right ear

^ Beginning of treatment, * Withdrawal of treatment

NOTE: Temperatures are expressed in degrees Centigrade

TABLE 5.27 (Cont.) Individual rectal temperatures of the control pigs (white tags) in Experiment 3

Pig	Day of Experiment					Group
Number	25	27	29	31	33	X
27						40.0
57						39.4
61						40.0
88	39.4	39.4	39.8	39.3	39.9	39.6
89	ND	ND	ND	ND	K	39.9
90(L)	40.1	40.1	39.9	40.1	40.7	40.1
90(R)						39.6
92	39.5	39.5	39.4	39.4	39.4	39.6
93	39.7	39.8	39.9	39.8	39.8	39.9
94	40.0	39.7	39.7	39.4	40.1	40.0
X	39.7	39.7	39.7	39.6	40.0	39.8

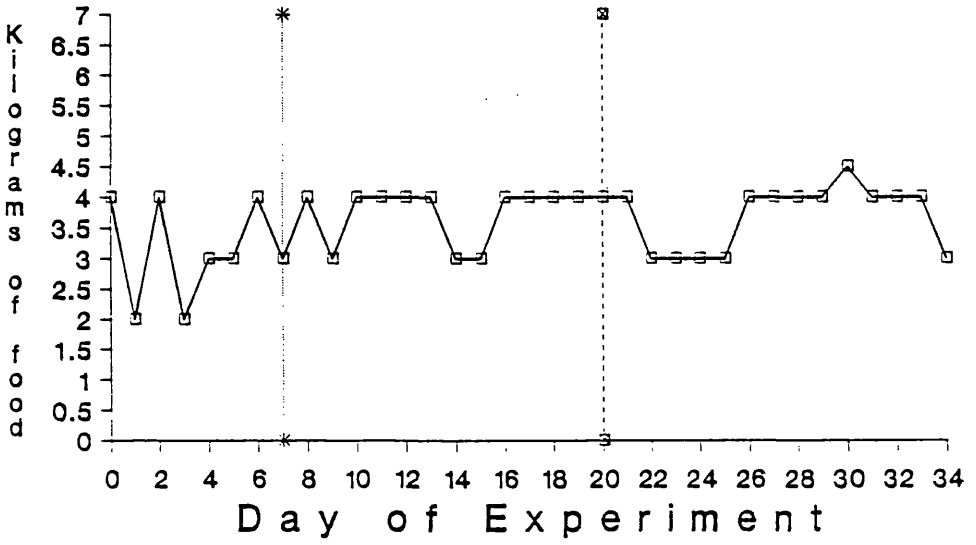
(L) = Left ear, (R) = Right ear,
ND = Not Determined (Not Done), K = Killed
NOTE: Temperatures are expressed in degrees Centigrade

There was marked variation in temperatures in individual animals. In both groups there were pigs with raised rectal temperatures such as pigs 65 (40.6°C), 84 (40.8°C), 87(40.7°C) and 88 (40.6°C) from the treated group, and pigs 90 (L) (40.7°C) and 94 (40.8°C) from the control group. Pig 90(L) was febrile with temperatures greater than 40°C on 10 out of 16 days of sampling, varying between 40.1°C(minimum) to 40.7°C (maximum). High temperatures were recorded in pig 94 on 6 out of 16 sampling days ranging from 40.0°C to 40.8°C.

Feed and water consumption: The daily feed consumption records from the treated and control groups in this Experiment are presented below in Figures XXVI and XXVII.

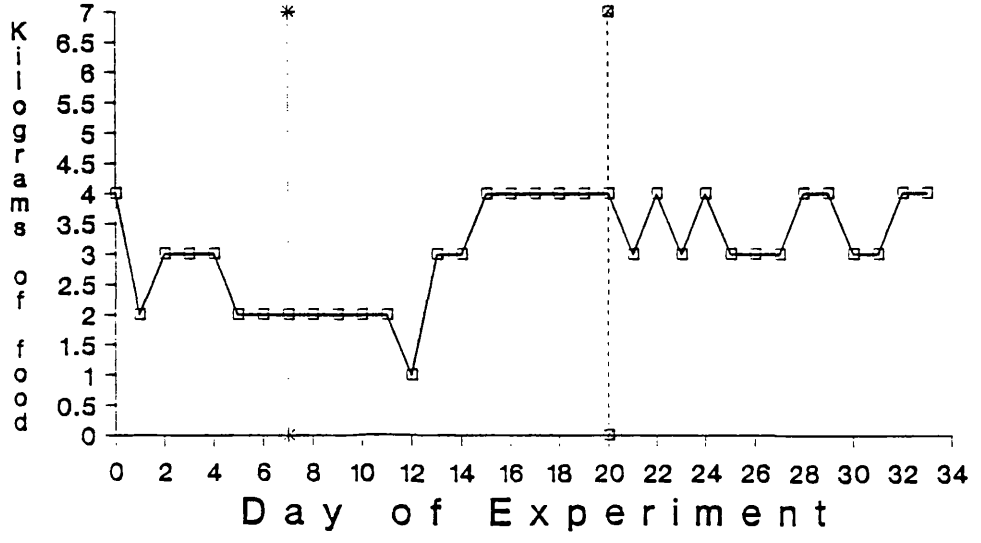
The water consumption records shown in Table 5.28 and Figures XXVIII and XXIX show that the treated animals drank more water during treatment. No spillage was observed in the treated groups.

FIGURE XXVI. Feed consumption of the treated pigs in Experiment three



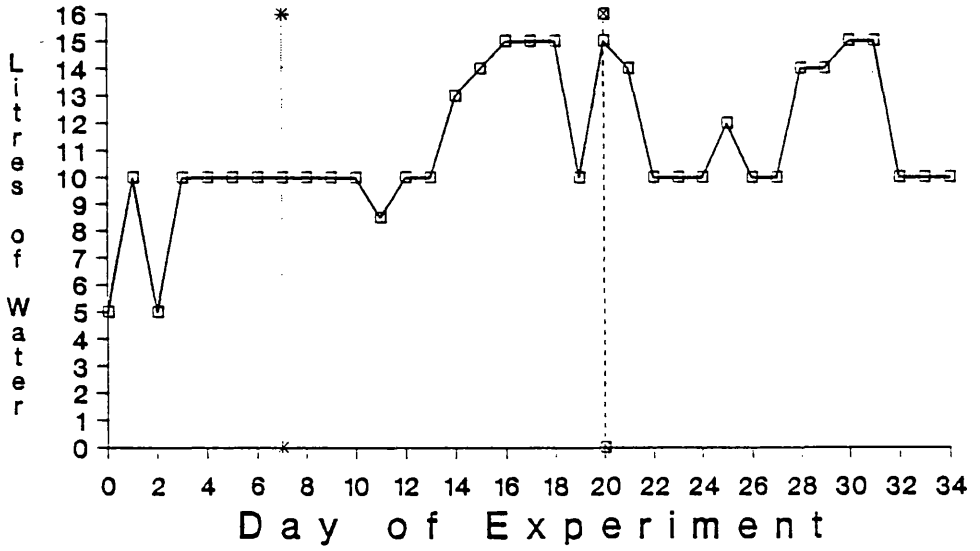
. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE XXVII. Feed consumption of the control pigs in Experiment three



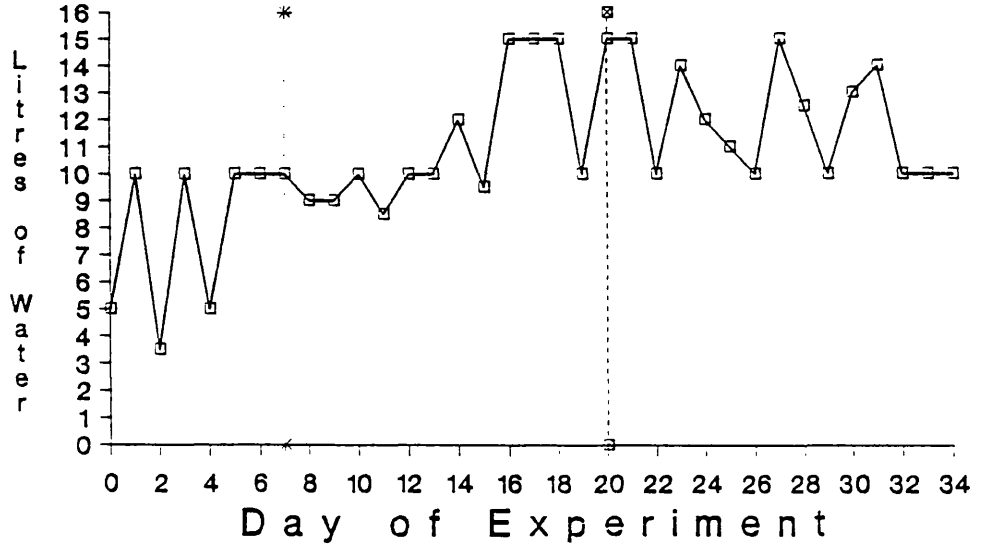
. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE XXVIII. Water consumption of the treated pigs in Experiment three



. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE XXIX. Water consumption of the control pigs in Experiment three



. . . . Beginning of treatment
---- Withdrawal of treatment

TABLE 5.28 Water consumption record from the treated and control groups in Experiment 3

Group	Days before treatment (7)	Days during treatment (13)	Days after treatment (14)
Treated	70	165.5	164
Control	63.5	148	170.5

NOTE: Water consumption is expressed in litres

Daily liveweight gain: The weekly individual live weights recorded from the pigs in Experiment 3 in both treated and control groups over the periods 0 to 7, 7 to 21 and 21 to 34 days are given below in Tables 5.29 and 5.30, and the individual weekly liveweight gains are presented in Tables 5.31 and 5.32.

TABLE 5.29 Individual liveweights of the treated pigs in Experiment 3, expressed in kilograms

Pig Number	Day of Experiment 0	7^	14	21*	27	34	Total Gain
84	5.2	6.8	9.3	10.9	14.7	18.9	13.7
86(63)	6.8	9.3	11.9	13.7	16.3	20.1	13.3
87	5.8	5.8	9.0	12.0	14.6	18.7	12.9
88	6.8	8.6	10.3	13.3	16.1	19.7	12.9
90	6.5	8.1	9.8	12.5	14.8	18.7	12.2
Total	31.1	38.6	50.3	62.4	76.5	96.1	65.0

^Beginning of treatment, *Withdrawal of treatment

TABLE 5.30 Individual liveweights of the control pigs in Experiment 3, expressed in kilograms

Pig Number	Day of Experiment 0	7^	14	21*	27	34	Total Gain
88	4.5	5.9	7.3	9.6	12.2	15.4	10.9
90(L)	5.7	6.8	9.2	12.4	15.6	19.0	13.3
92	5.9	7.6	9.7	10.9	13.5	17.3	11.4
93	5.6	8.5	11.3	14.0	17.2	19.9	14.3
94	6.3	8.4	10.6	13.7	16.8	21.1	14.8
Total	28.0	37.2	48.1	60.6	75.3	92.7	64.7

^Beginning of treatment, *Withdrawal of treatment

TABLE 5.31 Individual daily live weight gains of the treated pigs for days 0-7,7-21 and 21-34, in Experiment 3

Pig Number	Day of Experiment 0-7	7-21	21-34
84	0.228	0.293	0.571
86(63)	0.357	0.314	0.457
87	0.000	0.443	0.479
88	0.257	0.336	0.457
90	0.228	0.314	0.443
X	0.214	0.340	0.481

TABLE 5.32 Individual daily live weight gains of the control pigs for days 0-7,7-21 and 21-34, in Experiment 3

Pig Number	Day of Experiment 0-7	7-21	21-34
88	0.200	0.264	0.414
90(L)	0.157	0.400	0.471
92	0.243	0.236	0.457
93	0.414	0.393	0.421
94	0.300	0.379	0.529
X	0.263	0.334	0.458

NOTE: Weight gains are expressed in kilograms

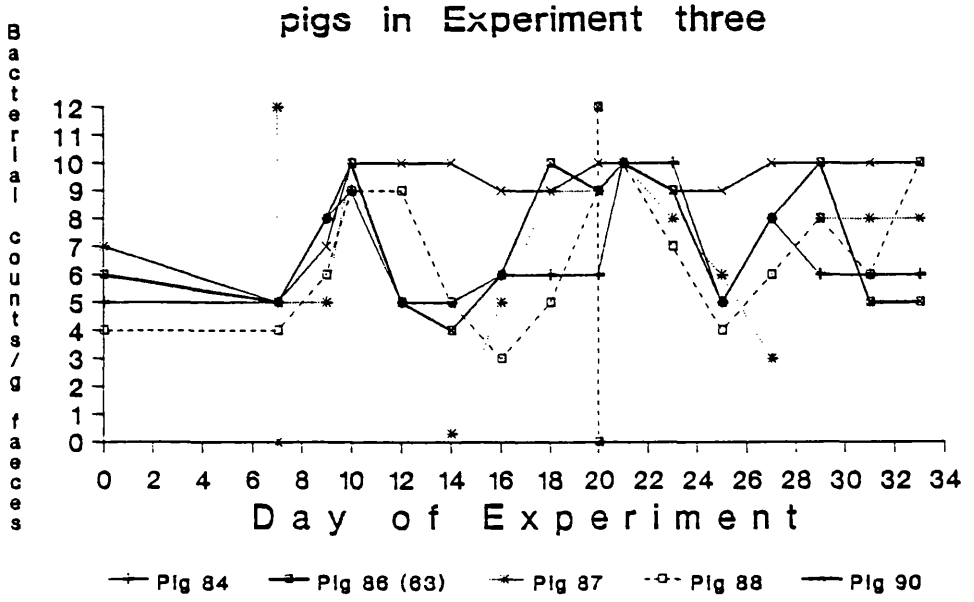
B) Bacteriological examinations of faeces

Over the periods before, during and after lincomycin treatment quantitative and qualitative bacteriological examinations were performed on faeces obtained individually from the pigs in both treated and control groups. The results of these examinations are presented in Tables 5.33 to 5.48 as arithmetic means, and in Figures XXX to XLV.

a) Aerobic organisms

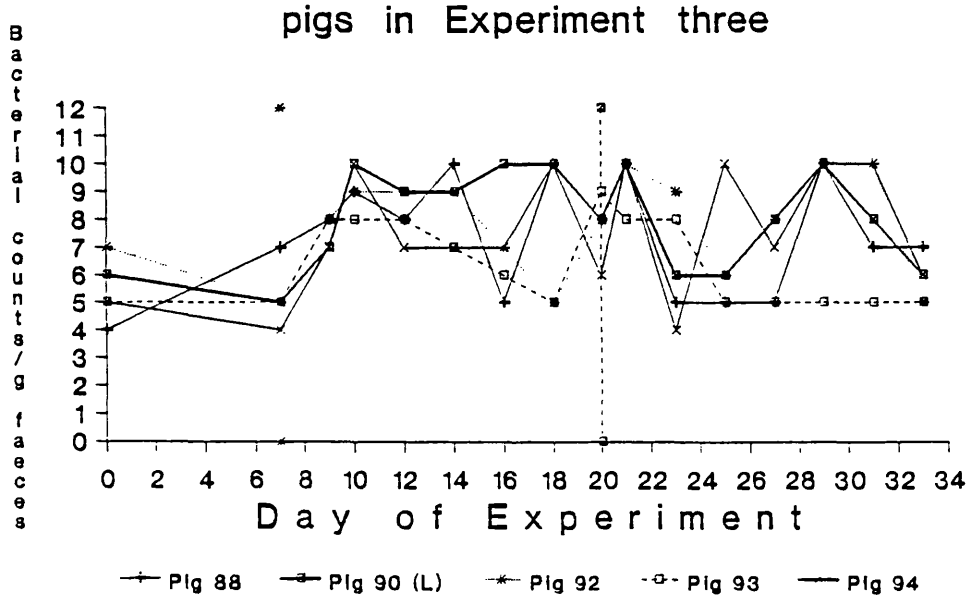
i) Total coliforms: The numbers of total coliforms (counted on MacConkey agar) in the treated group averaged 10^6/g before treatment, 10^9/g during treatment, and remained at 10^9/g until the end of the study. Similar figures were recorded from the

FIGURE XXX. Individual bacterial counts of total coliforms from the treated pigs in Experiment three



. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE XXXI. Individual bacterial counts of total coliforms from the control pigs in Experiment three



. . . . Beginning of treatment
---- Withdrawal of treatment

controls. The variation was from 10^3 to 10^{10} bacteria/g of faeces (Figure **XXX**). In the control group the numbers varied from 10^4 to 10^{10}/g as may be seen below in Figure **XXXI**. Total counts and means maybe found in Appendix **C**, Tables **5.33** and **5.34**.

ii) E. coli: The numbers of E. coli (counted on Sheep blood agar) resembled closely those counted on MacConkey agar (Tables **5.35** and **5.36**, Appendix **C**) Figs. **XXXII** and **XXXIII**, below. However, the counts of total coliforms obtained on MacConkey agar plates before treatment (10^6) were apparently lower than those of E. coli (10^7) isolated from Sheep blood agar plates.

In this Experiment the faecal samples were also examined for Beta haemolytic E. coli by culture on Sheep blood agar plates. Beta haemolytic colonies were found in the treated group only on days **27** (Pig **90**), and **29** (Pig **88**), and in the control animals only on days **0** (Pig **88**), and **27** (Pig **92**).

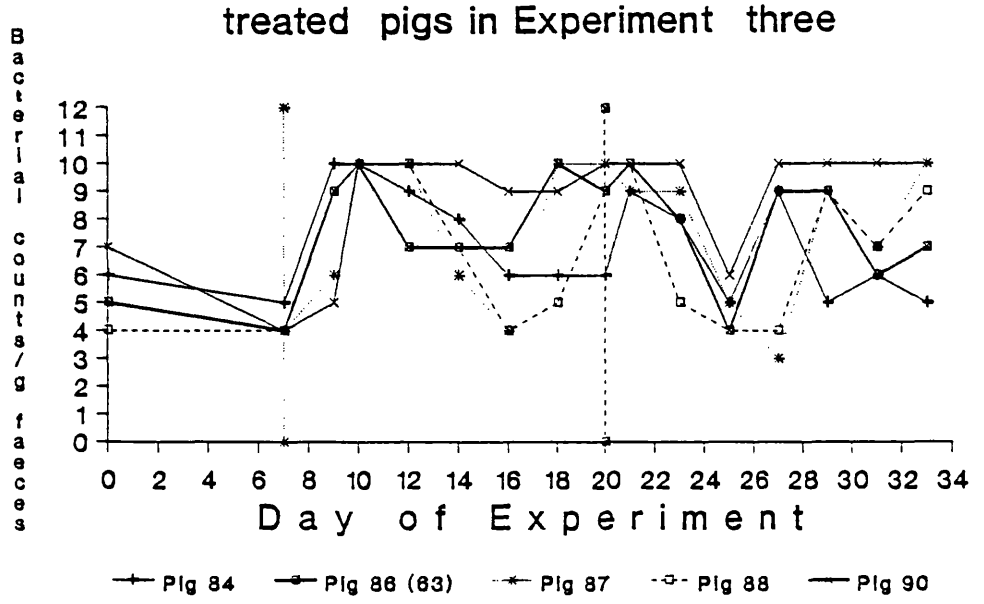
iii) Faecal streptococci: These organisms were isolated rarely from the Slanetz and Bartley agar plates inoculated with the faecal samples (Tables **5.37** and **5.38**, Appendix **C**). The numbers present were 10^3 bacteria/g before and during treatment. After withdrawal of treatment only 10^2/g were present (Figure **XXXIV**). 10^3/g were present in the control group at the beginning of the study and this level was maintained until day **21** of the study, but at the end as may be observed in Figure **XXXV** their numbers increased to 10^4 bacteria/g of faeces.

iv) Salmonella spp.: As in the previous Experiment, no salmonellae were isolated from any of the pigs in either group.

b) Microaerobic organisms

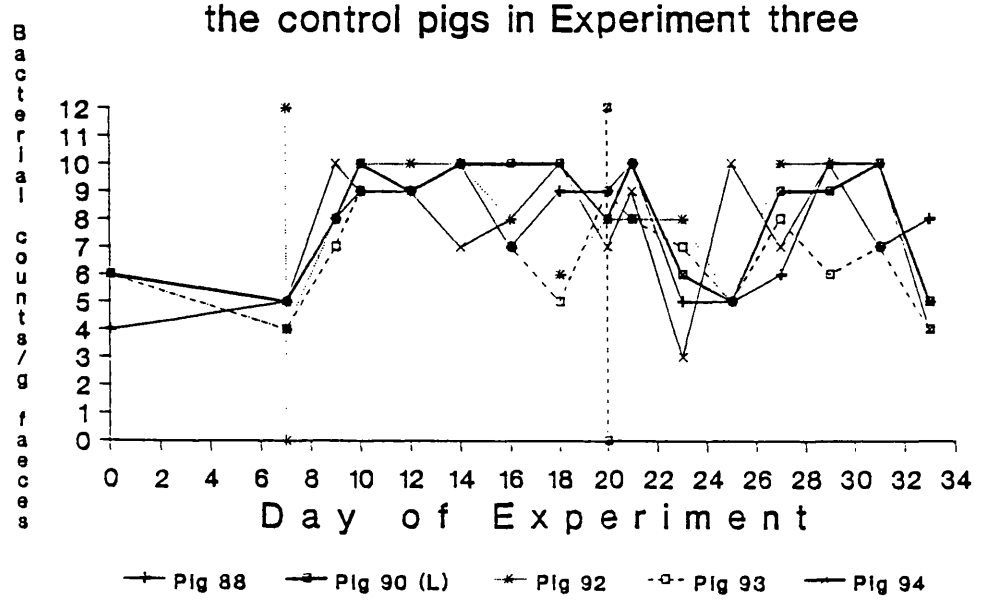
i) Campylobacter spp.: Counts of Campylobacter spp. found in the pigs from the treated group in this Experiment were 10^3/g before treatment, 10^4/g during treatment and remained at 10^4/g after the withdrawal of the antimicrobial (Figure **XXXVI**). Similar behaviour was recorded from the control group; 10^5/g before, 10^5/g during treatment, and 10^6/g after drug withdrawal, Figure **XXXVII** (Tables **5.39** and **5.40**, Appendix **C**).

FIGURE XXXII. Individual bacterial counts of total coliforms (E. coli) from the treated pigs in Experiment three



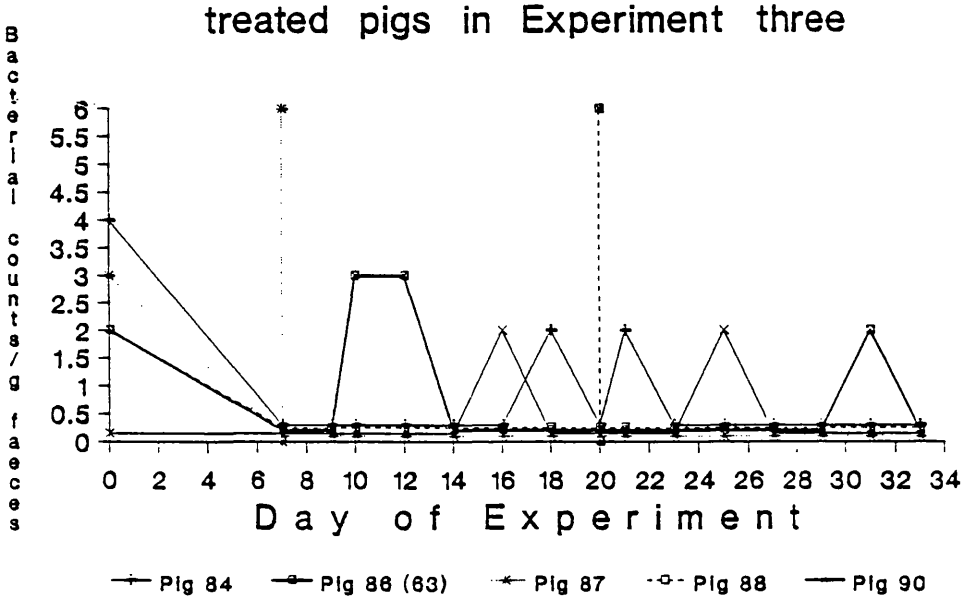
. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE XXXIII. Individual bacterial counts of total coliforms (E.coli) from the control pigs in Experiment three



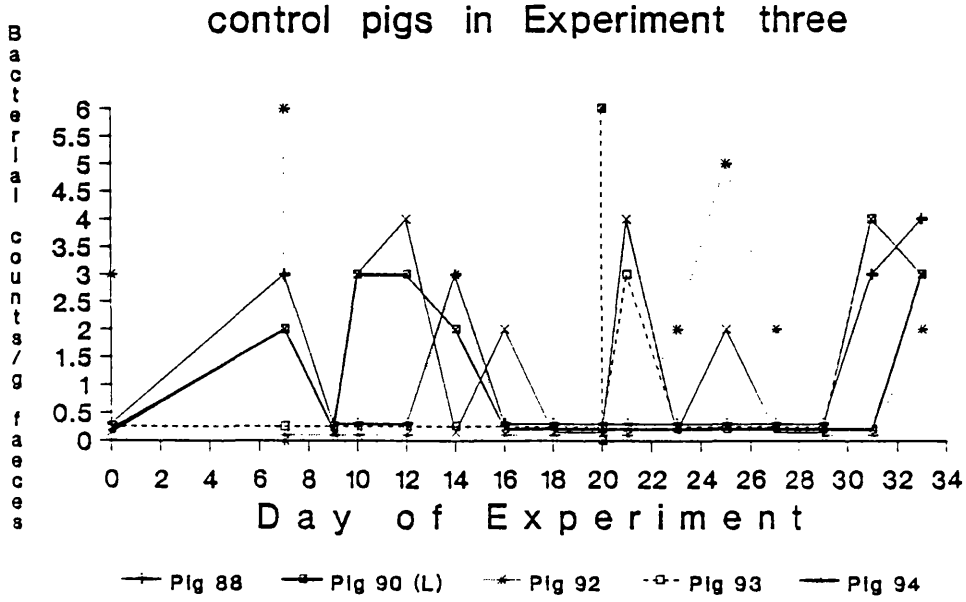
. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE XXXIV. Individual bacterial counts of faecal streptococci from the treated pigs in Experiment three



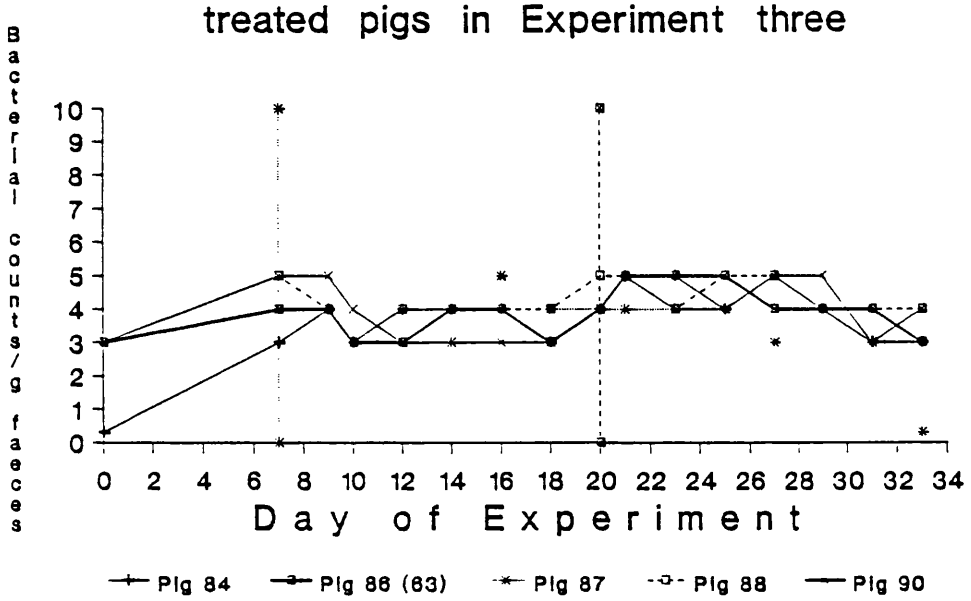
. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE XXXV. Individual bacterial counts of faecal streptococci from the control pigs in Experiment three



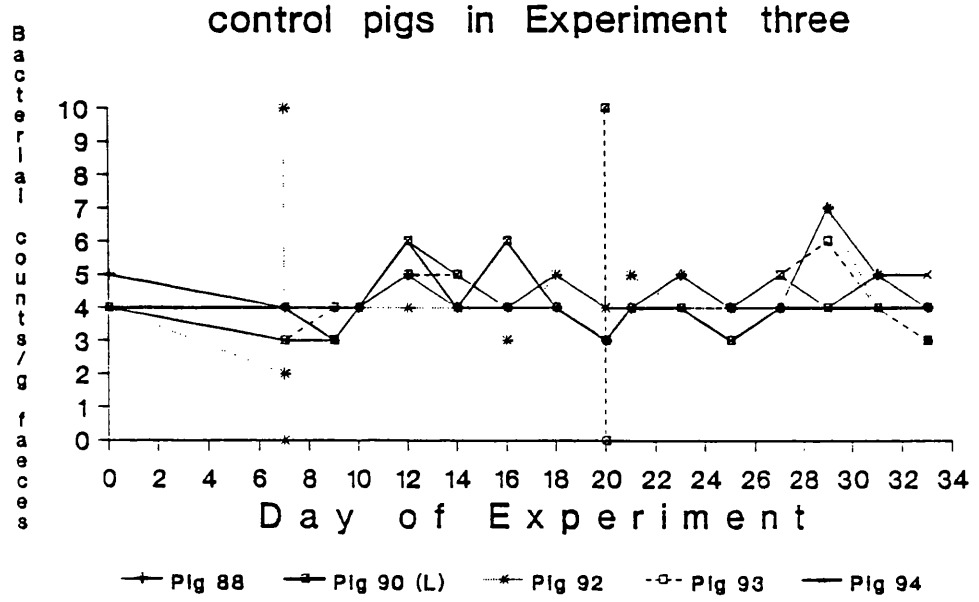
. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE XXXVI. Individual bacterial counts of Campylobacter spp. from the treated pigs in Experiment three



. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE XXXVII. Individual bacterial counts of Campylobacter spp. from the control pigs in Experiment three



. . . . Beginning of treatment
---- Withdrawal of treatment

ii) Lactobacilli: Populations of lactobacilli were of 10^5/g at the beginning, increased to 10^9/g during treatment and remained at 10^9/g after the withdrawal of lincomycin (Figure **XXXVIII**). The population of lactobacilli in the control group behaved in exactly the same way (Figure **XXXIX**). The individual counts of lactobacilli isolated are presented in Tables **5.41** and **5.42** (Appendix **C**).

c) Anaerobic organisms

i) Enteric spirochaetes: Spirochaetes such as *Serpulina hyodysenteriae* or *Serpulina innocens* were not isolated from any of the animals of either group in this Experiment.

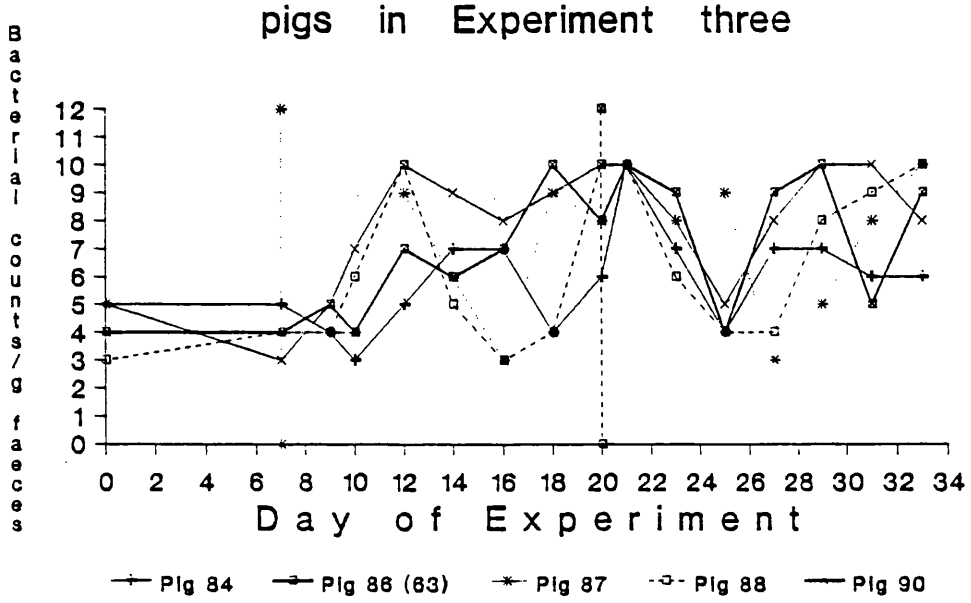
ii) *Bacteroides spp.*: Levels of *Bacteroides spp.* in the treated group dropped dramatically following treatment (Fig. **XL**) and this drop occurred, but to a lesser extent in the controls. Mean levels of 10^5/g were found prior to treatment, remained at 10^5/g during treatment and increased to 10^8/g after withdrawal of the antimicrobial. Populations rose earlier in the control group averaging 10^4/g at the beginning rising to 10^7/g during treatment and reached 10^8/g at the end of the Experiment (Fig. **XLI**). The variation in populations in both groups was between 10^2 to 10^9/g of faeces (Tables **5.43** and **5.44**, Appendix **C**).

iii) *Clostridium difficile* was not isolated from any of the faecal samples examined from the pigs examined. Colonies developed on the selective media, but after confirmatory testing the organisms proved not to be *C. difficile* in either vegetative or spore form.

iv) *Clostridium perfringens* type A (vegetative cells: *C. perfringens* type A was isolated from the treated group in numbers of 10^5/g at the beginning, 10^6/g during treatment and 10^7 organisms per gram of faeces after withdrawal of treatment. Their numbers varied from 10^2 to 10^8/g (Figure **XLII**). Populations of 10^4/g, increased to 10^8/g during treatment and remained at 10^8/g until the end of the study in the control animals. The variation in their populations was between 10^2 to 10^9/g (Figure **XLIII**). Actual counts are given in Tables **5.45** and **5.46**, Appendix **C**.

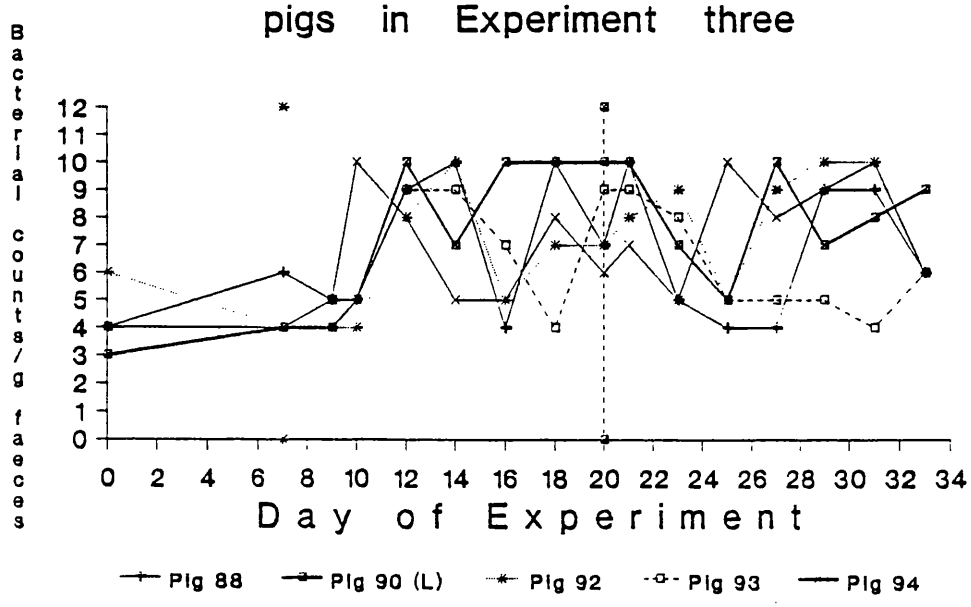
Spore-forming strains.- Only three pigs of the treated

FIGURE XXXVIII. Individual bacterial counts of lactobacilli from the treated pigs in Experiment three



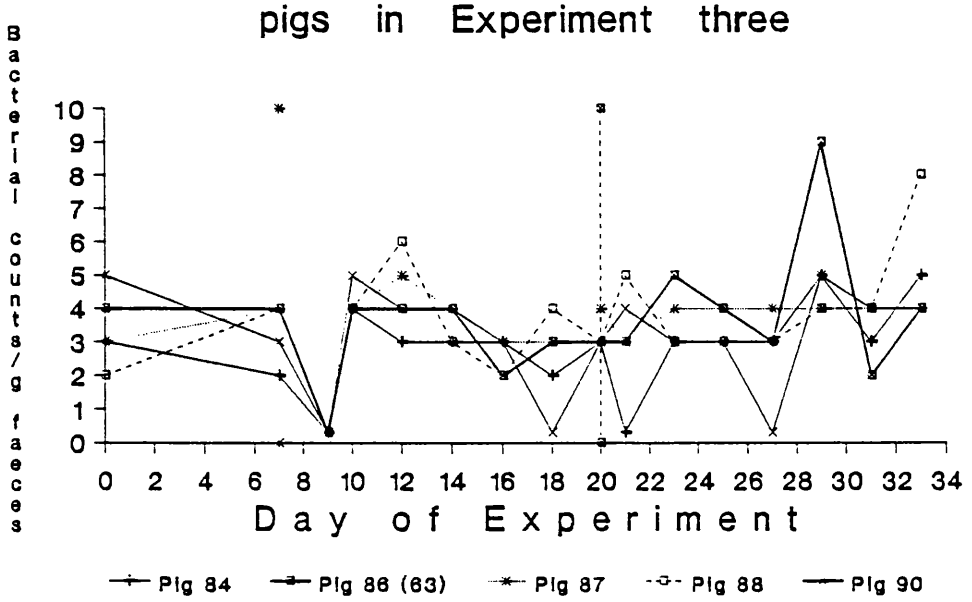
. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE XXXIX. Individual bacterial counts of lactobacilli from the control pigs in Experiment three



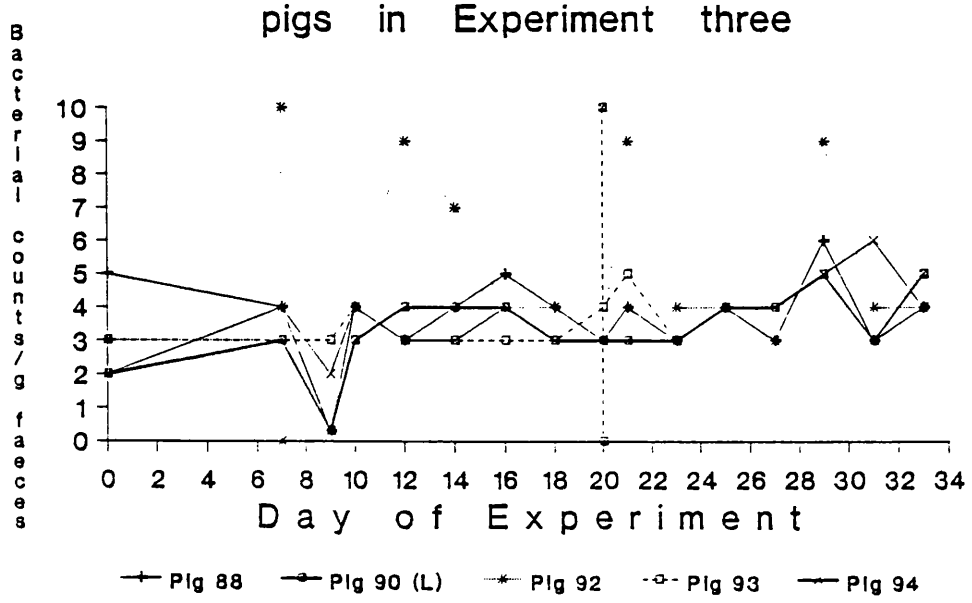
. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE XL. Individual bacterial counts of Bacteroides spp. from the treated pigs in Experiment three



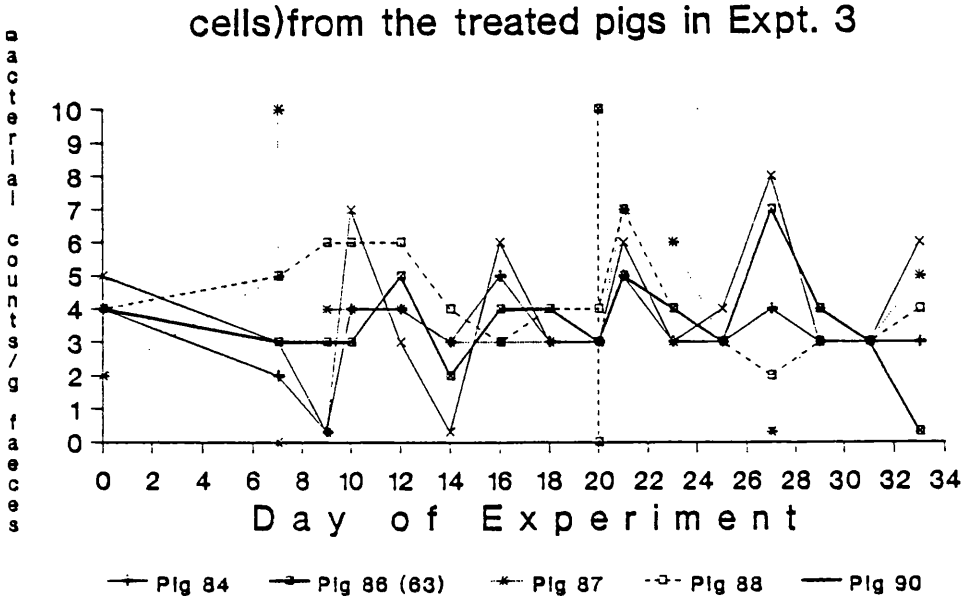
. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE XLI. Individual bacterial counts of Bacteroides spp. from the control pigs in Experiment three



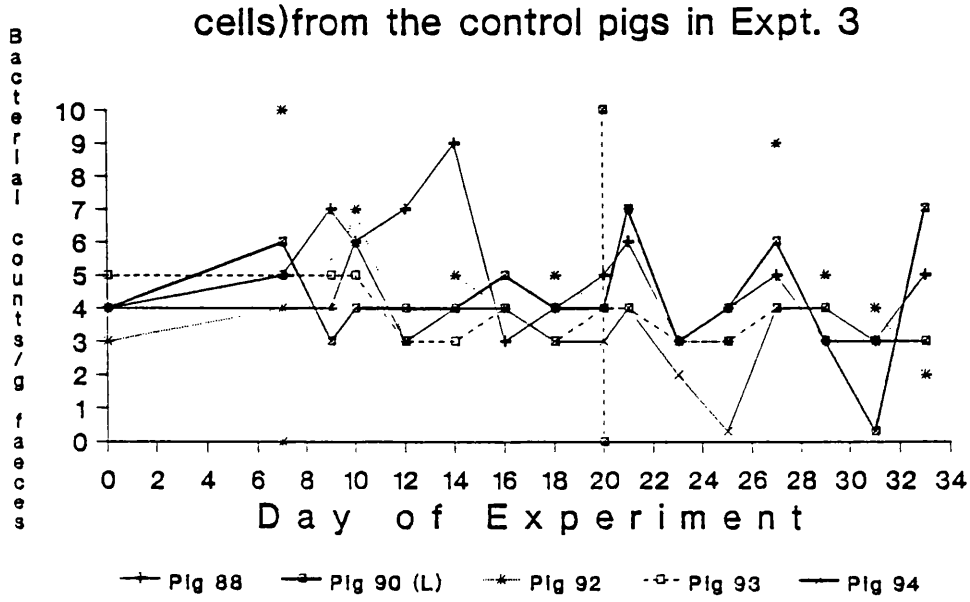
. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE XLII. Individual bacterial counts of Clostridium perfringens type A (veg. cells)from the treated pigs in Expt. 3



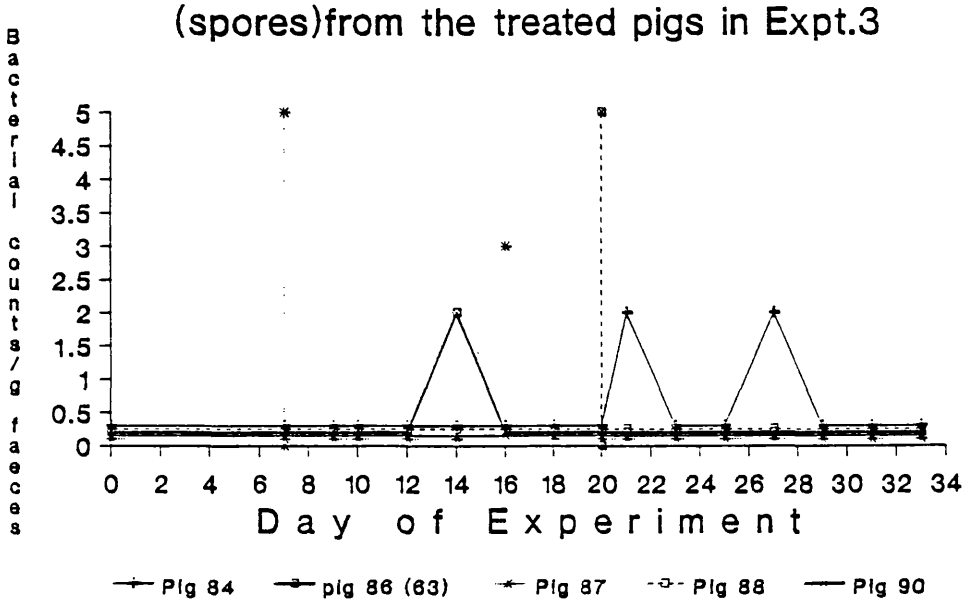
. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE XLIII.Individual bacterial counts of Clostridium perfringens type A (veg. cells)from the control pigs in Expt. 3



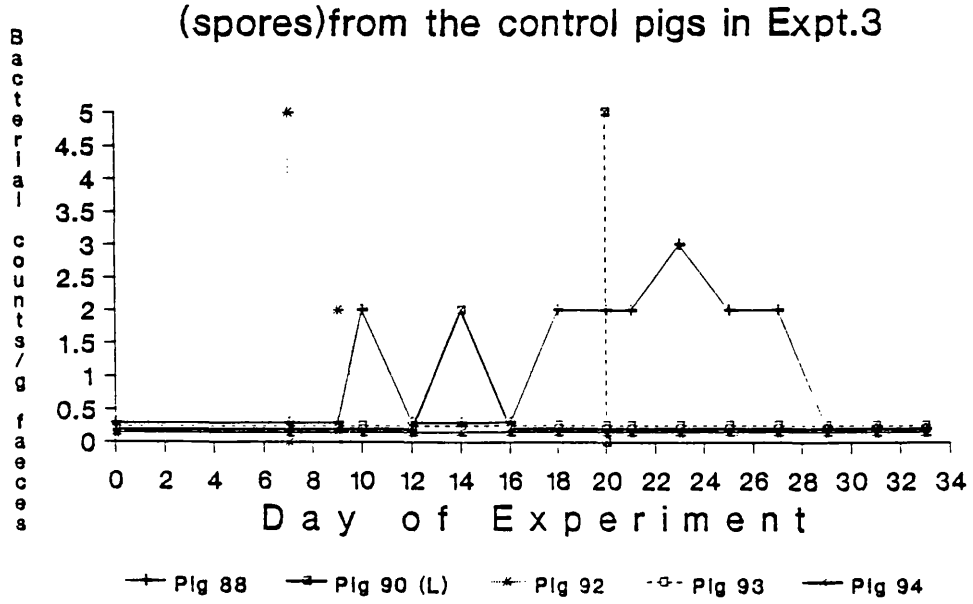
. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE XLIV. Individual bacterial counts of Clostridium perfringens type A (spores)from the treated pigs in Expt.3



. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE XLV. Individual bacterial counts of Clostridium perfringens type A (spores)from the control pigs in Expt.3



. . . . Beginning of treatment
---- Withdrawal of treatment

group yielded C. perfringens spores (Fig. XLIV) and then only in low numbers (10^3/g). Similar results were obtained from the controls, although one of the three pigs to yield spores did so on seven occasions (Pig 88) mainly after withdrawal of treatment (Figure XLV). Their numbers varied from 10^2 to 10^3 bacteria/g faeces (Tables 5.47 and 5.48, Appendix C).

