EPIDEMIOLOGIC AND BACTERIOLOGIC STUDIES OF CALF COLIBACILLOSIS IN HERDS OF CATTLE IN ZARIA

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DECLARATION

I hereby declare the originality of this piece of work which was carried out by me in the laboratories of the Department of Veterinary Microbiology and Pathology; Surgery and Medicine, and Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria under the supervision of Professors J.O. Adekeye, E.O. Gyang and J.U. Umoh.

The work of other investigators is duly acknowledged and referred to. No part of this thesis has previously been submitted for a degree or diploma.

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CERTIFICATION

This thesis by Lazarus Baba Tekdek meets the regulations governing the degree of Doctor of Philosophy of Ahmadu Bello University and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

To the Glory of God's Grace and Mercy

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ABSTRACT

A total of 479 calves in 27 herds were examined for diarrhoea and enteric pathogens. The overall prevalence of diarrhoea was 13.6%. The characteristics of diarrhoea faeces viz, the consistency, colour and smell were evaluated and related to the causative agents. A loose greenish non-smelly diarrhoea was suggestive of helminthic infections while whitish, bloody or mucoid diarrhoea was suggestive of coccidial and bacterial diarrhoea. The proportion of calves which survived for 120 days without diarrhoea was 91.3%. The probability of calves having diarrhoea was high among age-group 0-30 days. This indicated the high risk period. There was no significant difference between the occurrence of diarrhoea and either geographical locations of herd, year or season. Odds ratios indicated associations between some enteric pathogens and diarrhoea. These enteric pathogens were *E. Coli* + *Toxocara* sp; *E. coli* + *Coccidia; E. coli* + *Strongyloides* sp. (in older calves); and *E. coli* + *Toxocara* sp. + *Coccidia.* Important pathogens responsible for diarrhoea in the first 30 days of life were *E. coli* + Coccidia; *E, coli* + *Toxaocara* sp; and *E. coli* + *Toxocara* sp. + Coccidia. It is suggested that *E. coli* may be an important factor to be present for gut parasites to cause diarrhoea. The presence of *E. coli* enterotoxins were not related with the occurrence of diarrhoea. Thus if *E. coli* caused the diarrhoea observed, it might have done it by the

presence of other factors other than enterotoxins in *E. coli* responsible for diarrhoea. One of these factors was the ability of *E. coli* to haemagglutinate Ox RBC. *E, coli* strains isolated from 65 and 41 diarrhoeic and non-diarrhoeic calves respectively were characterized. In this work, serogroup 08 was among the commonest 0 serogroups identified. Mixed infections of enterotoxigenic *E. coli* (ETEC) and gastrointestinal parasites (GP) caused 11.8% of the diarrhoea, while mixed infection of *E. coli* strains which agglutinated Ox RBC caused 9.8% of the diarrhoea. While the combination of GP and *E.coli* strains contributed 23.5%; and *E. coli strains* alone contributed 5.9% of the diarrhoea. The freguencies of enterotoxins identified were ST (8.1%), and LT, (3.3%) from diarrhoeic calves while the freguencies of verocytotoxins were 3.3% and 9.8% from diarrhoeic and non-diarrhoeic calves respectively. The property of *E. coli* to haemagglutinate Ox RBC (16.4%) was of significant finding in diarrhoeic calves.

A total of 17 lambs were used for experimental reproduction of enteric colibacillosis. The lambs were divided into 3 groups of 3, 4, and 10 lambs each. The first 3 lambs were in group one which served as blank control lambs that were administered 5.0ml of sterile Brain Heart Infusion Broth; 4 lambs in group two were positive control lambs that were administered 5.0ml of Brain Heart Infusion Broth containing about 3 x 10^8 *E. coli* organism/ml, while 10 lambs in group three were experimental lambs that were

administered 5.0ml of Brain Heart Infusion broth containing about 3 x 10^8 *E. coli* organisms/ml. The average pack cell volume, protein levels, temperature (°C), pulse and respiratory rates were recorded before and after infection. From group one, lamb 01 developed mild non-smelly pasty diarrhoea probably due to change in feed. From group two, lambs 04 and 07 had severe smelly and pasty diarrhoea and latter died (50% case fatality rate) suggesting that the *E. coli* strain used was enterotoxigenic in lambs. From group three 5 lambs developed severe smelly and pasty diarrhoea and case fatality rate was 80%. Experimental infection of lambs suggested that the *E. coli* strain from diarrhoeic calf in Zaria was enterotoxigenic in lambs. Cross infections can easily occur between lambs and calves kept together. The average values of temperature, pulse and respiratory rates were not significantly different between groups before or after infections suggesting non-involvement of circulatory system.

In conclusion further studies are needed to evaluate the roles of viruses, parasites, bacteria and management practices on the prevalence of calf diarrhoea in Zaria. Experimental infections of calves with single and multiple combinations of common enteric pathogens is suggested to determine experimentally any correlation between the nature of diarrhoea and enteric pathogens. This information will be helpful in making a clinical diagnosis of diarrhoea in calves.

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ABBREVIATIONS AND SYMBOLS

ETEC	=	Enterotoxigenic *E. coli*
EPEC	=	Enteroinvassive *E. coli*
VTEC	=	Verocytotoxigenic *E. coli*
Ox RBC	=	Ox Red Blood Cells
PCV	=	Packed cell volume
"	=	Same as above
+	=	Plus/positive reactions/qualitative number of eggs per microscopic field
-	=	Negative reactions
x	=	Late and irregularly positive
±	=	Either positive or negative reaction
d	=	Different biochemical types
VRB	=	Violate Red Bile Agar
MaC	=	Mac'Conkey Agar
Des	=	Deoxycholate Agar
EMB	=	Eosin methylene Red Blue Agar
°C	=	Degrees celcius
<	=	Less than
>	=	More than
LT	=	Heat labile toxin
ST	=	Heat stabile toxin
cAMP	=	Cyclic Adenosine monophosphate
cGMP	=	Cyclic Guanylate monophosphate
Ig	=	Immunogglobulin
'O'	=	Somatic antigen
'K'	=	Capsular antigen

'H'	=	Flagellar antigen
BHI	=	Brain Heat Infusion Broth
%	=	Percentage
HC	=	Haemorrhagic Colitis
BD	=	Bloody diarrhoea
Frq/Fg	=	Frequency
GP	=	Gastrointestinal Parasites
Ml	=	Millitres
et al	=	and others
ZST	=	Zinc Sulphate Test
/	=	and, or
mg	=	Microgram
TRP	=	Temperature, Respiratory and Pusle Rates
=	=	Equals to
x	=	Multiply by
S/N	=	Serial Number
Sp.ag'	=	Means that the motile culture was spontaneoustly agglutinable
(.)	=	Means weak reaction
No/No.	=	Number
rpm	=	Revolution per minute
TSI	=	Triple sugar iron
eg	=	For example

CHAPTER ONE

INTRODUCTION

The cause of diarrhoea and the mechanism by which different entero-pathogens cause disease have been the subject of global interest in the last decade. Different research works led to the identification of a considerable number of entero-pathogens which were previously unrecognized or ill defined and whose roles were not clear in the development of diarrhoea in animals. These developments together with newly acquired biotechnology opened unlimited opportunity for more sensitive and rapid diagnostic tools, better understanding of the mode of the pathogenesis of entero-pathogen (especially *Escherichia coli* (*E. coli*) and other means of control measures adopted for each entero-pathogen.

One of the entero-pathogens which has received world-wide attention is *E. coli*. It has been studied more extensively than other bacteria. It causes colibacillosis in both man and animals (Blood and Radostits, 1989a).

Colibacillosis is a group of diseases caused by pathogenic strains *E. coli*. The disease has two major forms - the colisepticaemic form which is characterized by sudden death (mainly in lambs and foals), and enteric form which is characterized by varying degrees of diarrhoea, dehydration, acidosis and electrolytes imbalance (Barnum *et al.*, 1967; Radostits, 1986; Blood and Radostits, 1989a).

Colibacillosis in farm animals occurs more commonly among animals raised on intensive management system than animals raised on free range, and cause a significant economic loss in livestock industry (Tzipori, 1985b). Many epidemiological determinants (factors) such as weather, husbandry system, age and immune calves and other enteric pathogens influence the occurrence of the disease and these must be considered when evaluating and investigating the disease outbreaks (Barnum *et al.*, 1967; Tekdek, 1983; Blood and Radostits, 1989a). The disease is most common in animals under 3 days of age but can occur in animals 12 - 18 hours after birth and occasionally in calves up to 3 weeks of age and weaned piglets (Tzipori, 1965; Blood and Radostits, 1989a;).

Calves form the backbone of any cattle industry and so replacement of cattle for beef and milk production depend on their availability. If calf morbidity and mortality are high, the development of cattle industry is impeded. On the other hand, a reduction in calf morbidity and mortality would lead to an increased beef and milk production. A high rate of neonatal calf mortality would therefore markedly increase the costs of cattle production. Diseases associated with calf mortality are mainly those involving the gastrointestinal and respiratory systems in the form of diarrhoea and pneumonia (Tekdek, 1983).

Adetosoye *et al.* (1976a,b) reported on enteropathogenicity of hemolytic *E. coli* isolated from diarrhoeic

piglets and characterised those of them that are colicinogenic. Ojo and Moorwaw (1972) classified serologically 26 *E. coli* isolates from rectal swabs of piglets. A similar work was done on 13 porcine and bovine isolates, 12 which were classified as entero-pathogenic and 1 as non-pathogenic (Falade and Oluwadiya, 1973).

It is known that, although, *Escherichia coli* (*E. coli*) is a normal inhabitant of the intestinal tract, pathogenic ones cause enteric and septicaemic diseases in both man and animals. The components of the epidemiological triad viz: agent, host and environment are closely involved with the development of the disease. The disease, colibacillosis, is referred to as a group of diseases caused by *E. coli* in both man and animals (Blood and Radostits, 1989a). It occurs in all species of newborn animals and cause a major economic loss in this age group.

Calf diarrhoea is a symptom of a disease condition of enteric malfunction rather than a specific diagnosis. It has been long recognised as an important cause of economic loss of calves world-wide (Mills, 1776). In calves, diarrhoea is caused by a number of agents acting singly or in combination, but more so, by confused and complex aetiology which involves interactions between entero-pathogenic strains of *E. coli* (ETEC), *Salmonellia* spp., *Clostridium* spps, rotaviruses, corona-like viruses, gastrointestinal parasites, e.g. coccidia, Cryptosporidium spp, and helminths, level of colostral immunity, the nature

of diet and the level of management (Dun, 1978; Radostits, 1986; Blood and Radostits, 1989a). In the face of these etiologic agents there must be a general guide at arriving to a specific aetiologic diagnosis of diarrhoea in calves for effective treatment, prevention and control of the disease.

This work is designed to:

(1) Determine the prevalence of diarrhoea among calves in Zaria.

(2) Determine the effects of geographical location, year, season, age and enteric pathogen (*E. coli*, helminths and protozoa) on the frequency of calf diarrhoea in Zaria.

(3) Characterize *Escherichia coli* isolates from calves faeces in Zaria.

(4) Determine the pathogenicity of *E. coli* isolated from diarrhoeic calf on lambs.

CHAPTER TWO

LITERATURE REVIEW

2.1 Historical Background

Theobald Escherich (1885), described certain organisms which he isolated by examining the faces of newborn babies that were breast fed. He named one of these organisms *Bacterium coli commune* which is now accepted as *Escherichia coli*. He described it as a short , plump rod, growing readily on gelatin or agar; it also grew as a slimy mass on potatoes and coagulated milk with the production of acid (Sojka, 1965). Many years later, writers used the generic name *Bacterium coli*. It was in Denmark, in 1891, that Jensen first associated *E. coli* with calf scour or Kalberruhr and he distinguished between pathogenic and non-pathogenic *E. coli* by oral infection of *E. coli* culture to newborn calves. Jensen's work extended over a period of twenty years which provided a framework for the development and understanding of the disease.

Later in the 1920s, Theobaid Smith and others confirmed in the United States of America the importance of *E. coli* in white scours and the importance of colostrum in maintaining equilibrium between host and parasite in calves. Following this, Lovell (1937), was the first to distinguish serologically between calf strains of *E. coli* by precipitin tests.

The effect of the organism in other animal species came much later. For example, Adam (1927), isolated from patient with infantile gastroenteritis some strains of coliform bacilli marking the first epidemiologic study of human infantile gastroenteritis caused by *E. coli*. Further serological work on *E. coli* began to emerge by the use of serological investigation, such as Kauffman-Knipschildt-Vahine serological work (Barnum *et al.*, 1967). Kauffman (1947) established the *E. coli* antigenic scheme which was later expanded by Ewing and Orskov. This opened field for extensive scientific research of *E. coli* and its associated diseases in both man and animals. Wramby (1948) did the first large-scale use of the antigenic E. *coli* scheme among calves.

Interest was then focused on the detailed pathogenesis of the disease in neonates. The importance of adhesive factors in the development of enteric colibacillosis was established by Smith and Linggood (1971). Of recent, research work is focused on the characterization of *E. coli* and methods of diagnosis and the detection of *E. coli* enterotoxins. Detailed work on DNA structures and the use of gene probes for the detection of enterotoxins is receiving attention (Moseley *et al.*, 1982; Levine *et al.*, 1983; Tzipori, 1985a).

With the understanding of transformable plasmids in *E. coli*, the organism has been used for the production of hepatitis virus vaccine in humans (Smith and Linggood,

1971). A large number of hybrid DNA molecules comprising plasmid PBR 322 and various fragments of the HBV genome have been cloned and propagated in *E. coli* to produce the hepatitis vaccine. This raises the possibility of large scale antigen production for diagnostic purposes and development of the hepatitis vaccine (Burrell *et al.*, 1979).

2.2 Classification and Taxonomy

2.2.1 Characteristics of the Organism

The family Enterobacteriaceae is large heterogenous group of gram-negative rods, whose natural habitat is the intestinal tract of both man and animals (Edwards and Ewing, 1972; Cottral, 1978; Jawetz *et al.*, 1989). The family is subdivided into tribe and genera on the basis of biochemical tests giving 5 known tribes which are Escherichieae, Edwardsiellaeae, Salmonellaeae, Klebsiellaeae and Proteae. There are 12 genera with numerous species (Table 1). Some species are atrichous, and non-motile variants of motile species also occur. Motile forms are peritrichous and flagellated (Edward and Ewing, 1972; Jewets *et al.*, 1989).

2.2.2 The Tribe *Escherichieae*

These consist of organisms which give positive methyl-red reaction and negative Voges-Proskauer test. They do not reduce urease, phenylalanine, deaminase and hydrogen sulphide (Edwards and Ewing, 1972). They do not utilize sodium malonate, gelatin is not liquefied and there is no

growth neither on Simmon's citrate agar nor in medium containing potassium cyanide (Sojka, 1965; Edwards and Ewing, 1972). Sojka (1965), stated that according to the Enterobacteriaceae Subcommittee of the Nomenclature Committee of the International Association of Microbiological Societies 3rd report 1958, the Escherichia group consists of motile or non-motile bacteria that conform to the definition of the family Enterobacteriaceae and possesses the following biochemical characteristics:

Gas formation from glucose	+
Sucrose	+ or x
Mannitol	d
Dulcitol	+
Salicin	d
Adonitol	d
Inositol	-
Indole	+
Methyl-red	+
Voges-Proskauer	-
Ammonium citrate	-
Hydrogen sulphide	-
Urease	-
Gelatin liquefaction	-
Growth in KCN medium	-
Phenylalanine deaminase	-
Sodium malonate	-

Table 1: The Classification and Nomenclature of the Family Enterobacteriaceae.

Family:	**Enterobacteriaceae (Rehn)**
Tribe I:	Escherichieae (Bergery, Breed, Murry)
Genus 1:	1. *Escherichia coli* (Migula) Castellani and Chalmers.
Genus 2:	Shigella (Castellani and Chalmers)
	1. *Shigella dysenteriae* (Shig) Castellani and Chalmers.
	2. *Shigella flexneri* (Castellani and Chalmers)
	3. *Shigella boydii* (Ewing)
	4. *Shigella sonnei* (cevin Weldin)
Tribe II:	Edwardsielleae-Ewing and McWhoriter
	1. *Edwardsilla tarda-Ewing* & McWhorter
Tribe III:	Salmonelleae (Bergery, Breed & Murray)
Genus 1:	Salmonella (Lignieres)
	1. *Salmonella cholerae-suis* (Smith Weldin)
	2. *Salmonella typhi* (Schroeter) Warren and Scot
	3. *Salmonella enteritidis* Gaertner-Caste Uani and Chalmers.
Genus 2:	Arizona O-Ewing and Fife
	1. *Arizona hinshawii* (Ewing & Fife) Weing
Genus 3:	Citrobacter - Werkman and Gillon
	1. *Citrobacter freudii* (Bredak) Werkman and Gillon.

Table 1 (continued).

Family: Enterobacteriaceae (Rehn)	
Tribe IV:	Klebsielleae-Trevisan
Genus 1:	1. *Klebsiella pneumoniae* (Schroeter) Trevisan
	2. *Klebsiella ozaenae* (Abel) Bergery Bred and Murray.
	3. *Klebsiella rhinoschieromatis* - Trevisan
Genus 2:	Enterobacter-Hagmache and Edwards
	1. *Enterobacter cloacae* (Jordan) Hormaeche and Edwards
	2. *Enterobacter aerogenes* (Kruse) - Hormaeche and Edwards.
	3. *Enterobacter hafniae*- (Meellar)
	4. *Enterobacter liquefaciens* (Grimes and Hennerty) Ewing
Genus 3:	Pectobacterium - Waldae
	1. *Pectobacterium carotoverum* (Jones) Waldar
Genus 4:	Serratia (Bizid)
	1. *Serratia marcescene* (Bizid) (Serratia marcescens subspecies mercescens).
	1a *Serratia marceacens* - (Ewing Davis and Johnson)
Tribe V:	Proteeae - Castellani and Chalman
Genus 1:	Proteus - Hauser
	1. *Proteus vulgaris* (Hauser)
	2. *Proteus mirabilis* - (Hauser)

Table 1 (continued).

