The Role of Gut Microflora on the Pathogenicity of Enteric Coccidia of Sudanese goats

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Dedication

To the soul of my father, my brother, Dr. Abdel Malik and my son Aymen. To my husband, Mohammed Hamid and beloved children, Eiman, Sarra, Ammar, Selma and Mustafa. To my uncle Mahgoub and all my family members.
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Abstract

In the present study, three experiments were performed using 3-month old, 42 male goat kids. They were divided into two groups (A and B) which subdivided into three subgroups (A1, A2, A3; B1, B2, B3, B4) 6 kids each, to study gut microflora, their distribution, factors affecting its composition and the interaction with goat coccidia.

Group A1 was given normal ration (Leucerne and Sorghum hay), group A2 was injected with Gentamycin while group A3 was supplemented with protein ration. Whereas, group B1 fed normal ration; B2 injected with Gentamycin and B3 was given high protein ration. All group B (B1, B2, B3) were inoculated orally with 1.5X10⁶ sporulated oocysts of mixed eimeria spp., whereas group B4 remained as uninfected untreated control.

Viable counts of the microflora were made in different parts of small intestine and feces.

All flora observed were qualitatively similar in all kids of group A1 consisting principally of lactobacilli, streptococci species *Escherchia coli*, *Clostridium perfringens*, bacteroides and *Staphylococcus auerus*. These bacteria formed the major components of the alimentary flora in goat kids.

The presence of the aforementioned bacteria increased progressively from duodenum to ileum, being more numerous in the large intestine. This population pattern was observed in all parts of the gut; lactobacilli, *Escherichia coli* streptococci, *Clostridium*
perfringens; whereas, Staphylococcus aureus and bacteroides were confined to the large intestine only.

Clostridium perfringens was either present in small number, or completely absent. Staphylococcus aureus was usually found in small number. The pattern of bacterial microflora of the faeces closely resembled that of the large intestine.

In group A2 (injected with Gentamycin) Escherichia coli, Lactobacilli, Streptococcus species and Staphylococcus aureus showed a reduction in number compared with that of group A1.

In group A3 (high protein ration). Streptococci, clostridium and bacteroides increased in number while lactobacilli, staphlococcus and E. coli remained unchanged.

In group B interaction of coccidia with gut microflora showed that Eimeria species of goats is the main cause of the acute fatal disease observed in the experimental animals. The other organisms, (bacterial flora) however, may play a role in the severity of the disease. These bacteria may induce their effects when coccidiosis occur as a result of changes in the intestinal pH, transit, motility and permeability of the intestinal wall. These conditions are favourable for pathogenic flora to proliferate and interact with coccidian in the gut.

E. coli isolates recovered from apparently healthy goat kids are generally non toxigenic and may belong to a number of non pathogenic serogroup. However, Clostridium perfringens isolated from healthy kids were pathogenic to both mice and kids resulting in a peracute disease.
However, we conclude from the present study, that, the activity and the interaction of bacterial flora in the intestines is complex and very little was understood with the present isolates.
ملخص الأطروحة

أجريت في هذه الدراسة ثلاثة تجارب باستخدام 42 سخان الماعز عمر ثلاثة شهور. تم تقسيمها إلى مجموعتين (A و B) وكل مجموعة قسمت إلى: المجموعة A و A2 ، و B و كل مجموعة قسمت إلى المجموعة B و B1 ، و B2 ، و B3. سخان لكل مجموعة. وذلك لدراسة النبض و نوع النبض، المعايير، توزيع العوامل المثيرة في مكون المعي الطبيعي والتفاعل مع كوكسديبا الماعز.


توزيع النتائج وجدت أن كل النتائج الجرثومية يتشابه نوعي في كل السخانات الصغيرة في المجموعة Escherichia coli, Clostridium streptococci, lactobacilli. هذه البكتيريا تكون الأجزاء الرئيسية perfringens, bacteroides, Staphylococcus auerus للناتج المعوي في صغار سخان الماعز. وتلاحظ أن كل الناتج الجرثومي يتشابه نوعي في كل السخانات الصغيرة في المجموعة Escherichia coli, Clostridium streptococci, lactobacilli. هذه البكتيريا تكون الأجزاء الرئيسية perfringens, bacteroides, Staphylococcus auerus للناتج المعوي في صغار سخان الماعز.

توجد البكتيريا المذكورة أعلاه، يزداد تصاعديا من ileum إلى duodenum ووجدت الأعداد الكبيرة في الأمعاء الغليظة. و يوجد هذا النمط التوزيعي في كل أجزاء الإمعاء: Clostridium perfringens, streptococci, Escherichia coli, lactobacilli عادة تكون موجودة في الأمعاء الغليظة فقط. و Staphylococcus aureus لا يوجد في الأمعاء الغليظة. و Staphylococcus aureus لا يوجد في الأمعاء الغليظة.

توجد Staphylococcus aureus في الأمعاء الغليظة. و النتائج تشير إلى أن Staphylococcus aureus لا يوجد في الأمعاء الغليظة. و Staphylococcus aureus لا يوجد في الأمعاء الغليظة.

أظهرت المجموعة A2 (المقدمة بالمضادات الحيوية) انخفاضا في أعداد Escherichia coli و lactobacilli، Staphylococcus و streptococci، و ذلك عند مقارنتها بالمجموعة A1.
وجد في المجموعة A3 (وجبة بروتين عالية) تزايد في أعداد بقيت دون Escherchia coli و Staphylococcus, lactobacilli و bacteroides و Clostridium تغيير.

أظهرت المجموعة B (تفاعله بين الكوكسيديا و النيبت المعوي) بأن أنواع Eimeria في الماعز هي المسؤولة للمرض الحاد القاتل في حيوانات التجارب. بينما الأجسام الأخرى (النيبت البakterى) قد تلعب دورا في زيادة حدة المرض. هذه البكترىيا قد تتداخل عندما تحدث الكوكسيديا تغيراتها المرضية كنتيجة لحوضمة الأمعاء، الترحيل وقابلية الحركة و الامتصاص في جدران الإمعاء. حيث تكون الأحوال مواتية للنيبت الممرض للتكاثر و التفاعل مع الكوكسيديا.

أنواع ال Clostridium المزروعة من سخال الماعز الغير مريضة ظاهرا عادة غير محدودة السمية وقد تتسمى إلى عدة مجموعات مصلية غير مرضية. و لقد وجدت المعزولة من السخال الغير مريضة بأنها مرضية وتسبب مرض حاد في السخال و perfringens فنران التجارب.

و نتلمى من هذه الدراسة أن درجة نشاط وتفاعل النيبت البكتيري في الإمعاء ععقد و تم فهم القليل في الأنواع المزروعة حاليا.
INTRODUCTION

Goats are highly susceptible to coccidiosis. This is especially true in the youngs when brought into confined quarter. In spite of the fact that coccidiostats added to feed have reduced mortality, outbreaks of coccidiosis continue to occur. Factors involved in coccidiosis are not well understood and may involve in addition to different species of Eimeria, some other agents such as interaction between these Eimeria species and normal flora of intestines.

The most important component of these flora are bacteria. These bacterial flora at first develop remarkably in a similar manner in all animal species but as animals grow older considerable dissimilarities become apparent between the species according to environmental changes. Some of these interactions are beneficial to the host but some may be detrimental. However, the activity of bacteria in alimentary tract is complex.

Little is known about the factors which affect the change in microflora in young animals. The flora develop towards the adult flora and become more numerous with predominance of E. coli, Streptococcus, Clostridium spp. and Bacteroides species (Bullen et.al, 1976).

Little research has been done on the effect of coccidian infection and the composition of microflora of the gut. Most of the reported studies have been focused on the effect of E. tenella on caecal microflora.
Several workers suggested that there is synergism between the normal flora and *E. tenella*, resulting in clinical caecal coccidiosis (Morse, 1908; Visco and Burn, 1972). Naqi and Mathey (1972) observed a synergistic relationship between pathogenic *E. coli* and *Eimeria brunetti*.

Fantham (1910) was the first worker to suggest that merozoites and sporozoites of *Eimeria* species act as inoculating needles and tissue damage is the result of bacteria, found normally. Mann (1947) and Ott (1937) have isolated enteric organism, most frequently, *Escherichia coli*, from the livers of chickens suffering from coccidiosis.

The same type of interaction was found in mammals. Gouet and Yvore (1989) noted that *Eimeria* species is perfectly pathogenic for conventional lambs did not produce any symptoms in axenic lambs. Only few oocysts were excreted later on.

Gouet et al., (1984) studied the effect of *Eimeria ovinoidalis* infection in the development of enterotoxigenic strain of *E. coli* K99, and found that presence of coccidian favours the establishment and development of *Colibacillosis*.

Iwamalsu (1986) reported that sudden death in a calf was attributed to enterotoxaemia caused by *Clostridium perfringens* type A, which may have been induced by coccidial infection, as he found a large number of coccidian gametes and growth of rods beside other lesions in histopathological sections of the small intestine.
In the Sudan little so far is known about effect of coccidia on the intestinal microflora in animals. Renda (1997) has studied the effect of coccidian infection on goat microflora and she reported that a considerable alteration in various constituents of the gut microflora were observed before and after the onset of diarrhea. *E. coli* was the most dominant organism amongst the various constituents of gut microflora.

**Objectives:**

Our investigation was designed to observe the changes in gut microflora during acute coccidiosis in goats, together with effect of antibiotic and protein in both gut microflora and pathogenicity of coccidian. Also the study represents an attempt to determine the types and numbers of certain bacterial flora of faeces and different parts of the small intestines in goats.
CHAPTER ONE
LITERATURE REVIEW
1.1. Normal microflora of the gastrointestinal tract.

At birth, the gastrointestinal tract of animals is sterile, within minutes of birth the canal is flooded with microorganisms acquired from the immediate environment (Linton, 1982). The dam is the most important contributor to microbial environment. The flora of the gastrointestinal changes as newborns diet changes from milk to solid food. The change in flora is dramatically different depending upon the character and nature of the food ingested and takes place in sequential manner (Dubos et. al, 1965).

The organisms that colonized the alimentary tract of young were probably derived form faeces of their dams (Smith, 1965a). One factor affecting the speed of colonization was the degree of contact between the new born animal and sources of the colonization organisms. The newborn animals that were unable to walk and were kept in a nest under comparatively clean condition, like rats and puppies may have accounted for the rather longer time taken for organisms to colonize their alimentary tracts. The early colonization of these animals with organisms that were plentiful on the breasts of their dams, contact or ingestion of vomits contaminated with rumen organisms. Animals which were active at birth and were soon walking around in an environment heavily contaminated with faeces of their dams, may favour rapid entry of considerable number of bacteria into their alimentary tract leading to massive proliferation of organisms in their
stomach and flooding their intestines during a short period of time. Herbivores, such as cattle, sheep and goats, develop a rumen with flora and fauna that are quite distinct from the rest of the domestic species, though in many ways similar to the colon and caecum of non-ruminant herbivores. Omnivores and carnivores are much the same with respect to the flora of the gastrointestinal tract (Hirsh, 1980).

The gastrointestinal tract contains a vast variety of microorganisms. In colon there are $10^{11}$ organisms per gram of faeces (Moore, 1969). It has been estimated that 400 to 500 different species may be represented, many of which have not yet been cultured (Moore and Holdeman, 1974), most are Gram negative non-spore-forming microorganisms.

In ruminants the rumen is sterile at birth (pre-ruminant). The development of rumen and its flora and fauna depend greatly upon the diet of the animals (Hungate, 1966). In general there are about $10^9$-$10^{10}$ bacteria/gram.

The most numerous facultative anaerobes are *Streptococcus bovis* ($10^5$ to $10^7$/g) and the coliform (*Escherichia coli-Enterobacteior*) at concentrations of $10^3$ to $10^5$ /g of contents. Thus the facultative microorganisms comprise only about 1 to 2% of the rumen flora. The most numerous type of microorganisms in rumen is the obligate anaerobes. These organisms make it possible for the rumen to do its work. Of $10^9$ or so obligate anaerobes, there are only 6 to 10 dominant genera with no one
species dominating under normal conditions (Smith, 1965). The importance of these organisms depending upon the role played in the fermentation process. At weaning the flora of gastrointestinal tract (abomasums to rectum) are predominantly anaerobic 95 to 99%. The composition of flora anaerobes, Enterobacteriaceae (mainly E. coli) enterococci yeast and Lactobacillus spp. It is worth noting that members of the family Enterobacteriaceae notably E. coli can be found throughout the small intestines (Smith, 1965, Smith, 1971). The caecum and rectum contain a flora and fauna much like those seen in the rumen.

Viable counts were made of bacteria and yeast in different parts of stomach and small intestine and in the caecum, colon and the faeces of different species of animals. Smith (1965) found that in ox, sheep, horse, monkey, pig, dog, cat, guinea-pig, rat, mouse fowl and ducks they consist of the same type of organisms, but the numbers of microorganisms in certain parts of alimentary tract differed greatly between some of animals species.

Large numbers of organisms are found in the anterior part or the body of the stomach and much lower ones in the posterior parts of antrum, and the duodenum. The number increases progressively from duodenum to ileum and the highest numbers are found in the large intestine.

Six groups of organisms formed the major part of the alimentary flora in all these animals. These are Lactobacilli, E. coli, Streptococci and Clostridium pefringens (Welchi and
Veillonellae). Yeast were found in somewhat similar numbers in all regions of the alimentary tract and *bacteroides* were usually confined to large intestine (Coates, 1980). The first organisms to colonize the alimentary tract in most species of animals were *Escherichia coli, Clostridium perfringens* and *Streptococci*. These were followed by *Lactobacilli*, which became the commonest inhabitant of the chyma in the stomach and small intestine, except in dogs, cats and rabbits. *Bacteroides* were slower in colonizing the alimentary tract and appeared to be mainly restricted to the large intestines, of which they were the principal inhabitant in most of animals examined (Smith, 1965; 1971).

*Staphylococcus aureus* was common inhabitant of the alimentary tract in dogs and man. It was rarely found in the other species of animals. Yeasts were not often found in animal whose diet consisted solely of milk (Smith and Crabb, 1961).

Suckling rabbits were unique amongst the species studied in that the contents of the stomach and small intestines were often almost sterile (Smith, 1965). But the flora of the large intestine consisted almost of *bacteroides*.

The development and composition of the faecal flora during the first few weeks of life were similar in all different animals. By the end of the first day, large numbers of *E. coli, Cl. welchi (perfringes)* and *streptococci* are present and within another day or so they were joined by *bacteroides* and *lactobacilli*, these two
kinds of bacteria soon constituted the greater part of the faecal flora. As the animals grew older considerable quantitative and qualitative differences developed (Smith, 1965, Coates, 1980).

The flora of the gastrointestinal tract is remarkably stable. Fluctuations in numbers of bacteria that occurred as result of various stimuli are transient. So the relationship between the normal host and its flora is optimal i.e each location or site along the gastrointestinal tract is specially suited for particular species of microorganisms (Smith, 1961). Each microbe occupied a particular site or niche, to exclusion of all others. These microorganisms come in intimate contact with the host by certain structures by which adhesion that stick a particular microbe in its niche so that it will not be swept away by peristalsis. Adhesions may be classified under fimbria, agglutinins and capsules (Moore, 1969, Hirsh, 1980). Aside from these three categories, there are many other ways that bacteria may adhere to an epithelial surface (Moore, 1969).

The secretion of certain substances into the immediate environment may be important in keeping population size in check, in addition to making conditions undurable for other competing species of microorganisms. Three classes of bacterial metabolic by-products have greater or lesser influence on gut bacteria, bacteriocin, fatty acids and deconjugate bile salts. Bacteriocins secreted by enteric bacteria called colicin since their discovery, colicin were hypothesized to be substance that would
be responsible for stability of the gastrointestinal flora. Fatty acids are highly detrimental to members of the Enterobacteriaceae. Facultative anaerobes are important in maintaining the redox environment for strict anaerobes, by scavenging oxygen. It has been suggested that the product of anaerobic metabolism i.e. volatile fatty acids and low pH are the main factors responsible for the selective inhibition of growth of aerobic or facultative anaerobic bacteria (Meynell, 1963, Bohnhoff et.al, 1964 and Byrne and Dankert, 1979).

Host factors influencing gut flora include composition of diet, properties such as epithelial surface and substance bathing them are the structures in most intimate contact with microorganisms living in the gastrointestinal tract. The most probable sites for bacterial adhesion appear to be brush border and overlying mucus gel. Other property is peristalsis through which microorganisms that are not anchored would be swept distally. So the peristalsis plays a major role in host defense of intestinal tract (Hirsh, 1980; Jubb et.al, 1985).

1.2.1. Factors affecting number and kinds of microflora

The numbers and kinds of microorganism living in the alimentary tract are varied depending on a number of factors. Feeding habit, anatomy and physiology of the stomach. Different species of animals have profound influence in determining the composition of flora. These factors are summarized below.
1.2.2. Nutritional factors

The flora of gastrointestinal changes as the diet changes depending upon the character and nature of food taken by animals.

The composition of the diet had a profound effect on the composition of the flora (Smith, 1965). Diet was considered responsible for many of the differences between the homothermic species. Experimental studies indicated that the differences of the flora were largely a function of the different abilities of the diet to support multiplication of different alimentary tract organisms (Lee et.al, 1971).

*Lactobacilli* in most mammals and birds, particularly those whose diet was mainly cereal, constituted the major component of the flora of the stomach, small intestine and in some cases large intestine. They occupied a much less dominating position in the herbivore and carnivore (Lee, Gordon, Lee and Dubos, 1971).

*Streptococci* had somewhat similar distribution to lactobacilli in homothermic animals. They were however, present in much smaller numbers in species other than carnivore and herbivore (Lee and Gemmel, 1972).

*Clostridium perfringens* was either absent or present in only small numbers in animals other than carnivore. In carnivore, they are relatively plentiful, particularly in cat whose diet contained higher proportion of meat than in dog (Smith, 1965b). Experimentally *Cl. perfringes* was present in considerable numbers throughout alimentary tract in rat fed on raw pork where
it was usually absent or present only in very small numbers in other rats.

*Escherichia coli* except in rabbit and guinea pig, in which it was found infrequently. *E. Coli* was usually present in all regions of the alimentary tract in homothermic animals. It was most numerous in large intestine, but only in sheep it did constitute a large proportion of the flora of that region.

*Bacterioides* were found only in large intestine of most species and they commonly constituted the bulk of the flora in this organ. In ruminants, they were sometimes present also in the rumen, reticulum and omasum.

*Staphylococcus aureus* is not normally found in most of the animal species. It was found in large intestine of puppies, hamsters and rodents and the crop of several fowls and man. (Smith, 1965a).

Yeasts were commonly found in the alimentary tract in adult mammals fed on cereal diet (Smith, 1965). The absence of the yeast from the tract of their young suggested that a milk diet did not favour the development of yeast flora, although the presence of yeast in some animals indicated that a milk diet was not completely restricted in this respect. It is noteworthy that yeasts were a common inhabitant of the alimentary tract in young guinea-pigs but within a day or so of birth these animals were supplementing their diet with their mothers cereal food.
1.2.2. pH

In most animals of all species except rabbit, the pH of the contents of the anterior compartment of the stomach was sufficiently high to permit bacterial multiplication, (Tannock and Smith, 1970). By contrast, it was low in the posterior compartment, sufficiently low in some animals to kill many of the bacteria. The reaction of the small intestine in all species except in monkey remained alkaline in large intestine of the herbivora, the rodents and the rabbit (Smith, 1966).

