STUDIES ON CALF DISEASES WITH SPECIAL REFERENCE TO INFECTION WITH ESCHERICHIA COLI

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DECLARATION:

This thesis is my original work and has not been presented for a degree in any other University.

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This thesis has been submitted for examination with our approval as University supervisors.

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S U M M A R Y

The impetus for research on diseases affecting young calves has been the recognition of the important relationship between management of the dam prior to calving and post natal management of the calf soon after birth. There are large numbers of potentially pathogenic bacteria that can infect the calf during the first two weeks of life. These bacteria which include Escherichia coli, Claustridium perfrigens, Streptococci, lactobacillus and bacteroides are constantly present in the intestinal tract of the adult bovine. Most of these bacteria are not pathogenic to the adult cattle but the first mentioned bacteria, Escherichia coli, is of the greatest importance in the calf and the septicaemic or intestinal disorder with which it is associated is referred to as colibacillosis.

The purpose of this study was to inquire into the causes of calf mortality and to investigate the role of Escherichia coli in calf mortality in Kenya. The information about the management of the calf during the first few weeks of its life and calf losses in various parts of the country was obtained by means of a questionnaire sent to farmers by post. Results of post mortem examination of calf carcases submitted to a central laboratory were also analysed. Strains of Escherichia coli isolated from septicaemic or enteric cases were serologically examined. These strains were also examined for their resistance to seven antimicrobial drugs. Presence of infective drug resistance in these strains was also investigated.

In a majority of farms calves were fed colostrum from the bucket as they were removed from their dams soon after birth.

In those farms where calves were left with their dams to suckle colostrum, this period varied from one hour to five days. Out of 414 calves reported to have died, abortions and weak calves dying within 24 hours after birth accounted for 13.1 per cent and 56 per cent respectively. Other causes of calf mortality cited by farmers were scouring, pneumonia, tick borne diseases, bloat, sweating sickness and poisoning.

Of the 248 calf carcases necropsied, E. coli septicaemia was diagnosed in 8.5 per cent while enteritis was diagnosed in 26.2 per cent. Other conditions diagnosed were parasitic and bacterial pneumonia, coccidiosis, bloat, babesiosis, poisoning and traumatic injuries.

Only 110 (40.44%) of 272 Escherichia coli isolates were serotypable and represented ten serogroups: 05, 020, 039, 045, 078, 086, 0117, 0119, 0132 and 0153.

A total of 220 Escherichia coli isolates were tested for resistance to seven antimicrobial drugs. The majority (90%) of these isolates were resistant to Sulphafurazole alone or in combination with other drugs. Multiple resistance patterns most commonly found were Sulphafurazole and Streptomycin (22.72%), Sulphafurazole, Ampicillin and Streptomycin (11.11%) and Sulphafurazole, Streptomycin and Tetracycline (8.5%). None of the isolates were resistant to Furazolidone or Neomycin.

Of 198 E. coli isolates examined for their ability to transfer their resistance to Salmonella typhimurium, only 105 (53.3%) could transfer their resistance. In all cases when transfer of resistance occurred, the whole resistance pattern was transferred to Salmonella typhimurium.

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The purpose of this study was therefore

1. To investigate the causes of calf mortality in various parts of Kenya.
2. To investigate the role of *E. coli* in calf mortality in Kenya.

SECTION TWO

LITERATURE REVIEW

I. Incidence of Calf Mortality:

Several workers have surveyed the incidence and cause of calf mortality in various parts of the world. Smith (1934) reported that the total average death rate in calves, from all cases, on 25 farms in Ayrshire, Scotland, during the period 1931-1934 was 20 per cent of which "white scour" accounted for 40 per cent. Lovell and Hill (1940) working on data from 355 herds in England and Wales showed that 5.5 per cent of calves died before the age of six months and that 14.3 per cent of calf losses were attributed to stillbirth and abortions. Lovell (1945) also estimated that six per cent of calves died before they reached the age of six months in the northern Districts of England.

Withers (1952, 1953), who carried out a survey of diseases and mortality among calves up to 6 months of age in 44 herds in England, Wales and Southern Scotland, found that *E. coli* infection accounted for 27.9 per cent of the total number of deaths. *E. coli* septicaemia and scouring accounted for 56 per cent and 44 per cent respectively of all cases of *E. coli* infection. Abortions and stillbirth accounted for more than half the total losses of calves. Deaths occurred within the first week of life in 77 per cent of the cases. Withers also found that the death rate in pail-fed calves up to one month old was two and one third times greater than in calves allowed to obtain colostrum naturally.

Smith (1957) estimated that scouring associated with E. coli was responsible for 5 to 10 per cent of deaths of all calves born in Great Britain. In another study in England and Wales, Sellers, et al. (1968), found diarrhoea, navel ill and pneumonia to account for 22.7 per cent of all deaths during the first eight weeks of life. Analysis of post mortem results, of calves examined between 1959 to 1961, from data collected from various Veterinary Investigation centres in Britain (Anon., 1964), revealed that of the 3,667 calves examined, 24.5 per cent of deaths were due to septicaemia. Deaths during the first week accounted for 46.7 per cent while 53.2 per cent of deaths occurred in the first four weeks of life. E. coli infection accounted for 65 per cent while Salmonella infection accounted for 33 per cent of 1,400 calves which died of septicaemia and gastro-enteritis. Calves less than four weeks old accounted for 60 per cent of all cases examined. In Bashkir, USSR, Gribanov (1937) examined various factors associated with the mortality in calves and found that 25.7 per cent of losses were due to E. coli infection. Examination of 2423 calf carcases in Sweden by Nordlund (1939) revealed that in half of the cases E. coli was incriminated.

In Nigeria a report from Shika Livestock Farm (Anon., 1944) showed that "white scours" caused a mortality of 26.8 per cent in calves during a period of three years.

More recently, in Michigan, U.S.A., (Oxender, et al. 1973), a survey of 77 dairy herds revealed calf mortality of 17.7 per cent between birth and 60 days of age. Diarrhoea and pneumonia were reported as the main problems during the first four weeks of life.

Internal parasitism, haemorrhagic septicaemia and sweating sickness have been associated with calf mortality in Kenya (Daubney, 1927). In addition, E. coli gastro-enteritis in conjunction with nutritional disturbances, calf diptheria, pneumonia, anaplasmosis and a form of glomerulo-nephritis unassociated with a specific infection were found (Shirlaw, 1959).

II. Bacteriology

Escherichia coli is one of the bacteria belonging to the normal intestinal flora, and named after its discoverer Theobald Escherich. In 1885, Escherich isolated the bacteria in the faeces of newly-born babies and regarded it as a normal harmless seprophyte. Laruelle (1889) was the first person to suggest that E. coli might be a pathogen.

The form of diarrhoea in calves known as "white scours" caused by E. coli was first described by Jensen (1893). In the 1920's Smith and his colleagues studied E. coli infections in calves. These workers found that the disease might appear in either septicaemic or non-septicaemic form in which case the latter was localized in the small intestines (Smith and Little, 1922, a & b; Smith and Orcutt 1925). They were however unsuccessful in distinguishing pathogenic from non-pathogenic strains using biochemical tests. Lovell (1937) ascertained that E. coli infections in calves were caused by certain serological types using a precipitation test.

E. coli infection is fairly general among all types of domestic stock and recent monographs by Sojka (1965), and Barnum, Glanz and Moon (1967) have extensively reviewed E. coli

infection in domestic animals.

Escherichia coli are gram-negative, rod-shaped bacteria that conform to the definition of the family Enterobacteriaceae. They grow readily on most laboratory media at 15°C to 45°C, the optimum temperature being 37°C. The biochemical reactions which characterized E. coli are listed in Table I. Some E. coli strains fail to ferment lactose and non-gas forming strains (anaerogenic) are not uncommon. While identification is based on biochemical and serological characteristics (Edward and Ewing, 1962 and Kauffman, 1954), several workers have pointed out that identification based on fermentation of sugars appear to be unreliable as no single feature is particularly characteristic of Escherichia group (Sojka, 1965) and identification depends on a comparison of various tests.

Serologically, strains of E. coli are divided on the basis of their somatic heat stable "O" antigen, the capsular "K" antigen and the flagellar"H" antigen (Sojka, 1965 and 1971; Edward and Ewing, 1962; Kauffman 1954). The somatic O antigens in the smooth phase are thermostable (resisting 100°C for two and half hours) and are located in the bacterial cell wall and liberated on autolysis of the cell. The O antigen is a lipopolysaccharide which is responsible for the O specificity. The lipopolysaccharide can be liberated by physical and chemical manipulation including the use of solvents, heating and rapid freezing and thawing (Sojka 1965 and Orskov, et al, 1971). Antigenic relationship between and within O groups of E. coli and between other members of Enterobacteriaceae have been reviewed by Edward and Ewing (1962) and by Sojka (1965).