C) Pathological findings in sacrificed animals

Animals were euthanased on the days shown in Table 5.2. One control pig (Pig 57 white)was in poor condition with a low body weight (3.8 kg) and a hairy coat. The remainder appeared normal. Pig 67 orange was diarrhoeic and was sacrificed on the same day, day 7 immediately prior to treatment.

a) Gross post mortem findings

Few abnormalities were present in the pigs of this study. Changes were restricted to the gastroenteric tract and were mild.

Pigs 57 white and 67 orange from the control and treated groups respectively were sacrificed on day 7 of the study and found to have no external abnormalities or lesions. The stomach, duodenum, jejunum and ileum of Pig 67 were all normal but the caecum and colon showed evidence of mild inflammation. There was an excess of mucus, reddening of the mucosal surface and soft to fluid contents in the caecum and colon. There were no changes in either the thoracic or the abdominal cavities and organs of Pig 57 except for slight stickiness of the visceral serosal surfaces.

Pigs 86 orange and 90 (R) white, from the treated and control groups respectively, were killed on day 20 but neither showed external gross lesions. The gastroenteric tract of Pig 90 (R) was grossly normal. That of Pig 86 was also normal in appearance except for mild inflammation of the caecum and colon where some reddening of the mucosa was observed. The colonic contents were pasty in consistency.

On day 22 pigs 61 orange (treated) and 61 white (control) were sacrificed. They were in good bodily condition with no apparent external abnormalities or lesions, their internal

organs were also normal in appearance with the exception of the last portion of their gut in which evidence of inflammation was found. In the case of Pig 61 orange this was in the caecum and colon and in Pig 61 white there was ileitis and caecitis with attachment of contents as patches to the caecal epithelium.

Pigs 65 orange (treated) and 27 white (control) were sacrificed on day 24 of the study, and appeared normal upon external examination. When the carcasses were opened the organs and serosal surfaces of the thoracic and abdominal cavities appeared normal. No pathological changes were found on close examination of the gastroenteric tract of either pig.

At the end of the experiment pigs 64 orange (treated) and 89 white (control) were also sacrificed and there were no gross pathological changes in the abdominal or thoracic viscera. No gross changes were seen in the gastrointestinal tracts following detailed examination.

b) Histological examinations

The pathological changes noted during histological examinations of the small and large intestines were not striking. The detailed findings are presented below in the order in which the animals were sacrificed.

Pig 67 (orange) from the treated group was killed on day 7 of the study. The stomach mucosa was normal in appearance. In the duodenum the villi were relatively low particularly over the folds or rugae. There was some eosinophilic material over the brush border but no cell shedding was present. There were some neutrophils present in the lamina propria. Some fusion of the villi was present in the jejunum but no clear evidence of cell shedding or bacterial adhesion were noted. There were few neutrophils in the lamina propria. The ileum was relatively normal and Cryptosporidium spp., was seen on the epithelial brush borders but the villi were normal in length. In the caecum epithelial shedding was occurring. Mild caecitis was apparent. Balantidium coli were seen on the mucosal epithelium and some bacteria were present in dilated crypts. Bacteria were also found close to areas of mucosal discontinuity. In crypt lumens

some yeast cells were also seen. Plasma cells were present in the lamina propria. There was some evidence of mild colitis and bacteria were present attached to the lumenal epithelium. There was thinning and shedding of the lumenal epithelium. **Balantidium coli** and yeasts or trichomonas-like organisms were also observed.

In pig 57 (white) killed on day 7 the stomach was grossly normal except for few lymphocytic accumulations in the lamina propria and a few shed epithelial cells. The villi in the duodenum were relatively low. Some apical cells were rounded and vacuolated. There was mild inflammation, and capillary dilatation was observed in the lamina propria. The villi were low but little cell shedding was seen at their tips in the jejunum. No bacteria were attached to this epithelium. Large numbers of lymphocytes and/or yeasts some of them mitotic were seen in the lumen of the ileum. No bacteria were seen but eosinophils were present in lamina propria. In the caecum the mucosa was thin and crypts contained organisms, and some bacteria were seen on the epithelial surface but no other changes were found in the lumenal epithelium. Cocci were found locally on the colonic epithelium and a large number of mitotic cells were seen in the crypts.

Pig 86 (orange) was killed on day 20 of the study and found to have epithelial cell shedding from the gastric mucosa, and congestion and lymphocytic accumulation in the lamina propria. In the duodenum the villi were relatively low and there was cell desquamation. Some neutrophils were present in the lamina propria. Some **post mortem** changes, such as cell shedding, crypts containing cells and lymphocytes, and some eosinophils in lamina propria were seen in the jejunum. Debris, cells and bacteria were seen in the lumen and on the tips of the villi of the ileum. Eosinophils were present in the lamina propria. In the caecum cell desquamation was seen and the height of the epithelium was reduced. Bacteria were present in dilated crypts and local thickening of the lamina propria was seen in the caecum. In the colonic epithelium bacteria were seen attached on the mucosal epithelium, and epithelial cells were present in the crypts.

Pig 90 (R) (white) from the control group was killed on the same day (day 20) and the gastric mucosa was histologically

normal except for local epithelial cell shedding and congestion in the lamina propria. Some **post mortem** change was seen in the duodenum as well as a few shed cells, and there was some capillary dilation with congestion and mild oedema of the lamina propria. Some epithelial cell shedding and fusion of the villi was present in the jejunum. Congestion and mild oedema of the lamina propria were also seen. In the ileum there were few shed epithelial cells and few bacteria in the lumen or attached to the epithelium. Lymphocytes were abundant in the lumen. Congestion, mild oedema and a few eosinophils were all present in the lamina propria. There was some cell shedding from the epithelium of the caecum. Lymphocytes and bacteria were seen in the crypts which also showed mitotic figures. Mild capillary dilatation was present in the lamina propria. The colonic epithelium showed cell shedding and bacterial attachment. In the lumen bacteria and Balantidium coli were also present. Some crypts contained debris, bacteria and free cells.

Pig 61 (orange) was killed on day 22 of the study and a very large number of bacteria (rods) and plant material was seen in the folds and on the epithelium of its stomach. In the lamina propria lymphocytic accumulations were present. The duodenal villi were slightly stunted, bacteria (cocci) were present close to the mucosa and some cell desquamation was seen at the tip of the villi in the duodenum. In the jejunum there was some cell desquamation and a few bacteria were seen attached to the epithelium. Lymphocytes were seen in some crypts. There were some bacteria (rods) on the mucosa of the ileum. Cell shedding was also present and crypts contained cells and eosinophilic debris. There was oedema present in the lamina propria. In the caecum Balantidium coli were seen in the lumen and close to the epithelium which was thin and also showed bacteria on its surface. A crypt abscess was also present. Bacteria and debris were present in the lumen and on the epithelium of the colon. Cryptosporidium spp.-like organisms and crypts containing lymphocytes were also seen.

Pig 61 (white) from the control group was killed on day 22 of the study, and the changes observed in the gastric mucosa were mild. There were lymphocytic accumulations and mild oedema in the lamina propria, some cell shedding and debris were seen

in the gastric lumen and adjacent to the mucosa. Some cell desquamation and shedding were observed in the duodenal and jejunal epithelium, lymphocytic accumulations were also seen in the duodenal lamina propria. Large numbers of eosinophils were present in the ileal lamina propria. Cryptosporidium spp. were seen close to the epithelium and bacteria were seen adjacent to the mucosa. The caecal epithelium was affected **post mortem**, but a uniform bacterial layer was observed attached to the surviving caecal epithelium. High numbers of bacteria were seen in the crypts. In the colon bacteria and lymphocytes were present in the crypts and close to the mucosa. Some local epithelial cell desquamation and shedding was also observed. Lymphocytic accumulations were seen in the lamina propria.

Pig 65 (orange) from the treated group was sacrificed on day 24 of the Experiment and its stomach was largely normal with the presence of some debris and epithelial cells shed into the lumen. Few lymphocytic accumulations were observed in the lamina propria. No villi were seen in the duodenum. Epithelial cell shedding, some lymphocytes, debris and a few bacteria were all observed in the lumen. In the lamina propria focal lymphoid accumulations were present. The villi in the jejunum were fused and reduced in height and some tips had been lost. No bacteria were observed, but accumulations of lymphocytes were seen in the lamina propria. A few bacteria were seen locally and debris were seen close to the ileal epithelium. Disrupted villi and villous fusion were also present. Numerous Cryptosporidium spp. in the mucosa and eosinophils in the lamina propria of the caecum were also seen. Many bacteria (cocci) were seen attached to the epithelium and into the crypts. Debris were also seen close to the mucosa. The mucosal epithelium was severely damaged and shed cells were observed in the lumen adjacent to the mucosa. Balantidium coli were present apparently in the lamina propria together with lymphocytic accumulations. Shed cells and Balantidium coli were seen in the colonic lumen. Large numbers of bacteria were observed close to and attached to the epithelium where Cryptosporidium spp. were also present. The epithelium was largely lowered. The crypts were seen to contain bacteria, debris and lymphocytes. In the lamina propria eosinophils were observed.

The stomach of Pig 27 (white) from the control group killed

on day 24 of the study showed a low epithelium. Some debris was seen close to the mucosal epithelium and some cell shedding was also present. Mild localised oedema was observed in the lamina propria. Some epithelial cell shedding was observed at the tip of a stunted and fused duodenal villi. Few intraepithelial lymphocytes were observed. Some congestion, mild oedema, plasma cells and lymphocytic accumulations were all present in the lamina propria. The jejunal mucosa was lowered and cell shedding was apparent. In the lamina propria lymphocytic accumulations, congestion and mild oedema were observed. Mild ileitis was apparent. A few Cryptosporidium spp. were seen on the mucosal epithelium and curved bacteria in the epithelial associated contents were all present. Crypts were observed containing debris. Eosinophils, congestion, mild oedema, capillary dilatation and lymphocytic accumulations were all seen in the lamina propria. Slight **post mortem** changes such as lifted epithelium were observed in the caecum. The lumenal epithelium was lowered. Bacteria were seen close and adherent to the caecal epithelium. A few bacteria and debris were also observed in the crypts. The colonic mucosa was essentially normal, but the epithelium was lowered locally. Some crypts contained bacteria which were also present close to the colonic epithelium.

Pig 64 (orange) from the treated group was sacrificed on day 34 at the end of this Experiment. The gastric epithelium was slightly low. Slight cell shedding and debris were seen close the the epithelial surface. Mild congestion, oedema and capillary dilatation were all present in the lamina propria. The duodenal villi appeared stunted and fused. Characteristic **post mortem** changes such as epithelial lifting and shedding were also observed. Debris, some bacteria and plasma cells were present in the lumen, close to the mucosa, and inside crypts. Lymphocytic accumulations, mild oedema and neutrophils were observed in the lamina propria. There was some evidence of mild inflammation. Intraepithelial lymphocytes were seen in the jejunal mucosa and the villi appeared lowered, fused and cell shedding was also observed. Mild inflammation was apparent. Some **post mortem** changes were also present. Bacteria and debris were seen in contents associated with the epithelium. Lymphocytic accumulations, congestion and oedema were seen in the lamina propria. The ileal villi were low. Many Cryptosporidium spp.

were seen in the mucosal epithelium and inside crypts. Some crypts were also seen to contain few bacteria (rods). Some **post mortem** changes were also seen. Many eosinophils, congestion, oedema and lymphocytic accumulations were all present in the lamina propria. In the caecum numerous bacteria were seen inside the crypts, and close or attached to the mucosal epithelium. Some crypts also contained lymphocytes debris and eosinophils. Congestion, lymphocytic accumulations and mild capillary dilatation were seen in the lamina propria. Mild **post mortem** changes (lifted epithelium) were also seen. **Post mortem** changes were also observed in the colon. Numerous bacteria and debris were seen adjacent and or attached to the colonic epithelium and inside crypts. Mild congestion and oedema were observed between lymphocytic accumulations in the lamina propria.

From the control group Pig 89 (white) was sacrificed on day 34 of the study. Debris in the lumen and moderate **post mortem** changes were observed in the stomach. Localised congestion and lymphocytes were seen in the lamina propria. Shedding of epithelial cells and debris were seen close to the duodenal epithelium. Intraepithelial lymphocytes were seen in the mucosal villi which were fused and lowered. Lymphocytes were commonly seen inside crypts. Lymphocytic accumulations, plasma cells, many eosinophils, mild congestion and oedema were all present in the lamina propria of the duodenum. The villi in the jejunum were low and few were seen fused. A few bacteria were observed adjacent to the epithelial surface and debris were seen inside crypts.There were some intraepithelial lymphocytes in the mucosa. Capillary dilatation, lymphocytic accumulations and neutrophils were seen in the lamina propria. Fusion of the villi and many **Cryptosporidium spp.** were present in the ileal epithelium. A moderate degree of cell shedding and debris were seen in the lumen together with bacteria. Many eosinophils, congestion, oedema and lymphocytic accumulations were observed in the lamina propria. Some **post mortem** changes such as lifting of the mucosa were observed in the caecum. Thinning of the epithelium was also observed. In the lumen **Balantidium coli** and debris were present. Bacteria were seen adjacent and attached to the epithelial surface. There were also many crypts containing bacteria and lymphocytes. A moderate number of mitotic cells were seen in the crypts. In the lamina propria local congestion

and lymphocytic accumulations were also present. Bacteria, debris and inflammatory cells were seen in crypts and in the colonic lumen. They were also seen adjacent and attached to the epithelium. Few crypts also contained lymphocytes. Lymphocytic accumulations were seen in the lamina propria of the colon.

D) Bacterial examinations of gastrointestinal contents

The quantitative bacterial examinations carried out on contents obtained from the stomach, small and large intestines of both groups of sacrificed pigs over the periods before, during and after lincomycin treatment are given below in Tables 5.49 to 5.62 as arithmetic means and according to the individual bacterial species identified.

a) Aerobic organisms

i) Total coliforms and E. coli: The counts of total coliforms obtained from the contents of each section of the gastroenteric tract from pigs of this Experiment, are presented in Tables 5.49 and 5.50. Numbers of coliforms in most regions of the gut were highest in the two pigs sacrificed on day 20, when treatment had been established for two weeks. The phenomenon was noted in both treated and control groups (Pig 86 and 90 (R)). Tables 5.51 and 5.52 for E. coli counts on Sheep blood agar confirm these results. No Beta haemolytic E. coli were isolated.

TABLE 5.49 Coliform counts from gastrointestinal contents of the treated pigs in Experiment 3

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
67	7^	5.0×10^3	5.0×10^3	1.0×10^5	1.0×10^5	3.0×10^5	1.0×10^6
86	20*	1.5×10^{10}	5.0×10^9	4.0×10^{10}	1.0×10^7	5.0×10^6	1.0×10^8
61	22	5.0×10^3	5.0×10^2	1.5×10^4	1.0×10^8	1.5×10^4	5.0×10^5
65	24	5.0×10^4	1.0×10^5	1.0×10^6	7.5×10^{10}	1.0×10^6	1.5×10^6
64	34	1.0×10^3	1.0×10^4	1.0×10^8	1.0×10^6	5.0×10^6	1.0×10^4

^ Beginning of treatment, * Withdrawal of treatment

TABLE 5.50 Coliform counts from gastrointestinal contents of the control pigs in Experiment 3

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
57	7^	$2.0x10^5$	$5.0x\ 10^2$	$1.0x\ 10^3$	$5.5x\ 10^5$	$2.5x10^5$	$5.0x10^5$
90(R)	20*	$2.5x10^3$	$2.0x\ 10^4$	$4.0x10^{10}$	$1.5x10^{11}$	$1.0x10^8$	$3.5x10^5$
61	22	N G	$5.0x\ 10^3$	$1.0x\ 10^7$	$2.5x\ 10^6$	$1.0x10^7$	$5.0x10^5$
27	24	$5.0x10^3$	$1.0x\ 10^4$	$5.0x\ 10^5$	$5.5x\ 10^5$	$5.0x10^4$	$5.0x10^6$
89	34	$1.0x10^4$	$2.5x\ 10^3$	$1.5x\ 10^5$	$5.0x\ 10^5$	$1.5x10^5$	$1.0x10^7$

^ Beginning of treatment, * Withdrawal of treatment

N G = No Growth

TABLE 5.51 Escherichia coli counts from gastrointestinal contents of the treated pigs in Experiment 3

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
67	7^	$5.0x\ 10^3$	$5.0x\ 10^2$	N G	$1.0x\ 10^5$	$1.0x10^5$	$5.0x10^5$
86	20*	$3.0x10^{10}$	$5.0x\ 10^9$	$2.0x10^{10}$	$1.5x10^{10}$	$5.0x10^7$	$5.0x10^7$
61	22	$4.5x\ 10^3$	$2.5x\ 10^3$	$3.5x\ 10^4$	$5.0x\ 10^9$	$5.0x10^4$	$1.0x10^5$
65	24	$5.0x\ 10^5$	$5.0x\ 10^8$	$5.0x\ 10^5$	$8.0x10^{10}$	$2.5x10^5$	$5.0x10^7$
64	34	$5.0x\ 10^2$	$5.0x\ 10^4$	$5.0x\ 10^5$	$1.5x\ 10^4$	$5.0x10^6$	$5.0x10^6$

^ Beginning of treatment, * Withdrawal of treatment

N G = No Growth

TABLE 5.52 Escherichia coli counts from gastrointestinal contents of the control pigs in Experiment 3

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
57	7^	$5.0x10^4$	$5.0x\ 10^2$	$1.5x\ 10^2$	$1.0x\ 10^5$	$1.0x10^6$	$2.5x10^5$
90(R)	20*	$2.0x10^3$	$5.0x\ 10^6$	$7.5x10^{10}$	$6.0x10^{10}$	$5.0x10^7$	$4.5x10^5$
61	22	$1.5x10^3$	$1.0x\ 10^3$	$5.0x\ 10^4$	$2.0x\ 10^7$	$1.0x10^6$	$5.0x10^8$
27	24	N G	$5.0x\ 10^3$	$1.0x\ 10^4$	$1.0x\ 10^8$	$5.5x10^4$	$5.0x10^9$
89	34	$5.0x10^3$	$5.0x\ 10^2$	$1.0x\ 10^5$	$5.0x\ 10^4$	$1.5x10^5$	$1.5x10^6$

^ Beginning of treatment, * Withdrawal of treatment

N G = No Growth

ii) **Faecal streptococci:** Tables 5.53 and 5.54 shows the streptococcal counts isolated from the contents of the gastroenteric tracts of the pigs sacrificed from both treated and control groups. In the majority of cases, faecal streptococci could not be isolated. Highest levels were seen in Pig 65, treated group, day 24 when 10^8/g and 10^7 organisms/g were recovered from the stomach and caecum, respectively.

TABLE 5.53 Faecal streptococcal counts from gastrointestinal contents of the treated pigs in Experiment 3

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
67	7^	$5.0x10^2$	N G	N G	N G	N G	$1.5x10^3$
86	20*	$2.0x10^3$	N G	N G	N G	N G	N G
61	22	N G	N G	N G	$5.0x10^3$	N G	N G
65	24	$5.0x10^8$	N G	N G	$1.5x10^3$	$1.0x10^7$	$1.0x10^3$
64	34	N G	N G	N G	$5.0x10^2$	N G	N G

^ Beginning of treatment, * Withdrawal of treatment
N G = No Growth

TABLE 5.54 Faecal streptococcal counts from gastrointestinal contents of the control pigs in Experiment 3

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
57	7^	N G	N G	$2.5x10^3$	$4.0x10^3$	$1.0x10^3$	$1.5x10^3$
90(R)	20*	N G	N G	N G	$5.0x10^2$	N G	N G
61	22	N G	N G	N G	$3.5x10^4$	N G	N G
27	24	N G	N G	N G	$1.0x10^3$	N G	N G
89	34	N G	N G	$1.0x10^4$	N G	N G	$4.0x10^3$

^ Beginning of treatment, * Withdrawal of treatment
N G = No Growth

iii) Salmonella spp.: No salmonellae were isolated from the intestinal contents of any pig of either group in this Experiment.

b) Microaerobic organisms

i) Campylobacter spp.:The numbers isolated from each region

of the gut are shown in Tables 5.55 and 5.56. It may be noticed that they were not isolated regularly. Campylobacters were isolated from the gastric and duodenal contents more frequently in the treated group.

TABLE 5.55 *Campylobacter spp.* counts from the gastrointestinal contents of the treated pigs in Experiment 3

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
67	7^	$1.5x10^3$	$7.5x\ 10^4$	$5.0x10^2$	$1.0x10^4$	$5.0x10^4$	$5.0x10^3$
86	20*	$3.5x10^4$	$5.0x\ 10^4$	$3.5x10^3$	$1.0x10^6$	$1.0x10^5$	$5.0x10^4$
61	22	N G	$1.0x\ 10^3$	$5.0x10^4$	$5.0x10^7$	$5.0x10^4$	$4.5x10^4$
65	24	N G	$2.0x\ 10^3$	$5.0x10^2$	$1.5x10^4$	$5.0x10^3$	$1.0x10^4$
64	34	N G	$5.0x\ 10^2$,	$1.5x10^5$,	$5.0x10^2$,	$1.5x10^5$,	$5.0x10^4$

^ Beginning of treatment, * Withdrawal of treatment
N G = No Growth

TABLE 5.56 *Campylobacter spp.* counts from the gastrointestinal contents of the control pigs in Experiment 3

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
57	7^	N G	N G	$1.0x10^2$	$5.0x10^3$	$2.0x10^4$	$4.5x10^4$
90(R)	20*	N G	N G	$1.0x10^4$	$3.0x10^6$	$5.0x10^3$	$1.0x10^4$
61	22	N G	N G	$5.0x10^4$	$4.0x10^4$	$7.0x10^3$	$2.0x10^5$
27	24	N G	N G	N G	$5.0x10^3$	$5.0x10^4$	$1.0x10^5$
89	34	$5.0x10^2$,	$5.0x10^3$,	$1.0x10^3$,	$5.0x10^4$,	$2.0x10^4$,	$1.5x10^4$

^ Beginning of treatment, * Withdrawal of treatment
N G = No Growth

ii) Lactobacilli: Counts of these organisms are presented below in Tables 5.57 and 5.58, corresponding to the examination of gastroenteric contents of five pigs from both treated and control groups. The highest levels noted were once again in pigs 86 and 90 (R) sacrificed on day 20 just prior to withdrawal of lincomycin. The highest count obtained was 10^{10}/g from the jejunum of Pig 86.

TABLE 5.57 Lactobacillus spp. counts from the gastrointestinal contents of the treated pigs in Experiment 3

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
67	7^	$5.0x10^2$	N G	N G	$5.0x10^2$	$5.0x10^3$	$5.0x10^4$
86	20*	$5.0x10^8$	$2.0x10^8$	$1.0x10^{10}$	$2.5x10^5$	$5.0x10^5$	$1.0x10^8$
61	22	$1.0x10^3$	$5.0x10^2$	N G	$5.0x10^6$	$1.0x10^5$	$2.5x10^5$
65	24	N G	$1.0x10^3$	$5.0x\ 10^8$	$5.0x10^3$	$5.0x10^2$	$1.0x10^6$
64	34	$5.0x10^2$	N G	$5.0x\ 10^2$	$5.0x10^2$	$1.0x10^4$	$1.5x10^3$

^ Beginning of treatment, * Withdrawal of treatment
N G = No Growth

TABLE 5.58 Lactobacillus spp. counts from the gastrointestinal contents of the control pigs in Experiment 3

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
57	7^	$5.0x10^3$	N G	$3.0x10^3$	$1.5x10^6$	$5.0x10^4$	$5.0x10^4$
90(R)	20*	N G	$5.0x10^4$	$1.5x10^8$	$1.5x10^5$	$1.0x10^7$	$5.0x10^3$
61	22	N G	N G	N G	$1.0x10^5$	$1.0x10^5$	$5.0x10^6$
27	24	N G	N G	N G	$1.0x10^5$	$5.0x10^3$	N G
89	34	$2.0x10^3$	$1.0x10^5$	$6.5x10^3$	$5.0x10^2$	$5.0x10^4$	$1.0x10^5$

^ Beginning of treatment, * Withdrawal of treatment
N G = No Growth

c) Anaerobic organisms

i) Enteric spirochaetes: As in the faecal samples these bacteria were not isolated from contents of either gastric or intestinal regions.

ii) Bacteroides spp.: Colonies of Bacteroides spp. were isolated from contents in numbers which are reported below in Tables 5.59 and 5.60. As was expected, the organisms were detected most consistently and in greater numbers in the lower intestines. The exception to this was Pig 86 euthanased on day 20 from the treated group where the organisms were detected in all parts of the gut sampled. The highest counts (10^5/g) were seen in the controls.

TABLE 5.59 Bacteroides spp. counts from the gastrointestinal contents of the treated pigs in Experiment 3

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
67	7^	N G	N G	N G	$5.0x10^2$	$1.0x10^4$	$5.0x10^4$
86	20*	$5.0x10^3$	$5.0x10^4$	$1.5x10^4$	$1.5x10^3$	$5.0x10^3$	$2.0x10^3$
61	22	N G	N G	$5.0x10^3$	$1.0x10^4$	N G	$5.0x10^4$
65	24	N G	N G	N G	$5.0x10^3$	$5.0x10^3$	$5.0x10^4$
64	34	N G	N G	N G	N G	$2.0x10^4$	$5.0x10^4$

^ Beginning of treatment, * Withdrawal of treatment
N G = No Growth

TABLE 5.60 Bacteroides spp. counts from the gastrointestinal contents of the control pigs in Experiment 3

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
57	7^	N G	N G	$1.0x10^3$	$2.0x10^3$	$5.0x10^3$	$1.0x10^4$
90(R)	20*	N G	N G	$5.0x10^4$	$2.5x10^3$	$5.0x10^5$	N G
61	22	N G	N G	$9.5x10^3$	$5.0x10^3$	$5.0x10^2$	$4.5x10^3$
27	24	N G	N G	N G	$5.0x10^3$	$1.0x10^3$	$5.0x10^4$
89	34	N G	$5.0x10^2$	N G	$1.0x10^4$	$5.0x10^3$	$5.0x10^5$

^ Beginning of treatment, * Withdrawal of treatment
N G = No Growth

iii) Clostridium difficile: No vegetative or spore-forming strains of these bacteria were isolated from any of the contents examined from the gastroenteric tract of any sacrificed pigs.

iv) Clostridium perfringens type A was isolated from the gastroenteric contents of the sacrificed pigs in the numbers shown in Tables 5.61 and 5.62. Levels isolated were generally low and isolations were more frequent from the jejunum onward.

Spore-forming strains of these bacteria were found in the treated group only from the ileum during treatment withdrawal on day 22 (10^2/g) and from the caecum, before and during treatment in numbers of 10^2/g. From the control group counts of 10^3/g were isolated only from colonic contents and after the withdrawal of

the treatment by day 34 of the study.

TABLE 5.61 *Clostridium perfringens* type A (vegetative cells) counts from the gastrointestinal contents of the treated pigs in Experiment 3

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
67	7^	1.0×10^3	N G	N G	5.0×10^2	5.0×10^3	5.0×10^3
86	20*	5.0×10^2	N G	1.0×10^6	5.0×10^4	7.5×10^3	1.5×10^4
61	22	N G	5.0×10^2	N G	5.0×10^4	1.0×10^3	5.0×10^3
65	24	N G	5.0×10^2	N G	1.5×10^5	5.0×10^3	5.0×10^3
64	34	5.0×10^2	N G	1.5×10^3	5.0×10^2	2.5×10^4	3.0×10^4

^ Beginning of treatment, * Withdrawal of treatment
N G = No Growth

TABLE 5.62 *Clostridium perfringens* type A (vegetative cells) counts from the gastrointestinal contents of the control pigs in Experiment 3

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
57	7^	2.0×10^4	N G	1.0×10^3	5.0×10^2	2.0×10^4	5.0×10^3
90(R)	20*	N G	N G	N G	5.0×10^2	5.0×10^3	2.0×10^4
61	22	N G	N G	1.0×10^8	5.0×10^4	1.5×10^3	5.0×10^3
27	24	5.0×10^2	N G	N G	5.0×10^3	5.0×10^3	5.0×10^2
89	34	1.0×10^3	N G	5.0×10^3	4.0×10^3	3.0×10^4	1.0×10^6

^ Beginning of treatment, * Withdrawal of treatment
N G = No Growth

E) Virological examinations of faeces and contents

The examinations carried out for the detection of rotavirus and/or rotaviral particles in the faecal, ileal and colonic filtrates were all negative.

F) Parasitological examinations of faeces and contents

Although cryptosporidia were observed in histological sections they were not present in the Ziehl-Neelsen's stained smears examined from either samples of gastroenteric contents,

or faeces from the pigs of Experiment 3.

4. D I S C U S S I O N

Two experiments were carried out to assess the effects of treatment and withdrawal of lincomycin in weaned pigs. These experiments (2 and 3) were designed to supplement the results of Experiment 1, to use the drug over longer periods of time (11 and 20 days); to increase the number of pigs in each group and to supplement the observations in the treated animals with control untreated groups and to include post mortem examination of treated and untreated animals in Experiment 3.

At the level of lincomycin used (13.3 ppm) the effects of treatment and its withdrawal were slight in both Experiments. In both Experiments diarrhoea occurred in both treated and control groups. In some cases such as that of Pig 100 (white), control group, Experiment 2, the diarrhoea was present for long periods (Table 5.4). The presence of this diarrhoea affected the results but also provided a means of assessing the effects of treatment and its withdrawal. The cause of the diarrhoea present was not determined. There was no evidence for the presence of rotavirus, beta haemolytic pathogenic E. coli or parasites other than Cryptosporidium spp. and Balantidium coli which were identified only in histological sections. The only potentially enteropathogenic bacteria identified routinely were Campylobacter spp. which was present in all pigs and C. perfringens type A which was present in most animals in the vegetative form.

The results of Experiment 2 suggest that the introduction of lincomycin reduced the amount of diarrhoea. Treated pigs had 4 diarrhoea days compared with 7 in the controls. The number of diarrhoea days increased in the post-treatment observation period to 8 in the treated group (Table 5.3) and 10 (Table 5.4) in the untreated controls. These differences were not significant ($P>0.05$). The only unusual observation was the occurrence of blood in the faeces of Pig 68 (orange) (day 17) and excess of mucus in the faeces of Pig 98 (orange) (day 18) in the treated group following withdrawal of antimicrobial. The cause of these changes was not clear. Rectal temperatures were normal during treatment but rose slightly (Pigs 69 and 98

orange) following antimicrobial withdrawal (Table 5.5) whereas fever (>40^{o}C)occurred in the controls during both periods (Table 5.6). The differences between treated and control groups (rectal temperature), recorded during treatment were statistically significant (t=-11.22) after using a paired student's t-Test (Microsoft Excel, Version 4.0 (c) 1992). The mild nature of the differences between treated pigs during treatment and after withdrawal were underlined by the figures for daily live weight gain (Table 5.10) in which gain fell during the treatment period and rose following withdrawal. A similar pattern was seen in the control animals and cannot be attributed to the effects of lincomycin.

The bacterial findings were focused on the bacteria which in previous reports were closely related to gastrointestinal disorders in weaned pigs and were: Total coliforms, E. coli, lactobacilli, Campylobacter spp., Bacteroides spp., enteric spirochaetes, C. difficile, C. perfringens type A (vegetative and spore-forming organisms) and Salmonella spp.

Although the result of an individual faecal culture may be involved in the cause of a specific disease, critical evaluation must be done to determine whether it is the primary cause or is due to a disturbed intestinal flora secondary to another disease **e.g.** infections of the respiratory tract.

There were apparent alterations in the number of viable total coliforms, particularly in the treated group following the establishment of the treatment (Table 5.12). The changes were noticed when an average count of 10^{8}/g of faeces (before treatment) decreased to 10^{5}/g during the period of treatment but returned to 10^{8}/g after lincomycin withdrawal. The numbers of total coliforms isolated from the control group were respectively 10^{5}/g, 10^{8}/g and 10^{9}/g before, during and after the period of treatment (Table 5.13). The variation between individual counts made the statistical significance of these differences difficult to interpret using the software package employed here ("StatWorks^{TM} Data", Kruskal-Wallis Test[1991]).

Isolation of faecal streptococci was rare and never more than 10^{3}/g in both treated and untreated animals (Figures **XVII** and **XVIII**). This result was due perhaps to the efficacy of the

antimicrobial on Gram-positive organisms and the presence of tylosin phosphate and copper sulphate in the basic ration.

Another pattern of response to treatment and its withdrawal was seen with Campylobacter spp. and Lactobacillus spp. numbers. These remained at the same level following the introduction of treatment (campylobacters at 10^4/g, Table 5.14 [Appendix C] /Figure XIX, lactobacilli at 10^6/g, Table 5.16/Figure XXI) and then rose following withdrawal of treatment (campylobacters to 10^6/g, Table 5.14, lactobacilli 10^7/g, Table 5.16). This change was once again not statistically significant due to the variation in the individual counts. A similar pattern was seen in the controls but was exaggerated by the high counts of campylobacters found in pigs 99 and 100 (white) on day 16 (Table 5.15 [Appendix C]/Figure XX) and lactobacilli counts in Pig 95 on day 14 (Table 5.17/Figure XXII). The differences between groups were not great.

Bacteroides spp. recovery occurred in most pigs in this study although at levels which were lower than expected from the literature (Kenworthy and Crabb, 1963; Smith and Crabb, 1961; Smith and Jones, 1963). This lower level might have been due to delays in sampling or to the presence of antimicrobial growth promoters in the basic ration. Counts of these organisms from the treated group fell slightly from 10^4/g to 10^3/g during treatment (Table 5.18/Figure XXIII) and increased to 10^5/g following withdrawal of treatment. This recovery was influenced by the high counts found in pigs 99 and 68 on days 17 and 18 respectively (Table 5.18). A similar change occurred in the control group (Table 5.19/Figure XXIV) but on days 12 to 14, earlier than in the treated animals. There was therefore little difference between groups except in terms of the time at which the change took place.

The results of counts for C. perfringens type A were difficult to interpret because of individual variations (Tables 5.20 and 5.21/Figures XXV and XXVI). These organisms remained at the same level (10^4/g) in both treated and untreated groups during the whole period of sampling. However, in the treated group, pigs 97 and 98 on day 16, and Pig 99 on day 17 gave higher counts of 10^5-10^6/g after withdrawal. No such changes

were seen in the controls. The counts of sporulating organisms (Tables 5.22 and 5.23, Appendix C) were too low and irregular to associate with treatment or its withdrawal although lower counts were observed in the controls when they were found (Figures XXVII and XXVIII).

The two organisms associated in the literature with antimicrobial treatment; C. difficile and Salmonella spp. were not identified in this study and nor were Serpulina spp. which have been associated with the withdrawal of treatment (Olson, 1980; 1986; Olson and Rodabaugh, 1976a,b; 1977; 1978; 1984)

In Experiment 3 a similar format was adopted but the period of treatment was lengthened to 20 days and animals were examined post mortem. An attempt was made to differentiate E. coli counts from those of total coliforms but results proved so similar that only total coliform counts are discussed here. An additional complication was the erroneous selection for slaughter by the farm staff of Pig 86 from the monitored group. The bacterial counts and additional data were then continued with records from Pig 63, the animal previously designated for sacrifice. The inclusion of this data makes the interpretations offered below less satisfactory.

The major clinical abnormality recorded in this study was diarrhoea. Six pigs (67 and 88 orange, and 27, 88, 89, and 93 white) were diarrhoeic on day 0 (Tables 5.24 and 5.25) but had recovered by the first day of treatment (day 7). Diarrhoea occurred in both groups, both during the treatment period and after its withdrawal. No difference was seen in the controls (11 diarrhoea/days in the treatment period and 9 during the withdrawal period) but there was a marked difference in the treated group. Ten diarrhoea/days were recorded during the treatment period but only 4 in the withdrawal period (Figures XXIX and XXX). It is not clear whether the removal of Pig 86 (orange)had any influence on this finding and the results were statistically not significant ($P>0.05$). The only unusual observation was the occurrence of excess mucus and dark blood in the faeces of Pig 88 (white) (day 16) in the control group. These results are contrary to those of Experiment 2. The reason for the diarrhoea was not identified in faeces but the pathological findings in sacrificed members of the group showed that cryptosporidia were present in the ileum and colon in the

histological sections examined.

Rectal temperatures were raised in both groups in this study in contrast to the situation in Experiment 2. Nine pig/days at 40°C or over were recorded in the treated group during treatment and twelve pig/days following withdrawal. Similar findings were made in the control group with 10 pig/days in the treatment period and 10 pig/days during the withdrawal period (Tables 5.26 and 5.27), but the differences were not statistically significant ($P>0.05$). Feed consumption was similar in both groups (Figures XXXI and XXXII), but slightly higher (361g/pig/day) in the treated animals, than in the controls (359g/pig/day). Slightly more water was consumed by treated pigs when compared with untreated animals during the period of treatment (Table 5.28).

In spite of the increase in clinical signs in the pigs of this group, the daily liveweight gains in both groups were higher during the treatment period than prior to treatment (Tables 5.29 to 5.32) unlike the situation with the treated group in Experiment 2 (Table 5.10) in which a fall was recorded. As in Experiment 2, the treated group gained weight more successfully than the control group. However, the results were statistically not significant ($P>0.05$) by using a two sample paired student's t-Test (Microsoft Excel version 4.0 [(c) 1992).

Some changes in bacterial counts occurred following the introduction of treatment and its withdrawal in this study. One a dip in the numbers of anaerobes recovered on day 9 is considered to have been technical, possibly due to jar failure as it occurred only in Bacteroides spp. (Figures XLV and XLVI) and in some of the clostridial counts (Figure XLVII).

Treatment was associated with a rise in total coliforms and Lactobacillus spp. counts (Tables 5.33, 5.41 and Figures XXXV and XLIII), levels then remained at the higher level. In both types of bacteria a fall in numbers was noted in both treated and control animals in the post-treatment period. This occurred on day 23 in the control and 25 for the treated group for coliforms but was slightly less marked for lactobacilli. The changes were not considered statistically significant between

groups by the tests used because of individual variation.

Streptococcal and Campylobacter spp. counts were essentially similar throughout the study in both groups in this Experiment (Tables 5.37 to 5.40, Appendix C, and Figures XXXIX to XLII). Individual counts of streptococci raised markedly, particularly in the controls. Mean levels of C. perfringens type A also remained unchanged by treatment but as with streptococci, there was marked individual variation in numbers recovered (Tables 5.45, 5.46 and Figs. XLVII, XLVIII).