1.2.3. The anatomy and physiology of the stomach and small intestine

The feeding habit of the different species of the animals also had profound influence in determining the alimentary flora (Smith, 1965). Only small numbers of bacteria were present in the stomach and small intestine of rat that were denied food for 24 hours. After a very large meal the numbers in the anterior part of stomach were similar to those found in rats fed ad libitum. The numbers in the posterior part of the stomach and in the small intestine did not attain the relatively high levels found in the rats fed ad libitum because at seven hours the pH of the posterior compartment was much lower than in the rat fed ad libitum, sufficiently low to have pronounced bactericidal effect. Much lower concentrations of bacteria were reached in rats given a meal half the size of large meal or large that acidified pH 3.0 or amount of large meal divided into several small portions given at intervals during period of seven hours (Smith, 1965b).
The bacteria were usually much more numerous in the stomach and small intestine of calves, lambs, piglets and young rats than that of puppies and kittens, a situation that may be associated in part with the fact that in the former group of animals a large amount of food was always available in the stomach to support bacterial multiplication. The stomach of puppies and kittens, on the other hand, frequently contained only small amounts of food whose period of residence there would be short, a situation not conductive to proliferation of bacteria. It is conceivable too that stomachs of the puppies and kittens might empty occasionally and give rise to some degree of colonization. It is noteworthy in this respect that the total or partial absence of lactobacilli accounted in the main for the fact that the bacterial content of the stomach and small intestine was low in these animals as compared with that in calves and lambs; the lactobacilli were indeed rather slow in rate of progress of the contents through the large intestine and the fact that this organ was never empty were important reasons for the ease with which it was colonized, compared with stomach and small intestine. Bacteria had already colonized the large intestine in large numbers in some newly born animals, yet they were either scanty in or absent from the stomach or small intestine despite the fact they must have passed through these organs to reach the large intestine.
1.2.4. Age

In general, the development and composition of faecal flora during the first few weeks of life were similar in all different animals species. By the end of the first day, large number of *E. coli*, *Cl.welchi* and *Streptococci* were present, and within another day or so they were joined by *Bacteroides* and *Lactobacilli*, these two kinds of bacteria soon constitute the greater part of faecal flora. As the animal grew older, considerable quantitative and qualitative differences develop, until after several months the bacterial flora of the faeces of the different species were grossly dissimilar from each other, as well as from those of the very young of all the species. In calves, lambs and piglets, the total viable counts were commonly about $10^{10}/g$ during the first few weeks of life, slowly decreasing with age. Eventually, in calves over 26-weeks old, the total counts as low as $10^{6}/g$ were often recorded; this great decrease (10000 fold) was shared to greater or lesser extent by all kinds of bacteria initially present. The change in total count in lambs resembled that in calves, except that the decrease with age was slightly less (Smith and Crabb, 1961).

A general feature of aging in all animal species was gradual reduction in *E. coli* count from initially high to comparatively low numbers, with only moderate degree of fluctuation. Some variations occurred between species in rate of reduction and in the final levels attained. In faeces of calves, goats and lambs, *Cl. welchi* declined much more rapidly with age; only small numbers
of this organism were isolated after 1-3 weeks. Also total streptococcal count in all animals declined gradually with age. The decline with age in number of lactobacilli in faeces of calves and lambs was more easily traced than that of bacteroides. Eventually, it was not uncommon to fail to isolate lactobacilli from these animals (Smith, 1965). Differential counts were performed on the faeces of adult cattle, sheep, horses, pigs, rabbits, guinea pigs, mice, dogs, cats, chicken and man. The results for animals of the same species confirmed a common pattern and this differed markedly from species to species. Staph. aureus was only in human faeces.

1.2.5. Antibiotic

The commonly used antibacterial agents inhibit or kill the normal microflora. However, two members of the normal flora may not be affected as seriously as the rest, yeasts and fungi and members of the family Enterobacteriaceae. Smith (1966) reported that the complete absence of organisms from the stomach and small intestine in suckling rabbits was due to that the stomach contents of these animals contain antibacterial substances. Dietary antibiotic in small amounts was found to improve growth of the animals and did not cause nutrient deficiency. It was suggested that, this might be due to inhibition of bacteria commonly inhabiting gut despite that such bacteria are not strictly pathogenic, but cause depression in growth (Jukes and Williams, 1953 and Francois, 1962).
1.2.6. Stress

Stress be nutritional or emotional, appears to increase the likelihood of disease of any kind, but especially intestinal disease. Experimentally, stress will result in a change in the normal intestinal flora (Abrams, 1970; Tannock and Savage, 1974). These changes, at least in experimental animals, are related to a decrease in the anaerobic component of the normal flora. Subsequently, the numbers of coliform bacteria increase greatly. What appears to be occurring is a change in the major regulatory mechanism of the intestinal tract, the fatty acid production. Most if not all enteric pathogens belong to family Enterobacteriaceae. Without fatty acid control these pathogens are able to grow to numbers that would allow for successful competition for attachment to target cell site. If the stress was of sufficient magnitude and chronicity it would result in an increased concentration of corticosteroids that would be sufficient to decrease mucin production. Conceivably, the number of anaerobic bacteria that utilize the mucin as an energy source would also be reduced (Hoskin and Zamcheck, 1968; Savage et.al, 1971).

1.3.1. The Importance of microflora

The importance of the gut microflora to their host has been a matter of speculation. Since the end of last century. Pasteur, (1885) and Schottclius (1903) believed that the hosts may not be able to sustain life for long if deprived of their microflora. In contrast, Metchnikoff (1903) mentioned that some species of
these microflora may be harmful, produce toxins that reduce life span and interfere with host wellbeing. Generally, microflora may act positively to their host in three ways. First, they provide a source of energy from ingested materials. Secondly, they serve as host defense barrier in prevention of disease by agents seeking entry via the intestinal tract either by making the target cells unavailable to the pathogen or by creating an environment that is detrimental to the pathogen. Thirdly, the microbial inhabitants condition immunologic components of the gastrointestinal tract to respond to antigenic materials in a highly different manner (Hirsh, 1980).

1.3.2. Anatomical Importance

The microbial flora influences the anatomic structures that line gastrointestinal tract. Epithelial cell renewal has been shown to be faster in animals that have acquired a microbial flora (Lesher et.al, 1964; Abrams, 1970). Likewise, the crypts are longer and there is more surface area in normal intestine as compared to the germ-free state (Abrams, 1970). The microbial flora also aid or stimulate the formation of lymphatic tissue that lies beneath and in the tract. Specifically, the formation of lymphoid nodules take place very rapidly once the flora begins to establish (Crabbe, et.al, 1968) beside that also macrophages from conventional animals have been shown to digest, phagocytosed materials more efficiently than macrophages taken from germ-free animals. Macrophage mobilization and subsequent participation in immune
related phenomenon have been shown to be increased in animals possessing microbial flora (Bauer et.al, 1966).

1.3.3. Functional importance

It has been found that substance moves through the tract at a significantly faster rate when microbial flora are present i.e. increase in peristaltic activity. The increase in peristaltic activity may appear to be a detriment, for by decreasing the peristaltic activity (e.g by removing the normal flora). The absorption of poorly absorbed substances increases. The peristaltic activity of small bowel is host defense mechanism that sweeps microorganisms (e.g pathogenic) away (Hirsh, 1980).

1.3.3. Defense Importance

Bacterial pathogens attach to the “so-called target cells" as first step in producing disease. Following attachment to the target cells and multiplication, the pathogen either produces a toxin, invades and kills the target cell or invades and enters the lymphatics, ultimately gaining entry into the blood stream. Through one of these steps diseases occur.

The normal microbial flora act as a defense barrier either by making the target cells unavailable to the pathogen, or by creating an environment that is detrimental to the pathogen. In other words, if the normal inhabitants of the gastrointestinal tract are secure in their niche, then species of bacteria with pathogenic potential may not be able to compete successfully with them for a site of attachment. Without a site of attachment, the pathogen is
swept distally by peristalsis. (Hirsh, 1980, Linton, 1982). Thus anything that results in an upset in the balance between host and normal flora may give a pathogen easier access to its target cells, or allow it to multiply to high enough number so that competition for the cells is easier.

1.3.4. Nutritional importance

Coates (1980) reported that microflora are beneficial for the host, for example, synthesis of vitamins and aminoacids from simple precursors and production of enzymes, that contribute to digestion of dietary ingredients, is carried out by some species of microflora. On the other hand, microflora have activities which are detrimental to the host like converting some non-toxic component to toxins or render some nutrients unavailable to the host.

1.4.1. Importance of **Clostridium perfringens** (**Cl. perfringens**)

*Cl. perfringens* usually forms a part of the normal intestinal flora of man and animals and can be found in soil.

The organisms of this genus are large, anaerobic, spore-forming Gram’s positive rods,. They are motile except *Cl. perfringens*. Fermentative and catalase negative.

They reside in the soil and in the intestinal tract of animals and man. They are both pathogenic and non pathogen.

The most important species of clostridium as intestinal flora of man and animals is *Clostridium perfringens*. The organism is widespread in the soil and is found in alimentary tract of nearly all
species of warm blooded animals. It is frequently found as postmortem invader from the alimentary tract in the tissues of bloating cadavers of man and animals. It is also the most important cause of enteric and histotoxic diseases in both human and animals (Songer, 1997; Johnson and Gerding, 1997).

1.4.2. Morphology of *Clostridium Perfringens*:

It occurs as thick straight–sided rod either singly or in pairs seldom in chain. The individual cells are about 1.0 micron wide and 4-8 microns long (Buxton and Fraser, 1977).

The spores are oval and not form in highly acid media. Capsules are formed in tissues and in some types of culture media. No flagella, the organism is therefore non-motile. The organism has a quick generation time, as short as 8 minutes.

1.4.3. Culture appearance

In blood media it will be haemolysed. Sharp hemolytic zones are formed around colonies on plates. In broth there is excellent growth, the fluid becoming greatly clouded. Gelatin is rapidly liquified and coagulated egg medium is not liquified. There is good growth in cooked meat medium with considerable gas formation. The meat fragments are pinkish and not digested. A sour odor is emitted. A very characteristic reaction occurs in litmus milk “Stormy fermentation” the milk quickly coagulates and curd is fragmented by active gas formation. Acid and gas are formed from glucose, galactose, lactose, maltose, sucrose and xylose.
1.4.4. Strains of *Cl. Perfringens*

They show no consistent differences as for cultural characteristics. It is a very heterogeneous group of organisms with respect to their metabolic byproduct, toxin and pathogenic potential. The species is divided into five types from A to E (Guss, 1977, Niilo, 1980) as shown in table (1).

Each toxin produces a different type of lesion in different animals species (Radostitis et.al, 2000)

Type A intestinal commensals, are found in soil responsible for gas gangrene of man and animals. Avian necrotic enteritis, necrotizing colitis of equine, enterotoxaemia of lambs and goats, cattle and camels. Food poisoning of man and animals (Estrada Correa and Taylor, 1989 and Songer, 1996).

Type B causes lamb desentry, enterotoxaemia of calves, foal, sheep and goats (Melin et.al, 1997).

Type C causes enterotoxaemia of sheep (Struck), neonatal haemorrhagic enterotoxamia of calves, lamb and foal, enteroloxaemia of piglets and necrotic enteritis of man and foals (Gayle, Cohen and Chaffin, 1995; Kohler, Zabke, Reher and Rummler, 1997).

Type D causes enterotoxaemia of sheep, goats cattle, camel and man (Finnie and Hajdak, 1992; Buxton, 1981).

Type E induces enterotoxaemia of sheep and cattle, and enteritis in rabbits.
As these toxins are antigenic, typing is achieved by neutralization of lethal toxin with type specific antisera using mice or guinea pigs.

In addition, the various types may produce other antigenic substances, some of which possess pathogenic properties. Accordingly, varieties are known to exist within types A, B and C which produce certain combinations of antigens or toxin and are associated with clearly defined disease syndromes. These toxic and enzymatic substances have been studied in details because of their importance in relation to identification of the strain and pathogenesis of the disease (Hauschild, 1971). Sixteen antigenic substances were produced and designated by the Greek letters (Table 2 ) (Niilo, 1980 ; Hatheway, 1990). These antigens are rearranged and grouped according to their practical significance. Group 1 contains the major lethal toxin and minor toxins that are important, either in pathogenicity or for identification. Group 2 contains relatively unimportant substance that may have significance for research purposes (Brooks et.al,1959; Sterne and Warrck, 1964; McDonal, 1980). These antigens have been collectively referred to as toxin (Niilo, 1980).

It is evident that the pathology and clinical picture of a disease caused by Clostridium perfringens depends largely on the toxins they produces. Each toxin has its own pharmacological action which may be cumulative in producing the final effect. (Hatheway, 1990 ; Rood and Cole, 1991).
1.4.5. *C. perfringens* type A

It has been cited as the most common cause of enteroxaemia in goats and sheep (Sterne and Batty, 1975, Songer, 1996). Although it forms part of bacterial flora of the alimentary tract in many normal animals and in soil there are reports of highly fatal haemorrhagic disease caused by these organisms (McDonal, 1980.; Ardehali et.al, 1994). It has two varieties, one is characterized by alpha toxin production causing wound contamination, gas gangrene and enteric disease enterotoxaemias in animals. The other variety is enterotoxigenic or food poisoning which is characterized by production of enterotoxin capable of causing device food poisoning in man and enteritis in animals (Hatheway 1990; Songer, 1996 and Radostits et.al, 2000).

1.4.6. Pathogenicity

It produces alpha toxin which is produced by all Clostridial types but in large amount by type A. The toxin is an enzyme chemically known as phospholipase (Lecithinase-C) which hydrolyzes lecithin into diglyceride phosphorylcholine and diglyceride (Ispolatovskaya, 1971) As the cell membranes consist of lipoprotein complexes containing lecithin so alpha-toxin leads to destruction of these membranes and this, results in hemolysis, necrosis or death depending on the tissue accessible to the toxin (Niilo, 1971 ; 1972 ; 1977).
1.4.7. Pathology

The hemolytic disease is an acute onset of severe depression, collapse, mucosal pallor, jaundice, haemoglobinuria, dyspnea, protruding of the tongue with frothy discharge from mouth and nostril, staggering and convulsion (Russel 1970; Jansen and Swift, 1982; Popoff, 1984; Wernery, et.al, 1991). The disease is highly fatal; most of animals die within 12 hours of onset. At necropsy, the cardinal features are pallor, jaundice and haemoglobinuria. The kidneys are swollen dark brown and may contain infarcts. The liver is pale and swollen. There may be hydropericardium and pulmonary oedema (Niilo, 1980 Popoff, 1984; Barker and Van-Dreumel, 1985; Worrall et.al, 1987).

It is diagnosed by clinical, postmortem findings which are confirmed by presence of large numbers of clostridia dominating
**Table 1: Cl. perfringens toxic or antigenic component and some of their characteristics**

<table>
<thead>
<tr>
<th>Toxin or antigen</th>
<th>Produced by types</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I Alpha</td>
<td>A –E</td>
<td>Lecithinase (calcium dependent phospholipase) hemolytic, necrotizing, lethal leukocidal</td>
</tr>
<tr>
<td>Beta</td>
<td>B, C</td>
<td>Necrotizing- lethal, trypsin labile</td>
</tr>
<tr>
<td>Delta</td>
<td>C</td>
<td>Hemolytic</td>
</tr>
<tr>
<td>Epsilion (E)</td>
<td>B, D</td>
<td>Necrotizing, lethal, activated by protolytic enzymes, increases capillary permeability</td>
</tr>
<tr>
<td>Theta ( )</td>
<td>A-C, D, E</td>
<td>Hemolytic-oxygen labile</td>
</tr>
<tr>
<td>Iota (t)</td>
<td>E</td>
<td>Necrotizing lethal activated by protolytic enzymes</td>
</tr>
<tr>
<td>Enterotoxin</td>
<td>A, C, D</td>
<td>Sporulation-dependent enterotoxin resistant to prolytic enzymes.</td>
</tr>
<tr>
<td>Group II Kapps</td>
<td>(A, D), F</td>
<td>Collagenase, necrotizing</td>
</tr>
<tr>
<td>Lambda</td>
<td>(B, D) E</td>
<td>Non specific proteinase</td>
</tr>
<tr>
<td>Mu</td>
<td>A, E</td>
<td>Hyaluronidase</td>
</tr>
<tr>
<td>Nu</td>
<td>A, E</td>
<td>Desoxyribonuclease</td>
</tr>
<tr>
<td>Urease neutaminidase</td>
<td>A</td>
<td>Enzymes of no practical significance</td>
</tr>
<tr>
<td>Fibrinolysin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This table is base on data from several sources
<table>
<thead>
<tr>
<th>Types</th>
<th>Toxin</th>
<th>Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Alpha (α)</td>
<td>Yellow lamb, Gas gangrene</td>
</tr>
<tr>
<td>B</td>
<td>Beta</td>
<td>Lamb dysentery, Hemorrhagic enterotoxaemic IET of lambs, kids, foals</td>
</tr>
<tr>
<td>C</td>
<td>Beta (β)</td>
<td>Alpha, struck of young adult sheep, hemorrhagic enterotoxaemia in lambs, goats, calves, piglets</td>
</tr>
<tr>
<td>D</td>
<td>Beta (β)</td>
<td>Alpha epsilon, Enterotoxaemia of young feedlot sheep and goats (over eating, pulpy kidney diseases)</td>
</tr>
</tbody>
</table>
the bacterial population of the small intestine in the smears made from the contents of intestine, also by alpha toxin present in large quantities.

1.4.8. *Clostridium perfringens* type B & C.

It occurs commonly in soil and the alimentary tract of normal animals. Infection of *Clostridium perfringens* types B and C results in severe enteritis with diarrhoea and dysentery in young lambs, calves, goats, pigs, and foals (Jansen, 1961, Barker and Van-Dreumel, 1985, Stubbing 1990; Buergelt et.al, 1999).

1.4.8.1. Pathogenesis

Beta toxin (potent cytotoxic agent produced by *Clostridium* type B and C). It is trypsin and heat sensitive protein that causes mucosal cell inflammation and destruction in small intestine. The toxin causes haemorrhagic enteritis and ulceration of intestinal mucosa resulting in diarrhoea and dysentery (Manteca et.al, 2002). Lamb dysentery caused by *Cl. perfringens* type B occurs in young lambs up to 3 weeks of age, also it affects adult sheep (struck) (Barron, 1942; Blackwell and Bulter, 1992).

1.4.9. Clinical signs:

In peracute forms, it causes onset of bloat, abdominal pain, recumbency convulsion and diarrhea representing the major clinical signs. Infected animals may die of haemorrhagic enteritis before diarrhoea develops (Kimberling, 1988). The diarrhoea when occurs is explosive and yellow to dark brown in colour.
(digested blood). Neurologic signs are prominent in animals with more prolonged survival (Sterne and Bathy, 1975; Stubbing 1990).

1.4.10. Necropsy findings:

There is haemorrhagic enteritis, with ulceration of the mucosa in some cases. The intestinal mucosa is congested and dark red in colour, and there is an excess of serous fluid in the peritoneal cavity. Peritonitis and oedema of skeletal muscle may also be observed in sheep (Barker and Van-Dreumel, 1985).

1.4.11. Diagnosis:

The disease is rapidly fatal once clinical signs are noted, so diagnosis is often made on post-mortem. Excessive haemorrhage in small intestine and mesenteric lymph nodes with observation of large number of Gram-positive rods on a faecal smear support a diagnosis (Guss, 1977). However, definitive diagnosis is made with ELISA test that detects the beta toxin (β) (Martin et.al, 1988). Treatment is rarely successful due to the fatal nature of the disease. Intravenous fluid therapy; providing mixed electrolyte solution with bicarbonate are indicated to counter shock, dehydration and acidosis in sick animals. However, pencillin, anti-inflammatory (non-steroidal) and types C and D antitoxins are recommended to all neonates at risk. Prevention of the disease is through vaccination of pregnant animals in the final 4-6 weeks of pregnancy (Quarmby, 1947; King, 1980).
1.4.12. *Clostridium perfringens type D:*

It normally inhabits the alimentary tract of ruminants. The organism is the cause of acute enterotoxaemia which is also known as pulpy kidney, overeating disease (Bullen, 1970; Barker and van-Deumed, 1982; Niilo, 1993).

1.4.13. Pathogenesis:

It elaborates an epsilon toxin which is lethal necrotizing but not hemolytic. Commensal *Cl. perfringens* type D organisms reside in the gut without producing any harmful effect. Several conditions favour overgrowth of these organisms such as diet excessive in protein and carbohydrate, lambs on heavy-milking ewe are particularly susceptible (overeating) to the disease (Barker and Van-Dreumel, 1985) when large amounts of epsilon toxin are produced in the gut; it increases intestinal permeability hereby facilitating its own absorption into the general circulation, subsequently the vascular permeability increases in many organs mostly the central nervous system, (brain oedema), kidney (renal damage) and liver (Kellaway et.al,1940; Gardner, 1973, Rood and Cole, 1991). Hepatic metabolism of glycogen is altered resulting in hyperglycemia (Gardner, 1973).

1.4.14. Clinical signs:

In lambs, the illness is very short (less than 2 hours and never more than 12 hours). The first signs may be dullness, depression, yawning, facial movement, off food and convulsion. Cases which survive for a few hours show a green pasty diarrhea,
staggering, recumbency and convulsions. Adult sheep survive for up to 24 hours. They lag behind the flock staggering and knuckling, champing of the jaw, salivation with shallow respiration.

