STUDIES ON

The Prevalence of *Escherichia Coli* and *Salmonella* in the Intestinal Tract of Calves in Health and in Enteric Disorders

Thesis

Submitted to the Magadh University in Partial Fulfilment of the Requirements for the Award of Master of Science (Veterinary) Degree in BACTERIOLOGY

Md. Sayeed Ahmad Ansari,

B.V.Sc. & A.H., P.G. Bact. (IVRI)

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November,
1965.

I certify that this Thesis has been prepared under my supervision by Sri Md. Sayeed Ahmad Ansari, a candidate for the M.Sc.(Vet.) with Bacteriology as major subject, and that it incorporates the results of his independent study.

P. B. Kuppuswamy. 24.11.65

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November,
1965.

C O N T E N T S

C O N T E N T S

PART-II SALMONELLA

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

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S. A. ANSARI

PART - I

INTRODUCTION

(E.COLI)

I N T R O D U C T I O N

Escherich (1885) isolated the organism Escherichia coli from the faeces of breast fed infants.

Lehmann and Neumann (1896) described the organisms isolated by Escherich (1885) under the group Bacterium with type species as Bact. coli, where as Castellani and Chalmers (1919) included the Escherich's organisms under the group Escherichia with type species as Escherichia coli. Rahn (1937) described E.coli under the family Enterobacteriaceae. The Enterobacteriaceae subcommittee (Report, 1954) define the family as gram-negative, non-sporing rods, either motile with peritrichate flagella or non-motile, which grow on ordinary media and ferment glucose rapidly with or without gas production; nitrates are reduced to nitrites. The subcommittee recognised nine groups, Salmonella, Shigella, Arizona, Escherichia, Alkalescens-dispar, Klebsiella, Bethesda, Proteus and Providence, but does not imply that these should be regarded as genera. The Escherichia group, as defined by subcommittee, consists of organisms classified as E.coli and its varieties including E.coli mutabile and anaerogenic strains. Although the fermentation of lactose is regarded as a main character of the species E.coli, it is not requisite for inclusion in the Escherichia group. Kauffmann (1954) included in his serological classification those organisms which were non-lactose fermentor, but were giving other characteristics of E.coli. The subcommittee also supports the inclusion of these organisms in the Escherichia group. Breed et al (1957) divided

the family Enterobacteriaceae into five tribes, Escherichieae, Erwinieae, Serratieae, Proteeae and Salmonelleae. The Escherichieae has, further, been divided into five genera, Escherichia, Aerobacter, Klebsiella, Paracolobactrum and Alginobacter and the genus-Escherichia includes the species, E.coli, E.aurescens, E. freundii and E.intermedia. In the species E.coli, four varieties have been recognised on the basis of fermentation reactions of salicin and saccharose. The E.coli var communis ferments salicin but not saccharose, E.coli var communior (communius) ferments saccharose but not salicin, E.coli var neapolitana ferments both and E.coli var acidilactici ferments neither salicin nor saccharose. These varieties are not now regarded as significant.

E.coli is the predominant organism in the intestinal canal in man and animals. The organisms gain enterance to the intestinal canal shortly after birth and persist through out life. Sears and Brownlee (1952) and Sears et al (1956) studying E.coli in the intestinal tract, found that normal human individual and dogs harboured two kinds of strains: residents which persisted over long periods of time, and transients which were present only for a few days or a few months. In human subjects and dogs, strains ingested, occasionally appeared transiently in the faeces, but did not displace the current residents. A complete change of environment was often necessary in order to produce change in resident strains. All attempts by Sears et al (1956) to establish artificially new resident strains in dogs failed. They also demonstrated that this pattern of E.coli occurred in very young

babies as well as adults. Gant et al (1943) stated that Colon bacilli most probably serve a useful function in the body by suppressing growth of certain proteolytic organisms and by synthesizing appreciable amounts of vitamins of the B.complex group. Some strains of E.coli may,under certain conditions, assume the role of a pathogen, especially in the intestinal tract or organe anatomically related to it.