Levels of Bacteroides spp. fell slightly at the end of treatment (Tables 5.43 and Figure XLV) and recovered on withdrawal. The changes were not statistically significant between groups due to individual variation. In the control group one pig (Pig 92) was responsible for peaks in Bacteroides spp. counts on 3 occasions (Figure XLVI) which were related to but not identical with the occurrence of diarrhoea in that animal.

Spore-forming clostridia were recovered in low numbers (Figures XLIX and L) and was from only three animals in both groups. Pig 88 (white) from the control group yielded the organisms in seven occasions mainly in the withdrawal period.

As in Experiment 1 and 2 the organisms thought to be associated with antimicrobial treatment and withdrawal (C. difficile, Salmonella spp. and Serpulina spp.) were not isolated.

Post mortem findings did not indicate any major differences between the treated group and the controls or between the animal on treatment (Pig 86 orange, day 20) and the remainder of the group. All changes were slight and few differences between the treated group and the control were found.

All the sacrificed pigs in this Experiment were healthy and none of them showed external gross lesions. The macroscopic appearance of the internal viscera in the pigs sacrificed in this study was normal. However, evidence of enteritis was present through the findings of some localised reddish areas in the small and large intestines. The contents varied in consistency from firm to soft or fluid along the whole tract, in

some parts of the colon the contents were attached to the mucosa but no lesions were found beneath the contents after washing. The localised reddish areas were in some cases due perhaps to the manipulation of the segments during the process of examination. There was no clear difference between groups in intensity of the macroscopic lesions.

All pigs were found to have minor inflammatory changes in the intestinal mucosa at all levels. Villous atrophy and fusion were common and different degrees of inflammation were present in the caecum and colon. Fewest changes were seen in pigs 86 orange (treated group, on treatment, day 20), 61 white and 27 white (euthanased on days 22 and 24 respectively). The intensity of the changes was not clearly related to treatment or its withdrawal, but Pig 86 (orange) was the only member of its group killed while on treatment and had fewest lesions. Further comparison of pigs on treatment and following withdrawal would perhaps allow further assessment of the significance of this parameter. The origin of the inflammatory changes was not clear but those in the small intestine may have been associated with past cryptosporidial infection as organisms were seen in the ileal mucosa of many animals. The chronic nature of many of the large intestinal lesions was underlined by the presence of Balantidium coli.

Bacterial counts of the intestinal contents agreed with those of other workers (Briggs, et al., 1954; Fewins, et al., 1957; Fuller, et al., 1960; Horvath, et al., 1958; Pesti, 1962; Wilbur, et al., 1960; Willingale and Briggs, 1955) that populations of many organisms were lower in the stomach, duodenum and jejunum than the ileum and large intestine. It was of interest that campylobacters were found throughout the tract (Tables 5.55 and 5.56) and that Bacteroides spp. were only recovered at relatively low levels from the caecum and colon (Tables 5.59 and 5.60). It is not clear whether this reflects technical problems with maintenance of anaerobiosis in culture or during transport or reflects a genuinely low number in the population.

The most striking associations between treatment and numbers of bacteria were the counts obtained in Pig 86(orange) on day 20. This animal was under treatment and yielded higher numbers of total coliforms (Table 5.49) and lactobacilli (Table

5.57) in stomach and jejunum than the untreated animal sacrificed on day 7 (Pig 57 white) and those killed following withdrawal of treatment (Pigs 61 and 65 orange). The changes were shared to some extent by control Pig 90 (R) sacrificed on the same day (day 20) and may be a day effect rather than a direct effect of antimicrobial treatment (Tables 5.50 and 5.58).

Other changes were less obvious. The animals from which treatment had been withdrawn had increased numbers of bacteria in the anterior portion of the intestines. In particular, levels of total coliforms, lactobacilli, Bacteroides spp. and C. perfringens type A were all higher to a variable extent. It is not clear to what extent these changes are related to withdrawal of treatment.

The results of the studies described in this Chapter suggested that changes in the enteric flora were occurring and were particularly striking in individuals. It was concluded that the continued use of antimicrobial growth permitters in the feed and the presence of intercurrent disease might affecting post-antimicrobial effects. The study described in Chapter 6 was therefore carried out.

CHAPTER 6

THE EFFECT OF IN-FEED MEDICATION WITH CHLORTETRACYCLINE ON THE GASTROINTESTINAL BACTERIAL FLORA AND ITS RELATIONSHIP WITH ENTERITIS FOLLOWING ITS WITHDRAWAL IN WEANED PIGS

1. INTRODUCTION

In order to evaluate the effect on the gut flora of a broad spectrum antimicrobial effective against both Gram-positive and Gram-negative bacteria, an Experiment was carried out to assess the direct effects of treatment and withdrawal on the "normal" gastrointestinal flora of recently weaned pigs. In this study chlortetracycline hydrochloride (Aureomycin) was given in feed at 300 ppm for 21 days to weaned pigs from the high health status herd. These pigs were used in order to minimize the effects of diseases commonly present in conventionally reared pigs such as P.P.E. and swine dysentery. This study used feed prepared at the Veterinary School Farm with no antimicrobial growth promoter in order to maximize the effects of the antimicrobial and its withdrawal.

The effects of the antimicrobial and its withdrawal on the pigs were monitored by evaluating its relationship with clinical signs, changes in populations of specific bacteria present in both faecal samples and gastroenteric contents. Macroscopic and microscopic lesions were also evaluated in the pigs which were killed at specific times from both treated and control groups. Feed consumption and the effect of the drug on growth-productive parameters were also recorded and evaluated in this Experiment.

2. MATERIALS AND METHODS

The majority of the materials and methods used in this experiment have been described in detail in Chapter 2, and were carried out as in Chapters 4 and 5 of this dissertation.

A) Experimental animals

a) Source of the experimental animals

In this Experiment twenty, six-week-old minimal disease weaned pigs weighing between 5.8 and 10.8 kg were used. All these animals were obtained from the Veterinary School Animal Husbandry Department's Farm at Cochno described in Chapter 2.

b) Identification system

As in previous experiments, all the pigs were individually identified by the application of plastic, numbered, coloured tags in the right ear. After being individually identified and weighed they were assigned at random to one of two groups. Different coloured tags (white and yellow) were used to differentiate between pigs under treatment (yellow) and untreated controls (white). The individual identification of the pigs and their distribution in the two groups (treated and control) is presented below in Table 6.1.

TABLE 6.1 Individual identification of pigs in Experiment 4

Tag Number and Colour	Sex	Initial Weight(Kg)	Study Group	Killed on Study Day
1 yellow	M	10.8	T	ND
2 yellow	F	9.6	T	ND
3 yellow	M	7.0	T	24
4 yellow	M	9.2	T	ND
5 yellow	M	5.8	T	7
6 yellow	F	7.2	T	ND
7 yellow	M	7.3	T	35
8 yellow	F	6.2	T	22
9 yellow	M	9.2	T	ND
10 yellow	M	7.4	T	20
71 white	M	9.7	C	ND
72 white	M	7.3	C	24
73 white	F	8.3	C	ND
74 white	M	7.1	C	ND
75 white	F	7.8	C	35
76 white	M	6.3	C	7
77 white	F	8.5	C	ND
78 white	F	5.8	C	20
79 white	F	9.7	C	ND
80 white	F	7.5	C	22

M = Male, F = Female, T = Treated
C = Control, N D = Not Done

c) Maintenance of experimental animals

The pigs used in both treated and control groups were housed during the experimental period in separate cement floored pens. The pens were partially slatted and this area included the nipple drinker and was the wet-dirty area. At the front was a clean, dry and warm area which contained an automatic feeder and a lamp (150w bulb) positioned to provide an extra source of heat for the first weeks of the Experiment. The temperature of the whole building was controlled at 23°C-27°C. Water was freely available from the automatic drinker mentioned above. Except for the use of the medicated food, the pigs were reared under commercial conditions prevalent in the piggery of the Glasgow University Veterinary School at Cochno (details of the farm are presented in Chapter 2).

B) Food

a) Standard diet

All the pigs were fed until the beginning of the study with the existing diet which was provided by B.O.C.M. Silcock, Ltd. (B.O.C.M. 483 Growercare pellets + Tylamix, Chapter 2), and was available ad-libitum.

b) Experimental diet

In order to assess the effect of chlortetracycline on the gut flora of recently weaned pigs, a ration was prepared at the experimental block of Cochno farm to a formula provided by the Animal Husbandry Department, using raw materials present on the farm (Table 6.2). 300 ppm of chlortetracycline hydrochloride (Aurofac 100, Cyanamid) was included in this ration in 100 kg batches, the rest of the 19% crude protein ration (400 kg) prepared was used to feed the control group. After withdrawal of the medicated feed on day 0 the two groups were fed with the unmedicated ration until the end of the study.

TABLE 6.2 Formulation of the experimental farm-made ration

Ingredients	% in ration	Protein content	Kilograms
Barley	73.0	10% = 2550	255
Soya	11.4	40% = 1600	40
Fish meal	14.3	50% = 2500	50
Min. & Vitamins	1.4	0% = 0000	5
TOTAL	100.00%	= 6650 :	350=19%C.P.

C) Experimental procedures

a) General

The 20 pigs individually identified and divided at random into the two different groups were monitored once before treatment. During treatment sampling was carried out for two weeks beginning on day 7 and for 2 weeks after the withdrawal of the antimicrobial from the ration of the treated group.

b) Serum samples

Blood samples were collected from the external jugular vein of all pigs before treatment began, and from the pigs of both groups prior to euthanasia. Sera were obtained by the methods described in Chapter Two. 5 ml amounts of each were stored at $-20^{o}C$ in sterile plastic containers until required for further examination.

c) Post mortem examination

As soon as possible after death post mortem examinations were carried out by the methods described in Chapter Two. The pigs examined were from the treated and control groups and were killed on the 7th, 20th, 22nd, 24th and 35th day of the study. All ten pigs were examined for gross changes or lesions of the organs of the thoracic and abdominal cavities. Detailed investigation was carried out on the gastroenteric tract to which particular attention was paid.

D) Clinical observations

The general bodily condition of the animals used in this

study in either group was noted every time sampling was carried out at the farm during the three periods of adjustment to, during and after treatment, and in particular on the pigs which were killed on dates shown above at the Veterinary School-Post mortem room. The characteristics investigated are described in detail in Chapter Two. Sampling days are shown in Table 6.3.

a) Faecal consistency, rectal temperature, feed and water consumption and daily liveweight gain (DLWG)

i) Appetite and individual faecal consistency were noted at the time of sampling from both groups (Tables 6.3 and 6.4).

ii) Rectal temperatures were taken and recorded at the beginning of the Experiment from all twenty pigs and every third day afterwards from the pigs of the treated and control groups (Tables 6.5 and 6.6). Care was taken to obtain such measurements at a similar time of the day to avoid variations due to the regular management of the piggery.

iii) Feed consumption from both groups of this Experiment was measured daily and recorded.

iv) Body weights of the pigs from the treated and control groups were measured using a stationary weighing crate (Individual pig weights were recorded before treatment from the treated and control groups on day 0, and weekly after the establishment of treatment and its withdrawal from the five pigs selected in each group (Tables 6.7 and 6.8).

E) Diagnostic samples

a) Faecal samples and gastrointestinal contents

In this Experiment rectal faecal sampling was carried out every third day and the samples obtained were examined qualitative and quantitatively for bacteria. Qualitative examinations for protozoal parasites and rotaviral particles were also carried out by the methods described in Chapter Two. Similar examinations were carried out with samples from the contents of stomach, duodenum, jejunum, ileum, caecum and colon

obtained immediately after the gross post mortem examinations of the sacrificed pigs were finished.

Air-dried heat fixed smears of faeces, small intestinal mucosal scrapings, and contents from the intestinal portions mentioned above were made and stained by Gram's method and modified Ziehl-Neelsen's method for the identification of bacteria and for evaluation of the presence of cryptosporidial oocysts respectively.

b) Histological examinations

To evaluate the presence and distribution of pathological changes and/or lesions present in the different epithelial strata of the gastrointestinal tract, sections of stomach, duodenum, jejunum, ileum, caecum and colon were taken after completion of the gross post mortem examination. The sections obtained were fixed and processed by the methods used in the Pathology Department. Histological sections stained by H & E were examined by light microscopy to detect any abnormalities present in the gastrointestinal epithelium and for the presence of pig diarrhoea-related parasites such as Cryptosporidium spp., Isospora suis and Balantidium coli.

c) Examination for enteric agents and their products

For the demonstration of C. perfringens type A enterotoxin RPLA tests were carried out routinely throughout the experimental period. RPLA tests were also used in this study to demonstrate rotavirus particles. Both tests were carried out on filtrates of faeces and contents. The presumptive identity of C. perfringens type A was confirmed by lecithinase production, the Nagler reaction and by its double zones of haemolysis (alpha and beta). Subcultures of the presumptive colonies were carried out on egg yolk and sheep blood agar plates incubated anaerobically at 37^{o}C for 48 to 72 hours. Antigens of C. difficile were also screened using commercial RPLA kits available for agglutination from faecal dilutions by the methods described in Chapter 2.

F) Bacteriological examinations

The identification of the different bacteria present was

presumptive in most cases and was based on colonial morphology and effect on the media. Cellular morphology of the bacteria isolated was confirmed by light microscopical examination of Gram's stained smears. Further biochemical tests were carried out in some cases for the identification of specific bacterial genera and species. In the case of Beta-haemolytic colonies of E. coli, in situ hybridization of their nucleic acid was carried out by the staff of the Faculte de Medecine Veterinaire of the Universite de l'Etat a Liege, Brussels, Belgium to identify the specific pathotypes present in the pigs of either group used in this study. Isolates were tested for the presence of genes for VT, ODP, STaP, STaH, STb and LTp (enterotoxins) and F4, F5, F6 and F41 (adhesins).

The bacterial counts of aerobic, microaerobic and anaerobic organisms provided the number of Colony Forming Units (CFU), and were carried out by the methods described in detail in Chapter 2 and briefly mentioned in Chapters 4 and 5.

3. R E S U L T S

The results obtained from this study are presented below in the order in which routine examinations were carried out. The bacterial counts are presented for better understanding in both Tables and Figures summarizing the daily results obtained from the different bacteriological cultures.

A) Clinical signs

At the beginning of the Experiment none of the weaned pigs used in this study were suffering from any external abnormality or lesions such as hernias, wounds, haemorrhages, ectoparasites, or any apparent locomotor disorder. They were in good bodily condition and appeared healthy and active. Pig 2 (yellow) which had been chosen to live during the whole Experiment developed a swelling on day 19 on the knee of the left leg. The swelling was soft and approximately 5 cm long and 2.5 cm wide. There was no pain or increased temperature to the touch. On day 21 the swelling became hard in consistency and the area was moderately hot and was painful when touched. On day 23 there was no evidence of the swelling at all, the pig appeared totally recovered even from the slight lameness shown in previous days,

and its appetite, weight gain and conversion rates were maintained unaltered. In the control group Pig 74 (white) appeared constipated on two consecutive sampling days (days 21 and 23) when sampling was attempted. The rest of the pigs in both groups appeared to be in good health throughout the period of study except for the faecal changes described below.

a) Faecal consistency

In this Experiment diarrhoea was uncommon in either group of pigs. None of the animals from the treated group showed diarrhoeic faeces in any of the three periods of study. However, soft faeces were present in 4 out of 5 pigs following the withdrawal of chlortetracycline from the ration. The soft faeces of Pig 1 (yellow) of this group on day 13 contained a fleck of blood, and the faeces of Pig 4 contained excess of mucus on its firm faeces on day 19. Soft faeces which included a bloody strip and flecks of blood on the surface of the stool was recorded from Pig 71 (white) of the control group on day 0. Pigs 74, 77 and 79 were diarrhoeic on days 0, 21 and 19 respectively. They were the only affected animals during the whole period of study (Tables 6.3 and 6.4).

TABLE 6.3 Individual faecal consistency changes recorded from the pigs of the treated group in Experiment 4 before, during and after treatment

Pig	Day of Experiment															Total
Number	0	7^	9	11	13	15	17	19	21*	23	25	27	29	31	33	35	D/D
1	N	N	N	N	S	N	N	S	S	N	N	N	N	N	N	N	0/16
2	N	N	N	N	N	N	N	N	S	N	N	N	N	N	N	N	0/16
3	S	ND	ND	ND	ND	ND	ND	ND	ND	K							0/10
4	N	N	S	S	N	N	N	N	S	N	N	N	N	N	N	N	0/16
5	N	K															0/2
6	S	N	N	N	N	S	S	N	S	S	S	N	N	N	N	N	0/16
7	N	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	K	0/16
8	N	ND	ND	ND	ND	ND	ND	ND	K								0/9
9	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0/16
10	N	ND	ND	ND	ND	ND	ND	K									0/8

N = Normal, S = Soft, D = Diarrhoea, DD = Diarrhoea Days
ND = Not Determined (Not Done), K = Killed
^ Beginning of observations, * Withdrawal of treatment

TABLE 6.4 Individual faecal consistency changes recorded from the pigs of the control group in Experiment 4 before, during and after treatment

Pig	Day of Experiment																Total
Number	0	7^	9	11	13	15	17	19	21*	23	25	27	29	31	33	35	D/D
71	S	S	N	N	S	N	N	S	S	S	S	N	N	N	N	N	0/16
72	N	ND	ND	ND	ND	ND	ND	ND	ND	K							0/10
73	N	S	N	N	S	S	S	S	N	N	S	S	N	N	S	N	0/16
74	D	N	S	N	N	S	S	S	ND	S	S	N	N	S	S	N	1/16
75	S	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	K	0/16
76	N	K															0/2
77	N	N	N	N	S	N	N	S	D	S	S	N	S	S	N	N	1/16
78	S	ND	ND	ND	ND	ND	ND	K									0/8
79	N	N	N	N	N	S	N	D	S	N	S	N	N	N	N	N	1/16
80	N	ND	ND	ND	ND	ND	ND	ND	K								0/9

N = Normal, S = Soft, D = Diarrhoea, DD = Diarrhoea Days
ND = Not Determined (Not Done), K = Killed
^ Beginning of observations, * Withdrawal of treatment

b) Rectal temperature

The temperatures recorded every third day from individual pigs varied and did not always relate to the other clinical signs such as changes in faecal consistency, diarrhoea, or feed consumption. The detailed figures and the arithmetic means corresponding to each individual pig are given in Tables 6.5 and 6.6, below.

TABLE 6.5 Individual rectal temperatures of the treated pigs (yellow tags) in Experiment 4

Pig	Day of Experiment										
Number	0	7^	9	11	13	15	17	19	21*	23	25
1	39.8	39.7	39.7	39.8	40.4	40.2	39.9	39.9	40.0	40.3	39.7
2	39.8	39.8	39.6	39.9	40.3	39.8	39.7	39.7	40.2	40.0	39.8
3	39.6	ND	ND	ND	ND	ND	ND	ND	ND	K	
4	39.7	39.6	39.0	40.0	39.5	39.8	39.7	40.0	39.8	39.7	39.8
5	39.3	K									
6	39.3	39.5	39.7	39.7	40.4	40.2	40.1	40.0	39.9	40.3	40.1
7	39.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8	39.8	ND	ND	ND	ND	ND	ND	ND	K		
9	39.7	39.6	39.5	39.8	39.8	39.3	39.5	39.6	39.6	39.4	39.6
10	39.8	ND	ND	ND	ND	ND	ND	K			
X	39.6	39.6	39.5	39.8	40.1	39.8	39.8	39.8	39.9	39.9	39.8

ND = Not Determined (Not Done), K = Killed
^ Beginning of observations, * Withdrawal of treatment

TABLE 6.5 (Cont.) Individual rectal temperatures of the treated pigs (yellow tags) in Experiment 4

Pig	Day of Experiment					Group
Number	27	29	31	33	35	X
1	39.5	39.8	39.3	39.5	39.2	39.8
2	39.5	39.6	39.5	39.5	39.3	39.7
3						39.6
4	40.0	40.0	39.5	40.2	39.8	39.7
5						39.3
6	39.7	40.2	39.9	39.9	39.9	39.9
7	ND	ND	ND	ND	K	39.5
8						39.8
9	39.3	39.3	39.4	39.3	39.8	39.5
10						39.8
X	39.6	39.8	39.5	39.7	39.6	39.7

ND = Not Determined (Not Done), K = Killed

NOTE: Temperatures are expressed in degrees centigrade

TABLE 6.6 Individual rectal temperatures of the control pigs (white tags) in Experiment 4

Pig	Day of Experiment										
Number	0	7^	9	11	13	15	17	19	21*	23	25
71	39.9	39.8	40.2	40.0	40.2	39.7	39.8	39.7	40.3	40.2	39.8
72	40.0	ND	ND	ND	ND	ND	ND	ND	ND	K	
73	39.9	40.5	40.2	40.1	40.2	40.6	40.3	40.2	40.2	40.2	40.1
74	40.5	39.5	39.7	39.7	39.7	39.8	39.8	39.9	40.1	39.9	39.8
75	40.4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
76	39.9	K									
77	40.2	40.0	40.1	40.3	40.5	40.2	40.1	40.1	40.3	40.4	40 3
78	39.9	ND	ND	ND	ND	ND	ND	K			
79	40.1	39.6	39.9	40.4	39.8	40.0	40.1	39.3	39.9	40.0	39.9
80	40.0	ND	ND	ND	ND	ND	ND	ND	K		
X	40.1	39.9	40.0	40.1	40.1	40.0	40.0	39.8	40.1	40.1	40.0

ND = Not Determined (Not Done), K = Killed

^ Beginning of observations, * Withdrawal of treatment

TABLE 6.6 (Cont.) Individual rectal temperatures of the control pigs (white tags) in Experiment 4

Pig Number	Day of Experiment 27	29	31	33	35	Group X
71	39.6	39.6	40.0	39.5	39.0	39.8
72						40.0
73	40.0	39.8	39.7	39.9	39.7	40.1
74	39.7	40.0	39.8	39.8	39.3	39.8
75	ND	ND	ND	ND	K	40.4
76						39.9
77	39.5	40.1	40.2	39.9	39.5	40.1
78						39.9
79	39.8	39.6	39.8	39.6	39.5	39.8
80						40.0
X	39.7	39.8	39.9	39.7	39.4	39.9

ND = Not Determined (Not Done) K = Killed

NOTE: Temperatures are expressed in degrees centigrade

In this Experiment the variation in temperatures recorded showed differences in individual animals of up to one degree between one sampling day to the next but the variation in temperatures throughout the period of study in most pigs was a maximum half of a degree centigrade.

The rectal temperatures recorded from the pigs in the treated group gave an overall average of 39.7°C with a range between 39.0°C and 40.4°C. In the control group the average temperature was 39.9°C with a range between 39.0°C and 40.6°C. In this group Pig 77 (white) maintained temperatures of 40.0°C or over during 13 sampling days out of 16, varying from 40.0°C to 40.5°C and Pig 73 (white) showed a similar pattern on 11 out of 16 days of sampling varying between 40.0°C to 40.6°C. None of the pigs with a raised rectal temperature appeared to be abnormal in any other way other than Pig 2 (yellow) where raised rectal temperature was associated with the presence of a swelling on days 21 and 23 of the study.

c) Liveweight and daily liveweight gain

The weights of the individual pigs are given in Tables 6.7

and 6.8. Daily live weight gains and their means over the periods of 0 to 7, 7 to 21 and 21 to 35 sampling days, corresponding to the stages of adjustment to, during and after treatment are presented below in Tables 6.9 and 6.10. The mean of daily liveweight gains obtained from the treated and control groups were: Adjustment period; 346g/day (treated) and 254 g/day (control), during treatment; 556 g/day (treated) and 530 g/day (control) and after withdrawal of treatment; 693 g/ day (treated) and 670 g/day (control). The differences in weight gains present in each group during each period were considerably reduced by the end of the study.

TABLE 6.7 Individual live weights of the treated pigs in Experiment 4, expressed in kilograms

Pig	Day of Experiment						Total
Number	0	7^	14	21*	28	35	Gain
1	10.8	13.9	17.9	22.5	27.9	33.2	22.4
2	9.6	12.1	16.0	19.9	24.7	28.6	19.0
4	9.2	10.5	13.5	17.2	20.4	27.4	18.2
6	7.2	9.9	13.5	17.9	23.0	28.6	21.4
9	9.2	11.7	15.2	19.5	22.9	27.7	18.5
Total	46.0	58.1	76.1	97.0	118.9	145.5	99.5

^Beginning of observations, *Withdrawal of treatment

TABLE 6.8 Individual live weights of the control pigs in Experiment 4, expressed in kilograms

Pig	Day of Experiment						Total
Number	0	7^	14	21*	28	35	Gain
71	9.7	12.3	16.2	20.3	23.2	30.3	20.6
73	8.3	10.2	13.3	15.8	18.8	25.2	16.9
74	7.1	8.5	11.8	15.8	18.6	22.3	15.2
77	8.5	10.6	14.8	18.5	23.3	29.3	20.8
79	9.7	10.6	15.0	18.9	23.9	29.1	19.4
Total	43.3	52.2	71.1	89.3	107.8	136.2	92.9

^Beginning of observations, *Withdrawal of treatment

TABLE 6.9 Individual daily live weight gains of the treated pigs for days 0-7, 7-21 and 21-35

Pig Number	Day of Experiment 0-7	7-21	21-35
1	0.443	0.614	0.764
2	0.357	0.557	0.622
4	0.186	0.479	0.729
6	0.386	0.572	0.764
9	0.357	0.557	0.586
X	0.346	0.556	0.693

TABLE 6.10 Individual daily live weight gains of the control pigs for days 0-7, 7-21 and 21-35

Pig Number	Day of Experiment 0-7	7-21	21-35
71	0.371	0.571	0.714
73	0.271	0.400	0.671
74	0.200	0.521	0.464
77	0.300	0.564	0.771
79	0.128	0.593	0.729
X	0.254	0.530	0.670

d) Feed consumption

The feed consumption was measured daily from both treated and control groups and the results are presented below in Figures XLVI and XLVII.

B) Bacteriological examination of faeces

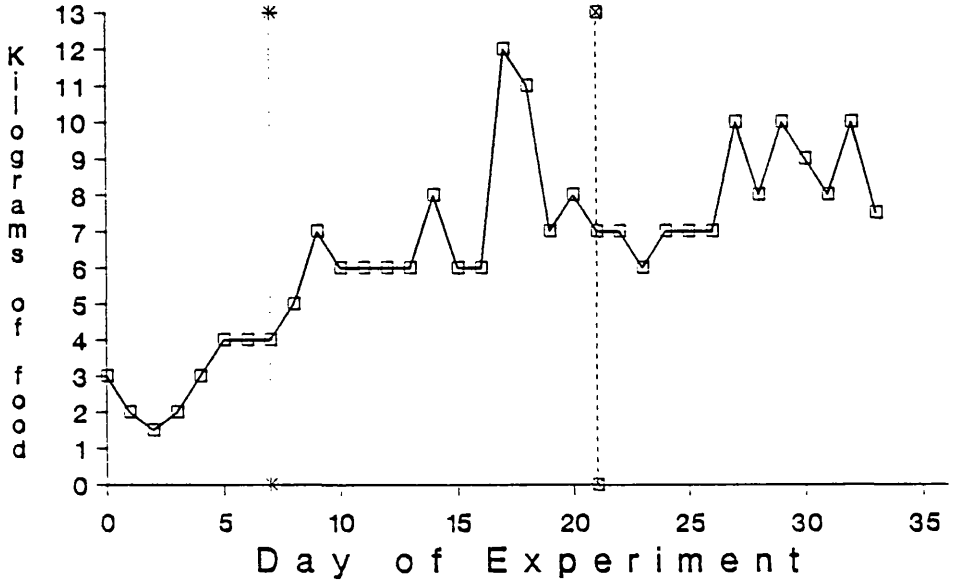
The results of the quantitative examination of faecal samples are presented in Tables 6.11 to 6.27 (Appendix D) as arithmetic means, graphically in Figures XLVIII to LXI, below.

a) Aerobic organisms

i) Total coliforms

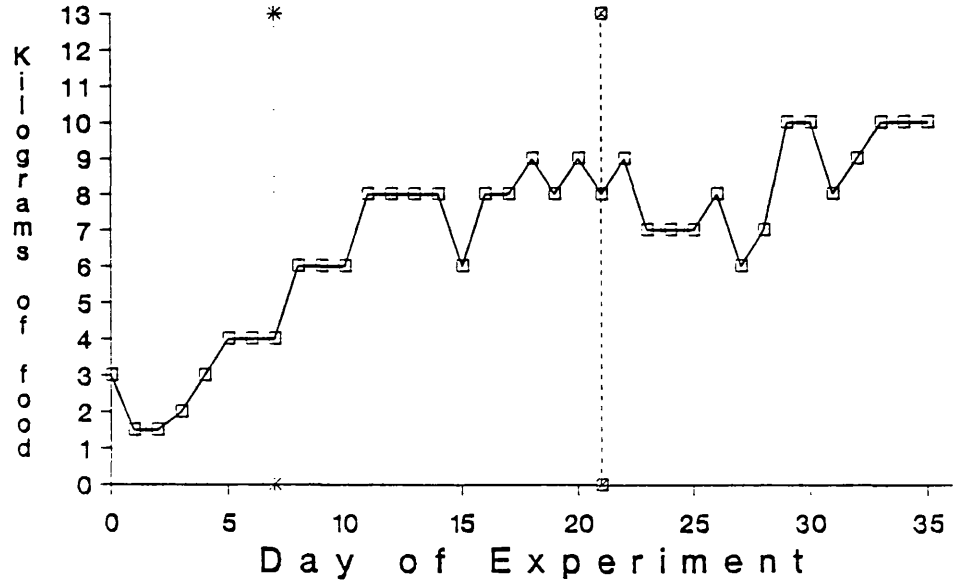
The bacterial counts obtained from the treated and control groups are presented in Tables 6.11 and 6.12 (Appendix D). The counts of total coliforms from the treated group before treatment were found to average 10^9/g, during treatment their numbers varied between 10^3 to 10^{10} bacteria/g of faeces giving an average of 10^9/g, and their numbers remained at 10^9/g after the withdrawal of treatment by day 35 of the study (Figure XLVIII). In the control group the mean numbers of total coliforms were 10^9/g before and during treatment and increased to 10^{10}/g by the end of the study (Figure XLIX). The range of

FIGURE XLVI. Feed consumption of the treated pigs in Experiment 4



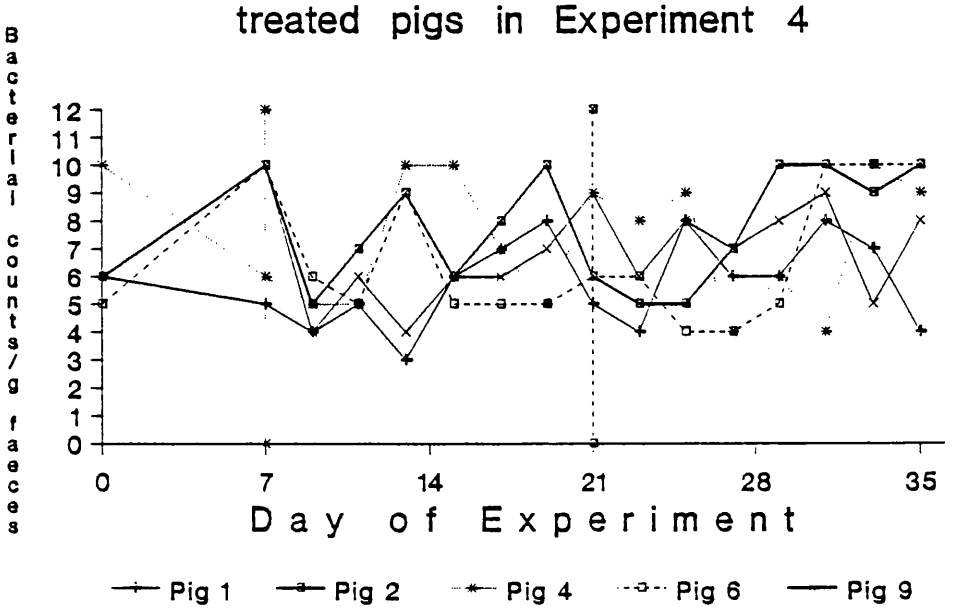
. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE XLVII. Feed consumption of the control pigs in Experiment 4



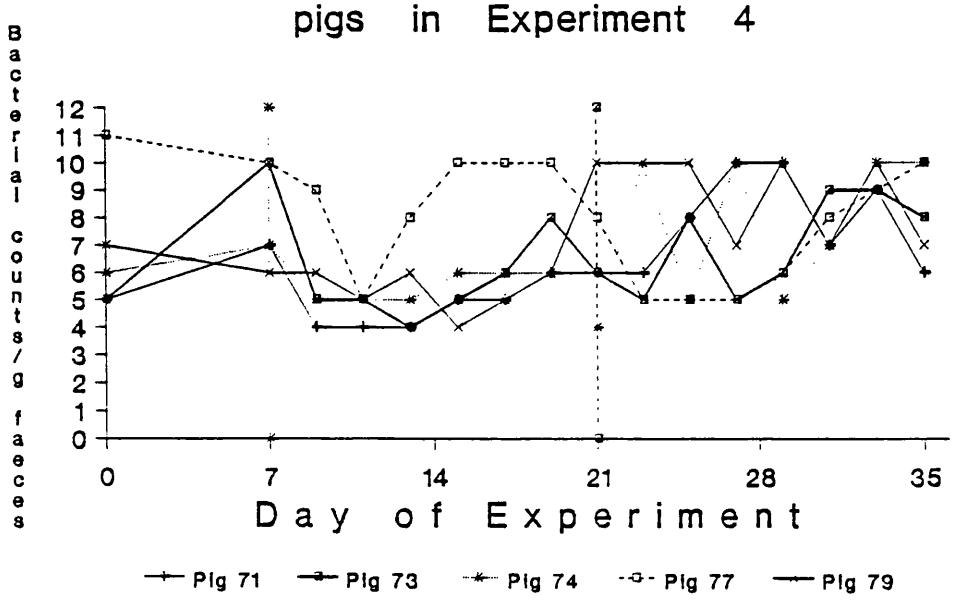
. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE XLVIII. Individual bacterial counts of total coliforms from the treated pigs in Experiment 4



. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE XLIX. Individual bacterial counts of total coliforms from the control pigs in Experiment 4



. . . . Beginning of treatment
---- Withdrawal of treatment

variation in populations in this group was found to be between 10^4/g and 10^{10}/g, during the whole period of examination.

ii) Escherichia coli

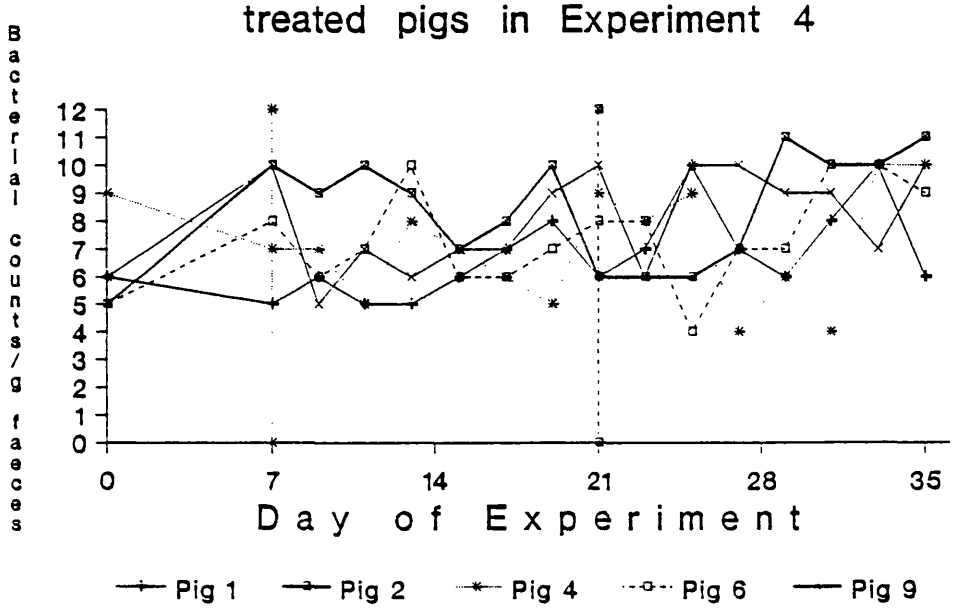
In this Experiment as in Chapter 5 the faecal samples were also examined for E. coli and the numbers isolated from each group are presented in Tables 6.13, 6.14 (Appendix D) and in Figures L and LI. Beta haemolytic colonies on sheep blood agar plates were examined further to determine their pathotype and the results are presented in Table 6.15. In the treated group the numbers of E. coli before treatment were of 10^9/g, during treatment they remained at 10^9/g, and after withdrawal of treatment they increased slightly to reach 10^{10}/g. The populations of E. coli in this group varied from 10^4 to 10^{10} bacteria/g of faeces. In the control group their mean numbers varied from 10^4/g to 10^{10}/g and they were found at 10^9/g before treatment. During the period of treatment their counts were 10^9/g and at the end of the screening period their numbers as in the treated group increased to 10^{10}/g.

TABLE 6.15 Pathotype of E. coli strains isolated from the pigs of both treated and control groups

Reference Number	Day of Expt.	Pig Number	Faeces Consist	Temp °C	Study Group	Source Sample	Findings VT-II	Findings ODP
5536	19	1 y	Soft	39.9	T	Faeces	+	+
5542	21	2 y	Soft	40.2	T	Faeces	+	+
5527	23	2 y	Normal	40.0	T	Faeces	+	+
5523	24	3 y	Soft	39.6	T	Ileum	+	+
5537	24	3 y	Soft	39.6	T	Colon	+	+
5529	21	4 y	Soft	39.8	T	Faeces	+	+
5530	23	4 y	Normal	39.7	T	Faeces	+	+
5532	21	6 y	Soft	39.9	T	Faeces	+	+
5534	21	9 y	Normal	39.6	T	Faeces	+	+
5522	19	71 w	Soft	39.7	C	Faeces	+	+
5543	19	73 w	Soft	40.2	C	Faeces	+	+
5539	31	73 w	Normal	39.7	C	Faeces	+	+
5540	15	74 w	Soft	39.8	C	Faeces	+	+
5535	15	77 w	Normal	40.2	C	Faeces	+	+
5541	19	77 w	Soft	40.1	C	Faeces	+	+
5524	31	77 w	Soft	40.2	C	Faeces	+	+
5525	33	77 w	Normal	39.9	C	Faeces	+	+
5531	15	79 w	Soft	40.0	C	Faeces	+	+
5528	21	79 w	Soft	39.9	C	Faeces	+	+

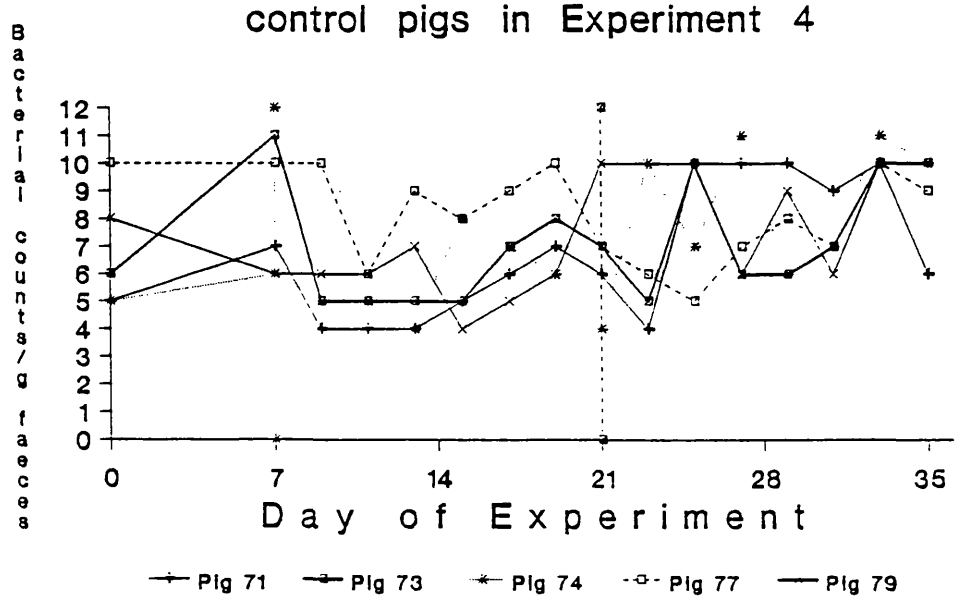
VT = Verotoxin, ODP = Oedema Disease Principle, + = Positive
T = Treated, C = Control, y = yellow, w = white
NOTE: None of the above Beta haemolytic E. coli strains showed the presence of either STaP, STaH, STb, LTp (enterotoxins) nor F4, F5, F6 or F41 (adhesins).

FIGURE L. Individual bacterial counts of total coliforms (E. coli) from the treated pigs in Experiment 4



. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE LI. Individual bacterial counts of total coliforms (E. coli) from the control pigs in Experiment 4



. . . . Beginning of treatment
---- Withdrawal of treatment

A further 19 isolates of Beta haemolytic E. coli were free from the above pathogenic determinants.

iii) Faecal streptococci

The numbers of faecal streptococci isolated from the faecal samples of pigs in both treated and control groups are given in Tables 6.16 and 6.17 (Appendix D). In the treated group they were present before treatment at 10^7/g of faeces, during treatment their counts increased to 10^9/g and remained at this level (10^9/g) after the withdrawal of treatment. In the control group 10^3/g were isolated before treatment, and as in the treated group their numbers increased to 10^9/g and at the end of the study their mean numbers remained at 10^9/g of faeces as may be observed on Figures LII and LIII, below.

iv) Salmonella spp.

The attempts to isolate Salmonella spp. from the cultures examined from either faecal samples or intestinal contents were unsuccessful.

b) Microaerobic organisms

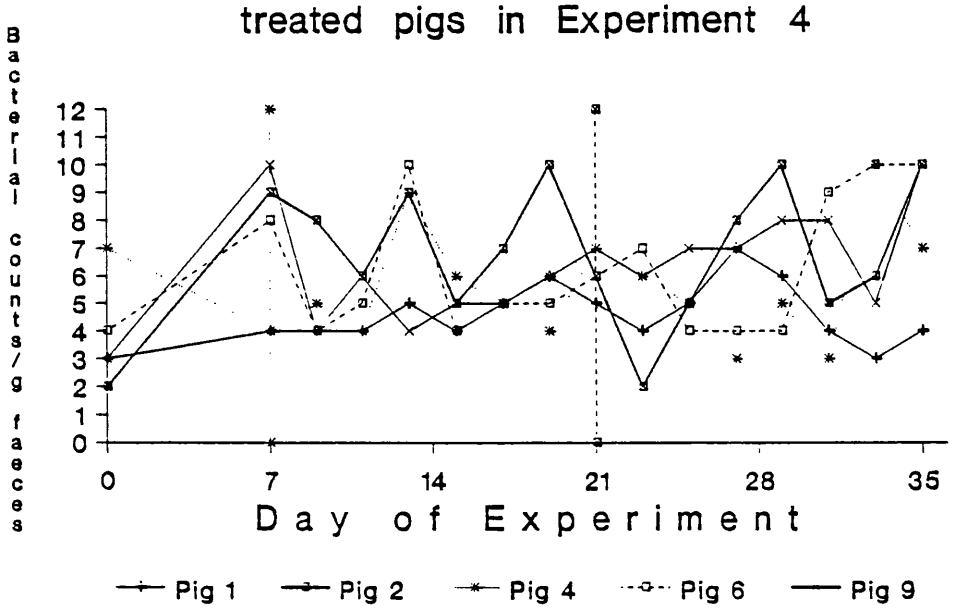
i) Campylobacter spp.