In calves the syndrome is similar to that of adult sheep (Mumford, 1961).

In goats, the peracute form occurs more frequently in young goats. The course is usually less than 24 hours and may go unnoticed. Clinical signs are loss of appetite, depression, marked abdominal pain with arching back, loud and painful screaming and profuse watery diarrhoea with blood shreds and mucus. They may show convulsions (Oxer, 1956, Blackwell and Bulter, 1992).

1.4.15. Necropsy findings:

The carcase is usually in good condition. In peracute cases there may be no gross lesions. There are clear straw-coloured pericardial fluids and petechial haemorrhage in the epicardium and endocardium. Patchy congestion of abomasal and intestinal mucosa. Liver is dark and congested.

1.4.16. Diagnosis

Circumstances, clinical and necropsy findings are diagnostic with observation of large number of Clostridium (Gram-positive rods). Definitive diagnosis is made by demonstration of epsilon toxin by ELISA test (Naylor et.al, 1987; 1997), which shows good specificity and sensitivity for epsilon-toxin (Elidrissi and Ward, 1992).
1.5.1. Importance of *Escherichia coli* (*E. coli*)

Being normal inhabitant of the intestinal tract of animal and man can also be associated with a variety of pathological conditions in animals. The organism has been considered to be a major etiological agent of diarrhoea of newly born of several species of animals particularly calves, lambs, kids, pigs and human beings (Acres, 1985).

*E. coli* is Gram negative, small non-sporing rod, usually motile and seldom capsulated. Aerobic or facultatively anaerobic and grow well on ordinary media forming large (2-3 m length and 0.6 in breadth) circular, low convex, smooth and colourless colonies in 18 hours on nutrient agar and large red colonies on MacConkey's lactose agar. On blood agar, there is discolouration around the growth and they may be haemolysis, they grow over a wide range of temperature (15-45°C); some strains are more heat resistant than other enterobacteria and will survive at 60°C for 15 minutes or 55 °C for 60 min. They are more sensitive to certain dyes particularly brilliant green (Carter, 1979).

It ferments glucose, maltose, mannitol, xylose, sorbitol lactose with acid and gas. All reduce nitrate to nitrites. Oxidase negative, catalase-positive, usually urease-negative, methyl red-positive.
1.5.2. Pathogenicity

*E. coli* is predominant among the aerobic commensal flora of the gut of animals and man. They are normal inhabitants of lower of all warm animals. Some strains of *E. coli* were found to be pathogenic (Bruner and Gillespie, 1973; Greenwood et.al, 1992).

The disease most commonly occurs in first 2 weeks of life. Neonates show signs of progressive depression and inappetence followed by watery diarrhoea. Signs of sepsis such as fever, hypothermia (terminal cases) and tachycardia. Disease progression depends on the virulence of *E. coli* serotype and the vulnerability of the patient so some may develop coma and death within 6 hours of the onset of clinical signs. In slowly progressing cases bacteraemia can result in signs of meningitis, phlebitis and synovitis.

1.5.3. Diagnosis

The postmortem lesions include petechial and ecchymotic haemorrhages on serosal surface. The *E. coli* often can be isolated from various organs. Faecal culture is of limited value because coliforms are normal inhabitants of intestinal tract.

1.5.4. Types of *E. coli* infection:

Blood et.al, (1989) recognized three syndromes associated with *E. coli* infection:

1. Septicaemia form (Coli septicaemia ) in which sudden death is associated with *E. coli* bacteraemia. The organism can
be isolated (usually as single serotype in pure culture) from all organs and tissues throughout the body.

2- Enterotoxaemic form characterized by collapse and sudden death, and associated with proliferation of specific strain of *E. coli* in the intestine without bacteraemia.

3- Enteric form (calf scours), if the physiological disturbances are severe the animals may die, but it is not always fatal.

The relatively few publications about *E. coli* infection in lambs and kids are concerned with septicaemia, entritis, meningitis and arthritis (Teriecki and Sojka, 1965).

Two forms of infection are recognized in lambs and kids, systemic and enteric form. These forms have become major problem in lambs. Roberts (1970) considered *E. coli* infection to be the most important cause of mortality in lambs.

Ugochukwn and Anukam (1988) found that *E. coli* consisted 73% of isolates from faecal swaps taken from goats with diarrhoea in Nigeria. It has been reported that *E. coli* was the dominant enterobacteria isolated from cases of enteritis in goat and kids (Roy et.al, 1986; Ramaswamy Ganersan, Ray Andrew, Sarvandara and Venugopalan, 1992).

1.6.1. Importance of *Streptococci*

It is known that relatively harmless parasite. *Streptococci* are frequently present in throat and intestinal tract of animals. Some of these cocci may assume a pathogenic role when normal resistance is markedly reduced (William and Dorsey, 1961).
Most *Streptococci* are Gram-positive. In old culture, Gram-negative forms are commonly found. Never acid fast. With rare exceptions (group D enterococci) they are non motile. A number of species form definite capsules when developing in tissue and media containing blood. Such strains form colonies that are mucoid and differ in appearance from the majority (Buxton and Fraser, 1977).

**1.6.1.1. Cultural character**

They develop into chain resembling string of bead. The chain length depends upon species differences and upon the media. Typical-chain formation is best seen in fluid media. On solid media, chain become so entangled.

*Streptococci* produce small, delicate, translucent colonies diameter of about 1mm on solid media. Heavy inoculations give confluent growth that are nearly transparent. The surface of growth is smooth and glistening. The individual colonies are perfectly circular. In fluid media, growth usually is a little more abundant than in solid. Most of them grow readily under aerobic as well as anaerobic condition. There are some strain however that grow only under anaerobic conditions. Sugars are fermented by all streptococci with end products of acid and gas. Gas is produced by only few strains. Temperature for growth is 10°C-45°C (Buxton and Fraser, 1977). Growth at 45°C and 50°C, growth in presence of 6.5% Nacl or 40% bile and growth at pH 6.9, all of which are characteristics of group D.
1.6.1.2. Classification

They are classified in various ways into four principal categories (Carter, 1979).

1- Pyogenic group contains the majority of the disease-producing strain.

2- Viridans group is a serologically heterogeneous group which produces greenish coloration of blood agar or alpha hemolysis.

3- Lactic group includes strain recovered from milk whey.

4- Enterococci are intestinal flora which are recovered from faeces of animals and man.

Enterococci generally occur as commensals in the intestinal tract of man and animals and have been incriminated in sporadic infections. *Streptococcus faecalis* has been recovered from lesions of endocarditis in chicken (Carter, 1979).

1.7.1. Importance of Lactobacillus spp.

*Lactobacilli* are straight or curved rods of varying length and thickness, arranged singly or in chain, sometimes filamentous or pleomorphic, without branching, clubbing, or bifid formation. Gram positive and non sporing. Usually non moltile and non pigmented. Growth on the surface media is poor. It is extremely fastidious, with complex nutritive requirements including amino acids, peptide, vitamins and fatty acid (John, 1998). Energy is obtained by anaerobic fermentation of sugars. Growth is favoured by microaerophilic or anaerobic conditions and by CO₂. Some
species grow at 45°C, but not at 15°C. Glucose is fermented and either lactic acid alone or lactic acid along with volatile acids, CO₂ and other by-products are formed. Little or no protolytic activity. No production of catalasae –oxidase, or indole and no reduction of nitrate. Readily killed by heat, but unusually resistant to acid. Non-pathogenic to man and animals (Sims, 1964). Sharpe et.al, (1973) reported 10 strains that had been isolated from cardiac, septicaemic and suppurative lesions in man, but how far they were primarily responsible for the lesion is open to question.

Widely distributed in fermenting vegetables and animal products and in the alimentary tract of man and animals. They are of paramount importance in food industry, both as beneficial organism and as spoilage organisms. They are used in the production of fermented milk products. If the growth of these bacteria is not controlled, they can be a major cause of food spoilage. The souring of milk and the greening of meat are common examples of spoilage resulting from the unchecked activity of these organisms (John, 1998).

1.8. Importance of Bacteroides

1.8.1. Morphology

They are rods, with rounded ends, occurring singly, in pairs or in short chains; sometimes pleomorphic, non motile or motile by peritrichous flagella, non-sporing and Gram-negative. Many require enriched media for growth. They are anaerobic.
Gas may be liberated from peptone water. Most species ferment a range of sugars with the production of acid and sometimes gas, butyric acid is not among the end product (Rosebury, 1962 and Reinhold, 1964). Final pH in glucose broth (pH 4.5).

Some species are non-saccharolytic. Threonine not dehydrogenated and nitrate is not reduced. A dark or black pigment is produced by a few species.

1.8.2. Pathogenicity

May be pathogenic, found in the alimentary and urogenital tracts of man and animals. Over twenty species recognized only three species are of significance B. nodosus, B. melaninogenicus, B. fragilis (Buchanan, and Gibbons, 1974). B. nodosus causes contagious foot rot in sheep (Beveridge, 1941). B. melaninogenicus was isolated from a variety of diseases and lesions in animals. Incidence was highest in cats and then cattle sheep, dog, pigs and horses (Biberstein et.al,1968). This organism was often present in association with other bacteria in disease conditions including meningitis, fistulous condition, peritonitis, pericarditis, suppurative arthritis and pneumonia. Also isolated from spleen abscess and skin abscess in dog (Carter, 1979). Berkhoff and Redenbarger (1977) reported several isolates of this organism, B. fragilis, from disease conditions in animals. It often causes serious and life-threatening infections. These conditions include abscesses in brain, lung, liver and other
internal organs, joint infection, septic abortions, postoperative bacteraemia and septicemias.

On subcutaneous inoculation in the rabbit, an extensive phlegmon with sloughing of the skin, and death in 6-7 days occurs. Intravenous inoculation of the rabbit is followed by death, but no organisms are found in tissues, probably death is due to toxaemia, for killed culture have the same effect (Carter, 1979).

1.9.1. Importance of Staphylococcus spp.

They are aerobic and facultatively anaerobic, catalase positive, non-motile, fermentative. Gram-positive cocci are seen in cluster and pairs, short chains may be seen in smears from fluid media. They occur commonly as commensals on the skin and mucous membranes of man and animals (Barrow and Feltham, 1993).

The species, which are found as intestinal flora in some animals species are *Staph. aureus* (*Staph. pyogenes*). Typical strains (*Staph aureus*) are coagulase positive. In order to qualify for identification as *Staph aureus*, strain must produce coagulase, ferment mannitol and produce clear beta haemolysis.

1.9.2. Cultural character

Blood agar colonies are round, glistening, convex smooth and opaque, beta haemolysis is produced by most of strains and frequently double zone is apparent in which the central clear zone is surrounded by a band of partial haemalysis. Colonies may possess white golden or lemon pigmentation.
1.9.3. Pathogenicity

*Staph. aureus* causes suppurative wound infections pyoderma of the dog and cat and other animals, abscesses in many animals. Secondary invaders are found in a wide variety of disease processes. Dennis (1966) found *Staph. aureus* to be the most important cause of neonatal infections in lambs. Staphylococcal enteritis occurs in man but has not yet been reported in animals.

Some strains of *Staph aureus*, including those responsible for staphylococcal food poisoning, produce a thermstable enterotoxin (Carter, 1979).

1.10.1. Coccidiosis:

Coccidiosis is a serious infectious disease of mammals and birds. It is the most common cause of diarrhoea in young animals including kids between 3 weeks and 5 months of age, particularly when goats are housed in confinement (Chhabra and Pandy, 1991).

Coccidiosis is caused by intracellular protozoan parasites of genus Eimeria. In the past the Eimeria species infecting goats and sheep were presumed to be the same. In 1979, however, well-controlled experiments, using *E. ninakohyakimovae* and *E. christensenii* demonstrated that each small ruminant host has its own host-specific Eimeria species that are not readily cross-infective (McDougald, 1979).

The nomenclature for the recognized Eimeria of goats is given in table (2), with information on comparable sheep species and oocyst morphology (Norton, 1986; Soe and Pomocy, 1992).
1.10.2. Life cycle:
This organism goes through a typical sporozoan life cycle and with exception of sporulation of oocyst, all stages occur within the host’s intestinal tract (Fig. 1). Oocysts passed in feces sporulated in animals environment to produce infective sporocysts containing sporozoites. These sporocysts are ingested by other animal. Ingested sporocysts release their sporozoites which then enter host cells to form schizonts. Schizonts undergo asexual reproduction to produce a first generation of daughter merizoites. Depending on species of Eimeria, anywhere from a few dozen to one hundred thousand merozoites may be formed by each schizont. The merozoites then break from the disrupted host cell and each is capable of invading a new host cell to form a second generation of schizonts. The number of cycles of schizogony varies among Eimeria species. The final cycle of schizogony leads to differentiation of merozoites into male and female gametes. Male gametes (microgametes) then are released from host cells to fertilize female gametes gamons (macrogametes) within their host cells. Zygote formation results in oocyst formation with release of oocysts from disrupted host cells and subsequent passage in feces (Hammond and Long, 1973; Fitzgerald, 1980; Ernest and Benz, 1981). Eimeria species usually parasitize the alimentary
### Table 2: Oocysts of coccidian in goats

<table>
<thead>
<tr>
<th>Species</th>
<th>Average dimensions (microns)</th>
<th>Morphology</th>
<th>Comparable species in sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. alijevi</strong></td>
<td>17x15</td>
<td>Subspherical, ellipsoidal or ovoid, colourless or pale yellow, Micopies sometimes discernible</td>
<td><strong>E. parva</strong></td>
</tr>
<tr>
<td><strong>E. aspsheronica</strong></td>
<td>31x23</td>
<td>Ovoid, distinct micropyre at narrow end, pale</td>
<td><strong>E. faurei</strong></td>
</tr>
<tr>
<td><strong>E. arloingi</strong></td>
<td>28x20</td>
<td>Ellipsoidal with micropyle cap, yellowish-brown</td>
<td><strong>E. bakuensis</strong></td>
</tr>
<tr>
<td><strong>E. caprina</strong></td>
<td>32x23</td>
<td>Ellipsoidal yellowish-brown. Micropyre present but no cap.</td>
<td><strong>E. caprovina</strong></td>
</tr>
<tr>
<td><strong>E. caprovina</strong></td>
<td>30x24</td>
<td>Broadly ellipsoidal, yellowish-brown. Micropyre present but no cap.</td>
<td><strong>E. caprovina</strong> (experimental infection)</td>
</tr>
<tr>
<td><strong>E. christenseni</strong></td>
<td>38x25</td>
<td>Ovoid to ellipsoidal, yellowish-brown. Micropyre cap</td>
<td><strong>E. ahsata</strong></td>
</tr>
<tr>
<td><strong>E. hirici</strong></td>
<td>23x18</td>
<td>Ellipsoidal to subspherical. Colourless to light yellow with or without a shallow micropyre cap</td>
<td><strong>E. crandallis</strong></td>
</tr>
<tr>
<td><strong>E. jolchijevi</strong></td>
<td>31x22</td>
<td>Ellipsoidal or ovoid. Micropyre cap at broad end. Yellowish-brown</td>
<td><strong>E. granulosa</strong></td>
</tr>
<tr>
<td><strong>E. kocharli</strong></td>
<td>47x32</td>
<td>Ellipsoidal. Thick brown wall, transversely striated. Yellowish-brown.</td>
<td><strong>E. intricata</strong></td>
</tr>
<tr>
<td><strong>E. ninakohlyakimovae</strong></td>
<td>24x19</td>
<td>Ellipsoidal. Thin-walled colourless or pale yellow, micropyre barely perceptible</td>
<td><strong>E. ovinoidalis</strong></td>
</tr>
<tr>
<td><strong>E. pallida</strong></td>
<td>14x10</td>
<td></td>
<td><strong>E. pallida</strong> (thought to infect both hosts)</td>
</tr>
</tbody>
</table>
Fig.1. Life cycle of coccidians.
canal, and liver in its lining epithelium.

The most common Eimeria pathogenic to goats in Sudan are *E. arloingi*, *E. alijevi*, *E. ninakohlyakimovae*, *E. christenseni*, *E. hirci*, *E. jolchijevi*, *E. pallia*, *E. apsheronica*, *E. arloingi* and *E. ninakahlyakimovae* (Singh and Pande, 1967; Levine and Iven, 1986; Nikam and Kamble, 1999; Balicka-Ramisz, 1999; Mubarak et.al, 2000).

1.10.2. Epizootiology

*Eimeria spp.* were isolated from goats worldwide. Epizootiological studies indicated that coccidian oocysts are widely present in feces of both normal and Coccidia infected goats, with prevalence ranging between 38% to 100% of all goats examined (Lima, 1980; Cornelissen et al., 1995). Concurrent infection with multiple *Eimeria spp.* is the rule. It is safe to assume that where there are goats, there are coccidians (Chhabra and Pandy, 1991; Svensson, 1993).

At any age, goats may be infected with Eimeria (Da Silva and Miller,1991), but numerous factors contribute to the highest prevalence of clinical coccidiosis occurring in young goats 3 weeks to 5 month old (De La Fauente and Alunda, 1992). These factors include host, parasite, management and environmental factors. Age-related resistance to clinical coccidiosis is reported in all ruminant specie (Fox et.al, 1991). The resistance is immunologic in nature and is maintained by continuous exposure to coccidial infection. There is a steady decline in oocyst
numbers from 6 months through 6 years of age followed by an increase in goats 7 years and older as immunity begin to wane (Kanyari, 1988).

Complete elimination of coccidial infection may lead to a failure of immunity and the development of clinical disease on re-exposure to pathogenic Eimeria. The immunity that develops is species specific and animals at any age may develop clinical disease if exposed to a population of different Eimeria species (Craig, 1986). Resistance may be impaired by stressors such as concurrent lactation, transport, feed change, weather change increase or exposure to new species of Eimeria (Kanyari, 1988).

Oocysts are quite resistant to environmental conditions, and are even more resistant when sporulation occurs. In general, with adequate moisture and oxygen, sporulation of oocysts occurs optimally within 2 to 5 days at temperature between 24°C to 32°C (75°F-90°F). (Pout, 1974).

The various species of Eimeria may also differ in the number of cycles of schizogony involved in reproduction. The more merozoites produced, the more intestinal epithelial cells are disrupted because every merozoite potentially can invade a target host cell. Prepatent periods also vary among goat Eimeria spp. (Craig, 1986).
1.10.3. Pathogenicity

The adverse effects of Eimeria infection result from destruction of gastrointestinal epithelium as these intracellular parasites complete their complex life cycle in the digestive tract of the host.

There are species specific differences in the host cells invaded by various Eimeria species. For example, sporozoites of *E. ninakohlykimovae* group of the most pathogenic of the goat species, enter basal epithelial cells in the crypts of lieberkuhn in villi of the small intestine. Many thousands of merizoites are produced in each schizont, these merizoites then invade epithelial cells in other areas. The subsequent gametogonous stage invades the ileum, cecum, and large intestine where oocysts are produced (Smith, 1980; Osman, 1988). Since multiple infections are common in goats disruption of gastrointestinal epithelium can be quite extensive.

Diarrhoea results from inflammation and disruption of the intestinal mucosa. In massive infections, severe haemorrhage into intestinal lumen can result in death caused by blood loss. In more typical acute form of the disease, fluid and electrolytes loss result from a compromise of the normal resorptive potential of the intestinal epithelium as well as leakage of plasma and lacteal constituents from the inflammed and disrupted mucosa. These losses can lead to dehydration and acidosis. Disruption of the mucosal integrity leads also to increased susceptibility to secondary bacterial infection and resultant septicemia, thus
increasing mortality rate (Smith and Sherman, 1994). The ill-thrift and poor growth commonly seen in the aftermath of clinical coccidiosis are manifestations of prolonged malabsorption and maldigestion (Hammond and Long, 1973; Fitzgerald, 1980; Ernest and Benz, 1981).

1.10.4. Pathology
Subclinical conditions should be suspected when complaints of poor growth, weight loss, or loss of well formed fecal pellets conditions are reported to young susceptible animals kept under management conditions conducive to the persistence and multiplication of coccidia (Long, 1973).

Peracute cases caused by severe blood loss in the intestinal lumen may present as sudden death before signs of diarrhoea or abdominal discomfort are seen (Richardson and Kendall, 1963). The intestinal lumen may be filled with blood due to massive destruction of intestinal mucosa (Richardson and Kendall, 1963).