Table 1: Biochemical Reaction of E. coli (Sojka, 1965)

Glucose	A/G
Lactose	+ or x
Sucrose	d
Mannitol	+
Dulcitol	d
Sahein	d
Adonitol	-
Inositol	-
Indole	+
Methyl Red	+
Voges Proskaur	-
Ammonium Citrate	-
Hydrogen Sulphide	-
Urease	-
Gelatin liquifaction	-
Growth in KCN	-
Phenylalanine deaminase	-
Sodium malonate	-

*A/G - Acid and Gas, + - Positive reaction, x - delayed reaction, d - differential biotypes, - negative, no reaction.

The K antigens which have the ability to inhibit the O agglutination of live or formalized bacteria, occur as envelopes, sheaths or capsules. Three types of K antigens (L, B and A) have been demonstrated. (Sojka, 1965 and Kauffman, 1954). The inhibiting effect is inactivated by heat at 100°C for L and B and at 121°C for the A variety of the K antigens. The flagellar antigens are thermolabile and are associated with the flagellar of the motile E. coli strains. The antigenic scheme for serotyping E. coli contains over 150 O groups, and the scheme remains incomplete with new O groups being added from time to time. There are over 90 K antigens and about 50 H antigens, which are likely to increase as more are found (Ørskov, et al. 1972; Furowicz, et al. 1972 and Orskov, et al. 1973). In addition to O, K and H antigens, Stirm, et al. (1967) and Bertschinger (1968) have described a fimbrial antigen (K88)L surrounding the bacterial cell. This antigen becomes inactivated at more than 60°C and can also be suppressed by culturing at 18°C (Bertschinger, 1968).

Various serological techniques that have been applied to investigate E. coli were reviewed by Sojka (1965). The tube and slide agglutination tests are accepted as the backbone of Enterobacteriaceae serology (Edward and Ewing 1962; Sojka, 1965 and Kauffman, 1954). Indirect haemagglutination test (Neter, et al. 1952; Kunin and Beard, 1963), immunoelectrophoresis (Bettelheim and Taylor, 1971; Orskov, et al. 1971) and precipitation technique (Ørskov and Ørskov, 1970) have also been used to characterize the E. coli antigens.

In addition to endotoxins (part of somatic antigens) responsible for toxic effect during colibacillosis, there is

another factor called "colicine" (Frederiq, 1957). It is toxic to heterologous organism and inhibit their growth (Frederiq, 1957). The colicines are thought to block oxidative phosphorylation, to retard protein synthesis, and to degrade deoxyribonucleic acid of other organisms. Colicines are not bacteriolytic. They are thought to attach directly to certain receptors of the susceptible organisms (Jacobs, Sminovitch and Wollman, 1952).

III. Escherichia Coli Infection in Calves

Although the role of E. coli in the etiology of calf scours and septicaemia was not clear for a long time, the work done by Smith and Crabb (1956); Dunne, et al. (1956); Glanz, et al. (1959, 1968) and reviews by Gay (1965) and Sojka (1971) have tended to confirm that it is partly responsible for the natural disease. At birth, there are generally no bacteria present in the content of the alimentary tract. The first organisms to colonize the alimentary tract of the normal calf are E. coli, Streptococci and Clostridium perfringens. These are followed by lactobacillus and bacteriodes which become the commonest organisms in the abomasum and small intestines (Smith and Crabb, 1961). The temporary dominance of the first organisms to colonize the alimentary canal is attributed to the high pH of the abomasal content at this time and their decline in the upper gastrointestinal tract is associated with the subsequent drop of pH.

E. coli infection occurs in all breeds of cattle during the first two weeks of life (Gay 1965, Sojka 1965 and Barnum, et al. 1967). Diarrhoea is the most common disorder in young calves (Withers, 1952 and Sojka, 1965) and may or may not

be a manifestation of E. coli infection. The clinical signs vary depending upon the form of infection present in the individual animal examined.

The septicaemic form occurs in the first few days of life, characterized by sudden death and associated with E. coli bacteremia. The organism can be isolated, usually as a single serotype, from organs and tissues throughout the body. This form of infection is most common in calves that have been deprived of colostrum or for some reason, are deficient in serum gammaglobulin (Gay, et al, 1965). The enteric form is manifested by collapse and sudden death. It is associated with proliferation of mucoid strains of E. coli in the small intestines (Gay, 1965 and Gay, et al, 1965). There is no bacteremia. The enteric form is characterized by diarrhoea, and may be accompanied by death if the physiological disturbances are severe but is not always fatal (Sojka, 1971).

Studies of calf colibacillosis in many parts of the world (Gay, 1965; Sojka, 1965; Barnum, et al. 1967), have established that the septicaemic form is associated with certain E. coli serogroups, among them are 06, 015, 026, 078, 086, 0115, 0117 and 0137 (Sojka, 1971). Shirlaw (1960) isolated serogroup 086 from calves that died of the septicaemic form of the disease in Kenya. There are relatively few reports of serological typing of enteropathogenic strains associated with calf diarrhoea (Sojka, 1971). Available information indicates that they are mucoid, possess the A type K antigen, and usually belong to 08, 09 and 011 (Gay, et al, 1964 a & b; Gay, 1965 and Smith and Hall, 1967a). In Kenya 07, 08, 011, 024 and 036 have been isolated

from calves with diarrhoea (Shirlaw, 1960). Glanz (1971) isolated 54 different serogroups from 277 newborn calves. Serogroups 02 and 09 were isolated from calves with enteritis while serogroups 08, 026, 055, 078, 0101, 0115, 0117 and 0119 were septicaemic. In addition one unclassified serogroup OX28 was isolated from a septicaemic case.

Several workers (Smith and Hall, 1967 a and Gyles and Barnum, 1969) have found that the enterotoxins produced by E. coli to be either heat stable or heat labile. The heat stable enterotoxin is dialyzable and nonantigenic while the heat labile enterotoxin is nondialyzable and antigenic (Smith and Gyles, 1970 a and b). While enteric colibacillosis is an expression of enterotoxic activity produced by enteropathogenic E. coli, it is not known whether the disease caused by the living organism is the result of in-vivo production of heat stable or heat labile enterotoxins, both, or neither (Whipp and Moon, 1973).

There are a number of test systems which have been used to study pathogenicity of E. coli. The best evidence that E. coli is pathogenic in a given disease syndrome is the experimental reproduction of the syndrome in the natural host. Several investigators (Darn, 1960 and Gay, et al. 1964a) have shown that when the organism is administered to calves which have received colostrum, diarrhoea or other illness seldom follows. When it is administered to calves that have not received colostrum, the calf may develop acute septicaemia with collapse and death within 36 hours (Smith, 1962). It may also develop diarrhoea. Fey and Margadant (1962) showed that

infection with certain serogroup such as O78:K8 was successful when the organism was given immediately after birth and if intake of colostrum had been postponed or if the calf lacked gammaglobulins despite ingestion of colostrum.

Smith and Hall (1967, a) have recognized pathogenicity of E. coli enteric strains by their ability to dilate ligated intestinal segments of the pig, rabbit and the calf. This method was first used to study the pathogenesis of human cholera by inoculating rabbit gut loops with Vibrio cholerae (De, Bhattacharya and Sarkar, 1956; Taylor, et al. 1961). Most enteropathogenic E. coli from animals do not consistently cause dilation of the rabbit gut loop (Smith and Hall, 1967, a). They however, cause dilation of gut loops in the host for which they are enteropathogenic (Moon, Sorensen and Sautter, 1966; Gyles and Barnum, 1967, Nielsen, et al. 1968).

Virulence to laboratory animals has also been used to test pathogenicity of E. coli strains. Jacks and Glanz (1967) showed that serotypes isolated from non-enteric, systemic sources were more virulent for mice inoculated intraperitoneally than did the enteric isolates. These workers also concluded that the ability of an E. coli to invade the system of its host appear to be associated with its virulence to mice. Hughes and Lovell (1966) tested strains of E. coli isolated from healthy and diseased calves for their virulence in 13 days old chick embryoes. These workers found that strains isolated from cases of septicaemia were more virulent than those isolated from the localized intestinal infection and from healthy calves.