E.coli has been found to be associated with pathological conditions both in man and animals, particularly with diseases affecting the gastrointestinal tract of the young mammals. In calves, E.coli causes white scours or there is an important association between this organism and the disease process. It is also associated with navel infection or joint infection (Jensen, 1893; Smith & Little,1927; Lovell,1937;Orskov,1951; Thomson,1954; Dunne et al,1956; Glantz et al,1956; Rees,1958; Smith,1960). In older animals (bovines) it is associated with cervicitis,cystitis, mastitis and metritis (Jensen,1896; Fey,1953; Merchant et al,1956). In lambs and monkeys, it produces diarrhoea (Charles,1957; Kater et al,1963; Ewing et al,1955), in pigs-oedema disease (Sojke et al,1957; Rees,1959;Szabo,1962) and it is pathogenic to poultry producing coli-granuloma(Hjarre & Wramby,1945; Osbourne et al,1946; Gross,1957; Glantz et al,1962;Bekajle et al, 1963). In human beings, it has been incriminated as the cause of infantile gastro-enteritis, infection involving uro-genital tract, peritonitis, gall bladder infection, pneumonia, appendicitis and meningitis (Dudgeon et al,1923; Meyer et al,1924; Bray,1945; Taylor & Charter,1952; Charter,1955;Rees,1957).

There are some similarities between white scours in calves and epidemic gastro-enteritis in babies and their possible association has been discussed by Lovell (1951, 1955); Fey (1955); Smith (1955); Rees (1957) and others. Although it is difficult to prove any connexion between the two diseases on epidemiological grounds even though, in a few instances, the same antigenic type of E.coli has been recovered from calves and babies. A number of well defined antigenic types is implicated in cases of white scours and in gastro-enteritis of babies. They are considered by many to be the primary aetiological agents and this receives support from the work of Dunne et al (1956) who have successfully reproduced white scours in susceptible calves by feeding cultures of E.coli to them, isolated from babies, under rigidly controlled condition.

The results of epidemiological investigation reported by several workers in relation to disease in young calves and other new born animals, show that the infection with this organism is the largest single cause of mortality in young calves during the first few days of life (Christiansen,1915,1917,1923; Meissner and Koser, 1931,1934; Mejlho,1934; Lovell and Hughes,1935; Khera et al,1963). Jordan (1933) described the death of 169 female calves out of 784 within a short time after birth. Smith (1934) described the death rate of female calves as 20% and Lovell & Hill (1940) described as 5.5% within six months of age. Withers (1952) described the death from 6.8 to 11.9% . Nearly half the death took place during the first week of life and over four-fifth in the first month and nearly a third of the death was due to white scours associated with E.coli infection. His

results confirmed the previous observations of the high death rate during the early spring months of the year and the important factors seem to be climate, feeding of colostrum and the personal attention given to calves. Minett (1946) in India, observed the mortality in female calves from 23.4 to 26.5% during the year 1938 - 44. He also concluded that the extreme climatic conditions, the grouping together of young calves and their nutrition play a part in this comparatively high mortality.

The epidemiology of the disease was also studied under experimental conditions (Aschaffenburg et al, 1949, 1951; Lovell, 1951; Ingram et al, 1953, 1956; Wood, 1955; Smith, 1960). Their results indicated that white scours developed spontaneously and mainly in those calves which were denied colostrum and the strains of E.coli were recovered from the tissues of the animals which were examined serologically. A septicaemia was more frequent in calves deprived of colostrum and a localised intestinal infection in those receiving some colostrum. It was also clear from their experiment that special races of E.coli tended to dominate the flora in the calf-pens at any one time. There were frequent changes in the bacterial flora with transient domination by one strain for a short period of time, after which another takes its place. This type of change has also been reported in infants by Linzenmeier (1962) in Munich infants clinic.

Various workers have emphasized the negative correlation between white scours in calves and feeding of colostrum which contains the antibodies against the E.coli and vitamin-A , besides other essential substances (Smith & Little, 1922; Stewart & McCallum, 1938; Ingram et al, 1953, 1956).