The pathology of coccidiosis has been reported (Lotze, 1952; Long, 1973). The onset and severity of the disease may be influenced by various factors including pathogenicity of coccidian, animal species involved, management, nutritional status and age (Hammond and Long, 1973). Young animals usually develop the acute disease which always terminates fatally.

In adult animals, a chronic debilitating disease is more frequently observed and such animals are considered as carriers (Levine, 1973 and Soulsby, 1982). The early signs include
anorexia, loss of appetite, listlessness, and weakness. Severely affected animals will pass watery faeces that might contain fresh blood mucus fibrin cast and mucosal strands. Anaemia, hypoproteinaemia and dehydration can develop as result of destruction and loss of intestinal epithelium, which allows loss of fluid, plasma protein (albumin) and blood into lumen (Howe, 1980).

The severity of the disease seen in an individual depends on the number of intestinal cells invaded and the amount of tissue adversely affected. If a large number of cells is invaded they are eventually destroyed and lesions may be produced in the intestine. The surrounding tissue react by sloughing of the dead cells. If enough cells have been destroyed, the mucosa may be removed down to the muscular layer (or deeper) of the intestine. This bares the small vessels in the capillary bed and profuse bleeding into the lumen occurs. According to the number of cells invaded, clinical effect may range from no effect to death of animals (Fitzgerald, 1980).

At necropsy the carcases are pale, emaciated, dehydrated and oedematous and marked congestion and haemorrhage in the intestinal mucosa are common findings. Circumscribed grayish white spots, measuring 0.5-2mm in diameter, were scattered irregularly on the mucosal surface all over the small intestine, (mainly the jejunum and ileum). These lesions are seen on both serosal and mucosal surface. The mesenteric lymph nodes are
often enlarged, haemorrhagic and edematous (Mugera, 1968 and Osman, 1988).

Microscopically, there were focal erosions on the intestinal mucosa. The villous structure is lost but the superficial lining was mostly intact. There was diffuse mononuclear infiltration, the epithelium of tubular glands was hyperplastic and the individual cells were hypertrophic. Various coccidial stages e.g. meronts, gamonts and developing oocysts were seen in the epithelium of villi, glands and even muscle layer (Osman, 1988; Gregory and Catchpole, 1990).

1.10.5. Diagnosis

For diagnosis observation of large (uncountable) number of oocysts in faecal samples during routine faecal floatation reflects a severe infection. This finding in the presence of clinical signs support a diagnosis of coccidiosis. It is important to remember that the acute cases might not yet have a patent infection, a negative faecal examination does not rule out coccidiosis. It is also important to realize that the presence of a small number of oocysts in faeces is a normal finding in ruminant, many immune animals shed them fairly constantly. Also, not all species of coccidia are pathogenic. Postmortem (gross and microscopic) findings are diagnostic (Lisa Williamson, 2002).
1.10.6. Treatment and prevention

Clinical coccidiosis is man-made problem that occur primarily in non-immune, stressed animals that are crowded together in lots or stables that are heavily contaminated with oocysts. Coccidiosis is a self-limiting disease, and spontaneous recovery without specific treatment occurs commonly when the multiplicative stages of coccidia have passed.

Supportive care is principal therapeutic intervention in active cases of coccidiosis. Diarrheic goats should be removed from the group and given oral or parenteral balanced electrolyte solution depending on the degree of dehydration.

Milk should be fed only in small amounts as disruption of the intestinal mucosa will produce maldigestion and may promote osmotic diarrhoea from indigested lactose. Broad antibiotics are indicated in severe cases to prevent bacterial septicemia secondary to disruption of the intestinal mucosa (Guss, 1977).

The use of anti-coccidial drugs in individual, active cases may have limited value. Most anti-coccidial drugs are coccidiostats that inhibit but not eliminate coccidial reproduction. The main goal in using coccidiostats is to reduce the number of additional cases developing in a group of animals at risk rather than curing existing cases. (Guss, 1977; Osman, 1988; Radostits et al, 2000).

Drugs including sulfonamide, nitrofurazone, inophores and amprolium are reported in the treatment of caprine coccidiosis (Guss, 1977; Dash and Misra, 1988). Measures should be taken to
control coccidiosis before the observation of clinical disease. The goals of control are to reduce oocysts numbers in the environment sufficiently to avoid massive challenge to susceptible animals while allowing sufficient exposure so that resistance and immunity can properly develop. Hygiene and management are essential for the infection control. Weaning of kids should be as unstressful as possible and they should have access to grain well before weaning.

We must do our best for the control of coccidiosis because beside the loss in animals and the cost of treatment, the main economic impact of the disease is failure of young stock to grow and gain weight to their full potential. When animal recover from coccidiosis, gut function and appetite do not return to normal for many weeks. Recovering animals remain unthrifty and fail to gain weight (Smith, 1980; Foreyt, 1990).

1.11.1. Interaction Between Coccidiosis and Gut Microflora

Enteric microflora play a role in the pathogenesis and pathophysiology of inflammatory bowel disease syndrome.

Intestinal parasites differ widely in their relationship to microbiological flora, present in host digestive tract for example *Histomonas meleagrisida* requires the presence of specific microorganisms for the production of pathological change typical of the disease (Franker and Doll, 1964; Bradley and Reid, 1966). On the other hand, the tapeworm *Raillietina cesticillus*, presents an entirely different picture in which the parasite appears to be
unaffected by the presence or absence of bacterial microflora (Reid and Botero, 1967).

Renault et.al, (1973) demonstrated different pathogenic *E. coli* serotypes in caecal flora of rabbit suffering from dysentery while low in healthy rabbits.

Nematode parasites infections have been demonstrated to be modified by previous infection with viruses or bacteria (Gaafar et.al, 1973; Wade and Gaafar, 1981).

Coccidia and helminth infection may occur concurrently in goats of varying ages, but nematode infections do not occur in kids (Borgsteede and Dercksen, 1996). It has been shown that the establishment of the protozoan *Entamoeba histolytica* in the guinea pig and *Histomonas meleagridis* in the turkey is associated with the presence of intestinal bacteria (Phillips et.al, 1955; Doll and Franker, 1963 ; Bradley and Reid, 1965).

Little attention has been given to the interaction between coccidia and gut microflora in animals. Few studies have focused on the effect of *Eimeria tenella* on the caecal microflora.

Some early investigators suggested that the intestinal microflora related to mortality and morbidity occurred during caecal coccidiosis (Morse, 1908; Fantham, 1910). Because *Coliform* bacteria were isolated from blood of *Eimeria tenella* infected chick, it was believed that the normal microflora traversed the injured caecal mucosa during coccidiosis and established itself in various internal organs. Ott (1937)
demonstrated that the livers of *E. tenella* infected chicks became contaminated with gastrointestinal tract bacteria. Some workers (Tyzzer, 1929; 1937; Mann, 1947) have suggested that, beyond secondary invasion, the gut flora are associated directly with severe caecal pathology during *E. tenella* infections. Burns and Challey (1959) have reported that the presence of caecal flora is possibly associated with clinical manifestation of the infection. Investigations into *E. necatrix* have shown, however, that neither *Salmonella typhimurium* nor *Escherichia coli* or *Streptococcus faecalis* affected the clinical manifestation or intestinal pathology of this coccidial infection. Stephens et.al, (1964) and Stephens and Vestal (1966) reported that *S. typhimurium* was recovered from internal organs.

It has been shown that clinical manifestations and mortality do not occur in bacteria-free chicken infected with surface-sterilized *E. tenella* oocysts, and that chickens with one of two species of bacteria develop more severe lesions of coccidiosis than do bacteria-free counterparts (Johnoson and Reid, 1972; Radhakrishnan ,1971; Visco and Burn, 1972).

It was also known that during the course of caecal coccidiosis the growth of *Clostridium perfringens* and *coliform* (especially *Escherichia coli*) is stimulated and the growth of *Lactobacillus* spp. is suppressed (Johnson and Sarles, 1948 ;Rahhakrishnan, 1971).
Several studies were carried out to clarify the interrelationship between caecal coccidiosis and caecal flora. In these studies comparisons were made among germ-free, monoflora, diflora and conventional chickens on the severity of caecal coccidiosis. Significant changes in microflora of conventional chickens influenced by *E. tenella* (Kimura et.al, 1976; Lafont et.al, 1983) depicted that inoculation of small number of *E. tenella* oocysts enables the development of *Salmonella* population.

The interaction of *E. tenella* and *E. coli* infection in chickens was investigated. Specific pathogen free chickens inoculated orally with *E. tenella* and challenged four days later with *E. coli* via the air sac showed more severe acute septicaemic lesions and subacute serositis than chickens given *E. coli* alone (Nakamura et.al, 1990). Moreover, caecal lesions induced by *E. tenella* were more severe in chickens given both *E. tenella* and *E. coli* than in those given *E. coli* alone (NaKamura et.al, 1990).

Licosis and Guillot (1980) noted that in young rabbits experimentally infected with *E. intestinalis* induced an increase in the number of *Escherichia coli* isolated from the faeces of these rabbits.

Baba and Gaafar (1984) demonstrated that *Isospora suis* infection interfered with establishment and/or development of *Salmonella typhimurium* in the intestine of pigs.
Iwamalsu (1986) reported that one-month-old calf died suddenly and the cause of death was diagnosed to be enterotoxaemia caused by \textit{Cl. perfringens} type A, which might have been induced by coccidial infection. The author found a large number of coccidial gametes and growth of rods beside other lesions in histopathological sections of the small intestine. Experimental infection in calves with corona virus and \textit{E. bovis} may result in clinical disease and lesions that are more severe than those by either infection alone (Hoblet et.al, 1992).

Gouet et.al, (1984) reported that \textit{E. ovinoidalis} was perfectly pathogenic to conventional lambs and specific pathogen free (SPF) lambs, inoculated with 63 pure bacterial strains representative of dominant digestive microflora and free of all pathogenic organisms. By contrast, clinical signs including diarrhoea were not observed in axenic lambs (Gouet et al, 1984).

In lambs Gouet and Yvore (1989) studied the effect of \textit{E. ovinoidalis} infection on the development of an enterotoxinogenic strain of \textit{E. coli} K99, a serotype that is the cause of acute neonatal diarrhoea in calves, lambs and kids, and they suggested that the presence of coccidian infections favours the establishment and development of colibacillosis.

In kids and goats Mohamed et.al, (2000) reported that noticeable alterations were observed in the normal constituents of the gut microflora in coccidian-infected kids. These changes were characterized by progressive decrease of Gram-positive organisms.
and an increase of Gram-negative organisms after the onset of diarrhoea. *E. coli* was the most dominant organism.
CHAPTER TWO
MATERIALS AND METHODS
2.1 Parasitological Methods:

2.1.1. Preparation of coccidial oocysts:

The same *Eimeria* species was used throughout the study, which have been recently isolated from goats with coccidia. A whole day collected faeces was separated from faecal debris first by seiving through graduated seive aperture (100-200µm). The filtrate was transferred into one-litre cylinder and allowed to stand overnight. The supernatant was discarded and the sediment was centrifuged at 1500 r.p.m. for 3 minutes. The supernatant was then discarded and the sediment was emulsified with 2.5% potassium dichromate and left for sporulation in shallow layer in Petri dishes at room temperature and examined at intervals until sporulation was completed.

The sporulated oocysts were centrifuged at 1500 r.p.m. for 3 minutes and this was repeated for several times to wash up the potassium dichromate. Oocysts in cultures were counted by taking 5ml samples, made up to 10ml, mixed thoroughly and counted into two chambers of McMaster (Fuchs-Rosenthal) using 5 mm coverslip in each. The calculation was done as follows:

No. of oocysts/ml of culture =

Average No. oocysts/chamber x5x10x100

Area counted per chamber

10 = dilution

1000 = conversion of cu. mm to 1ml.
The cultures were then stored in a refrigerator at 4-5°C and were used before 4 weeks. However, immediately before using in experimental trials, the oocysts were surface sterilized with 0.5% solution of per acetic acid (Harleman and Meyer, 1984).

2.1.2 Faecal oocysts counts:

Oocysts were counted in each faecal sample by using the McMaster counting chamber technique and sodium chloride as floatation solution (MAFF, 1986). Three grams of faecal samples were taken at 3 days intervals, mixed thoroughly with 42 ml of tap water. The homogenate was then strained through a 100 mesh sieve and centrifuged for 3 minutes at 1500 r.p.m. and the supernatant was discarded. The sediment was emulsified in saturated sodium chloride solution and the centrifuge tube was shaken and inverted several times to obtain an even suspension. The two chambers of McMaster slide were filled using a clean Pasteur pipette. The average number of oocysts counted in these chambers was multiplied by 100 to obtain the number per gram of faeces.

2.1.3. Identification of oocysts:

After sporulation, the faecal material was concentrated by coverslip floatation method using saturated sodium chloride solution. Five slides were made from each sample and the oocysts present in these slides were described morphologically. Measurement of the length and width of different types of the oocysts were taken and the average values of those were used to
identify the species of coccidia present. The oocysts dimension were measured by eye-piece micrometer directly through a calibrated microscope, adopting the criteria of Levine, Ivens and Fritz, (1962).

2.2. Haematological methods:

2.2.1. Haemoglobin concentration (Hb):

Hb was determined by cyanomethaemoglobin method (Schalm, 1971), 0.2 ml of well mixed blood was taken into 4 ml of Darbkin’s solution (0.2 gm potassium ferricyanide, 1g sodium bicarbonate per litre distilled water). After 10 minutes Hb concentration was measured using Corning Haemoglobinometer against cyanomethaemoglobin standard and values were recorded in g/100ml.

2.2.2. Packed Cell Volume (PCV):

Blood was drawn in plain capillary tubes sealed at one end with plasticine and centrifuged for 5 minutes at 8000 r.p.m. using Haematocrit centrifuge (Hawksley and Sons Ltd., England). The PCV value (%) was read in Hawksley microhaematocrit reader and results.

2.3. Pathological methods:

Thorough post-mortem examination was carried out on animals that died of infection or scarificed. All organs were examined carefully for any pathological lesions, then pieces from liver, lung, kidney, brain, duodenum, jejunum, ileum and large intestine were taken aseptically for bacteriological isolation.
Tissues for histopathology were taken from different parts of the small intestine and all other organs, fixed in 10% formal saline, processed in an automatic tissue processor (Schendon), embedded in paraffin, was sectioned at 4-6µ and were stained with haematoxylin and Eosin (H &E). The processing and staining techniques were done according to the method described by Clayden (1971).

2.4. Bacteriological methods:

2.4.1. Sterilization:
The media and solutions used were sterilized and autoclaved at 121°C and under 1.06 kg/cm² for 15 minutes or at 115°C under 0.69kg/cm² for 20 minutes. Equipment and Petri dishes were sterilized in an oven under temperature of 100°C for 60 minutes.

2.4.2 Collection of Samples for Bacteriological examination:
Faecal specimens freshly removed from rectum, with sterile plastic bags, were collected with care to prevent contamination. Faeces was weighed and placed in a glass tube, to each volume of faeces 9 volumes of sterile saline solution were added and emulsion was made. Further dilutions were made from this initial faecal stock by serial 10 fold dilutions up to the appropriate dilutions for each bacteria.

At necropsy the whole alimentary tract was removed directly and unraveled. The small intestine was divided into duodenum, jejunum, ileum and large intestine and the contents of
each segment were removed with sterile precautions. To each volume of intestinal content 9 volumes of sterile saline solution were added and emulsion was made and further serial dilutions were made as described in case of faeces.

From faeces and intestinal contents, dilutions selected for each bacterium and 0.1ml volume of suspension was spread on plates of different selective media, using a modified method of Miles et al, (1938).

2.5. Bacteriological media used:

2.5.1. Reinforced Clostridial Agar (RCM Agar Oxoid CM151):

It is solid version of Oxoid Reinforced Clostridial medium CM 149, suitable for cultivation and enumeration of clostridia and anaerobes. RCM was also employed for investigation of intestinal flora. Perry et al, (1955). Smith and Crabb (1961) used the media with added sodium chloride for counts of animal faeces.

Smith (1961) used oxoid RCM agar with added blood for total counts of Lactobacillus spp of animal faeces and with blood and neomycin for determination of Bacteroides and total counts for streptococci.

The RCM agar was made by suspension of 52.5 g in 1 litre of distilled water, boiled to dissolve completely and sterilized by autoclaving at 115°C for 20 minutes. Counts were made on the following selective media:
Salt agar (Oxoid RCM Agar, CM151), with 8% sodium chloride after inoculation and incubated aerobically at 37°C 48 hours.

2.5.3. Blood agar (Oxoid RCM agar, CM 151) with 55 citrated sheep blood and incubated anaerobically in an atmosphere of 95% hydrogen and 5% carbon dioxide for 48 hours at 37°C.

2.5.4. Neomycin blood agar
(Blood agar with 70ug per ml of neomycin sulphate) incubated anaerobically in an atmosphere of 95% hydrogen and 5% carbon dioxide for 48 hours.

2.5.5. Neomycin Naglar agar (Lowbury and Lilly, 1955),
100ml of sterile nutrient agar (Blood based CM 55) were recommended, melted and cooled to 50°C, 5ml of Fildes Extract (prepared by action of enzyme pepsin on defibrinated sheep blood according to Fildes (1920), were added and then concentrated egg yolk emulsion (SR 47) and used in 5% concentration. After inoculation, the plates were incubated for 24 hours at 37°C.

2.5.6. Rogosa agar (Oxoid modification)
Formulated as follows:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trylone (oxoid)</td>
<td>10.0 gm</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>5.0 gm</td>
</tr>
<tr>
<td>Dextrose</td>
<td>20 gm</td>
</tr>
<tr>
<td>Tween 80</td>
<td>1.0gm</td>
</tr>
<tr>
<td>Ingredient</td>
<td>Quantity</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Potassium dihydrogen sulphate</td>
<td>6.0gm</td>
</tr>
<tr>
<td>Ammonium citrate</td>
<td>2gm</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>25gm</td>
</tr>
<tr>
<td>Glacial acetic acid</td>
<td>0.1575</td>
</tr>
<tr>
<td>MgSO$_4$·7 H$_2$O</td>
<td>0.12</td>
</tr>
<tr>
<td>MnSO$_4$·2 H$_2$O</td>
<td>0.034</td>
</tr>
<tr>
<td>Agar</td>
<td>20.0gm</td>
</tr>
<tr>
<td>Distilled water completed to</td>
<td>1 litre</td>
</tr>
</tbody>
</table>

The pH was adjusted to 5.4 approximately.

Suspend 74.5 g/litre, and adjust the pH to 5.5. with acetic acid 96% (approx. 1.3 ml/litre). Do not autoclave. The plates were then incubated up to 48 hours at 37°C under anaerobic condition in 5% carbon dioxide atmosphere.

2.5.7. McConkey agar (Oxoid CM7).

It is a differential medium for detection, isolation and enumeration of coliform which may be present in various specimens such as urine, faeces and others.

Formula

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>20.0 gram/litre</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.0</td>
</tr>
<tr>
<td>Bile salts</td>
<td>5.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0</td>
</tr>
<tr>
<td>Neutral red</td>
<td>0.075</td>
</tr>
<tr>
<td>Agar No. 3</td>
<td>12.0</td>
</tr>
<tr>
<td>PH 7.4 (approximately</td>
<td></td>
</tr>
</tbody>
</table>
It was made by suspending 52 gm in 1 litre of distilled water, boiled to dissolve completely, then sterilized by autoclaving at 121°C for 15 minutes and the surface plate was dried before inoculation. The plates were finally incubated aerobically at 37°C for 24 hours.

2.5.8. Reinforced Clostridial Medium (RCM)

Thirty-eight grams of RCM (Oxoid, England) were suspended in one litre of distilled water (DW) and heated in controlled water bath until dissolved completely. The medium was then sterilized by autoclaving at 121°C for 15 minutes.

2.5.9. Cooked Meat Media:

Ten grams of granules of cooked media (Oxoid, England) were transferred into MaCarteny bottles, and 10ml of D.W. were added. The media were then autoclaved at 121°C for 15 minutes for sterilization.

2.6. Biochemical test

Primary tests include: growth aerobically and anaerobically, Gram’s stain, glucose fermentation motility, oxidase and catalase tests.

2.6.1. Gram’s stain:

A smear from a well separated colony was made, Gram's stained (Barrow and Feltham, 1993) and examined microscopically. It was used to differentiate between Gram-negative and Gram-positive isolates and to study the morphology of the bacterial cell.
2.6.2. **Motility:**

This was examined by the hanging drop method. (Cruikshank et al, 1975). A part of separated colony was taken from culture on nutrient agar, emulsified in a drop of normal saline, placed on a clean slide, then covered with a thin cover glass and examined microscopically.