IV. Control of Colibacillosis

Although poor feeding and husbandry practices are important predisposing factors for development of E. coli infection (Henning, 1956) the importance of colostrum for the survival of the calf is an established and an accepted fact (Smith and Little 1922, a; Smith, 1930 and Lovell, 1955). A high percentage of colostrum deprived calves die with septicaemia whereas those fed colostrum survive at a significantly high rate (Aschaffenburg, et al. 1951). It has been shown that specific agglutinating antibody against the K antigens of E. coli is the factor in colostrum which protects the calf against colisepticaemia and other forms of E. coli infection (Aschaffenburg, et al. 1951; Briggs, 1951 and Lovell 1955). This opinion is not shared by Gay and colleagues who contend that it is most unlikely that K-agglutinins are the factors in colostrum protecting calves against E. coli septicaemia, for it has been shown in field studies that although calves receive O-agglutinins in the colostrum they do not usually receive agglutinins against the K-antigens of the same E. coli strains (Gay, et al. 1964b; Gay, et al. 1965 and Gay, 1965). Gay, McKay and Barnum (1964b) found that calves fed with colostrum are resistant to experimental E. coli septicaemia, regardless of the presence or absence of specific agglutinating antibody in their sera against the challenge strain. Smith (1962) similarly found no relationship between E. coli infection in colostrum fed calves and the presence of demonstrable agglutinins in the serum. It therefore seems unlikely that specific antibody directed against every pathogenic serotype could be present in colostrum (Gay, 1975).

The serum of new-born calf either does not contain gammaglobulins or contains only traces of it. Gammaglobulins appear in the calf's serum after the ingestion of colostrum (Fey, 1971). The absorption of colostral antibodies is limited to the first 24 to 36 hours after birth with only traces being able to pass afterwards (Gay, 1965). Absorption is greatest during the first 6 hours, gradually getting less by 12 hours. The quantity of immune lactoglobulin absorbed by the new-born calf is governmed by three factors: time of first feed, amount of lactoglobulin presented and maternal presence (Fisher, Selman and McEwan, 1971). However, several workers have shown that in spite of having been fed colostrum on the first day of life some calves died of E. coli septicaemia. Gay, et al. (1964) examined the gammaglobulin levels of 178 calves and found that 53 calves (29 per cent) were markedly deficient in serum gammaglobulins. Thirty-one of these calves (17 per cent) died, twenty with E. coli septicaemia. Smith, O'Neil and Simmons (1967) found low levels of immunoglobulins in 52 calves that had spent two days with their dams and consequently had access to colostrum. This was also confirmed by Fey and Margadant who found that 175 out of 191 calves (91.6 per cent) with E. coli infection were found to be hypo- or agammaglobulinemic in spite of colostrum intake on the first day of parturition (Fey and Margadant 1961; 1962). In a survey carried out by Gitter, Boarer and Brand (1970) low levels or absence of immunoglobulins were found in 124 out of 239 serum samples from farms with "scour" problems in Kenya where calves were removed from their dams at birth and fed at the next milking.

Smith and Little (1922) were the first people to suggest that bovine sera can be used as an alternative to colostrum to protect new-born calf against colibacillosis. Since then many investigations have been made in which a variety of blood-derived products such as citrated whole blood, normal and hyperimmune sera, and serum fractions have been used for this purpose (Anderson, DuPre and LaMaster, 1952; Christian and Segard, 1953; McDonald and Oakley, 1961; Lotan, et al, 1964; Watt, 1967 and Dam, 1973). The major immune components present in the bovine serum having antibacterial activity, especially against Gram negative bacteria which include pathogenic E. coli serotypes (Penhale, 1965 and Logan, et al, 1974), are Immunoglobulin M(IgM) and Immunoglobulin G(IgG).

IgM and IgG are found in the bovine colostrum where they are found in higher concentration than in the serum (Penhale and Christian, 1969; Logan and Penhale, 1971). For this reason, these components have been considered to play a major role in conveying passive immunity to septicaemic colibacillosis (Penhale, et al, 1970). Parenteral immunization of the dam, to control colibacillosis, would appear to be the method of choice directed at stimulating high levels of IgG and IgM in the sera of the pregnant female in order to obtain maximum transfer in the colostrum. Gay, McKay and Barnum (1964) who conducted several field trials found that immunization of cows markedly reduced incidence of white scours in calves. Other workers (Sellers, Smith and Spook, 1962; Top, 1969) using a similar method found that immunization did not reduce the incidence of calf scours but that the mortality rate was lowered.

The efficacy of vaccination against enteric colibacillosis has not been determined. However, there are reports (Gay, 1971) giving inconclusive results or indicating lack of protection. Recent investigations (Gay, 1975) have shown that in utero vaccination with a single serotype of E. coli can result in heterogenic protection against colisepticaemia.

Various chemotherapeutic agents and antibiotics have been used in treatment of calf scours. Thorp, et al. (1944) found sulphonamides, especially sulphamethazine, effective against scours in calves. Udall (1946) and Candlin (1949) found good results when sulphonamides were administered four times daily until diarrhoea ceased. But Pearson (1954) found that while large doses of oxytetracycline were effective in treatment of scours, E. coli was not eliminated despite clinical improvement. Smith and Crabb (1956) claimed that oral administration of streptomycin had a very significant effect in controlling white scours. Chloramphenicol, tetracyclines and nitrofurans have also been used in treatment of calf scours (Reisinger, 1965).

While antibiotics and other chemotherapeutic agents are used mainly for treatment of pathogenic bacteria, they are also used, especially in calves, as feed additive for prevention of diseases or for "nutritional purpose" (Roy, 1970). Smith (1957, 1967) and Smith and Crabb (1957) have shown that the use of antibiotics during treatment of clinical disease or as a feed additive has lead to increased resistance to antibiotics in strains of E. coli isolated from calves. Smith (1967) in his review has stated that there is no difference between resistance pattern in strains of E. coli as a result of treatment of clinical disease

or as a result of antibiotic use as feed additive for prevention of disease.

Examination of E. coli isolated from the small intestines of calves fed diet containing tetracyclines at "nutritional level" showed that resistance pattern of these strains was of the infective type and transmissible to E. coli and Salmonella pathogenic to man and animals (Smith and Hall, 1966). These findings were also observed by Harry (1962) and by Locken, et al. (1971) who have showed most of the resistance passed by E. coli isolated from calves, pigs and birds is of the infective type. Smith and Hall (1966) found that despite serial passage only a minority of E. coli strains lost their resistance pattern.

The phenomenon of infective drug resistance has been reviewed by Watanabe (1965 and 1967) and by Walton (1966). This phenomen is important because, first, bacteria whether pathogenic or not may acquire resistance without contact with the antibiotic and secondly the resistance can be transferred to every genus of Kauffmann's family Enterobacteriaceae and even to Vibrio, Aromonas, Pasteurella and Pseudomonas species (Kampelmancher, 1969 and Watanabe, 1971).

SECTION THREE

MATERIAL AND METHODS

Collection of data on calf losses from Farms

Data relating to calf management and calf diseases in various farms (Table 2) were derived from a questionnaire sent by mail to farmers (Appendix I & II). An endeavour was made to include both large and small scale farms, well managed as well as less efficiently managed farms. Some had experienced heavy calf losses in the past. No attempt was made to select farms on a statistical basis as the primary object was to elucidate factors causing calf mortality. Some of the information requested in the questionnaire was: the breed of the cattle kept, management of the dam prior to calving, pasture and feed provided to the calf, type of calf houses, methods of feeding colostrum, whether whole or artificial milk is fed, calf losses occurring within four weeks after birth and presumed reasons for such losses.

Calf carcases for post mortem examination

Calves were submitted to the laboratory by their owners shortly after death and post mortem examination was made on arrival. Detailed necropsy was carried out and cultures prepared from the liver, spleen, duodenum and mesenteric lymph nodes of each carcase. In addition, a long bone was sectioned, the cut surface seared, and cultures made from the bone marrow. When fresh carcases (not more than 4 hours old) were received cultures were made from heart blood.

Faecal specimens

Faecal specimens were collected from clinical cases of diarrhoea in calves varying in age from a few days to two weeks.

Table 2: Distribution of Farms from which questionnaires were received

Province	District	Number of Farms
Central	Kiambu	14
	Nyeri	4
	Nyandarua	12
Rift Valley	Nanyuki	9
	Kitale	10
	Nakuru	18
Western	Kakamega	7
	Bungoma	1
Others	Embu	2
	Lamu	1
	Nairobi	6
Total		84

The samples were taken from the rectum using sterile swabs and placed immediately in Stuart's transport media. When not processed immediately they were stored in the refrigerator at 4°C.

Bacteriological examination

Using aseptic techniques, faecal swabs and tissues were plated on dry 10 per cent bovine blood agar and MacConkey (Oxoid) agar plates. After 24 hours incubation at 37°C, where there was a predominance of lactose fermenting colonies, selected colonies were inoculated into peptone water. Mortility tests were conducted using hanging drop technique and sub-inoculation made into glucose phosphate broth for methyl red and Voges-Proskauer tests, into liquid MacConkey broth and to Christansen's urea agar. Carbohydrate media containing one per cent glucose, lactose, sucrose, mannitol, inositol, maltose, dulcitol, salicin, were inoculated to determine fermentation. The enterobacteria group of each isolate was recorded and typical E. coli cultures were clearly and individually labelled, and examined serologically. The cultures were maintained on Dorset egg at 4°C.