The changing of the environment of the calf shortly after birth has long been recognized as a dangerous procedure. The reasons are now becoming clear that it may be equally hazardous to change the environment of the mother shortly before the birth of the calf. The colostrum contains protective substances against potential pathogenic bacteria which are prevalent in the neighbourhood where the cow has been living. The potentially pathogenic bacteria in a new neighbourhood may be different from those in the old and in the colostrum, though effective against those in the old environment, is of no avail in the new. The calf, therefore, acquires protection against the hazards of the old home but not against the new (Lovell, 1959).

The chemical basis of disease associated with increased numbers of E.coli has yet to be elucidated. The endotoxins of a wide range of gram-negative bacteria have similar chemical, physical and biological properties (Thomas, 1954). The lipopolysaccharide endotoxins of E.coli and salmonella have the same order of toxicity for mice and there was no apparent difference between the endotoxins of strains of E.coli recovered from scouring calves and those from other bovine sources. The virulence of a special strain is probably an attribute of the living organisms and intimately associated with it and emphasis in outlook is shifting from toxicity to the relationship of enzymes of organisms and their substrates (Ingram & Lovell, 1960; Harvey et al, 1960). The available evidence indicates that the E.coli produce their harmful

effects by proliferating in the small intestine, when there is intestinal failure and accumulation of unabsorbed fermentable material in the lower part of the intestinal tract. The tissue invasion occurs producing septicaemia,if the calf does not have the immunity against that organism; on the other hand, tissue invasion may not occur, if the calf has the immunity against that strain. The products of their metabolism are absorbed in part and are in part diluted by the inflow of water and electrolyte from the tissues. The subsequent loss of unabsorbed food materials in the faeces is undoubtedly severe, but more serious is the depletion of electrolyte which may result in dehydration and death (Blaxter et al, 1953).

Various workers have classified the strains of E.coli isolated from calf scours by serological methods (Orskov,1951; Fey,1957; Rees,1957,1960; Gould,1958; Ulendeev,1963), phage typing (Smith & Crabb,1956; Smith,1958,1960; Kasatiya and Singh, 1961) and tested them with antibiotics (Merlin et al,1955; Smith & Crabb,1956; Gould,1958; Glantz,1962). Their results indicate that various serotypes and phage types are involved in calf scours. Some of the strains of E.coli associated with white scours are resistant to various antibiotics either singly or multiply. The increased percentage of resistant strains have been detected in those farms where the antibiotics are used as feed supplements or as prophylactic measures against the white scours.

In the present study, an attempt was made to examine various physiological characters, serological behaviour,phage

typing and action of antibiotics on E.coli, isolated from calves. This small survey was undertaken with a view to find out the various biochemical types, serotypes and phage types of E.coli prevalent in the normal as well as diseased calves at Government Cattle Farm, Patna and to correlate the E.coli prevalent with that of pathogenic E.coli already isolated and incriminated as the cause of white scours in calves and infantile gastro-enteritis in babies. It was also viewed to find out the serotype of E.coli of public health importance. Further, attention was directed to find out the possible resistant strains prevalent, which is the basis of modern chemotherapy, so that a suitable measures may be undertaken in future to prevent the white scours and its clinical syndrome.

REVIEW OF LITERATURE

(E.COLI)

REVIEW OF LITERATURE

(A) Relationship between Escherichia coli and white scours in calves:

Escherichia coli is the predominant organism in the intestinal canal in men and animals and was first isolated from faeces by Escherich in 1885. He considered the E.coli as saprophytic micro-organism.

As early as 1965, Obich pointed out that the E.coli was associated with white scours in young calves, but the first bacteriological study of the disease was,however, carried out by Jensen in 1892 -93. He reported that the white scour type of disease had been known in Denmark for 100 years and the death rate might be as high as 66%. He showed that the condition could be reproduced by feeding calves with the faeces of diseased ones and with cultures of E.coli isolated from them. He also concluded that the special races of E.coli were responsible for the disease. Jensen (1896) concluded that E.coli was responsible for peritonitis and mastitis in cattle, and endometritis, pyelonephritis and abscess of the prostate gland in dogs. Christiansen (1915,1917,1923) concluded that the E.coli was responsible for heavy mortality in young calves during the first few days of life. Smith & Little (1922) described that particular biochemical types of E.coli was associated with calf scours. Carpenter and Wood (1924) found large numbers of Colon bacilli in the abomasal, duodenal and jejunal contents of scouring calves than of healthy calves. Smith and Orcutt (1925) came to

similar conclusion as that of Carpenter et al (1924) and pointed out that the more severe the clinical condition, the further was the extent of the E.coli invasion up the intestinal tract from the lower regions in which they normally resided in the healthy calves. They also expressed the view that white scour was a result of an upset of the equilibrium which exists between the animal (calf) and E.coli, which may be normally present. They believed that special races of E.coli might develop by passage through calves which were weak and especially in new born calves which had received no colostrum or had received it after some delay.