Family: Enterobacteriaceae (Rehn)	
Genus 2:	3. *Proteus morganii* - Wimslow Kligher and Rothbery) Rauis 4. *Proteus rettegeri* (Hadley *et al.*). Husffgian and Stuart. Previndencia - Ewing 1. *Previndencia alcalifaciens* (De-Sellas cromes) Ewing

The first species listed in each genus is the type species.

Source: Edwards and Ewing (1972).

2.3 Growth Media

A number of growth media have been described and numerous modifications have been devised for growing *E. coli*. A few of such media include Violate Red Bile Agar (VRB). Mac'Conkey (MaC) Agar, Deoxycholate agar (Des), Eosin Methylene Blue (EMB) agar and Blood Agar (Barnum *et al.*, 1967; Edwards and Ewing, 1972; Cottral, 1978). Specific growth media for the enhancement of the growth of some *E. coli* strains (K88 and K99 strain) have been described by Guinee *et al.* (1976, 1977) using Minca agar for K99.

2.4 Growth Characteristics and Morphology

Acid is produced by a typical lactose fermenting *E. coli*, with a characteristic change in the indicator in differential media (Barnum *et al.*, 1967). On VRB agar and MaC., *E. coli* is known to produce acid from the lactose present (Barnum *et al.*, 1967), while on EMB, the colonies are black with a green metallic sheen and is characteristic of *E. coli*. On VRB, MaC and Des, the colonies are red, as a result of acid which is produced (Sojka, 1965; Barnum *et al.*,1967; Edwards and Ewing, 1972). Whenever growth of *E. coli* is over-crowded, the colonies are small, pink, and even colourless for slow lactose fermenters (Cottral, 1978). On Blood agar, the colonies are usually grey and hemolytic strains are identified. On VRB colonies are entire or irregular, flat or raised, pink or deep-red and can have a

solid colour or a red centre with transparent edge. *E. coli* colonies are rarely mucoid or watery (Conttral, 1978; Sojka, 1965). A very thick mucoid growth is usually associated with capsular strains (Cottral, 1978).

When *E. coli* is grown on plate, more than one type of *E. coli* may be on the differential medium in addition to other gram negative bacteria (Sojka, 1965; Edwards and Ewing, 1972).

When *E. coli* is examined on direct smear, the rods are gram-negative rods; many are short or long, single or paired, thin or flat and occasionally bipolar (Cottral, 1978). Pleomorphic forms occur and actively motile forms have flagella (Cottral, 1978).

2.5 Serology

The serological identification of *E. coli* cultures is based on their O (Somatic), K (Capsular) and if motile H (Flagella) antigens (Sojka, 1965; Barnum *et al.*, 1967; Edwards and Ewing, 1972; Cottral, 1978; Jawetz *et al.*,1989).

(a) O antigen

The O antigens are heat-stable somatic antigens that are not inactivated by heat at 100 or 120°C and are also resistant to alcohol (Edwards and Ewing, 1972). Lipopolysaccharide forms the most external part of the bacterium cell wall, which consists of repeating units of polysaccharide. It consists of polysaccharide-phospholipid

complex (Sojka, 1965; Jawetz *et al.*, 1989). O antigens are detectable by agglutinating antibodies to O antigens, which are basically and predominantly IgM. Some enteric organisms may share one or more O antigens. For example, *Shigalla* spp share one or more O antigen with *E. coli* and so cross reaction do occur between these organisms (Jawetz *et al.* 1989).

O antigens occur in the smooth forms (S-forms) of *E. coli* (Sojka, 1965). In 1947, Kauffmann identified 25 O antigens, 55 K antigen and 20 H antigens. Many workers, Kauffmann, 1947; Kauffmann and Porch, 1948; Sojka, 1965; Glantz *et al.*, 1968, have studied serotypes of *E. coli* and by 1972, Edwards and Ewing had reported 148 O antigens, 92 K antigens, and 51 H antigens. Presently, there are about 164 O antigens reported (Acres, 1985; Jawetz *et al.*, 1989).

(b) K antigens

These are envelope or capsule antigens which are thermolabile polysaccharide (Sojka, 1965). These antigens are external to O antigen of *E. coli* (Jawetz *et al.*, 1989). The K antigens interfere with agglutination by O antisera and may be associated with virulence of the organism (Sojka, 1965). Based on physical properties, there are at least three varieties of K antigens - L, A and B (Sojka, 1965; Edwards and Ewing, 1972). The differences are presented in Table 2. More than 100 K antigens have now been identified (Jawetz *et al.*, 1989).

(c) H antigens

These are thermolabile protein antigens located on the flagella of motile strains of *E. coli* and are denatured or removed by heat at 100°C or alcohol. They are preserved by treating the motile strains of bacteria with formalin (Sojka, 1965; Edwards and Ewing, 1972; Jawetz *et al.*, 1989). There are more than 50 H antigens identified (Jawetz *et al.*, 1989). Most of the H antigens are specific and show no significant cross-relationship with other H antigens (Sojka, 1965).

Various methods of determining each of these antigens have been described by Edwards and Ewing (1972). Their use in the diagnosis of colibacillosis by serotyping has also been described by Sojka (1965); Barnum *et al.* (1967); Edwards and Ewing (1972); Jawetz *et al.*(1989).

2.6 Physiology and Nutrition

Lactose was one of the first substances used to determine the biochemical properties of *E. coli* by Chantemesse and Wildal (1887; 1890). Theobaid Smith discovered that *E. coli* was capable of fermenting lactose, whereas *S. typhi* failed to do so, thereby forming a strong point of difference between *Salmonella* spp and *E. coli* (Sojka, 1965). However, the failure of an organism to split lactose does not elude it from the Esherichia group, provided that the other biochemical reactions are typical

Table 2. The Different Types of K Antigens of *E. coli* and their characteristics.

Variety of K antigen	Characteristics
L	1. Agglutinability of L antigen in L antiserum is inactivated by heat at 100°C for 1 hour. 2. Suspensions are rendered agglutinable in O antiserum by heat at 100°C for 1 hour. 3. Antibody binding power is inactivated by heat at 100°C for 1 hour 4. Antigenicity is inactivated by heat at 100°C for 1 hour. 5. Occurs as envelops or sheath, occasionally as capsule.
A	1. Agglutinability of A antigen in A antiserum is inactivated by heat at 121°C for 2½ hrs. 2. Suspensions are rendered agglutinable in O antiserum by heat at 100°C for 2½ hrs. 3. Antibody binding power are not inactivated by heat at 100°C for 2½ hrs. or at 121°C for 2½ hrs. 4. Antigenicity is inactivated by heat 120°C for 2½ hrs. 5. Occurs as capsules.

Table 2 (continued)

Variety of K antigen	Characteristics
B	1. Agglutinability of B antigen is B antiserum is inactivated by heat at 100°C for 1 hour. 2. Suspensions are rendered agglutinable in O antiserum by heat at 100°C for 1 hour. 3. Antibody binding power not inactivated by heat at 100°C for 2½ hrs. and at 121°C for 2 hrs. 4. Antigenicity inactivated by heat at 100°C for 1 hour. 5. Occurs as envelops or sheaths or as capsule.

Source: Edwards and Ewing (1972).

for the group (Kauffmann and Perch, 1948; Kauffmann, 1954). Fermentation of glucose with production of acid was first noticed by Chantemesse and Wildal (1891).

E. coli is known to form indole in a medium containing oeptone which depends on the presence of tryptophan in the medium (Sojka, 1965; Edwards and Ewing, 1972). In addition to indole production, valerianic, caproic and succinic acids, hydrogen phenol, creatinine and ammonia are formed (Blumenthal, 1895). However, there are strains which fail to produce indole (Kauffmann and Perch, 1948). *E. coli* is capable of breaking formic, succinic and tartaric acids with the formation of hydrogen, carbon-dioxide and methane (Sojka, 1965; Barnum *et al.*, 1967; Edwards and Ewing, 1972). *E. coli* does not grow on ammonium substrate containing sodium citrate but grows very well on Simmons' ammonium glucose agar although strains which are citrate positive occur (Kauffmann and Perch, 1948).

E. coli does not attack the purine ring in medium containing glycerine. Simple salts, uric acid, uracil, xanthine and adenine can be utilized by *E. coli* as sole source of nitrogen (Sojka, 1965). Some *E. coli* strains which contain the enzyme urease may hydrolyze urea to ammonia as a sole source of nitrogen for their metabolism (Orskov *et al.*, 1962).

Voges-Proskauer (1898), described the production of a pink colour when a strong solution of potassium hydroxy (KOH) is mixed with some bacteria. The pink colour is

produced by acetyl-methyl carbinol (acetoin) a product of glucose breakdown by bacteria which is oxidized by KOH to diacetyl which in turn reacts with a substance in peptone probably quinidine group of arginine to produce a fluorescent pink colour. *E. coli* strains do not produce this reaction (Sojka, 1965; Jawetz *et al.*, 1989). *E. coli* gives a positive red colour to methyl-red reaction. Methyl-red detects the hydrogen concentration produced during bacterial fermentation of dextrose (Sojka, 1965; Edwards and Ewing, 1972; Cottral, 1978).

E. coli reduces nitrates to nitrites (Sojka, 1965). Potassium cyanide test (KCN) is useful for dividing members of the Entero-bacteriaceae into two main groups. For example *E. coli* fails to grow in a simple medium containing 1% peptone without sugar, while Klebsiella grows in the presence of KCN (Sojka, 1965). Medium containing sodium malonate is used to distinguish *Escherichia* from *Aerobacter* spp. *Escherichia* strains produce no change in colour (remains green) while *Aerobacter* gives Prussian blue colour (Barnum *et al.*, 1967; Sojka, 1965).

When *E. coli* is tested for amino-acid decarboxylases, there are various patterns obtained from lysine, ornithine and glutamic acid (Edwards and Ewing, 1972; Barnum *et al.*, 1967; Sojka 1965; Kauffman and Perch, 1948).

2.7 Diseases Caused by *E. Coli*

Although *E. coli* is known to be a normal commensal organism of the gastrointestinal tract, field experiences have shown that some strains of *E. coli* have been associated with neonatal and adult diseases in both man and other animals. These diseases are summarised in Table 3.

Field experiences by veterinarians have shown that there is no single cause of calf and piglet diarrhoea but rather the cause is complex usually involving interactions between entero-pathogenic bacteria and viruses, helminth and protozoa, the immunity of the animal and the effects of the environment (Blood and Radostits, 1989a; Radostits, 1981).

The distribution and occurrence of entero-pathogens in faeces of both diarrhoeic and non-diarrhoeic calves vary according to age, management practices, type of calves being investigated and extent to which the diagnostic laboratories are capable of isolating and characterising the pathogens (Tzipori, 1985).

Tzipori (1985a) reviewed the relative importance of some enteric pathogens affecting neonates of domestic animals world wide. The four most common pathogens causing diarrhoea in neonates include rotavirus, *Cryptosoporidium* spp., coronavirus and enterotoxigenic *E. coli*. These entero-pathogens cause 75 - 95% of infections in neonatal calves world wide (Tzipori, 1985a; Moon *et al.*, 1978). These pathogens have been shown to differ between locations

Table 3. Diseases caused by Pathogenic *E. coli* in Man and Animal.

Category	Diseases	Species	References
A Neonates	Colisepticaemia (*ETEC*)	Lambs Foals Calves	Blood & Radostits, 1989a; Sojka, 1965
	Neonatal Diarrhoea (ETEC)	Calves Piglets	Blood & Radostits, 1989a; Sojka, 1965.
	Oedema disease (NETEC) (Vasotoxin)	Weaned Piglets	Blood & Radostits, 1989b.
	Coliform gastroenteritis (*E. coli*)	Swine (post weaning diarrhoea)	Blood & Radostits, 1989b.
	Cerobrospinal Angiopathy (*E. coli*)	Swine-weaned Pigs	Blood & Radostits, 1989b.
	Toxaemia due to: endotoxin, enterotoxin, necrotoxin, and unidentified toxins	Lambs, Piglets, Foals, Calves	Blood & Radostits, 1989a.
	Pneumonia	Calves	Tekdek, 1983.
	Coliform meningo-encephalitis	Calves	Glantz & Rotenbacher, 1965.

Table 3 (continued).

Category	Diseases	Species	References
B Adult Animals	Coliform mastitis	Bovine Swine Ovine	Bramley, 1981.
	Endometritis	Rats Bovine	Yoshikazu & Tsuyoshi, 1965
	Abortion	Bovine Ovine Swine	Blood & Radostits, 1989a
C Poultry	Air succulitis Salpingitis Pericarditis (colisepticaemia) Embryonic deaths & chicks Enteritis Coli granuloma Hjarre's disease	Poultry	Sojka, 1965
D Man	Urinary Tract infection	Man	Sanberg *et al.* 1988; Ljungh & Wadstrom,
	Peritonitis Dysentery Diarrhoea Travellers Diarrhoea	Man	1983. "
	Hemorrhagic colitis Hemolytic uremic syndrome	Man	Bowe *et al.*, 1987

and between seasons and years (Acres, 1975). Any one of the most common pathogens may predominate or be absent in a certain group of animals. For instance rotavirus was found to be most common in dairy calves than beef calves (Reynolds *et al.*, 1986; Snodgrass *et al.*, 1986). Coronavirus was found to be predominant in beef calves in some countries and not in others, while *Cyryptosporidium* and coronavirus occur in 25 - 40% of the diarrhoeic calves world wide (Bulgin *et al.*, 1982).

In some situations the combination of *Cryptosporidium* sp. and rotavirus may predominate (Pohjola *et al.*, 1986), while in other reports *Cryptosporidium* sp. was the second most commonly detected pathogens next to rotavirus and ETEC may not be detectable in up to 30% of diarrhoeic calves (Reynolds *et al.*, 1986). The age distribution of common enteropathogenes associated with diarrhoea in neonates is indicated in Table 4.

Some studies of diarrhoeic and healthy calves from the same management groups show that entero-pathogens commonly found in diarrhoeic calves can be found in healthy calves though at a lower frequency of occurrence with the exception of rotavirus which may be excreted by up to 50% of healthy calves (Myers *et al.*, 1984). It appears healthy calves may be infected more often with ETEC, *Cryptosporidium* spp, coronaviruses and rotavirus in herds in which some calves recently had enteric disease than in herds free from major enteric disease (Myers *et al.*, 1984). Also a study

Table 4. Age Distribution of the Common Enterophogens Associated with diarrhoea in Neonates.

Enteropathogens	Age (days)
ETEC	< 3
Rotavirus	5 - 15
Coronavirus	5 - 21
Cryptosporidium spp	5 - 35
Salmonella spp	5 - 42
Clostridium perforanges type B & C	5 - 15

Source: Reynolds et al. (1986).

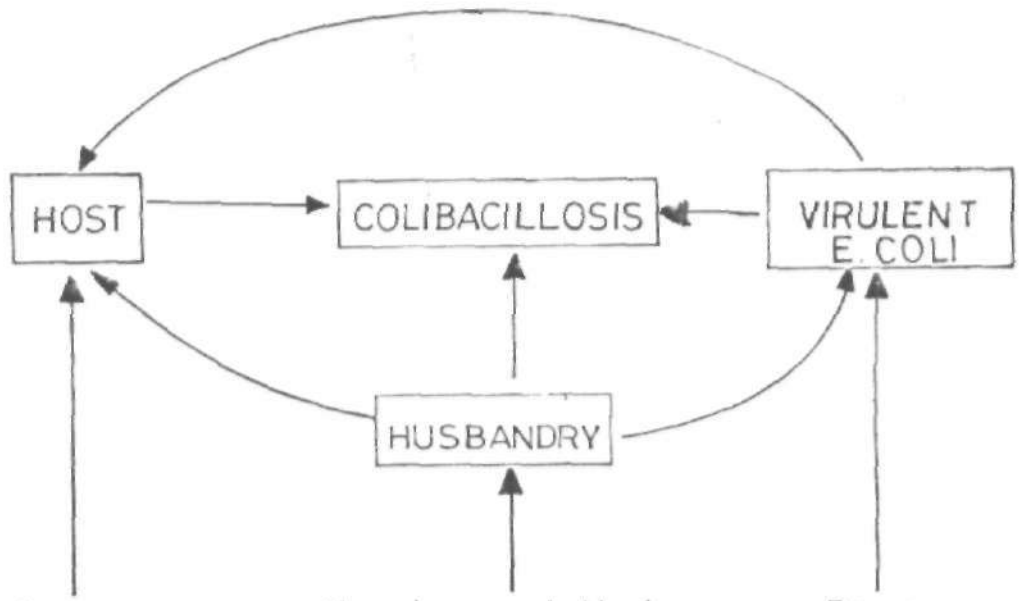
indicates that rotavirus and coronavirus occur with almost equal frequency in the intestinal tracts of both normal and diarrhoeic calves (Morin *et al.*, 1978).

Certain management practices may be associated with differences in the prevalence of enteropathogens. For example, dairy calves fed on nipple feeders showed increased probability of shedding *Salmonella* spp., *E. coli*, rotavirus and coronavirus (Waltner-Toeus *et al.*, 1986). The use of grouped pens was associated with increased odds of *Campylobactor jejuni*, rotavirus and K99^{+} *E. coli* (Myers *et al.*, 1984 ; Tekdek, 1983). Factors contributing to colibaciliosis are varied. The schematic presentation of contributing factors to colibacillosis in neonates (Tekdek, 1983; Barnum *et al.*, 1967; Inglis, 1960), is shown in Figure I.

2.7.1 Aetiology of Enteric Colibacillosis

Specific serotypes of *E. coli* are known to cause enteric colibacillosis in calves and piglets (Blood and Radostits, 1989a). These serotypes of *E. coli* are called entero-pathogenic *E. coli* (EPEC) and enteroptoxigenic *E. coli* (ETEC). Most EPEC possess specific pili known as adhesive factors which attach on to specific brush boarder receptors of the enterocytes (Blood and Radostits, 1989a). These adhesive factors are designated as K88, K99, 987P and F41 on *E. coli* strains which affect piglets, K99 and F41 on

Figure I: Schematic Presentation of Contributory Factors to Colibacillosis in neonates.