Preparation of hyperimmune sera

Isolates selected for serum preparation were from carcases derived from premises where virulent form of the coli-bacillosis was present. Eight E. coli cultures isolated from such premises from scouring or calf carcases were submitted to WHO International Escherichia Centre, Copenhagen and were sero-grouped as 0153, 0132, 05, 020, 039, 045 and 0119. Hyperimmune sera was also prepared against serogroups 08, 09, 055, 078, 086 and 0117 kindly supplied by Dr. I. Ørskov of International Escherichia Centre, Copenhagen.

In all instances, cultures for hyperimmune serum production were plated in 10 per cent sheep blood agar plates and incubated for 24 hours at 37°C. A portion of a well isolated colony was inoculated into peptone broth. After 24 hours incubation at 37°C, the broth cultures were heated at 100°C for one hour, and after cooling it was preserved by addition of 10 per cent formalin to give a final concentration of 3.2×10^9 organisms per ml (Sojka, 1965). Half grown rabbits were selected for serum production, the absence of any natural agglutinins being determined by slide agglutination test using pre-inoculation serum and the strain used for inoculation. The prepared E. coli suspension was inoculated intravenously into rabbits marginal ear vein in the following regime: 0.5ml, 0.5ml, 1.0ml, 1.5ml and 1.5ml at three day intervals. Six days after the last inoculation blood samples were taken and if titres proved adequate the rabbits were exsanguinated. The serum was separated and stored at -20°C until required.

Slide agglutination test

Selected colonies, emulsified in saline and boiled at 100°C for one hour, were initially tested in a pooled serum representing five specific serogroups. When a positive reaction was evident agglutination was continued with each serum incorporated in the group. Positive agglutinations were rapid occurring in less than 30 seconds. Following the slide agglutinations test, 'O' antigen for the tube agglutination test was prepared.

Tube agglutination test

The 'O' antigen for the tube agglutination test was prepared as described by Schipper, Kelling, Ebeltoft and Graves (1973).

Smooth colonies grown in nutrient agar for 24 hours at 37°C were removed from the agar plate with sterile saline. Centrifuged at 3,000 r.p.m. and diluted to 1 ml of organism to 9 ml of sterile phosphate buffered saline (pH 7.3). The diluted cell suspension was heated at 100°C in a waterbath for 1 hour. Serial two-fold dilution of the test serum diluted in saline was dispensed in Dreyers conical agglutination tubes in quantities of 0.3 ml and an equal volume of homologous 'O' antigen suspension was added. The mixture was shaken and incubated in a waterbath at 50°C and read after 48 hours.

Sulphonamide and Antibiotic sensitivity tests

These tests were performed using commercially prepared multodisks (Oxoid No. 3372E). Each multodisk contained the following drugs: 50 mg of oxytetracycline; 50 mg of chloramphenicol; 300 mg sulphafurazole (sulphadiazole, sulphadiazine and sulphamenthazine); 50 mg furazclidone, 30 mg neomycin; 25 mg ampicillin; 25 mg streptomycin. The culture to be tested was inoculated into nutrient broth and incubated for 6 hours at 37°C. Using a sterile pasteur pipette the surface of a dry Oxoid Sensitivity Agar (D.S.T. agar, oxoid Cm 261) plate was flooded with a small quantity of the 6 hour broth culture and the surplus fluid removed. After drying (approximately for 15 minutes at 37°C) the surface of the inoculated plate was overlaid with the multodisk and incubated for 24 hours at 37°C. Organisms were considered resistant when the zone of inhibition extended less than 2 mm from the edge of the disk.

Detection of Resistance-factors

Possession of Resistance-factors was assessed by the ability of resistant E. coli isolates to transfer resistance in vitro to completely antibiotic-sensitive receipient: Salmonella typhimurium. The strain of Salmonella typhimurium was isolated from a calf. The Salmonella organism was first made resistant to Nalidixic acid* by spreading a large amount of the organism on to a solid media containing 50 ug of Nalidixic-acid per ml and selecting the resistant colony that grew after 24 hours incubation at 37°C.

Each of the E. coli resistant isolates isolated (donor) was cultured in nutrient broth for 24 hours at 37°C. The nalidixic-acid resistant Salmonella typhimurium (recipient) was cultured under the same conditions. Approximately 3 ml of nutrient broth was then seeded with 0.02 ml of the donor and 0.01 ml of the recipient. After 24 hours incubation at 37°C, a loopful of the mixture was streaked on a selective MacConkey agar plate containing nalidixic-acid, streptomycin, chloramphenicol and oxytetracycline. The concentration of these drugs in the selective plates were as follows: 50 ug nalidixic-acid, 25 ug streptomycin; 50 ug chloramphenicol; 50 ug oxytetracycline. These plates were incubated at 37°C for 24 hours. One to three colonies of Salmonella typhimurium which had acquired resistance-factor were re-isolated and purified on a selective plate of the same composition. The purified colony was then examined for resistance to other drugs by the same method as already described.

*Winthrop Products Incl.

SECTION FOUR

RESULTS

Factors associated with calf mortality

Out of the 245 questionnaires sent to farmers in various parts of the country, 101 were returned. Seventeen of the questionnaires were incorrectly completed and were discarded. The 84 questionnaires on which the main conclusions are based supply data for the year 1972. Most of these questionnaires (Table 2) came from farmers in Central and Rift Valley Provinces while the rest came from other Provinces.

The cattle breeds represented in these farms (Table 3) include Ayrshire (12), Friesian (12), Guernsey (10), Jersey (5), Boran (7), Red Poll (1) and mixed breeds (34). The majority of small scale farmers (21) keep cross breeds. Very few farmers gave information regarding the size of their herds but the sizes of these herds vary from 2-35 on small scale farms, and up to and more than 800 on large scale farms.

On 32 farms (39.5%) calves were removed from their dams immediately after birth. The majority of these farms were small scale (30.9%). On 49 farms (60.5%) calves were left with their dams for various lengths of time. The majority of those who let the calf suckle its dam 38 (46.9%) were large scale farmers while 11 (13.6%) were small scale farmers. Tables 4 and 5 show the methods of feeding the calf colostrum and the length of time the calves were left with their dams to suckle colostrum. The length of time the calf was left with the dam ranged from 1 hour to five days.

In the questionnaire, farmers indicated the number

Table 3: Cattle breeds kept in large* and small** scale farms

Breed	Large Scale Farms	Small Scale Farms	Number of Farms
Ayrshire	7	5	12
Jersey	4	1	5
Friesian	9	3	12
Guernsey	5	5	10
Red Poll	-	1	1
Boran	7	-	7
Mixed Breeds	16	21	37

*Large scale farms = average acrage of 2,300 acres.

**Small scale farms = average acrage of 9 acres

Table 4: Methods of feeding colostrum to calves in 84 farms

Method of administering colostrum	Large scale farms	Percentage of total	Small scale farms	Percentage of total	Total number of farms	Percentage of total
Calf removed immediately	8	9.52	25	29.75	33	39.28
Calf left with dam for various lengths of time	40	47.61	11	13.09	51	60.71
Total	48	-	36	-	84	-

Table 5: Farms on which calves were left with their dams to suckle colostrum for various length of time

Length of time	Large scale farms	Small scale farms	Total
5 days	16	-	16
4 days	8	-	8
3 days	4	1	5
1 day	5	4	9
12 hours	5	2	7
6 hours	-	1	1
1-2 hours	2	3	5
Total	40	11	51

Table 6: Classification of causes of calf losses in both large and small scale farms

Disease	Number of calves lost on Large scale farms	Number of calves lost on Small scale farms	Total
Infectious conditions:			
Scours	53	17	70
Pneumonia	7	1	8
Helminths	14	-	14
Abortions	54	1	55
Tickborne diseases	12	5	17
Sweating sickness	24	-	24
Total	164	24	188
Non-infectious conditions:			
Weak calves dying within 24 hours after birth	149	-	149
Accidents	46	2	48
Bloat	16	1	17
Poisoning	3	-	3
Unknown	9	-	9
Total	223	3	226
Total deaths			414

isolated from bone marrow, spleen, mesenteric lymph nodes and gall bladder. In the remaining cases, E. coli was isolated from the mesenteric lymph nodes, duodenum and from rectal swabs.

Pneumonia mainly of bacterial origin accounted for 8.9 per cent of the cases. The commonest bacteria encountered being Corynebacterium pyogenes which was isolated in 14 cases. In two cases, parasitic pneumonia associated with Dictyocaulus viviparus was the cause of death. In the remaining cases, death was due to inhalation pneumonia.

Table 7 shows the other disease conditions diagnosed. Apart from E. coli infection and pneumonia, coccidiosis, bloat, babesiosis, liver abscesses, internal parasites, spinal abscess, leukosis, traumatic injuries were diagnosed. No definite diagnosis was made in 18.9 per cent of the carcases submitted. These carcases arrived in a state of advanced decomposition or no specific cause of death could be found.