These bacteria were isolated from the pigs of the treated group in numbers which varied from 10^3 to 10^7 organisms/g of faeces (Table 6.18, Appendix D). Before treatment their mean counts were 10^4/g. During treatment and after its withdrawal their mean numbers increased to 10^5/g and remained in the same level until the end of the study (Figure LIV). The mean numbers of Campylobacter spp. in the control group were 10^7/g before treatment, 10^4/g during treatment and 10^7/g after the withdrawal of treatment. The range fluctuated between 10^3/g and 10^9/g, as may be observed in Table 6.19 and Figure LV.

ii) Lactobacilli

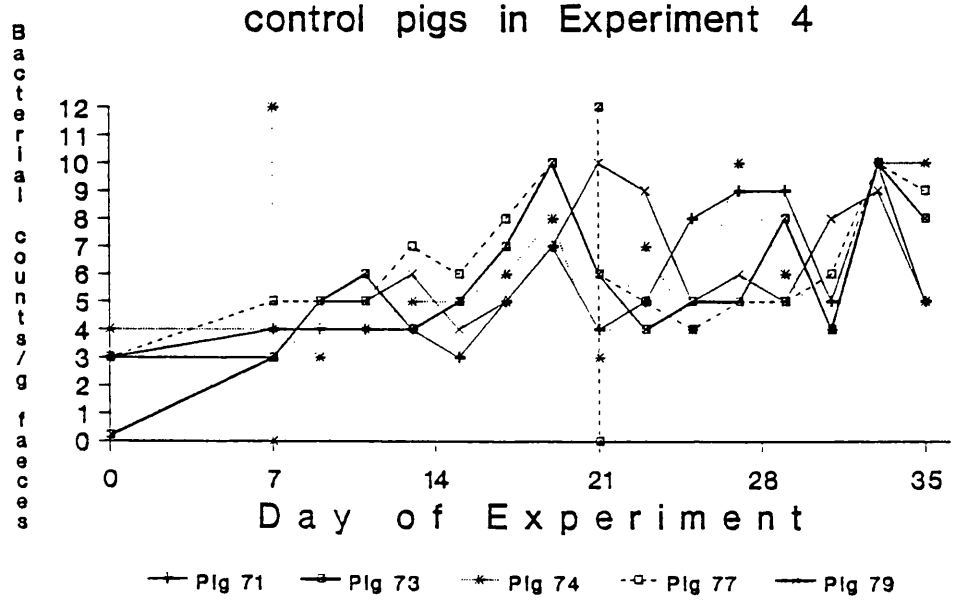
Tables 6.20 and 6.21 (Appendix D) and Figures LVI and LVII give the bacterial counts of lactobacilli isolated from the faecal samples of pigs in both treated and control groups. The

FIGURE LII. Individual bacterial counts of faecal streptococci from the treated pigs in Experiment 4



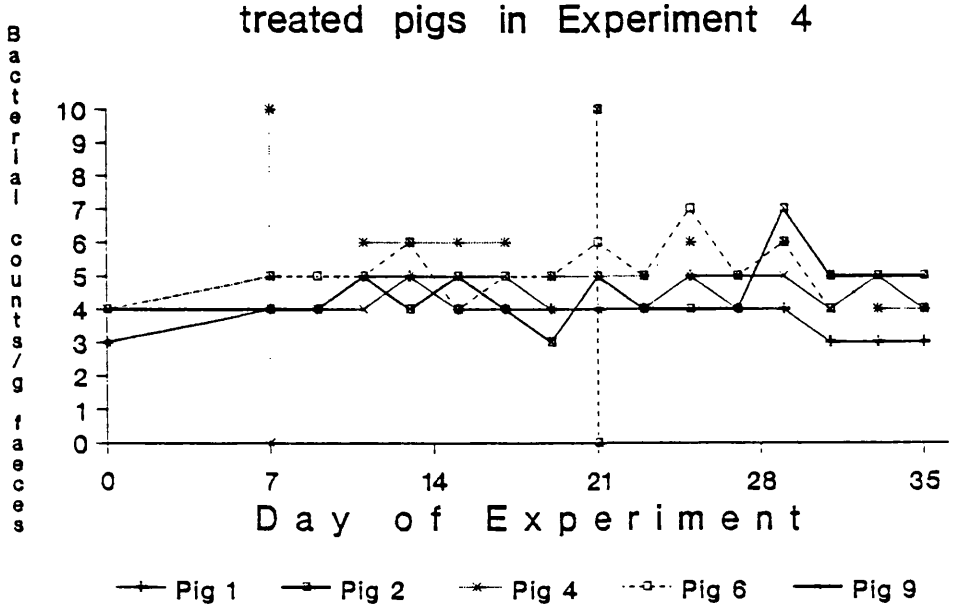
. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE LIII. Individual bacterial counts of faecal streptococci from the control pigs in Experiment 4



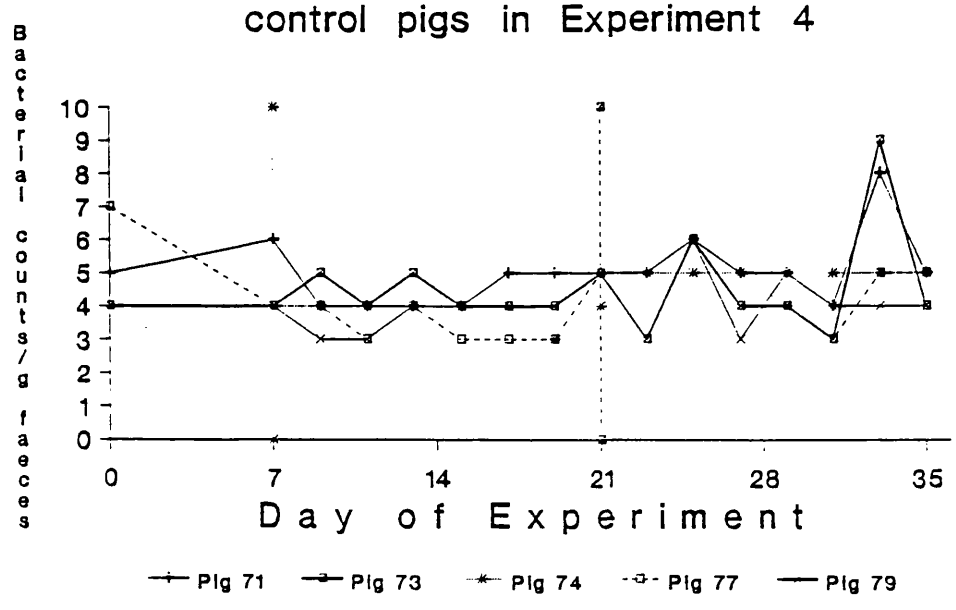
. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE LIV. Individual bacterial counts of Campylobacter spp. from the treated pigs in Experiment 4



. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE LV. Individual bacterial counts of Campylobacter spp. from the control pigs in Experiment 4



. . . . Beginning of treatment
---- Withdrawal of treatment

mean number of these bacteria before treatment, during treatment and after withdrawal of treatment in the treated group was 10^9/g, and they were found to vary between 10^3 and 10^{10} organisms/g of faeces. In the control group lactobacilli were also found at 10^9/g before and during treatment but increased to 10^{10}/g after the withdrawal of the drug. The variation in this group was from 10^4 and 10^{10} bacteria/g.

c) Anaerobic organisms

i) Bacteroides spp.

Bacteroides spp. were isolated in numbers which varied from 10^2 to 10^8/g in the treated group (Table 6.22, Appendix D). In this group these bacteria maintained populations of 10^5/g over the periods before and during treatment but after its withdrawal their numbers increased to 10^6/g (Figure LVIII). Similar patterns were seen in the control group in which their numbers were 10^4/g before and during treatment and increased to 10^5/g by the end of the study (Table 6.23, Appendix D). The bacteria were not isolated on two days of the Experiment in this group and their populations varied between 10^2 and 10^6 organisms/g of faeces (Figure LIX).

ii) Enteric spirochaetes

No enteric spirochaetes were isolated from any of the faecal samples obtained from the pigs of either treated or control groups.

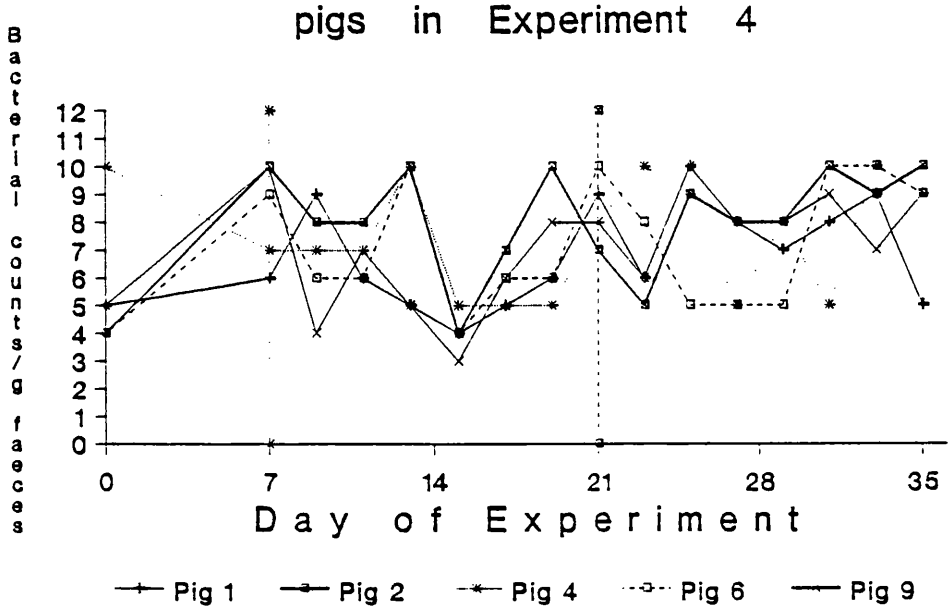
iii) Clostridium difficile

There was no confirmed evidence for the presence of this organism in the samples from the pigs used.

iv) Clostridium perfringens type A

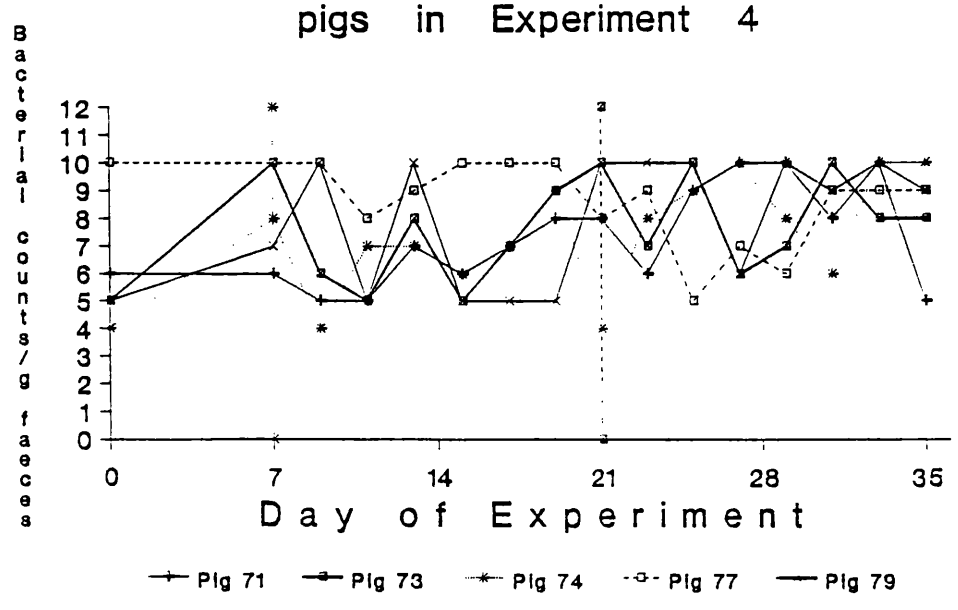
Vegetative cells.- The arithmetic means of C. perfringens type A obtained in this study varied between 10^3 and 10^{10} bacteria/g of faeces, and showed mean populations of 10^8/g in the treated group over the periods before, during and after

FIGURE LVI. Individual bacterial counts of lactobacilli from the treated pigs in Experiment 4



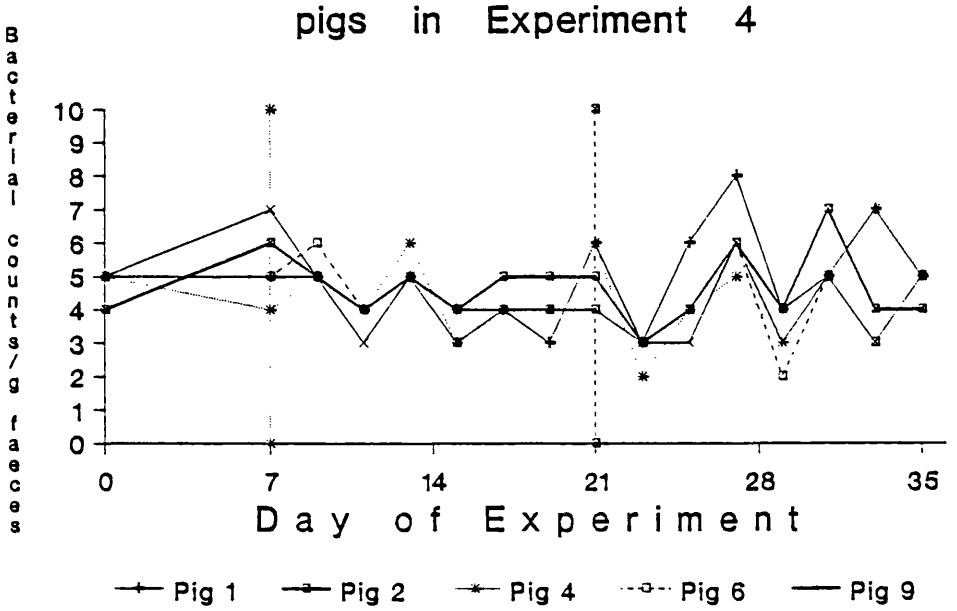
. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE LVII. Individual bacterial counts of lactobacilli from the control pigs in Experiment 4



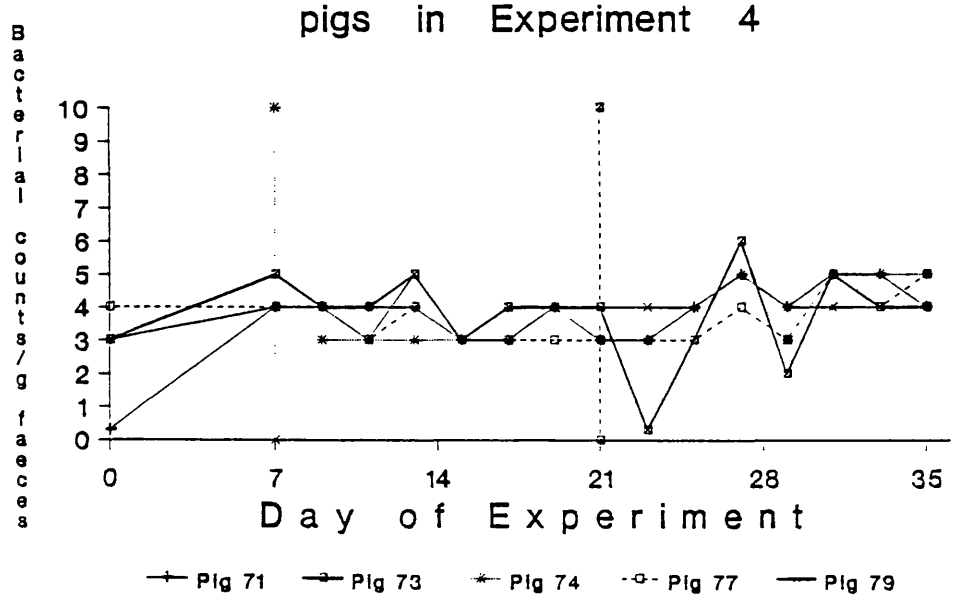
. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE LVIII. Individual bacterial counts of Bacteroides spp. from the treated pigs in Experiment 4



. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE LIX. Individual bacterial counts of Bacteroides spp. from the control pigs in Experiment 4



. . . . Beginning of treatment
---- Withdrawal of treatment

withdrawal of treatment (Figure LX). In this treated group increases occurred in individual pigs after chlortetracycline withdrawal (Table 6.24, Appendix D). Their numbers varied in the control group, giving means of 10^5/g before treatment, and increasing to 10^8/g during treatment and at the end of the Experiment. After withdrawal of treatment their numbers were found to be 10^9 organisms/g (Table 6.25 and Figure LXI).

Spore-forming strains.- Sporulating C. perfringens were isolated on a few occasions from either treated or control groups (Tables 6.26 and 6.27, Appendix D). Spore-forming bacteria were not isolated before treatment in either the control group or the treated group.

C) Findings in sacrificed animals

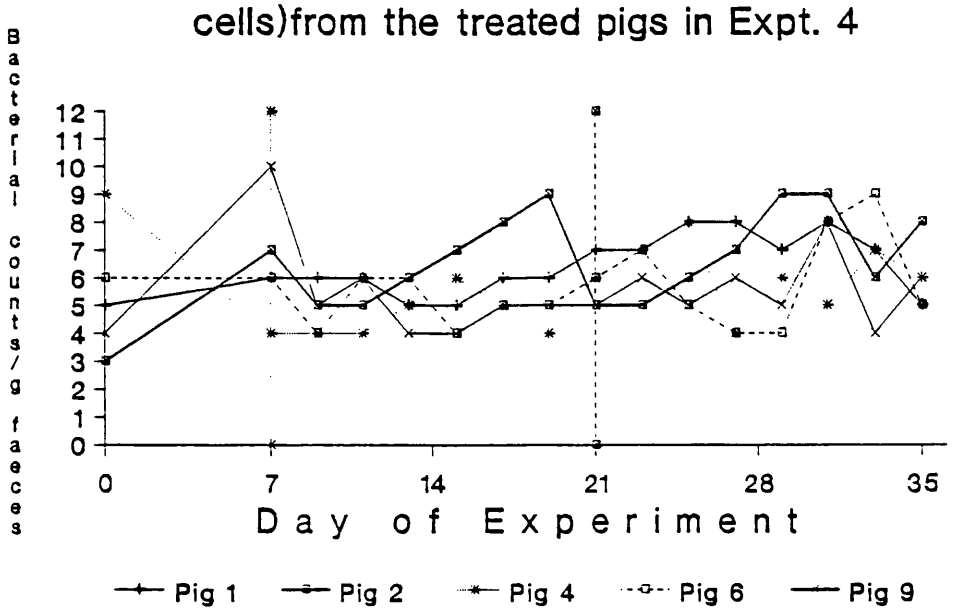
a) Gross post mortem findings

Few or no gross pathological changes were found in the ten pigs sacrificed in this Experiment. The majority of pigs killed on days which agreed with the activities in the post mortem room of the Veterinary School (Table 6.1) had stomachs empty or half filled with feed, the duodenum was always partially empty and usually contained a mucoid yellowish material containing gas bubbles. The jejunum and ileum were most frequently half filled with contents, and the caecum and colon were at all times filled with contents which varied in colour and consistency.

The first two pigs (5 yellow and 76 white) sacrificed on day 7 of the study were found to have no apparent external abnormalities or lesions. Their internal viscera were also normal in appearance with no lesions. After the opening of the gastrointestinal tract no gross changes were seen which suggested the presence of any infection or pathological process except for villi slightly reduced in height in one or two segments of the small intestinal mucosa.

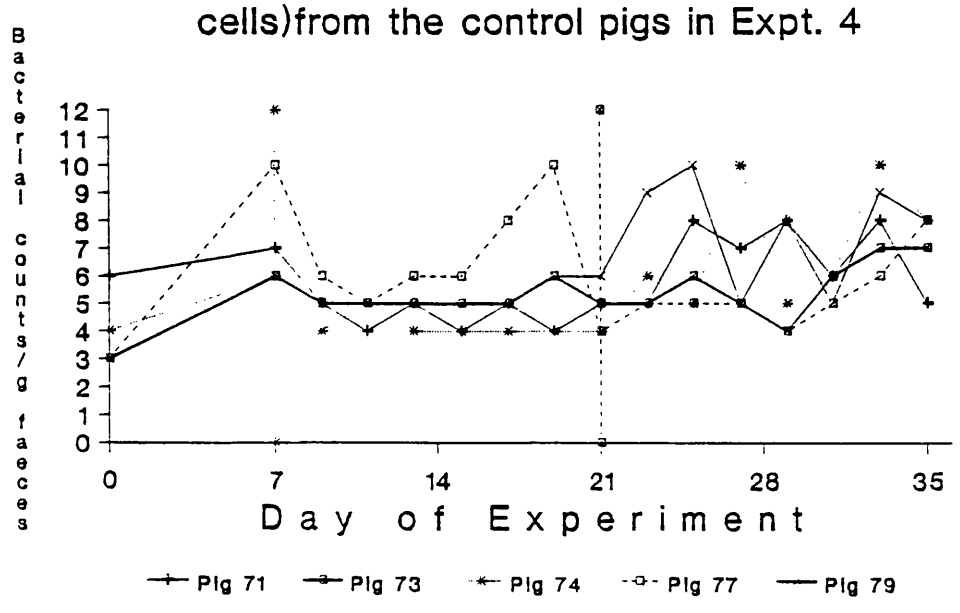
There were no gross pathological changes in the viscera of both thoracic and abdominal cavities from pigs 10 (yellow) and 78 (white) killed on day 20 of the study and from pigs 8 (yellow) and 80 (white) which were sacrificed on day 22 of the

FIGURE LX. Individual bacterial counts of Clostridium perfringens type A (veg. cells)from the treated pigs in Expt. 4



. . . . Beginning of treatment
---- Withdrawal of treatment

FIGURE LXI. Individual bacterial counts of Clostridium perfringens type A (veg. cells)from the control pigs in Expt. 4



. . . . Beginning of treatment
---- Withdrawal of treatment

Experiment. On day 24 two more pigs 3 (yellow) and 72 (white) were killed. These pigs were also found to have no obvious external changes or lesions at the time of slaughter. The gastrointestinal tract was normal and no apparent lesions or pathological changes were seen on detailed macroscopic examination.

Pigs 7 (yellow) and 75 (white) were killed on day 35 of the Experiment and were in good bodily condition. Neither of them showed any external gross lesions. There were no gross pathological changes seen in the abdominal or thoracic viscera during the examination of their carcases.

D) Bacterial examinations of gastrointestinal contents

The results of quantitative and qualitative bacterial examinations performed before,during and after chlortetracycline treatment on the contents obtained from the gastrointestinal tract of the sacrificed pigs in both treated and control groups are presented below (Tables 6.28 to 6.41).

a) Aerobic organisms

i) Total coliforms and Escherichia coli

The bacterial counts of total coliforms isolated from the contents of each section of the gastroenteric tract of the sacrificed pigs are presented in Tables 6.28 and 6.29. Counts of E. coli from the same samples are given for the treated and control groups in Tables 6.30 and 6.31 respectively.

Stomach.- Total coliform counts from the treated group were 10^6/g before treatment, when treatment was established they decreased to 10^3/g, their numbers remained at 10^3/g after its withdrawal by day 22, and similar levels were found at the end of the study on day 35. The counts in the control group were 10^3/g before treatment. No coliforms were isolated during treatment but they were 10^3/g after its withdrawal and increased to 10^4/g by day 24 of the study, remaining at this level until the end of the Experiment.

Counts of E. coli of 10^5/g were found in the treated group

before and during treatment, their population decreased to 10^3/g on day 22 after withdrawal of treatment, returned to 10^5/g on day 24 and finished at 10^3/g. E. coli counts in the control group were 10^4/g before treatment but, no isolations were made during treatment. After withdrawal on day 22 their numbers were 10^3/g, increased to 10^5/g and ended 10^4/g.

Duodenum.- In the treated group no coliforms were found before treatment or at the end of the study, but during treatment their numbers were 10^2/g and increased to 10^3/g after withdrawal of the drug by day 22 of the study. The counts of total coliforms in the control group were recorded only before treatment and after withdrawal of the treatment in numbers of 10^2 organisms/g of contents.

E. coli was found in the treated group only after withdrawal of treatment in numbers of 10^3/g on day 22 and 10^2/g by day 24. Similar levels of 10^3/g and 10^2/g were found in the control group, on the same days after the withdrawal of treatment. Their counts at the end of the study by day 35 were 10^3 organisms/g of contents.

Jejunum.- Prior to treatment total coliforms were found in numbers of 10^3/g, decreased to 10^2/g during treatment and returned to 10^3/g after withdrawal of treatment on day 22. No coliforms were isolated by the end of the Experiment. Coliforms from the jejunal contents in the control group were isolated only after withdrawal of treatment (10^3/g) and at the end of the Experiment by day 35 their population remained at 10^3/g.

The numbers of E. coli isolated in the treated group before treatment, after treatment and up to the end of the Experiment were 10^3/g. No Escherichia coli were isolated during treatment from either group. From the control group counts of 10^3/g were found only after withdrawal of treatment and at the end of the study.

Ileum.- In the treated group coliforms were present at 10^4/g before and during treatment, their populations increased dramatically to 10^{10}/g just after withdrawal of treatment and returned to 10^4/g by day 24. At the end of the study on day 35

their numbers increased to finish at 10^5/g. From the control group 10^5/g were isolated before treatment, during treatment their numbers increased to 10^6/g and finally low populations of 10^4/g were found after treatment and at the end of the Experiment.

The counts of E. coli obtained from the ileal contents in the treated group were 10^4/g before treatment, increased numbers (10^8/g) were found during treatment and after withdrawal (10^{10}/g). Their numbers on day 24 fell to 10^3/g and recovered to 10^5/g at the end of the study. In the control group similar counts of 10^4/g were found before treatment, during treatment the numbers rose to 10^7/g and dropped back to 10^3/g after withdrawal. On day 24 10^4/g were isolated and remained at this level until the end of the study by day 35.

Caecum.- Total coliform counts from the treated group were 10^6/g before treatment, during treatment and after its withdrawal they were 10^5/g. By day 24 after withdrawal of treatment they were 10^4/g and increased to 10^{10}/g by the end of the Experiment. Counts of 10^5/g were found in the control group before and during treatment, following withdrawal these counts increased dramatically to 10^{10}/g, and by day 24 decreased to 10^6/g. Numbers of 10^3/g were found at the end of the study.

E. coli were found in the treated group at 10^7/g before treatment. During chlortetracycline treatment their numbers fell to 10^4/g, and increased to 10^6/g after its withdrawal. Populations of 10^5 organisms/g were found on day 24, and by day 35 their numbers increased to 10^{10}/g. From the control group 10^4/g were isolated before treatment, an increased number of 10^5/g were found during treatment and after withdrawal of treatment higher populations of 10^{10}/g were isolated. E. coli counts of 10^6/g were found on day 24 and by day 35 10^4 organisms/g contents were present.

Colon.- Total coliforms were isolated at 10^{10}/g from the colonic contents of the treated group before and during treatment. After withdrawal of treatment their populations decreased to 10^9/g and further down to 10^5/g on day 24. Their numbers returned to 10^{10}/g at the end of the study. The samples

from the control group gave higher counts of 10^{10}/g before treatment. During treatment they decreased to 10^{8}/g, after the withdrawal of treatment 10^{6}/g were isolated and by the end of the study counts were 10^{4}/g.

10^{9} Escherichia coli/g were isolated from the colonic contents of the treated group before treatment. Populations of 10^{10}/g were isolated during treatment, after its withdrawal and at the end of the study. On day 24 of the study numbers of E. coli dropped to 10^{5}/g. Counts of 10^{10}/g were found in the control group before treatment. During treatment they decreased to 10^{8}/g. Following withdrawal of treatment their numbers were 10^{6}/g and later increased to 10^{10}/g. The counts found at the end of the study were 10^{5} organisms/g of contents.

TABLE 6.28 Coliform counts from gastrointestinal contents of the treated pigs in Experiment 4

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
5	7^	1.0×10^{6}	N G	5.0×10^{3}	3.0×10^{4}	2.0×10^{6}	1.5×10^{10}
10	20*	5.0×10^{3}	5.0×10^{2}	5.0×10^{2}	5.0×10^{4}	1.0×10^{5}	2.0×10^{10}
8	22	1.0×10^{3}	2.5×10^{3}	1.0×10^{3}	3.5×10^{10}	3.5×10^{5}	5.0×10^{9}
3	24	5.0×10^{3}	1.0×10^{3}	N G	2.0×10^{4}	1.0×10^{4}	1.0×10^{5}
7	35	5.0×10^{3}	N G	N G	1.0×10^{5}	2.0×10^{10}	1.0×10^{10}

^ Beginning of observations, * Withdrawal of treatment
N G = No Growth

TABLE 6.29 Coliform counts from gastrointestinal contents of the control pigs in Experiment 4

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
76	7^	5.0×10^{3}	5.0×10^{2}	N G	6.0×10^{5}	5.0×10^{5}	3.5×10^{10}
78	20*	N G	N G	N G	4.0×10^{6}	1.5×10^{5}	5.0×10^{8}
80	22	5.0×10^{3}	5.0×10^{2}	3.0×10^{3}	1.0×10^{4}	1.5×10^{10}	5.0×10^{6}
72	24	1.0×10^{4}	N G	N G	N G	5.0×10^{6}	5.0×10^{6}
75	35	1.0×10^{4}	N G	5.0×10^{3}	5.0×10^{4}	5.0×10^{3}	5.0×10^{4}

^ Beginning of observations, * Withdrawal of treatment
N G = No Growth

TABLE 6.30 *Escherichia coli* counts from gastrointestinal contents of the treated pigs in Experiment 4

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
5	7^	$5.0x10^5$	N G	$1.5x10^3$	$5.0x\ 10^4$	$1.0x\ 10^7$	$1.0x10^9$
10	20*	$1.0x10^5$	N G	N G	$1.0x\ 10^8$	$6.0x\ 10^4$	$3.0x10^{10}$
8	22	$5.0x10^3$	$3.0x10^3$	$5.0x10^3$	$1.5x10^{10}$	$1.0x\ 10^6$	$1.0x10^{10}$
3	24	$1.5x10^5$	$5.0x10^2$	N G	$4.5x\ 10^3$	$5.0x\ 10^5$	$5.0x10^5$
7	35	$3.0x10^3$	N G	$1.5x10^3$	$1.0x\ 10^5$	$1.0x10^{10}$	$1.0x10^{10}$

^ Beginning of observations, * Withdrawal of treatment
N G = No Growth

TABLE 6.31 *Escherichia coli* counts from gastrointestinal contents of the control pigs in Experiment 4

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
76	7^	$1.5x10^4$	N G	N G	$2.0x10^4$	$5.0x\ 10^4$	$3.0x10^{10}$
78	20*	N G	N G	N G	$2.0x10^7$	$5.0x\ 10^5$	$1.5x10^8$
80	22	$1.5x10^3$	$5.0x10^3$	$2.0x10^3$	$5.0x10^3$	$2.0x10^{10}$	$2.5x10^6$
72	24	$5.0x10^5$	$5.0x10^2$	$1.0x10^3$	$1.0x10^4$	$5.0x\ 10^6$	$1.5x10^{10}$
75	35	$2.0x10^4$	$1.0x10^3$	$1.0x10^3$	$4.5x10^4$	$3.5x\ 10^4$	$1.5x10^5$

^ Beginning of observations, * Withdrawal of treatment
N G = No Growth

ii) Faecal streptococci

The streptococcal counts isolated from the gastroenteric contents of the pigs sacrificed from either group in this Experiment are presented below in Tables 6.32 and 6.33.

Faecal streptococci were isolated from the gastric contents of the treated group before treatment in numbers of 10^3/g. The control group yielded streptococci before treatment (10^3/g), and at the end of the Experiment (10^2/g). The duodenum of the treated group yielded 10^2 organisms/g following withdrawal of the antimicrobial, but no further isolations were made in this group. In the control group faecal streptococci were isolated at 10^2/g before treatment and at the end of the study on day 35.

Faecal streptococci were isolated from the jejunal contents

of the treated group at 10^3/g before treatment. In the control group, populations of these organisms were found only after the withdrawal of treatment in numbers of 10^2/g. Streptococci were isolated on several occasions from ileal samples at levels of 10^4/g before treatment, 10^3/g during treatment, 10^5/g after withdrawal, 10^2/g on day 24 and they remained at this level until the end of the study. Streptococci were isolated from the control group at 10^4/g before treatment and increased to 10^6/g during treatment. There were no isolations following withdrawal of treatment but by day 35 10^2/g were present.

10^4 organisms/g were isolated from the caecum of the treated group before treatment, during treatment their counts decreased to 10^3/g, returning to 10^4/g after withdrawal and remaining at this level until the end of the study. Counts of 10^4/g were recorded before and during treatment in the control group. Increased numbers of 10^8/g were isolated after withdrawal, but on day 24 and until the end of the Experiment their populations were 10^5/g.

The numbers of streptococci isolated from the colon in the treated group were 10^7/g before treatment, decreased to 10^6/g during treatment and following treatment withdrawal they were found to be 10^5/g. Their numbers rose to 10^9/g by day 35 of the study. The control group gave isolations of 10^6/g before treatment, during treatment their populations were 10^5/g, decreased to 10^4/g on day 22, increased to 10^5/g on day 24 and returned to 10^4/g by the end of the study.

TABLE 6.32 Faecal streptococcal counts from gastrointestinal contents of the treated pigs in Experiment 4

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
5	7^	3.0×10^3	N G	1.0×10^3	5.0×10^4	2.0×10^4	5.0×10^7
10	20*	N G	N G	N G	1.0×10^3	1.5×10^3	2.0×10^6
8	22	N G	5.0×10^2	N G	5.0×10^5	1.5×10^4	2.0×10^5
3	24	N G	N G	N G	5.0×10^2	N G	5.0×10^5
7	35	N G	N G	N G	5.0×10^2	5.0×10^4	5.0×10^9

^ Beginning of observations, * Withdrawal of treatment

N G = No Growth

TABLE 6.33 Faecal streptococcal counts from gastrointestinal contents of the control pigs in Experiment 4

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
76	7^	$5.0x10^3$	$5.0x10^2$	N G	$5.0x10^4$	$1.5x10^4$	$1.5x10^6$
78	20*	N G	N G	N G	$1.0x10^6$	$1.0x10^4$	$2.0x10^5$
80	22	N G	N G	$5.0x10^2$	N G	$5.0x10^8$	$5.0x10^4$
72	24	N G	N G	N G	N G	$5.0x10^5$	$1.5x10^5$
75	35	$5.0x10^2$	$5.0x10^2$	N G	$5.0x10^2$	$1.0x10^5$	$2.0x10^4$

^ Beginning of observations, * Withdrawal of treatment

N G = No Growth

iii) Salmonella spp.

There were no isolations of these bacteria from any of the samples examined throughout the whole Experiment.

b) Microaerobic organisms

i) Campylobacter spp.

The counts shown below in Tables 6.34 and 6.35 gave the numbers of the isolations made from the samples examined. No campylobacters were isolated from the stomach of the pigs from the treated group. In the control group they were isolated in numbers of 10^3/g, only at the end of the study on day 35. Duodenal contents of the treated group gave populations of 10^3/g before treatment, after withdrawal of treatment their numbers decreased to 10^2/g. No further isolations were made in this group. There were no Campylobacter spp. isolated from the control group.

No campylobacters were isolated from the contents of the jejunum in either groups. Ileal contents from the treated group yielded 10^3/g before treatment, during treatment numbers decreased to 10^2/g and then returned to 10^3/g following withdrawal. Counts of 10^2/g were found on day 24 and increased levels of these organisms (10^4/g) were finally isolated by the end of the study on day 35. As in the treated group 10^3/g were isolated in the control group at the beginning of the study. Counts of 10^5/g were found during the treatment period. Lower counts of 10^3/g were present after treatment had been withdrawn

on day 24 of the study.

Campylobacter spp. were isolated from the caecum of the treated animals in numbers of 10^3/g, before treatment. During chlortetracycline treatment their numbers increased to 10^6/g, decreased to 10^5/g after withdrawal and to 10^2/g by day 24 of the Experiment. When the Experiment ended 10^3/g were found. Similar counts of 10^3/g were isolated from the control group before treatment. During treatment their numbers increased to 10^5/g, remained at this level following withdrawal and decreased to 10^3/g by day 24. 10^4/g were finally isolated at the end of the study. Levels of 10^5/g were isolated from the colon of the treated group before, during and after withdrawal of treatment. Later, on day 24, their populations were 10^3/g and were 10^4/g on day 35. Similar levels of 10^4 organisms/g of contents were found in the control group before treatment, when treatment was established raised numbers of 10^6/g were found. After withdrawal of treatment they decreased to 10^5/g, and by day 35 their counts remained at 10^5/g.

TABLE 6.34 Counts of Campylobacter spp. from the gastroenteric contents of the treated pigs in Experiment 4

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
5	7^	N G	$2.0x10^3$	N G	$5.0x10^3$	$3.5x10^3$	$5.0x10^5$
10	20*	N G	N G	N G	$5.0x10^2$	$1.0x10^6$	$2.0x10^5$
8	22	N G	$5.0x10^2$	N G	$4.0x10^3$	$3.5x10^5$	$1.0x10^5$
3	24	N G	N G	N G	$5.0x10^2$	$5.0x10^2$	$5.0x10^3$
7	35	N G	N G	N G	$5.0x10^4$	$5.0x10^3$	$1.5x10^4$

^ Beginning of observations, * Withdrawal of treatment
N G = No Growth

TABLE 6.35 Counts of Campylobacter spp. from the gastroenteric contents of the control pigs in Experiment 4

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
76	7^	N G	N G	N G	$1.0x10^3$	$5.0x10^3$	$5.0x10^4$
78	20*	N G	N G	N G	$5.0x10^5$	$1.0x10^5$	$1.5x10^6$
80	22	N G	N G	N G	N G	$5.0x10^5$	$4.5x10^4$
72	24	N G	N G	N G	$3.5x10^3$	$1.0x10^3$	$1.0x10^5$
75	35	$5.0x10^3$	N G	N G	N G	$1.0x10^4$	$3.0x10^5$

^ Beginning of observations, * Withdrawal of treatment
N G = No Growth

ii) Lactobacilli

Counts of lactobacilli isolated from gastroenteric contents of the sacrificed pigs from both groups are presented below in Tables 6.36 and 6.37. Counts of 10^4/g were isolated from gastric contents of the treated pigs before treatment. During treatment they were found to be 10^5/g, following withdrawal of treatment they decreased to 10^3/g but returned to 10^5/g by day 24. 10^3 organisms/g of contents were isolated when the Experiment ended. In the control group, lactobacilli were isolated at 10^4/g before treatment, during treatment their numbers decreased to 10^2/g. Increased populations of 10^3/g and 10^5/g were isolated after withdrawal of treatment on days 22 and 24 respectively. By the end of the study their numbers remained at 10^5/g.

The duodenum of the treated group yielded these organisms only after withdrawal of treatment in numbers of 10^3/g and 10^2/g. Samples from the control group gave 10^3/g before treatment and after withdrawal of treatment. No isolations were made during treatment but 10^2/g were found at the end of the study. In jejunal contents of the treated group they were found before treatment, following withdrawal and at the end of the study in numbers of 10^3/g. During treatment and on day 24 their populations were found to be 10^2/g of contents. There were no isolations of these organisms in the control group before and during treatment, but regular counts of 10^3/g were found after withdrawal of treatment and at the end of the study.

From the ileum of the treated group lactobacilli were isolated at 10^4/g before treatment, during treatment they rose to 10^9/g but fell to 10^5/g and 10^4/g after withdrawal. Counts of 10^5/g were recorded on day 35. The control group gave counts of 10^3/g before treatment, higher counts of 10^{10}/g were found during treatment but they dropped to 10^4/g by day 22 and to 10^3/g by day 24. Lactobacilli were isolated at the end of the Experiment in numbers of 10^5/g. Bacterial counts of 10^8/g were isolated from the caecum of the treated pigs before treatment, decreased counts of 10^5/g were found during treatment but their numbers increased to 10^9/g following the withdrawal of treatment on day 22. By day 24 they returned to 10^5/g, and when the study ended they were 10^7/g of contents. Samples from the control

group yielded numbers of 10^4/g before treatment, during treatment they were 10^6/g, increased to 10^{10}/g following withdrawal of treatment and fell to 10^5/g by day 24. At the end of the Experiment their populations remained at 10^5/g.

Lactobacillus spp. were isolated from the colonic contents of the treated group at levels of 10^{10}/g, before treatment. During and following treatment withdrawal their numbers decreased to 10^9/g. Their counts fell to 10^5/g on day 24 but recovered to 10^8/g by the end of the study. Numbers found in the control group were 10^9/g before treatment, during the period of treatment their populations decreased to 10^8/g and following withdrawal of treatment 10^5 organisms/g were isolated. On day 24 of the study the counts obtained were 10^8/g and on day 35 10^6/g were present.

TABLE 6.36 Counts of Lactobacillus spp. from the gastroenteric contents of the treated pigs in Experiment 4

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
5	7^	$1.5x10^4$	N G	$5.0x10^3$	$3.5x10^4$	$1.0x10^8$	$2.0x10^{10}$
10	20*	$5.0x10^5$	N G	$5.0x10^2$	$5.0x10^9$	$1.0x10^5$	$5.0x10^9$
8	22	$2.5x10^3$	$5.0x10^3$	$1.0x10^3$	$5.0x10^5$	$5.0x10^9$	$5.0x10^9$
3	24	$1.5x10^5$	$5.0x10^2$	$5.0x10^2$	$5.0x10^4$	$1.0x10^5$	$5.0x10^5$
7	35	$2.5x10^3$	N G	$1.0x10^3$	$5.0x10^5$	$5.0x10^7$	$5.0x10^8$

^ Beginning of observations, * Withdrawal of treatment
N G = No Growth

TABLE 6.37 Counts of Lactobacillus spp. from the gastroenteric contents of the control pigs in Experiment 4

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
76	7^	$1.0x10^4$	$1.5x10^3$	N G	$5.0x\ 10^3$	$5.0x\ 10^4$	$5.0x10^9$
78	20*	$5.0x10^2$	N G	N G	$1.0x10^{10}$	$5.0x\ 10^6$	$2.0x10^8$
80	22	$2.5x10^3$	$2.0x10^3$	$2.0x10^3$	$5.0x\ 10^4$	$1.5x10^{10}$	$5.0x10^5$
72	24	$5.0x10^5$	$1.5x10^3$	$5.0x10^3$	$3.0x\ 10^3$	$5.0x\ 10^5$	$5.0x10^8$
75	35	$1.0x10^5$	$5.0x10^2$	$5.0x10^3$	$1.5x\ 10^5$	$2.0x\ 10^5$	$1.0x10^6$

^ Beginning of observations, * Withdrawal of treatment
N G = No Growth

c) Anaerobic organisms

i) Enteric spirochaetes

These bacteria were not isolated from any of the samples of the gastrointestinal contents obtained from the sacrificed pigs.

ii) Bacteroides spp.