2.6.3. **Glucose:**

To test glucose breakdown by the isolates, peptone water containing 1% glucose was used, incubation was done at 37°C. The colour of Andrades indicator changes to pink to indicate a positive result. The production of gas is evidenced by its accumulation in the inverted Durham tube.

2.6.4. **Oxidase test:**

A freshly prepared solution of teteramethyl-p-phenylenediamine dihydrochloride was used to wet a filter paper placed in a clean sterile petri dish. Using a glass rod or a swab stick, a part of a separated colony was streaked on the soaked paper. A positive test was evidenced by development of dark purple colour in few seconds (Barrow and Feltham, 1993).

2.6.5. **Catalase test:**

A drop of 3% aqueous solution of H₂O was dropped onto a separated colony on nutrient agar. Evolution of gas immediately or few seconds later, indicates a positive test.
2.6.6. **Oxidation/fermentation of glucose (O/F test):**

Inoculation of isolates, each into duplicate tube, with straight wire loop was done in the O/F medium. To one of the tubes a layer of sterile paraffin oil was added to seal it from air before incubation of the 2 tubes and 37°C. The change of the indicator colour to yellow in both tubes means attack of sugar by fermentation, colour change in the uncovered tube only indicates oxidation, whereas no change of colour implies negative result.

2.6.7. **Eijkman test:**

Incubate MacConkey's broth warmed to 37°C and incubate in a water bath at 44°C±0.1 for 48 hours. Regard the production of both acid and gas as positive results.

2.6.8. **Coagulase slide test: (William and Harper, 1946)**

Emulsify colony in a drop of water or saline or microscope slide to produce a thick suspension. Stir the bacterial suspension with a straight wire which has been dipped into plasma. A positive result is indicated by microscopic clumping within 5 seconds.

2.7. **Preparation of inocula**

2.7.1. **E. coli** inoculum:

*E. coli* stock cultures maintained in MacConkey's broth were used for preparation of inoculum. Then they were subcultured in MacConkey's agar plates and incubated aerobically at 37°C 24 hours. Pure colonies from each plates were cultured in MacConkey's broth and incubated for 24 hours aerobically.
2.7.2. *Clostridium perfringens* toxins:

The toxins were prepared using the method of Bullen and Batty (1956), 8 hours cultures of *Clostridium perfringens* grows in RCM liquid media were centrifuged at 6000 r.p.m. for 20 minutes. The supernatant was aseptically collected in sterile McCartney bottles.

The toxin epsilon (Type D) was activated by adding trypsin 0.1ml of 10% trypsin solution to 2.5 ml of toxin filtrate and incubated at 37°C for 45 minutes.

2.7.3. *Cl. perfringens* inocula for experimental infection:

*Cl. perfringens* stock culture (whole culture) maintained in RCM liquid media, were used for preparation of the inoculum. Then strains were subcultured in blood agar plates and incubated anaerobically at 37°C for 24 hours. Pure colonies from each plate were cultured in 50 ml of toxin media.

2.7.4. Mice inoculation:

This experiment was done to detect the lethal effect of *Cl. perfringens*. A sterile disposable syringe was used to inoculate 0.5 ml of trypsin activated and inactivated toxin intraperitoneally. The mice were kept under close observation.

2.8. Experimental procedure:

Surgical techniques were employed for intraduodenal inoculation of whole culture. The experimental animals (3-month-old kids) were kept off feed for 24 hours before surgery. The area in the right side flank ventral to the third lumber
vertebral transverse process was cleaned and prepared for surgery. The incision was made through skin, muscular layer and peritoneum, the duodenum was exposed and subsequently the animals were dosed with a whole culture which was infused gradually and intraduodenally. The abdomen and skin were then sutured. All experimental animals were kept under close observation.

2.8.1. Experimental animals:
Forty-two male goats kids (2-3) month old were obtained from Khartoum State. They were housed in thoroughly cleaned and disinfected pens at the Central Veterinary Research Laboratories at Soba. The kids were initially checked for clinical fitness. They were confirmed to be negative for the presence of coccidian oocysts and other internal parasites in their faeces by repeated parasitological faecal examination. The animals were constantly fed on sufficient amount of green Lucerne and Sorghum hay and had free access to water.

The high protein ration formulated as follows: each 100 kg contain 50 Sorghum, 17.5 wheat bran, 10 cotton seed cake, 20 Ground nut cake, 1.0 Sodium chloride and 1.5 Vitamins and Minerals.

2.8.2. Experimental Design:
Three experiments were performed using a total of 42 goat kids.
2.8.3. Experiment 1:
Eighteen (18) kids were randomly divided into 3 equal groups A1, A2 and A3, to study the factors affecting the normal composition of the gut microflora. Group A1 was given the normal diet (Green Leucerne and Sorghum hay), Group A3 was given high protein ration, while group A2 was given antibiotic injection (1 cc gentamycin I/m every other day). Faecal samples were collected at 3-day-interval for bacterial isolation and characterization of faecal microlflora. Clinical observations were recorded daily. After necropsy or death of animals, bacteriological isolation from different parts of the small intestine (duodenum, Jejunum, ileum and large intestine) was done.

2.8.4. Experiment 2:
The interaction of coccidia with gut microflora was tried, 24 goats kids were allotted to 4 groups, each of 6 animals (group B1, B2, B3 and B4); group B1 was given normal diet, group B3 was given high protein diet, group B2 was given injectable antibiotic every other day (1 cc of gentamycin). These 3 groups (B1,B2 and B3) were orally inoculated with 1.5 million sporulated oocysts of mixed Eimeria species. While group B4 was kept as uninfected untreated control. During the course of the experiment the following parameters were done:

1- Clinical observation record
2- Faecal examination of oocysts
3- Haematological findings (Hb, PCV and Differential WBC count).
4- Bacteriological examination of faecal samples.
5- Postmortem examination of morbid animals for histopathological and bacteriological examination of the various parts of intestinal tract at necropsy or slaughter.

2.8.5. Experiment 3:
To study the pathogenicity of the most potentially pathogenic bacteria *E. coli* and *Clostridium perfringens* isolated by inoculation in experimental animals.

Pathogenicity of *E. coli* and *Clostridium perfringens* isolates from normal healthy goat kids.
Pathogenicity of *E. coli*: 2 trials (A &B) were done.

Trial A, 2 male goat kids, deprived from clostrum by taking them immediately after birth and kept on goat milk.

After one week these kids were given orally whole pure culture of *E. coli* (have clostrum) \((26 \times 10^8)\) viable colonies. Another 2 male kids were given the same dose of *E. coli* culture orally and the two groups were kept under close observation. For control one kid was given sterile MaConkey's broth.

Experiment No. B:
Two male kids 3-month-old were given *E. coli* inocula (previously prepared) intraduodenally, according to the aforementioned procedure.
Pathogenicity of *Clostridium perfringens*:

Two trials were performed. (C and D)

**Trial No. C toxigenicity in mice:**

Mice were inoculated in this trial. Using 1ml disposable syringes 1ml of trypsin activated trypsin and unactivated toxin were given intraperitonealy, (each in mice) and kept under observation.

**Trial No. D:**

Two male goat kids 3-month-old were inoculated intraduodenally with pure *Clostridium perfringen* whole culture as prepared previously.

**Statistical analysis:**

The MedCalc® Version 7.3.0.1 software was used for calculation of Anova, Student t-test and correlation coefficient,
CHAPTER THREE
RESULTS
3.1. Parasitological results

The morphological features of the identified Eimeria species are summarized in table (4).

The species identified, in the material collected from the natural infection, were: *Eimeria arloingi* (20%), *Eimeria ninakhyakmovae* (25%), *Eimeria chrislensen* (18%), *Eimeria alijevi* (15%), *Eimeria hirci* (8%), *Eimeria apsheronal* (5%) *Eimeria jolchijevi* (4%) and *Eimeria pallida* (5%).

Eight species of Eimeria were identified from the natural infection and used for inoculation of our experimental animals.

Oocysts of these species were shown in figures (2 and 3).

3.2 Identification of faecal flora:

The principal faecal bacteria identified and counted (Table 6) on selective media, were *Escherichia coli*, *Clostridium perfringens*, *Streptococi*, *lactobacilli*, bacteroides, but on a few occasions small number of *Staphyllococcus aureus* was also isolated. The incidence and frequency of other organisms did not appear to be significant.

With some of these bacteria the colonial appearance on certain of the selective media and the microscopical examination
Table (4): Oocysts used for inoculation of discernible experimental kids.

<table>
<thead>
<tr>
<th>Species</th>
<th>Average dimensions (microns)</th>
<th>Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. arloingi</strong></td>
<td>27.9x20.1</td>
<td>Ellipsoidal with micropylar cap yellowish-brown</td>
</tr>
<tr>
<td><strong>E. ninakoh lyakimovae</strong></td>
<td>(33-29.7)x(22.1-19.8)</td>
<td>Ellipsoidal, thin walled colourless micropyle barely perceptible.</td>
</tr>
<tr>
<td><strong>E. christensen</strong></td>
<td>(37.6x25.2)</td>
<td>Ovoid to ellipsoidal yellowish-brown micropylar cap.</td>
</tr>
<tr>
<td><strong>E. alijevis</strong></td>
<td>16.5x16.5</td>
<td>Subspherical or ovoid colourless – no Micropyler cap discernible</td>
</tr>
<tr>
<td><strong>E. hirci</strong></td>
<td>23.5x17.4</td>
<td>Ellipsoidal to subspherical colorless, to high yellow with or without shallow micropylar cap.</td>
</tr>
<tr>
<td><strong>E. jolchijevi</strong></td>
<td>(37.9x24.7)</td>
<td>Ovoid to ellipsoidal, yellowish to brown micropyle present.</td>
</tr>
<tr>
<td><strong>E. pallida</strong></td>
<td>14x10</td>
<td>Ellipsoidal, thin wall, colorles no micropyle cap.</td>
</tr>
<tr>
<td><strong>E. aspheronica</strong></td>
<td>(31.4x22.8)</td>
<td>Subspherical</td>
</tr>
<tr>
<td></td>
<td>(33-31.9)x (21.1-20.8)</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 2. Different Eimeria species oocysts. H&E X40

Fig. 3. Different Eimeria species oocysts. H&E X40
were sufficiently characteristic to permit instant identification. With others it was often necessary to perform further tests.

3.2.1. *Escherichia coli*

The organism was estimated from the number of typical lactose-fermenting colonies appearing on the plates of MacConkey's agar. These colonies were different in size (2.3mm) with entire or irregular edges, produced red colonies indicating lactose fermentation. Gram-negative bacilli with varying lengths but the majority were short rods.

Representative colonies were subjected to the Eijkman test for confirmation. Production of both acid and gas is positive for *E. coli*.

3.2.2. Lactobacilli

The colonial forms most commonly isolated on Rogosa medium were large grey rough colonies and small white colonies varying from pin-point to several millimeters in diameter.

On Gram’s stain smear: straight or curved rod varying in length, arranged singly or in chains, Gram-positive organism was seen. Non-motile.

3.2.3. Bacteriodes:

These were identified on the media containing neomycin (R.C.M. and Blood agar) to suppress the growth of *E. coli* and lactobacilli and allowed the small colonies of bacteroides to develop, they appeared as small grey colonies up to 0.1 mm in diameter.

Stained smears from colonies revealed Gram-negative rods with rounded ends, arranged singly or in pairs and sometimes short chain was found.
Table 5 Media and incubation period used for the enumeration of the bacterial flora.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Incubation at 37°C</th>
<th>Organisms enumerated</th>
</tr>
</thead>
<tbody>
<tr>
<td>MaConkey’s Agar</td>
<td>Aerobic 24 hours</td>
<td>E. coli</td>
</tr>
<tr>
<td>*RCM +5% citrated blood</td>
<td>Anaerobic 24 hours</td>
<td>Streptococcus sp.</td>
</tr>
<tr>
<td>Salt agar-RCM+8% Nacl</td>
<td>Aerobic 48 hours</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>Neomycin Naglar Agar</td>
<td>Anaerobic 48 hours</td>
<td>Clostridium perferingens</td>
</tr>
<tr>
<td>RCM+neomycin</td>
<td>Anaerobic 48 hours</td>
<td></td>
</tr>
<tr>
<td>Rogosa **</td>
<td>Anaerobic 48 hours</td>
<td>Lactobacillus spp.</td>
</tr>
</tbody>
</table>

* Reinforced Clostridial Medium (Oxoid C.M. 149)

Table No.6. Experiment No.1. Number of different kinds of bacteria in faeces.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Log$_{10}$ viable count of organism per gm of faeces</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A$_1$</td>
</tr>
<tr>
<td></td>
<td>(normal diet)</td>
</tr>
<tr>
<td>E. coli</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>6.5-8.5</td>
</tr>
<tr>
<td>Streptococcus sp.</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>7.0-7.9</td>
</tr>
<tr>
<td>Cl. perfringens</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>N-2.5</td>
</tr>
<tr>
<td>Lactobacillus spp.</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td>7.0=10.0</td>
</tr>
<tr>
<td>Bacteroids</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>7.0-9</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>N-3.6</td>
</tr>
</tbody>
</table>
3.2.4. Streptococci identified on the RCM+blood media

Colonies appearance: about 1mm in diameter, circular granular and greyish in colour. They are gram positive cocci arranged in pairs or short chains non-motile positive cocci arranged in pairs, chain or in varying length. Catalase and oxidase negative.

3.2.5. Clostridium perfringens:

It was estimated from the lecithinase producing colonies of appropriate appearance on the neomycin nagaler agar incubated anaerobically for 48 hours.

Clostridium perfringens showed cloudy zones around the colonies on the media.

Smears from these cultures stained with Gram’s stain showed thick straight –sided rod either singly or in pairs. Spore-forming organism oval in shape and non-motile forming a circular, entire colony was observed.

3.2.6. Staphylococcus aureus

It was estimated from salt agar incubated anaerobically for 24 hours. Gram positive cocci were seen in clusters. Coagulase slide test: (clumping factor) showed positive reaction clumping within 5 minutes,) and indicated presence of Staphylococcus aureus.
3.3. Experimental No (1)

3.3.1. Clinical observation:
In experiment No 1 group (A1, A2 and A3) all animals showed no signs of disease, they behaved normally with normal appetite and temperature and had no diarrhoea or any type of secretions.

Faecal examination was done weekly and no coccidial oocysts were seen in faeces of these animals.

Two animals from each group were slaughtered for postmortem examination. On performing autopsy, no gross lesions were seen on the intestinal segments or any other organs.

Histopathological examination of sections from small intestines and other organs also showed no histopathological changes.

3.3.2. Distribution of bacterial flora:
The results of enumerating the *Escherchia coli*, *Clostridium perfringens*, *Streptococci*, *Lactobacilli*, *Bacteroides* and *staphylococci* in the contents of different regions of the alimentary tract of kids and those of their faecal materials are summarized on tables 7, 8 and 9.

These organisms were the principal ones found in alimentary tract of kids used in these experiments.

Although there were some variations in viable number of microflora in some animals, the mean counts were nearly similar.

In group A1, lower numbers of organisms were present in the anterior parts of the small intestine (duodenum). The numbers then increased progressively in subsequent portion of the small intestine (Jejunum and ileum) and the highest numbers were found in the large intestines (Table 7).
The distribution pattern was in general, shown by all types of organisms identified except the Bacteriodes which were found only in the large intestine, Bacteroides commonly constituted the bulk of the flora in the large intestine.

Lactobacilli in most animals constituted the major component of the flora of small intestine. Streptococci had a somewhat similar distribution to lactobacilli but in lower number. *Escherichia coli* was usually present in all region of the small intestines. It was most numerous in large intestine. *Clostridium perfringens* was either absent or present in small number. *Staphylococcus* spp was frequently found in small numbers.

In group A2, (injectable antibiotic) *E. coli* Lactobacilli, *Streptococci* and *Staphylococcus* spp showed reduction in number compared with that of group A1, Bacteroides, Clostridium spp showed no changes.

In group A3 (high protein diet). *Streptococci clostridium perfringes, bacteoides*, population was increased compared with that of group A1, while *E. coli, lactobacilli and staphylococcus* showed no change.

3.4 Experiment No 2 (interaction of coccidia with gut flora groups B1, B2 and B3)

The results are shown in tables No. 10, 11, 12, 14, 15, 16, 17, 18).

3.4.1. Clinical observation:

All animals in (B1 and B3) showed acute form of coccidiosis except 2 in group B3 which showed peracute form.
Table 7. Experiment No.1: Numbers of different kinds of bacteria in the intestinal content. Group A1

<table>
<thead>
<tr>
<th>Organism</th>
<th>log$_{10}$ viable count of organism per gm content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>E. coli</td>
<td>6.5</td>
</tr>
<tr>
<td>Streptococcus spp</td>
<td>3.4</td>
</tr>
<tr>
<td>Clostridium spp</td>
<td>N</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>5.2</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>N</td>
</tr>
<tr>
<td>Staphylococcus spp</td>
<td>N</td>
</tr>
</tbody>
</table>

N0. 1 = Duodenum
2= Jejunum
3= Ileum
4= Large intestine
Table 8: Numbers of different kinds of bacteria in the intestinal content. Group A$_2$

<table>
<thead>
<tr>
<th>Organism</th>
<th>log$_{10}$ viable count of organism per gm content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>2</td>
</tr>
<tr>
<td><em>Streptococcus spp</em></td>
<td>2.7</td>
</tr>
<tr>
<td><em>Clostridium spp</em></td>
<td>N</td>
</tr>
<tr>
<td><em>Lactobacilli</em></td>
<td>4.3</td>
</tr>
<tr>
<td><em>Bacteroides</em></td>
<td>N</td>
</tr>
<tr>
<td><em>Staphylococcus spp</em></td>
<td>N</td>
</tr>
</tbody>
</table>

N0. 1 = Duodenum  
2= Jejenum  
3= Ileum  
4= Large intestine
Table 9: Numbers of different kinds of bacteria in the intestinal content. Group A₃

<table>
<thead>
<tr>
<th>Organism</th>
<th>log₁₀ viable count of organism per gm content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>E. coli</td>
<td>3.2</td>
</tr>
<tr>
<td>Streptococcus spp</td>
<td>3.4</td>
</tr>
<tr>
<td>Clostridium spp</td>
<td>1.7</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>5.0</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>N</td>
</tr>
<tr>
<td>Staphylococcus spp</td>
<td>N</td>
</tr>
</tbody>
</table>

1 = Dodenum  
2 = Jejenum  
3 = Ileum  
4 = Large intestine
During the first 7 days post infection, all animals showed no signs of disease. Coccidial oocysts were detected in the faeces of kids in these groups on the 8th day of infection. From the 8th to 10th day of infection, all animals gradually became dull, and had reduced appetite, accelerated respiration with slight increase in body temperature in the first days of signs appearance. Two goats from group B3 died before diarrhoea appearance with severe enteritis. Soft faeces appeared 9-11 days post infection later on, the diarrhoea was profuse and tinged with blood and had an offensive odour. Progressive loss of condition was evident in all animals accompanied by staggering, dyspnea dehydration and recumbency was observed shortly before death which occurred within 13-18 days of infection.

In group B2 (injectable antibiotic) oocysts were detected in faeces in this group 12-14 days i.e increase in the prepatent period and the length of diseases started from 13 days to 22 days post infection.

The clinical signs were similar to those of group B1 and group B3.

3.4.2. Pathological changes:
At necropsy, all animals were anaemic, emaciated and dehydrated, carcases body fat was depleted and that of mesentery and the heart became gelatinous. Hydrothorax, hydropericardium and hydroperitoneum were constant features. Hearts were flabby and lungs were normal.
The most characteristic gross changes were observed in the small intestines. Lesions in the jejunum and ileum of all animals were similar although varied in intensity, so they were described together. Round whitish foci (1-2 mm in diameter) were seen on the mucosal surface and also visible even through the serosa (Fig. 4, 6). The wall of small intestine was oedematous and thick (Fig. 5) and the contents were watery, mucoid, and bloody. The mucosae of abomasum in most cases were severely oedematous and haemorrhagic with very small ulcers (Fig. 7, 8). Caeca in some cases were haemorrhagic with thin wall. Large intestine, rumen and omasum were normal. The mesenteric lymph nodes were oedematous and enlarged. The cut surfaces were watery and haemorrhagic (Fig. 9). The pancreas, liver, kidney, adrenal glands and brain were congested and haemorrhagic (Fig. 33, 34, 35, 36, 37).