Table 8 shows yearly distribution of the commonest diseases diagnosed while Figure 1 shows monthly distribution of calf carcases received during the period under investigation. Calves for post mortem examination received evenly throughout the year except in the year 1970 when 21 carcases were received in the month of March.

Serological study of E. coli isolated from calves with Septicaemia and/or diarrhoeaing calves.

At the outset of the study it was merely proposed to ascertain serogroups associated with colibacillosis in calves from material submitted from or collected from farms in various parts of the country. It was also realized that a calf may harbour a variety of serogroups and therefore more isolates were

Table 7: Etiological diagnosis in calves examined at post mortem between 1970-73

Cause	Number	Percentage of Total
E. coli septicaemia and enteritis	86	34.7
Pneumonia	22	8.9
Coccidiosis	33	13.3
Bloat	28	11.3
Salmonellosis (S. enteritidis (S. dublin, S. typhimurium)	4	1.6
Babesiosis	5	2.0
Liver abscesses	3	11.2
Poisoning	6	2.4
Internal parasites	7	2.8
Injuries	4	1.6
Spinal abscess	1	0.4
Leukosis	2	0.8
No diagnosis	47	18.9
Total	248	

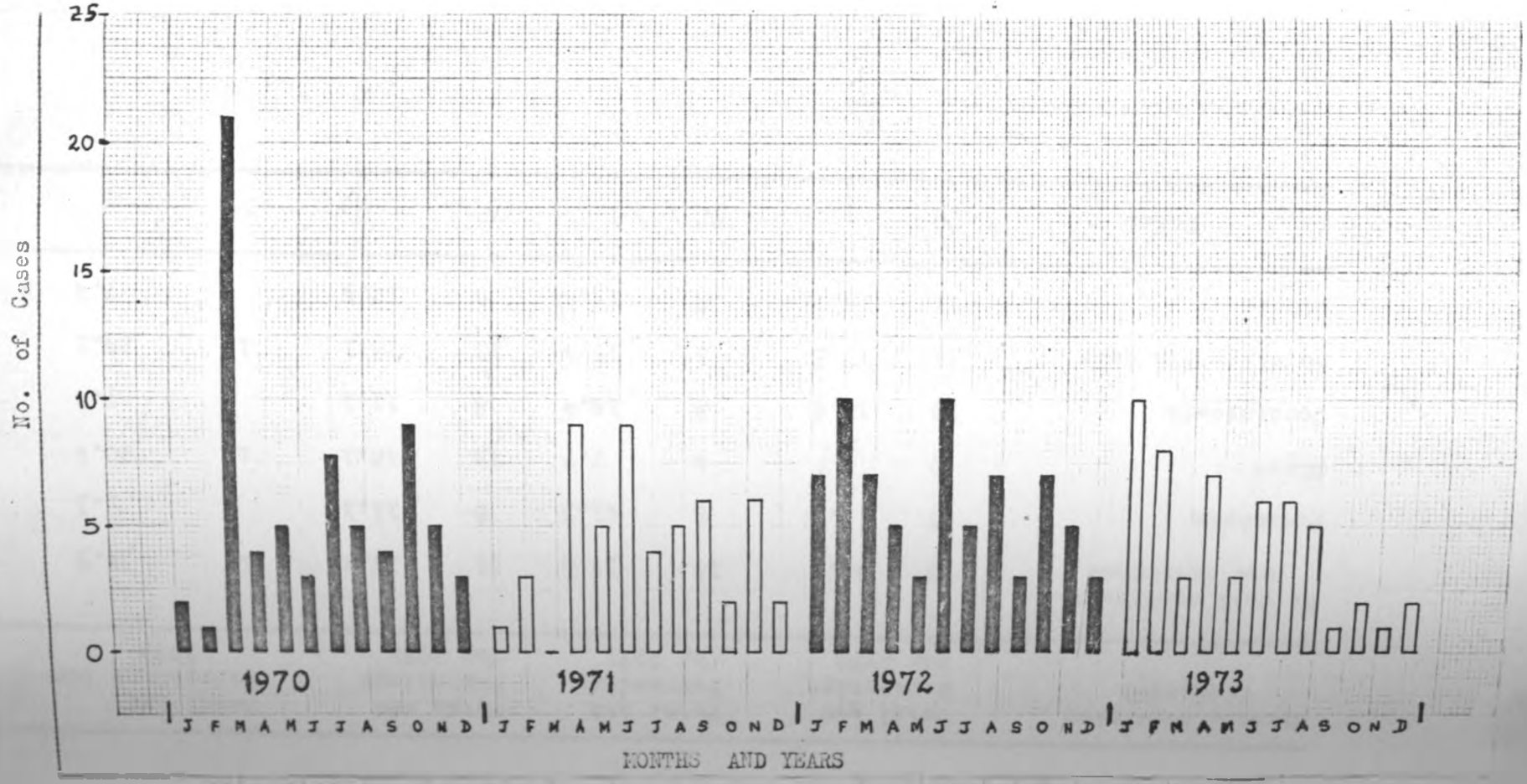

Fig 1: Monthly distribution of calf carcases received for autopsy between 1970 and 1973

Distribution of commonest diseases diagnosed at autopsy in calves during 1970-73

Disease condition diagnosed	Total and Percentage for 1970		Total and Percentage for 1971		Total and Percentage for 1972		Total and Percentage for 1973	
E. coli septicaemia and diarrhoea	30	42.8	18	34.6	21	29.2	17	31.5
Pneumonia	3	4.3	6	11.5	8	11.1	5	9.3
Bloat	0	0	4	7.7	13	18.1	11	20.4
Coccidiosis	13	18.6	8	15.4	8	11.1	4	7.4
No diagnosis made	13	18.6	8	15.4	13	18.1	13	24.1
Others	11	15.7	8	15.4	9	12.5	4	7.4
Total	70		52		72		54	

made from each case. All isolates which were from the same case and culture and not distinguishable serologically were counted as one. Cases were termed septicaemic when there was growth from bone marrow culture and the carcase was not more than 4 hours old.

A total of 415 isolates were examined biochemically. Out of this number 272 isolates were E. coli, the rest belonging to Klebsiella, Enterobacter, Proteus, Pseudomonas, Citrobacter and Alkaligenes genera.

Of the 272 E. coli isolates examined serologically it was possible to serogroup 110 with the E. coli O group sera. The remainder 173 were untypable with the hyperimmune sera prepared. The reason why these were untypable was because of the few strains available in the hyperimmune sera. Table 9 shows the origin of the 110 E. coli isolates grouped with the sera. It is realized that had more isolates been sent to Denmark for typing instead of 8 isolates, more would have been typed locally.

There was no predominant serogroup in the material examined. Serogroups 078, 086 and 0119 were frequently isolated from septicaemic cases while isolates from the faeces were frequently associated with serogroup: 05, 039, 045, 0132 and 0153. Serogroup 045, 0132, 039 and 015 were also isolated in septicaemic cases. Serogroup 05 and 020 were isolated in faeces only. In septicaemic cases the serogroups were isolated in organs (bone marrow, gall bladder, mesenteric lymph nodes and the spleen) and in faeces. In enteric cases, the serogroups were isolated in the faeces and not in the organs.

Table 9: Frequency of Serogroups and sites from which the 110 isolates were made.

Serogroups	Septicaemia*	Faeces	Total
045	11	20	21
0132	3	19	22
039	2	11	13
0153	1	8	9
05	-	9	9
020	-	10	10
0119	11	-	11
086	4	-	4
078	10	-	10
0117	1	-	1
Total	33	77	110

*Isolates were made from the bone marrow, gall bladder, mesenteric lymph nodes and the spleen.

Incidence of Drug Resistance and Infective Drug Resistance Amongst E. coli strains isolated from scouring calves

The summary of drug resistance found amongst 220 strains of E. coli isolated from calves, with diarrhoea, to individual drugs is shown in Table 10. Most of the strains were resistant to sulphafurazole (90.00%), streptomycin (55.45%) and tetracycline (24.09%). The percentage of strains which were resistant to ampicillin and chloramphenicol were 12.72 and 10.00 respectively. Only 32 (14.00%) strains were sensitive to all drugs tested. None of the strains were resistant to furazolidone or neomycin.

Detailed multiple drug ressistance shown by 198 strains which were resistant to sulphafurazole alone or in combination with other drugs is shown in Table 11. The most common multiple resistance pattern were sulphafurazole and streptomycin (22.72%), sulphafurazole, streptomycin and ampicillin (11.11%), sulphafurazole and tetracycline (9.59%) sulphafurazole, streptomycin and tetracycline (8.58%). Only 9 and 12 strains were resistant to more than four and five drugs respectively.