The counts of these organisms are presented below in Tables 6.38 and 6.39, corresponding to the examination of gastrointestinal contents from the pigs of both treated and control groups. No Bacteroides spp. were isolated from the samples of gastric, duodenal and jejunal contents obtained from the sacrificed pigs of the treated and control groups. They were not isolated from the ileum of the treated group before treatment but during treatment they were present at 10^3/g and similar counts were found at the end of the Experiment. The control group gave numbers of 10^4/g during treatment and 10^3/g following withdrawal by day 22. As in the treated group further isolations were not achieved.

The caecal contents of the treated and control groups were also negative for the isolation of Bacteroides spp. before treatment, but during treatment these bacteria were isolated from the treated group at levels of 10^4/g, decreased numbers of 10^3/g were found following withdrawal of treatment. On day 24 their numbers returned to 10^4/g and finally counts 10^3/g were isolated at the end of the study. The control group gave counts of 10^3/g during treatment, after withdrawal they increased to 10^4/g but by day 24 they fell to 10^2/g. Counts of 10^3/g were isolated when the Experiment ended.

Counts of 10^3/g were made from the colon of the treated pig before treatment. During treatment, after withdrawal of treatment and until the end of the study the numbers of these organisms were regularly found at levels of 10^4/g. Populations of 10^3/g of contents were isolated in the control group before treatment, during treatment and following withdrawal of treatment. On day 24 increased numbers of 10^5 organisms/g of contents were isolated from the samples obtained. When the

Experiment ended on day 35 the numbers of Bacteroides spp. isolated were, on average 10^4/g.

TABLE 6.38 Counts of Bacteroides spp. from the gastrointestinal contents of the treated pigs in Experiment 4

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
5	7^	N G	N G	N G	N G	N G	$1.5x10^3$
10	20*	N G	N G	N G	$1.5x10^3$	$1.0x10^4$	$2.0x10^4$
8	22	N G	N G	N G	N G	$5.0x10^3$	$5.0x10^4$
3	24	N G	N G	N G	N G	$5.0x10^4$	$1.5x10^4$
7	35	N G	N G	N G	$1.5x10^3$	$5.0x10^3$	$5.0x10^4$

^ Beginning of observations, * Withdrawal of treatment
N G = No Growth

TABLE 6.39 Counts of Bacteroides spp. from the gastrointestinal contents of the control pigs in Experiment 4

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
76	7^	N G	N G	N G	N G	N G	$1.0x10^3$
78	20*	N G	N G	N G	$5.0x10^4$	$5.0x10^3$	$1.5x10^3$
80	22	N G	N G	N G	$1.0x10^3$	$5.0x10^4$	$3.0x10^3$
72	24	N G	N G	N G	N G	$5.0x10^2$	$1.5x10^5$
75	35	N G	N G	N G	N G	$5.0x10^3$	$5.0x10^4$

^ Beginning of observations, * Withdrawal of treatment
N G = No Growth

iii) Clostridium difficile

Clostridium difficile was not isolated from any of the gastrointestinal contents of the sacrificed pigs in either treated or control groups.

iv) Clostridium perfringens type A

Colonies of C. perfringens type A were found in the contents of the sacrificed pigs in the numbers presented below in Tables 6.40 and 6.41. Samples from the stomach of the treated group yielded counts of 10^3/g during treatment, higher counts of

10^4/g were isolated after withdrawal of treatment on day 24. No clostridia were isolated before treatment or at the end of the study. 10^3/g were isolated from the control group before treatment and at the end of the Experiment. No isolations were made during treatment but after withdrawal on day 24, 10^4 organisms/g of contents were present.

No growth was found from the duodenum of the pigs from the treated group. From the control group populations of 10^2/g were isolated only on day 24 after withdrawal of treatment but no further isolations were made. Throughout the whole period of study these bacteria were not isolated from the jejunum of either experimental group. C. perfringens type A was isolated from the ileal contents of the treated group in numbers of 10^2/g before treatment, and increased to 10^4/g by the end of the study. No growth was found either during treatment or after its withdrawal. Counts of 10^4/g were found in the control group during treatment and on day 35 at the end of the Experiment they dropped to 10^2/g.

Levels of 10^4/g were isolated from the caecum of the treated group before treatment, 10^5/g were recorded during treatment, and 10^4/g after withdrawal of treatment with 10^5/g on day 35. Populations of 10^4/g were found in the control group before treatment. During treatment they were isolated at levels of 10^3/g but rose to 10^6/g following the withdrawal of treatment. On day 24 they decreased to 10^5/g and finally their counts were found to be 10^4/g by day 35.

Samples from the colon of the treated group yielded counts of 10^6/g before treatment, during treatment their numbers decreased to 10^5/g but returned to 10^6/g following the withdrawal of treatment. By day 24 the numbers of C. perfringens type A were 10^4/g but increased to 10^5/g by the end of the experiment on day 35. The numbers of these bacteria in the control group were 10^4/g before treatment, during treatment and following withdrawal of treatment. On day 24 higher counts of 10^7/g were isolated and by day 35 their populations decreased to be 10^5 organisms/g.

The presence of spore-forming strains were demonstrated only after withdrawal of treatment at levels of 10^2/g from the colonic contents of the control group. No other isolations were

made from any of the remaining samples in this study.

TABLE 6.40 Counts of Clostridium perfringens type A vegetative cells from the gastrointestinal contents of the treated pigs in Experiment 4

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
5	7^	N G	N G	N G	5.0×10^2	2.0×10^4	1.0×10^6
10	20*	1.5×10^3	N G	N G	N G	5.0×10^5	5.0×10^5
8	22	N G	N G	N G	N G	3.0×10^4	1.0×10^6
3	24	5.0×10^4	N G	N G	N G	1.5×10^4	3.5×10^4
7	35	N G	N G	N G	4.0×10^4	1.0×10^5	3.0×10^5

^ Beginning of observations, * Withdrawal of treatment
N G = No Growth

TABLE 6.41 Counts of Clostridium perfringens type A vegetative cells from the gastrointestinal contents of the control pigs in Experiment 4

Pig Number	Study Day	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon
76	7^	5.0×10^3	N G	N G	N G	2.0×10^4	5.0×10^4
78	20*	N G	N G	N G	1.0×10^4	5.0×10^3	5.0×10^4
80	22	N G	N G	N G	N G	1.0×10^6	2.0×10^4
72	24	5.0×10^4	5.0×10^2	N G	N G	1.0×10^5	1.0×10^7
75	35	1.0×10^3	N G	N G	5.0×10^2	5.0×10^4	1.5×10^5

^ Beginning of observations, * Withdrawal of treatment
N G = No Growth

E) Pathological findings

i) Histological examinations

The histological changes noted in the stomach, small and large intestines from all the sacrificed pigs in this study were mild and are presented below in detail, accordingly with the date, pig number and section of the gastrointestinal tract examined.

The changes observed from the stomach of Pig 5 (yellow) killed before treatment on day 7 of the study were restricted to

the presence of small foci of plasma cells in the glandular portion of this organ. The duodenum and jejunum were normal in appearance. In the ileum the villi were normal and some debris was present between them and the crypts. The lamina propria of the caecum was normal but organisms as well as inflammatory cells were seen in the lumen of many crypts. The colonic mucosa was generally normal but the epithelium was low. As in the caecum, inflammatory cells were present in some crypts and organisms were seen in others.

Pig 76 (white) from the control group was also killed on day 7 of the study, the stomach was normal, the duodenal villi were slightly reduced in height and neutrophils were seen at the apex. The jejunal villi were also slightly reduced in height. In the ileum some cell exudate was seen in the crypts and between the villi. Bacilli were seen adjacent to mucosal epithelium, and in the lamina propria neutrophils were visible in some vessels and large numbers of monocytes were also seen. Large number of crypts in the caecum contained inflammatory cells and some also contained organisms. Bacteria and debris were adjacent to the epithelial mucosa. Large numbers of Cryptosporidium spp. were present inside many caecal crypts. Bacteria were adherent to the colonic epithelium and Balantidium coli was seen in the lumen. Cryptosporidium spp. and bacteria were seen in crypts. There were locally dilated capillaries and vessels containing many neutrophils in the lamina propria.

In Pig 10 (yellow) killed on day 20 the gastric mucosa was largely normal but pink homogeneous contents were present in the crypts of the whole section. Some vessels in the lamina propria were congested and mild oedema was present. Post mortem changes were also present. The duodenum showed some plasma cells in lamina propria. No neutrophils or inflammation were seen but post mortem changes were present. The jejunal villi were normal in length but disrupted mainly due to post mortem change. The lamina propria was also grossly normal. The ileum was normal except for Cryptosporidium spp. present locally. The caecal epithelium and lamina propria were very thin. Some bacteria were seen in crypts and 1 to 2 crypts also contained inflammatory cells. Balantidium coli were seen adjacent to bacteria on the epithelial mucosa. The colonic mucosa was thin but no

inflammation was present. Some bacteria were seen in the crypts. The epithelium was intact except for post mortem change.

The stomach of Pig 78 (white) killed on day 20 of the study was normal but many epithelial lymphocytes were observed. The duodenal villi were low and fused. Some intraepithelial lymphocytes were also present. The jejunum was apparently normal. Large numbers of eosinophils and capillary dilatation were present in the ileal lamina propria. Some inflammatory cells were seen in the Peyer's patches. Locally there was some exudate between the villi and the crypts. Bacteria were present adjacent to the mucosal epithelium and in some crypts. Some inflammatory cells were also seen in the crypts. Post mortem changes were also present. The colonic mucosa contained some dilated capillaries. Balantidium coli and bacteria were seen adjacent to the mucosal epithelium in some areas. Inflammatory cells and bacteria were seen in some crypts. Pavementing of neutrophils in some vessels was seen locally in the lamina propria.

The stomach of Pig 8 (yellow) killed on day 22 was grossly normal. Duodenum and jejunum were normal in appearance. Their lamina propria was un-inflamed. Some bacteria were adjacent to the ileal epithelium but no obvious inflammatory changes were seen. Large numbers of Cryptosporidium spp. and locally some bacteria (rods) were present inside crypts. The caecal mucosa was thin, the epithelium was largely intact but lower in 1-2 areas where neutrophils were present. Bacteria were seen adherent to the epithelium and in crypts. Inflammatory cells were seen in the lumen of some crypts and Balantidium coli were seen on the surface in some areas. The colon was apparently normal except for the presence of bacterial adhesion to the epithelium.

In Pig 80 (white) killed on day 22 the stomach was normal in appearance. The duodenum was autolysed and some lymphocytic accumulations were seen in the lamina propria. The villi of the jejunum were disorganized and post mortem destruction resembled inflammation. In the ileum eosinophils were common in the lamina propria. Tissue debris were seen in crypt lumens and adjacent to the villi. No Cryptosporidium spp. were seen. The caecum was normal but the colon showed local capillary dilatation in the lamina propria with some inflammatory cells often associated

with bacterial adhesion to the epithelium.

The section of the stomach of Pig 3 (yellow) killed on day 24 of the study was poor in quality but from an intact area slight capillary dilatation, congestion, oedema and many intraepithelial lymphocytes were seen in the lamina propria. The duodenum was grossly normal. Some villi of the jejunum were forked and fused but they were mostly normal. No adherent bacteria were seen. In the ileum the villi were slightly reduced in height. Cryptosporidium spp. were seen in several crypts and tissue debris was present locally in some crypts. The caecum presented areas of mild caecitis with epithelial cell shedding, neutrophil accumulation and adherent bacteria. In one area some crypts contained bacteria but the rest was normal in appearance. The colon was largely normal, only one area of local inflammation and cell shedding was seen. Some bacteria were observed inside colonic crypts.

The stomach of Pig 72 (white) killed on day 24 of the study was grossly normal. In the duodenum the villi were slightly reduced in height and prominent intraepithelial lymphocytes were seen. The jejunum was largely normal with some autolysis. Short villi and some debris on the epithelium were seen in the ileum. The caecal mucosa was normal but bacteria were present in some crypts and locally close to the epithelium. The mucosa in the colon was normal although some bacteria were present adjacent to the epithelium.

On day 35 of the Experiment Pig 7 (yellow) was killed and the histological section from the stomach was normal in appearance. There was some post mortem loss of the duodenal epithelium and large numbers of intraepithelial lymphocytes, but no neutrophils were present. The submucosa was thin and only one Peyer's patch was seen. In the jejunum some villi were fused and the mucosa, submucosa and muscularis were all thin. Some rod shaped bacteria were seen adjacent to the ileal epithelium which was largely normal. The villi were intact but Cryptosporidium spp. were present adjacent to Peyer's patches in a crypt. The colonic epithelium was coated locally with adherent bacteria and minor capillary dilatation was present in the lamina propria. Some bacteria could be seen adjacent to the mucosa and inside crypts.

Pig 75 (white) was killed on day 35 of the study and the stomach was normal. The duodenum presented numerous intraepithelial lymphocytes. The villi and the lamina propria were normal. In the jejunum there were some villous fusion and inflammatory debris in an otherwise normal crypt. There were many eosinophils in the ileal lamina propria and the villi were distorted. Some cell debris were seen between villi and in crypts. Bacilli were adjacent to epithelial cells possibly adherent to the mucosa locally. The caecal mucosa was normal in spite of Balantidium coli and some bacteria being adjacent to the surface. In the colon some bacteria were seen in crypt lumens and adjacent to the epithelium. Some cell debris was present in the lumen and in one crypt inflammatory cell debris was present.

F) Virological examinations of faeces and contents

No rotavirus or rotaviral antigens were detected in any of the faecal samples and filtrates from gastroenteric contents of the sacrificed pigs using the RPLA test.

G) Parasitological examination of faeces and contents

All the smears of faeces and gastrointestinal contents were negative for the presence of parasitic oocysts, however histological sections stained with H & E showed the presence of Cryptosporidium spp. and Balantidium coli on the mucosa and crypts of the ileum, caecum and colon of the sacrificed pigs.

4. D I S C U S S I O N

In this Experiment, the effects of therapeutic levels of chlortetracycline hydrochloride (Aureomycin) and its withdrawal on gut flora in weaned pigs were monitored. This broad spectrum antimicrobial was chosen to complement the observations obtained previously with Avoparcin and Lincomycin and to determine whether adverse effects were present in pigs. Such effects have been reported previously in horses, where profuse foul smelling diarrhoea, apathy, anorexia, increased pulse-rates, liver damage and sensitization reactions have occurred following use of tetracyclines. In these cases the horses died 4 to 5 days after

drug administration, and the autopsy picture was characterised by acute necrotizing nephrosis, acute catarrhal to haemorrhagic typhlitis and colitis with a markedly oedematous mucous membrane and oedema and haemorrhages in various organs (Andersson, et al., 1971). Overgrowth of resistant bacteria and fungi in the mouth and in the gastrointestinal tract have also been reported following treatment.

Chlortetracycline was the first of the tetracycline antibiotics discovered in 1944 (Aronson, 1980; Kilroy, et al., 1990). Since the early 1950's it has been used extensively in the pig industry as a growth promotant and as a therapeutic agent. It is excreted to a great extent in the bile of pigs. It inhibits the growth of practically all Gram-positive and Gram-negative pathogenic bacteria by inhibiting protein synthesis, but depending on the amount used it may be bacteriostatic or bactericidal. Chlortetracycline is given by mouth because it is relatively insoluble but is absorbed from the gastrointestinal tract reaching peak blood levels in about six hours. Absorption may be inhibited by the presence of calcium ions. In this study it was given included in the feed at 300 ppm for 21 days. The whole period of observation in this Experiment was of 36 days. As a result of the work load experienced in the earlier experiments, clinical observations and faecal sampling were carried out every third day to permit better and more accurate laboratory examinations. This reduction in the frequency of observation may have affected the ability to detect changes.

The use of the antimicrobial was initiated one week previous to the regular sampling to allow the establishment of the treatment. However, the standard diet available to all the pigs prior to the beginning of the study contained 40 mg/kg of Tylosin phosphate which could have affected the results obtained from the bacteriological examinations at the beginning of the study.

The observation of clinical signs in this Experiment provided little evidence of enteric disorders (Tables **6.3** and **6.4**). In the treated group none of the animals showed diarrhoeic faeces. However, soft faeces were present in 4/5 pigs after withdrawal of treatment. Although soft faeces were more frequently observed in the control group (Tables **6.3** and **6.4**),

diarrhoea was seen only once in three different animals (pigs 74 [day 0], 79 [day 19] and 77 [day 21]), and Pig 74 (white) appeared constipated on two consecutive sampling days (days 21 and 23). During this Experiment rectal temperatures varied considerably in individual animals and they were considerably higher in the animals from the control group (Tables 6.5 and 6.6). Pigs 73 (white) and 77 (white) of this group maintained temperatures of 40°C or over during 13 and 11 sampling days out of 16, respectively. The differences between groups were statistically significant (t=-3.06), during the period of treatment.

Daily liveweight gains were higher in the chlortetracycline treated animals (Tables 6.9-6.10), but they were statistically not significant (P>0.05). Differences were particularly marked in the period of adaptation to the new ration (days 0 to 7) when the antimicrobial-treated group had a daily liveweight gain of 346 g/day compared with the 254 g/day of the controls. The differences were less marked in the week following withdrawal of treatment and were reversed in the last week of the study. The increased rates of growth in the controls during the last week of the study may be associated with the increase in food consumption (Figures XLVI and XLVII).

The results of daily liveweight gain monitoring suggest that periods to which particular attention should be paid are those immediately following introduction of antimicrobial (days 0 to 7)and the period following withdrawal (days 21-35). Any readjustment of the animals to the normal flora may be occurring during these two periods. The first period is not documented in this study and was not the primary aim. The second period, however, was studied in more detail.

The bacterial counts were examined in the periods of adaptation to treatment (days 0 to 7) and withdrawal of treatment (days 21 to 35), in both treated and control groups. Changes in total coliforms were affected only very slightly (Figures XLVIII and XLIX). Mean numbers in both groups were of the order of 10^9/gram throughout the study. There was a slight increase to 10^{10}/g of faeces in the control group in the last part of the study (Tables 6.11 and 6.12, Appendix D).

The levels of E. coli (Tables 6.13 and 6.14, Appendix D) were similar to those of total coliforms. What was unexpected from the earlier studies was the presence of Beta haemolytic E. coli which had not been seen previously. These were pathotyped

(Table 6.15) as containing VT-IIv (the oedema disease principle, ODP). No other pathogenic determinants were identified, but F107 probes were not available. Most isolates were recovered in the later part of the treatment period and after withdrawal. Equal numbers were recovered from both treated and control groups (Figures L and LI). In the treated group isolation was most common around the period of withdrawal (days 19 to 24), but in the controls, isolations were spread over a longer period (days 15 to 33). The organism was recovered from Pig 77 (white) on 4 occasions. As this animal was not examined post mortem, the relationship between the organism and any pathological changes remains unknown. Pig 3 (yellow) was examined post mortem while infected and no changes suggestive of bowel oedema were recorded on gross post mortem examination or on histological examination.

The recovery of faecal streptococci/enterococci was much greater in this study than in the other three Experiments. This increased recovery may be due to the lack of growth permitters in the basal ration used. It is interesting to note (Figures LII and LIII) that the levels of faecal streptococci on day 0 were lower than later in the study. On day 0 the medicated feed was withdrawn, levels were at their lowest, suggesting that this hypothesis may be correct. Chlortetracycline did not appear to have affected recovery of this organism and levels on day 7 were markedly higher in treated animals than those in the controls. Mean levels of faecal streptococci were not affected by withdrawal of chlortetracycline (Tables 6.16 and 6.17, Appendix D).

Bacteroides spp. were also recovered more consistently in this study than in the previous Experiments, perhaps for a similar reason (Tables 6.22, 6.23 [Appendix D] and Figures LIV and LV). Mean numbers recovered were higher and every pig yielded the organism on every day of the study. Recovery was still lower than that reported by other workers (Kenworthy and Crabb, 1963; Smith and Crabb, 1961; Smith and Jones, 1963). A similar effect was noted with Clostridium perfringens type A where the organism was also recovered on every day of sampling. As sampling, transport and cultural procedures were similar in every study it seems unlikely that they affected recovery in this case.

Examination of the faecal bacterial counts during the period of adjustment to chlortetracycline administration on days 0-7 suggests that chlortetracycline had no effect on total coliforms, E. coli, Lactobacillus spp. and clostridial counts (Tables 6.11 to 6.14, 6.20, 6.21, 6.24, 6.25 [Appendix D] and

Figures XLVIII to LI, LVI, LVII, LX and LXI). The position with regard to faecal streptococci, campylobacters and Bacteroides spp. is less clear. Mean levels of these species were higher in one group or the other at day 0 and this difference continued. Levels of faecal streptococci and Bacteroides spp. were higher in the chlortetracycline treated group than in the controls (Tables 6.16, 6.17, 6.22 and 6.23 [Appendix D] and Figures LII, LIII, LVIII and LIX) but in Campylobacter spp. the position was reversed and the levels remained lower in the chlortetracycline treated group.

Mean bacterial counts over the period of withdrawal were broadly similar to those found in the treatment period for total coliforms, E. coli, faecal streptococci, lactobacilli and clostridia. The differences found between groups and periods for the other two species were slight and were not considered important. When mean counts for the days immediately following withdrawal were compared with those for the last day of treatment (day 21), no change was seen for E. coli or Clostridium perfringens type A. Minor changes were seen for total coliforms and lactobacilli. Levels of faecal streptococci rose in the treated group from 10^6/g (day 23) to 10^8/g (day 27) and 10^{10}/g on day 35, while levels in the controls remained more stable. An effect was also evident in the counts of Bacteroides spp. These fell from 10^6/g on day 21 to 10^3/g on day 23 and recovered gradually to 10^7/g on day 27. These changes can be seen most clearly in Figures LVIII and LIX. Apparent changes in numbers of other bacteria such as total coliforms and lactobacilli for individual pigs can also be seen in the relevant Figures for this period, but are not fully reflected in the means.

No evidence for the growth of C. difficile, Salmonella spp. or Serpulina spp. was found. The antibiotic associated growth of these organisms reported in the literature (Bartlett, et al., 1978c; George, et al., 1978; Price, et al., 1979; Linton, et al., 1975; Wilcock and Olander, 1978; Olson, 1980; 1986; Olson and Rodabaugh, 1976a, b; 1977; 1978; 1984) could therefore not be documented. This result agrees with those of the earlier studies.

All the pigs sacrificed in this study were killed during the periods of treatment and after withdrawal (Table 6.1). Changes occurring during adaptation to antimicrobial could only be observed by comparing the pigs killed on day 7 with the control. No gross differences were observed but more inflammatory changes and the presence of Cryptosporidium spp.

and Balantidium coli were observed by histological examination in the control pig (Pig 76, white). Differences in bacterial counts were also slight. Perhaps the most obvious were the failure to isolate Bacteroides spp. from the caecum in either pig (Tables 6.38 and 6.39) killed on this day (day 7) in contrast to those killed later and the slightly higher numbers of total coliforms, E. coli and lactobacilli isolated from the caecae of the sacrificed animals of the treated group (Tables 6.28 to 6.31 and 6.36, 6.37).

The possibility that there might be a difference between animals maintained on chlortetracycline and controls was also examined. The minor differences between pigs 5 (yellow) and 76 (white) on day 7 have already been discussed above. The findings in the second pair of pigs, pigs 10 (yellow) and 78 (white) killed on day 20 were similar. Slightly more inflammatory change was present in the gastrointestinal tract of the control pig (Pig 78) especially in the large intestine. Bacterial counts in the two groups were broadly similar.

The last area for comparison of the sacrificed pigs was the withdrawal period. The pigs sacrificed from the treated group were first compared with their contemporary controls. No gross changes were seen in the pigs killed on day 22, but histological examination suggested that the mild inflammatory changes in ileum and large intestine were more severe in the treated pig (Pig 8 yellow) than in the control (Pig 80 white). This reversal of the findings from the earlier post mortem examination was accompanied by the isolation of 10^{10} total coliforms/g from the ileal contents of Pig 8 (yellow) compared with 10^{4}/g from Pig 80 (white). E. coli counts were similar. Coliform counts in the caecum of the treated animal were lower than those in the control while colonic levels were the reverse (Tables 6.28 and 6.29). Other differences were few. Faecal streptococci and Campylobacter spp. were present in the ileum of the treated pig, but absent from the control (Tables 6.32 to 6.35). The significance of this finding is not clear as all the other controls yielded these organisms in this site.

By day 24 the only differences between treated (Pig 3 yellow) and control (Pig 72 white) were histological. More inflammation was present in the ileum and large intestinal mucosa of Pig 3 (yellow) than in the control. Differences in the bacterial flora were minor with slightly lower levels of E. coli, Campylobacter spp, lactobacilli and clostridia in the

colonic contents (Tables 6.30-6.31, 6.34-6.37 and 6.40-6.41).

By day 35 the mild histological differences were reversed with slightly more inflammation in the ileum, caecum and colon of control Pig 75 (white) when compared with treated Pig 7 (yellow). This finding is not related to the counts of intestinal bacteria which were generally higher in Pig 7 (yellow) than in the control. Differences were most marked in the ileum, caecum and colon and occurred in total coliforms, E. coli and Lactobacillus spp. populations (Tables 6.11-6.14 and 6.36, 6.37). Ileal populations of Campylobacter spp., Bacteroides spp. and Clostridium perfringens type A were also higher (Tables 6.34, 6.35 and 6.38 to 6.41).

When comparison was made between treated animals killed on days 7 and 20 and those following withdrawal, an apparent increase in mild inflammation of ileum, caecum and colon was noted. This had disappeared by day 35. The most obvious change in the flora was the high total coliform numbers found in Pig 8 (yellow) on day 22 and in Pig 7 (yellow) on day 35. Whether these were sufficient to account for the differences in performance was not clear from the number of animals examined in this study. It is possible that the changes following withdrawal of antibiotic are most obvious in the 1 to 4 days following antimicrobial withdrawal and in this series, were most apparent in the coliform populations.

The mild changes seen were identified in pigs which were essentially disease free. Cryptosporidium spp. and Balantidium coli were present but their presence did not appear to be related to antimicrobial usage. The only potential enteric pathogens isolated were VT-II +ve E. coli, Campylobacter spp. and C. perfringens type A and no clear association between them and withdrawal of treatment was found. C. difficile, Serpulina spp. and salmonellae were all absent in this study. In conclusion, the changes pathology and bacterial counts following withdrawal were slight. The most interesting findings were associated with weight gain, both after adaptation to and withdrawal of antimicrobial. The absence of clinical disease and significant changes in the bacterial species monitored suggests the presence of an as yet undiscovered reason for this effect.

CHAPTER 7

GENERAL DISCUSSION

1. INTRODUCTION

The primary aims of this research were to determine whether or not diarrhoea and enteritis occurred following the withdrawal of antimicrobials used for growth promotion or therapy in pig farms in the United Kingdom, and to study the effects of selected antimicrobials on the gastrointestinal tract of experimental pigs.

It was postulated that the use of antimicrobial therapy over the period when maternal antibody levels to a variety of organisms was declining should prevent the establishment of known pathogens and the development of active immunity to them as in the case of proliferative enteritis (Rowland and Lawson, 1992; Love and Love, 1979). It was also postulated (Chapter 1) that elements of the normal flora would be suppressed and that reinfection with them or the establishment of other organisms of the normal flora not usually considered to be pathogens might lead after the withdrawal of antimicrobial to clinical, pathological or microbiological evidence of abnormality. The results of this study (Chapters 3, 4, 5 and 6) should be viewed in terms of the findings of studies which have involved enteric disease, antimicrobial therapy and its withdrawal.

At the present time antimicrobial therapy is most commonly associated in all species with the development of disease whilst they are receiving antimicrobial. Review of the literature in Chapter 1 provided evidence for the effects of treatment on the microbial flora of the gut (Gall, 1970; Haenel,1970; Henderickx, *et al.*, 1982; Hentges, 1970; Kenworthy, 1973; Linton, *et al.*, 1975; Parker, 1974). Chief amongst these are the antimicrobial-associated enteric conditions in man, hamster, rabbit and horses. These are associated with a number of bacterial species, but the chief agent concerned is the toxin producing, spore-forming, Gram-positive, anaerobic rod, *Clostridium difficile*. Pseudomembranous colitis (PMC) in man is associated with the use of therapeutic ampicillin, amoxycillin, clindamycin or

lincomycin and with overgrowth of C. difficile and production of its A and B toxins (Bartlett, et al., 1977; 1978a, b; Borriello and Larson, 1981; George, et al., 1977; 1978; Larson, et al., 1978; Lee and Olson, 1986; Trnka and Lamont, 1984).

Antimicrobial therapy has not been identified as a primary cause of enteritis in the pig. C. difficile has only been identified by Jones and Hunter, (1983) who isolated it from animals with diarrhoea but gave no details of any association with antimicrobials. Its possible implication in swine dysentery was reported by Lysons, et al. (1980). The other record of the organism (Lysons and Hall, 1982) is from a gnotobiotic pig, once more with no history of antimicrobial use. The major cause of enteritis during treatment in pigs is resistance of the organism being treated to the antimicrobial used or the use of inadequate levels of antimicrobial.

Little clear evidence for post-antimicrobial enteritis in the pig or any other species exists. Mackinnon, (1987) citing Walton, (1986), suggested that such an effect can occur. The circumstances they described affected gilts selected after a long period on antimicrobial growth promoters and transferred to rations without them. No history of background disease was given. In most cases post-antimicrobial disease is associated with the recurrence of or reinfection with the original disease after inadequate treatment or hygienic measures.

Typical examples of this recurrence are the accounts of recurrence of swine dysentery after treatment by Olson, (1980; 1986) and Olson and Rodabaugh, (1976a, b; 1977; 1978; 1984). The treatment regimes described would not have been considered adequate by most veterinarians and the whole series of papers may therefore be of limited use.

The phenomenon of a reduction in the rate of growth following the withdrawal of growth promoters from the ration is well documented (Bridges, et al.,1952; 1953; Henderickx, et al., 1982; Kenworthy, 1973). Some evidence exists for a rise in the numbers of potential pathogens in the faeces at this time, and the development of harmful resistant strains of pathogens (Braude, et al., 1955; Fuller, 1989; Langlois, et al., 1978b;

Linton, et al., 1977; 1988; Mackinnon, 1985; 1987; Parker, 1974; Van der Molen, et al., 1988; 1989; Van-Houweling and Gainer, 1978; Visek, 1978; Walton, 1979). Clear evidence for the occurrence of disease during this period is absent. The picture is complicated by the fact that the early literature describes the growth promoting effects of compounds now restricted to therapeutic use (Braude, 1978; Bridges, et al., 1953; Fuller, et al., 1960; Langlois, et al., 1978a; Linton, et al., 1975).

2. POST-ANTIMICROBIAL ENTERITIS IN THE FIELD

The farm study described in Chapter 3 revealed that reports from the field of post-antimicrobial enteritis of the type postulated in this thesis were largely inaccurate.

Disease occurring in association with treatment was poorly documented. It frequently occurred following inadequate or inappropriate treatment (farms 1, 2, 3 and 5). In two cases (Farm 1, Visit 1 and Farm 6) the problem appeared to have resolved, although microscopic examination of gastrointestinal tracts on both farms suggested that histopathological evidence of enteritis was still present.

The limitations of a field study of the kind described here were discussed briefly in Chapter 3. The inability to follow the progress of individual pigs and to examine appropriate animals post mortem limited the amount of information which could be gained. The pathological changes were only studied in pigs which had died (Farm 1, visit 2), and in 5 pigs from Farm 1, and 1 pig from Farm 6 which had been sent to the Veterinary School for examination. More data had been obtained from faecal samples. Primary pathogens were recovered from a number of incidents. Beta haemolytic E. coli from disease investigated on Farm 1, (visits 1, 3) and Farms 2, 3, 5 and 6 (Tables 3.1, 3.3, 3.4, 3.6 to 3.9, and 3.12), Salmonella kedougou from Farm 5, Campylobacter spp. from Farm 1, (visits 1, 2, 3 and 4) and Farms 2, 3, 5 and 6 (Tables 3.3 to 3.9, and 3.12), C. perfringens type A from Farm 1, (visits 1 and 2) and Farms 2, 3 and 6 (Tables 3.1, 3.6, 3.7, 3.9 and 3.12) and S. hyodysenteriae from Farms 2 and 3. In some cases their presence was related to clinical signs (Farm 3, S. hyodysenteriae, Farm 5, S. kedougou and beta

haemolytic E. coli.

In most cases where post mortem examination was carried out, macroscopic lesions were inapparent, the mucosa which was grossly normal was abnormal when examined histologically. However, the lesions were not enough to identify the cause of the changes microscopically observed.

The bacteriological examinations carried out were of limited value as the results of Chapter 3 indicate. It is clear from the literature that S. hyodysenteriae is in most cases isolated with many other bacteria. Robinson, et al. (1982; 1984) listed 14 species of bacteria which could be isolated from the lesions of swine dysentery and Alexander, et al. (1976), Joens, et al.(1981) and Lysons, et al. (1980), reported the isolation of various anaerobic organisms other than S. hyodysenteriae which may contribute to the lesions of swine dysentery. These reports were comprehensive but their isolates did not include organisms such as campylobacters identified in this study. The cultural conditions used in this study and, in particular in Chapter 3 did not allow the isolation and identification of the full range of species to be expected in a lesion of that type.

The bacteria isolated also differ in some respects from those isolated from the porcine enteric tract by workers such as Horvath, et al. (1958), Kenworthy, (1973), Pesti, (1962), Smith and Jones, (1963), Szynkiewicz, et al. (1982) and Willingale and Briggs, (1955). However, it is possible that the bacteria isolated in this study reflect the population actually present. This view was supported by the relationship between the bacteria seen in the direct smears and the results of bacterial isolation. This was particularly clear with morphologically distinctive organisms such as campylobacters, streptococci, C. perfringens and spirochaetes.

The findings related to other pathological agents such as viruses and parasites were in most cases limited. This made it difficult to say with certainty that one or more of these types of agents was or had been present in the enteric tracts or faecal samples of the pigs examined. Their involvement can only be confirmed by further studies.

The limitations imposed by the availability of information about treatment, disease status and technical difficulties in recovering all organisms which might be involved also extended to uncertainty about the presence of non-bacterial agents such as viruses and parasites. Rotavirus was not demonstrated but cryptosporidia were prominent in Farm 1. Their role in the continuation of disease during and following the withdrawal of antimicrobial treatment was not clear.

The study of post-antimicrobial enteritis reported in the literature and evaluated by examination of field cases proved to be difficult to confirm for reasons discussed in Chapter 3 of this thesis where the following problems were identified:

1) Poor history
2) Problem gone away
3) Incorrect levels of treatment and reappearance of original disease
4) Bacterial resistance
5) Uncontrolled studies
6) Adoption of another mechanism of treatment

In the only case (Farm 1, visit 2) where the condition had appeared to have occurred it seemed to be due to none of the classic agents of pig diarrhoea.

The attempt to define the postulated syndrome in the field was unsuccessful and the experimental work was developed to test the possibility that post-antimicrobial changes did occur.

3. EXPERIMENTAL STUDIES OF POST-ANTIMICROBIAL ENTERITIS

The experimental studies were intended to evaluate the effect of 3 pharmacologically different antimicrobials on the gut flora of recently weaned pigs (Chapters 4, 5 and 6), following their oral administration to recently weaned pigs. Studies of this type do not appear to have been caried out in any species.

All 3 compounds chosen had been identified as causes of disturbance to the gut flora or to induce disease in individual animals or species.

The results of each study have been presented and discussed briefly in Chapters 4, 5 and 6. The work described in those chapters identified a number of factors common to all the Experiments which affected both the results obtained and the conclusions drawn. These common factors included the absence of pathogens which had been reported from apparent antimicrobial-associated disease with the antimicrobials used. In the field studies described in Chapter 3 much post-antibiotic change was associated with pathogens. Salmonella spp., C. difficile and Serpulina hyodysenteriae were all absent. The association of these species with the individual antimicrobials is discussed below. Organisms not susceptible to antimicrobials such as Cryptosporidium spp. and Balantidium coli were identified in pigs in all 4 studies and rotavirus was identified in 2 pigs from Experiment 1. These three organisms may have initiated or contributed to enteritis and diarrhoea which could not be related to antimicrobial administration. The results of Chapter 6 suggested that the inclusion of routine growth promoters in the basic ration had had a profound effect on the results obtained.

In Chapters 4 and 5 the low counts of Bacteroides spp. and Clostridium spp. was attributed largely to problems with the culture of these anaerobes and with transit times. The higher counts of these organisms and of faecal streptococci in Experiment 4 (Chapter 6) suggested that the growth promoters included had been important in the failure to recover these organisms in Experiments 1 to 3. The failure to recover levels of these organisms reported by previous authors (Kenworthy and Crabb, 1963; Smith and Crabb, 1961; Smith and Jones, 1963) may have been due in part to bacteriological technique but was also clearly associated with the presence of the growth promoters. It is also widely recognised that one factor which may explain the rise and fall in the counts of enteric bacterial populations is the changing populations of gut organisms with time. Only repeated sampling and comparing data from various periods of time will reveal significant shifts in bacterial populations resulting from the continuous feeding of a particular growth promoter or therapeutic antimicrobial as it was intended in these studies.

Throughout the study the workload required to carry out the counts presented meant that the numbers of animals monitored was

very small and that animals to be sacrificed could not be studied in the same detail as those being continually monitored. The final common problem was the variation in bacterial counts which made statistical significance difficult to calculate.

4. AVOPARCIN IN POST-ANTIMICROBIAL ENTERITIS

Avoparcin suppresses Gram-positive organisms and is a glycopeptide inhibitor of cell-wall, peptidoglycan formation in routine use in the E.E.C. as a growth promoter, and does not require withdrawal prior to slaughter. It has been used as a growth promoter for chickens, cattle, sheep and pigs in the U.K. for many years and has been known to inhibit populations of Gram-positive bacteria in the gut. However, its use has been reported in chickens to stimulate the shedding of pathogenic strains of *Salmonella spp.* and to contribute to the presentation of clinical disease (Barrow, 1987; Barrow, *et al.*, 1984; 1987; Hinton, *et al.*, 1986; Linton, *et al.*, 1985; Matthes, 1984; Smith and Tucker, 1978; 1980).

The absence of salmonellae from the pigs used in this study precluded the detection of *Salmonella spp.* shedding and questions the significance of studies in which salmonellae were isolated. The effects noted were slight (Chapter 4), possibly because of the presence of other growth permitters (olaquindox and copper sulphate) in the basal ration. However, there appeared to be an association between the frequency of diarrhoea and the withdrawal of treatment (Table 4.3). This association was not confirmed statistically (Pradal-Roa and Taylor, 1988; 1989). The rise in faecal coliforms, streptococci, *Bacteroides spp.*, campylobacters and *C. perfringens* type A which occurred after withdrawal (Tables 4.6 to 4.8, 4.10 and 4.11 and Figures III to VII) may be caused by a similar mechanism to that reported for *Salmonella spp.* by Barrow, 1987; Hinton, *et al.*, 1986; Linton, *et al.*, 1985; Smith and Tucker, 1978; 1980. The individual serotypes of *E. coli* were not recorded in this study but work by Hinton, *et al.* (1985) in unweaned and weaned pigs suggests that while the total numbers of a species in faeces may rise and fall, far more dramatic changes may occur with individual O-serotypes or biotypes.

All of these apparent changes were difficult to assess in the absence of a control group of equal size. The importance of such control groups became obvious when bacterial counts from faeces were examined in the subsequent experiments.

5. LINCOMYCIN IN POST-ANTIMICROBIAL ENTERITIS

Lincomycin is a monoglycoside with good activity against both aerobic and anaerobic Gram-positive bacteria, L-forms of Gram-positive cocci and mycoplasmas. Generally, it has little activity against Gram-negative bacteria, viruses and fungi. It is bacteriostatic, and at high concentrations is bactericidal (Hamdy, 1975). The mode of action of lincomycin is inhibition of protein synthesis via interference of the translocation step (Burrows, 1980). Lincomycin has a powerful effect on the gastrointestinal flora of ruminants and horses.

When erroneously included in the ration it has been reported several times by the Veterinary Investigation Service to be related to anorexia, agalactia, digestive upsets (ruminal atony and diarrhoea varying from slight to profuse and watery) and metabolic disorders (ketosis) in dairy cattle (Anon, 1979; Anon, 1984b; Anon, 1984c; Bulgin, 1988; Lang, 1979; Lyne, 1984; Plenderleith, 1988; Rice, et al., 1983; Woodger, 1979). Administration of lincomycin via the drinking water to ruminants alters the flora of the rumen and subsequently gives rise to gastrointestinal disturbances (Okoh and Ocholi, 1986; Schmit, 1986). In horses, lincomycin interferes with the gut flora and causes digestive upsets and signs of colic, founder, and/or endotoxic shock associated with a haemorrhagic caeco-colitis (Bulgin, 1988; Plenderleith, 1988; Raisbeck, et al., 1981). These are not caused by direct drug irritation but by encouraging the preferential growth of toxin producing bacteria (Burrows, 1980; Rifkin, et al., 1978). Unfortunately their identity was not specified.

The results of Experiments 2 and 3 suggested that the administration of lincomycin in the drinking water at the concentration used had little overall effect on the pigs. There was no evidence for major gastrointestinal upset in either experiment and the results of the two experiments in terms of

diarrhoea days (Tables 5.3, 5.4, and 5.24, 5.25) and effects on rectal temperatures ($P<0.05$) differed (Tables 5.5, 5.6, and 5.26, 5.27). Changes in the faecal flora were noted in Experiment 2 with a rise in total coliform numbers after withdrawal of treatment (Table 5.12 and Figure XV). This was not statistically significant and was not repeated in Experiment 3 (Table 5.33 and Figure XXXV).

Results from the pigs sacrificed during the Experiment 3 showed that many of the changes were shared by controls sacrificed on the same day.

Organisms such as C. difficile were absent from these pigs and the severe enteritis described in the literature as occurring in antimicrobial associated enteritis (Bartlett, et al., 1977; 1978a, b; Borriello, et al., 1987; George, et al., 1978; Larson, et al., 1978; Lee and Olson, 1986; Price, et al., 1979) was not seen.

The conclusions drawn from these studies were that lincomycin did not cause post-antimicrobial enteritis in the pig under the conditions used in Experiments 2 and 3.

6. CHLORTETRACYCLINE IN POST-ANTIMICROBIAL ENTERITIS

Chlortetracycline is a broad-spectrum antibiotic which has a widespread effect against both Gram-positive and Gram-negative bacteria. It has been used as an antiprotozoal and against rickettsias, Mycoplasma spp. and Ehrlichia spp. The mechanism of action of this compound is to inhibit protein synthesis reversibly. In the U.S.A. it is used as a growth promoter (MacKinnon, 1984) as well as for therapy.

The effects of therapeutic levels of chlortetracycline hydrochloride on gut flora of weaned pigs were monitored in Experiment 4. This broad spectrum antimicrobial was chosen to complement the observations obtained previously with Avoparcin and Lincomycin, and to determine whether any of the adverse effects previously reported in other species were a feature of treatment with chlortetracycline in pigs.