Smith (1927) and Smith & Little (1927) found that broth cultures of strains of E.coli obtained directly from the ileum of scouring calves were highly toxic for cows and calves when given intraveinously. The results of investigation reported by several workers in relation to disease in young calves and other new born animals show that the infection with this organism is the largest single cause of mortality in young calves during the first few days of life (Meissner and Koser,1931,1934; Mejlbo,1934). Jordon (1933) described that the high incidence of death in calves is seasonal, the late winter and early spring months being the bad periods. Lovell and Hughes (1935), in Great Britain, examined 100 dead calves and attempted to classify their diseases on an aetiological basis. They found that E.coli infection was the one most frequently encountered; it was involved in 43 calves, being the only organism concerned in 37 calves. Lovell and Hill (1940) came to the similar

conclusion as that of Jordon (1933) that the high incidence of death in calves is seasonal, the late winter and early spring months being the bad periods.

Light & Hodes (1943) in the U.S.A. produced diarrhoea in calves by infecting them with a filterable agent recovered from the stools of infants during an epidemic of infantile diarrhoea. However, they failed to recover the filterable agent from natural outbreaks of diarrhoea among calves. Baker (1943) reported a rather mild type of pneumo-enteritis affecting calves less than a month old in U.S.A. caused by a filterable virus transmissible to mice as well as calves. His work did not indicate that the disease under observation possessed the usual characteristics and efforts, in general, to establish proof of a virus as a cause of diarrhoea have been negative.

McEwan (1950) had found that when a large number of E.coli isolated from dead calves were fed to normal calves, although the strain fed could be recovered from the lymph glands draining intestine, the calves remained healthy.

Vanpelt et al (1953) examined the bacterial flora of the faeces of calves daily for the first 19 days of life; their studies being confined to the coliform and the total aerobic count. Blaxter et al (1953) reported that scouring of calves was associated with high intestinal tract failure, and the bacteria played a secondary role by multiplying in the unabsorbed material present in the gut. The central feature was the abnormal presence of large amounts of fermentable material in the lower

third of the intestinal tract. The coliform organisms multiply in the lower part of the gut and then tissue invasion occurs, if the calf does not have the immunity against that organism; on the other hand, if the calves have immunity against that strain, the tissue invasion may not occur. The bacteria increase in number, spreading higher and higher up the intestinal tract. The products of their metabolism are absorbed in part and are in part diluted by the inflow of water and electrolyte from the tissues. The subsequent loss of unabsorbed food materials in the faeces is undoubtedly severe, but more serious is the depletion of electrolyte which may result in dehydration and death.

Thomas (1954) reviewing the physiological effects of lipopolysaccharide endotoxins, noted that a wide range of gram-negative endotoxins, had similar chemical,physical and biological properties. The chief toxic componant of smooth strains of E.coli was a polysaccharide-phospholipid complex, possibly with a protein component. It was attached to the bacterial bodies of which it appeared to be an integral part and seemed to conform to the classical definition of an endotoxin.

Roy et al (1955) following observations made under experimental conditions suggested that build up of infection through a period of use of a calf-pen might be partially responsible for the known increase in scouring and mortality in calves in the late winter. Wood (1955) concluding his experimental findings of 333 calves, said that the white scour infections arose spontaneously among new born calves kept under defined experimental conditions in a closed community for periods of

up to three weeks (21 days), during the autumn,winter and spring of 1950-53. The diarrhoea was observed soon after the beginning of each season and was later accompanied by the death of many young calves and the mortality increased as the season progressed. 153 calves died during the experimental period and 134 revealed E.coli faecal type I. A septicaemia was the most common finding, although in some cases the infection was restricted to the intestinal tract and adjoining lymph nodes; a septicaemia was more frequent in calves deprived of colostrum and a localised intestinal infection in those receiving colostrum. He,further, suggested that the observed "build up of infection" was related to the presence of special serological types of E.coli introduced into the calf house and that by a process of natural selection the most virulent strains became predominant.