Examination of Table 11 shows that resistance to sulphafurazole was observed in all strains except O group 020. Resistance to tetracycline alone was also observed in O groups 05 and 0119 only. Multiple drug resistance involving sulphafurazole, streptomycin, tetracycline and ampicillin was observed in O group 045 only. Multiple drug resistance involving five drugs, sulphafurazole, streptomycin, tetracycline, ampicillin and chloramphenicol was observed in O group 0153 and ten other isolates.

Table 10: Incidence of drug resistance in E. coli strains isolated from scouring calves.

Drug	Number Resistant	Percentage
Sulphafurazole	198	90.00
Streptomycin	122	55.45
Tetracycline	53	24.09
Ampicillin	28	12.72
Chloramphenicol	22	10.00
Furazolidone	-	-
Neomycin	-	-
Total number tested*	220	

*32 strains were sensitive to all drugs tested.

In the present investigation, all the 198 strains of *E. coli* which were resistant to sulphafurazole alone or in combination with other drugs were examined for the presence of infective drug resistance. Of the 198 strains examined, 105 (53.30%) could transfer their resistance to *Salmonella typhimurium*. In all cases, when transfer of resistance occurred, the whole resistance pattern was transferred (Table 11).

SECTION FIVE

DISCUSSION AND CONCLUSION

In an attempt to assess the cause of calf mortality, as observed by farmers, the aetiology of the diseases must be considered on its widest sense. Calf scouring, pneumonia, helminths, bloat, tick-borne diseases were the main causes of calf losses as observed by farmers. The results of the 248 calf carcases examined confirm these findings. The present study, however, confirms findings of other workers who have studied calf mortalities in a similar manner. The investigation carried by Lovell and Hill (1940) showed that still-births and abortions accounted for 14.3 per cent of calf losses. Withers (1952, 1953) found that abortions and still-births accounted for 6.5 per cent of total births in England and Wales. In the present study, calf losses due to weak calves dying within 24 hours after birth and abortions accounted for 39.9 per cent and 13.1 per cent respectively. While there are no comparable figures, regarding early deaths in calves in this country, these figures suggest that abortion and still-births or deaths within the first 24 hours of life are causing a considerable loss to the cattle industry.

The results reported in the present study also confirm findings of workers in other countries and the increasing role of *Escherichia coli*. Lovell and Hughes (1935) isolated a number of bacteria from 100 necropsies which they regarded as significant. These bacteria included *E. coli*, *C. pyogenes*, *Salmonella typhimurium* and naemolytic

coccobacillus. By far the most important bacteria which they isolated was E. coli. E. coli was isolated in 37 cases, C. pyogenes in 12 cases and Salmonella typhimurium was isolated in 2 cases. In 31 cases, no bacteria were isolated and no diagnosis could be made. Shirlaw (1959) in a survey of 33 farms in Kenya, colibacillosis, Salmonella dublin and Salmonella typhimurium were isolated in calves but the total number of cases examined were not given. However, Shirlaw stated that of the 90 strains of Salmonella isolated from cases of calf paratyphoid, 83 were typed as Salmonella dublin and the remainder as Salmonella typhimurium. The present investigation show that in 248 calf carcases examined, Salmonella dublin and Salmonella enteritidis were isolated in one case each while Salmonella typhimurium was isolated in two cases.

The importance of E. coli infection in this country cannot be under-estimated. In the present study, out of 248 calf carcases examined, E. coli septicaemia and gastro enteritis was diagnosed in 34.7 per cent of the carcases. This is in agreement with findings of other workers. Anon (1964) in Britain analysed post mortem results of 3,367 calf carcases and found that E. coli septicaemia and gastro enteritis accounted for 24.5 per cent and 29.4 per cent, respectively, of all the cases.

A study of 272 E. coli strains isolated from cases of colibacillosis in calves from several parts of Kenya, using 13 different hyperimmune E. coli sera,

indicates that a variety of serogroups could be associated with the disease. Only 110 could be identified using the sera utilized. One feature of the results in the present study (Table 9) is the presence of serogroups 039, 045, 0132, 0152 which were isolated from both septicaemic and enteric cases. The other observation is the absence of serogroups 07, 08, 011 and 024 which have been isolated by Shirlaw (1960) in Kenya previously.

Serological examination of E. coli strains isolated from cases of colibacillosis in the calf have been carried by various authors in many parts of the world. In Sweden, Wramby (1948) isolated O group 04, 078 in cases of septicaemia and O group 08, 015, 09, 013 in scouring calves. Serogroup 08 was reported by Bokhari and Orskov (1952) in Denmark. These authors isolated serogroups 09, 015, and 0115 from calves as did Wood (1955). These strains have also been isolated in Germany, Switzerland, Belgium, Japan, America and in Britain (Sojka, 1965). E. coli serogroups 08, 015, 026, 035, 078 and 086 occur most frequently in E. coli septicaemia. In the present study both serogroups 078 and 086 were isolated from septicaemic cases. Serogroup 078 was isolated in ten cases while serogroup 086 was isolated in four cases. Other E. coli strains isolated in septicaemic cases were serogroups 0117, 0119 and 0137. These strains have been isolated by workers in other countries (Sojka, 1971).

Under the present systems of husbandry, diarrhoea was the commonest condition seen in young calves and in the past there has been a considerable difficulty in establishing that a given strain of E. coli is responsible for a given outbreak of scouring. E. coli could be isolated from the intestine of any scouring calf regardless of the etiology of the diarrhoea. To establish that a given E. coli strain is associated with an outbreak of scouring in a group of calves, it is therefore necessary to serotype all isolates of E. coli from both healthy and scouring calves in the group over a period of time. Such a task is generally beyond the scope of many laboratories. Where such work has been attempted (Smith and Crabb, 1956; Gossling, et al, 1964 and Glanz, et al, 1968) serogroups 08, 09, 015 and 0115 have been isolated. In the present study, serogroups 05 and 020 were isolated in cases of diarrhoea. These strains have previously been isolated by Glanz (1971) in America and by Varga and Farid (1974) in Hungary. Serogroups 039, 045, 0132 and 0153 were isolated in septicaemic and enteric cases. E. coli serogroup 0153 has recently been isolated in cases of diarrhoea in children (Ørskov, et al, 1972) and in calves (Varga and Farid, 1974). The significance of serogroups 039, 045 and 132 is difficult to interpret as the K antigens were not determined. These isolates have however been isolated in different countries (Barnum, et al 1967 and Glanz, 1971) but their pathogenicity is unknown at present.

Nutrition of the dam during pregnancy is an important contributary factor to pre-natal and post-natal calf losses. A lack of adequate feed intake, during pregnancy, especially minerals and vitamins is probably one of the greatest factors contributing to early calf losses. Vitamin A deficiency occurs in cattle during the last half of gestation and is characterized by abortion and birth of weak or dead calves (Robers, 1956). This is more likely to occur in Kenya after or during drought or when cattle are fed low quality hay.

The commonest method of feeding colostrum to the calf is either by allowing the calf to suckle its dam or by bucket feeding. The present survey indicate that calves are removed from their dams and fed colostrum from the bucket by a majority of small scale farmers. In the large scale farms and in ranches, farmers allow the calves to suckle their dams. Those small scale farmers who allow the calves to suckle the dam, the calf was allowed to remain with its dam for relatively short period of one to 12 hours. Smith and Little (1922) showed that calves which did not receive colostrum either died or grew poorly due to the effect of scouring. The results of the present survey show that in 32 farms where calves were removed from their dam immediately after birth, more than 50 per cent experienced calf losses due to scouring. In those farms where calves were allowed to suckle their dams for a period of time, calf losses due to diarrhoea was experienced in those farms where calves were left with the dam for shorter periods of between one to 12 hours.

Prior to calving, the udder selectively concentrates the immunoglobulins from the maternal serum into colostrum (Pierce and Feinstein, 1965). After ingestion by the calf, the colostral immunoglobulins are absorbed from the small intestine and carried in the lymph to the general blood circulation. These colostral immunoglobulins also spread through the intestinal tract limiting the growth and invasion of E. coli and other pathogenic micro-organisms. Balfour and Comline (1959a,b) found that the process of immunoglobulin absorption, amongst other substances, by the small intestine occurred during the first 24 hours of life.