Adverse reactions related to the gut have been documented most fully in the horse. Profuse foul smelling diarrhoea, apathy, anorexia, increased pulse-rates, liver damage and sensitization reactions as occur following the administration of tetracycline. These have been called Colitis "X" (Andersson, et al., 1971; Baker and Leyland, 1973; Cook, 1973; Harries and Strother, 1969; Kelly, 1972; MacKellar, et al., 1973; O'Brien, 1985; Prescott, et al., 1988; Rooney, et al., 1963; Schiefer, 1981; Wierup and DiPietro, 1981). In these cases the horses died 4-5 days after administration of the drug, and the autopsy picture was characterised by acute necrotizing nephrosis, mild catarrhal enteritis of the small intestine, acute catarrhal to haemorrhagic typhlitis and colitis with a markedly oedematous mucous membrane and watery contents. Oedema and haemorrhages in various organs (mainly the liver) were also present. No significant lesions were found in the stomach, duodenum, jejunum, pancreas, or urogenital tract (Andersson, et al., 1971; Harries and Strother, 1969; Rooney, et al., 1963; Schiefer, 1981). These authors mention that an overgrowth of resistant bacteria and fungi may also occur in the mouth and in the gastrointestinal tract.

Experimental administration of tetracyclines to horses by Horspool, (1992) failed to produce 'Colitis X' but did cause minor changes in faecal consistency. The bacteriological effects of tetracycline administration are considered below.

Conventional dosages of tetracycline and oxytetracycline given orally or intravenously to dogs and oxytetracycline given intravenously to cattle seem to be tolerated well. In contrast, therapeutic doses of these drugs given orally to cats often produce serious gastrointestinal upsets. In cats, clinical signs consist of diarrhoea, colic, emesis, depression, fever and anorexia. It is not known whether gastrointestinal irritation or alteration of the intestinal flora, or both, is responsible for the high occurrence of gastrointestinal upsets in cats (Aronson, 1980; Schiefer, 1981).

Similar upsets clearly occur in cattle. The data sheets for tetracyclines (Anon, 1993-94) record that rumen flora may be depressed and that cellulolytic digestion may be reduced. There

is a contra-indication for the use of tetracycline in feed for ruminants receiving root vegetables and this is associated with colic, apathy, anorexia, foul smelling diarrhoea and abnormalities in rumen function and occasionally tetracycline causes death (Taylor, 1993, Personal Communication).

Some reports of gastrointestinal disturbance occur in the medical literature. Finger and Wood, (1955) reported a case of acute gastroenteritis in humans due to Salmonella muenchen in which the infection appeared to have been activated by prophylactic therapy with oxytetracycline. Such infections are assumed to be due to disturbances in the normal microflora where organisms insensitive to the antimicrobial, but normally held in check by factors such as bacterial antagonism may thus be allowed to grow luxuriantly and produce active infection. Pseudomembranous colitis in humans is an example in which antibiotic therapy makes the host susceptible to a toxin-mediated bacterial infection with C. difficile and invariably develop a fatal enterocolitis (Larson, et al., 1978).

There is little evidence for these adverse effects in pigs. The tetracyclines are routinely used in pigs and few side effects are reported at the levels used for therapy. One recent study of respiratory disease (Burch, et al., 1986) recorded a marked increase in performance during medication and a dramatic reduction in performance after withdrawal of chlortetracycline. This change in productivity was attributed to reinfection with respiratory disease and the effect on the gastroenteric tract was not reported.

The widespread use of tetracyclines for therapy and growth promotion has given rise to antimicrobial resistance particularly in the enterobacteria such as Salmonella spp. and E. coli (Smith, 1974; Smith and Crabb, 1957; Wilcock and Olander, 1978).

Chlortetracycline was fed at 300 ppm in Experiment 4. None of the above clinical signs were noted and performance in the treated animals was greater than in the untreated controls during the period of treatment. Following withdrawal, the rate of weight gain in the treated animals slowed down (Tables 6.9 and 6.10) although it still remained higher than that in the

controls.

When bacteriological changes in the faecal flora were examined over these periods, data from the period of adaptation to the medicated ration were sparse. The levels of coliforms remained similar in control and treated groups in contrast to the changes reported by Andersson, et al. (1971) and Horspool, (1992) for the horse, where a rise was recorded. No clear differences in the other bacterial species were recorded at this period and at post mortem examination of the pigs euthanased at day 7 and day 20, fewer changes were seen in the intestines of medicated animals than in controls.

These findings are in marked contrast to those reported above for the other species. It is possible that they arise because of the young age of the animals and that a well-developed large intestinal flora may be required for changes to occur. The horse and cattle studies have largely been carried out with animals with such a mature flora (Andersson, et al., 1971; Baker and Leyland, 1973; Cook, 1973; Horspool, 1992; MacKellar, et al., 1973; Rollin, et al., 1986; Rooney, et al., 1963; 1966; White and Prior, 1982). Even the changes described by MacKinnon, (1987) citing Walton, (1986) were in gilts, considerably older than the animals used here.

The variation between groups was different in the post-withdrawal period. The differences between treated and untreated groups were most marked during the period following withdrawal and had largely disappeared by the end of the study on day 35. They consisted of high coliform counts in treated animals on days 22 and 35 and a different pattern in the frequency of isolation of VT-IIv positive E. coli. These were isolated over a longer period before and after withdrawal in the untreated controls compared with the treated group. The most obvious changes were histological and were found on days 22 and 24 of the study immediately following withdrawal. It is not clear whether these mild inflammatory changes were associated with any particular agent and they were not linked with any being studied in the Experiment.

It appears from the results of the chlortetracycline study that there is a difference in performance in pigs following the

withdrawal of chlortetracycline. This mirrors the results of Burch, et al. (1986) but could not be related to any effect on respiratory disease. It therefore seems that a post-antimicrobial effect does exist in the pig in the absence of any defined disease and this effect may result from recolonization with elements of the gut flora. In the studies described here E. coli and coliforms seemed to be affected most consistently. Since E. coli could not be linked to the mild inflammatory gastroenteric changes recorded, it is possible that other species of bacteria were responsible and these have yet to be identified. It is not clear to what extent background or earlier infection with non-antimicrobial-sensitive agents such as Cryptosporidium spp., Balantidium coli and rotavirus was responsible for the changes seen.

7. LIMITATIONS OF THIS STUDY AND FUTURE WORK

This study represents an attempt to examine the effects of withdrawal of antimicrobial on the gut flora of healthy pigs. Observations from the field (Burch, et al., 1986; MacKinnon, 1985; 1987) had suggested that withdrawal of an antimicrobial was associated with reduction in performance. This reduction in performance appeared as disease when pathogens such as S. hyodysenteriae and Ileal symbiont intracellularis were present (Olson, 1980; 1986; Olson and Rodabaugh, 1976a, b; 1977; 1978; 1984; Gebhart, 1987; Lawson, et al., 1977; 1979; Love and Love, 1979; Love, et al.,1977; Rowland and Lawson, 1975; 1986; 1992; Yates, et al., 1979) and when respiratory disease was being treated (Burch, et al., 1986). However, the absence of respiratory disease from these animals and the close association between withdrawal of treatment, depression of rate of gain and the gut flora in the absence of known bacterial disease agents is of interest. It suggests that part of the effect noted following withdrawal is attributable to bacteria not usually considered enteropathogenic for the pig. Such agents could be of widespread importance in both animal and human medicine.

The results of Experiment 4 in which chlortetracycline at therapeutic level was given to animals on diets containing no growth permitter for the longest period were of most interest. Further work should concentrate on the changes associated with

adaptation to antimicrobial treatment of the pig over days 0 to 7 post introduction and the period of 0 to 7 days post withdrawal but monitoring at least until day 15 post withdrawal. Increased numbers of animals would have to be observed during this period to confirm that a statistically-significant change in growth rate occurred during the post withdrawal period. Increasing the antimicrobial levels given and beginning the treatment at an earlier age might also exaggerate the effects.

The faecal flora and the lumenal flora were monitored in this study. It may be that future studies should examine organisms other than those studied here such as Streptococcus hyointestinalis and strict anaerobes of the type described as colonizers of the S. hyodysenteriae lesions (Alexander, et al., 1976; Lysons, et al., 1980; Robinson, et al., 1982; 1984). Use of an anaerobic cabinet and specialized media would make this study more worthwhile. Organisms which appeared to be involved could then be followed in the same way as the established pathogens. It is possible that some some Bacteroides spp. might be important. High numbers of these organisms were recovered from diarrhoeic animals in Experiment 3 and enterotoxigenic strains of B. fragilis have now been identified.

It is probable that the effects would be more obvious if conventional healthy pigs were used. These need not be infected with major pathogens, but it may be that, as with the failure of growth permitters to act in high health pigs these animals are not ideal for monitoring changes of this type.

This study must be regarded as preliminary but has shown that there is an effect following withdrawal of antimicrobial. Further experimental studies may define the phenomenon better and enable it to be detected with confidence in the field.

ENGLISH

api 20 E

≠ 2 010 0

NTIFICATION SYSTEM FOR
EROBACTERIACEAE AND OTHER
AM-NEGATIVE RODS

ruction Manual
sion A

20 E is a standardized identification system for Ente-
acteriaceae and other Gram-negative rods which
s 23 miniaturized biochemical tests and a data base

nciple

e API 20 E strip consists of 20 microtubes containing
ydrated substrates. These tests are inoculated with a
terial suspension which reconstitutes the media
ing incubation, metabolism produces colour changes
t are either spontaneous or revealed by the addition
reagents.

e reactions are read according to the **Interpretation**
ble and the identification is obtained by referring to
Identification Table, the **Analytical Profile Index** or the
APILAB software.

The **API 20 E** kit allows the performance of 25 identifi-
cations. It consists of

- 25 **API 20 E** strips
- 25 incubation boxes
- 25 **report sheets**
- 1 **API 20 E** instruction manual

To use **API 20 E**, the following are necessary

- **Suspension Medium**, 5 ml (≠ 2 011 0)
- **Reagent kit** (≠ 2 012 0) or the individual reagents (≠ 7 040 0 to 7 046 0 and 7 054 0)
- Zn reagent (≠ 7 038 0)
- **Mineral oil** (≠ 7 010 0)
- **Pipettes** (≠ 7 030 0) or **PSIpettes** (≠ 7 025 0)
- **Ampoule rack** (≠ 7 020 0)
- **API 20 E Analytical Profile Index** (≠ 2 019 0) or *APILAB* software (≠ 3 101 0, 3 104 0)

The following additional products when necessary

- **API OF Medium** (≠ 5 011 0). Test for the determination of fermentative or oxidative metabolism
- **API M Medium** (≠ 5 012 0). Test for motility of facultative anaerobic bacteria.

Plus the following general laboratory equipment :

- incubator (35-37°C), refrigerator, Bunsen burner, marker pen.

Storage

API 20 E strips should be stored at 2-8°C on arrival. The expiry date is indicated on the packaging.
The reagents should also be stored at 2-8°C. The **OX** reagent should be stored in the dark and wrapped in aluminium foil to protect it from light.

Composition of media and reagents

- **Suspension Medium** (≠ 2 011 0) : distilled water

- TDA reagent (≠ 7 040 0) for the detection of tryptophane deaminase :

ferric chloride	3.4 g
distilled water	100 ml

- Reagents for the detection of indole :

JAMES Reagent (≠ 7 054 0)

Compound J 2183	0.5 g
HCl N acid	100 ml

or

IND reagent (≠ 7 041 0)

paradimethylaminobenzaldehyde	5 g
isoamyl alcohol	75 ml
HCl 37 %	25 ml

- Voges Proskauer reagents for the detection of acetoin :

VP 1 (≠ 7 042 0)

potassium hydroxide	40 g
distilled water	100 ml

VP 2 (≠ 7 043 0)

alpha naphthol	6 g
ethanol	100 ml

- Griess reagent for the detection of nitrites :

NIT 1 (≠ 7 044 0)

sulfanilic acid	0.8 g
acetic acid 5N	100 ml

NIT 2 (≠ 7 045 0)

N-N-dimethyl-1-naphthylamine	0.6 g
acetic acid 5N	100 ml

- OX (≠ 7 046 0) for the detection of oxidase :

tetramethyl-p-phenylenediamine	1 g
isoamyl alcohol	100 ml

Instructions for use

1. **Preparation of the strip**

- prepare an incubation box, tray and lid, and distribute about 5 ml of water into the honeycombed wells of the tray to create a humid chamber
- record the strain reference on the elongated tab of the tray
- remove the strip from its packaging and place it in the tray

2 **Preparation of the inoculum**

- open an ampoule of **Suspension Medium** (ref 2 011 0) or use any tube containing 5 ml of sterile distilled water without additives
- with the aid of a pipette remove a single well-isolated colony from an isolation plate
- carefully emulsify to achieve a homogeneous bacterial suspension
- at the same time, perform an oxidase test on one identical colony as follows
  - place a piece of filter paper onto a glass slide
  - moisten the paper with 1 drop of water
  - take the chosen colony with a wooden or glass applicator stick and rub it onto the moistened filter paper
  - add one drop of **OX** reagent
  - a deep PURPLE coloration that appears within 1 or 2 minutes indicates a POSITIVE REACTION

3 **Inoculation of the strip**

- with the same pipette fill both the tube and cupule of tests CIT, VP, GEL with the bacteria suspension
- fill only the tubes (and not the cupules) of the other tests
- create anaerobiosis in the tests ADH, LDC, ODC, URE and H_2S by overlaying with **mineral oil**.
- close the incubation box and incubate at 35-37°C for 18-24 hours

4 **Reading of the strip**

- after 18-24 hours at 35-37°C, read the strip by referring to the **Interpretation Table.**
- record all spontaneous reactions on the **report sheet.**
- **if the glucose is positive** and/ or 3 tests or more are positive reveal the tests which require the addition of reagents

  - **VP Test** add 1 drop of **VP 1** and **VP 2** reagents. Wait at least 10 minutes. A BRIGHT PINK or RED colour indicates a POSITIVE reaction to be recorded on the **report sheet.**

  - **TDA Test** add 1 drop of **TDA** reagent. A DARK BROWN colour indicates a POSITIVE reaction to be recorded on the **report sheet.**

  - **IND Test** :

    add 1 drop of **JAMES** reagent (ref 7 054 0). The reaction takes place immediately : a PINK colour developed in the whole cupule indicates a POSITIVE REACTION to be recorded on the **report sheet.**
    or
    add 1 drop of **IND** reagent. Wait 2 minutes. A RED RING indicates a POSITIVE reaction to be recorded on the report sheet

  - **NO_2 Test** add 1 drop of each of **NIT 1** and **NIT 2** reagents to the GLU tube. Wait 2 to 3 minutes. A RED colour indicates a POSITIVE reaction. A negative reaction (yellow) may be due to the reduction to nitrogen (as sometimes evidenced by gas bubbles) : add 2 to 3 mg of **Zn** (ref 7 038 0) to the GLU tube. After 5 minutes, if the tube remains YELLOW this indicates that (N2) is POSITIVE and is to be recorded on the **report sheet**. If the test turns PINK-RED, this is a NEGATIVE reaction ; the nitrates still present in the tube have been reduced by the Zinc

- **if the glucose is negative** and the number of positive tests is less than or equal to 2, do not add reagents
  - inoculate 2 **API OF Medium** (ref 5 011 0) to confirm the metabolism of glucose
  - streak a MacConkey agar plate
  - check for motility by inoculating 1 **API M Medium** (ref 5 012 0) for fermentative organisms or by microscopic observation
  - reincubate 24 hours
  - add the reagents as described above
  - record the strip and supplementary test results on the **report sheet** by referring to the **Interpretation Table.**

5 **Identification**

- using the **IDENTIFICATION TABLE** compare the results recorded on the **report sheet** with those given in the table by sliding the sheet down the table. Their dimensions are identical in order to facilitate this comparison
- with the **Analytical Profile Index** or the ***APILAB*** software the pattern of the reactions obtained must be coded into a NUMERICAL PROFILE

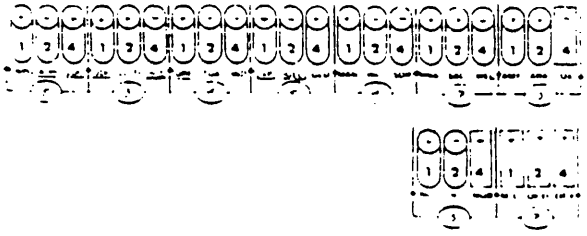
On the **report sheet**, the tests are separated into groups of 3 and a number 1, 2, or 4 is indicated for each. By adding the numbers corresponding to POSITIVE reactions within each group, a 7-digit profile number is obtained for the 20 tests of the **API 20 E** strip. The oxidase reaction constitutes the 21st test and has a value of 4 if it is positive.

In some cases, the 7-digit profile is not discriminatory enough and supplementary tests should be carried out

- reduction of nitrates to nitrites (NO_2)
- reduction of nitrates to N_2 gas (N_2)
- motility (MOB)
- growth on MacConkey agar medium (McC)
- oxidation of glucose (OF-O)
- fermentation of glucose (OF-F)

The corresponding reactions are coded according to the same principle which results in a 9-digit numerical profile

Example :



5 315 173-57 : Enterobacter gergoviae

the Analytical Profile Index and the *APILAB* disk allow the numerical profiles to be identified, along with the following information :

- name of the species identified.
- the identification percentage and the T index which takes into consideration the typical aspect of the profile.
- tests against the identification, if any, with the percentage of positive reactions normally observed for that species.
- comments on the quality of the identification.
- in the case of " low discrimination " between 2 or 3 taxa, a note refers to a TABLE where differential tests, extracted from the literature, are given
- for some profiles, the need for reincubation is indicated.

the *APILAB* software includes DOUBTFUL and UNACCEPTABLE numerical profiles along with the forementioned information.

the **API Computer Service** : allows the bacteriologist to consult our laboratory for any unlisted profiles. In all cases, features of interest such as source of the specimen, colonial and microscopic morphology, patient history, serology etc., must be taken into consideration for the identification obtained.

6. Disposal

After use, all ampoules, strips and incubation boxes must be autoclaved, incinerated, or immersed in a disinfectant prior to disposal.

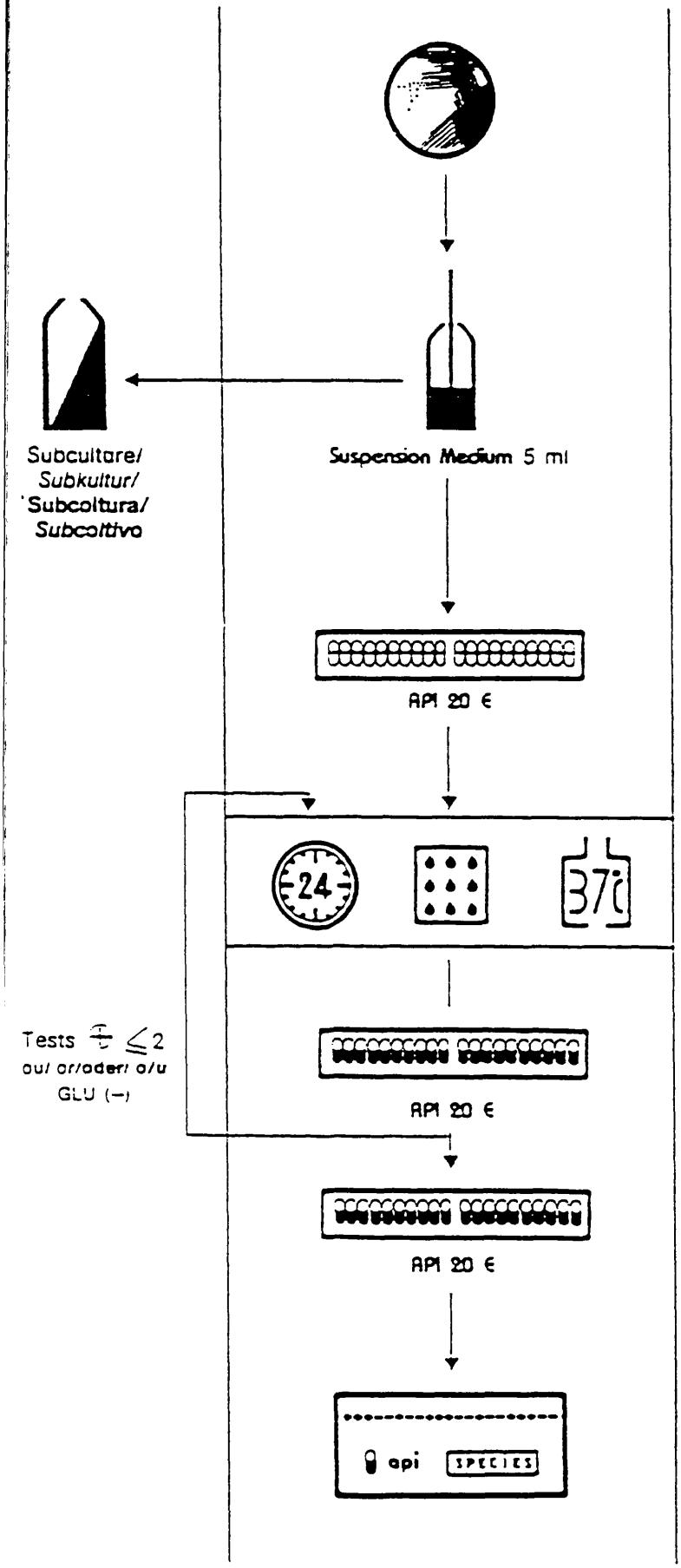
Quality Control

The media, strips and reagents are systematically quality controlled at various stages of their manufacture. For those who wish to perform their own quality control tests, it is recommended that the following stock cultures be used, to obtain the results below.

1. *Klebsiella pneumoniae*	NCTC 8172
2. *Enterobacter cloacae*	ATCC 13047
3. *Proteus vulgaris*	ATCC 13315
4. *Pseudomonas aeruginosa*	ATCC 10145

	ONPG	ADH	LDC	ODC	CIT	H2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	OX
1.	+	−	+	−	+	−	+	−	−	+	−	+	+	+	+	+	+	+	+	+	−
2.	+	+	−	+	−	−	−	−	−	+	−	+	−	−	−	+	−	−	−	−	−
3.	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−	+	−	−	−	−
4.	−	+	−	−	−	−	+	−	−	−	+	+	−	−	−	−	−	−	−	−	+

MÉTHODOLOGIE/PROCEDURE/METHODIK
PROCEDIMENTO/TECNICA



SELECTION/AUSWAHL
SELEZIONE/SELECCION

INOCULUM/INOKULUM
SOSPENSIONE/INOCULO

1 colonie
colony
Kolonie
colonia

INOCULATION/BEIMPFEN
INOCULO/INOCULACION

- CIT
- VP
- GEL
- ADH ➡ ODC
- H2S - URE

INCUBATION/INKUBIEREN
INCUBAZIONE/INCUBACION

LECTURE/READING/ABLESEN
LETTURA/LECTURA

- TDA TDA
- IND JAMES
  ou/ or/ oder/ o IND
- VP VP1 + VP2
- NO_3 NIT 1 + NIT 2
  in GLU
- OX OX

INTERPRETATION/INTERPRETAZIONE
INTERPRETACION

- Tab. Identif.
- Cat. Analy.
- *APILAB*

ENGLISH

api 20 A

2 030 0

OR THE IDENTIFICATION
F ANAEROBES

istruction manual
ersion A

he API 20 A system enables 21 tests to be carried out uickly and easily for the biochemical identification of naerobes. Other tests such as colonial and microscopic norphology, Gram stain, gas-liquid chromatography of netabolic products ..., should be performed and the esults used to confirm or complete the identification.

dditional tests using API ZYM (= 2 520 0) or API 50 CH = 5 030 0) may also assist in the identification of the rganism.

Principle

he API 20 A system consists of 20 microtubes containing ehydrated substrates. The bacterial suspension ispensed into the tubes reconstitutes these substrates.

he metabolites produced are revealed by pH indicators r by the addition of reagents after an incubation period f 24 or 48 hours at 35-37° C.

- The API 20 A kit allows the performance of 25 tests. It consists of:

- 25 API 20 A strips
- 25 incubation boxes
- 25 ampoules of API 20 A medium
- 25 report sheets
- 1 instruction manual

- To use API 20 A, the following are necessary:

- anaerobic jar or anaerobic chamber
- inoculating loop or sterile swabs (= 7 061 0)
- mineral oil (= 7 010 0)
- plastic petri dish (9 cm in diameter)
- EHR reagent (= 7 052 0)
- BCP reagent (= 7 051 0)
- XYL reagent (= 7 053 0)
- Hydrogen peroxide (3 %)
- Ampoule rack (= 7 020 0)
- sterile Pipettes (= 7 030 0) or
- PSIpettes (= 7 025 0)
- Ultra violet lamp (365 nm) (= 7 080 0)
- API 20 A Analytical Profile Index (= 2 039 0) or
- *APILAB software* (= 3 101 0, 3 104 0)

Plus standard laboratory equipment

- incubator, refrigerator, Bunsen burner, marker pen

Storage

The API 20 A strips and the API 20 A medium should be stored at 2-8° C. The expiry date is printed on the packaging.

The EHR reagent has to be stored at 2-8° C while BCP and XYL reagents can be stored at room temperature.

Composition of media and reagents

- API 20 A medium included in the API 20 A kit.

Tryptic case	5.0 g
Yeast extract	5.0 g
Sodium chloride	2.5 g
L-tryptophan	0.2 g
L-cystine	0.4 g
Haemin	0.01 g
Vitamin K_3	0.01 g
Sodium sulphite	0.10 g
Distilled water qsp	1000 ml
pH 7.0-7.2	

- Reagents

EHR : p-dimethylaminobenzaldehyde	8.75 g	
Ethanol	825	ml
HCl 37 %	175	ml

BCP : a 0.02 % aqueous solution of Bromocresol purple

XYL : xylene

Instructions for use

1. **Preparation of the inoculum :**

- Open an ampoule of **API 20 A medium**.
- Using a sterile swab, harvest all the growth obtained on the anaerobic blood agar plate. Check that the strain is pure.
- Hold the ampoule upright and emulsify the organisms by rotating the swab and rubbing it against the side of the ampoule without taking it out of the suspension medium. The final turbidity must be greater than or equal to that of **tube 3 on the McFarland opacity scale** Slow growing organisms may require more than a single blood agar plate to achieve this inoculum density

NOTE : To maintain anaerobic conditions, do not stir the inoculation medium excessively otherwise air may be dissolved in it.

2. **Preparation of the strip**

The method of using the strip depends on the method chosen to create anaerobic conditions :

- Incubation in an anaerobic chamber or in individual sachets.
  - Take an **API 20 A** strip out of its packaging and place it in an individual incubation box moistened with about 5 ml of water beforehand.
  - Using a pipette, inoculate the strip with inoculated **API 20 A medium**, avoiding the formation of bubbles and tilting the strip slightly forwards.
    - For the test GEL, fill both the tube and cupule
    - For the test IND, fill just the tube with **API 20 A medium** and fill the cupule with **mineral oil** to prevent the indole from evaporating.
  - Close the incubation box and place it at 35-37° C
- Incubation in an anaerobic jar
  - Take an **API 20 A** strip out of its packaging and bend it so that it fits into an empty Petri dish ; seal the base and lid together with two strips of adhesive tape, one between the two ends of the strips, the other in the middle between the ARA and GEL tubes.
  - Note the strain reference on the Petri dish
  - Using a pipette, inoculate the strip with inoculated **API 20 A medium** avoiding the formation of bubbles Only fill the tubes. The IND tube should be slightly underfilled and overlaid with **mineral oil**
  - Place it in the jar and incubate at 35-37° C.
- Incubation with **mineral oil**
  (not sufficient for strict anaerobes).
  - Take an **API 20 A** strip out of its packaging and place it in an individual incubation box moistened with about 5 ml of water beforehand.
  - Note the strain reference on the lateral flap.
  - Using a sterile pipette, inoculate the strip with the suspension in the **API 20 A medium** avoiding the formation of bubbles and tilting the strip slightly forwards.
  - Fill just the tubes with **API 20 A medium**
  - Fill all the cupules with **mineral oil**
  - Close the box and incubate at 35-37° C
- The surplus **API 20 A medium** can be used
  - To check the purity and viability of the strain by inoculating a set of 2 Petri dishes ; one inoculated aerobically and the other anaerobically.
  - to perform additional tests
    - inoculate a glucose broth for Gas liquid chromatography of the fatty acids produced.
    - add 1 or 2 drops of KNO_3 (6 mg/ml) to the ampoule to study the reduction of nitrates. Incubate anaerobically for 24-48 hours and read the reaction after adding **NIT 1** and **NIT 2** reagents.

3. **Reading the strips**

Many anaerobic bacteria produce reactions which are clear and easy to read within 24 hours, but some strains grow slowly and can only be identified after 48 hours of incubation

After a 24-hour incubation period, observe the reactions which do not require the addition of any reagents (see Reading Table)

- If the reactions have given a satisfactory result, add the reagents, read the results according to the **Reading Table**, complete the **report sheet** and carry out the final identification of the organism concerned.
- Otherwise, reincubate the strip and perform the identification only after incubating for 48 hours

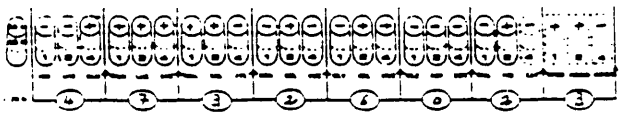
4 **Identification**

- Identification can be made using the **Identification Table**. Compare the biochemical profile obtained with the average profiles of the taxa in the data base, by sliding the report sheet down the Table Their dimensions are identical in order to facilitate this comparison.
- Identification can also be made by using the **Analytical Profile Index** or the ***APILAB software***. To do this, the pattern of reactions is coded into a NUMERICAL PROFILE.

Coding the profiles

- The **report sheet** reproduces the outline of the API 20 A strip with its 20 tests, plus the catalase reaction and 3 morphological characteristics. SPOR for spore (+,–), GRAM (+,–) and COCC for coccus (+,–). These tests are divided into groups of three and a number (1, 2 or 4) is assigned to each test. By adding, in each group, the numbers corresponding to POSITIVE reactions, eight figures are obtained which form the numerical profile.

Example :



4 732 602-3 *Clostridium septicum*

- The **API Computer Service** allows the bacteriologist to consult our laboratory for any unlisted profiles.

5. Disposal

After use, ampoules, boxes, pipettes and strips must be autoclaved, incinerated or immersed in disinfectant prior to disposal.

Quality control

The media, strips and reagents are systematically quality controlled at various stages of their manufacture.

For those who wish to perform their own quality control tests, the following stock cultures are recommended to obtain the results below :

1. *Bacteroides thetaiotaomicron*	ATCC 8492
2. *Clostridium perfringens*	ATCC 13124
3. *Clostridium tertium*	ATCC 19405

	IND	URE	GLU	MAN	LAC	SAC	MAL	SAL	XYL	ARA	ESC	GLY	CEL	MNE	MLZ	RAF	SOR	RHA	TRE	CAT
1.	±	–	+	–	+	+	+	–	+	+	–	+	–	V	+	±	+	–	+	+	+
2.	–	–	+	–	+	+	+	–	–	–	+	–	+	–	+	–	–	±	–	+	–
3.	–	–	+	+	+	+	+	+	V	–	–	+	–	+	+	+	–	–	–	+	–

api 20 A — READING TABLE

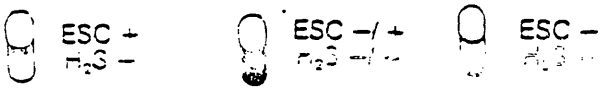
TESTS	SUBSTRATES	REACTIONS/ENZYMES	RESULTS	
			NEGATIVE	POSITIVE
IND	Tryptophane	Indole formation	1 drop of XYL to mineral oil. Mix and leave for 2-3 mins. Add 1 drop of EHR reagent. The reagent should float on the xylene/ mineral oil. Read within 5 mins.	
			Yellow	Red
URE	Urea	Urease	Yellow-orange	Red
GLU	Glucose	Acidification	BCP (1)	
MAN	Mannitol	Acidification		
LAC	Lactose	Acidification		
SAC	Saccharose	Acidification		
MAL	Maltose	Acidification	Purple	Yellow green-Yellow
SAL	Salicin	Acidification		
XYL	Xylose	Acidification		
ARA	Arabinose	Acidification		
GEL	Gelatin	Hydrolysis (protease)	No pigment diffusion (2)	Diffusion of black pigment (2)
			Yellow (3)	Brown-Black (3)
	Esculin		in U.V. (365 nm)	
ESC	Ferric Citrate	Hydrolysis (β glucosidase)	Fluorescence	No fluorescence
GLY	Glycerol	Acidification	BCP (1)	
CEL	Cellobiose	Acidification		
MNE	Mannose	Acidification		
MLZ	Melezitose	Acidification		
RAF	Raffinose	Acidification	Purple	Yellow green - Yellow
SOR	Sorbitol	Acidification		
RHA	Rhamnose	Acidification		
TRE	Trehalose	Acidification		
			After 30 mins in air 2 drops H_2O_2 to the MAN tube	
CAT		Catalase	No bubbles	Bubbles
SPOR		Spores	None	Present
GRAM		Gram reaction	Pink	Violet
COCC		Morphology	Rod	Coccus

) The BCP present in each tube may be discoloured by reduction. In this case, add 1 drop of BCP reagent (= 7 051 0) to each tube efore reading. Do not read discoloured tests as positive.

!) With incubation in a round jar, the pigment only diffuses in the lower part of the tube.

!) The brown-black colour sometimes only develops after the strip is exposed to air and this should be taken into consideration when ading.

black colour may be due to the formation of Ferric Sulphide due to H_2S reacting with the Ferric citrate. This does not indicate Esculin ydrolysis. The two may be distinguished by the fact that Ferric sulphide forms a black precipitate at the base of the tube whereas Escu n hydrolysis results in a brown-black area at the top of the tube. If the tube is completely black, and in case of doubt, the test should b ead by examining for fluorescence in U.V. light.



ENGLISH

api 20 STREP

Ref. 20 600

IDENTIFICATION
OF STREPTOCOCCI

Instruction Manual
Version 3

API 20 STREP is a standardized method combining 20 biochemical tests that offer widespread applications.

It enables a genus or species identification to be made of most Streptococci encountered in medical or veterinary bacteriology within 4 or 24 hours. The list of species which can be identified can be found in the identification table on p. 22.

Principle

API 20 STREP consists of a strip of 20 microtubes containing dehydrated substrates for the demonstration of enzymatic activity or the fermentation of sugars. A dense suspension is made from a pure culture and used to rehydrate the enzymatic substrates. The metabolic endproducts produced during the incubation period are either revealed through spontaneous coloured reactions or by the addition of reagents. The fermentation tests are inoculated with an enriched medium which reconstitutes the sugar substrates. Fermentation of carbohydrates is detected by a shift in the pH indicator.
These reactions are read by referring to the **Interpretation Table** and identification is obtained using the **Identification Table**, the **API 20 STREP Analytical Profile Index** or the identification software.

- The API 20 STREP kit enables 25 identifications and consists of :
  - 25 **API 20 STREP** strips
  - 25 incubation trays
  - 25 ampoules of **GP Medium**
  - 25 **report sheets**
  - 25 sterile swabs
  - 1 instruction manual
- In order to use API 20 STREP, the following are also required :
  - **NIN** reagent (ref. 70 490)
  - **VP** 1 and **VP** 2 reagents (ref. 70 420 and 70 430)
  - **ZYM A** and **ZYM B** reagents (ref. 70 470 and ref. 70 480)
  - **Suspension Medium** 2 ml (ref. 70 600)
  - **Mineral oil** (ref. 70 100)
  - **Pipettes** (ref. 97 560) or **PSIpettes** (ref. 70 250)
  - **Ampoule rack** (ref. 70 200)
  - **API 20 STREP Analytical Profile Index** (ref. 20 690) or identification software
  - **McFARLAND STANDARD** (ref. 70 900), point 4.
  - Shaedler broth (optional)
  - Columbia Blood Agar plates

Plus the following laboratory equipment

- 35-37°C incubator, refrigerator, Bunsen burner, marker pen, anaerobic jar.

Storage

GP Medium should be stored at 2-8°C. The expiry date is marked on the packaging. The reagents are also stored at 2-8°C except **ZYM A** and **VP 1** which are stored at 3-30°C.
NOTE : the reagents must be in perfect condition

- **NIN** reagent is very sensitive to traces of water and air. Be sure to use only dry pipettes for transfer and keep the bottle tightly closed.
- **ZYM A** produces a precipitate upon refrigeration which does not alter its properties and dissolves at 60°C.
- **ZYM B** is light-sensitive. Any tint of pink indicates deterioration. Only take it out at the time of use and do not leave it on the bench for prolonged periods of time. Protect it from light by wrapping aluminium foil around the bottle.

Composition of media and reagents

- **GP Medium** included in the kit :

Cystine	0.50	g
Tryptone	20	g
Sodium chloride	5	g
Sodium sulfite	0.50	g
Phenol red	0.17	g
Demineralized water	qsp 1000	ml
final pH = 7.3		

- **Suspension medium**

Demineralized water	2	ml

NIN reagent

Ninhydrin	7	g
2-methoxy ethanol	qsp 100	ml

- Reagents for the detection of acetoin

VP 1

Potassium hydroxide	40	g
Demineralized water	qsp 100	ml

VP 2

Alpha naphthol	6	g
Ethyl alcohol	qsp 100	ml

- Reagents for the detection of enzymatic activity :

ZYM A

Tris-hydroxymethyl-amino-methane	25	g
Hydrochloric acid 37 %	11	ml
Lauryl sulfate	10	g
Demineralized water	qsp 100	ml

ZYM B :

Fast blue BB	0.35	g
2-methoxy-ethanol	qsp 100	ml

tions for Use

ony Selection

he microorganism to be identified has been isolat- verified to be a member of the genus Streptococ-

e the type of haemolysis (this constitutes the 21st).

a well-isolated colony (1) and suspend it in 0.3 ml istilled water. Homogenize well.

pd a Columbia sheep blood agar plate (2) with this pension (or aseptically swab the entire surface of agar plate).

ubate for 18-24 hours at 35-37°C in anaerobic ditions.

ta-haemolytic streptococci and enterococci pro- sufficiently large colonies after 24 hours of growth. her streptococci it is preferable to select a colony 48 hours of incubation. For fastidious strains (pro- g minute colonies after 48 hours), the following dure is recommended.

ulture the colony in 1 ml of Schaedler broth at 5-37°C for 5 hours.

lood a Columbia sheep blood agar plate with the ntire culture.
liminate any excess liquid.

ncubate anaerobically for 18 hours at 35-37°C.

the case of suspected pneumococci, it is advisable epare 2 plates in order to obtain sufficient growth.

paration of the strip

epare an incubation box, tray and cover and add out 5 ml of distilled water into the tray in order to cre- a humid atmosphere.

cord the strain reference on the lateral flap of the y.

move the strip from its packaging and place it in the y.

eparation of the inoculum

pen an ampoule of **Suspension Medium** or use any tube ntaining 2 ml of sterile distilled water without addi- es.

sing a sterile swab, harvest all the culture from the eviously prepared subculture plate. Make a dense spension with a turbidity greater than 4 on the cFarland scale.

4. Inoculation of the strip

- In the first half of the strip (tests VP to ADH) : distribute the suspension with a sterile pipette, avoiding the formation of bubbles (tilt the strip slightly forwards) :
  - for the tests VP to LAP : distribute approximately 150 µl in each cupule (3 drops with a Pasteur pipette or 6 drops with a **PSIpette**).
  - for the test ADH : fill the tube portion only.

- In the second half of the strip (tests RIB to GLYG) :
  - Open an ampoule of **GP Medium** and transfer the remaining suspension into it (about 0.5 ml). Homogenize well.
  - Distribute this new suspension into the tubes only.

- Overlay the cupules of the tests ADH to GLYG with **mineral oil** forming a convex meniscus.
- Close the incubation box.
- Incubate at 35-37°C for 4 hours to obtain a first reading and if necessary, 24 hours to obtain a second reading.

5. Reading and Interpretation

- After 4 hours of incubation :
  - Add the reagents :
    - **VP** test : 1 drop of **VP1** and **VP2**.
    - **HIP** test : 2 drops of **NIN**
    - **PYRA**, **αGAL**, **βGUR**, **βGAL**, **PAL**, **LAP** tests : 1 drop of **ZYM A** and **ZYM B**.
  - Wait 10 minutes, then read the reactions by referring to the **Colour chart** or the **Identification table** (if necessary, expose the strip to a strong light (10 seconds with a 1000 W lamp) to decolorize any excess reagents in tubes PYRA to LAP.

- Reincubation is necessary when the profile cannot be found in the **API 20 STREP Analytical Profile Index** (see section 6) or when the profile is given with the following note : "Identification not valid before 24 hours of incubation". After 24 hours reread the reactions ESC, ADH, and RIB to GLYG without rereading the enzymatic reactions. Note the reactions on the **report sheet.**

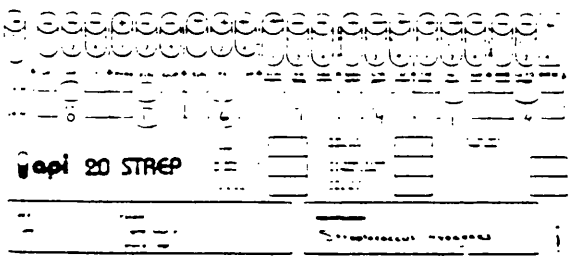
6. Identification

- Identification can be made by using the **Analytical Profile Index** or the identification software. To do this, the pattern of reactions is coded into a PROFILE NUMBER.

  On the **report sheet**, the tests are separated into groups of 3 and a number 1, 2, or 4 is indicated for each. By adding the numbers corresponding to positive reactions within each group, 7 digits are obtained which constitute the profile number.

- Identification can also be made using the **Identification Table**. Compare the reactions recorded on the **report sheet** with those given in the table by sliding the sheet down the table.

Example :



0161414 *Streptococcus pyogenes*

NOTE : The haemolytic reaction constitutes the 21st test ; beta-haemolysis is considered as positive with a numerical value of 4. All other haemolytic reactions are considered as negative with a numerical value of 0. Nevertheless this test may be of discriminant value for certain species.

- The **Computer Service** offers assistance for profiles not listed in the index.

7. Disposal

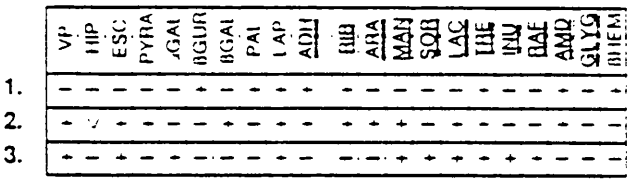
After use, all ampoules, strips, pipettes and incubation trays must be autoclaved, incinerated or immersed in a disinfectant for decontamination prior to disposal.

8. Quality Control

The media, strips and reagents are systematically quality controlled at various stages of their manufacture.

For those who wish to perform their own quality control tests, the following stock cultures are recommended to obtain the results below :

1. *Streptococcus equisimilis*	ATCC 35666
2. *Enterococcus faecium*	ATCC 35667
3. *Streptococcus mutans*	ATCC 35668



Limitations

The **API 20 STREP** system is designed solely for the identification of *Streptococcus*, *Enterococcus*, *Listeria*, *Gemella*, *Gardnerella*, and *Aerococcus* species found in the data base (see identification table p. 22) and for them alone.