- Age
- Species
- Genetics
- Primary disease
- Nutrition of the dam and host.

- Housing and Hygiene
- Weather
- Feeds and Feeding
- Equipments

— Drugs
- Gut microflora

Arrows indicate direction of influence from each determinants.

Source : Inglis(1960); Barnum et al., (1967); Tekdek (1983).

E. coli strains which affect calves and lambs and CFA I, II, III, IV and E877C on *E. coli* which humans (Blood and Radostits, 1989a; Hirsh, 1985; Tzipori, 1985b; Thomas *et al.*, 1985; Qrskov and Qrskov, 1984; Evans *et al.*, 1978, 1975).

E. coli strains which cause coliform gastroenteritis in weaned piglets are predominantly hemolytic and produce enterotoxin at the time of proliferation (Blood and Radostits, 1989a). In weaner and growing pigs, the strain of *E. coli* which produce gut oedema is non-enterotoxigenic, but is known to produce vasotoxin (Blood and Radostits, 1989b).

Enteric colibacillosis in animals can occur without actual invasion of the body system by the organism. Wray and Thomlinson (1972) administered *E. coli* endotoxins through subcutaneous, intravenous and oral routes and observed gross lesions and histological findings resembling those which occur in natural colibacillosis in calves.

2.7.2 Sources, ecology and trasmission of *E. coli*

In farm animals, major sources of infection is the infected animals (Blood and Radostits, 1989a). Others include vagina and uterus of sows which serve as potential sources of infection to piglets (Blood and Radostits, 1989a). Calving and farrowing pens are heavily infested with *E. coli* (Barnum *et al.*, 1967; Blood and Radostits, 1989a). Young animals obtain the organism from contaminated

environments, pens, beddings, diarrhoeic calves, overcrowded calving grounds, milk from cows with coliform mastitis and from the skin and perineum and udder of adult animals (Blood and Radostits, 1989a; Barnum *et al.*, 1967; Inglis, 1960). Normal calves which are subclinically infected and adult cattle serve as reservoirs of infection (Blood and Radostits, 1989a; Acres, 1985).

2.7.3 Spread of ETEC among Calves

The spread of the disease agent is through infected calves and all inanimate objects which are contaminated by faeces. Infected animals are the major reservoirs for enterotoxigenic *E. coli* and their faeces are major sources of environmental contamination with the bacteria (Blood and Radostits, 1989a). The shedding of ETEC through faeces causes the multiplier effect. For example, diarrhoeic calve pass 10 kg ETEC within 12 hours and recovered calves continue to shed the organisms for months (Acres, 1985).

Carrier animals introduced into an uninfected herd were believed to be one of the main causes of natural outbreaks (Acres, 1985).

2.8 Prevalence and Incidence of ETEC

The prevalence of ETEC varies widely in diarrhoeic calves from country to country. The prevalence can be 50 - 60% in diarrhoeic calves under 30 days of age and only 5 -

10% in diarrhoeic calves at 8 days of age (Krogh, 1983) and 5 - 8% in some countries (Sherwood *et al.*, 1983).

The incidence of calf diarrhoea and mortality increases as a result of natural selection of virulent *E. coli* strain and as long as a calf house is occupied (Wray and Thomlinson, 1975). In some other countries, such as USA, *E. coli* K99+ antigen may cause 30 - 40% of diarrhoea in calves, while in countries like the UK the incidence may be as low as 3 - 6% (Reynolds *et al.*, 1986; Sherwood *et al.*, 1983).

2.9 Virulence of ETEC

The severity of enteric colibacillosis is influenced by the immune status of the host, presence of adhesive factors in ETEC, and the dose of ETEC ingested (Blood and Radostits, 1980a; Radostits, 1981). In calves, the pili antigen which induces fluid secretion is K99$^+$. It produces a heat stable toxins (ST) (Isaascson *et al.*, 1978). The predilection site for these pili antigens is the ilieum enterocytes on to which they adhere. Non-pathogenic *E. coli* do not adhere to the enterocytes (Hadad and Gyles, 1982). The commonest O antigens in calves which cause diarrhoea are 08, 09, 020 and 0101 (Tzipori, 1985b).

Escherichia coli strains particularly those of porcine origin may have heat labile toxin (LT) and Heat stable toxin (ST). The heat stable toxin is of two forms, the methanol-soluble (STa) which is active in infant mice and in neonatal piglets but not weaned pigs and the methanol-insoluble *E.*

coli (STb) which is active in weaned pigs and rabbits but not in infant mice (Eidels *et al.*, 1983). *E. coli* K99 and 987p+ produce STa in infant mice and some neither produce ST or STa but STb only (Moon *et al.*, 1980).

2.10 Pathogenesis of ETEC

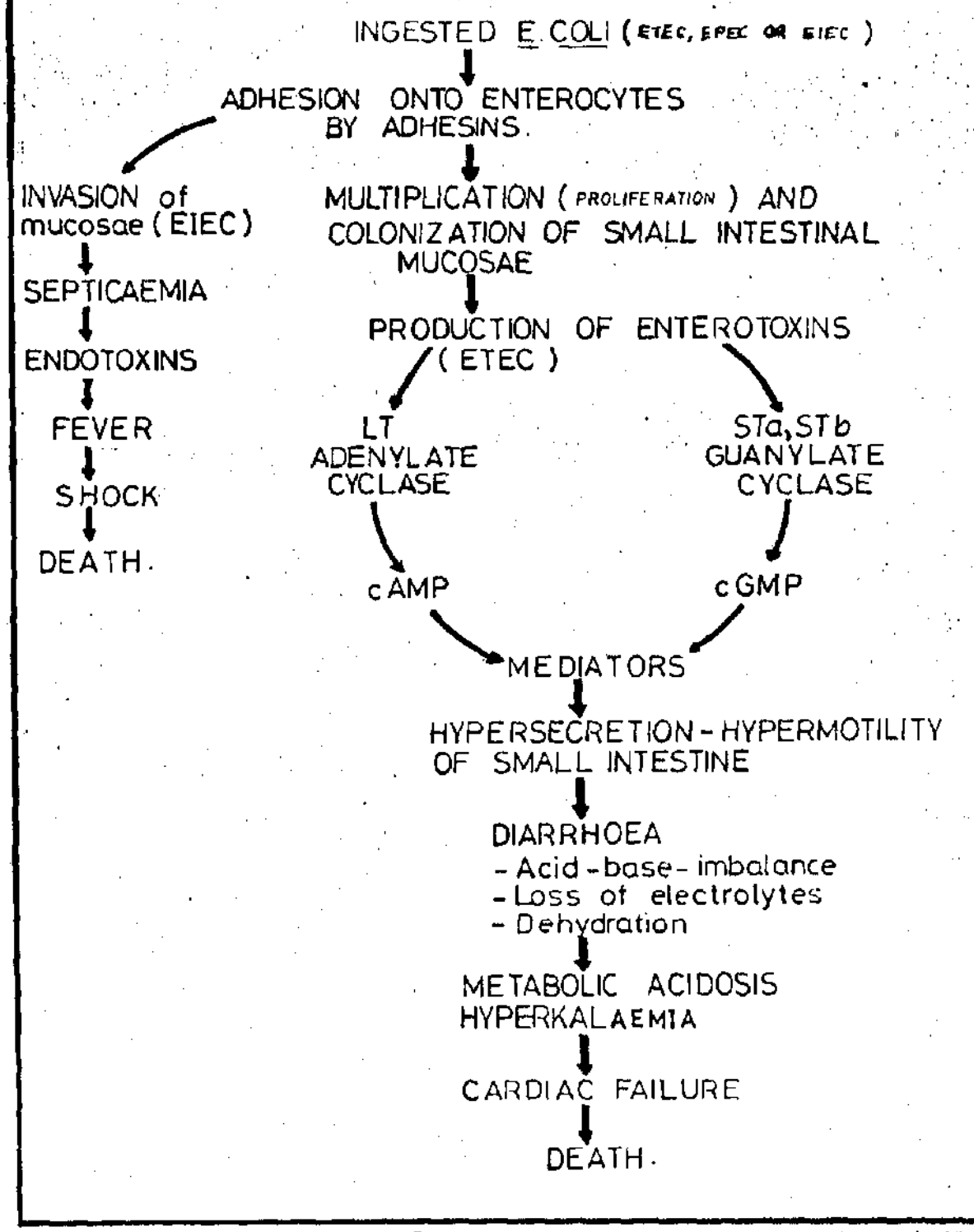
Major factors responsible for the pathogenesis of colibacillosis in farm animals, are the immune status of the animal, and the strain of *E. coli* either invasive producing septicaemia or non-invasive producing enteritis which cause diarrhoea (Moon, 1974). Ingested ETEC possess the ability to adhere to specific enterocytes, colonize, proliferate and produce enterotoxin in the upper part of the intestine (Moon, 1974; Smith and Halls, 1967). These enterotoxins increase the secretion of fluid and electrolytes from system circulation. The pathogenesis of diarrhoea is summarized in Figure II (Yeoman, 1980; Gyang and Aliu 1989).

For ETEC to cause enteric colibacillosis, it has to be ingested orally together with contaminated feeds, water or by licking of the body of infected animal or inanimate objects.

Adhesins on the surface of microorganisms enable a microbe to become established on a particular enterocyte (Duguid, 1959).

During the early 1970s, there was a surge of interest on the mechanism by which bacteria colonise a mucosal surface. Plasmid encoded fimbrial antigen K88 is an

FIGURE II: Pathway of the Pathogenesis of Colibacilosis



SOURCE Yeoman, (1980). GYANG AND ALIU (1989)

essential virulence factor for the majority of *E. coli* strain causing diarrhoea in piglets and K88 mediates the adhesion of these *E. coli* to the mucosal surface of the small intestine of piglets (Smith and Linggood, 1971; Jones and Rutter, 1972).

Adhesion is mediated by the bacterial pili and its mechanisms are complex (Acres, 1985; Dougton and Morrissory, 1985). The bacterial fimbriae attach to specific receptors on the enterocytes and multiply to form microcolonies that cover the surface of the villi cells (Blood and Radostits, 1989a).

A number of factors mediating adhesion and thus colonisation of the host have been identified and their specificities for various molecules present on eukaryotic cell surface have been established (Smith and Linggood, 1971).

Intestinal surface is covered by mucus layer which protects the epithelial cells. Mucus glycoproteins (mucin) are macro-molecules forming the gel and are rich in carbohydrate which occurs as oligo-saccharides which provide potential receptors for bacterial adhesions (Hamphal and Byle, 1983). Certain proteins (lectins) on the surface of a host cell have affinity for carbohydrates and the carbohydrates in the capsule of certain bacteria are attracted to these lectins (Magnussion *et al.*, 1980). The process of bacterial adhesion to the receptor sites may include chemotactic attraction of bacteria to mucus gel,

penetration of and trapping within the mucus gel, adhesion to receptors in the mucus, adhesion to the epithelial cells surface and multiplication of mucus - associated bacteria (Tzipori, 1985a; Freter, 1981).

Different species of enterobacteria possess proteineous appendages in the form of type I fimbriae which mediate the mannose specific adherence of the organism to eukaryotic cells and can be considered as lectins by virtue of their ability to agglutinate cells (Firon *et al.*, 1984). Most bacterial capsules are composed of acidic poly-saccharide which impart hydrophilic character to the bacterial cell wall and lipopolysaccharide line just below the capsule and impart hydrophilic nature to bacteria. However, the role of lipopolysaccharide as an adhesive structure is not clear. It has been shown that without other adhesive structures bacteria expressing only lipopolysaccharide on their surfaces will not adhere to the eukaryotic cell membranes (Magnussion *et al.*, 1980).

Fimbriated bacteria were estimated to possess 2.3×10^3 binding sites per cell (Lindahl and Carlstedt, 1980). Further studies indicate that K99 fimbrial adhesin is a sialoglyco-conjugate-binding lectin in the small intestine - the fimbriae recognise both mucin and glycolipids (Mouricout and Julien, 1987).

Research work on pig small intestine reports that the binding sites for purified K99 is located in mucus layer but not in the submucosal connective tissues (Lindahl and

Carlstedt, 1990). Mucin-glycopeptides found in pig small intestine binds with K99 fimbriated ETEC in contrast to non-fimbriated cells (Lindahl and Carlstedt, 1990). Also sialic acid specificity for K99 fimbriae was also demonstrated by the significant reduction in binding upon desialylation of mucin glycopeptides (Lindahl and Carlstedt, 1990).

Piglet intestine contains a receptor-active glycolipid - the monosialo- ganglioside GM3GC (Seignole *et al.*, 1991). Piglets susceptible to K99 fimbriae had a higher level of sialylated glycolipids than those resistant to K99 attachment, because the proneness to adhere to brush boarders from piglet intestinal cells correlated positively with the degree of sialylation of the intestinal epithelium (Seignole *et al.*, 1991).

It has been found that the adhesion of K99 fimbriae of ETEC to enterocytes is dependent on the age of the host (Hunnels *et al.*, 1980). This finding may explain the age susceptibility to infection in animals (Smith and Halls, 1967).

Enterotoxin Production

After ETEC adheres and colonises the mucosae of the small intestine, they liberate enterotoxins which stimulate the development of diarrhoea. ETEC strains produce one or more enterotoxins which are broadly divided into heat-labile toxin (LT) and heat-stable toxin (ST).

(a) Heat Labile Toxin

These consist of two polypeptide subunits A and B. Subunit A with a molecular weight of 25,000 stimulates adenylate cyclase activity increasing the level of cyclic adenosine monophosphate (cAMP) which causes increase of intestinal fluid secretion (Acres, 1985; Tzipori, 1985a). The secretion primarily originates from the intestinal crypts, although the villous epithelium has also a secretory function (Whipp and Moon, 1988). The cAMP inhibits the absorption of Na+ and hence of C1- and water molecules in the villous cells, while in the crypts cAMP exerts a direct secretory effect by increasing the concentration and consequently, causing loss of C1- and water (Tziport, 1985a). The net result of these activities is the influx of fluid into the intestinal lumen (Tzipori, 1885a; Barnum *et al.*, 1967).

The B subunits of LT have a molecular weight of 11,500 (Tziport, 1985) and adsorb to Y - 1 adrenal cells which is used in LT tests (Sack and Sack, 1975a,b).

(b) Heat stable toxin

Heat stable toxins have no antigenic cross-reactivity to heat labile toxins (Tzipori, 1985a). ST does not have any influence on the concentration of cAMP, but stimulates the activity of guanylate cyclase resulting in an increase in cGMP only in the intestinal epithelial cells and not in other body tissues or cell lines, which may suggest that

there are specific toxin receptors in the small intestine for ST (Tzipori, 1985a; Newsome *et al.*, 1978). ST lacks the secretory activity of LT and cholera toxin (CT) (Tzipori, 1985a).

(c) Diarrhoea

The production of enterotoxins result in fluid secretion and electrolytes loss from the systemic circulation into the lumen of the gut, resulting in varying degrees of diarrhoea, dehydration, electrolytes imbalances, acidosis, hyperkalaemia (when the acidosis is severe), circulatory failure, shock and death (Blood and Radostits, 1989; Acres, 1985).

The fluid secreted is alkaline compared to serum; is also isotonic, low in protein and high in Na+ and HCO3-ions (Moon, 1974).

2.11 The Role of Colostrum in the Prevention of Colibacillosis

The importance of colostrum in preventing neonatal diarrhoea was recognised as early as 1893. The use of serum instead of colostrum feeding was investigated in 1930 with the hypothesis that serum contains at least as much antibodies. The challenge tests indicated a much higher level of antibody in colostrum than in serum (Smith, 1930). These feedings led to an established fact of the tremendous importance of colostral intake for positive immunity in animals.

Neonatal mammals are born with well developed lymphoid neutrophil and macrophage populations, but are not immunologically competent (Logan and Penhale, 1971; 1972; Radostits, 1986). Due to the absence of transplacental transfer in food animals, newborn animals are born hypo-or agammaglobulinaemic (Radostits, 1981; McEvan *et al.*, 1970).

Feeding of colostrum neonates is very essential for the following reasons as stated by Radostits (1986) and Logan and Penhale (1971; 1972):

(1) Is an important source of immunoglobulin part of which when ingested is observed to protect neonates against systemic disease (IgG1, IgG2 mainly, and part of which remains in the lumen of the intestine to prevent the development of enteric colibacillosis (diarrhoea), mainly of which is IgA by neutralizing the pathogens.

(2) It enhances neonatal phagocytosis due to impaired serum complement concentration.

(3) Colostrum stimulates neutrophils and enhances the neutrophilic response to endotoxin challenge.

(4) It contains transferrin and lactoferrin which bind iron and thus restrict bacterial growth.

Immunoglobulin IgG is the major immunoglobulin in colostrum, others are IgM and IgA (Blood and Radostits, 1989a). Absorption of colostral immunoglobulins takes place within the first 24 hours of life of the new born farm animals with the peak of absorption within 6 - 8 hours of age (Blood Radostits, 1989a; Haggard and Sherman, 1984).

The absorption of the immunoglobulins across the epithelial cells of the small intestine is by the process of micropinocytosis and are transported via the lymphatic system to the blood (Blood and Radostits, 1989a; Gay and Besser, 1985). In the calf, there is evidence of differential absorption between classes of immunoglobulins and their duration of absorption. About 90% of IgM is absorbed within 27 hours, 48% of IgA and 59% IgA are absorbed in 16 and 22 hours respectively (Blood and Radostits, 1989a).

IgA is synthesized by mammary gland of sow throughout lactation and serves as an important defence mechanism against enteric disease in the pig (Snodgrass *et al.*, 1986), whereas only low concentrations of IgA are secreted into the milk of the ruminant (Porter, 1973).