Professor M.G.Fincher (1956) wrote as follows,"calves may be born at full term from apparently normal cattle and die from acute septicaemia in 12 to 76 hours, many scours profusely for several days or may have milder diarrhoea for a variable period. Several of a group of affected calves may survive, only to terminate in unthriftiness with or without pneumonia". With regard to aetiology Fincher said "this defies accurate description, but the condition is assumed to be due to a great variety of known and no doubt, several undiscovered factors". Dunne et al (1956) described that the E.coli had been isolated more often than any other organisms from calves dying from scours. Calf scours was produced by feeding small amounts of a pathogenic

strains of E.coli in the first milk consumed after birth. Control calves remained healthy. The absence of pneumonia in calves dying from scours caused by E.coli suggested that it was different from the pneumo-enteritis of viral aetiology. Ingram et al (1956) described the relationship between the colostral antibodies and white scours. They examined, by simple agglutination test, more than 150 strains of E.coli, that were associated with the death of calves from white scour infection, with colostral whey to detect the presence of agglutinin in the colostrum against them and then they correlated their findings with colostrum fed calves and colostrum deprived calves that died. Out of 59 colostrum fed calves that died, 45 had received colostrum that was devoid of agglutinins against the strains associated with their death. The strains RVC 118 A, RVC 330 and RVC 95 A were usually isolated from the dead calves. Out of 94 colostrum deprived calves that died, 66 deaths were associated with strains against which agglutinins could be demonstrated in the colostrum given to contemporary calves that survived. The strains isolated from this group of calves that died were more heterogeneous. However, two particular strains (RVC 101 and RVC 51) were isolated from dead calves on 28 occasions.

Podkopaev (1957) studied the relationship between gram-positive and gram-negative organisms from healthy and sick calves isolated from abomasum, duodenum and large intestine. In healthy un-weaned calves the gram-positive organisms were 60 - 90% while in sick calves it was 10 - 40% only. In sick calves the

remainder of the microflora was composed of Bact.cloacae, Bact.liquefaciens, Ps.pyocyanea, E.coli, Bact.aerogenes and Bact.faecalis-aerogenes. In healthy calves this group accounted for 10 - 40% of the microflora.

Glantz et al (1959) described that when colostrum deprived calves were fed certain strains of E.coli, fatal scours developed and that one strain caused scours even in calves which had suckled their dams. Osborne et al (1959) reported that the E.coli are associated with the condition known as calf-scours, white scours and calf-enteritis. The disease occurs in three forms: an acute rapidly fatal diarrhoea; a sub-acute debilitating diarrhoea and a chronic, localized joint or middle ear infection. Roy et al (1959) studied the sodium and potassium concentration in the serum of calves suffering from white scours. They found that with increasing incidence of scouring, the fall in serum-sodium values after birth became greater with a concomitant but slight rise in potassium values and concluded that the calves with E.coli infection might succumb to cardiac arrest as a result of the high concentration of potassium in the serum.

Smith (1960) said that much of the work that had led to the better understanding of calf scours had been carried out on colostrum deprived calves in which the disease usually terminated in a E.coli bacteraemia. Of course nearly all calves receive colostrum and in such calves, scours only infrequently terminate in bacteraemia. The available evidence indicates that in these calves the E.coli produce their harmful effects by proliferating in the small intestine, a region of the alimentary