In the peracute cases (2 animals from group B3) beside, these lesions severe haemorrhage in nasal cavity, the heart, abomasum, liver, pancreas, kidney, adrenal glands and the brain were seen (Fig. 36, 37).

In group B2 (injectable antibiotic + coccidia). The lesions in small intestines were similar to those described previously. But the haemorrhagic enteritis was less in intensity (Fig. 12).
Table 10: Experiment No.2. Number of different kinds of bacteria in faeces of group B₁

<table>
<thead>
<tr>
<th>Organism</th>
<th>Log₁₀ viable count/gm of faeces</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (uninfected)</td>
<td>Infected with coccidial oocysts</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>9.1</td>
<td>10.9</td>
<td>8.3-12.6</td>
</tr>
<tr>
<td></td>
<td>7.8-10.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus spp</td>
<td>7.3</td>
<td>8.2</td>
<td>6.0-9.0</td>
</tr>
<tr>
<td></td>
<td>3.8-8.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium spp</td>
<td>3.4</td>
<td>6.3</td>
<td>2.4-6.6</td>
</tr>
<tr>
<td></td>
<td>N-3.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>9.2</td>
<td>9.2</td>
<td>7.3-10.3</td>
</tr>
<tr>
<td></td>
<td>8.3-10.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteroides</td>
<td>10.0</td>
<td>11.8</td>
<td>7.0-11.8</td>
</tr>
<tr>
<td></td>
<td>8.0-11.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus spp</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
Table 11: Number of different kinds of bacteria in the intestinal content in Group B₁ (infected)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Log₁₀ viable count/gm faeces</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group B₁</td>
</tr>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td>3.0</td>
</tr>
<tr>
<td><strong>Streptococcus spp</strong></td>
<td>3.6</td>
</tr>
<tr>
<td><strong>Clostridium spp</strong></td>
<td>N</td>
</tr>
<tr>
<td><strong>Lactobacilli</strong></td>
<td>5.1</td>
</tr>
<tr>
<td><strong>Bacteroides</strong></td>
<td>N</td>
</tr>
<tr>
<td><strong>Staphylococcus spp</strong></td>
<td>-</td>
</tr>
</tbody>
</table>

1 = Duodenum  
2 = Jejunum  
3 = Ileum  
4 = Large intestine
<table>
<thead>
<tr>
<th>Organism</th>
<th>Group B₁</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Log₁₀ viable count/gm faeces</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>E. coli</td>
<td>4.6</td>
<td>3.7</td>
<td>8.1</td>
<td>9.1</td>
</tr>
<tr>
<td>Streptococcus spp</td>
<td>5.7</td>
<td>8.2</td>
<td>8.2</td>
<td>9.2</td>
</tr>
<tr>
<td>Clostridium spp</td>
<td>N</td>
<td>3.4</td>
<td>5.2</td>
<td>6.0</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>5.1</td>
<td>6.0</td>
<td>6.2</td>
<td>8.1</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>1.2</td>
<td>N</td>
<td>9.3</td>
<td>10.9</td>
</tr>
<tr>
<td>Staphylococcus spp</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

1 = Duodenum
2 = Jejunum
3 = Ileum
4 = Large intestine
Table 13: Normal flora in group B2 (injected antibiotic) (Faecal samples)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Control (uninfected)</th>
<th>Injected by antibiotic</th>
<th>Infected with coccidial oocyts +antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. coli</strong></td>
<td>6.3</td>
<td>7.0-8.0</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7.3-9.0</td>
</tr>
<tr>
<td><strong>Streptococcus spp</strong></td>
<td>8.0</td>
<td>8.3-9.3</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6.5-8.2</td>
</tr>
<tr>
<td><strong>Clostridium spp</strong></td>
<td>1.7</td>
<td>N-2</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N-3.0</td>
</tr>
<tr>
<td><strong>Lactobacilli</strong></td>
<td>3.4</td>
<td>N-6.7</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.2-10</td>
</tr>
<tr>
<td><strong>Bacteroides</strong></td>
<td>8.7</td>
<td>N-8.6</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6.5-10.9</td>
</tr>
<tr>
<td><strong>Staphylococcus spp</strong></td>
<td>N</td>
<td>N-N</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N-4.0</td>
</tr>
</tbody>
</table>

N= viable counts not done
Table No.14. Normal flora in group B₂ from different parts of intestine (control).

<table>
<thead>
<tr>
<th>Organism</th>
<th>Log₁₀ viable count/gm of content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td>2.7</td>
</tr>
<tr>
<td><strong>Streptococcus</strong></td>
<td>N</td>
</tr>
<tr>
<td><strong>Clostridium sp</strong></td>
<td>N</td>
</tr>
<tr>
<td><strong>Lactobacilli</strong></td>
<td>5</td>
</tr>
<tr>
<td><strong>Bacteroides</strong></td>
<td>N</td>
</tr>
<tr>
<td><strong>Staphylococcus</strong></td>
<td>N</td>
</tr>
</tbody>
</table>

N0. 1 = Duodenum  
2 = Jejunum  
3 = Ileum  
4 = Large intestine  
N = viable counts not done
Table 15: Normal flora (group B2 infected with coccidial oocysts +antibiotic) intestinal content.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Log(_{10}) viable count/gm of content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td>3.3</td>
</tr>
<tr>
<td><strong>Streptococcus spp</strong></td>
<td>N</td>
</tr>
<tr>
<td><strong>Clostridium spp</strong></td>
<td>N</td>
</tr>
<tr>
<td><strong>Lactobacilli</strong></td>
<td>5.9</td>
</tr>
<tr>
<td><strong>Bacteroides</strong></td>
<td>N</td>
</tr>
<tr>
<td><strong>Staphylococcus spp</strong></td>
<td>N</td>
</tr>
</tbody>
</table>

N0. 1 = Duodenum  
2= Jejunum  
3= Ileum  
4= Large intestine  
N= viable counts not done
Table 16. Normal flora in group B3 (coccidia + high protein Diet) (Faecal samples)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Control (uninfected)</th>
<th>Infected with (high protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>log$_{10}$ viable count of organism per gm content</td>
<td></td>
</tr>
<tr>
<td></td>
<td>With protein diet</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>9.8</td>
<td>10.3</td>
</tr>
<tr>
<td></td>
<td>7.3-10.8</td>
<td>9.3-12.9</td>
</tr>
<tr>
<td>Streptococcus spp</td>
<td>11.7</td>
<td>11.8</td>
</tr>
<tr>
<td></td>
<td>7.3-12.6</td>
<td>10.7-12.9</td>
</tr>
<tr>
<td>Clostridium spp</td>
<td>2.4</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>N-2.5</td>
<td>4.8-5.6</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>8.3</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>7.1-10.1</td>
<td>5.3-10.9</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>10.0</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>10.0-12.6</td>
<td>10.2-12.9</td>
</tr>
<tr>
<td>Staphylococcus spp</td>
<td>N</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>N-1.9</td>
<td>3.3-4.9</td>
</tr>
</tbody>
</table>

N= viable counts not done
Table 17: Normal flora of intestinal content in group B3 (coccidia + high protein Diet) control.

<table>
<thead>
<tr>
<th>Organism</th>
<th>log₁₀ viable count of organism per gm content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>E. coli</td>
<td>4.1</td>
</tr>
<tr>
<td><em>Streptococcus</em> spp</td>
<td>3.1</td>
</tr>
<tr>
<td><em>Clostridium</em> spp</td>
<td>N</td>
</tr>
<tr>
<td><em>Lactobacilli</em></td>
<td>4</td>
</tr>
<tr>
<td><em>Bacteroides</em></td>
<td>N</td>
</tr>
<tr>
<td><em>Staphylococcus</em> spp</td>
<td>N</td>
</tr>
</tbody>
</table>

1 = Duodenum
2 = Jejunum
3 = Ileum
4 = Large intestine
N = viable counts not done
Table 18: Normal flora of intestinal content in group B_3 (coccidian + high protein ration).

<table>
<thead>
<tr>
<th>Organism</th>
<th>log_{10} viable count of organism per gm content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td>3.6</td>
</tr>
<tr>
<td><strong>Streptococcus spp</strong></td>
<td>3.0</td>
</tr>
<tr>
<td><strong>Clostridium spp</strong></td>
<td>3.6</td>
</tr>
<tr>
<td><strong>Lactobacilli</strong></td>
<td>5.0</td>
</tr>
<tr>
<td><strong>Bacteroides</strong></td>
<td>N</td>
</tr>
<tr>
<td><strong>Staphylococcus spp</strong></td>
<td>N</td>
</tr>
</tbody>
</table>

1 = Duodenum
2 = Jejunum
3 = Ileum
4 = Large intestine
N = viable counts not done
Fig. 4. Intestine. Note whitish nodules on mucosa visible from serosa.

Fig. 5. Intestine. Note whitish nodules on mucosa visible from serosa.
Fig. 6. Intestine. Note haemorrhage and thickening of intestinal wall.

Fig. 7. Abomasum. Haemorrhages on the mucosal surface.
Fig.8. Abomasum. Haemorrhages on the mucosal surface

Fig.9. Lymph node. Haemorrhagic and oedematous.
Fig. 10. Intestine. Severe haemorrhages.

Fig. 11. Intestine. Severe haemorrhages in small intestine.
Fig. 12. Small intestine. Haemorrhage is less intense (upper group B2 antibiotic) than the lower (group B1).

Fig. 13. Small intestine. Hyperplasia of epithelial cells with individual cells showing hypertrophy. H&EX10
Microscopically. In small intestine, severe villous atrophy was seen with complete loss of intestinal villi, crypts epithelial showed hydroplasia while individual cells were hypertrophied (Fig.13).

Sloughing of epithelial cells caused focal erosion and ulceration of the villi (Fig.14,15). Epithelial cells covering the villi were flattened and irregular in size and shape. Severe necrosis was seen in mucosa(Fig. 17, 18).

Various coccidial stages seen in the epithelial cells of the villi and glands. Merozoites found in cells displacing the nucleus of the host cells surrounded by clear plasmatic halo. Different size and stages of schizonts were also present and surrounded by distinct walls (Fig.16 ). In some cases epithelial cells showed hyperplasia, while individual cells showed hypertrophy (Fig.20). All individual cells in one case were occupied by different size and stages of schizonts and no other stages of coccidia were seen (Crowding phenomenon) (Fig. 21, 22).

A sexual stages (Macro- & microgametocytes) appeared in section as glistening orange-red globular bodies (macrogametes) and as slender slightly pointed basophilic bodies (microgametes) (Fig.23,24 ). The developing oocyst exhibited an eosinophilic wall and had bluish pink granular content (Fig. 25).

The lamina propria and submucosa showed variable degrees of congestion (Fig.27), haemorrhages and marked cellular infiltration of lymphocytes, plasma cells, macrophages and sometimes eosinophils (Fig.28 )
In some sections, abomasal and caecal muscosae showed congestion and hemorrhage.

Mesenteric lymph nodes showed depletion of lymphocytes and loss of cortical lymphoid follicle structure schizonts of different stages and sizes were seen in pericapsular connective tissues in the capsule and marginal subscapular sinuses and in side lymphatic (Fig.29,30,31,32). Also schizonts were seen in cortex and medulla.

Microscopically, appearance in the peracute cases is similar to above in addition to Nasal cavity haemorrhage.

In group B2, the picture was the same as the one mentioned above. The coccidial schizonts were seen in the kidney of only one kid in group B1(Fig.33).
Fig. 14. Small intestine. Sloughing of epithelial cells causing focal erosion with haemorrhages and cellular infiltration. H&EX40

Fig. 15. Small intestine. Sloughing of tips of villi cells in lumen and invaded with schizonts. H&EX40
Fig. 16. Small intestine. Sloughing of epithelial cells in lumen of small intestine and heavily invaded with coccidial stages. H&EX40

Fig. 17. Small intestine. Severe necrosis of villi. H&E.X40
Fig. 18. Small intestine. Severe necrosis of villi with congestion. H&E. X40

Fig. 19. Small intestine. Gametocytes are predominant, though other stages are also seen. H&E. X10.
Fig. 20. Small intestine. Note hypertrophied epithelial cells invaded with coccidial stages and developing oocysts. H&E. X40.

Fig. 21. Small intestine. All the epithelial cells and crypts cells are invaded only with different stages of schizonts and no other stages of coccidia seen. H&E. X40.
Fig. 22. Small intestine. Different stages of schizonts and no other stages of coccidia seen (Crowding phenomenon). H&E. X40.

Fig. 23. Small intestine. Gametocytes invading almost all epithelial cells (micro, macro and developing oocysts). H&E. X40.
Fig. 24. Small intestine. Gametocytes invading almost all epithelial cells (micro, macro and developing oocysts). H&E. X40.

Fig. 25. Small intestine. Developing oocysts with gametocytes. H&E. X40.
Fig. 26. Small intestine. Developing oocysts in the lumen. H&E. X40.

Fig. 27. Small intestine. Lamina propria and submucosa showing variable degrees of congestion, haemorrhages and cellular infiltration. H&E. X40.
Fig. 28. Small intestine showing cellular infiltration of lymphocytes and plasma cells. H&E. X40.

Fig. 29. Mesenteric lymph node. Coccidial schizonts in the subscapular sinuse. H&E X40
Fig. 30. Mesenteric lymph node. Coccidial schizonts in cortex. H&E X40.

Fig. 31. Mesenteric lymph node. Coccidial schizonts in the internodular trabeculae. H&E X10.
Fig. 32. Mesenteric lymph node. Coccidial schizonts in the lymphatic vessel. H&EX10

Fig. 33. Kidney. Coccidial schizont in the kidney. H&EX40
Fig. 34. Kidney. Degenerated tubules congestion and haemorrhage. H&E X40

Fig. 35. Liver, Fatty cytoplasmic vacuolation. H&E X40
Fig. 36. Brain. Severe congestion with inflammatory cells infiltration. H&E X10.

Fig. 37. Brain. Severe congestion with inflammatory cells infiltration. H&E X10.
3.5.1. **Haematological parameters:**

The mean Hb concentrations in group B1, B2 and B3 are shown in tables (19, 20 and 21). The values in these groups show slight variation during the first 9 days of experiment and thereafter exhibited a gradual increase till the end of observation. The two kids showing peracute disease in group B3 have low Hb concentration and low PCV%.

The PCV% mean values in group B1 B2 and B3 are summarized in tables (19, 20, and 21). The changes were also similar to those of Hb concentration values. The mean values of PCV were maintained around 25-26 during the first 9 days of experiment but increased to 28-37% from day 12 to the end of the experiment. In the control groups the values fluctuated between 21-24%.

The mean Hb concentrations in group B1, B2 & B3 are shown in tables (19, 20, and 21). The values in these groups showed slight variation during the first 9 days of the experiment. And thereafter exhibited a gradual increase till the end of the observation period. Two kids showing peracute disease in group B3 had low Hb concentration and low PCV values, compared with those of the control animals. The Hb value in the control animals fluctuated within the normal values. The PCV mean values in groups B1, B2 and B3 are summarized in tables (19, 20, and 21).

The changes were also similar to those of Hb concentration. The mean values were maintained around 25-26% during the first 9 days but increased to 28-33% by day 12 to the end of the experiment. In the control, the values fluctuated at 21-24%.
3.6. Results of Pathogenicity experiments No.3.

3.6.1. Results of Experiment A:
All kids in this trial remained apparently healthy with normal temperature and manifested no diarrhoe or any other clinical signs.

3.6.2. Results of experiment B:
The two kids in this trial showed acute form of the disease, which ended fatally without apparent clinical signs within 18-20 hours except general weakness and recumbency prior to death.

1- On necropsy, severe haemorrhages enteritis affecting almost all small and large intestines was observed (Fig. 10 and 11). Congestion and haemorrhage in liver, kidneys, heart and mesenteric lymph nodes (Histopathologically (Fig. No. )).

2- Histopathological impression smears from the intestines, stain with Gram staining revealed pure population of shorts thick Gram-positive organism. On culturing intestinal content in MaConkey's agar plate incubated at 37°C for 24-48 hours, no growth obtained. When culturing these materials on blood agar (Sheep blood), and Incubated anaerobically for 24 hours, these cultures developed greyish opaque colonies and double zone of haemolysis, inner narrow zone, followed by wide zone of incomplete haemolysis.

In RCM media incubated anaerobically for 24 hours. The isolates demonstrated production of turbidity and gas. These isolates were non motile and changed the colour of LM agar from red to yellow and produced gas which indicative for lactose fermentation.
On Naglar agar, the isolate demonstrated the opalescence in egg yolk. On culturing the intestinal content on blood agar and incubated aerobically no growth obtained.

So the organism obtained from these intestinal contents were identified as *Clostridium perfringens*.

### 3.6.3. Results of Experiment C:

Toxigencity in mice. It was found that toxins extracted from clostridium spp isolated from intestinal content were lethal to mice (7 out of 8). Affected mice demonstrated a clinical signs including dullness, reluctant to move, tremor rough coat, abdominal respiration and death occur within 8-24 hours.

### 3.6.4. Results of Experiment D:

The two kids in this trial developed peracute form of disease which ended fatally. The kids died within 18-20 hours without any clinical signs except that the animals became weak and depressed with accelerated respiration and recumbency before death.

At necropsy the intestines and caecum were hyperaemic and edematous with widespread haemorrhages on the serosal surface. There was congestion of liver, kidneys and brain (Fig.34, 35, 36, and 37).

There was severe haemorrhage on the intestinal section, abomasum, kidneys, liver and adrenal gland.

Impression smears from the intestinal mucosa and stain by Gram stain it reveal an almost pure population of short, thick, Gram-positive rod. When cultured these intestinal content on blood agar (sheep blood) and incubated anaerobically for 24 hours these cultures revealed greyish opaque colonies. These colonies developed a double zone of
haemolysis: inner complete zone of haemorrhage and incomplete outer layer. In RCM media incubated anaerobically, turbidity and gas were noticed. These organism were non motile, changed the LM agar from red to yellow with production of gas which was indicative for lactose fermentation. On Naglar agar, the isolates demonstrated the opalescence in egg yolk. Accordingly the organism was identified as *Clostridium perfringens*.
Table 19. Experiment No. 2 group B1 (normal diet) Haemoglobin (Hb) concentration and packed cell volume (PCV) values.

<table>
<thead>
<tr>
<th>Day</th>
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<th>Control group</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>PCV%</td>
<td>Hb gm/100ml</td>
</tr>
<tr>
<td>3 days Before</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>26.3</td>
<td>8.5</td>
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<tr>
<td></td>
<td>21-35</td>
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<td>8.9</td>
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<tr>
<td></td>
<td>22-34.6</td>
<td>6.6-10</td>
</tr>
<tr>
<td>12</td>
<td>28</td>
<td>9.3</td>
</tr>
<tr>
<td></td>
<td>24-38.0</td>
<td>7.2-11</td>
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<td>37</td>
<td>11</td>
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<td></td>
<td>29-40</td>
<td>8-12</td>
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Table.20. experiment No. 2 group B2 (injectable Gentamicin) (Hb) and (PCV) values.

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<td>27</td>
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Table.21. experiment No. 2 group B3 (High protein diet) Hb and PCV values.

<table>
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<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb (gm/100ml)</td>
<td>Hb(gm/100 ml)</td>
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<tr>
<td></td>
<td>PCV%</td>
<td>PCV%</td>
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<td></td>
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<tr>
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<td>22.6 21-24</td>
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<td>26.8 19-37</td>
<td>23.9 23-24</td>
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<td>9</td>
<td>6.8 4.2-10</td>
<td>7.6 7.2-7.8</td>
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<td>23 22-24</td>
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CHAPTER IV
DISCUSSION
Animals and human beings have involved in intimate and constant association with a complex microflora, under natural condition, the development and functions of their tissue are influenced by countless, microorganism which are always present in the digestive and respiratory tract and probably in other organs. The anatomical structures and physiological needs have been determined in part by the microflora which prevailed during evolutionary developments and that many manifestation of the body at any given time are influenced by microflora now present.

We can say that microflora is part of the environment to which animals and man have had to become adapted, and to which they have come to depend.

The factors which determine the initial colonization are not fully understood, but must be related to environment, including birth canal and to diet Fidler (1996) reported that bacterial colonization of digestive tract commences already at birth when interaction between gut microflora and diet starts. It changes with age and state of health. Subsequent factors such as, natural diet during pregnancy, method of delivery, diet of neonatal and gestational age, influence the initial colonization of the gut.