Newborn calves must, therefore, ingest approximately 50ml/kg body weight within the first 8 - 12 hours (Blood and Radostits, 1989a). Multiple small intakes of colostrum give significantly great levels of serum immunoglobulins than a large single intake and calves with continuous access to their dams are in the most favourable position (Blood and Radostits, 1989a).

CHAPTER THREE

THE EFFECTS OF GEOGRAPHICAL LOCATION, YEAR, SEASON, AGE AND ENTERIC PATHOGENS ON THE DISTRIBUTION OF CALF DIARRHOEA IN ZARIA

3.1 Summary

A total of 479 calves in 27 herds were examined for diarrhoea and enteric pathogens. About 13.6% had diarrhoea.

Loose greenish non-smelly diarrhoea was suggestive of helminthic infections, while whitish, bloody or mucoid diarrhoea was suggestive of coccidial and bacterial diarrhoeas.

The proportion of calves which survived for 120 days without diarrhoea was 91.3%. The probability of calves having diarrhoea was highest during the first month of life.

There was no statistically significant differences between the occurrence of diarrhoea and geographical locations of herds, year and season.

The presence of enteric pathogens in diarrhoeic calves did not indicate that they caused diarrhoea. There were associations between some enteric pathogens and the presence of diarrhoea. These enteric pathogens were *E. coli* + *Toxocara* sp; *E. coli* + Coccidia; *E. coli* + *Strongyloides* (in older calves); and *E. coli* + *Toxocara* sp + Coccidia. Important pathogens responsible for diarrhoea in the first 30 days of life were *E. coli* + Coccidia; *E. coli* + *Toxocara* sp. and *E. coli* + *Toxocara* sp. + Coccidia. It is suggested

that *E. coli* may be an important factor to be present for gut parasites to cause diarrhoea.

The presence of *E. coli* enterotoxins was not related to the occurrence of diarrhoea. The ability of *E. coli* to haemagglutinate Ox RBC may be an important factor to look for from *E. coli* isolated from diarrhoeic calf in Zaria.

3.2 Introduction

Diarrhoea is the passage of water in the faeces in relative excess in proportion to faecal dry matter (Jubb *et al.*, 1985). It usually reflects absolute faecal loss of fluid which usually leads to loss of electrolytes, acid-base imbalance and dehydration (Sojka, 1965; Barnum *et al.*, 1967; Jubb *et al.*, 1985; Tzipori, 1985a,b; Blood and Radostits, 1989a). It is a symptom of enteric malfunction rather than a specific diagnosis because there is usually no single cause but a complex confused aetiology which involves two or more interactions between entero-pathogenic strains of *E. coli*, *Salmonella sp.*, corona virus, corona-like viruses, *Cryptosporidium sp. Emeria spp.*, the level of colostral immunity, the nature of diet and the level of management (Radostits, 1981; Tekdek, 1983; Tzipori, 1985b; Blood and Radostits, 1989a), and parasitic infections such as *Toxocara vitulorum* (Dun, 1978).

Young calves are constantly faced with the challenges of life from the environment and disease agents. Of particular interest are the entero-pathogens which are

mostly ingested through colostrum and milk, contaminated feeds and water. In addition to the colonization of the gut, as calves grow, different stages of parasites are ingested into the gut with the occurrence of either single or multiple infections. These infections may or may not result in diarrhoea (Myers *et al.*, 1978; Tekdek and Ogunsusi, 1987). Some studies on diarrhoeic calves showed little or no differences in the types of entero-pathogens observed in healthy and sick calves (Morin *et al.*, 1978; Myers *et al.*, 1984). Isolation of some entero-pathogens from diarrhoeic calves may not necessarily mean that the entero-pathogens are the cause of the diarrhoea.

Calf morbidity and mortality world wide, have been closely associated with some factors such as management systems, weather or environmental conditions, age and infectious agents (Speicher and Hebb, 1973; Martin *et al.*, 1975; Tekdek, 1983). The first two risk factors are closely associated with enteric infections which gradually build up in the calf pens and cause enteric diseases in neonates with low immunological status (Leech *et al.*, 1968; Blood and Radostits, 1989a).

In Nigeria, some studies have shown that in our indigenous herd, calf mortality rate has been estimated to be 11.2% (Pullan, 1980) and 5.5% (Tekdek, 1983). Both reports indicated that gastrointestinal parasitism caused the highest toll of calf mortality due to diarrhoea. There is no known, documented report on the effects of geogra-

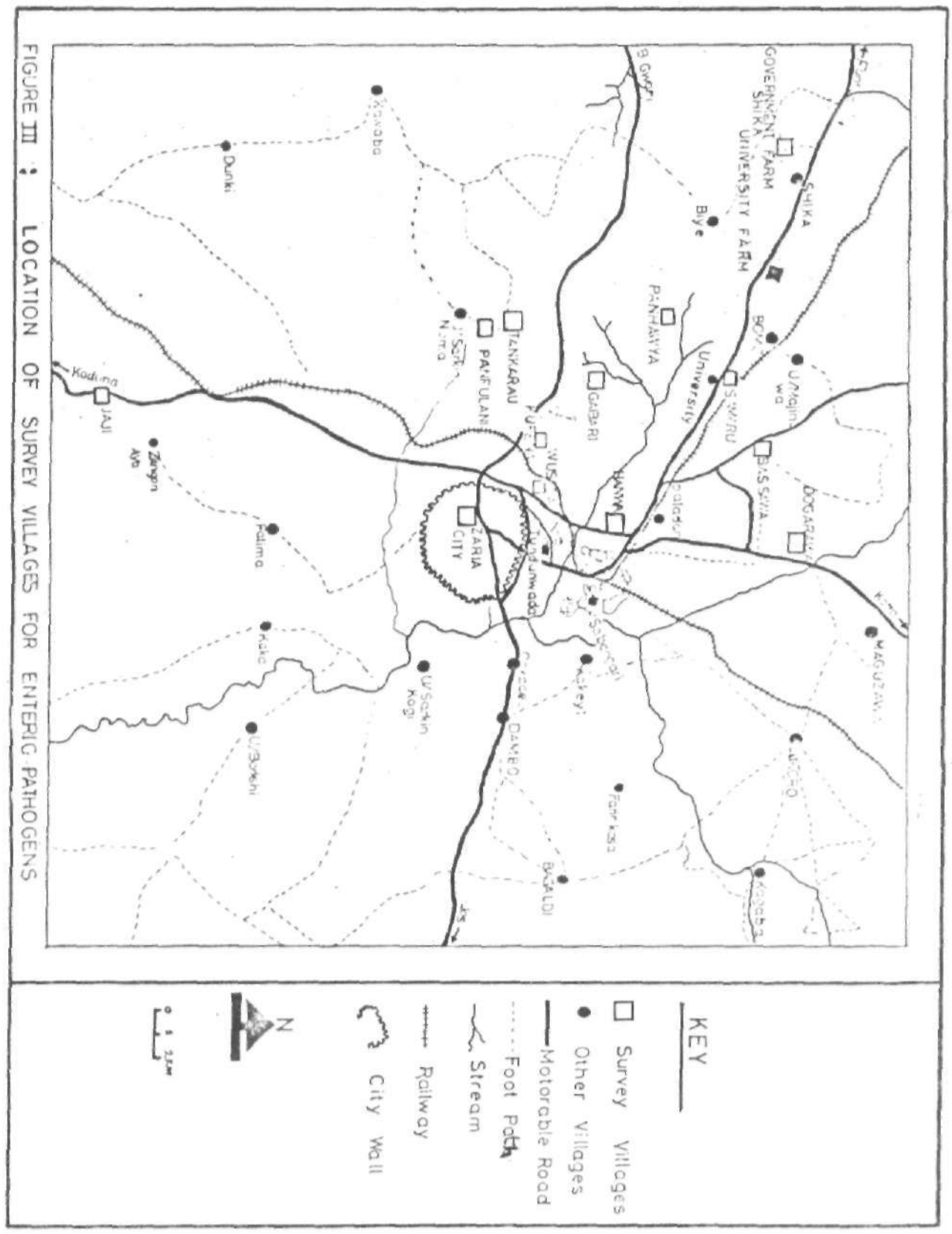


FIGURE III : LOCATION OF SURVEY VILLAGES FOR ENTERIC PATHOGENS

Aging of the calves was done by information from the owners. All ages were recorded in days.

Sample collection

Faecal samples and two rectal swabs were collected from each available diarrhoeic and selected non-diarrhoeic calves on each occasion. Rectal swabs were inoculated into test tubes of sterile *MacConkey and Selenite Broths* respectively and were then inoculated for 24 hours at 37°C.

Sample examination

Faecal samples were checked for the presence of protozoan oocysts and helminths eggs by floatation method using 33% zinc sulphate solution as described by Dun (1978). Oocysts and eggs count per field was recorded as follows:

NPF = No parasite found = Equivalent to zero eggs
* = Light infection = Equivalent to 1-10 eggs
** = Moderate infection = Equivalent to 10-20 eggs
*** = Heavy infection = Equivalent to 20-50 eggs
**** = Packed heavy infection = Equivalent to 50-100 eggs.

Bacterial Culture

Each samples was subcultured on to EMB and MacConkey agar for 24 hours, at 37°C. Any colony with greenish metallic sheen on EMB was considered to be *E. coli*. Confirmatory test were carried out using biochemical test as follows:

Methyl red +

Indole +

Voges Proskauer -

Citrate -

Lactose fermenter +

The growth on MacConkey agar indicated whether the organisms were lactose fermenters or non-lactose fermenters. Any non-lactose fermenters were subcultured onto Shigella-Salmonella Agar (SS Agar) and Brilliant green agar. The biochemical tests which indicated *Salmonella spp* were as follows:

- TSI - Alkaline/Acid ± H_2S
- Urea -- negative
- Lysine decarboxylation + ve

All confirmed *E. coli* isolates and suspected Salmonella isolates were subcultured onto nutrient agar slants and stored at 4°C for serotyping. Other bacteria and viruses were not looked for.

Probability of calves with or without diarrhoea and the proportion of calves surviving unto 4 months of age were calculated as described by Remington and Schork (1970); Martins and Wiggins (1973). Chi-square tests and Odds ratios were used to test for associations where appropriate.

3.4 Results

Sixty-five of the 479 calves from 27 herds examined had diarrhoea (13.6%).

The faeces from the diarrhoeic calves were loose greenish non-smelly (72.3%), whitish pasty and smelly (13.8%), watery and smelly (9.2%) and bloody/mucoid smelly (20.0%) (Table 2). The diarrhoea characteristics were suggestive of the various enteric pathogens ($P<0.05$).

A total of 535 calves were followed up from birth to 120 days of age. The proportion of calves which survived for the 120 days of age without diarrhoea was 73.8%. The probability of calves having diarrhoea was highest (9.9%) in age-group 0 - 30 days and lowest (4.0%) in age group 61 - 90 days (Table 6).

No statistically significant association existed between geographical location of the herds, year and season and frequency of diarrhoea ($P>0.05$)(Tables 7-9).

Age distribution of calf diarrhoea showed that the frequency was highest (23.2%) among calves of age group 0 - 30 days and lowest (7.8%) among calves of age group 61-90 days (Fig. IV).

Table 10 shows the frequency of enteric pathogens among diarrhoeic and non-diarrhoeic calves up to 4 months of age. Although, there was no significant difference in the frequency of enteric pathogens between diarrhoeic and non-diarrhoeic calves ($P>0.05$), the frequency of enteric pathogens was lower (13.1%) in diarrhoeic calves than in non-diarrhoeic calves (86.3%).

Table 11 shows the distribution of enteric pathogens occurring either single or in combination. There was

significant difference in the distribution of enteric pathogens between diarrhoeic and non-diarrhoeic calves. It was observed that single helminthic infection in calves without *E. coli* infections did not cause diarrhoea; while single helminthic infection in the presence of *E. coli* caused diarrhoea.

Multiple helminthic infection caused diarrhoea in 11.1% of the calves, while multiple helminthic infection in the presence of *E. coli* or coccidia sp. caused diarrhoea in 16.5% and 44.4% of the calves respectively.

Light and heavy infection load of coccidia and *Strongyloides* sp. in calves were not associated with diarrhoea, while heavy infection with *Toxocara* sp. in calves was associated with diarrhoea (Appendix A).

Odds ratios indicated association between some enteric pathogens and the presence of diarrhoea. Infections with *E. coli* + *Toxocara* sp; *E. coli* + Coccidia; and *E. coli* + *Toxocara* sp + Coccidia (Appendix B) were 2.3, 1.9, 3.2 times more likely to cause diarrhoea than calves without these parasites respectively.

Figure V shows the distribution of enteric pathogens among non-diarrhoeic and diarrhoeic calves by age-group (Appendix C). There was no significant difference between different age-groups. Figure VI shows the distribution of enteric pathogens in non-diarrhoeic and diarrhoeic calves up to 30 days of age. The enteric pathogens that were significantly associated with diarrhoea in this age-group

were *E. coli* + Coccidia; *E. coli* + *Toxocara* sp; and *E. coli* + Toxocara + Coccidia. The presence of *Strongyloides* sp. had no significant effect on the occurrence of diarrhoea in this age-group as was in older age group.

Table 12 shows the frequency of *E. coli* enterotoxins extracted from diarrhoeic calves up to 4 months of age. The isolation of *E. coli* was not associated with the presence of enterotoxins. Among the *E. coli* isolated from diarrhoeic calves, 85.2% did not show the presence of enterotoxins.

3.5 Discussion

The prevalence of diarrhoea in this study (13.6%) is lower than the 32.2% reported earlier by Tekdek and Ogunsusi (1987) for the same study area. This suggests the prevalence of calf diarrhoea in Zaria is variable.

The characteristics of the diarrhoeic faeces observed in this study may help in the clinical diagnosis but are not confirmatory. Similar descriptions of diarrhoea are given by other workers (Fitzgerald and Mansfield, 1972; Dun, 1978; Shah-Fischer and Say, 1989; Blood and Radostits, 1989a). Chanter *et al*. (1984, 1986) and Hall *et al*. (1985), reported that *E. coli* strains caused dysentry in calves.

The probability of calves surviving up to 4 months without diarrhoea was calculated using Life Table methods as described by Remington and Schork (1970). The probability of calves being without diarrhoea was lowest (90.0%) in age-group 0 - 30 days and highest (96.0%) in age group 61 - 90

days. This finding is similar to the reports of Blood and Radostits (1989a) that critical periods of calf diarrhoea is the first 21 days of life. In this study, the proportions of calves surviving without diarrhoea up to 4 months of age was 91.3%. This may indicate that at any given time about 10% of calves would have diarrhoea by the time they are 4 months of age. The high probability of calves having diarrhoea at the 91 - 120 days age group could be due to the fact that by the time calves were 4 months of age they will be in the rainy season when fresh grass is available. Calves grazing on fresh grass do come down with nutritional diarrhoea (Blood and Radostits, 1989a).

The results in this study indicate that diarrhoea in calves was not due to the location of herds. This finding indicates the uniformity of management conditions for calves in the villages studied. Reports elsewhere indicate that occurrence of diarrhoea was dependent on management systems (Martin *et al.*, 1975; Speicher and Hebb, 1973; Leech *et al.*, 1968).

The yearly distribution of calf diarrhoea suggests uniformity of calf diarrhoea over these years.

The reason why there was no significant difference in seasonal occurrence of diarrhoea in calves in this study may indicate uniformity of diarrhoea determinants in both dry and rainy seasons.

The frequency of diarrhoea was not dependent on age although there was a slightly higher rate of occurrence in

younger calves. This may suggest that younger calves have less developed immune systems than older calves (Radostits, 1981; Blood and Radostits, 1989).

The study shows that the presence of enteric pathogens in diarrhoeic calves does not necessarily imply that they are associated with diarrhoea, since calf diarrhoea is caused by complex and confusing aetiology which may involve two or more enteric pathogens, the colostral immunity level, the nature of diet and the management practices (Radostits, 1981; Tekdek, 1983; Tzipori, 1985a; Blood and Radostits, 1989a).

Since single and multiple helminthic infections in combination infected with *E. coli* caused a higher proportion of diarrhoea than single and multiple helminthic infections without *E. coli*, it could be inferred that for calves to have diarrhoea *E. coli* may be a factor. Among the enteric pathogen associated with diarrhoea, *E. coli* appeared to be consistent pathogen except where calves are infected with three or more parasites.

Infection load of parasites determined quantitatively or qualitatively indicates the level at which each parasite can cause disease (Dun, 1978). In this study, heavy infection load of *Toxocara* sp. in calves was associated with diarrhoea. This may suggest that the combinations of moderate to heavy infections of *Toxocara* sp. and *E. coli* in a calf is likely to cause diarrhoea.

The distribution of enteric pathogens among non-diarrhoeic and diarrhoeic calves were not significantly different among different age groups. This observation is contrary to the reports of Tekdek and Ogunsusi (1987), and Shah-Fischer and Say (1989), which state that younger calves have lesser infections than older calves.

Calves in the 0-30 day age-group were at a high risk of diarrhoea. The distribution of enteric pathogens in this group was associated with the presence of diarrhoea. This may suggest that the occurrence of diarrhoea in this age group is caused by enteric pathogens.

This ability of *E. coli* to haemagglutinate Ox RBC implicate the organism to cause diarrhoea (Tzipori, 1989a). As inferred from this study, this ability of *E. coli* isolated from diarrhoeic calve to haemagglutinate Ox RBC is suggested to be the major factor to look for among diarrhoeic calves in Zaria.

Fig. IV: The distribution of diarrhoea among calves up to 4 months of age, in Zaria, by Age.