tract in which they are present in only small numbers in healthy calves. Smith and Crabb (1960) carried out the work on faecal bacterial flora initially with 15 healthy calves, whose faecal bacteria were counted daily for the first 14 days of life; the period during which calf scours usually develops. They were impressed by the relatively constant manner in which the faecal bacterial flora developed from birth, the form of development varying surprisingly little from calf to calf irrespective of breed, sex, method of management and environment. Little difference was noted between suckling Aryshires and bucket-fed Jerseys kept under different conditions on premises 10 miles apart. The kinds of bacteria that were most prevalent in the faeces were E.coli, Clostridium welchii, Streptococci, Lactobacilli and bacteroides (fusiform like organisms). E.coli Cl. welchii and Streptococci were the first bacteria to appear in the faeces, often being present in large numbers a day or so after birth. Within a few days, bacteroides and lactobacilli had colonised the faeces in large numbers and outnumbered the other bacteria. It was not unusual for the bacteroides, in particular, to be present in numbers one hundred times greater than E.coli. The Cl.welchii content of the faeces declined rapidly and usually these organisms were present in small numbers only after the first 7 to 14 days. After 14 days the E.coli count usually decreased slowly and at 4 weeks might be present in numbers of one million per gramme of faeces compared with 10,000 million per gramme in the first week of life. By contrast, the bacteroides, lactobacilli and, to a lesser extent,

the streptococcal counts remained high.

Bacterial counts had also been carried out on the faeces of scouring calves before, during and after the scour period and they were not able to demonstrate any significantly constant difference between the faecal flora of scouring calves and healthy calves. They were unable to demonstrate any constant change occuring in the bacterial flora of the faeces that coincided with the onset of scours. The E.coli count, infact, some time decreased at that time.

Ingram and Lovell (1960) reported that the lipopolysaccharide endotoxins of E.coli and Salmonella had the same order of toxicity for mice and there was no apparent difference between the endotoxins of strains of E.coli recovered from scouring calves and those from other bovine sources. The virulence of a special strain is probably an attribute of the living organisms and emphasis in outlook is shifting from toxicity to the relationships of enzyme and their substrates. For example, when hyaluronic acid is present in a tissue, then the production of hyaluronidase by an organism is assumed to affect its virulence. A mucinase present in filtrates of Vibrio cholerae is able to destroy the viscosity and hence the mechanical protective and lubricating properties of intestinal mucus; this may play a part in facilitating desquamation of the intestinal epithelium. These and other relationships and their varying significance may aid us to the fuller understanding of the pathogenesis in coli-bacillosis and allied diseases. In the same year, Harvey et al (1960) reported that

the relatively simple analytical techniques had failed to reveal, in the 12 E.coli strains of bovine origin, any significant differences, or the presence of any especially toxic product other than a lipopolysaccharide. This substance is present in the virulent and non-virulent strains in concentration that do not appear to bear any relation to virulence. Quite clearly the property of virulence of E.coli strains are intimately associated with the viability of the organisms and not directly with the presence of a toxic lipopolysaccharide.

Fey et al (1961) studied the distribution of E.coli in the body of 37 calves that had died from coli-septicaemia. In 12 calves, the pathogenic type was isolated in pure culture from all parts of the body, but in 25 calves, it was missing from one part or other or whole of the digestive tract and in 17 calves the rectum and faeces were free from it. Massive infection of the urinary bladder was found in all cases. In the same year they described the isolation of E.coli type O_{78} : B_{80} from faeces and nose of 20 calves , in 12 of 16 herds where there had been outbreaks of coli-septicaemia; from 5 adult cows of three herds and from faeces and hands of attendants, and also from broom bundles, buckets and other utensils. On one farm strain $26:B_6$ was isolated simultaneously from an infant, a calf and a bull. Ibragimor (1961) isolated E.coli strains from mesenteric lymph nodes of 22 calves that had died from toxic dyspepsia (calf scour). The strains belonged to O111:B4; O55:B5; O26:B6; O86 ; O180 and O145.

Burki et al(1962) examined 88 dead calves of 5-7 days old and found that 61 had bacterial septicaemia of which 33 had

coli-septicaemia. Ouchterlony test confirmed that absence or deficiency of gamma globulin in the blood was related to septicaemia. The cyto pathogenic entero-virus could not be demonstrated in calf kidney tissue cultures from liver,spleen, kidney or rectal faeces of any calf and concluded that cytopathogenic entero-virus do not,therefore, influence coli-septicaemia in calves.