Interpretation of the test results should be made a competent microbiologist who should also take into consideration the patient history, the source of the specimen, colonial and microscopic morphology and, if necessary, the results of any other tests performed, particularly the antimicrobial susceptibility patterns.

api 20 STREP

INTERPRETATION TABLE

TESTS	SUBSTRATES	REACTIONS/ENZYMES	RESULTS			
			NEGATIVE		POSITIVE	
VP	Pyruvate	Acetoin Production	VP 1 + VP 2/ wait 10 min Colourless		Pink-Red	
HIP	Hippurate	Hydrolysis	NIN/ wait 10 min Colourless/ Pale blue		Dark blue/ Violet	
ESC	Esculin	β-glucosidase	4 hrs Colourless Pale yellow	24 hrs Colourless Pale yellow Light grey	4 hrs Grey Black	24 hrs Black
PYRA	Pyrrolidonyl 2 naphthylamide	Pyrrolidonylarylamidase	ZYM A + ZYM B/ 10 min (1) if necessary, decolorize with intense light Colourless or very pale orange		Orange	
αGAL	6-Bromo-2-naphthyl α-D-Galactopyranoside	α-galactosidase	Colourless		Violet	
βGUR	Naphthol AS-BI β-D-glucuronate	β-glucuronidase	Colourless		Blue	
βGAL	2-naphthyl-6-D galactopyranoside	β-galactosidase	Colourless or very pale violet		Violet	
PAL	2-naphthyl phosphate	Alkaline Phosphatase	Colourless or very pale violet		Violet	
LAP	L-leucine-2-naphthyl-amide	Leucine arylamidase	Colourless		Orange	
ADH	Arginine	Arginine dihydrolase	Yellow		Red	
			4 hrs	24 hrs	4 hrs	24 hrs
RIB	Ribose	Acidification	Red	Orange/ Red	Orange/ Yellow	Yellow
ARA	L-Arabinose	Acidification	Red	Orange/ Red	Orange/ Yellow	Yellow
MAN	Mannitol	Acidification	Red	Orange/ Red	Orange/ Yellow	Yellow
SOR	Sorbitol	Acidification	Red	Orange/ Red	Orange/ Yellow	Yellow
LAC	Lactose	Acidification	Red	Orange/ Red	Orange/ Yellow	Yellow
TRE	Trehalose	Acidification	Red	Orange/ Red	Orange/ Yellow	Yellow
INU	Inulin	Acidification	Red	Orange/ Red	Orange/ Yellow	Yellow
RAF	Raffinose	Acidification	Red	Orange/ Red	Orange/ Yellow	Yellow
AMD	Starch (2)	Acidification	Red	Orange/ Red	Orange/ Yellow	Yellow
GLYG	Glycogen	Acidification	Red or orange		Bright yellow	

(1) During a second reading after 24 hours of incubation, a deposit may be noticed in the tubes where the ZYM A and ZYM B reagents have been added. This phenomenon is normal and should not be taken into consideration.

(2) The acidification of starch is frequently weaker than that of other sugars.

TABLE 5.14 Daily individual bacterial counts of *Campylobacter spp.*/g faeces from the treated group in Experiment 2

Study							
Date	Days	Pig No. 68	Pig No. 69	Pig No. 97	Pig No. 98	Pig No. 99	$\bar{X}$
05.03.88	0	N D	N D	N D	N D	N D	N D
08.03.88	3^	5.0×10^3	5.0×10^4	5.0×10^3	1.5×10^4	3.5×10^3	1.5×10^4
14.03.88	9	3.0×10^4	2.5×10^4	4.5×10^3	5.0×10^4	5.5×10^3	2.3×10^4
15.03.88	10	2.0×10^4	1.0×10^4	N G	1.0×10^4	4.0×10^4	2.0×10^4
16.03.88	11	5.0×10^2	N G	N G	1.5×10^4	N G	7.7×10^3
17.03.88	12	1.5×10^4	1.5×10^4	3.0×10^4	3.5×10^4	3.0×10^4	2.5×10^4
18.03.88	13*	5.0×10^3	1.0×10^4	5.0×10^5	5.0×10^3	N G	1.3×10^5
19.03.88	14	5.0×10^3	2.0×10^3	4.5×10^3	1.0×10^4	N G	5.3×10^3
20.03.88	15	5.0×10^3	5.5×10^4	1.0×10^5	5.0×10^3	5.0×10^3	3.4×10^4
21.03.88	16	5.0×10^7	5.0×10^4	5.0×10^6	5.0×10^6	5.0×10^4	1.2×10^7
22.03.88	17	1.5×10^4	5.0×10^3	5.0×10^5	5.0×10^3	2.5×10^3	1.0×10^5
23.03.88	18	2.5×10^4	2.5×10^5	2.5×10^5	5.0×10^5	1.0×10^4	2.0×10^5

N D = Not Done (Determined) N G = No Growth

^ Beginning of treatment, * Withdrawal of treatment

TABLE 5.15 Daily individual bacterial counts of Campylobacter spp./g faeces from the control group in Experiment 2

Study							
Date	Days	Pig No. 50	Pig No. 95	Pig No. 97	Pig No. 99	Pig No. 100	$\bar{X}$
05.03.88	0	N D	N D	N D	N D	N D	N D
08.03.88	3^	1.0×10^3	5.0×10^4	1.0×10^4	4.5×10^4	5.0×10^3	2.2×10^4
14.03.88	9	1.0×10^3	5.0×10^4	1.0×10^4	5.0×10^3	1.0×10^4	1.5×10^4
15.03.88	10	5.0×10^5	2.0×10^4	2.0×10^5	2.0×10^4	2.5×10^5	1.9×10^5
16.03.88	11	N G	4.0×10^3	9.0×10^3	5.0×10^3	1.0×10^4	7.0×10^3
17.03.88	12	1.0×10^5	1.5×10^4	6.0×10^4	1.5×10^4	5.0×10^5	1.3×10^5
18.03.88	13*	5.0×10^2	2.0×10^3	1.0×10^3	5.0×10^4	5.0×10^5	1.1×10^5
19.03.88	14	N G	N G	4.0×10^3	6.0×10^3	5.0×10^4	2.0×10^4
20.03.88	15	N G	2.0×10^3	1.0×10^4	5.0×10^5	5.0×10^4	1.4×10^5
21.03.88	16	5.0×10^5	5.0×10^4	3.5×10^5	1.0×10^{10}	2.5×10^{11}	5.2×10^{10}
22.03.88	17	N G	1.0×10^4	1.0×10^4	5.0×10^4	5.0×10^4	3.0×10^4
23.03.88	18	5.0×10^3	5.0×10^4	2.0×10^4	5.0×10^5	5.0×10^4	1.2×10^5

N D = Not Done (Determined) N G = No Growth

^ Beginning of treatment, * Withdrawal of treatment

TABLE 5.22 Daily individual bacterial counts of *Clostridium perfringens* type A (spores)/g faeces from the treated group in Experiment 2

Study							
Date	Days	Pig No. 68	Pig No. 69	Pig No. 97	Pig No. 98	Pig No. 99	$\bar{X}$
05.03.88	0	N D	N D	N D	N D	N D	N D
08.03.88	3^	5.0 x10^2	N G	N G	N G	5.0 x10^2	5.0x10^2
14.03.88	9	N G	N G	N G	N G	5.0 x10^2	5.0x10^2
15.03.88	10	N G	N G	N G	N G	N G	N G
16.03.88	11	N G	N G	N G	N G	5.0 x10^2	5.0x10^2
17.03.88	12	N G	N G	N G	N G	N G	N G
18.03.88	13*	N G	N G	N G	N G	N G	N G
19.03.88	14	5.0 x10^2	N G	N G	N G	N G	5.0x10^2
20.03.88	15	N G	N G	N G	N G	N G	N G
21.03.88	16	N G	N G	5.0 x10^2	N G	N G	5.0x10^2
22.03.88	17	N G	N G	N G	N G	N G	N G
23.03.88	18	N G	N G	N G	N G	N G	N G

N D = Not Done (Determined) N G = No Growth

^ Beginning of treatment, * Withdrawal of treatment

TABLE 5.23 Daily individual bacterial counts of Clostridium perfringens type A (spores)/g faeces from the control group in Experiment 2

Study							
Date	Days	Pig No. 50	Pig No. 95	Pig No. 97	Pig No. 99	Pig No. 100	$\bar{X}$
05.03.88	0	N D	N D	N D	N D	N D	N D
08.03.88	3^	5.0×10^3	1.0×10^3	N G	N G	5.0×10^2	2.1×10^3
14.03.88	9	N G	N G	5.0×10^2	N G	N G	5.0×10^2
15.03.88	10	N G	5.0×10^2	N G	N G	N G	5.0×10^2
16.03.88	11	N G	N G	N G	N G	N G	N G
17.03.88	12	N G	N G	N G	N G	N G	N G
18.03.88	13*	N G	N G	N G	N G	N G	N G
19.03.88	14	N G	N G	N G	N G	N G	N G
20.03.88	15	N G	N G	N G	5.0×10^2	N G	5.0×10^2
21.03.88	16	N G	N G	N G	N G	N G	N G
22.03.88	17	N G	N G	N G	N G	N G	N G
23.03.88	18	N G	N G	5.0×10^2	N G	N G	5.0×10^2

N D = Not Done (Determined) N G = No Growth

^ Beginning of treatment, * Withdrawal of treatment

TABLE 5.33 Individual bacterial counts of total coliforms/g faeces from the treated group in Experiment 3

Study							
Date	Day	Pig No. 84	Pig No. 86(63)	Pig No. 87	Pig No. 88	Pig No. 90	$\bar{X}$
31.05.88	0	2.0×10^5	2.0×10^6	1.5×10^7	8.5×10^4	2.5×10^7	8.4×10^6
07.06.88	**7^**	**3.5×10^5**	**1.5×10^5**	**2.5×10^5**	**5.0×10^4**	**1.5×10^5**	**1.9×10^5**
09.06.88	9	5.0×10^8	1.0×10^8	1.0×10^5	5.0×10^6	5.0×10^7	1.3×10^8
10.06.88	10	5.0×10^9	3.5×10^{10}	2.5×10^{10}	2.0×10^9	5.0×10^{10}	2.3×10^{10}
12.06.88	12	4.0×10^5	1.5×10^5	5.0×10^5	1.5×10^9	2.0×10^{10}	4.3×10^9
14.06.88	14	3.0×10^5	5.0×10^4	N G	1.0×10^5	1.0×10^{10}	2.5×10^9
16.06.88	16	1.0×10^6	5.0×10^6	5.0×10^5	9.0×10^3	1.0×10^9	2.0×10^8
18.06.88	18	1.0×10^6	2.5×10^{10}	5.0×10^9	5.0×10^5	5.0×10^9	7.0×10^9
20.06.88	**20***	**5.0×10^6**	**5.0×10^9**	**1.0×10^9**	**2.0×10^9**	**1.0×10^{10}**	**3.6×10^9**
21.06.88	21	2.0×10^{10}	7.0×10^{10}	3.0×10^{10}	1.5×10^{10}	2.5×10^{10}	3.2×10^{10}
23.06.88	23	2.0×10^{10}	2.0×10^9	5.0×10^8	5.0×10^7	5.0×10^9	5.5×10^9
25.06.88	25	2.0×10^5	2.0×10^5	5.0×10^6	3.5×10^4	5.0×10^9	1.0×10^9
27.06.88	27	2.0×10^8	1.0×10^8	5.0×10^3	1.0×10^6	1.5×10^{10}	3.0×10^9
29.06.88	29	1.5×10^6	1.0×10^{10}	1.5×10^8	1.0×10^8	3.0×10^{10}	8.0×10^9
01.07.88	31	1.0×10^6	5.0×10^5	1.5×10^8	3.0×10^6	4.0×10^{10}	8.0×10^9
03.07.88	33	5.0×10^6	5.0×10^5	5.0×10^8	2.5×10^{10}	1.0×10^{10}	7.1×10^9

N G = No Growth

^ Beginning of treatment, * Withdrawal of treatment

TABLE 5.34 Individual bacterial counts of total coliforms/g faeces from the control group in Experiment 3

Study							
Date	Day	Pig No. 88	Pig No. 90(L)	Pig No. 92	Pig No. 93	Pig No. 94	$\bar{X}$
31.05.88	0	1.0×10^4	1.0×10^6	1.0×10^7	2.0×10^5	5.0×10^5	2.3×10^6
07.06.88	**7^**	**1.0×10^7**	**5.0×10^5**	**5.0×10^5**	**1.5×10^5**	**5.0×10^4**	**2.2×10^6**
09.06.88	9	2.5×10^8	2.0×10^7	2.0×10^8	2.5×10^8	2.5×10^7	1.4×10^8
10.06.88	10	3.5×10^9	1.5×10^{10}	5.0×10^9	5.0×10^8	1.0×10^{10}	6.8×10^9
12.06.88	12	1.0×10^8	4.5×10^9	1.0×10^9	5.0×10^8	5.0×10^7	1.2×10^9
14.06.88	14	3.5×10^{10}	5.0×10^9	1.0×10^9	2.0×10^7	1.0×10^7	8.2×10^9
16.06.88	16	5.0×10^5	1.5×10^{10}	5.0×10^7	5.0×10^6	1.0×10^7	3.0×10^9
18.06.88	18	1.0×10^{10}	1.0×10^{10}	5.5×10^5	2.0×10^5	1.5×10^{10}	7.0×10^9
20.06.88	**20***	**5.0×10^8**	**1.5×10^8**	**5.0×10^8**	**2.0×10^9**	**3.0×10^6**	**6.3×10^8**
21.06.88	21	3.0×10^{10}	8.0×10^{10}	2.5×10^{10}	5.0×10^8	1.5×10^{10}	3.0×10^{10}
23.06.88	23	5.0×10^5	1.5×10^6	5.0×10^9	1.5×10^8	5.0×10^4	1.0×10^9
25.06.88	25	2.0×10^5	5.0×10^6	5.0×10^6	5.0×10^5	2.0×10^{10}	4.0×10^9
27.06.88	27	1.0×10^5	1.0×10^8	3.5×10^8	1.5×10^5	5.0×10^7	1.0×10^8
29.06.88	29	1.5×10^{10}	1.5×10^{10}	2.5×10^{10}	1.5×10^5	2.0×10^{10}	1.5×10^{10}
01.07.88	31	5.0×10^7	5.0×10^8	2.0×10^{10}	1.0×10^5	2.5×10^{10}	9.1×10^9
03.07.88	33	5.0×10^7	2.5×10^6	5.0×10^5	1.0×10^5	5.0×10^6	1.1×10^9

^ Beginning of treatment, * Withdrawal of treatment

TABLE 5.35 Individual bacterial counts of *Escherichia coli*/g faeces from the treated group in Experiment 3

Study							
Date	Day	Pig No. 84	Pig No. 86(63)	Pig No. 87	Pig No. 88	Pig No. 90	$\bar{X}$
31.05.88	0	5.0×10^6	5.0×10^5	1.5×10^5	2.5×10^4	5.0×10^7	1.1×10^7
07.06.88	**7^**	**3.5×10^5**	**5.0×10^4**	**5.0×10^4**	**5.0×10^4**	**2.0×10^4**	**1.0×10^5**
09.06.88	9	2.0×10^{10}	1.0×10^9	5.0×10^6	5.0×10^9	5.0×10^5	5.2×10^9
10.06.88	10	1.0×10^{10}	3.5×10^{10}	3.0×10^{10}	2.5×10^{10}	4.0×10^{10}	2.8×10^{10}
12.06.88	12	5.0×10^9	5.0×10^7	1.0×10^{10}	2.0×10^{10}	2.5×10^{10}	1.2×10^{10}
14.06.88	14	1.5×10^8	1.0×10^7	1.5×10^6	5.0×10^7	1.5×10^{10}	3.0×10^9
16.06.88	16	5.0×10^6	5.0×10^7	1.5×10^4	1.5×10^4	5.0×10^9	1.0×10^9
18.06.88	18	1.0×10^6	2.5×10^{10}	1.0×10^{10}	1.5×10^5	2.0×10^9	7.4×10^9
20.06.88	**20***	**2.0×10^6**	**1.0×10^9**	**1.0×10^{10}**	**5.0×10^9**	**1.0×10^{10}**	**5.2×10^9**
21.06.88	21	2.5×10^9	2.5×10^{10}	2.5×10^9	1.0×10^{10}	1.5×10^{10}	1.1×10^{10}
23.06.88	23	5.0×10^8	4.0×10^8	5.0×10^9	2.0×10^5	1.5×10^{10}	4.1×10^9
25.06.88	25	5.0×10^5	5.0×10^4	1.5×10^5	2.5×10^4	5.0×10^6	1.1×10^6
27.06.88	27	5.0×10^9	5.0×10^9	5.0×10^3	5.0×10^4	3.5×10^{10}	9.0×10^9
29.06.88	29	1.0×10^5	5.0×10^9	1.0×10^9	5.0×10^9	3.5×10^{10}	9.2×10^9
01.07.88	31	2.0×10^6	5.0×10^6	1.0×10^7	5.0×10^7	5.0×10^{10}	1.0×10^{10}
03.07.88	33	5.0×10^5	5.0×10^7	2.0×10^{10}	5.0×10^9	1.0×10^{10}	7.0×10^9

^ Beginning of treatment, * Withdrawal of treatment

TABLE 5.36 Individual bacterial counts of *Escherichia coli*/g faeces from the control group in Experiment 3

Study							
Date	Day	Pig No. 88	Pig No. 90(L)	Pig No. 92	Pig No. 93	Pig No. 94	$\bar{X}$
31.05.88	0	5.0×10^4	2.0×10^6	3.0×10^6	1.5×10^6	5.0×10^6	2.3×10^6
07.06.88	**7^**	**5.0×10^5**	**5.0×10^5**	**5.0×10^4**	**3.5×10^4**	**2.0×10^5**	**2.5×10^5**
09.06.88	9	2.0×10^8	1.5×10^8	2.0×10^8	2.5×10^7	2.0×10^{10}	4.1×10^9
10.06.88	10	5.0×10^9	1.5×10^{10}	1.0×10^{10}	5.0×10^9	8.0×10^9	8.6×10^9
12.06.88	12	2.5×10^9	5.0×10^9	1.5×10^{10}	5.0×10^9	5.0×10^9	6.5×10^9
14.06.88	14	2.5×10^{10}	1.0×10^{10}	1.0×10^{10}	1.0×10^{10}	2.0×10^7	1.1×10^{10}
16.06.88	16	5.0×10^7	3.5×10^{10}	1.0×10^8	5.0×10^7	2.5×10^8	7.1×10^9
18.06.88	18	5.0×10^9	4.0×10^{10}	5.0×10^6	1.0×10^5	1.5×10^{10}	1.2×10^{10}
20.06.88	**20***	**5.0×10^9**	**5.0×10^8**	**5.0×10^8**	**5.0×10^9**	**1.0×10^7**	**2.2×10^9**
21.06.88	21	2.0×10^{10}	4.5×10^{10}	5.0×10^8	5.0×10^8	5.0×10^9	1.4×10^{10}
23.06.88	23	2.0×10^5	1.5×10^6	5.0×10^8	5.0×10^7	5.0×10^3	1.1×10^8
25.06.88	25	1.0×10^5	3.0×10^5	2.5×10^5	5.0×10^5	2.5×10^{10}	5.0×10^9
27.06.88	27	1.0×10^6	1.5×10^9	1.0×10^{10}	1.0×10^8	1.0×10^7	2.3×10^9
29.06.88	29	1.0×10^{10}	5.0×10^9	2.0×10^{10}	5.0×10^6	1.5×10^{10}	1.0×10^{10}
01.07.88	31	5.0×10^7	1.0×10^{10}	2.0×10^{10}	1.0×10^7	5.0×10^{10}	1.6×10^{10}
03.07.88	33	1.5×10^8	5.0×10^5	5.0×10^5	5.0×10^4	5.0×10^4	3.0×10^7

^ Beginning of treatment, * Withdrawal of treatment

TABLE 5.37 Individual bacterial counts of faecal streptococci/g faeces from the treated group in Experiment 3

Study							
Date	Day	Pig No. 84	Pig No. 86(63)	Pig No. 87	Pig No. 88	Pig No. 90	$\bar{X}$
31.05.88	0	1.0×10^4	5.0×10^2	5.0×10^3	5.0×10^2	N G	4.0×10^3
07.06.88	7ˆ	N G	N G	N G	N G	N G	N G
09.06.88	9	N G	N G	N G	N G	N G	N G
10.06.88	10	N G	2.5×10^3	N G	N G	N G	2.5×10^3
12.06.88	12	N G	1.0×10^3	N G	N G	N G	1.0×10^3
14.06.88	14	N G	N G	N G	N G	N G	N G
16.06.88	16	N G	N G	N G	N G	5.0×10^2	5.0×10^2
18.06.88	18	5.0×10^2	N G	N G	N G	N G	5.0×10^2
20.06.88	20*	N G	N G	N G	N G	N G	N G
21.06.88	21	5.0×10^2	N G	N G	N G	N G	5.0×10^2
23.06.88	23	N G	N G	N G	N G	N G	N G
25.06.88	25	N G	N G	N G	N G	5.0×10^2	5.0×10^2
27.06.88	27	N G	N G	N G	N G	N G	N G
29.06.88	29	N G	N G	N G	N G	N G	N G
01.07.88	31	N G	5.0×10^2	N G	N G	N G	5.0×10^2
03.07.88	33	N G	N G	N G	N G	N G	N G

N G = No Growth

ˆ Beginning of treatment, * Withdrawal of treatment

TABLE 5.38 Individual bacterial counts of faecal streptococci/g faeces from the control group in Experiment 3

Study							
Date	Day	Pig No. 88	Pig No. 90(L)	Pig No. 92	Pig No. 93	Pig No. 94	$\bar{X}$
31.05.88	0	N G	N G	4.5×10^3	N G	N G	4.5×10^3
07.06.88	**7^**	**1.0×10^3**	**5.0×10^2**	**N G**	**N G**	**5.0×10^2**	**6.6×10^2**
09.06.88	9	N G	N G	N G	N G	N G	N G
10.06.88	10	N G	5.0×10^3	N G	N G	2.5×10^3	3.7×10^3
12.06.88	12	N G	1.0×10^3	N G	N G	1.0×10^4	5.5×10^3
14.06.88	14	1.0×10^3	5.0×10^2	5.0×10^3	N G	N G	2.1×10^3
16.06.88	16	N G	N G	N G	N G	5.0×10^2	5.0×10^2
18.06.88	18	N G	N G	N G	N G	N G	N G
20.06.88	**20***	**N G**	**N G**	**N G**	**N G**	**N G**	**N G**
21.06.88	21	N G	N G	N G	2.0×10^3	5.0×10^4	2.6×10^4
23.06.88	23	N G	N G	5.0×10^2	N G	N G	5.0×10^2
25.06.88	25	N G	N G	1.0×10^5	N G	5.0×10^2	5.0×10^4
27.06.88	27	N G	N G	5.0×10^2	N G	N G	5.0×10^2
29.06.88	29	N G	N G	N G	N G	N G	N G
01.07.88	31	1.0×10^3	N G	N G	5.0×10^4	5.0×10^4	3.3×10^4
03.07.88	33	1.0×10^4`	1.5×10^3`	5.0×10^2`	1.0×10^3`	1.5×10^3`	2.9×10^3

N G = No Growth

^ Beginning of treatment, * Withdrawal of treatment

TABLE 5.39 Individual bacterial counts of *Campylobacter spp.*/g faeces from the treated group in Experiment 3

Study							
Date	Day	Pig No. 84	Pig No. 86(63)	Pig No. 87	Pig No. 88	Pig No. 90	$\bar{X}$
31.05.88	0	N G	5.0×10^3	1.0×10^3	2.0×10^3	2.0×10^3	2.5×10^3
07.06.88	**7^**	**4.0×10^3**	**4.5×10^4**	**5.0×10^4**	**1.0×10^5**	**1.0×10^5**	**5.9×10^4**
09.06.88	9	2.0×10^4	1.5×10^4	1.5×10^4	5.0×10^4	1.0×10^5	4.0×10^4
10.06.88	10	5.0×10^3	5.5×10^3	3.0×10^3	5.0×10^3	5.0×10^4	1.3×10^4
12.06.88	12	5.0×10^4	3.0×10^3	4.5×10^3	2.0×10^4	5.0×10^3	1.6×10^4
14.06.88	14	3.0×10^4	3.0×10^4	5.0×10^3	3.5×10^4	3.5×10^3	2.0×10^4
16.06.88	16	2.5×10^4	5.0×10^4	1.0×10^5	1.0×10^4	4.5×10^3	3.7×10^4
18.06.88	18	5.0×10^3	5.0×10^3	1.0×10^4	1.5×10^4	5.0×10^3	8.0×10^3
20.06.88	**20***	**4.0×10^4**	**5.0×10^4**	**1.0×10^4**	**5.0×10^5**	**5.5×10^4**	**1.3×10^5**
21.06.88	21	2.5×10^5	5.0×10^5	5.0×10^4	5.0×10^5	1.5×10^5	2.9×10^5
23.06.88	23	2.0×10^5	2.0×10^5	3.0×10^4	2.0×10^4	1.5×10^4	9.3×10^4
25.06.88	25	5.0×10^4	1.0×10^5	1.0×10^4	1.0×10^5	1.0×10^4	5.4×10^4
27.06.88	27	5.0×10^5	1.0×10^4	11.5×10^3	1.0×10^5	1.0×10^5	1.4×10^5
29.06.88	29	2.5×10^4	5.0×10^4	5.0×10^4	1.5×10^4	1.0×10^5	4.8×10^4
01.07.88	31	5.0×10^3	5.0×10^4	1.0×10^4	5.0×10^4	1.0×10^3	2.3×10^4
03.07.88	33	1.0×10^3	5.0×10^3	N G	2.5×10^4	2.5×10^4	1.4×10^4

N G = No Growth

^ Beginning of treatment, * Withdrawal of treatment

TABLE 5.40 Individual bacterial counts of *Campylobacter spp.*/g faeces from the control group in Experiment 3

Study							
Date	Day	Pig No. 88	Pig No. 90(L)	Pig No. 92	Pig No. 93	Pig No. 94	$\bar{X}$
31.05.88	0	5.0×10^5	1.5×10^4	3.5×10^4	7.0×10^4	5.0×10^4	1.3×10^5
07.06.88	**7^**	**1.0×10^4**	**1.0×10^4**	**5.0×10^2**	**5.0×10^3**	**5.0×10^3**	**6.1×10^3**
09.06.88	9	5.0×10^4	5.0×10^3	2.0×10^3	2.5×10^4	4.5×10^3	1.7×10^4
10.06.88	10	5.0×10^4	1.0×10^4	1.5×10^4	1.0×10^4	1.5×10^4	2.0×10^4
12.06.88	12	2.5×10^5	5.0×10^6	4.0×10^4	1.5×10^5	5.0×10^6	2.0×10^6
14.06.88	14	1.0×10^4	4.5×10^4	1.0×10^4	1.5×10^5	1.0×10^5	6.3×10^4
16.06.88	16	3.5×10^4	5.0×10^6	5.0×10^3	5.0×10^4	2.0×10^4	1.0×10^6
18.06.88	18	5.0×10^4	5.0×10^4	1.0×10^5	1.0×10^4	1.0×10^5	6.2×10^4
20.06.88	**20***	**6.5×10^3**	**5.0×10^3**	**4.0×10^4**	**1.5×10^3**	**3.0×10^4**	**1.6×10^4**
21.06.88	21	1.5×10^4	2.0×10^4	1.5×10^5	2.5×10^4	5.0×10^4	5.2×10^4
23.06.88	23	1.5×10^5	5.5×10^4	5.0×10^4	1.5×10^4	1.0×10^5	7.4×10^4
25.06.88	25	4.0×10^4	3.0×10^3	4.0×10^4	1.0×10^4	4.5×10^4	2.7×10^4
27.06.88	27	1.5×10^4	5.0×10^4	5.0×10^4	1.0×10^5	2.5×10^5	9.3×10^4
29.06.88	29	5.0×10^7	5.0×10^4	1.0×10^7	1.0×10^6	5.0×10^4	1.2×10^7
01.07.88	31	1.0×10^5	1.0×10^4	5.0×10^4	2.0×10^4	1.0×10^5	5.6×10^4
03.07.88	33	1.0×10^4	1.5×10^4	5.0×10^3	1.0×10^3	1.0×10^5	2.6×10^4

^ Beginning of treatment, * Withdrawal of treatment

TABLE 5.41 Individual bacterial counts of lactobacilli/g faeces from the treated group in Experiment 3

Study							
Date	Day	Pig No. 84	Pig No. 86(63)	Pig No. 87	Pig No. 88	Pig No. 90	$\bar{X}$
31.05.88	0	5.0×10^5	5.0×10^4	3.5×10^4	5.0×10^3	2.0×10^5	1.5×10^5
07.06.88	**7^**	**1.5×10^5**	**2.5×10^4**	**5.0×10^4**	**2.5×10^4**	**5.0×10^3**	**5.1×10^4**
09.06.88	9	2.0×10^4	1.0×10^5	5.0×10^4	5.0×10^4	1.5×10^5	7.4×10^4
10.06.88	10	5.0×10^3	5.0×10^4	2.0×10^4	1.0×10^6	3.0×10^7	6.2×10^6
12.06.88	12	3.0×10^5	5.0×10^7	5.0×10^9	1.0×10^{10}	1.5×10^{10}	6.0×10^9
14.06.88	14	1.0×10^7	5.0×10^6	5.0×10^6	1.5×10^5	5.0×10^9	1.0×10^9
16.06.88	16	1.0×10^7	1.0×10^7	1.0×10^3	5.0×10^3	2.5×10^8	5.4×10^7
18.06.88	18	6.0×10^4	2.5×10^{10}	5.0×10^9	5.0×10^4	2.5×10^9	6.5×10^9
20.06.88	**20***	**1.5×10^6**	**1.5×10^8**	**5.0×10^8**	**1.0×10^{10}**	**1.0×10^{10}**	**4.1×10^9**
21.06.88	21	2.0×10^{10}	4.0×10^{10}	3.5×10^{10}	1.0×10^{10}	1.5×10^{10}	2.4×10^{10}
23.06.88	23	5.0×10^7	1.5×10^9	5.0×10^8	5.0×10^6	5.0×10^8	5.1×10^8
25.06.88	25	4.5×10^4	5.0×10^4	5.0×10^9	5.0×10^4	1.5×10^5	1.0×10^9
27.06.88	27	1.5×10^7	5.0×10^9	5.0×10^3	5.0×10^4	5.0×10^8	1.1×10^9
29.06.88	29	1.0×10^7	1.0×10^{10}	5.0×10^5	1.5×10^8	2.5×10^{10}	7.0×10^9
01.07.88	31	1.0×10^6	1.0×10^5	5.0×10^8	5.0×10^9	5.5×10^{10}	1.2×10^{10}
03.07.88	33	5.0×10^6	5.0×10^9	2.0×10^{10}	1.0×10^{10}	1.5×10^8	7.0×10^9

^ Beginning of treatment, * Withdrawal of treatment

TABLE 5.42 Individual bacterial counts of lactobacilli/g faeces from the control group in Experiment 3

Study							
Date	Day	Pig No. 88	Pig No. 90(L)	Pig No. 92	Pig No. 93	Pig No. 94	$\bar{X}$
31.05.88	0	1.0×10^4	5.0×10^3	1.0×10^6	5.0×10^4	5.0×10^4	2.2×10^5
07.06.88	**7^**	**2.0×10^6**	**5.0×10^4**	**1.5×10^4**	**5.0×10^4**	**5.0×10^4**	**4.3×10^5**
09.06.88	9	2.0×10^5	2.0×10^4	3.0×10^4	2.0×10^5	1.0×10^5	1.1×10^5
10.06.88	10	2.0×10^5	1.0×10^5	5.0×10^4	5.0×10^5	1.0×10^{10}	2.0×10^9
12.06.88	12	1.0×10^9	2.0×10^{10}	4.5×10^8	1.5×10^9	1.5×10^8	4.6×10^9
14.06.88	14	2.5×10^{10}	5.0×10^7	1.5×10^{10}	1.0×10^9	1.0×10^5	8.2×10^9
16.06.88	16	3.5×10^4	2.5×10^{10}	1.5×10^5	1.0×10^7	5.0×10^5	5.0×10^9
18.06.88	18	1.0×10^{10}	1.5×10^{10}	5.0×10^7	1.0×10^4	5.0×10^8	5.1×10^9
20.06.88	**20***	**5.0×10^7**	**1.0×10^{10}**	**1.0×10^7**	**2.0×10^9**	**5.0×10^6**	**2.4×10^9**
21.06.88	21	2.5×10^{10}	3.5×10^{10}	1.5×10^8	2.0×10^9	1.5×10^7	1.2×10^{10}
23.06.88	23	2.5×10^5	5.0×10^7	2.0×10^9	5.0×10^8	5.0×10^5	5.1×10^8
25.06.88	25	5.0×10^4	5.0×10^5	1.5×10^5	1.0×10^5	1.0×10^{10}	2.0×10^9
27.06.88	27	4.0×10^4	1.0×10^{10}	1.5×10^9	5.0×10^5	1.0×10^8	2.3×10^9
29.06.88	29	5.0×10^9	5.0×10^7	2.5×10^{10}	1.0×10^5	5.0×10^9	7.0×10^9
01.07.88	31	5.0×10^9	5.0×10^8	1.5×10^{10}	5.0×10^4	1.0×10^{10}	6.1×10^9
03.07.88	33	5.0×10^6	5.0×10^9	1.5×10^6	5.0×10^6	5.0×10^6	1.0×10^9

^ Beginning of treatment, * Withdrawal of treatment

TABLE 5.43 Individual bacterial counts of *Bacteroides spp.*/g faeces from the treated group in Experiment 3

Study							
Date	Day	Pig No. 84	Pig No. 86(63)	Pig No. 87	Pig No. 88	Pig No. 90	$\bar{X}$
31.05.88	0	5.0×10^3	1.0×10^4	3.0×10^3	5.0×10^2	5.0×10^5	1.0×10^5
07.06.88	**7^**	**5.0×10^2**	**1.5×10^4**	**1.0×10^4**	**2.0×10^4**	**2.5×10^3**	**9.6×10^3**
09.06.88	9	N G	N G	N G	N G	N G	N G
10.06.88	10	1.0×10^4	1.0×10^4	1.0×10^4	4.0×10^4	1.0×10^5	3.4×10^4
12.06.88	12	5.0×10^3	5.0×10^4	1.0×10^5	5.0×10^6	2.0×10^4	1.6×10^6
14.06.88	14	2.0×10^3	1.5×10^4	2.0×10^4	1.0×10^3	2.5×10^4	1.2×10^4
16.06.88	16	1.5×10^3	5.0×10^2	1.5×10^3	5.0×10^2	1.0×10^3	1.0×10^3
18.06.88	18	5.0×10^2	5.0×10^3	2.5×10^3	1.0×10^4	N G	4.5×10^3
20.06.88	**20***	**2.5×10^3**	**5.0×10^3**	**4.0×10^4**	**5.0×10^3**	**5.0×10^3**	**1.1×10^4**
21.06.88	21	N G	1.0×10^3	7.0×10^3	1.0×10^5	1.0×10^4	2.9×10^4
23.06.88	23	5.0×10^3	5.0×10^5	5.0×10^4	5.0×10^3	5.0×10^3	1.1×10^5
25.06.88	25	2.5×10^3	1.0×10^4	2.5×10^4	2.5×10^3	5.0×10^3	9.0×10^3
27.06.88	27	4.5×10^3	6.5×10^3	1.0×10^4	6.5×10^3	N G	6.8×10^3
29.06.88	29	1.5×10^5	5.0×10^9	5.0×10^4	5.0×10^4	5.0×10^5	1.0×10^9
01.07.88	31	4.5×10^3	5.0×10^2	1.5×10^4	2.0×10^4	1.0×10^4	1.0×10^4
03.07.88	33	1.0×10^5	1.5×10^4	5.0×10^4	1.0×10^8	5.0×10^4	2.0×10^7

N G = No Growth

^ Beginning of treatment, * Withdrawal of treatment

TABLE 5.44 Individual bacterial counts of Bacteroides spp./g faeces from the control group in Experiment 3

Study							
Date	Day	Pig No. 88	Pig No. 90(L)	Pig No. 92	Pig No. 93	Pig No. 94	$\bar{X}$
31.05.88	0	1.0×10^5	5.0×10^2	5.0×10^3	1.5×10^3	5.0×10^2	2.1×10^4
07.06.88	**7^**	**2.5×10^4**	**5.0×10^3**	**1.0×10^3**	**2.5×10^3**	**5.0×10^4**	**1.6×10^4**
09.06.88	9	N G	N G	N G	5.0×10^3	5.0×10^2	2.7×10^3
10.06.88	10	3.0×10^4	2.0×10^3	1.5×10^4	5.0×10^4	5.0×10^4	2.9×10^4
12.06.88	12	5.0×10^3	5.0×10^4	1.0×10^9	5.0×10^3	5.0×10^3	2.0×10^8
14.06.88	14	2.5×10^4	1.0×10^4	5.0×10^7	5.0×10^3	4.0×10^3	1.0×10^7
16.06.88	16	2.0×10^5	2.0×10^4	2.0×10^4	5.0×10^3	5.0×10^4	5.9×10^4
18.06.88	18	1.5×10^4	5.0×10^3	1.5×10^4	1.0×10^3	5.0×10^3	8.2×10^3
20.06.88	**20***	**4.0×10^3**	**2.5×10^3**	**3.0×10^3**	**3.0×10^4**	**5.5×10^3**	**9.0×10^3**
21.06.88	21	1.0×10^4	1.5×10^3	5.0×10^9	1.5×10^5	1.5×10^4	1.0×10^9
23.06.88	23	2.0×10^3	2.5×10^3	5.0×10^4	5.0×10^3	6.0×10^3	1.3×10^4
25.06.88	25	4.0×10^4	5.0×10^4	5.0×10^4	5.0×10^4	6.5×10^4	5.1×10^4
27.06.88	27	3.5×10^3	5.0×10^4	3.0×10^3	5.0×10^4	1.0×10^4	2.3×10^4
29.06.88	29	1.0×10^6	5.0×10^5	5.0×10^9	1.0×10^5	5.0×10^5	1.0×10^9
01.07.88	31	5.5×10^3	5.0×10^3	5.0×10^4	5.0×10^3	5.0×10^6	1.0×10^6
03.07.88	33	1.0×10^4	1.0×10^5	3.0×10^4	2.0×10^4	5.0×10^4	4.2×10^4

N G = No Growth

^ Beginning of treatment, * Withdrawal of treatment

TABLE 5.45 Individual bacterial counts of Clostridium perfringens type A (vegetative cells)/g faeces from the treated group in Experiment 3

Study							
Date	Day	Pig No. 84	Pig No. 86(63)	Pig No. 87	Pig No. 88	Pig No. 90	$\bar{X}$
31.05.88	0	5.0×10^4	5.0×10^4	5.0×10^2	2.0×10^4	5.0×10^5	1.2×10^5
07.06.88	**7^**	**5.0×10^2**	**5.0×10^3**	**1.0×10^5**	**1.5×10^5**	**2.5×10^3**	**5.1×10^4**
09.06.88	9	N G	5.0×10^3	5.0×10^4	5.0×10^6	N G	1.6×10^6
10.06.88	10	1.0×10^4	5.0×10^3	1.0×10^4	5.0×10^6	1.5×10^7	4.0×10^6
12.06.88	12	5.0×10^4	5.0×10^5	5.0×10^4	1.0×10^6	5.0×10^3	3.2×10^5
14.06.88	14	2.0×10^3	5.0×10^2	2.0×10^3	2.0×10^4	N G	6.1×10^3
16.06.88	16	1.0×10^5	5.0×10^4	1.0×10^3	5.0×10^3	5.0×10^6	1.0×10^6
18.06.88	18	5.0×10^3	5.0×10^4	5.0×10^3	5.0×10^4	1.0×10^3	2.2×10^4
20.06.88	**20***	**3.5×10^3**	**1.0×10^3**	**1.5×10^3**	**1.0×10^4**	**4.5×10^3**	**4.1×10^3**
21.06.88	21	1.0×10^5	1.0×10^5	5.0×10^7	5.0×10^7	1.0×10^6	2.0×10^7
23.06.88	23	1.5×10^3	5.0×10^4	5.0×10^6	5.0×10^4	5.0×10^3	1.0×10^6
25.06.88	25	1.0×10^3	2.0×10^3	5.0×10^3	1.5×10^3	1.0×10^4	3.9×10^3
27.06.88	27	5.0×10^4	5.0×10^7	N G	5.0×10^2	5.0×10^8	1.1×10^7
29.06.88	29	5.0×10^3	1.0×10^4	1.5×10^4	5.0×10^3	2.0×10^3	7.4×10^3
01.07.88	31	1.5×10^3	1.0×10^3	3.5×10^3	5.0×10^3	2.0×10^3	2.6×10^3
03.07.88	33	4.5×10^3	N G	5.0×10^5	5.0×10^4	5.0×10^6	1.3×10^6

N G = No Growth

^ Beginning of treatment, * Withdrawal of treatment

TABLE 5.46 Individual bacterial counts of Clostridium perfringens type A (vegetative cells)/g faeces from the control group in Experiment 3

Study							
Date	Day	Pig No. 88	Pig No. 90(L)	Pig No. 92	Pig No. 93	Pig No. 94	$\bar{X}$
31.05.88	0	1.0×10^4	5.0×10^4	5.0×10^3	1.0×10^5	2.0×10^4	3.7×10^4
07.06.88	**7^**	**3.5×10^5**	**1.0×10^6**	**5.0×10^4**	**1.0×10^5**	**2.0×10^4**	**3.0×10^5**
09.06.88	9	1.0×10^7	5.0×10^3	5.0×10^4	1.0×10^5	5.0×10^4	2.0×10^6
10.06.88	10	1.0×10^6	5.0×10^4	5.0×10^7	5.0×10^5	5.0×10^6	1.1×10^7
12.06.88	12	1.0×10^7	5.0×10^4	5.0×10^3	5.0×10^3	4.5×10^3	2.0×10^6
14.06.88	14	5.0×10^9	5.0×10^4	5.0×10^5	5.0×10^3	1.0×10^4	1.0×10^9
16.06.88	16	5.0×10^3	5.0×10^5	2.5×10^4	1.0×10^4	1.5×10^4	1.1×10^5
18.06.88	18	5.0×10^4	1.0×10^4	5.0×10^5	1.0×10^3	5.0×10^3	1.1×10^5
20.06.88	**20***	**1.0×10^5**	**5.0×10^4**	**5.0×10^4**	**1.0×10^4**	**3.0×10^3**	**4.2×10^4**
21.06.88	21	5.0×10^6	5.0×10^7	5.0×10^7	1.0×10^4	1.5×10^4	2.1×10^7
23.06.88	23	5.0×10^3	1.5×10^3	5.0×10^3	2.5×10^3	5.0×10^2	2.9×10^3
25.06.88	25	5.0×10^4	1.0×10^4	5.0×10^3	5.0×10^3	N G	1.7×10^4
27.06.88	27	5.0×10^5	5.0×10^6	5.0×10^9	5.0×10^4	5.0×10^4	1.0×10^9
29.06.88	29	5.0×10^3	3.0×10^3	1.0×10^5	1.0×10^4	1.0×10^4	2.5×10^4
01.07.88	31	1.0×10^3	N G	5.0×10^4	1.5×10^3	5.0×10^3	1.4×10^4
03.07.88	33	1.5×10^5	1.0×10^7	5.0×10^2	1.0×10^3	3.0×10^3	2.0×10^6