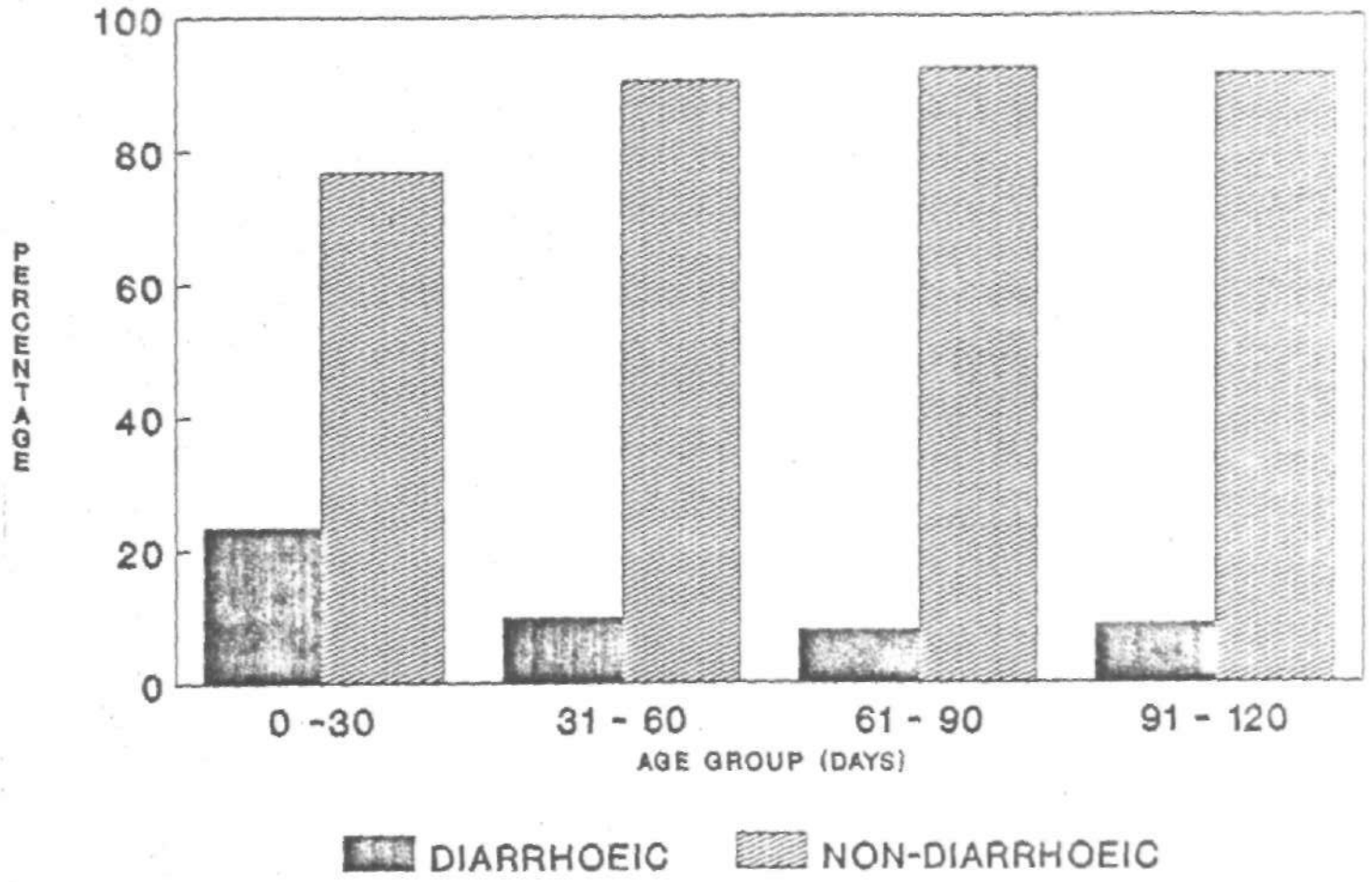
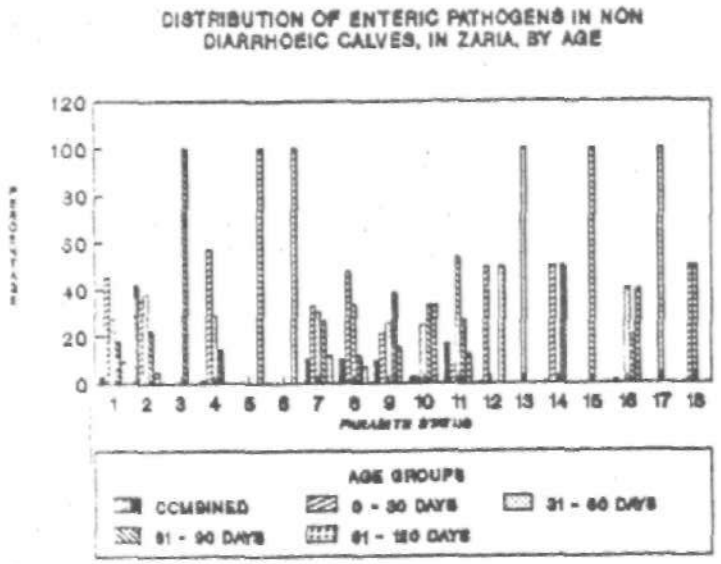
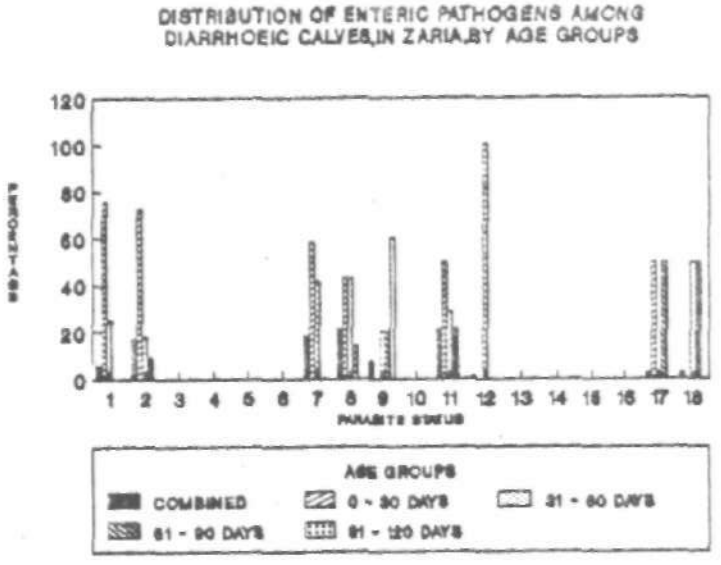


Fig. V: Distribution of Enteric Pathogens in Non-diarrhoeic and Diarrhoeic Calves in Zaria, by Age.





Key:-

1 = Negative for Pathogens
2 = E. coli alone
3 = Strongyloides alone
4 = Coccidia alone
5 = Strongyles alone
6 = E. coli + Moniezia
7 = E. coli + Coccidia
8 = E. coli + Toxocara
9 = E. coli + Strongyloides
10 = E. coli + Strongyles
11 = E. coli + Coccidia + Toxocara
12 = Toxocara + Strongyloides
13 = Toxocara + Strongyles
14 = Coccidia + Strongyloides
15 = Coccidia + Strongyles
16 = Strongloides + Strongyles
17 = Coccidia + Strongyles + Strongyloides
18 = Coccidia + Strongules + Toxocara

Fig. VI: Distribution of Enteric Pathogens in Non-diarrhoeic and Diarrhoeic Calves of up to 30 days of age, in Zaria

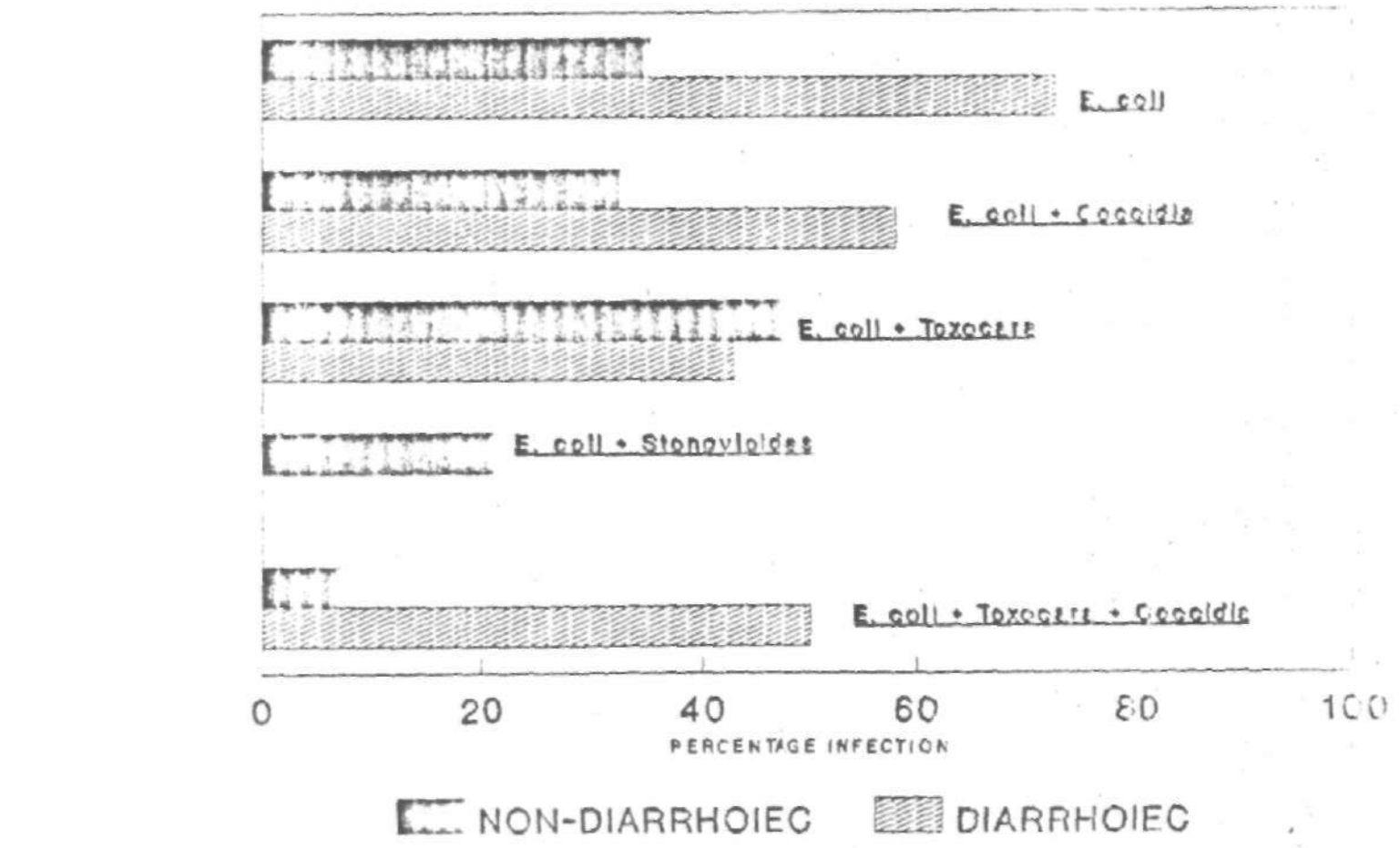


Table 5. Characteristics of Diarrhoea Produced by Different Enteric Pathogens among Diarrhoeic Calves up to 4 Months of Age, in Zaria.

Enteric Pathogens	No. of calves with Diarrhoea	Characteristics of Diarrhoea							
		Loose Greenish non-smelly		Whitish pasty and smelly		Watery and Smelly		Bloody/ Mucoid	
		Fq	%	Fq	%	Fq	%	Fq	%
Negative for Pathogens	4	0	0.0	1	25.0	2	50.0	1	25.0
Multiple helminths alone	1	1	100.0	0	0.0	0	0.0	0	0.0
E. coli alone	11	7	63.0	3	27.3	1	9.1	0	0.0
E. coli + Coccidia	12	1	8.3	3	25.0	1	8.3	7	58.3
E. coli + Single Helminths	19	16	84.2	1	5.3	1	5.3	1	5.3
E. coli + Multiple Helminths	1	9	64.3	1	7.1	1	7.1	3	21.4
Multiple Helminths + Coccidia	4	3	75.0	0	0.0	0	0.0	1	25.0
Total	65	47	72.3	9	13.8	6	9.2	13	20.0

Table 6. Life Table Showing the Probability of Calves Surviving up to 4 Months (120 days) of age without Diarrhoea, in Zaria.

X	Ox	nWx	ndx	nqx	npx	Px
0 - 30	535	0	53	0.0991	0.9009	1.000
31 - 60	535	5	35	0.0654	0.9346	0.9009
61 - 90	530	3	21	0.0396	0.9604	0.8420
91 -120	527	3	46	0.0873	0.9127	0.8086
> 120		-	-	-	-	0.>380

Key: X = number in days following birth.
Ox = number of calves alive at least after birth.
nWx = number of calves withdrawn by deaths.
ndx = number of calves having diarrhoea between the xth + 30 days for those alive at time x.
nqx = probability of having diarrhoea at the xth to the xth + 30 days for those alive at time x.
npx = probability of those calves without diarrhoea at the time x surviving until the xth + 30 days. Thus npx = 1 - nqx.
Px = The proportion of calves surviving x 30 days.

Table 7. The Distribution by Village of up to 4 Month Old Diarrhoeic Calves, in Zaria.

Survey Villages	No. of Herds	No. of Calves Examined	Frequency of Diarrhoea	% Frequency of Diarrhoea
A	4	47	10	21.3
B	5	58	7	12.1
C	2	64	6	9.4
D	3	50	7	14.0
E	4	111	14	12.6
F	8	110	16	14.5
G	1	39	5	12.8
Total	27	479	65	13.6

Key: A = Tankarau
Kufena
Pan-Fulani

B = Wusasa
Zaria City

C = Government Farm Shika
University Farm Shika

D = Bassawa
Dogarawa
Samaru

E = Hanwa
Gabari

F = Panhauya

G = Jaji/Barin Yaro

Table 8. The Yearly Distribution of Diarrhoea in Calves Up to 4 Months of Age, in Zaria.

Year	No. of Calves	No. of Calves with Diarrhoea	Diarrhoea Year specific Rate
1984	70	8	11.4
1985	209	23	11.0
1986	200	34	17.0
Total	479	65	13.6

Table 9. Seasonal Distribution of Diarrhoea in Calves Up to 4 Months of Age in Zaria.

Season	No. of Calves Examined	No. of calves with Diarrhoea	%
Dry	1434	105	7.3
Wet	543	37	7.2

Table 10. The Frequency of Enteric Pathogens among Diarrhoeic and non-Diarrhoeic Calves Up to 4 Months of Age, in Zaria.

	No. of Calves with Diarrhoea	No. of Calves without Diarrhoea	Total
No. of calves positive for enteric pathogens	61(13.1%)	403(86.3%)	464
No. of calves Negative for enteric pathogens	4(26.7%)	11(73.3%)	15
Total	65(13.6%)	414(86.4%)	479

Table 11. The Distribution of Enteric Pathogens Among Diarrhoeic and Non-Diarrhoeic Calves Up to 4 Months of Age in Zaria.

Enteric Pathogen	Diarrhoea Status		
	Presence of Diarrhoea	Absence of Diarrhoea	Total
Negative for pathogens	4(26.7)	11(73.3)	15
Single Helminths alone	0(0.0)	2(100)	2
Multiple helminths alone	1(11.1)	8(88.9)	9
Coccidia alone	0(0.0)	7(100)	7
Bacteria alone (*E. coli*)	11(6.0)	172(94.0)	183
Coccidia + Bacteria (*E. coli*)	12(21.8)	43(78.2)	55
Single Helminths + Coccidia	0(0.0)	3(100)	3
Single Helminths + Bacteria (*E. coli*)	19(20.7)	92(79.3)	111
Multiple helminths + Bacteria (*E. coli*)	14(16.5)	71(83.5)	85
Multiple helminths + Coccidia	4(44.4)	5(55.6)	9
Total	65(13.5%)	414(86.4%)	479

Table 12. The Frequency of E. coli Enterotoxin Extracted from Diarrhoeic Calves Up to 4 Months of Age, in Zaria.

Cases of Diarrhoea	Enterotoxin Status		
	Presence of *E. coli* Enterotoxins	Absence of *E. coli* Enterotoxins	Total
1. With *E. coli*	0(0.0)	10(100)	10
2. With *E. coli* and other pathogens	6(14.6)	35(85.4)	41
3. With Ox RBC (*E. coli*)	1(33.3)	2(66.7)	3
4. With Ox RBC (*E. coli*) and other pathogens	2(28.6)	5(71.4)	7
Total	9(14.8)	52(85.2)	61

CHAPTER FOUR

CHARACTERIZATION OF *ESCHERICHIA Coli* ISOLATED FROM CALVES FAECES IN ZARIA

4.1 Summary

A total of 102 *E. coli* strains isolated from 65 and 41 diarrhoeic and non-diarrhoeic calves respectively were characterized. In this work a known enterotoxigenic strain, serogroup 08, was among the commonest 0 serogroups identified. Other common serogroup was 026 which is known to be associated with infant haemorrhagic colitis and bloody diarrhoea in humans and it is of public health significance.

Mixed infections of enterotoxigenic *E. coli* (ETEC) and gastro-intestinal parasites (GP) caused 11.8% of the diarrhoea, while mixed infection of GP and *E. coli* strains which agglutinated Ox RBC caused 9.8% of the diarrhoea. While the combination of GP and *E. coli* strains contributed 23.5%, and *E. coli* strains alone contributed 5.9% of the diarrhoea.

The frequencies of enterotoxins identified were, ST, 8.1% and LT, 3.3% from diarrhoeic calves while the frequencies of Verocytotoxins were 3.3% and 9.8% from diarrhoeic and non-diarrhoeic calves respectively. Although the role of VTEC in calf diarrhoea is not yet clear, VTEC is sometimes associated with calf diarrhoea.

The property of *E. coli* to haemagglutinate Ox erythrocytes (16.4%) was a significant finding in diarrhoeic

calves. The factor that may be incriminated with *E. coli* isolated from diarrhoeic calves is the ability of *E. coli* to haemagglutinate Ox RBC.