The studies of quantitative and qualitative bacteriological of intestinal contents and faceces must show considerable error due to complex materials used. To minimize such error, highly selective media were used for enumerating these organism (Smith, 1965, 1965b and Lorna, 1968) and the studies have concerned chiefly with
more common organisms, *E. coli*, streptococci, lactobacilli, clostridia, bacteroides and staphylococci. The emphasis placed on these organism is explainable in large part, if not completely, by the fact that they tend themselves to *in vitro* studies because they grow readily on the artificial culture media. Yet it is certain that they represent but a very small part of the total indigenous microflora and may be the most important and in large population.

Although there were similarities in age of animal, diet and environmental condition, considerable differences in bacterial count among these animals were observed. These differences can be attributed to errors introduced in experimental procedures, to normal animal variations such as food intake or rate of passage of digesta between individuals or other unknown factors.

Our results for counting different types of bacteria in small intestine, large intestine and faeces, resembled in general, those obtained by workers using different techniques (Haemel and Muller-Beulhow, 1956; 1957) and also with those using the same techniques (Smith, 1961, Lorna, 1968).

In experiment No.1 group A, the distribution pattern of microflora showed by all types of organism identified from the alimentary tract was that the organism except Bactericides was present in small number in duodenum and then increased progressively in subsequent portion of small intestines till it reached the highest number in large intestine and faeces (Draser and Hill, 1974 and Filder, 1996). The bacterial populations in the faeces are representative of those of large intestine (Moore et al, 1978). Bacteroides were found only in large intestine and faeces.
in most cases and this found by most of investigators (Draser and Hill, 1974; Fidler, 1996) except Graber et al, (1966) who isolated bacteroides from the anterior part of the intestine and attributed their findings to vigorous swabbing to the mucosa to elute the entrapped bacteria.

The highest number of bacteria found in large intestine mostly due to that much slower rate of progress of contents through the large intestine and it was never empty. These results show a fairly close similarity to those reported by (Smith, 1965, Smith, 1965b, Lorna, 1968 and Fidler, 1996).

In addition to that distribution the bacterial flora remained essentially the same throughout the period observation (4 weeks) and those may be remained essentially the same throughout the life span of animals as long as these were maintained under favourable environmental and physiological conditions (Lee and gemmel, 1972, Rotimi and Duerden, 1981).

The normal physiological development of the intestinal flora still requires extensive research. The microbial colonization of the intestinal tract differs substantially from part of the tract to another due to different physical and chemical conditions and differences in substrate availability. So this distribution is mostly controlled by the pH in different parts, from low pH in the anterior part to high pH in posterior parts. The pattern of development of the bacterial population was as followed. Lactobacilli, streptococcus, *E. coli* and *Clostridium perfringens*, though this different degrees depending on properties as their acid tolerance (Smith, 1965b; Lorna, 1968).
Variation in populations size corresponded closely to pH change with the highest population in the distal jejunum or proximal ileum. The highest number of viable bacteria in these regions of the small intestine probably is result of pH (6.6-8) (Ben-Ghedalia et al, 1974; Drasar and Hill, 1974).

Dukes (1947) stated that the pH increased along the length of small intestine, starting from duodenum until its peak in the jejunum and ileum. Then, declined in the posterior ileum and caecum and then increased in other parts of large intestine. The increase of pH result from bicarbonate in pancreatic and intestinal secretion. As well as diffusion urea into the intestine from blood (Nolan, 1975).

Accordingly, the population lactobacilli, streptococcus spp and Bacteroides remained at high level as indicated in table (4). Whereas *E. coli*, clostridium spp and staphylococcus spp remained at low levels as compared to the above. These findings are in agreement with those reported by Schaedler et al, (1965) and with group A₂ which given gentamicin (as injectable antibiotic). Gentamicin commonly used for treatment of infectious diseases influenced gut microflora in that, it altered the negative aerobes enteric organisms *E. coli*, Streptococcus spp and staphylococcus while no change in anaerobic organisms because the drug need to actively transported across the cell membrane and this does not occur in anaerobic organism which are therefore resistant (Peter and Tom, 1996).

Methes (1988) found that administration of penicillin ampicillin and lincomycin altered microflora composition in favour of Gram-negative bacteria causing diarrhoea and death while
tetracycline, chloramphenicol, erythromycin and spectinomycin had no effect on gut microflora.

Treatment with penicillin oxteteracycline brought about a rapid and often lasting disappearance of these dominant bacterial group (Streptococci, bacteroides lactobacilli) (Dubos et al, 1965).

In group A3 (with high protein diet) streptococi, Clostridium perfringens and Bacteroides populations were increased compared with that of group A, while E. coli lactobacilli and staphylococcus spp remained as that of group A1.

The present results provided further emphasis on relationship between high protein diet and proliferation of microflora especially clostridium perfringens. Lisa Williamson, (2002) reported that among several condition favoured overgrowth of Clostridium perfringens is diet excessive in protein and carbohydrate. Allori et al, (2000) have demonstrated that in malnourished mice’s a decrease in number of lactobacillus spp and anaerobic microorganisms was observed, where as there was an increase in number of enterobacteriaceae. These mice when treated with dried skim milk a significant improvement in the microflora in small and large intestine was found.

Dubos and Schaedler (1962) reported that the composition of the diet affects profoundly the faecal population of lactobacilli, both, qualitatively and quantitatively. They also indicated that the casein diet brought about a sharp decrease in the numbers of lactobacilli.

On the other hand, Bornside, (1978) and Hill (1981) found that the dietary change on the bacterial population had shown no real effect. Generally the intestinal microflora and nutrition of the host
have several complicated but important interaction. Some of these interactions are beneficial to the host but some may be detrimental. However, the activity of the bacteria is complex and very little understood at present (Christine Edwards, 1993).

The adverse effects of Eimeria infection result from destruction of the gastrointestinal epithelium as these intracellular parasites exhibit their complex life cycle in the digestive tract. Because of the multiple cycles of a sexual and then sexual reproduction that can occur, successive host cell disruption occurred. The severity of the disease depends on pathogenicity of *E. species* and, in turn, the pathogenicity of Eimeria species depends on the number of microzoites that may be formed by each schizont.

The most pathogenic species of Eimeria is *E. ninakohly a kimovae, E. arloingi* and *E. christensi* (Norton, 1986; Githigia et al, 1992). These species of Eimeria when enter the epithelial cells many thousands of merizoites may be formed in each schizonts. The microzoites are released from the cells and invade a new epithelial cells and this reported for several cycles before sexual cycle. So the disruption and damage of intestinal epithelia can be wide spread. Guss (1981) stated that severity of coccidiosis directly related to the degree of damage to the gut, number of coccidial oocysts administered and immunologic resistance of the animals. Elgezuli et al, (1979). However, reported no correlation between the number of oocysts administered and manifestation of clinical signs. Pout (1974) stated that the resistance and the nutritional status may adversely be affected in spontaneous cases but not in experimental
ones. Stuart et al, (1982) reported that severity of experimental disease is dose and age-related.

Larger doses of oocysts would induced more extensive lesions in small intestines during the asexual phase leading to insufficient number of intact villi to support the gametogenic phase of the cycle. These were seen in figure (21 and 22), where all the intestinal epithelia were occupied by large number of different size and stages of schizonts only and no gametocytes or oocysts seen. A similar phenomenon occur in Eimeria acervulina infection in chickens is called the “crowding effect” (Krassner, 1963). It is also possible that interferon or interferon-like substance produced in response to coccidia might intervene in this phenomenon (Hammond and long, 1973). The number of oocysts is less as compared to the lesions found in this animal. It appears also that the age of the inoculum might have an important influence on the oocyst discharge.

In our study, although animals in all groups have been infected with similar infected doses the number of oocysts discharged has shown mark variations at the beginning but terminated in uncountable number, this may indicate that no correlation appeared to exist between infective dose and oocysts production (Marquardt, 1960, Pout, 1974; Meyer, 1982).

The individual variation in oocysts production may be due to variation exystation within the animals which in turn depends on intestinal pH, CO₂ tension, bile salt and trypsin level (Vercruysse, 1982).

Mixed infections with two or more species is a rule rather than exception (Osman, 1988). Chhabra and Pandey (1991) reported
very high populations of goats were infected with Eimeria species and identified 12 species. They reported that most of positive cases were mixed infection.

Excretion of oocysts in all groups started from (8-10) days post infection. In literature the excretion started (10-12) days post infection (Yvore and Besonard, 1980, Osman, 1988,). This period appeared longer than our reports. The differences may be due to the pathogenicity of species, number of schizonts generated or to the mixed infection or probably other unknown reasons.

In the present study, kids in all group of experiment number 2 developed acute or peracute disease which terminated fatally within (2-4) days after appearance of clinical signs (mortality 100%). It evident from these results that young goat kids are highly susceptible to enteric coccidiosis as already stated by (Osman, 1988, Bowman and Lynn, 1995).

Diarrhoea was the main manifestation of the disease severely affected animals passed watery, faeces that contained mucus in some cases the faeces became bloody with fibrin casts and mucosal strands. Other signs included dullness, inappetence and progressive loss of condition (Opaka-Pare and Chineme, 1979; Dent, 1980; Mohan Kumar, 1980; Osman, 1988 and Lisa Williamson, 2002).

Diarrhoea in young ruminants is a syndrome of great aetiological complexity, in addition to influence of varied environmental, managerial, nutritional and physiological factors, the infective agents capable of causing diarrhoea in these animals are numerous. Thus while reports of occurrence of individual microorganisms as cause of diarrhoea are common, concurrent
infections with two or more microorganisms have occurred. Dehydration, acidosis, impaired growth or death are the major consequences of diarrhea. Diarrhoea may result from hypermotility, increased permeability, hypersecretion and malabsorption (Moon, 1978).

In acute coccidiosis, diarrhoea may be due to severe enteritis caused by developing of the parasites within the intestinal epithelial cells till its rupture and new cells invaded for several times. These process ended in destruction of intestinal mucosa and sloughing of the epithelial lining which in turn lead to incomplete digestion and mal absorption. In contrast, following destruction of villous epithelium, crypt-epithelium increase in rate of proliferation and expanded in size so secretary capacity increased (Buller et al, 1978; Moon, 1978; Rahinson et al, 1983).

When ulceration of mucosa becomes extensive it leads to more fibrin-necrotic enteritis the increase of permeability of the intestinal mucosa secondary to inflammation will also contribute to diarrhoea and less of protein and repair will be more difficult (Moon, 1978).

Thus if total secretion increases beyond absorption capacity or if the absorption is impaired, net secretion results and the excess will expelled resulting in diarrhoea (Bywater, 1970, Blood et al, 1989). Davis et al, (1963) mentioned that diarrhoea was the most common sign of coccidiosis in various mammals and its severity depends upon the species of coccidia involve. Opka-Pare and Chineme (1979) reported watery diarrhoea in 4-7 days in kids before death.
Guss (1981) observed passage of bloody or tarry diarrhoea associated with straining in kids showing acute coccidiosis.

Clinical signs of coccidiosis are probably related to severity and extend of the villus atrophy and cell replacement induced the coccidia in small intestine. These changes cause a mal digestion and mal absorption of nutrients because of loss of digestive absorptive surface of small intestine (Moon, 1978). Villous atrophy with secondary mal digestion and mal-absorption has also been suggested as the pathogenesis of infection with coccidiosis in chicken and in lambs (Pout, 1974) and Isospora infection in dog (Dubey, 1978).

Our investigations have shown that the animals in group B2 given antibiotic (gentamicin at the time of infection and subsequently for a week) had only delayed the appearance of oocyst in faeces and clinical signs. Except these, all the animals in group B2 showed the same clinical picture of acute coccidiosis. Antibiotic was given to suppress proliferation of bacterial flora during the infection of coccidia to compare the interaction of coccidia and normal flora. The delay in appearance of clinical signs may attributed to suppression of the drug on the coccidial reproduction (Guss, 1977; Yvore, 1984; Dash and Misra, 1988). So the gentamicin administration in group B2 might have also coccidiostatic action which suppress only the development for a time and the disease flared up again (Osman, 1988). But the real action of the antibiotic and delay of the clinical signs is unknown and need further studies.

The pathological changes observed were confined mainly to the small intestines. Grossly, signs of catarrhal to haemorrhagic
enteritis. In the peracute cases, the intestinal lumen may contain fresh blood. The most consistent and characteristic lesions is the occurrence of multiple raised white nodules on the intestinal mucosa. These nodules represent sites of active gametogony. These were similar to those described by Helfer and Koller, (1976); Yvore and Besnard, 1980; Ernest and Benz, (1981), Osman,(1988) and Gregory and Catchpole (1990).

In some cases there severe haemorrhage in the abomasum with erosion and ulcer. Sivadas et al, (1965) also reported oedema and hyperaemia of the abomasum. Extensive involvement of all small intestine may be due to the infection by a number of Eimeria species Pout, 1974).

Histopathologically, the most prominent lesions observed in the small intestine particularly in jejunum and ileum were atrophic villi, degeneration, necrosis and desquamation of epithelial lining. Most of mucosal glands, crypts of lieberkuhn, were vacuolated and their epithelial cells were fragmented and occupied by different stages of coccidia. These results are in line with previous findings (Pane, Bahah’s chanhan, 1972, Singh and Rhagwan, 1974; Deb and Ansari, 1981 ; Gasmir et.al, 1998).

Hyperplasia of both crypts and villi would explain the grossly observed thickened wall. (Kent and Moon, 1973). Large destruction of epithelial cells with reaction with surrounding tissues by sloughing off the dead cells. If enough cells have destroyed the mucosa may be removed down to the muscular layer (Or deeper) of the intestine. This bares the small vessels in the capillary bed and
leads to profuse bleeding into the lumen (Gregory and Catchpole, 1990).

The mucosal blood capillaries were congested and there were superficial haemorrhages and necrosis (Blood et al, 1989).

Indigenous bacterial flora is known to be of substantial benefit to the host (Finegold et al, 1983; Hentages et al, 1985; Wilson and Freter, 1986; Wilson et al, 1988). It develops as result of the influence of the intestinal physiology on the interaction between the bacteria that contaminate the body (Draser and Barrow). Bacteria in gut may affect digestion and absorption, the products of bacterial fermentation may provide nutrients or affect the well-being of the host, but diet may also affect survival and metabolism of the bacteria.

In group B2 (high protein diet) populations of *E. coli* and *Clostridium perfringens* were increase. The proliferation of bacteria in this group may increase the severity of coccidial lesions where peracute disease were observed in two cases. This is not confined to this group, but it may true in all groups of experiment 2.

In acute coccidiosis, the condition occurring in intestinal lumen, was due to change in the intestinal pH (alkaline), transit, motility and permeability of the intestine, lesions with modification of the villi and crypts and often haemorrhage and necrosis, these will be suitable and encouraged the proliferation of bacteria found there especially the most pathogenic ones like *E. coli* and *Clostridium perfringes*.

*E. coli* and *Clostridium perfringens* were isolated from liver, intestinal content, mesentery lymph nodes and nasal cavity. Berg
and Fuller (1992) have shown that certain bacteria can pass from the gastrointestinal tract to partial blood and the liver when the permeability of the intestinal mucosa is increased due to destruction of intestinal mucosa. Johnson and Sarles (1948) and Bardley and Rodhokrishnan (1972) stated that the growth of Clostridium spp was stimulated by infection of *Eimeria tenella*. Also growth of *Clostridium perfringens* has been found to be favoured by decreased intestinal motility and intestinal injury.

To the best our knowledge, there are no records on the experimental studies of goats or other ruminants and their interaction with microflora of the gut. All information regarding such studies was derived from observation on *Eimeria tenella* and cecal microflora (Visca and Burns, 1972; Bradley et al, 1972; Nakamura et al, 1990).

The role of cecal microflora in the development of cecal coccidiosis has long been suspected (Ott, 1937). Bacteria belonging to coliform (*E. coli*) and spore-forming anaerobic group have been frequently isolated from liver, spleen, heart and blood of chicken which succumbed to coccidiosis (Rahhakrishnan, 1971).

It was also shown that clinical manifestation and mortality do not occur in bacterial-free chicken infected with *Eimeria tenella* oocysts and that chickens with one or two species of bacteria developed more severe lesions of coccidiosis than do bacteria free counterparts (Visco and Burns, 1972; Turk and Littlejohn, 1987; Nakamura, Isobe, Narito, 1990).

It is also known that during the course of cecal coccidiosis the growth of *Clostridium perfringens* and coliforms especially *E. coli* is
stimulated. Baba et al, (1990) concluded that intestinal bacteria increase the severity of coccidial lesions without causing bacteraemia and toxaemia.

In sheep, the absence of bacterial flora reduced coccidia development and suppressed the pathological consequences. It was also noted that parasite development was slowed down, the prepatent period was increased by around 4 days, the exact reasons and mechanism involved were due to lack of understanding only hypothesis can be proposed. Among those are the structural properties of the digestive mucosa, the fact that they are poorer in lymphoid elements, and some media characteristics such as pH, bile salts, softening of the faeces and slow transit rate several of the characteristics of the axenic animals could affect the parasite development and host reactivity. From these we can concluded that microflora was essential to the development and expression of pathogencity of parasites (lafont et al, 1975; Owen 1975; Yvore, 1989). Although limited work was carried out in lamb and rabbit (Yvore, 1989) but the overall results were almost similar in both avian and mammalian species; these results indicated an accelerated growth and multiplication of certain types of enteropathogenic organisms such as *E. coli* and other members of Gram-negative enterobacteriaceae (Strombeck and Guilford 1991 ; Mohamed et al, 2000).

Histopathological results indicate that the presence of viable intestinal bacteria was not essential for the development of *Eimeria tenella* in fowl, but since first and second generation schizonts were found in ceca of bacteria free chick, it appeared that presence of the
flora might favourably affect the excystment, penetration, or development of the early stages of the parasites. Also no change in oocyst discharge (Long, 1970; Visco and Burns, 1972). In contrast, Hegde et al, (1969) stated that *Eimeria brunetti* is able to produce the typical disease independently of the presence of intestinal microbial flora.

In this report we consider coccidia the principle cause of major intestinal lesions and significant clinical diseases and this can be prove histologically by severe destruction of all epithelial cells of villi and of the glands which were completely replaced by different stages of coccidian. However we must put in mind that bacterial flora may facilitate the development of lesions by invading the lamina propria denuded by coccidia. This was emphasised by the occurrence of peracute case of coccidiosis in group B3 (high protein diet) in which protein diet enriched the medium that already occurred in the intestine and which is favourable for bacterial proliferation. Also in group B2 (injectable antibiotic) where less haemorrhagic enteritis was seen comparing to other groups.

Still this is not restrictedly perfect because no solid evidence of such interaction between coccidia and normal flora. Further investigation must be done using ideal bacterial free animals for real comparison.

In experiment 3, trial A: animals in the two groups and control, remained normally and no any clinical signs of disease developed. Although many investigators believed that *E. coli* is a cause of diarrhoea in young animals experimental evidence is inconclusive, especially when administered to those which have
received Clostrum before (Gay, 1965; Sojka and Rutler 1975 and Besser, 1993).

Terlecki and Sojka (1965) stated that although the natural route of infection may be by mouth, oral administration of *E. coli* had no effect in kids 3-month-old or more. Fey and Margadant (1962) and Smith (1962) showed that experimental infection failed if the calf ingested *clostrum* immediately after birth and with calf with a normal concentration of gamma globulin in their serum. These results is similar to our results in group that allowed to take clostrum normally. But in the group that deprived from clostrum, no clinical signs were observed, in contrast to that found by Penhale et al, (1970), Mellor and Murray(1986) and Aldridge et.al,(1993),who mentioned that gamma globulin is probably the most important component required for prevention of *Coli septicaemia*. Smith and Halls (1968) reported that Strains of *E. coli* from cases of bacteraemia in calves will invade the blood stream when given orally to calves that are deficient in immune globulin’s but not when given to normal calves correspondingly they will grow in vitro in globulin deficient calf serum but not in normal calf serum.

According to smith and Halls (1968) and Besser (1993), the principal means of defense of animal again bacteriaemia is antibodies absorbed from Colostrum which sensitize coli bacteria to phagocytosis by cells of reticulendotheilial system.