N G = No Growth

^ Beginning of treatment, * Withdrawal of treatment

TABLE 5.47 Individual bacterial counts of *Clostridium perfringens* type A (spores)/g faeces from the treated group in Experiment 3

Study							
Date	Day	Pig No. 84	Pig No. 86(63)	Pig No. 87	Pig No. 88	Pig No. 90	$\bar{X}$
31.05.88	0	N G	N G	N G	N G	N G	N G
07.06.88	**7^**	**N G**	**N G**	**N G**	**N G**	**N G**	**N G**
09.06.88	9	N G	N G	N G	N G	N G	N G
10.06.88	10	N G	N G	N G	N G	N G	N G
12.06.88	12	N G	N G	N G	N G	N G	N G
14.06.88	14	N G	5.0×10^2	N G	N G	N G	5.0×10^2
16.06.88	16	N G	N G	1.0×10^3	N G	N G	1.0×10^3
18.06.88	18	N G	N G	N G	N G	N G	N G
20.06.88	**20***	**N G**	**N G**	**N G**	**N G**	**N G**	**N G**
21.06.88	21	5.0×10^2	N G	N G	N G	N G	5.0×10^2
23.06.88	23	N G	N G	N G	N G	N G	N G
25.06.88	25	N G	N G	N G	N G	N G	N G
27.06.88	27	5.0×10^2	N G	N G	N G	N G	5.0×10^2
29.06.88	29	N G	N G	N G	N G	N G	N G
01.07.88	31	N G	N G	N G	N G	N G	N G
03.07.88	33	N G	N G	N G	N G	N G	N G

N G = No Growth

^ Beginning of treatment, * Withdrawal of treatment

TABLE 5.48 Individual bacterial counts of *Clostridium perfringens* type A (spores)/g faeces from the control group in Experiment 3

Study							
Date	Day	Pig No. 88	Pig No. 90(L)	Pig No. 92	Pig No. 93	Pig No. 94	$\bar{X}$
31.05.88	0	N G	N G	N G	N G	N G	N G
07.06.88	**7^**	**N G**	**N G**	**N G**	**N G**	**N G**	**N G**
09.06.88	9	N G	N G	5.0×10^2	N G	N G	5.0×10^2
10.06.88	10	5.0×10^2	N G	N G	N G	N G	5.0×10^2
12.06.88	12	N G	N G	N G	N G	N G	N G
14.06.88	14	N G	5.0×10^2	N G	N G	N G	5.0×10^2
16.06.88	16	N G	N G	N G	N G	N G	N G
18.06.88	18	5.0×10^2	N G	N G	N G	N G	5.0×10^2
20.06.88	**20***	**5.0×10^2**	**N G**	**N G**	**N G**	**N G**	**5.0×10^2**
21.06.88	21	5.0×10^2	N G	N G	N G	N G	5.0×10^2
23.06.88	23	1.0×10^3	N G	N G	N G	N G	1.0×10^3
25.06.88	25	5.0×10^2	N G	N G	N G	N G	5.0×10^2
27.06.88	27	5.0×10^2	N G	N G	N G	N G	5.0×10^2
29.06.88	29	N G	N G	N G	N G	N G	N G
01.07.88	31	N G	N G	N G	N G	N G	N G
03.07.88	33	N G	N G	N G	N G	N G	N G

N G = No Growth

^ Beginning of treatment, * Withdrawal of treatment

TABLE 6.11 Individual bacterial counts of total coliforms/g faeces from the treated group in Experiment 4

Study Date	Day	Pig No. 1	Pig No. 2	Pig No. 4	Pig No. 6	Pig No. 9	$\bar{X}$
25.10.88	0	2.5×10^6	1.0×10^6	3.0×10^{10}	5.0×10^5	5.0×10^6	6.0×10^9
01.11.88	**7^**	**5.0×10^5**	**4.0×10^{10}**	**1.5×10^6**	**1.0×10^{10}**	**3.0×10^{10}**	**1.6×10^{10}**
03.11.88	9	1.5×10^4	5.0×10^5	2.5×10^5	1.0×10^6	2.5×10^4	3.5×10^5
05.11.88	11	5.0×10^5	1.5×10^7	1.5×10^5	1.0×10^5	2.5×10^6	3.6×10^6
07.11.88	13	5.0×10^3	1.5×10^9	1.0×10^{10}	2.0×10^9	6.0×10^4	2.7×10^9
09.11.88	15	5.0×10^6	1.0×10^6	1.0×10^{10}	1.5×10^5	2.5×10^6	2.0×10^9
11.11.88	17	3.0×10^7	6.0×10^8	4.0×10^7	2.0×10^5	5.0×10^6	1.3×10^8
13.11.88	19	1.0×10^8	7.5×10^{10}	5.0×10^5	5.0×10^5	2.5×10^7	1.5×10^{10}
15.11.88	**21***	**5.0×10^5**	**2.0×10^6**	**5.0×10^9**	**2.0×10^6**	**1.0×10^9**	**1.2×10^8**
17.11.88	23	5.0×10^4	1.5×10^5	1.0×10^8	5.0×10^6	1.0×10^6	2.1×10^7
19.11.88	25	5.0×10^8	2.0×10^5	5.0×10^9	1.0×10^4	5.0×10^8	1.1×10^9
21.11.88	27	5.0×10^6	1.5×10^7	5.0×10^4	2.0×10^4	2.0×10^7	8.0×10^8
23.11.88	29	2.5×10^6	5.5×10^{10}	5.0×10^6	5.0×10^5	1.0×10^8	1.1×10^{10}
25.11.88	31	5.0×10^8	1.0×10^{10}	5.0×10^4	7.5×10^{10}	5.0×10^9	1.8×10^{10}
27.11.88	33	5.0×10^7	1.0×10^9	1.5×10^{10}	4.0×10^{10}	5.0×10^5	1.1×10^{10}
29.11.88	35	3.0×10^4	4.5×10^{10}	5.0×10^9	1.5×10^{10}	5.0×10^8	1.3×10^{10}

^ Beginning of treatment, * Withdrawal of treatment

TABLE 6.12 Individual bacterial counts of total coliforms/g faeces from the control group in Experiment 4

Study							
Date	Day	Pig No. 71	Pig No. 73	Pig No. 74	Pig No. 77	Pig No. 79	$\bar{X}$
25.10.88	0	4.0×10^5	5.0×10^5	1.5×10^6	1.0×10^{11}	1.5×10^7	2.1×10^9
01.11.88	**7^**	**1.5×10^7**	**3.5×10^{10}**	**5.0×10^7**	**5.5×10^{10}**	**1.0×10^6**	**1.8×10^{10}**
03.11.88	9	5.0×10^4	5.0×10^5	2.0×10^5	5.0×10^9	3.0×10^6	1.0×10^9
05.11.88	11	4.0×10^4	1.0×10^5	5.0×10^5	5.0×10^5	5.0×10^5	3.3×10^5
07.11.88	13	3.5×10^4	5.0×10^4	2.0×10^5	5.0×10^8	5.0×10^6	1.0×10^8
09.11.88	15	1.0×10^5	3.5×10^5	2.0×10^6	2.0×10^{10}	7.5×10^4	4.0×10^9
11.11.88	17	5.0×10^5	2.5×10^6	3.0×10^6	4.0×10^{10}	2.0×10^5	8.0×10^9
13.11.88	19	5.0×10^6	5.0×10^8	5.0×10^6	8.0×10^{10}	5.0×10^6	1.6×10^{10}
15.11.88	**21***	**1.0×10^6**	**4.5×10^6**	**1.5×10^4**	**1.0×10^8**	**3.5×10^{10}**	**7.0×10^9**
17.11.88	23	1.0×10^6	5.0×10^5	1.5×10^{10}	2.5×10^5	5.5×10^{10}	1.4×10^{10}
19.11.88	25	5.0×10^8	1.0×10^8	5.0×10^5	1.5×10^5	1.0×10^{10}	2.1×10^9
21.11.88	27	1.5×10^{10}	1.5×10^5	6.0×10^{10}	5.0×10^5	5.0×10^7	1.5×10^{10}
23.11.88	29	1.5×10^{10}	5.0×10^6	5.0×10^5	1.5×10^6	1.0×10^{10}	5.0×10^9
25.11.88	31	5.0×10^7	1.5×10^9	5.0×10^7	2.0×10^8	1.0×10^7	3.6×10^8
27.11.88	33	5.0×10^9	5.0×10^9	6.0×10^{10}	1.5×10^9	5.0×10^{10}	2.4×10^{10}
29.11.88	35	5.0×10^6	2.5×10^8	2.5×10^{10}	2.5×10^{10}	5.0×10^7	1.0×10^{10}

^ Beginning of treatment, * Withdrawal of treatment

TABLE 6.13 Individual bacterial counts of *Escherichia coli*/g faeces from the treated group in Experiment 4

Study							
Date	Day	Pig No. 1	Pig No. 2	Pig No. 4	Pig No. 6	Pig No. 9	$\bar{X}$
25.10.88	0	1.0×10^6	5.0×10^5	5.0×10^9	5.0×10^5	5.0×10^6	1.0×10^9
01.11.88	**7^**	**1.0×10^5**	**2.5×10^{10}**	**5.0×10^7**	**5.0×10^8**	**4.0×10^{10}**	**1.3×10^{10}**
03.11.88	9	1.5×10^6	5.0×10^9	1.0×10^7	1.0×10^6	3.5×10^5	1.0×10^9
05.11.88	11	3.5×10^5	1.0×10^{10}	1.0×10^5	1.0×10^7	5.0×10^7	2.0×10^9
07.11.88	13	5.0×10^5	5.0×10^9	1.0×10^8	1.5×10^{10}	1.0×10^6	4.0×10^9
09.11.88	15	5.0×10^6	2.5×10^7	1.5×10^7	1.0×10^6	1.0×10^7	1.1×10^7
11.11.88	17	3.5×10^7	5.0×10^8	4.0×10^6	5.0×10^6	2.0×10^7	1.1×10^8
13.11.88	19	1.5×10^8	2.5×10^{10}	5.0×10^5	1.0×10^7	5.0×10^9	6.0×10^9
15.11.88	**21***	**4.0×10^6**	**2.0×10^6**	**5.0×10^9**	**2.5×10^8**	**1.0×10^{10}**	**3.0×10^9**
17.11.88	23	1.0×10^7	5.0×10^6	1.0×10^8	5.0×10^8	2.5×10^6	1.2×10^8
19.11.88	25	2.0×10^{10}	1.5×10^6	5.0×10^9	5.0×10^4	1.0×10^{10}	7.0×10^9
21.11.88	27	5.0×10^7	5.0×10^7	5.0×10^4	1.0×10^7	1.0×10^{10}	2.0×10^9
23.11.88	29	5.0×10^6	2.0×10^{11}	5.0×10^6	5.0×10^7	1.5×10^9	2.4×10^{10}
25.11.88	31	5.0×10^8	7.0×10^{10}	5.0×10^4	8.5×10^{10}	1.0×10^9	3.1×10^{10}
27.11.88	33	1.0×10^{10}	2.5×10^{10}	3.0×10^{10}	6.5×10^{10}	1.0×10^7	2.6×10^{10}
29.11.88	35	1.0×10^6	6.5×10^{11}	1.0×10^{10}	1.0×10^9	1.0×10^{10}	3.7×10^{10}

^ Beginning of treatment, * Withdrawal of treatment

TABLE 6.14 Individual bacterial counts of *Escherichia coli*/g faeces from the control group in Experiment 4

Study							
Date	Day	Pig No. 71	Pig No. 73	Pig No. 74	Pig No. 77	Pig No. 79	$\bar{X}$
25.10.88	0	4.0×10^5	2.0×10^6	1.0×10^5	4.5×10^{10}	1.0×10^8	9.0×10^9
01.11.88	**7^**	**3.0×10^7**	**2.5×10^{11}**	**1.0×10^6**	**5.0×10^{10}**	**5.0×10^6**	**3.5×10^{10}**
03.11.88	9	5.0×10^4	1.5×10^5	5.0×10^5	1.5×10^{10}	5.0×10^6	3.0×10^9
05.11.88	11	4.5×10^4	5.0×10^5	5.0×10^5	1.5×10^6	1.5×10^6	8.1×10^5
07.11.88	13	5.0×10^4	1.5×10^5	5.0×10^4	5.0×10^9	1.0×10^7	1.0×10^9
09.11.88	15	5.0×10^5	2.5×10^5	5.0×10^8	1.0×10^8	5.0×10^4	1.2×10^8
11.11.88	17	3.0×10^6	2.0×10^7	5.0×10^7	3.5×10^9	4.0×10^5	7.1×10^8
13.11.88	19	1.0×10^7	1.0×10^8	5.0×10^6	6.5×10^{10}	1.0×10^6	1.3×10^{10}
15.11.88	**21***	**5.0×10^6**	**5.0×10^7**	**5.0×10^4**	**5.0×10^7**	**6.5×10^{10}**	**1.3×10^{10}**
17.11.88	23	8.0×10^4	2.0×10^5	1.5×10^{10}	5.0×10^6	9.0×10^{10}	2.1×10^{10}
19.11.88	25	1.0×10^{10}	3.0×10^{10}	1.0×10^7	1.0×10^5	2.0×10^{10}	1.2×10^{10}
21.11.88	27	2.0×10^{10}	5.0×10^6	1.0×10^{11}	1.0×10^7	5.0×10^6	6.2×10^9
23.11.88	29	3.5×10^{10}	3.5×10^6	5.0×10^6	5.0×10^8	5.0×10^9	8.1×10^9
25.11.88	31	1.0×10^9	5.0×10^7	5.0×10^7	5.0×10^7	5.0×10^6	2.3×10^8
27.11.88	33	3.5×10^{10}	1.5×10^{10}	3.0×10^{11}	1.5×10^{10}	5.0×10^{10}	4.9×10^{10}
29.11.88	35	1.5×10^6	1.0×10^{10}	3.0×10^{10}	5.0×10^9	1.0×10^{10}	1.1×10^{10}

^ Beginning of treatment, * Withdrawal of treatment

TABLE 6.16 Individual bacterial counts of faecal streptococci/g faeces from the treated group in Experiment 4

Study							
Date	Day	Pig No. 1	Pig No. 2	Pig No. 4	Pig No. 6	Pig No. 9	$\bar{X}$
25.10.88	0	1.5×10^3	5.0×10^2	5.0×10^7	5.0×10^4	3.0×10^3	1.0×10^7
01.11.88	**7^**	**5.0×10^4**	**5.0×10^9**	**5.0×10^4**	**5.0×10^8**	**1.0×10^{10}**	**3.1×10^9**
03.11.88	9	2.0×10^4	5.0×10^8	3.0×10^5	5.0×10^4	3.0×10^4	1.0×10^8
05.11.88	11	2.5×10^4	5.0×10^6	2.0×10^4	5.0×10^5	5.0×10^6	2.1×10^6
07.11.88	13	1.5×10^5	1.0×10^9	5.0×10^9	1.5×10^{10}	5.5×10^4	4.2×10^9
09.11.88	15	3.0×10^4	2.0×10^5	1.5×10^6	5.0×10^4	5.0×10^5	4.5×10^5
11.11.88	17	2.0×10^5	4.5×10^7	5.0×10^5	1.0×10^5	2.5×10^5	9.2×10^6
13.11.88	19	5.0×10^6	2.5×10^{10}	3.0×10^4	5.0×10^5	5.0×10^6	5.0×10^9
15.11.88	**21***	**5.0×10^5**	**1.0×10^6**	**1.0×10^7**	**1.0×10^6**	**5.0×10^7**	**1.2×10^7**
17.11.88	23	5.0×10^4	5.0×10^2	5.0×10^6	1.0×10^7	1.0×10^6	3.2×10^6
19.11.88	25	1.0×10^5	5.0×10^5	1.0×10^5	1.0×10^4	1.0×10^7	2.1×10^6
21.11.88	27	2.0×10^7	5.0×10^8	4.5×10^3	1.5×10^4	1.0×10^7	1.0×10^8
23.11.88	29	5.0×10^6	2.0×10^{10}	5.0×10^5	5.0×10^4	1.5×10^8	4.0×10^9
25.11.88	31	6.5×10^4	2.0×10^5	5.0×10^3	5.0×10^9	5.0×10^8	1.1×10^9
27.11.88	33	2.5×10^3	1.5×10^6	1.0×10^{10}	2.0×10^{10}	3.0×10^5	6.0×10^9
29.11.88	35	5.0×10^4	2.5×10^{10}	5.0×10^7	1.0×10^{10}	1.5×10^{10}	1.0×10^{10}

^ Beginning of treatment, * Withdrawal of treatment

TABLE 6.17 Individual bacterial counts of faecal streptococci/g faeces from the control group in Experiment 4

Study							
Date	Day	Pig No. 71	Pig No. 73	Pig No. 74	Pig No. 77	Pig No. 79	$\bar{X}$
25.10.88	0	3.0×10^3	N G	2.5×10^4	2.5×10^3	2.0×10^3	8.1×10^3
01.11.88	**7^**	**3.0×10^4**	**2.5×10^3**	**1.5×10^4**	**1.0×10^5**	**5.0×10^3**	**3.0×10^4**
03.11.88	9	5.0×10^4	1.0×10^5	1.5×10^3	1.0×10^5	5.0×10^5	1.5×10^5
05.11.88	11	5.0×10^4	5.0×10^6	5.0×10^4	1.5×10^5	5.0×10^5	1.1×10^6
07.11.88	13	1.5×10^4	2.0×10^4	2.0×10^5	1.0×10^7	5.0×10^6	3.0×10^6
09.11.88	15	5.0×10^3	1.0×10^5	3.5×10^5	5.0×10^6	5.0×10^4	1.1×10^6
11.11.88	17	2.0×10^5	5.0×10^7	4.0×10^6	7.0×10^8	3.5×10^5	1.5×10^8
13.11.88	19	5.0×10^7	1.0×10^{10}	5.0×10^8	3.0×10^{10}	1.0×10^7	8.1×10^9
15.11.88	**21***	**2.5×10^4**	**2.0×10^6**	**5.0×10^3**	**3.0×10^6**	**3.0×10^{10}**	**6.0×10^9**
17.11.88	23	1.5×10^5	5.0×10^4	1.0×10^7	1.5×10^5	5.0×10^9	1.0×10^{10}
19.11.88	25	1.0×10^8	1.5×10^5	1.5×10^4	2.5×10^4	5.0×10^5	2.0×10^7
21.11.88	27	5.0×10^9	5.0×10^5	1.0×10^{10}	5.0×10^5	5.0×10^6	3.0×10^9
23.11.88	29	5.0×10^9	1.0×10^8	5.0×10^6	1.5×10^5	5.5×10^5	1.0×10^9
25.11.88	31	3.0×10^5	4.0×10^4	5.0×10^4	1.0×10^6	1.5×10^8	3.0×10^7
27.11.88	33	1.5×10^{10}	1.0×10^{10}	4.0×10^{10}	1.0×10^{10}	5.0×10^9	1.6×10^{10}
29.11.88	35	5.0×10^5	1.0×10^8	1.5×10^{10}	5.0×10^9	2.5×10^5	4.0×10^9

N G = No Growth

^ Beginning of treatment, * Withdrawal of treatment

TABLE 6.18 Individual bacterial counts of Campylobacter spp./g faeces from the treated group in Experiment 4

Study							
Date	Day	Pig No. 1	Pig No. 2	Pig No. 4	Pig No. 6	Pig No. 9	$\bar{X}$
25.10.88	0	5.0×10^3	5.0×10^4	5.0×10^4	6.0×10^4	5.0×10^3	3.4×10^4
01.11.88	**7^**	**3.5×10^4**	**4.0×10^4**	**1.0×10^5**	**5.0×10^5**	**5.0×10^4**	**1.4×10^5**
03.11.88	9	1.0×10^4	2.5×10^4	5.0×10^4	3.0×10^5	1.5×10^4	8.0×10^4
05.11.88	11	1.0×10^5	3.0×10^5	1.0×10^6	5.0×10^5	5.5×10^4	4.9×10^5
07.11.88	13	2.5×10^5	5.0×10^4	5.0×10^6	2.0×10^6	1.5×10^5	1.4×10^6
09.11.88	15	3.0×10^4	2.0×10^5	1.5×10^6	5.0×10^4	5.0×10^5	4.5×10^5
11.11.88	17	5.0×10^4	3.5×10^4	2.0×10^6	5.0×10^5	1.0×10^5	5.3×10^5
13.11.88	19	1.0×10^4	5.0×10^3	1.0×10^5	1.0×10^5	5.0×10^4	5.3×10^4
15.11.88	**21***	**1.5×10^4**	**5.0×10^5**	**3.5×10^5**	**1.0×10^6**	**5.0×10^4**	**3.8×10^5**
17.11.88	23	1.0×10^4	5.0×10^4	3.0×10^5	2.5×10^5	5.0×10^4	9.8×10^4
19.11.88	25	1.5×10^5	5.0×10^4	1.5×10^6	1.0×10^7	5.0×10^5	2.4×10^6
21.11.88	27	1.5×10^4	5.0×10^4	2.5×10^5	5.0×10^5	1.0×10^5	1.8×10^5
23.11.88	29	1.5×10^4	1.0×10^7	2.0×10^6	1.0×10^6	5.0×10^5	2.7×10^6
25.11.88	31	4.0×10^3	5.0×10^5	1.0×10^5	5.0×10^4	5.0×10^4	1.4×10^5
27.11.88	33	5.0×10^3	1.0×10^5	5.0×10^4	5.0×10^5	2.0×10^5	1.7×10^5
29.11.88	35	5.0×10^3	5.0×10^5	5.0×10^4	5.0×10^4	5.0×10^4	1.3×10^5

^ Beginning of treatment, * Withdrawal of treatment

TABLE 6.19 Individual bacterial counts of *Campylobacter spp.*/g faeces from the control group in Experiment 4

Study							
Date	Day	Pig No. 71	Pig No. 73	Pig No. 74	Pig No. 77	Pig No. 79	$\bar{X}$
25.10.88	0	5.0×10^5	5.0×10^4	1.0×10^4	5.0×10^7	2.0×10^4	1.0×10^7
01.11.88	**7^**	**5.0×10^6**	**5.0×10^4**	**5.0×10^4**	**5.0×10^4**	**6.0×10^4**	**1.0×10^6**
03.11.88	9	2.5×10^4	1.0×10^5	2.5×10^4	1.5×10^4	5.0×10^3	3.4×10^4
05.11.88	11	5.0×10^4	5.0×10^4	3.0×10^4	5.0×10^3	5.0×10^3	2.8×10^4
07.11.88	13	5.0×10^4	2.0×10^5	5.0×10^4	2.0×10^4	3.5×10^4	7.1×10^4
09.11.88	15	5.0×10^4	1.0×10^4	2.0×10^4	5.0×10^3	2.5×10^4	2.0×10^4
11.11.88	17	5.0×10^5	2.0×10^4	5.0×10^4	2.5×10^3	3.0×10^4	1.2×10^5
13.11.88	19	2.5×10^5	3.5×10^4	5.0×10^3	5.0×10^3	1.5×10^4	6.2×10^4
15.11.88	**21***	**5.5×10^5**	**1.0×10^5**	**4.5×10^4**	**1.5×10^5**	**1.0×10^5**	**1.8×10^5**
17.11.88	23	3.0×10^5	5.0×10^3	5.0×10^5	5.0×10^3	5.0×10^5	2.6×10^5
19.11.88	25	5.0×10^6	1.5×10^6	9.0×10^5	5.0×10^6	5.0×10^6	3.4×10^6
21.11.88	27	1.5×10^5	5.0×10^4	5.0×10^5	5.0×10^4	3.0×10^3	1.5×10^5
23.11.88	29	1.0×10^5	1.5×10^4	2.5×10^4	1.5×10^4	1.0×10^5	5.1×10^4
25.11.88	31	5.0×10^4	3.0×10^3	1.0×10^5	5.0×10^3	1.0×10^4	3.3×10^4
27.11.88	33	5.0×10^8	1.0×10^9	2.5×10^5	5.0×10^5	5.0×10^4	3.0×10^8
29.11.88	35	1.5×10^5	5.0×10^4	3.0×10^5	1.5×10^5	3.0×10^4	1.3×10^5

^ Beginning of treatment, * Withdrawal of treatment

TABLE 6.20 Individual bacterial counts of lactobacilli/g faeces from the treated group in Experiment 4

Study Date	Day	Pig No. 1	Pig No. 2	Pig No. 4	Pig No. 6	Pig No. 9	$\bar{X}$
25.10.88	0	3.0×10^5	2.5×10^4	1.5×10^{10}	5.0×10^4	5.0×10^5	3.0×10^9
01.11.88	**7^**	**1.5×10^6**	**6.5×10^{10}**	**5.0×10^7**	**5.0×10^9**	2.5×10^{10}	**1.9×10^{10}**
03.11.88	9	5.0×10^9	2.0×10^8	2.0×10^7	1.0×10^6	6.5×10^4	1.0×10^9
05.11.88	11	1.0×10^6	1.5×10^8	5.0×10^7	1.0×10^6	5.0×10^7	5.0×10^7
07.11.88	13	1.0×10^5	2.5×10^{10}	5.0×10^{10}	4.0×10^{10}	5.0×10^5	2.3×10^{10}
09.11.88	15	6.0×10^4	2.0×10^4	2.0×10^5	2.5×10^4	5.0×10^3	6.2×10^4
11.11.88	17	3.0×10^5	2.5×10^7	5.0×10^5	4.5×10^6	3.5×10^6	6.7×10^6
13.11.88	19	5.0×10^6	6.5×10^{10}	1.0×10^5	1.5×10^6	5.0×10^8	1.3×10^{10}
15.11.88	**21***	**5.0×10^9**	**5.0×10^7**	**5.0×10^8**	**2.5×10^{10}**	**1.0×10^8**	**6.1×10^9**
17.11.88	23	1.0×10^6	5.0×10^5	1.5×10^{10}	1.5×10^8	3.0×10^6	3.0×10^9
19.11.88	25	2.5×10^{10}	1.0×10^9	5.0×10^9	1.0×10^5	2.0×10^{10}	1.0×10^{10}
21.11.88	27	1.5×10^8	3.0×10^8	5.0×10^5	1.0×10^5	2.0×10^8	1.3×10^8
23.11.88	29	1.0×10^7	1.5×10^8	1.0×10^8	2.0×10^5	5.0×10^8	1.5×10^8
25.11.88	31	5.0×10^8	3.5×10^{10}	5.0×10^5	3.0×10^{10}	1.0×10^9	1.3×10^{10}
27.11.88	33	1.0×10^9	1.5×10^9	2.0×10^{10}	3.5×10^{10}	1.0×10^7	1.1×10^{10}
29.11.88	35	5.0×10^5	3.5×10^{10}	1.5×10^9	1.0×10^9	5.0×10^9	8.5×10^9

^ Beginning of treatment, * Withdrawal of treatment

TABLE 6.21 Individual bacterial counts of lactobacilli/g faeces from the control group in Experiment 4

Study							
Date	Day	Pig No. 71	Pig No. 73	Pig No. 74	Pig No. 77	Pig No. 79	$\bar{X}$
25.10.88	0	1.0×10^{6}	5.0×10^{5}	5.0×10^{4}	3.5×10^{10}	5.0×10^{5}	7.0×10^{9}
01.11.88	**7^**	**5.0×10^{6}**	**3.0×10^{10}**	**2.0×10^{8}**	**7.5×10^{10}**	**5.0×10^{7}**	**2.1×10^{10}**
03.11.88	9	5.0×10^{5}	1.0×10^{6}	5.0×10^{4}	4.5×10^{10}	2.5×10^{10}	1.4×10^{10}
05.11.88	11	5.0×10^{5}	5.0×10^{5}	1.0×10^{7}	1.0×10^{8}	5.0×10^{5}	2.2×10^{7}
07.11.88	13	1.5×10^{7}	5.0×10^{8}	5.0×10^{7}	1.0×10^{9}	1.5×10^{10}	3.3×10^{9}
09.11.88	15	1.0×10^{6}	5.0×10^{5}	1.5×10^{6}	1.0×10^{10}	1.5×10^{5}	2.0×10^{9}
11.11.88	17	3.0×10^{7}	1.0×10^{7}	2.0×10^{7}	2.5×10^{10}	6.0×10^{5}	5.0×10^{9}
13.11.88	19	5.0×10^{8}	1.5×10^{9}	5.0×10^{9}	7.0×10^{10}	5.0×10^{5}	1.5×10^{10}
15.11.88	**21***	**1.5×10^{8}**	**2.0×10^{10}**	**5.0×10^{4}**	**5.0×10^{8}**	**4.5×10^{10}**	**1.3×10^{10}**
17.11.88	23	5.0×10^{6}	5.0×10^{7}	2.0×10^{8}	1.0×10^{9}	6.0×10^{10}	1.2×10^{10}
19.11.88	25	5.0×10^{9}	1.5×10^{10}	5.0×10^{9}	1.0×10^{5}	4.0×10^{10}	1.3×10^{10}
21.11.88	27	4.0×10^{10}	5.0×10^{6}	5.5×10^{10}	5.0×10^{7}	1.5×10^{6}	1.9×10^{10}
23.11.88	29	3.5×10^{10}	1.0×10^{7}	5.0×10^{8}	5.0×10^{6}	1.5×10^{10}	1.0×10^{10}
25.11.88	31	1.0×10^{8}	1.5×10^{10}	5.0×10^{6}	5.0×10^{9}	5.0×10^{9}	5.0×10^{9}
27.11.88	33	3.0×10^{10}	5.0×10^{8}	8.0×10^{10}	2.0×10^{9}	6.0×10^{10}	3.4×10^{10}
29.11.88	35	5.0×10^{5}	2.0×10^{8}	4.0×10^{10}	5.0×10^{9}	5.0×10^{9}	1.0×10^{10}

^ Beginning of treatment, * Withdrawal of treatment

TABLE 6.22 Individual bacterial counts of *Bacteroides spp.*/g faeces from the treated group in Experiment 4

Study							
Date	Day	Pig No. 1	Pig No. 2	Pig No. 4	Pig No. 6	Pig No. 9	$\bar{X}$
25.10.88	0	1.0×10^5	5.0×10^4	5.0×10^5	1.0×10^5	1.0×10^5	1.7×10^5
01.11.88	**7^**	**5.0×10^5**	**1.0×10^6**	**4.0×10^4**	**1.0×10^5**	**1.0×10^7**	**2.3×10^6**
03.11.88	9	2.5×10^5	2.0×10^5	5.0×10^5	3.0×10^6	1.5×10^5	8.2×10^5
05.11.88	11	5.0×10^4	2.0×10^4	2.5×10^4	5.0×10^4	5.0×10^3	3.0×10^4
07.11.88	13	1.0×10^5	2.0×10^5	1.5×10^6	5.0×10^5	1.5×10^5	4.9×10^5
09.11.88	15	5.0×10^4	1.0×10^4	2.5×10^3	2.0×10^3	1.5×10^3	1.3×10^4
11.11.88	17	1.0×10^4	3.0×10^5	3.5×10^4	5.0×10^4	2.0×10^4	8.4×10^4
13.11.88	19	5.0×10^3	5.0×10^5	1.5×10^4	2.5×10^4	3.5×10^4	1.1×10^5
15.11.88	**21***	**1.5×10^6**	**1.0×10^5**	**5.0×10^6**	**5.0×10^4**	**1.0×10^4**	**1.3×10^6**
17.11.88	23	1.0×10^3	1.5×10^3	5.0×10^2	4.5×10^3	5.0×10^3	2.5×10^3
19.11.88	25	2.5×10^6	5.0×10^4	4.0×10^4	1.5×10^4	5.0×10^3	5.2×10^5
21.11.88	27	1.0×10^8	1.5×10^6	5.0×10^5	1.0×10^6	2.5×10^6	2.1×10^7
23.11.88	29	1.5×10^4	1.0×10^4	5.0×10^3	5.0×10^2	5.0×10^3	7.1×10^3
25.11.88	31	5.0×10^5	1.0×10^7	7.0×10^5	2.5×10^5	5.0×10^5	2.3×10^6
27.11.88	33	1.0×10^7	1.0×10^4	1.0×10^7	5.0×10^3	5.0×10^3	4.0×10^6
29.11.88	35	5.0×10^5	5.0×10^4	1.5×10^5	1.0×10^5	1.5×10^5	1.9×10^5

^ Beginning of treatment, * Withdrawal of treatment

TABLE 6.23 Individual bacterial counts of *Bacteroides spp.*/g faeces from the control group in Experiment 4

Study							
Date	Day	Pig No. 71	Pig No. 73	Pig No. 74	Pig No. 77	Pig No. 79	$\bar{X}$
25.10.88	0	N G	5.0×10^3	5.0×10^3	5.0×10^4	1.0×10^3	1.5×10^4
01.11.88	**7^**	**7.5×10^4**	**1.0×10^5**	**1.5×10^4**	**4.5×10^4**	**5.0×10^4**	**5.8×10^4**
03.11.88	9	5.0×10^4	4.0×10^4	7.0×10^3	1.0×10^4	4.5×10^4	3.0×10^4
05.11.88	11	2.0×10^4	2.5×10^4	5.0×10^3	5.0×10^3	3.5×10^3	1.1×10^4
07.11.88	13	5.0×10^4	5.0×10^5	5.0×10^3	2.5×10^4	1.0×10^5	1.3×10^5
09.11.88	15	1.5×10^3	4.5×10^3	3.0×10^3	7.0×10^3	5.0×10^3	4.2×10^3
11.11.88	17	7.0×10^3	5.0×10^4	1.5×10^4	3.0×10^3	2.0×10^3	1.5×10^4
13.11.88	19	3.0×10^4	2.5×10^4	5.0×10^4	5.0×10^3	1.5×10^4	2.5×10^4
15.11.88	**21***	**5.0×10^3**	**2.5×10^4**	**5.0×10^3**	**3.0×10^3**	**3.0×10^4**	**1.3×10^4**
17.11.88	23	5.0×10^3	N G	5.0×10^3	1.5×10^3	3.0×10^4	1.0×10^4
19.11.88	25	1.0×10^4	5.0×10^3	5.0×10^4	5.0×10^3	2.0×10^4	1.8×10^4
21.11.88	27	2.5×10^5	1.0×10^6	5.0×10^5	2.5×10^4	1.5×10^5	3.8×10^5
23.11.88	29	1.0×10^4	5.0×10^2	2.0×10^3	5.0×10^3	2.5×10^4	4.0×10^3
25.11.88	31	1.5×10^5	5.0×10^5	5.0×10^4	5.0×10^5	4.5×10^4	2.4×10^5
27.11.88	33	5.0×10^5	3.5×10^4	5.0×10^5	5.0×10^4	1.0×10^4	2.2×10^5
29.11.88	35	5.0×10^4	5.0×10^4	1.0×10^5	1.0×10^5	5.0×10^4	7.0×10^4

N G = No Growth

^ Beginning of treatment, * Withdrawal of treatment

TABLE 6.24 Individual bacterial counts of *Clostridium perfringens* type A (vegetative cells)/g faeces from the treated group in Experiment 4

Study							
Date	Day	Pig No. 1	Pig No. 2	Pig No. 4	Pig No. 6	Pig No. 9	$\bar{X}$
25.10.88	0	1.0×10^5	4.5×10^3	5.0×10^9	5.0×10^6	5.0×10^4	1.0×10^8
01.11.88	**7^**	**1.0×10^6**	**3.0×10^7**	**4.0×10^4**	**1.0×10^6**	**1.0×10^{10}**	**2.0×10^9**
03.11.88	9	1.0×10^6	5.0×10^5	3.5×10^4	3.0×10^4	1.5×10^5	3.4×10^5
05.11.88	11	5.0×10^6	5.0×10^5	5.0×10^4	1.0×10^6	1.0×10^6	1.5×10^6
07.11.88	13	3.0×10^5	1.0×10^6	5.0×10^5	3.0×10^6	2.0×10^4	9.6×10^5
09.11.88	15	5.0×10^5	1.0×10^7	5.0×10^6	5.0×10^4	5.0×10^4	3.1×10^6
11.11.88	17	2.0×10^6	1.5×10^8	2.5×10^5	3.0×10^5	4.5×10^5	3.0×10^7
13.11.88	19	5.0×10^6	5.0×10^9	5.0×10^4	1.5×10^5	1.5×10^5	1.0×10^9
15.11.88	**21***	**5.0×10^7**	**2.5×10^5**	**1.0×10^6**	**1.5×10^6**	**1.5×10^5**	**1.0×10^7**
17.11.88	23	1.5×10^7	5.0×10^5	1.0×10^7	5.0×10^7	1.0×10^6	1.5×10^7
19.11.88	25	5.0×10^8	5.0×10^6	5.0×10^8	1.5×10^5	5.0×10^5	2.0×10^8
21.11.88	27	1.0×10^8	1.0×10^7	5.0×10^4	5.0×10^4	5.0×10^6	2.3×10^7
23.11.88	29	5.0×10^7	1.5×10^9	1.0×10^6	2.5×10^4	1.5×10^5	3.1×10^8
25.11.88	31	1.0×10^8	1.5×10^9	1.0×10^5	5.0×10^8	1.0×10^8	4.4×10^8
27.11.88	33	5.0×10^7	1.0×10^6	1.5×10^7	1.0×10^9	3.0×10^4	2.1×10^8
29.11.88	35	5.0×10^5	1.5×10^8	5.0×10^6	5.0×10^5	5.0×10^6	3.2×10^7

^ Beginning of treatment, * Withdrawal of treatment

TABLE 6.25 Individual bacterial counts of *Clostridium perfringens* type A (vegetative cells)/g faeces from the control group in Experiment 4

Study							
Date	Day	Pig No. 71	Pig No. 73	Pig No. 74	Pig No. 77	Pig No. 79	$\bar{X}$
25.10.88	0	1.0×10^6	5.0×10^3	5.0×10^4	5.0×10^3	5.0×10^3	2.1×10^5
01.11.88	**7^**	**2.0×10^7**	**5.0×10^6**	**5.0×10^6**	**1.0×10^{10}**	**5.0×10^6**	**2.0×10^9**
03.11.88	9	3.5×10^5	2.0×10^5	5.0×10^4	1.5×10^6	1.0×10^5	4.4×10^5
05.11.88	11	1.5×10^4	2.0×10^5	1.0×10^5	1.5×10^5	5.0×10^5	1.9×10^5
07.11.88	13	5.0×10^5	5.0×10^5	6.5×10^4	1.0×10^6	2.5×10^5	4.6×10^5
09.11.88	15	3.0×10^4	1.5×10^5	5.0×10^4	1.0×10^6	4.0×10^4	2.5×10^5
11.11.88	17	5.0×10^5	2.0×10^5	1.0×10^4	5.0×10^8	3.5×10^5	1.0×10^8
13.11.88	19	5.0×10^4	1.5×10^6	2.0×10^4	2.0×10^{10}	1.0×10^6	4.0×10^9
15.11.88	**21***	**2.5×10^5**	**5.0×10^5**	**5.0×10^4**	**5.0×10^4**	**5.0×10^6**	**1.1×10^6**
17.11.88	23	3.0×10^5	2.0×10^5	1.5×10^6	1.5×10^5	5.0×10^9	1.0×10^9
19.11.88	25	5.0×10^8	5.0×10^6	1.0×10^5	5.0×10^5	1.0×10^{10}	2.1×10^9
21.11.88	27	5.0×10^7	5.0×10^5	2.0×10^{10}	1.0×10^5	2.5×10^5	4.0×10^9
23.11.88	29	1.0×10^8	5.0×10^4	2.0×10^5	4.0×10^4	5.0×10^8	1.2×10^8
25.11.88	31	5.0×10^6	2.0×10^6	1.0×10^6	1.0×10^5	2.0×10^5	1.6×10^6
27.11.88	33	5.0×10^8	5.0×10^7	2.0×10^{10}	5.0×10^6	5.0×10^9	5.1×10^9
29.11.88	35	1.0×10^5	5.0×10^7	1.0×10^8	1.0×10^8	5.0×10^8	1.5×10^8

^ Beginning of treatment, * Withdrawal of treatment

TABLE 6.26 Individual bacterial counts of *Clostridium perfringens* type A (spores)/g faeces from the treated group in Experiment 4

Study Date	Day	Pig No. 1	Pig No. 2	Pig No. 4	Pig No. 6	Pig No. 9	$\bar{X}$
25.10.88	0	N G	N G	N G	N G	N G	N G
01.11.88	**7ˆ**	**5.0 x10^2**	**1.0 x10^4**	**1.0 x10^4**	**5.0 x10^2**	**N G**	**5.2x10^3**
03.11.88	9	N G	N G	N G	N G	1.0 x10^3	1.0x10^3
05.11.88	11	5.0 x10^2	N G	N G	N G	N G	5.0x10^2
07.11.88	13	N G	5.0 x10^2	5.0 x10^2	N G	N G	5.0x10^2
09.11.88	15	N G	N G	N G	N G	N G	N G
11.11.88	17	N G	N G	N G	N G	N G	N G
13.11.88	19	N G	N G	N G	N G	N G	N G
15.11.88	**21***	**N G**	**N G**	**N G**	**N G**	**N G**	**N G**
17.11.88	23	N G	N G	N G	N G	N G	N G
19.11.88	25	4.0 x10^3	N G	N G	N G	N G	4.0x10^3
21.11.88	27	N G	N G	N G	N G	5.0 x10^2	5.0x10^2
23.11.88	29	2.5 x10^3	N G	N G	N G	N G	2.5x10^3
25.11.88	31	N G	N G	N G	N G	N G	N G
27.11.88	33	N G	N G	N G	N G	N G	N G
29.11.88	35	N G	N G	N G	N G	N G	N G

N G = No Growth

ˆ Beginning of treatment, * Withdrawal of treatment

TABLE 6.27 Individual bacterial counts of *Clostridium perfringens* type A (spores)/g faeces from the control group in Experiment 4

Study							
Date	Day	Pig No. 71	Pig No. 73	Pig No. 74	Pig No. 77	Pig No. 79	$\bar{X}$
25.10.88	0	N G	N G	N G	N G	N G	N G
01.11.88	7^	1.0×10^3	N G	1.5×10^3	5.0×10^2	5.0×10^3	2.0×10^3
03.11.88	9	N G	N G	N G	5.0×10^2	N G	5.0×10^2
05.11.88	11	N G	N G	N G	N G	N G	N G
07.11.88	13	N G	N G	N G	N G	N G	N G
09.11.88	15	N G	N G	N G	5.0×10^2	N G	5.0×10^2
11.11.88	17	N G	N G	N G	N G	N G	N G
13.11.88	19	N G	N G	N G	N G	N G	N G
15.11.88	21*	N G	N G	N G	N G	N G	N G
17.11.88	23	N G	N G	N G	N G	N G	N G
19.11.88	25	N G	N G	N G	N G	2.5×10^3	2.5×10^3
21.11.88	27	N G	N G	1.0×10^3	N G	N G	1.0×10^3
23.11.88	29	N G	N G	N G	N G	N G	N G
25.11.88	31	N G	N G	N G	N G	N G	N G
27.11.88	33	N G	N G	N G	N G	N G	N G
29.11.88	35	N G	N G	N G	N G	N G	N G

N G = No Growth

^ Beginning of treatment, * Withdrawal of treatment

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