4.2 Introduction

Escherichia coli has been shown to be a normal inhabitant of the gastrointestinal tract of animals and man (Sojka, 1965; Barnum *et al.*, 1967, Cottral, 1978; Jawetz et al., 1989; Blood and Radostits, 1989a). There are multiple strains of *E. coli* in the gut and are constantly varying (Sherwood *et al.*, 1983). This suggests that the diagnosis of diarrhoea caused by *E. coli* may be difficult to confirm.

Earlier surveys of calf mortality incriminated *E. coli* as a major cause of death due to 'White scours' (Jensen, 1893, Lovell and Hughes, 1935; Withers, 1952). Later, Smith and Halls (1967) showed that certain strains of *E. coli* isolated from calves and piglets caused dilation of ligated gut loops whose effect correlated with the ability of the strains to cause diarrhoea. These *E. coli* strains are known to produce enterotoxins and were later known as enterotoxigenic *E. coli* (ETEC) (Tzipori, 1985a).

In Nigeria, Adetosoye and Ojo (1983) reported the demonstration of LT from *E. coli* isolated from diarrhoeic kids and goats suffering from PPR while Agbonlahor *et al.* (1984) reported a prevalence of ETEC from diarrhoeic calves in Jos, Plateau State of Nigeria as 58.3% (ST) and 9.5% ST

and LT. Adetosoye (1985) reported 11 *E. coli* serotypes isolated from 12 diarrhoeic calves, three of which were known to dilate intestinal loop of rabbit with accumulation of fluid indicating that the strains were enterotoxigenic (Adetosoye, 1980).

This study is designed to determine the prevalence of heat stable (ST) and heat labile toxins, verocytotoxins (VT), the ability of Ox RBC to agglutinate Ox RBC, somatic (O), capsulla (K) and flagella antigens and adhesive factor ($K99^+$) from *E. coli* isolated from diarrhoeic and non-diarrhoeic calves.

4.3 Materials and Methods

4.3.1 Collection and Culture of Faecal Samples

Faecal materials were collected from 106 (65 diarrhoeic and 41 non-diarrhoeic) calves out of 479 calves in 27 indigenous herds in Zaria. Rectal swabs were collected from all the calves up to 4 months of age in the herds and transported to the laboratory in sterile universal collection bottles containing sterile MacConkey broth and incubated for 24 hours at 37°C. These were then subcultured on EMB agar at 37°C for 24 hours. Biochemical tests were performed on any colony showing black greenish metalic sheen to confirm if the colony is that of *E. coli*. All the *E. coli* isolates were grown on nutrient agar slant and were sent to *E. coli* Reference laboratories for O,K,H antigen

determination, screening for K99+ *E. coli* and for enterotoxins assay, LT, ST, VT and Ox RBC agglutination tests.

The *E. coli* reference laboratories are:

1. Statens Serumins-tituts, Copenhagen, Denmark (Drs. I and F. Orskov).
2. Nattriumwea 7, Post Box 184, 3800. Ad Amersfoort, The Netherlands (Dr. P.A. Guinee).
3. Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, New Haw, Weybridge Surrey, London (Mr. C. Wray).

4.3.2 Determination of ETEC

a. Extraction of Toxins

E. coli enterotoxins were extracted as described by Myers *et al.*, 1975). Each *E. coli* isolate was grown overnight at 37°C in 20ml of Brain Heart Infusion Broth in a conical flask oscillating at 40 oscillations per minute. Then the broth was centrifuged at 1600g for 20 minutes at 4°C. The supernatants were collected and the pellets discarded. Then each supernatant was filtered through millipore of 0.45um pole diameter, divided into two and stored at - 20°C for further use.

b. Heat stable toxin determination (ST)

One half of the stored endotoxin extract was thawed in a water bath at 65°C for 30 minutes.

Rabbits were starved for 24 hours. Each rabbit was anaesthetized by intramuscular injection of 0.04ml of ketamine (4mg). The ventral aspect of the abdomen was shaved from the xiphoid cartilage to the loin area, 1.0ml of 2% xylocaine was infiltrated at the mid-line surgical site. The area was washed with soap and disinfected with chlorhaxidate solution. A mid-line incision was made 5 to 6 cm from the xiphoid cartilage caudally. The intestine was exposed and the illeo-caecal junction was identified, 1.0ml of Brain Heart Infusion (BHI) broth was used to flush the lumen of the intestine and caudally milked along the length. A total of 14 loops each about 2.5cm to 3cm long were made using 3.0 plain gut. The first loop (nearer to illeocaecal junction) was injected with 1.0ml of toxin from *E. coli* 0141: K85, K99 ST (supplied by C. Wray, Weybridge, London) to serve as positive control while the middle loop was injected with 1.0ml of BHI broth to serve as negative control. Each of the remaining loops was injected with 1.0ml of toxin extract from different isolates of *E. coli*. The intestine was returned to the abdominal cavity. The peritonium and abdominal muscles were sutured together with interrupted suture pattern using 3.0 silk. The rabbits were kept for 6-7 hours and were then sacrificed and the intestines removed and examined. The length and volume of loops were measured. The ratio of the volume to length was calculated. Any loop with a ratio of volume to length of 1 or greater than 1.0ml/1cm was recorded as positive for ST.

c. Determination of Heat Labile Toxins (LT)

The second half of the stored supernatant (endotoxin) was thawed at room temperature.

The rabbits were prepared as for ST assay. After making the loops, the first loop was injected with *E. coli* 0101: K99, F41 extract supplied by C. Wray from Veterinary Laboratory Weybridge, London. After the closure of the abdominal cavities the rabbits were kept for at least 18 hours before they were killed. The volume to length ratio of the loops were calculated.

4.3.3 Verocytotoxin Tests

This cytotoxic substance was evaluated using either gene probes - Weybridge (London) or the use of vero cells, Denmark and Netherlands laboratories.

4.3.4 Ox erythrocytes Haemagglutination

All the *E. coli* isolates from diarrhoeic calves and non-diarrhoeic calves were tested for Ox RBC agglutination test. A direct haemagglutination experiments was conducted as follows:

> Whole blood was collected from the jugular vein of old calf into saline solution and washed twice in saline. Then a 3% erythrocytes suspension in saline was used for direct agglutination of bacteria on a slide. A colony of *E. coli* was usually picked by a sterile broom stick and suspended directly in the blood. Both positive and blank control were also prepared. After a mild shaking of the slide the result was read in about 10 - 30 seconds.

4.3.5 Determination of 'O', 'K', 'H' antigens

Sixty-one of *E. coli* isolates from diarrhoeic calves and 41 from non-diarrhoeic calves were sent to Denmark, Netherlands and London reference laboratories for the purpose of serotyping.

4.3.6 *Screening for* K99 antigen

All *E.coli* isolates were tested for K99 antigen by slide agglutination tests using rabbit anti-K12: K99 serum absorbed with strain K12.

4.4 Results

E. coli was isolated from 61 of the 65 diarrhoeic calves whilst four of them were negative for the organism.

Table 13 shows the frequencies of *E. coli* enterotoxins and *E. coli* strains which haemagglutinate Ox RBC isolated from the diarrhoeic and non-diarrhoeic calves. Heat stable (ST) and heat labile (LT) with frequencies of 8.1% and 3.3% respectively were observed from diarrhoeic calves only, while the frequencies of verocytotoxin producing *E. coli* (VTEC) observed from diarrhoeic and non-diarrhoeic calves were 3.3% and 9.8% respectively. Ox RBC haemagglutination was observed from *E. coli* strains isolated from diarrhoeic and non-diarrhoeic calves at the rate of 16.4% and 4.8% respectively. Appendices D1 and D2 show the raw results of ligated loop test for ST and LT respectively.

Different O, K, and H antigens were serotyped (Appendix E). The common 'O' antigens are shown in Table 14. These were, 08 (5.9%), 0.5 (4.9%), 075 (3.9%), 026 (3.9%) and 06 (2.9%). There were some 'O' antigens that did not belong to the known 'O' group 01 - 170 known. These were expressed as O?.. (6.9%).

There was no K99 *E. coli* antigen identified from the calves. About 13.7% of the isolates were untypeable meaning that they did not react with the available 170 'O' sera for *E. coli* serotyping. There was a wide variety of *E. coli* serotypes found and many of these did not possess 'K' or 'H' antigens.

Mixed infections of enterotoxigenic *E. coli* (ETEC) and gastrointestinal parasites (GP) was associated with 11.8% of the diarrhoea, while mixed infection of *E. coli* strains which agglutinated Ox erythrocytes (Ox RBC) and GP caused 9.8% of the diarrhoea (Table 15).

4.5 Discussion

By ileal loop ligation method, low frequency of ST(8.1%) was detected from diarrhoeic calves. Using DNA hybridization gene probe technique on the same isolates, no ST was detected. The difference could not be readily explained.

The results indicate that *E. coli* serotypes, in Zaria, vary from herd to herd and even within herds. The common 'O' serogroups known to cause diarrhoea in cattle include

08, 09, 020 and 0101, which contain the pilus antigens K99 and produce enterotoxin ST, and tend to be mucoid (Harnett and Gyles, 1983). In this report, the commonest 'O' serogroups observed was 08. This serogroup has the greatest potential of causing diarrhoea in calves in Zaria. Other common serogroups include 06 which is known to cause urinary tract infection in man (Edwards and Ewing, 1972) and 026 which is known to be associated with infant haemorrhagic colitis (HC) and bloody diarrhoea (BD) (Craviole *et al.*, 1987). These findings of common serogroup between man and calves is of public health importance. The other common serogroups 015 and 075 are not associated with diseases in calves and man. The occurrence of 'O?' serogroup which did not belong to any of the common 'O' serogroups indicates that these are serogroups to which no antisera are currently available. One *E. coli* serotype 078 : K80 isolated in this study is known to cause severe disease in calves (Logan and Penhale, 1971; 1972).

The heat labile enterotoxin is not commonly detected in calves (Tzipori *et al.*, 1981; Isaacson *et al.*, 1978). Thus the low frequency in this report is expected.

Some *E. coli* strains that produce verocytotoxins are called verocytotoxin *E. coli* (VTEC). Their frequencies were 3.3% and 9.8% from diarrhoeic and non-diarrhoeic calves respectively. Some reports indicate that VTEC is associated with diarrhoea in man and animals and is of public health importance (Giugliano and Giugliano, 1987; Clarke *et al.*,

1987; Scotland *et al.*, 1987). In another study, Peiris *et al.*, (1987) reported VTEC production in diarrhoeic and healthy humans, cattle, pigs and goats and concluded that verocytotoxin production may be one of the mechanisms of diarrhoea production by many EPEC strains. Some reports show 88.9% VTEC from healthy calves and some of the serotypes were 01:H20 and 0113:H21. These 'O' serogroups were also identified among the *E. coli* isolates in this report indicating the presence of VTEC in calves.

The ability of an *E. coli* strain from calf diarrhoea to haemagglutinate ox erythrocytes (Ox RBC) implicate the organism in the disease. This indicate that *E. coli* from diarrhoeic cases that agglutinate Ox RBC are probably associated with the diarrhoea. Awad-Masalmeh *et al.*, (1982), reported similar findings using porcine strain of enterotoxingenic *E. coli* lacking K88, K99, and 987P antigens.

Absence of K99 or F41 positive isolates among the *E. coli* associated with diarrhoea in Zaria may indicate that other pathogenic factors of *E. coli* may be the cause of diarrhoea among calves such as the ability of *E. coli* to haemagglutinate Ox RBC.

Table 13. *E. coli* Characteristics Isolated from 102 Diarrhoeic and Non-Diarrhoeic Calves up to 4 Months of Age in Zaria.

E. coli Characteristics	No. of *E. coli* Examined		No. of *E. coli* Strains Positive			
	From Diarrhoeic Calves	From Non-Diarrhoeic Calves	Diarrhoeic Calves		Non-Diarrhoeic Calves	
			Freq.	%	Freq.	%
K99 or F41	61	41	0	0	0	0
Heat stable (ST)	61	41	5	8.1	0	0
Heat labile (LT)	61	41	2	3.3	0	0
Verocytotoxin (VTEC)	61	41	2	3.3	4	9.8
Ox RBC Haemagglutination	61	21	10	16.4	1	4.8

Table 14. Common 'O' Antigens among 102 *E. coli* isolates from Diarrhoeic and Non-Diarrhoeic Calves up to 4 Months of Age in Zaria.

'O' Antigen	Frequency from Diarrhoeic and Non-Diarrhoeic Calves		Frequency from Diarrhoeic Calves	
	Frequency	%	Frequency	%
O?	7	6.9	6	85.7
O8	6	5.9	4	66.7
O15	5	4.9	3	60.0
O75	4	3.9	4	100.0
O26	4	3.9	3	75.0
O6	3	2.9	2	66.7
Total	29	2.8	22	75.9

O? = O antigens not belonging to known 'O' group O1 to O170.

Table 15. Frequency of Enteric Pathogens in 65 Diarrhoeic Calves up to 4 Months of Age in Zaria.

Enteropathogen	Frequency	%
1. Gastrointestinal Parasites alone (GP)	32	62.7
2. GP + ETEC	6	11.8
3. GP + Ox RBC (*E. coli*)	5	9.8
4. Ox RBC alone (*E. coli*)	2	3.9
5. GP + ETEC + Ox RBC (*E. coli*)	1	2.0
6. ETEC + Ox RBC (*E. coli*)	1	2.0
7. No Enteropathogen isolated	4	7.8
Total	51	100

Key:

GP = Gastrointestinal Parasites.
ETEC = Enterotoxigenic *E. coli* either heat stable (ST) or heat labile (LT) or verocytotoxin producing *E. coli* (VTEC).
Ox RBC = Ox erythrocytes haemagglutination by *E. coli* strain.

CHAPTER FIVE

EXPERIMENTAL INFECTION OF LAMBS WITH PATHOGENIC *E. COLI* ISOLATED FROM DIARRHOEIC CALF

5.1 Summary

Of a total of 17 lambs used for experimental reproduction of enteric colibacillosis, 15 were hypogammaglobulinaemic and 2 were hypergammaglobulinaemic. The lambs were divided into 3 groups with 3, 4 and 10 lambs each. The 3 lambs in group one served as blank control and were administered 5.0ml of sterile Brain Heart Infusion Broth. Four lambs in group two were positive control lambs and were administered 5.0ml of Brain Infusion Heart Broth containing 3 x 10^8 enteropathogenic *E. coli* organisms/ml isolated from diarrhoeic calves in Britain. While 10 lambs in group three were experimental lambs and were administered 5.0ml of Brain Heart Infusion Broth containing 3 x 10^8 enteropathogenic *E. coli* organisms/ml isolated from diarrhoeic calves in Zaria. The average packed cell volume (pcv), protein levels, pulse and respiratory rates were recorded before and after infection.

From group one, lamb 01, developed non-smelly mild pasty diarrhoea indicating changes in feed. From group two, lambs 04 and 07 had diarrhoea and later died (100% case fatality rate) indicating that the *E. coli* strain was enteropathogenic. From group three, 5 lambs (lambs 09, 13,

14, 15 and 17) developed diarrhoea and 4 of them died (80% case fatality rate) indicating that the *E. coli* strain from diarrhoeic calf, in Zaria was entero-pathogenic in lambs. Cross infection can easily occur between lambs and calves kept together. The mean value of temperature, pulse and respiratory rates were not significantly different between groups and before and after experimental infections.

5.2 Introduction

Colibacillosis in lambs is caused by the same *E. coli* strains which causes the disease in calves (Blood and Radostits, 1989a). The enteric form is the commonest of the disease in calves while the colisepticaemic form is the commonest in lambs (Blood and Radostits, 1989a). *E. coli* K99+ antigen causes both forms of the disease in calves and lambs (Sojka, 1965; Tzipori, 1985a; Blood and Radostits, 1989a).

Majority of cattle owners in Nigeria keep sheep and cattle together. One of the common diseases in both species is colibacillosis (Blood and Radostits, 1989a). Several workers have attempted to reproduce enteric colibacillosis, with varying degrees of success in both cattle and sheep (Smith and Halls, 1967; Logan and Penhale, 1971; 1972; Schoenaers and Kaeckenbeeck, 1975). The successes of these experimental infections depended on the choice of *E. coli* strain, the infecting dose, the age and specie of the

experimental animal and the absence of passive immunity acquired from colostrum.

The objective of this study is to attempt reproducing diarrhoea by experimental infection of lambs with an enteropathogenic strain of *E. coli* 078 : K80, isolated from a diarrhoeic calf in Zaria.

5.3 Materials and Methods

5.3.1 Experimental Design

A total of 17 lambs of up to 3 days of age were obtained from their dams. The lambs were fed with sterile preparation of NAN milk three times a day (commercially prepared baby milk powder).

For each batch of lambs there were three groups namely:

(i) Group 1 consisted of 3 lambs used as negative control group. During each experiment, they were orally administered with 5.0ml of sterile Brain Heart Infusion Broth (BHI).

(ii) Group 2 consisted of 4 lambs in positive control group that were orally administered with 5.0ml of BHI broth containing approximately 3 x 10^8 organisms/ml *E. coli*, 0101: K99, F41 kindly supplied by Dr. C. Wray of Vet Central Lab., Waybridge.

(iii) Group 3 consisted of 10 lambs in the experimental group that were orally administered with 5.0ml of BHI broth containing approximately 3 x 10^8 organisms/ml of *E. coli*, 078; K80, an enterotorogenic ST positive strain with ability to agglutinate Ox RBC isolated from diarrhoeic calf in Zaria.

All the lambs were kept in aluminium cages which were washed and disinfected at the arrival of each batch.

5.3.2 Measurement of Immunoglobulins

Serum sample from each lamb was measured using ZST as described by Pfeiffer *et al.*, (1977) and used at Worcester Central Veterinary Laboratory, London (see Appendix F).

5.3.3 Determination of the PCV and Protein Levels

At the beginning and on the 3rd day of each experiment, 1.0ml of blood was aseptically collected from the jugular vein for determination of packed cell volume and protein levels.