Fey et al (1962) studied the artificial infection of new born calves with E.coli type O78:B80, which was very common in material from septicaemic calves and rare in healthy calves and cattle. Calves deprived of colostrum were easily infected with the serotype, but normal calves were resistant. Fey et al(1962) described that the E.coli septicaemia was produced by oral or intra-nasal infection with type O78:B80 in 3 new born colostrum deprived calves, despite ligation and section of the oesophagus. From a fourth calf with the oesophagus left intact E.coli was recovered from the blood within 20 hours after intra-nasal infection,but not from contents of the small intestines, collected by laprotomy, or from bile. They concluded that the infection of the intestine was by the blood stream rather than by the digestive system. Walser (1962) described the clinical pictures of meningitis associated with coli-septicaemia. There was cerebral involvement in 12 calves 3-6 days old with E.coli septicaemia. Nervous symptoms included somnolence, paresis, opisthotonus, ataxia, spasms and mydriasis. There was no enteritis, but rhinitis, sinusitis and iritis were observed. Streptomycin, tetracycline, chloramphenicol and sulphonamides

probably prolonged life but failed to cure. He advocated gamma globulin to new born calves as a prophylactic measures.

Khera et al (1963) described that the infection with coli-aerogenes group had taken the largest toll i.e. 40 lives out of 117 dead calves they examined. Most of the calves in that group (75%) had died within two weeks of birth and all within three weeks. In 21 calves, the coliform organisms were isolated in pure culture from blood and various organs; and from others, pure cultures were isolated from different organs. Infection with proteus spp. and Ps. aeruginosa accounted for 15 and 6 cases respectively. Systemic infection with other organisms were few and were responsible for six cases in all.

(B) Relationship between white scours in calves and other factors:

I. Nutrition of Dam

Stewart and McCallum (1938) obtained statistically significant results showing that calves born of mothers whose colostrum were low in vitamin-A, were more liable to infections such as white scour, naval-ill and joint-ill than calves from dams whose colostrum had relatively higher vitamin-A content. The same authors found that the vitamin-A content of the liver of cows slaughtered in S.W. Scotland was liable to fall steeply from November to May, although they found a good deal of variation from year to year. The minimum values occurred in April and May within a short time after the animals were being transferred from stalls to pasture and they clearly demonstrated that there was a sharp fall during February and March. The April

figures for vitamine-A were only about a quarter of those of November. Williams (1940) stated that in his experience the early health of the calf was influenced by the nutrition of the embryo. Scour was observed in calves at birth and a foetal scour was described. He also suggested that many calves born without diarrhoea might be upon the brink of that phenomenon. Spielman et al(1946) said that some factor or factors were present in summer skimmed milk or in intrauterine life or both which favourably influenced survival rates of calves dropped from June 1 to January 1. Sheeby (1948) stated that the cows suffering from a chronic deficiency of phosphate in their diet gave birth to weak calves, similarly the calves from cows whose diet provided inadequate iodine might be born dead or goitrous and cows whose food intake during pregnancy was seriously deficient in vitamine-A or carotene produced weak calves which were highly susceptible to scour and pneumonia and among which casualties were numerous. Squibb et al (1940) made an interesting observation in this connexion when they showed that certain legume seeds, i.e. raw soyabeans, soyabean oil, lowered the blood plasma carotene than in cows fed a control ration containing no soyabean products. Difference was observed after 6 to 8 weeks. The ration also caused small variations with no particular trend in the vitamine-A. Payne (1949) reported that nutritional scours in calves were less frequent when cows were fed large amounts of grass silage or artificially dried grass than where a high proportion of imported concentrates was fed. Spielman et al(1949) found that the incidence of scours was lower in calves

born from the cows which were fed vitamine-A (1 million I.U. per day) for 30 days before parturition. They claimed that their data furnished additional evidence of the importance of the pre-partum ration in the subsequent performance of the new born calves. Dr. Alan Fraser (1953) said that probably the nutrition of the cow, while pregnant will influence the development of the unborn calf,like sheep, but that effect would be confined mainly in the last three months or so of the cows pregnancy. Mackintosh (1953) said that the size and vigour of the calf was also influenced by the feeding of the dam before calving. When the cows diet is seriously deficient the calf will be under nourished and weak. Withers (1953) also concluded that the nutrition of the dam during pregnancy and the standard of housing and hygiene had some relationship to calf mortality but that these were of secondary importance to the effects of climate, the methods of feeding colostrum and the personal factor. He emphasised that the intake of green food appeared to exercise some influence on the viability of the calf and that several herds with high calf mortality records were deficient in this respect. He suggested that probably the combined effects of adverse weather condition and a low vitamine-A content of the colostrum was responsible for much of the mortality in the early months of the year. Withers also noted that the calf mortality in dairy herds was much greater than in beef herds where more natural management was the rule.