The explanation of our results may be most due to that our strain isolated from normal animals are not pathogenic.Schuman,(1990) and Frank et al, (1994) mentioned that *E.coli* strain isolated from diarrhoeic lambs and goat kids are not
generally toxigenic and belong to a large number of O serogroup. Also may be due to other factor that inhibit the proliferation of bacteria such as the pH of the stomach, which is low enough to suppress bacterial growth. Sojka (1971) suggested that the stomach pH is sufficiently low to suppress multiplication of *E. coli*.

It is important to remember that Colostrum is not only a source of passive immunity for animals but also a food containing protein and vitamin A will do that young ruminant on a diet which induce vitamin A and also suckling ruminant born to dams which have been on a diet rich in protein and vitamin A will pass a *clostrum* rich in vitamin which give good passive immunity for their younger. Radostits et al, (2000) stated that factors important in understanding the pathogenesis of colibacillosis are immune status of animals and the virulence attributes of the strains of *E.coli*, particularly its capacity to invade tissue and produce a septicaemia or to produce enterotoxin which causes varying degree of severity of diarrhea.

In the other group given the *E. coli* broth culture intraduodenally, the animals died within 20-48 hours with extensive haemorrhage all over the intestines, mesenteric lymph nodes, liver, kidneys heart adrenal gland and pancreas. The disease is diagnosed as clostridial infection rather than *E. coli* infection. The explanation of this results may be due to the fact that growth of *Clostridium perfringens* is favoured by the decreased intestinal motility resulted from stress of surgical operation. In other words, the proliferation of *Clostridium perfringens* in conjunctions with reduced peristalsis enhances the concentration and pathogenic potential of the toxin.
produced by the organism (Kimberling, 1988). Beside, the overgrowth of *E. coli* administered in the anterior part of the intestine caused imbalance in microflora of the intestines and favoured medium suitable for *Clostridium perfringens* by utilization of more oxygen and the stress due to operation done to infect the animal.

These results are compatible with those of Bradley and Radhakrishnan (1972) who reported that clostridium growth in man and animals has found to be favoured by decreased intestinal motility and intestinal injury. Also Freter et al, (1983) observed that the population of most indigenous anaerobes are controlled by one or more nutritional substrate, the prevailing condition of pH and anaerobiosis. Styeffanus (1997) reported that stress can alter the balance of gut microflora through a decrease in motility of the intestinal tract. In contrast, Savage et al, (1971) found that experimentally induced stress will result in change in normal intestinal flora leading to decrease intestinal anaerobes with subsequent increase in coliform bacteria but the reasons are not known.

In trial D, kids developed per acute disease, which ended fatally without apparent clinical signs. Shanks(1949), Oxey (1956); Wanasinghe (1973); Blackwell et al, (1991) and Blackwell and Butter (1992) reported that peracute form of enterotoxaemia in goat is similar to the disease in sheep with animals often found dead without observed clinical signs , and the death occur within 24 hours.
Blackwell et al. (1991) mentioned that in kids infused intraduodenally with culture of *C. perfringens*, lethargy, diarrhoea, abdominal distension and sudden death were observed within 6 hours after infusion began.

Uzal, Pasini, Olaechea, Robies and Elizondo (1994), reported small foci of malacia and perivascular oedema in 3 out of 10 goats dying of enterotoxaemia. However, there was a simultaneous heavy infestation with coccidia in these animals. Similarly, it was also suggested by Graig (1986) that the mucosal cell disruption produced by coccidiosis affects the permeability of the intestinal mucosa and hence the absorption of toxin is facilitated. Aumont et al, (1986) reported similar results.

In study by Uzal et al, (1994) found that the most striking histopathological changes in the gut were severe congestion, haemorrhage and necrosis of colonic epithelium and of the tips of villi in the duodenum and ileum.

In an experimental study of Blackwell et al, (1991) no histological lesions were found in brain liver, adrenal gland, kidney mesenteric lymph nodes, abomasum and duodenum. In contrast to our findings, the reason may be that the dose, amount of toxin absorbed or any other unknown reason that facilitate the concentration and absorption of toxin in our experiment. So intraduodenal inoculation used in this study presented a successful production of enterotoxaemia in animals and induced pathological changes comparable to those of natural infection. Inoculation of mice by supernatant *Clostridium perfringens* culture (toxin) was found to be fatal and produced pathological changes in these mice.
Although this method is sensitive, some limitations due to variations in individual sensitivity and non specific toxicity from other substance may be present in the intestinal content (Henderson, 1984).

CONCLUSIONs AND RECOMMENDATION

The major economic impact of coccidiosis in production animals may be reduced growth rate and weight gains after clinical or subclinical infection. The aftermath of clinical coccidiosis are manifestation of the prolonged mal absorption and mal-digestion because the regeneration of the normal epithelial lining is not complete.

Among apparently healthy animals a high percentage may pass coccidial oocysts and this act as potential source of infection, such animals are difficult to detect. The economic significance of such latent infection can not be easily assessed but it is believed that the general health, growth rate and productively of animals may be adversely affected. This may also predispose animals to other infections which may result in apparently serious losses. So the parasitism without any pathogenic power or low degree of infection can not be neglected due to the indirect consequence that they may have.

Diarrhoea in young mammals is syndrome of great aetiological complexity in addition to influence of varied environmental managemental, nutritional and physiological factors, the infectious agents capable of causing diarrhoea in neonate are numerous.
Most flocks have exposed to diarrhoea causing pathogens and management practices. These pathogens either affect the animals and causes disease only or may be interacted with each other.

To evaluate the role of coccidia and their interaction with other agents in production of enteric diseases in kids or other young animals, we must use bacteria free animals for perfect comparison, bacteria-free animals has been useful tool in assessing the interaction parasites and gut flora.

In addition perfect knowledge of pathogenesis of infections and contribute towards better understanding of epizootiology which is essential for more efficient control of important disease of neonates.

Determination of causative pathogens by isolation and identification, putting in mind that more than one organisms may be involved.

So establishment of fully equipped laboratory is very important to establish quick diagnosis.

Alleviation of the coccidiosis and other enteric disease by good hygiene and thoughtful management are essential effective diarrhoeal disease control. Young should be as un-stressful as possible and should be separated from adult, provided feed and water in devices that minimize contamination with feces. Animal should never be fed on the ground.

In situation where outbreaks of coccidiosis can be reliably Anticipated, measures should be taken to control coccidiosis before the development of clinical disease. Proper control is balancing act. The goals are to reduce oocysts number in environment sufficiently to avoid massive challenge to susceptible animals while allowing
sufficient exposure so that immunity and resistance can properly develop.
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Annex

**Table 6. Experiment 1**

**Group A:**

<table>
<thead>
<tr>
<th>Data</th>
<th>Factor codes</th>
<th>Sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>G1</td>
<td>6</td>
</tr>
</tbody>
</table>

**Source of variation**

<table>
<thead>
<tr>
<th>Sum of squares</th>
<th>D.F.</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>55.8533</td>
<td>3</td>
</tr>
<tr>
<td>Within groups</td>
<td>0.0000</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>55.8533</td>
<td>5</td>
</tr>
</tbody>
</table>

**F-ratio:** Cannot be assessed

**Student-Newman-Keuls test for all pairwise comparisons**

<table>
<thead>
<tr>
<th>Factor</th>
<th>n</th>
<th>mean</th>
<th>Different (P&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) 2</td>
<td>2</td>
<td>2.0000</td>
<td>(2)(3)(4)</td>
</tr>
<tr>
<td>(2) 7.5</td>
<td>1</td>
<td>7.5000</td>
<td>(1)(3)(4)</td>
</tr>
<tr>
<td>(3) 8.2</td>
<td>2</td>
<td>8.2000</td>
<td>(1)(2)(4)</td>
</tr>
<tr>
<td>(4) 9.5</td>
<td>1</td>
<td>9.5000</td>
<td>(1)(2)(3)</td>
</tr>
</tbody>
</table>

**Comparison of group A1 & Group A2**

**Sample 1**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sample size</th>
<th>Lowest value</th>
<th>Highest value</th>
<th>Arithmetic mean</th>
<th>95% CI for the mean</th>
<th>Variance</th>
<th>Standard deviation</th>
<th>Standard error of the mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>= 6</td>
<td>= 2.0000</td>
<td>= 9.5000</td>
<td>= 6.2333</td>
<td>= 2.7259 to 9.7408</td>
<td>= 11.1707</td>
<td>= 3.3423</td>
<td>= 1.3645</td>
</tr>
</tbody>
</table>

**Sample 2**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sample size</th>
<th>Lowest value</th>
<th>Highest value</th>
<th>Arithmetic mean</th>
<th>95% CI for the mean</th>
<th>Variance</th>
<th>Standard deviation</th>
<th>Standard error of the mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2</td>
<td>= 6</td>
<td>= 1.9000</td>
<td>= 8.7000</td>
<td>= 5.3333</td>
<td>= 2.5890 to 8.0777</td>
<td>= 6.8387</td>
<td>= 2.6151</td>
<td>= 1.0676</td>
</tr>
</tbody>
</table>

**Variance ratio test (F-test)**

Variance ratio = 1.6335
P = 0.603
### Table 6.

**Correlation coefficient**

<table>
<thead>
<tr>
<th>Variable Y</th>
<th>Variable X</th>
<th>Sample size</th>
<th>Correlation coefficient r</th>
<th>95% Confidence interval for r</th>
<th>5% Confidence interval for r</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 (Log)</td>
<td>A2 (Log)</td>
<td>6</td>
<td>0.9538 P=0.0031</td>
<td>0.6298 to 0.9951</td>
<td>0.4750 to 0.9923</td>
</tr>
<tr>
<td>A1 (Log)</td>
<td>A3 (Log)</td>
<td>6</td>
<td>0.9354 P=0.0061</td>
<td>0.5143 to 0.9931</td>
<td></td>
</tr>
<tr>
<td>A1 (Log)</td>
<td>A2 (Log)</td>
<td>6</td>
<td>0.9386 P=0.0055</td>
<td>0.5333 to 0.9934</td>
<td></td>
</tr>
<tr>
<td>A1 (Log)</td>
<td>A3 (Log)</td>
<td>6</td>
<td>0.8452 P=0.0341</td>
<td>0.1071 to 0.9827</td>
<td></td>
</tr>
</tbody>
</table>

**Viable counts in the large intestines of groups A1, A2 and A3 (Table 7,8,9)**

<table>
<thead>
<tr>
<th>Variable Y</th>
<th>Variable X</th>
<th>Sample size</th>
<th>Correlation coefficient r</th>
<th>95% Confidence interval for r</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 (Log)</td>
<td>A2 (Log)</td>
<td>6</td>
<td>0.9386 P=0.0055</td>
<td>0.5333 to 0.9934</td>
</tr>
<tr>
<td>A1 (Log)</td>
<td>A3 (Log)</td>
<td>6</td>
<td>0.8452 P=0.0341</td>
<td>0.1071 to 0.9827</td>
</tr>
</tbody>
</table>
### Table 10

<table>
<thead>
<tr>
<th>Variable Y  : control (Log)</th>
<th>Variable X  : infected (Log)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>5</td>
</tr>
<tr>
<td>Correlation coefficient r</td>
<td>0.9214, P=0.0261</td>
</tr>
<tr>
<td>95% Confidence interval for r</td>
<td>0.2094 to 0.9949</td>
</tr>
</tbody>
</table>

### Table 11, 12

Viable counts in ileum:

<table>
<thead>
<tr>
<th>Variable Y  : control (Log)</th>
<th>Variable X  : infected (Log)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>5</td>
</tr>
<tr>
<td>Correlation coefficient r</td>
<td>0.4746, P=0.4193</td>
</tr>
<tr>
<td>95% Confidence interval for r</td>
<td>-0.7014 to 0.9564</td>
</tr>
</tbody>
</table>

Viable count in large intestine

<table>
<thead>
<tr>
<th>Variable Y  : control (Log)</th>
<th>Variable X  : infected (Log)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>5</td>
</tr>
<tr>
<td>Correlation coefficient r</td>
<td>0.9176, P=0.0281</td>
</tr>
<tr>
<td>95% Confidence interval for r</td>
<td>0.1853 to 0.9946</td>
</tr>
</tbody>
</table>
**Table.13:**
Variable Y : control (Log)  
Variable X : infected (Log)  
Sample size = 5  
Correlation coefficient $r = 0.8788$  $P=0.0497$  
95% Confidence interval for $r = -0.0155$ to $0.9920$

**Table.16**
Variable Y : control (Log)  
Variable X : infected (Log)  
Sample size = 5  
Correlation coefficient $r = 0.9690$  $P=0.0065$  
95% Confidence interval for $r = 0.5976$ to $0.9980$

**Table.17, 18 (Viable count in Jejunum)**
Sample 1  
Variable : control  
Sample size = 5  
Lowest value = 2.0000  
Highest value = 8.0000  
Arithmetic mean = 6.1000  
95% CI for the mean = 3.1525 to 9.0475  
Standard deviation = 2.3738  
Standard error of the mean = 1.0616

Sample 2  
Variable : infected  
Sample size = 5  
Lowest value = 3.6000  
Highest value = 8.1000  
Arithmetic mean = 7.0000  
95% CI for the mean = 4.5939 to 9.4061  
Standard deviation = 1.9378  
Standard error of the mean = 0.8666

---

Paired t-test

Mean difference : $-0.9000$  
Standard deviation : 0.5612  
95 % CI : $-1.5969$ to $-0.2031$  
t=$-3.586$  $DF=4$  $P = 0.0231$
Comparison by using Correlation coefficient

Variable Y : control (Log)
Variable X : infected (Log)

Sample size = 5
Correlation coefficient r = 0.9956  P=0.0003
95% Confidence interval for r = 0.9325 to 0.9997

Viable count in large intestine.

Sample 1
Variable : control
Sample size = 6
Lowest value = 2.3000
Highest value = 11.0000
Arithmetic mean = 7.2500
95% CI for the mean = 3.1393 to 11.3607
Standard deviation = 3.9170
Standard error of the mean = 1.5991

Sample 2
Variable : infected
Sample size = 6
Lowest value = 3.1000
Highest value = 10.6000
Arithmetic mean = 8.1167
95% CI for the mean = 4.8941 to 11.3392
Standard deviation = 3.0708
Standard error of the mean = 1.2536

Paired t-test
Mean difference : -0.8667
Standard deviation : 1.2420
95 % CI : -2.1701 to 0.4368
t=-1.709  DF=5  P = 0.1481

Comparison by using Correlation coefficient

Variable Y : control (Log)
Variable X : infected (Log)
Sample size = 6
Correlation coefficient r = 0.9313  P=0.0069
95% Confidence interval for r = 0.4903 to 0.9926
Table 19: PCV
Sample 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>7</td>
</tr>
<tr>
<td>Lowest value</td>
<td>24.3000</td>
</tr>
<tr>
<td>Highest value</td>
<td>37.0000</td>
</tr>
<tr>
<td>Arithmetic mean</td>
<td>27.4571</td>
</tr>
<tr>
<td>95% CI for the mean</td>
<td>23.4209 to 31.4933</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>4.3642</td>
</tr>
<tr>
<td>Standard error of the mean</td>
<td>1.6495</td>
</tr>
</tbody>
</table>

Sample 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>uninfected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>7</td>
</tr>
<tr>
<td>Lowest value</td>
<td>23.9000</td>
</tr>
<tr>
<td>Highest value</td>
<td>26.0000</td>
</tr>
<tr>
<td>Arithmetic mean</td>
<td>24.7000</td>
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<tr>
<td>95% CI for the mean</td>
<td>24.0331 to 25.3669</td>
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<td>Standard deviation</td>
<td>0.7211</td>
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<tr>
<td>Standard error of the mean</td>
<td>0.2726</td>
</tr>
</tbody>
</table>

Paired t-test

Mean difference : 2.7571
Standard deviation : 4.7651
95% CI : -1.6498 to 7.1641
\( t = 1.531 \)  DF=6  P = 0.1767

Table 19: Hb.
Sample 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>7</td>
</tr>
<tr>
<td>Lowest value</td>
<td>7.6000</td>
</tr>
<tr>
<td>Highest value</td>
<td>11.0000</td>
</tr>
<tr>
<td>Arithmetic mean</td>
<td>8.8286</td>
</tr>
<tr>
<td>95% CI for the mean</td>
<td>7.8102 to 9.8469</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>1.1011</td>
</tr>
<tr>
<td>Standard error of the mean</td>
<td>0.4162</td>
</tr>
</tbody>
</table>

Sample 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>uninfected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>7</td>
</tr>
<tr>
<td>Lowest value</td>
<td>7.4000</td>
</tr>
<tr>
<td>Highest value</td>
<td>7.7000</td>
</tr>
<tr>
<td>Arithmetic mean</td>
<td>7.5714</td>
</tr>
<tr>
<td>95% CI for the mean</td>
<td>7.4685 to 7.6743</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.1113</td>
</tr>
<tr>
<td>Standard error of the mean</td>
<td>0.0421</td>
</tr>
</tbody>
</table>

Paired t-test

Mean difference : 1.2571
Standard deviation : 1.1267
95 % CI : 0.2151 to 2.2992
t=2.952  DF=6  P = 0.0255

Table20: Hb
Sample 1
Variable : Infected

Sample size = 9
Lowest value = 6.5000
Highest value = 9.0000
Arithmetic mean = 7.3556
95% CI for the mean = 6.6701 to 8.0410
Standard deviation = 0.8918
Standard error of the mean = 0.2973

Sample 2
Variable : Control

Sample size = 9
Lowest value = 6.8000
Highest value = 7.9000
Arithmetic mean = 7.4222
95% CI for the mean = 7.1768 to 7.6676
Standard deviation = 0.3193
Standard error of the mean = 0.1064

Paired t-test

Mean difference : -0.0667
Standard deviation : 1.1769
95 % CI : -0.9713 to 0.8379
t=-0.170  DF=8  P = 0.8693

Table20: PCV
Sample 1
Variable : Infected

Sample size = 9
Lowest value = 21.3000
Highest value = 38.0000
Arithmetic mean = 27.4000
95% CI for the mean = 22.5270 to 32.2730
Standard deviation = 6.3396
Standard error of the mean = 2.1132

Sample 2
Variable : Control

Sample size = 9
Lowest value = 25.0000
Highest value = 28.0000
Arithmetic mean = 26.1111
95% CI for the mean = 25.3978 to 26.8244
Standard deviation = 0.9280
Standard error of the mean = 0.3093
Paired t-test

Mean difference: 1.2889
Standard deviation: 6.8375
95% CI: -3.9669 to 6.5446
t=0.566 DF=8 P = 0.5872

Table21: Hb
Sample 1
Variable: Control

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Lowest value</th>
<th>Highest value</th>
<th>Arithmetic mean</th>
<th>95% CI for the mean</th>
<th>Standard deviation</th>
<th>Standard error of the mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>7.2000</td>
<td>7.7000</td>
<td>7.4143</td>
<td>7.2338 to 7.5948</td>
<td>0.1952</td>
<td>0.0738</td>
</tr>
</tbody>
</table>

Sample 2
Variable: Infected

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Lowest value</th>
<th>Highest value</th>
<th>Arithmetic mean</th>
<th>95% CI for the mean</th>
<th>Standard deviation</th>
<th>Standard error of the mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>5.4000</td>
<td>7.8000</td>
<td>6.8571</td>
<td>6.1612 to 7.5530</td>
<td>0.7525</td>
<td>0.2844</td>
</tr>
</tbody>
</table>

Paired t-test

Mean difference: 0.5571
Standard deviation: 0.7807
95% CI: -0.1649 to 1.2792
t=1.888 DF=6 P = 0.1079

Table21: PCV
Sample 1
Variable: Control

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Lowest value</th>
<th>Highest value</th>
<th>Arithmetic mean</th>
<th>95% CI for the mean</th>
<th>Standard deviation</th>
<th>Standard error of the mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>21.0000</td>
<td>23.9000</td>
<td>22.8571</td>
<td>21.9671 to 23.7472</td>
<td>0.9624</td>
<td>0.3637</td>
</tr>
</tbody>
</table>

Sample 2
Variable: Infected

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Lowest value</th>
<th>Highest value</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>17.8000</td>
<td>26.8000</td>
</tr>
</tbody>
</table>
Arithmetic mean = 24.4000
95% CI for the mean = 21.3574 to 27.4426
Standard deviation = 3.2899
Standard error of the mean = 1.2435

Paired t-test
Mean difference : -1.5429
Standard deviation : 3.2690
95% CI : -4.5662 to 1.4804
t=-1.249  DF=6  P = 0.2583