5.3.4 Bacterial Culture

E. coli 078:K80 strain was used to infect 10 lambs in group 3. For the positive control, *E. coli*, 0101:K99:F41 was used to infect 4 lambs in Group 2.

The 2 *E. coli* strains to be used in the experiment were grown on EMB agar and typical colonies were picked and

subcultured on to BHI broth for 6 hours at 37°C. The culture on the EMB agar was subcultured every week and stored at 4°C till lambs were available. The approximate number of bacteria in the broth was estimated using McFarland nephelometer barium sulphate standard as described by Blair *et al.* (1970). Briefly at the end of 6 hours of incubation, the broth was serially diluted at the rate 10^{-1} dilution ratio in 10 test tubes. These were incubated for another 6 hours at 37°C. Then 0.1ml of the broth from the selected test tube which contained approximately 3.0×10^9 organisms estimated by McFarland nephelometer method was cultured onto 3 nutrient agar plates for 24 hours at 37°C. The colonies were counted using colony counter and averaged. The approximate number of colonies were 3×10^8/ml.

5.3.5 Experimental Infection

From the serially diluted broth containing approximately 3×10^8 organisms/ml, one mililitre was withdrawn and made up to 5.0ml with sterile broth. The diluted borth culture was orally administered to lambs in Groups 2 and 3. The lambs were observed for the development of diarrhoea for 5 days. One mililitre of blood was collected 2 days after the onset of diarrhoea for the measurement of PCV and protein levels.

On each day of the experiment, temperature, pulse and respiratory rates were taken and average values were recorded.

5.3.6 Post-mortem

Post-mortem examination was conducted on the 6 lambs which died.

5.3.7 Data Analysis

The PVC and protein levels, temperature, pulse and respiratory rates were compared between groups for both before and after infections using analysis of variance. After infection PCV and protein values were compared between groups using pre-infection values as covariates.

5.4 Results

Immunoglobulin Values and Production of Diarrhoea

The immunoglobulin concentration and production of diarrhoea are shown in Table 16. From Groups 1, 2 and 3, a total of 3, 4, and 8 lambs respectively were hypogammaglobulinaemic, while 2 lambs from group 3 were hypergammaglobulinaemic. Diarrhoea was not observed in Group 1 except lamb 01 which developed mild non-smelly, diarrhoea, on the 5th day of the experiment, which latter recovered (33.3%). In Group 2, 50% of the lambs had diarrhoea which was observed about 24 hours after infection in lamb 07 and on the third day in lamb 04. Both lambs died by the 5th day of infection. In Group 3, 4 lambs (lambs 09, 13, 14 and 17) developed diarrhoea on the 3rd day of infection, while 1 lamb (lamb 15) had diarrhoea on the 5th day. On the 5th day, lambs 09, 13, 14 and 17 died in Group

3. The diarrhoea observed were from hypogammaglobulinaemic lambs.

Average Values of Packed Cell Volume (PCV), Protein Levels, Temperature, Pulse and Respiratory Rates

The average values of lambs' PCV and protein levels, temperature pulse and respiratory rates are shown in Table 17. There was no significant difference between groups, of the average values of PCV before and after infection ($P>0.05$). The mean protein levels before and after infection showed no significant difference between groups 1 and 3, while the mean protein levels before and after infection were significantly higher in group 2 than group 1 ($P<0.05$) before and after infection. Between the three groups, there was no significant difference between the average temperatures before and after infection ($P>0.05$).

Case fatality rates for groups one to three were, 0%, 100% and 80% respectively.

Post-mortem findings revealed no gross lesions.

5.5 Discussion

The lambs in Group 1 were not infected yet one had mild diarrhoea. This observation could have been due to change in feed, from colostrum feeding to a commercially prepared milk (NAN). Roy (1980), reported that commercial milk replacers cause neonatal diarrhoea due to denatured protein content fed to neonates that are removed from their dams.

It might be that the milk used in feeding the lambs in this experiment was denatured. In Group two, 2 lambs had some diarrhoea and later died (50% case fatality rate). This indicates that the *E. coli* strain, of calf origin, was enteropathogenic for lambs. These two lambs came down with diarrhoea due to poor protection offered by immunogglobulins. Logan and Penhale (1971, 1972); Radostits (1981); Blood and Radostits (1989a), stated that low or absence of passive immunity from colostrum is a major factor in increased prevalence of neonatal diarrhoea. From group three, 62.5% of the lambs had diarrhoea and case fatality rate was 80%. This findings may indicate that the *E. coli* strain, 078: K80 from diarrhoea calf, was enteropathogenic for lambs which were poorly protected by maternal immunity due to lack of inadequate ingestion of colostrum. Reports indicate that, this *E. coli* strain is pathogenic world-wide (Logdn and Penhale 1971, 1972). The diarrhoea caused by those two *E. coli* strains were typical of *E. coli* diarrhoea in consistency, smell and colour (whitish pasty and offensive smell).

No differences among groups were observed for PCV and serum protein levels and this may indicate that the diarrhoea was not severe enough to cause significant changes within the circulatory system as reported elsewhere (Blood and Radostits, 1989a). However, there was significant increase of protein levels between groups1 and 3, indicating that the diarrhoea caused by *E. coli* 078: K99, was severe

enough to cause the change but not severe enough to cause changes in PCV values. There was no significant differences between groups considering temperature, pulse and respiratory rates before and after infection. This may indicate that the *E. coli* strains are entero-pathogenic and not enteroinvasive (Sojka, 1965; Blood and Radostits, 1989a) although since the major form of the disease in lambs is the colisepticaemic form (Blood and Radostits, 1989a).

The absence of gross lesions is an expected feature of enteric-colibacillosis (Blood and Radostits, 1989a).

Table 16. The frequency of diarrhoea and immunoglobulin concentration in lambs.

Group	No. of Lambs in Group	Concentration of Immunoglobulin		Diarrhoea Status		Case Fatality Rate		
		Concen-tration	Frq.	Frq.	%	No. of Lambs Diarrhoea	No. Dead	%
1	3	Hypo Hyper	3 0	1 0	33.3 0.0	1 0	0 0	0 0
2	4	Hypo Hyper	4 0	2 0	50.0 0.0	2 0	2 0	100.0 0
3	10	Hypo Pyper	8 2	5 0	52.5 0.0	5 0	4 0	80.0 0

Key: Hypo = Hypogammaglobulin
Hyper = Hypergammaglobulin.

Table 17. Mean Pack Cell Volume (PCV), Protein, Temperature (T°C), Pulse and Respiratory Rates of Lambs before and after Infection with enteropathogenic *E.coli* from Diarrhoeic Calf.

Group	No. of Lambs	Average Values Before Infection					Average Values After Infection					No. of Dead Lambs
		PCV (%)	Protein mg/100 ml	T°C	P	R	PCV (%)	Protein mg/100 ml	T°C	P	R	
One	3	41	6.6	38.2	117	42	42	6.4	38.3	115	41	0
Two	4	38.5	7.7	38.8	118	44	41.5	7.7	39.0	125	50	2
Three	10	44.2	7.4	38.7	114	44	46.3	7.2	38.7	112	44	4

Key: T°C = Degrees Celcius.

CHAPTER SIX

CONCLUSION

This study has determined the overall prevalence of calf diarrhoea in 27 herds to be 13.6% in Zaria.

Geographical location of herds, year and season had no effects on the occurrence of calf diarrhoea while age and some enteric pathogen had some effects on its occurrence. The probability of calves having diarrhoea was higher in age group 0 - 30 days than age group 61 - 90 days and the proportion of calves surviving up to 4 months without diarrhoea was 73.8%. The economic importance of these findings need to be studied.

The present study observed that both diarrhoeic and non-diarrhoeic calves have equal chances of being infected with enteric pathogens and so their presence in diarrhoeic calves did not imply that they caused the diarrhoea. The study determined the causes of diarrhoea by a combination of factors, viz, nature of diarrhoea (consistency, colour and smell), infection load of each parasites and determination of *E. coli* serotypes, enterotoxins and its ability to haemogglutinate Ox RBC. Odds ratio indicated association between some enteric pathogens of *E. coli* + *Toxocara* sp; *E. coli* + Coccidia; *E. coli* + *Toxocara* sp + Coccidia. Of these enteric pathogen, *E. coli* + *Strongyloides* sp. infections did not cause diarrhoea in younger calves of age group 0 - 30 days. The study determined that light infections of single

helminths and coccidia did not cause diarrhoea, while bacteria alone and in combinations with single or multiple helminths infections, and multiple infections of helminths and coccidia caused diarrhoea. The proportion of diarrhoea caused by gastrointestinal parasites (GP) when alone; GP in combination with ETEC and *E. coli* which haemagglutinate Ox RBC were 62.7% and 29.4% respectively.

The role of *E. coli* in diarrhoeic calves were determined by evaluating the presence of $K99^{+}$ antigen responsible for adhesion of *E. coli* onto enterocytes, enterotoxins, ST, LT and VTEC, and the ability of *E. coli* to haemagglutinate Ox RBC. The study observed no $K99^{+}$ antigen and the prevalences of ST, LT and VTEC were 8.1%, 3.3% and 3.3% respectively. The ability of *E. coli* to haemagglutinate Ox RBC was 16.4%. These findings suggest that the ability of *E. coli* to haemagglutinate Ox RBC is an important factor to look for when making a diagnosis of enteric colibacillosis in addition to other factors.

The present study determined a wide range of O, K, H antigen. The common ones included 08, 026, 015, 075 and 06. Of all the *E.coli* serotyped only one was a pathogenic strain - 078: K80, which causes diseases in calves world wide and by experimental infections caused enteric colibacillosis in lambs.

Further studies are needed to evaluate the roles of viruses, parasites, bacteria and management systems on the prevalence of calf diarrhoea in Zaria. Experimental

infections of calves with single and multiple combinations of common enteric pathogens is suggested to determine experimentally any correlation between the nature of diarrhoea and enteric pathogens. This information will be helpful in making a clinical diagnosis of diarrhoea in calves.

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APPENDIX A

THE FREQUENCY OF INFECTION (LOAD) OF GASTROINTESTINAL PARASITISM AMONG DIARRHOEIC CALVES OF UP TO 4 MONTHS OF AGE, IN ZARIA

Gastrointestinal Parasites	Frequency of Infection Rate (Load) (Eggs per Field of Microscope)				Total
	Non-Diarrhoeic Calves		Diarrhoeic Calves		
	Frequency	%	Frequency	%	
No parasite found	11	73.3	4	26.7	15
Coccidia					
+	61	79.2	16	20.8	77
++	33	86.8	5	13.2	38
+++	15	51.7	14	48.3	29
++++	0	0.0	0	0.0	0
Strongyloids					
+	41	71.9	16	28.1	57
++	29	100.0	0	0.0	29
+++	20	100.0	5	20.0	25
++++	0	0.0	0	0.0	0
Strongyles					
+	40	93.0	3	7.0	43
++	20	100.0	0	0.0	20
+++	2	100.0	0	0.0	2
++++	0	0.0	0	0.0	0
Moniezia					
+	1	100.0	0	0.0	1
++	0	0.0	0	0.0	0

Appendix A (continued)

Gastrointestinal Parasites	Frequency of Infection Rate (Load) (Eggs per Field of Microscope)				Total
	Non-Diarrhoeic Calves		Diarrhoeic Calves		
	Frequency	%	Frequency	%	
+++	0	0.0	0	0.0	0
++++	0	0.0	0	0.0	
Toxacara					
+	56	82.4	12	17.6	68
++	59	96.7	2	3.3	61
+++	3	60.0	5	62.5	8
++++	0	0.0	2	100.0	2

Key: + indicates number of eggs per microscophic field.
+ = light in infection
++ = Moderate infection
+++ = Heavy infection
++++ = Packed heavy infection

APPENDIX B

THE DISTRIBUTION OF ENTERIC PATHOGENS AMONG DIARRHOEIC CALVES OF DIFFERENT AGE GROUPS UP TO 4 MONTHS OF AGE, IN ZARIA

Parasite Status	No. of calves positive		Age Group (Days)								Odd Ratio
			0 - 30		31 - 60		61 - 90		0 - 120		
	Fq	%	Fq	%	Fq	%	Fq	%	Fq	%	
Negative for pathogens	4	6.2	3	75.0	1	25.0	0	0.0	0	0.0	
Bacteria alone (*E. coli*)	11	16.9	8	72.7	2	18.2	1	9.1	0	0.0	0.4
E. coli + Coccidia	12	18.5	7	58.3	5	41.7	0	0.0	0	0.0	1.9
E. coli + Toxacara	14	21.5	6	42.9	6	42.9	2	14.3	0	0.0	2.3
E. coli + Strongyloides	5	7.7	0	0.0	1	20.0	1	20.0	3	60.0	1.1
E. coli + Coccidia + Toxacara	14	21.5	7	50.0	4	28.6	3	21.4	0	0.0	1.3
Toxacara + Strongyloids	1	1.5	0		1	100.0	0	0.0	0	0.0	3.2
Coccidia + strongyles Strongyloids	2	3.0	1	50.0	0	0.0	1	50.0	0	0.0	11.4
Coccidia + Strongyles + Toxacara	2	3.0	0	0.0	1	50.0	1	50.0	0	0.0	
	65		32	49.2	21	32.3	9	13.8	3	4.6	

Odd ratio was compared using those calves with diarrhoea to those without diarrhoea.

APPENDIX C

THE DISTRIBUTION OF ENTERIC PATHOGENS AMONG CALVES OF DIFFERENT AGE GROUPS UP TO 4 MONTHS OF AGE, IN ZARIA

Parasite Status	No. of Calves Positive		Age Group (Days)							
			0 - 30		31 - 60		61 - 90		90 - 120	
	Fq	%	Fq	%	Fq	%	Fq	%	Fq	%
Negative for pathogen	15	3.1	8	53.3	4	26.7	2	13.3	1	6.7
Bacteria alone (*E. coli*)	183	38.2	69	37.7	67	36.6	39	21.3	8	4.4
Strongyloids alone	1	0.2	0	0.0	0	0.0	1	100.0	0	0.0
Coccidia alone	7	1.5	4	57.1	2	28.6	1	14.3	0	0.0
Strongyles alone	1	0.2	0	0.0	0	0.0	0	0.0	1	100.0
E. coli + Moniezia	1	0.2	0	0.0	0	0.0	0	0.0	1	100.0
E. coli + Coccidia	55	11.5	21	38.2	18	32.7	11	20.0	5	9.1
E. coli + Toxacara	56	11.7	26	46.4	20	35.7	7	12.5	3	5.4
E. coli + Strongyloides	44	9.2	8	18.2	11	25.0	16	36.4	9	20.5
E. coli + Strongyles	12	2.5	1	8.3	3	25.0	4	33.3	4	33.3
E. coli + Coccidia + Toxacara	72	15.0	12	16.7	42	58.3	22	30.6	9	12.5
Toxacara + Strongyloids	3	0.6	1	33.3	1	33.3	0	0.0	1	33.3
Toxacara + Strongyles	1	0.2	0	0.0	1	100.0	0	0.0	0	0.0
Coccidia + Strongyloides	2	0.4	1	50.0	0	0.0	1	50.0	0	0.0
Coccidia + Strongyles	1	0.2	0	0.0	1	100.0	0	0.0	0	0.0
Strongyloids + Strongyles	5	1.0	0	0.0	2	40.0	1	20.0	2	40.0
Coccidia + Strongyles + Strongyloids	3	0.6	1	33.3	1	33.3	1	33.3	0	0.0
Coccidia + Strongyles + Toxacara	4	0.8	1	25.0	2	50.0	1	25.0	0	0.0
	479		163	31.9	176	36.5	107	22.3	44	9.2

APPENDIX D1

LIGATED INTESTINAL LOOP TEST FOR PATHOGENICITY OF *E. COLI* IN RABBITS FOR ST

S/No	Calf	*E. Coli* strain	Volume (ml)			Length (cm)			Volume/ length ratio	Remark
			Initial	Final	Difference	Initial	Final	Difference		
1.	19	080:H26	1.0	0.9	0.1	3.0	2.6	0.4	0.25	
2.	181	093:H19	1.0	0.7	0.3	3.0	2.6	0.4	0.75	
3.	218	061:H19	1.0	0.5	0.5	3.0	3.0	0.0	0.0	
4.	219	022:H16	1.0	0.7	0.3	3.0	2.6	0.4	0.75	
5.	408a	0105:H20	1.0	0.5	0.5	3.0	3.0	0.0	0.0	
6.	409	0132:H32	1.0	1.8	0.8	3.0	2.4	0.6	1.33	+ ve
7.	412	0132:H21	1.0	0.8	0.2	3.0	2.7	0.3	0.67	
8.	412	*O-7H25:K48*	*1.0*	*0.7*	*0.3*	*3.0*	*3.0*	*0.0*	*0.0*	
9.	456	08:K + :H9	1.0	0.3	0.7	3.0	3.0	0.0	0.0	
10.	457a	045:H19	1.0	1.0	0.0	3.0	2.8	0.2	0.0	
11.	111	068:H12	1.0	0.6	0.4	3.0	2.4	0.6	0.67	
12.	14	(015a):H3	1.0	0.8	0.2	3.0	2.7	0.3	0.67	
13.	16	011ab:H21	1.0	0.8	0.2	3.0	2.7	0.3	0.67	
14.	21	0102:018ac sp. aq +	1.0	1.3	0.3	3.0	3.0	0.0	0.0	
15.	30	06:H21	1.0	0.9	0.1	3.0	2.8	0.2	0.5	
16.	47	0128ab:H31	1.0	1.0	0.0	3.0	3.0	0.0	0.0	
17.	51	07:H34	1.0	0.9	0.1	3.0	2.8	0.2	0.5	
18.	66	07:H8	1.0	1.1	0.1	3.0	3.0	0.0	0.0	
19.	69	07:H16	1.0	0.8	0.2	3.0	2.7	0.3	0.67	
20.	*69*	*0153:H45*	*1.0*	*0.9*	*0.1*	*3.0*	*3.0*	*0.0*	*0.0*	
21.	49	0'K40':H6	1.0	0.7	0.3	3.0	2.6	0.4	0.75	
22.	76	06:H26:H9	1.0	0.9	0.1	3.0	2.3	0.7	0.14	

Appendix D1 (continued.)