Several studies have been carried out to quantitate colostral immunoglobulins in relation to E. coli infection in the calf. In 1949, Aschaffenburg, et al, demonstrated that the prophylactic action of colostrum was confined to the whey fraction of the colostrum. More recently, in a series of experiments (Logan and Penhale, 1971, a, b, c and Logan, Stenhouse, Ormrod and Penhale, 1974) the role of individual immunoglobulins in the immunity of the calf was examined. These authors demonstrated that IgM was the principal class of immunoglobulin in the serum responsible for protection against colisepticaemia. In their opinion IgG and IgA plays little or no part in preventing colisepticaemia in the bovine. Colostral immunoglobulins, as well as being active in the serum following absorption, have a local protective action within the small intestines (Logan, et al. 1974). However, no single class of immunoglobulins prevented intestinal form of colibacillosis. Individually colostral immunoglobulin (IgG, IgM and IgA) preparations when given orally prevented death but were found to be individually less effective

than colostrum in relation to the prevention of diarrhoea. Thus it would appear that no class of immunoglobulin alone provided the full spectrum of protection obtained with colostrum and it seems probable that all three immunoglobulins may have a separate prophylactic qualities which may act in synergism when combined in colostrum.

Despite the many advances in our knowledge of aetiology of colibacillosis and the prophylactic role of colostrum, a large percentage of calves in this country receive little or no colostrum. The present study has shown that a majority of farmers remove calves from their dams before suckling and calves are fed colostrum several hours later. Where calves are left with their dams, the period vary from 1 hour to five days. The present study has also shown that scouring (which may be due to infectious conditions, dietetic or internal parasites) accounted for 16.9 per cent of calf losses in the farms investigated. However it is more likely that scouring in calves less than two weeks was due to E. coli infection. This was confirmed by post mortem examination of 248 calf carcases in which colibacillosis was diagnosed in 34.7 per cent of the cases. Since both systemic and intestinal colostral immunoglobulins are necessary for complete protection of the calf against colibacillosis, colostrum must be fed during the absorptive phase. This is optimum at birth but adequate for practical purposes up to six hours post partum. Merely to allow the calf to remain with its dam does not necessarily guarantee that the calf will obtain colostrum and ideally, each parturation should be supervised to ensure that the calf sucks to satisfaction.

Development of antibiotic resistance by bacteria is either by production of extra cellular inactivating enzymes, adaptation or by mutation (Smith, 1957). Enzymes are produced during normal metabolism of some bacteria, and are capable of neutralizing the action of an antibiotic. Adaptation is a non-hereditary change in the metabolism of bacteria whereby they gain the ability to survive in an unfavourable environment presented by the chemotherapeutic agent. On the other hand mutation is a hereditary change represented by the emergence in a sensitive bacteria of a small number resistant bacterial cells. Following mutation, natural selection operates in the presence of chemotherapeutic agent leading to the suppression of sensitive bacteria and their replacement in the environment by the resistant mutants.

In a recent paper (Kariuki, 1974) it was shown that the majority of E. coli strains isolated from scouring calves in Kenya, are resistant to the commonly used antitherapeutic agents. The majority of the strains tested were resistant to sulphafurazole (90.00%), streptomycin (62.7%) and tetracycline (21.4%). It was also shown that the commonest multiple resistance pattern involved sulphafurazole in combination with other chemotherapeutic agents. The present study does not only confirm these findings but also indicate that the resistance possessed by the E. coli strains isolated from diarrhoeaing calves is of the infective type. More than 40 per cent of the E. coli cultures examined transferred their resistance pattern to Salmonella typhimurium and in all cases the whole resistance pattern was transferred.

The high incidence of drug resistance amongst E. coli strains examined from the calves arises mainly from the frequent use of drugs in attempting to control diarrhoea. Several workers (Gossling, et al. 1964) have shown that it is in only a small proportion of cases does E. coli play an etiological role in calf diarrhoea and in most of cases calf diarrhoea is probably managemental problem. Therefore the practice of using antibacterial drugs in treating all cases of diarrhoea or in trying to prevent it probably has little beneficial effect on the calf. This type of treatment gives rise to development resistance in non-pathogenic E. coli population whose resistance may be transferred to strains that are trully enteropathogenic for the calf and to other pathogens such as Salmonella.

In Kenya, the control and the use of antibiotics and sulphonamides by non-professional persons has been difficult to implement on a country wide basis. A majority of farmers can acquire antibiotics and other chemotherapeutic agents under permit in accordance with the Pharmacy and Poisons Rules (1957). No doubt this system whereby chemotherapeutic agents are used without proper laboratory evaluation has contributed to the incidence of resistance in E. coli isolated from calves.

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A P P E N D I X I

Q U E S T I O N A I R E "A"

Name of the Farm.....

Address.....

.....

L.R. No.....

Telephone Number.....

G E N E R A L

1. (a) Breed of cattle kept.....

(b) Size of herd.....

(c) How long have you been on this Farm.....

2. Management of the dam before calving.....

.....

.....

3. Procedure at calving (supervised, special paddocks etc.)

.....

4. Treatment of the calf immediately after birth.....

.....

5. Is the calf removed from the dam immediately or allowed to suckle .....

6. If allowed to suckle, for how long is the calf left with the mother e.g. 1 hour, 4 hours, 12 hours or 24 hours etc.

.....

7. If the calf is removed from the dam how is colostrum fed and for how long.....

8. State the number of feeds per day.....

9. Give details of milk substitutes if used (e.g. denkavit etc.)

.....

10. When are calves introduced to concentrate (e.g. hay, weaner pencils etc).....

11. Are calves reared indoors, outdoors or partly indoors and partly outdoors.....

12. If indoors, when are they turned out.....
.....

13. (a) State type of pens used: permanent.....
Semipermanent or temporary.....

(b) If permanent or semipermanent have you

(i) Concrete floor with stone walls.....

(ii) Concrete floor with timber wall.....

(iii) Murrum floor with timber wall.....

(c) How often are the calf pens cleaned?.....
.....

14. What type of posture is made available to calves when turned out (special paddocks etc).....
.....

15. When are the calves weaned from

(a) Milk.....

(b) Concentrates.....

16. At what age are your heifers bred.....

17. Do you use A.I. or bull for service.....

18. What do you do with bull calves.....
.....

19. Any other relevant information concerning your methods of rearing which may be of interest.....
.....
.....
.....
.....

A P P E N D I X II

Q U E S T I O N A I R E "B"

DETAILS OF CALVES WHICH HAVE DIED DURING THE YEAR

(1) Calf: Breed.....

Sex.....

Age in weeks.....

(2) Cause of death

1. Slaughtered
2. Difficult birth
3. Death within 24 hours
4. Abortion
5. Accident
6. Poisoning
7. Weakness
8. Scouring
9. Internal parasites
10. Pneumonia
11. Bloat
12. Abnormalities
13. Other causes
14. Total deaths

A P P E N D I X III

DISTRIBUTION OF SEROGROUPS AS RELATED TO PLACE OF ORIGIN

Place of origin	Specimen from where cultures were isolated		E. coli Serogroup
Ngong	Faeces	619	045
Njoro	Faeces	317	untyped
Kitale	Faeces	2254	020
Kitale	Faeces	9205	untyped
Kitale	Faeces	2272	untyped
Ngong	Faeces	6242	untyped
Ngong	Faeces	8053	020
South Kinangop	Faeces	SK32	05
Njoro	Faeces	3182	0153
Kabete	Organs	1150	086
Kitale	Faeces	234	020
South Kinangop	Faeces	SK31	039
Ngong	Faeces	8121	untyped
Kabete	Organs	1475	0153
Njoro	Organs	614	078
Ngong	Faeces	6233	untyped
Ngong	Faeces	6252	untyped
Ngong	Faeces	921	045
Njoro	Faeces	3013	untyped
Kitale	Faeces	2281	untyped
Ngong	Faeces	8123	020
Kitale	Faeces	2265	039
Ngong	Organs	9203	086

South Kinangop	Faeces	SK15	untyped
Ngong	Faeces	6244	untyped
Kabete	Organs	1425	045
Ngong	Faeces	8143	045
South Kinangop	Faeces	SK13	039
Ngong	Faeces	6331	045
South Kinangop	Faeces	SK22	05
Subukia	Faeces	3623	untyped
Ngong	Faeces	9134	039
Ngong	Faeces	8131	045
Njoro	Faeces	3013	untyped
Ngong	Organs	6194	078
Ngong	Faeces	6235	039
Subukia	Organs	149	078
South Kinangop	Faeces	SK14	039
Ngong	Faeces	8132	045
Ngong	Organs	8124	078
Ngong	Faeces	6233	045
Ngong	Faeces	9204	untyped
Kitale	Faeces	2284	05
South Kinangop	Faeces	SK10	untyped
Ngong	Faeces	8125	0153
Kitale	Faeces	2312	020
Kitale	Faeces	2183	untyped
Ngong	Faeces	9131	0153
Kitale	Faeces	2343	020
Kitale	Faeces	2282	0153
Ngong	Organs	8135	086
Njoro	Faeces	3011	untyped