S/No	Calf	*E. Coli* strain	Volume (ml)			Length (cm)			Volume/ length Ratio	Remark
			Initial	Final	Difference	Initial	Final	Difference		
23.	275	Lost	-	-	-	-	-	-	-	
24.	277	038:H21	1.0	0.5	0.5	3.0	3.0	0.0	0.0	
26.	311	07H51	1.0	0.5	0.5	2.0	1.4	0.6	0.83	
26.	230	025:H34	1.0	1.4	0.4	3.0	3.5	0.5	0.80	
27.	434	07H34	1.0	1.1	0.1	3.0	3.2	0.2	0.5	
28.	140	068:H12	1.0	0.9	0.1	3.0	3.0	0.0	0.0	
29.	287	(0166): H49	1.0	0.5	0.5	3.0	2.0	1.0	0.5	
30.	299	0118:H12	1.0	0.5	0.5	3.0	2.0	1.0	0.5	
31.	34	0 7H20	1.0	0.8	0.2	2.5	2.8	0.3	0.67	
32.	390a	070:H8	1.0	0.2	0.8	3.0	3.0	0.0	0.0	
33.	390b	018ac:H32	1.0	0.8	0.2	3.0	2.5	0.5	0.4	
34.	70	08:1	1.0	0.7	0.3	3.0	2.6	0.4	0.75	
35.	223	08	1.0	0.8	0.2	3.0	2.7	0.3	0.67	
36.	346	untypeable	1.0	0.8	0.2	3.0	2.7	0.3	0.67	
37.	348	075	1.0	0.8	0.2	3.0	2.7	0.3	0.67	
38.	350	026:K60	1.0	1.0	0.0	3.0	2.5	0.5	0.0	
39.	46	untypeable	1.0	1.0	0.0	3.0	3.0	0.0	0.0	
40.	50	026	1.0	1.1	0.1	3.0	2.8	0.2	0.5	
41.	377	016:K'RVC 383'	1.0	1.0	0.1	0.0	3.1	0.1	0.0	
42.	281	012ab:H31	1.0	1.0	0.0	3.0	3.0	0.0	0.0	
43.	142	075	1.0	0.95	0.05	3.0	2.8	0.2	0.25	
44.	464	075	1.0	0.7	0.3	3.0	2.6	0.4	0.75	
45.	465	016:K'RVC 383'	1.0	0.3	0.7	3.0	2.0	1.0	0.7	

Appendix D1 (continued.)

S/No	Calf	*E. Coli* strain	Volume (ml)			Length (cm)			Volume/ length Ratio	Remark
			Initial	Final	Difference	Instial	Final	Difference		
46.	264	Lost	-	-	-	-	-	-	-	
47.	455	076	1.0	1.1	0.1	3.0	3.0	0.0	0.0	
48.	286	015	1.0	0.9	0.1	3.0	3,1	0.1	1.0	+ ve
49.	296	untypeable	1.0	0.9	0.1	3.0	3.0	0.0	0.0	
50	318	08		1.6	0.4	3.0	3.0	0.0	0.0	
51.	322	untypeable	1.0	1.5	0.5	3.0	3.2	0.2	2.5	+ ve
52.	100	untypeable	1.0	0.9	0.1	3.0	2.8	0.2	0.5	
53.	374	Lost	-	-	-	-	-	-	-	
64.	227	untypeable	1.0	0.5	0.5	3.0	2.3	0.7	0.71	
55.	224	026	1.0	0.9	0.1	2.9	0.2	0.2	0050	
66.	108	045:K'E65'	1.0	1.6	0.4	3.0	3.0	0.0	0.0	
67.	422	0112ab	1.0	1.2	0.2	3.0	2.9	0.1	2.0	+ ve
68.	408b	09K-	1.0	1.0	0.0	3.0	3.0	0.0	0.0	
69.	429	08K?	1.0	1.0	0.0	3.0	3.0	0.0	0.0	
60.	433	0128K-	1.0	0.6	0.4	3.0	2.5	0.5	0.75	
61.	458	078:K80	1.0	1.4	0.4	3.0	3.2	0.2	2.0	+ ve
62.	Control	076	1.0	0.8	0.2	3.0	2.7	0.3	0.67	

APPENDIX D2

LIGATED INTESTINAL LOOP TEST FOR PATHOGENICITY OF E. COLI IN RABBITS FOR LT

S/No	Calf	*E. Coli* strain	Volume (ml)			Length (cm)			Volume/ length Ratio	Remark
			Initial	Final	Difference	Initial	Final	Difference		
1.	18	080:H26	1.0	1.6	0.6	3.0	3.4	0.4	1.26	+ ve
2.	181	083:H19	1.0	0.9	0.1	3.0	2.8	0.2	0.50	
3.	218	061:H19	1.0	1.1	0.1	3.0	3.0	0.0	0.0	
4.	219	022:H16	1.0	0.9	0.1	3.0	2.7	0.3	0.33	
5.	408a	0105:H20	1.0	1.0	0.0	3.0	2.9	0.1	0.0	
6.	409	0132:H32	1.0	1.1	0.1	3.0	3.2	0.2	0.5	
7.	412	0132:H21	1.0	0.3	0.7	3.0	3.0	0.0	0.0	
8.	412	07H25:K48	1.0	0.2	0.8	3.0	3.9	0.9	0.89	
9.	456	08:K + :H9	1.0	0.3	0.7	3.0	3.0	0.0	0.0	
10.	457a	045:H18	1.0	0.7	0.3	3.0	2.6	0.4	0.76	
11.	111	068:H12	1.0	1.1	0.1	3.0	3.2	0.2	0.60	
12.	14	(015a):H3	1.0	0.9	0.1	3.0	2.8	0.2	0.5	
13.	16	011ab:H21	1.0	0.26	0.75	3.0	3.0	0.0	0.0	
14.	21	0102:018ac sp.ag +	1.0	1.0	0.0	3.0	3.0	0.0	0.0	
16.	30	06:H21	1.0	1.1	0.1	3.0	3.2	0.2	0.5	
16.	47	0128ab:H31	1.0	0.8	0.2	3.0	2.7	0.3	0.67	
17.	61	O ? H34	1.0	0.9	0.1	3.0	2.8	0.2	0.5	
18.	66	O ? H8	1.0			3.0				
19.	59	O ? H16	1.0			3.0				
20.	69	0153:H45	1.0	0.3	0.7	3.0	3.0	0.0	0.0	
21.	49	O.K40:H5	1.0	2.2	1.2	3.0	4.3	1.3	0.92	
22.	76	08:H25:H9	1.0	0.7	0.3	3.0	2.6	0.4	0.76	

Appendix D2 (continued.)

S/No	Calf	*E. Coli* strain	Volume (ml)			Length (cm)			Volume/ length Ratio	Remark
			Initial	Final	Difference	Initial	Final	Difference		
23.	276	Lost	-	-	-	-	-	-	-	
24.	277	038:H21	1.0	0.8	0.2	3.0	3.0	0.0	0.0	
25.	311	07:H51	1.0	0.4	0.6	3.3	3.3	0.0	0.0	
26.	230	026:H34	1.0	0.2	0.3	4.5	5.0	0.6	0.6	
27.	434	07:H34	1.0	1.0	0.0	3.0	3.1	0.1	0.0	
28.	140	068:H12	1.0	1.1	0.1	3.0	2.8	0.2	0.5	
29.	287	(0166):H49	1.0	0.8	0.2	3.0	2.7	0.3	0.67	
30.	299	0118:H12	1.0	0.8	0.2	3.0	3.3	0.3	0.67	
31.	34	07: H20	1.0	0.6	0.4	3.0	2.5	0.5	0.80	
32.	390a	070:H6	1.0	0.8	0.2	3.0	2.75	0.25	0.8	
33.	390b	018ac:H32	1.0	0.9	0.1	3.0	2.8	0.2	0.5	
34.	70	018	1.0	0.7	0.3	3.0	3.0	0.0	0.0	
35.	223	06	1.0	1.0	0.0	3.0	3.1	0.1	0.0	
36.	346	untypeable	1.0	0.7	0.3	3.0	2.6	0.4	0.75	
37.	348	075	1.0	0.8	0.2	3.0	2.7	0.3	0.67	
38.	360	026:K60	1.0	1.0	0.0	3.0	3.0	0.0	0.0	
39.	46	untypeable	1.0	1.1	0.1	3.0	2.8	0.2	0.5	
40.	50	026	1.0	1.1	0.1	3.0	2.8	0.2	0.5	
41.	377	015:K'RVC 383'	1.0	0.4	0.6	3.0	3.0	0.0	0.0	
42.	281	012ab:H31	1.0	0.5	0.5	3.0	2.4	0.6	0.83	
43.	142	075	1.0	1.1	0.1	3.0	3.3	0.3	0.33	
44.	464	075	1.0	0.9	0.1	3.0	2.8	0.2	0.5	
45.	465	015:K'RVC 383'	1.0	1.0	0.0	3.0	3.0	0.0	0.0	

Appendix D2 (continued.)

S/No	Calf	*E. Coli* strain	Volume (ml)			Length (cm)			Volume/ length Ratio	Remark
			Instial	Final	Difference	Initial	Final	Difference		
46.	264	Lost	-	-	-	-	-	-	-	
47.	466	075	1.0	1.1	0.1	3.0	2.7	0.3	0.33	
48.	295	015	1.0	0.9	0.1	3.0	2.8	0.2	0.5	
49.	295	untypeable	1.0	0.8	0.2	3.0	2.7	0.3	0.67	
50	318	08	1.0	1.2	0.2	3.0	3.3	0.3	0.67	
51.	322	untypeable	0.8	1.3	0.5	3.0	3.4	0.4	1.25	+ve
52.	100	untypeable	1.0	1.1	0.1	3.0	3.2	0.2	0.5	
53.	374	Lost	-	-	-	-	-	-	-	
54.	227	untypeable	1.0	0.8	0.2	3.0	2.7	0.3	0.67	
55.	224	026	1.0	0.8	0.2	3.0	3.0	0.0	0.0	
56.	108	045:K"E66"	1.0	0.76	0.26	3.0	2.7	0.3	0.83	
57.	422	0112ab	1.0	0.3	0.7	3.0	3.0	0.0	0.0	
58.	408b	09K-	1.0	0.2	0.3	3.0	2.6	0.4	0.75	
59.	429	09K-	1.0	0.7	0.3	3.0	2.5	0.5	0.60	
60.	433	0128K-	1.0	0.8	0.2	3.0	3.0	0.0	0.0	
61.	468	078:K80	1.0	1.2	0.1	3.0	3.3	0.3	0.33	
62.	Control	0141:K85, K99	1.0	1.3	0.3	3.0	3.1	0.1	3.0	

APPENDIX E

E. COLI SEROTYPES ISOLATED FROM DIARRHOEIC AND NON-DIARRHOEIC CALVES, UP TO 4 MONTHS OF AGE IN ZARIA

Laboratory	Location of Herds	Calf	Age	E. coli serotypes	S/No
From Diarrhoeic Calves					
Dr.I.& F. Orskov	Zaria City	19	4	080:H26	1
		181	8	093:H19	2
International Escherichia d Klebsiolla Centre, Denmark		218	8	061:H19	3
		219	5	022:H16	4
	Hanwa	408(a)	3	0105:H20	5
		409	4	0132:H32	6
		412	8	0132:H21	7
		414	12	0?H25:K48	8
		456	12	08:K5:H0	9
		476a	5 days	045:H19	10
		111	12	068:H12	11
	Tankarau	14	4	0159:H3	12
		16	8	0112ab:H21	13
		21	8	0102:018ac sp.ag°	14
		30	1	06:H21	15
	Panhauya	47	16	0128ab:H31	16
		51	6	0?:H34	17
		56	6	0? H8	18
		59	12	0? H16	19
		69	12	0153:H45	20
		49	8	0'K40':H5	21

Appendix E (continued).

Laboratory	Location of Herds	Calf	Age	*E. coli* serotypes	S/No
	Dogarawa	76	4	O8:425:H9	22
		275	8	lost	23
		277	5	O38:H21	24
		311	3 days	O?:H51	25
	University Farm, Shika	230	4	O25:H34	26
		434	8	O?H25	27
		140	8	O68:H122	28
	Gabari	287	4	(O166):H49	29
	Government Farm, Shika	299	4	O118:H128	30
	Wusasa	34	4	O?:H20	31
		390a	4	O70:H8	32
		390b	16	O18ac:H32	33
	Tankarau	14	4	O159:H3	12
		16	8	O112ab:H21	13
		21	8	O102:O18ac sp.Og	14
		30	1	O6:H21	15
	Panhauya	47	16	O128ab:H31	16
		51	6	O?:H34	17
		56	6	O? H8	18
		59	12	O? H16	19
		69	12	O153:H45	20
From Non-Diarrhoeic Calves					
	Wusasa	55	3 days	VT,O86:Hsp.og.	1

Appendix E (continued).

Laboratory	Location of Herds	Calf	Age	*E. coli* serotypes	S/No
	Panhanya	60	2 days	015:H21	2
	Samaru	71	4	093:H19	3
	Samaru	72	4	024:H41	4
	Panhanya	97	8	015:H21	5
	Panhanya	101	8	27:H10	6
	University Farm, Shika	146	8	0103:H21	7
	Tankaran	225	4	01:47	8
	University Farm, Shika	213	8	018ac:H10	9
	Panhanya	374	8	01:H7	10
	Hanwa	450	12	06:H4	11
	Kufena	466	8	0118:H128	12
	Panhauya	477	8	091:H14	13
	Panhauya	478	8	VT, 0168:H8	14
	Panhauya	370	16	018ac:H10	16
From Diarrhoeic Calves					
Dr. C.Wray, Veterinary Laboratory Weybridge London	Tankarau	70	1	018	1
		223	8	06	2
	Jaji	346	4	untypeable	3
		348	3	075	4
		350	3	026:K60	5
	Panhauya	46	8	untypeable	6
		50	6	026	7
		377	4	015:K'RVC 383'	8

Appendix E (continued)

Laboratory	Location of Herds	Calf	Age	E. coli serotypes	S/No
	Dogarawa	281	12	012ab:H31	9
	University Farm, Shika	142		075	10
	Kufena	464	16	075	11
	Kufena	465	8	015:K'RVC383'	12
	Gabari	264	3	lost	13
	Gabari	455	4	075	14
	Shika Govt. Farm	293	4	015	15
	Shika Govt. Farm	295	4	untypeable	16
	Basawa	318	4	08	17
	Basawa	322	8	untypeable	18
	Panhauya	100	8	untyeable	19
	Panhauya	374	8	lost	20
	Tankaran	227	4	untypeable	21
	Tankaran	224	4	026	22
	Hanwa	108	1	045:K'E64'	23
	Hanwa	422	8	0112ab:	24
From Non-Diarrhoeic Calves					
	Dogarawa	314	16	untypeable	1
	Basawa	327	4	032	2
	Basawa	328	8	related to 088	3
	Jaji	346	4	untypeable	4
	Jaji	360	16	064	5
	Hanwa	410	8	related to 064	6

Appendix E (continued)

Laboratory	Location of Herds	Calf	Age	*E. coli* serotypes	S/No
	Hanwa	413	12	026	7
	Hanwa	416	8	related to 06	8
	Hanwa	419	8	related to 08	9
From Diarrhoeic Calves					
Dr. P.A. Guinee Belthoren *Netherlands*	Hanwa	408b	1	09K-	1
	Hanwa	429	8	08K?	2
	Hanwa	433	8	0:28K?	3
	Kufena	459	16	078:K80	4
From Non-Diarrhoeic Calves					
	Gabari	282	16	untyeable	1
	Dogarawa	316	16	0153K-	2
	Jaji	353	14	05K-	3
	Jaji	361	3	025K?	4
	Hanwa	436	12	0132K?	5
	Hanwa	439	16	08K?	6
	Hanwa	449	12	untypeable	7
	Hanwa	454	12	untypeable	8
	Hanwa	458	12	0113K-	9
	Kufena	461	8	0138K-	10
	Kufena	463	8	0113K-	11
	Kufena	469	8	untypeable	12
	Basawa	334	8	08K?ted to 064	13
	Jaji	360a	8	untypeable	14
	Basawa	330	8	085K-	15
	Hanwa	437	12	08K?	16

Appendix E (continued).

Common 'O' Antigens:

1. 07 = 7
2. 08 = 6
3. 015 = 5
4. 026 = 4
5. 06 = 3
6. 036 = 4
7. untypeables = 14

APPENDIX F

PROCEDURE FOR ZINC SULPHATE TURBIDITY TEST FOR IMMUNOGLOBULINS, WORCESTER CENTRAL VETERINARY LABORATORY, LONDON

Reagents

1. 0.208g of Hydrated Zinc Sulphate dissolve in 1 litre of distilled water.

2. Barium chloride Standard:

   1.15% solution of Hydrated Barium chloride is prepared 3ml of this is made up to 100ml with 0.2N Sulphuric acid. This solution is equivalent to 20 Z.S.T. units.

3. Positive Control Serum - used sample of serum from adult cattle.

Procedure

1. Add 0.1ml of Serum to 6ml of Zinc Sulphate - mix.

2. Blank Reagent: 6ml distilled water to 0.1ml serum - Relative.

3. 6ml of working standard. use parafilm or bone tubes - stand them for 1 hour.

4. Set Cecil Spectrophotometer for 495nms. Read standard last - Traces of Barium chloride affects test readings.

5. Calculation = $20 \times = \frac{\text{Reading of Test}}{\text{Reading of Standard}}$

   = Concentration of Immunoglobulins.

Normal Values **(Z.S.T. Units)**

0 - 5	-	Little or no absorption and protection
6 - 15	-	Poor absorption and/or minimal protection
16 - 20	-	Improving absorption and moderate protection
> 20	-	Efficient absorption and protection.