South Kinangop	Faeces	SK51	untyped
Ngong	Faeces	8103	05
Ngong	Organs	8112	078
Kitale	Faeces	2323	045
Ngong	Organs	6251	078
South Kinangop	Faeces	SK11	untyped
Ngong	Faeces	8054	05
Njoro	Organs	3771	086
Kabete	Faeces	412	untyped
South Kinangop	Faeces	SK14	untyped
Njoro	Faeces	653	untyped
Kitale	Faeces	2311	020
Ngong	Faeces	8144	untyped
Njoro	Faeces	3273	045
Kabete	Faeces	162	untyped
Kitale	Faeces	2361	untyped
Ngong	Faeces	9235	045
Njoro	Faeces	3272	05
Kitale	Faeces	2331	020
Kitale	Faeces	2321	045
Ngong	Faeces	8104	untyped
Kitale	Faeces	2250	039
Kabete	Organs	813	039
Kitale	Faeces	2334	untyped
Njoro	Faeces	660	untyped
Kitale	Faeces	2321	039
Kabete	Organs	4761	039
Kitale	Faeces	2274	untyped
Njoro	Faeces	612	untyped

Subukia	Faeces	617	0153
Ngong	Faeces	9212	045
Njoro	Faeces	614	039
South Kinangop	Faeces	SK5	045
Kabete	Faeces	1611	untyped
South Kinangop	Faeces	SK2	untyped
Ngong	Faeces	6245	untyped
Kitale	Faeces	2211	039
Kabete	Faeces	4762	untyped
Kabete	Organs	1565	078
Ngong	Faeces	8142	045
Subukia	Faeces	715	untyped
Ngong	Faeces	6252	untyped
South Kinangop	Faeces	SK57	045
South Kinangop	Faeces	SK11	untyped
Njoro	Faeces	3183	untyped
Njoro	Faeces	3183	untyped
South Kinangop	Faeces	SK12	05
Kitale	Faeces	2291	039
Kabete	Organs	1849	020
Kabete	Faeces	484	untyped
Ngong	Faeces	6191	045
Njoro	Faeces	69	untyped
Ngong	Faeces	8101	untyped
South Kinangop	Faeces	SK6	untyped
South Kinangop	Faeces	SK13	0153
Kitale	Faeces	3275	untyped
Kabete	Faeces	151	0153
Ngong	Faeces	8051	045

Kabete	Organs	1882	039
Kabete	Faeces	1862	0153
Kitale	Faeces	2264	untyped
Kitale	Faeces	2333	untyped
Kabete	Faeces	1532	045
Ngong	Faeces	9132	05
Njoro	Faeces	631	untyped
Ngong	Faeces	8055	045
Njoro	Faeces	641	untyped
Ngong	Faeces	9135	untyped
Njoro	Faeces	615	untyped
Ngong	Faeces	6195	045
Kitle	Faeces	2342	020
South Kinangop	Faeces	SK8	untyped
Njoro	Faeces	613	untyped
Njoro	Faeces	611	untyped
Kitale	Faeces	2262	05
Ngong	Faeces	6243	untyped
Njoro	Faeces	3173	untyped
Njoro	Faeces	9201	untyped
Ngong	Faeces	9133	untyped
Kabete	Faeces	2001	untyped
Kitale	Faeces	314	untyped
Kabete	Organs	1150	0132
Ngong	Faeces	8131	untyped
Ngong	Faeces	9134	untyped
Ngong	Faeces	6231	untyped
Kitale	Faeces	2344	untyped

Ngong	Faeces	8211	untyped
Ngong	Faeces	6235	0132
Ngong	Faeces	8132	untyped
South Kinangop	Faeces	SK10	untyped
Ngong	Faeces	8141	untyped
Ngong	Organs	8104	0119
Kitale	Faeces	2343	untyped
Kitale	Faeces	2265	032
Kabete	Organs	1155	078
Ngong	Faeces	6232	untyped
Kitale	Faeces	2331	0132
South Kinangop	Organ	SK151	0119
Kitale	Faeces	2281	untyped
Ngong	Faeces	9211	untyped
Ngong	Faeces	6253	0132
Ngong	Faeces	8135	untyped
Ngong	Faeces	9135	untyped
Ngong	Faeces	8124	untyped
Kitale	Faeces	232	0132
Ngong	Faeces	9204	untyped
Kitale	Faeces	231	untyped
Ngong	Faeces	923	0132
Ngong	Faeces	6251	untyped
Njoro	Faeces	3272	untyped
Kitale	Faeces	225	untyped
Ngong	Faeces	8143	0132
Njoro	Faeces	6141	untyped
Kitale	Faeces	221	untyped
Ngong	Faeces	9203	untyped

Ngong	Faeces	227	0132
Njoro	Faeces	3013	untyped
South Kinangop	Faeces	SK121	untyped
South Kinangop	Faeces	SK62	untyped
South Kinangop	Faeces	SK31	untyped
Ngong	Faeces	9212	untyped
Kabete	Faeces	813	untyped
Njoro	Faeces	3275	untyped
Njoro	Organs	318	0119
Kabete	Faeces	6244	untyped
Kabete	Faeces	1425	untyped
Ngong	Faeces	8125	0132
Kitale	Faeces	2271	0132
Kabete	Faeces	891	untyped
Kitale	Faeces	225	untyped
Ngong	Organ	9131	0119
Njoro	Organ	8121	0119
Njoro	Faeces	841	untyped
Njoro	Organ	817	0119
Kitale	Organ	2321	0132
South Kinangop	Faeces	SK5	0132
Kabete	Faeces	21	untyped
Kitale	Faeces	227	0132
Kabete	Faeces	162	untyped
Kitale	Faeces	233	untyped
Kabete	Organ	849	0119
Ngong	Faeces	619	untyped
Njoro	Faeces	651	untyped

Ngong	Faeces	8051	untyped
Ngong	Faeces	652	untyped
Ngong	Organ	8010	0119
Kabete	Faeces	476	untyped
Kabete	Faeces	532	untyped
South Kinangop	Faeces	SK20	untyped
Ngong	Faeces	8055	untyped
Njoro	Faeces	624	untyped
Kitale	Faeces	227	0132
Kabete	Faeces	484	untyped
Kabete	Faeces	476	untyped
South Kinangop	Faeces	132	0132
Kabete	Faeces	156	untyped
Kabete	Faeces	184	untyped
Kitale	Faeces	226	untyped
Ngong	Faeces	142	untyped
Kitale	Faeces	342	untyped
South Kinangop	Faeces	SK58	untyped
South Kinangop	Faeces	SK111	0132
Ngong	Faeces	195	untyped
Ngong	Faeces	243	untyped
Ngong	Organs	252	0119
Njoro	Faeces	131	untyped
Kabete	Faeces	155	untyped
Njoro	Faeces	301	untyped
Kabete	Faeces	161	0132
Kabete	Organs	186	078
Ngong	Faeces	9201	untyped
Kitale	Faeces	2335	untyped

Njoro	Faeces	3193	untyped
Kabete	Organs	882	0132
Kabete	Faeces	2006	untyped
Kitale	Faeces	2264	untyped
Njoro	Faeces	614	untyped
Njoro	Faeces	3183	untyped
South Kinangop	Faeces	SK81	untyped
South Kinangop	Faeces	SK53	0132
Ngong	Faeces	9131	0132
Kabete	Faeces	1162	untyped
Kabete	Faeces	13	untyped
Kitale	Faeces	3011	0132
Kabete	Organs	681	078
Kitale	Faeces	2282	untyped
Kitale	Faeces	3271	untyped
Ngong	Faeces	8052	untyped
Kitale	Faeces	2323	untyped
Ngong	Faeces	8145	untyped
Njoro	Faeces	3771	untyped
Kitale	Faeces	2311	untyped
Kitale	Faeces	2314	untyped
Njoro	Faeces	2322	untyped
Njoro	Faeces	651	untyped
Ngong	Faeces	9133	untyped
Kabete	Faeces	154	untyped
Njoro	Organ	6151	0119
Njoro	Organ	6111	0119
Ngong	Faeces	9205	untyped
South Kinangop	Faeces	SK131	untyped

Ngong	Faeces	8122	untyped
Ngong	Faeces	6194	untyped
Ngong	Faeces	6193	untyped
South Kinangop	Faeces	SK32	untyped
Kabete	Organs	41	0119
Ngong	Faeces	624	untyped
Ngong	Faeces	8053	untyped
Kabete	Faeces	1425	untyped
Kitale	Faeces	2284	untyped
Ngong	Faeces	3015	untyped
South Kinangop	Faeces	SK41	untyped
Kitale	Faeces	3171	untyped
Kitale	Faeces	3022	0132
Ngong	Faeces	8123	untyped
Ngong	Faeces	8143	untyped
Ngong	Faeces	6233	untyped
Kabete	Faeces	651	untyped
South Kinangop	Faeces	Sk143	untyped
Kitale	Faeces	3412	untyped
South Kinangop	Faeces	521	untyped
Kitale	Faeces	2631	untyped