Inglis (1960) concluded that vitamine-A deficiency in the prepartum diet played a part in calf enteritis. This fits well with the field observations.

II. Nutrition of the calf

Smith and Little (1922) showed that calves which did not receive their colostrum either died or grew poorly on account of scouring. Since then numerous workers have confirmed the protective effect of colostrum and also extended the knowledge of the nature of the protective mechanism. Ingham et al (1930) claimed that too little milk would lead to scouring, specially in older calves. Turner (1931) found that the calves of prepartum milked cows scoured and died more readily than those from dams not so treated. Stewart and McCallum (1938) suggested that calves fed colostrum with a high vitamin-A potential were better able to resist scours than those fed colostrum which was poor in vitamin-A. Lovell and Hill(1940) suggested after field observations that the mortality in the herds was not influenced by the 3 methods of feeding (suckling, bucket-fed undiluted milk, and bucket-fed with diluted milk), but their work suggested that there was an advantage in allowing the calf to take the colostrum naturally from its mother. Hibbs and Kraus (1947) concluded that vitamin supplements containing vitamin-A, niacin and vitamin-C were of doubtful value in preventing scour where colostrum was fed and Nevens and Kendall(1947) found vitamin-A,D,and C and nicotinic acid all of doubtful value when colostrum has been fed. Poundon and Hibbs (1947) stated that over feeding with milk can initiate scour and that a serious predisposing cause of scour is a too high protein content of the diet. Miles et al (1948) claimed benefits from comprehensive vitamin supplement. Blakemore et al(1948) had

found that vitamin-A was not effective in preventing scours in calves which had been denied colostrum. Esh et al (1948) found that vitamin-A supplement and lecithin was beneficial where calves did not get colostrum, while a supplement of vitamin-A alone was not satisfactory. Sheeby (1948) found advantages in the treatment of calf diarrhoea in diluting milk with water previously boiled, and he claimed advantages in feeding water alone for one day and then milk and water in equal parts for the next 2 days. He also found dilution of milk with 25% water was a preventive against calf enteritis, but that odd animals required to have the milk more extensively diluted. Another findings were that calves could tolerate the undiluted milk from freshly calved cows better than that from animals in later lactation. He also found some advantage in feeding 3 times daily instead of the usual twice, but this did not by any means eliminate scouring. Shanks (1950) encountered diarrhoea in calves coincident with having the cows producing milk for them on luscious pastures. He considered there was a possibility of substances in the rich pasture passing through the mother's milk to the calves. He succeeded in reproducing this condition experimentally on a small scale with controls which did not scour. It is now accepted that the antibodies are absorbed intact in the intestine during 6 to 27 hours after the birth of a calf (Comline et al,1951). Aschaffenburg et al (1951) found that the calves fed on milk of prepartum milked cows gave a proper performance and Hill et al (1951) experienced no difficulty in rearing calves from prepartum milked cows where the importance

of hygiene was observed. Blaxter et al (1951) found that deliberate over loading of the abomasum by suddenly giving the whole daily allowance of food in one feed induced scours. Aschaffenburg et al (1953) working at Reading found no benefit from feeding vitamin-A and lecithin or vitamin-A alone where colostrum has been denied. Roy (1959) stated," without doubt the main predisposing cause of scouring is the quality and quantity of the diet given after the colostrum feeding period". Inglis (1960) concluded that omitting the feeding of colostrum was not a common cause of enteritis, the calves scour and die inspite of receiving colostrum and in some cases even when they are getting the colostrum by suckling. He said over feeding of the calves was dangerous from the point of view of enteritis and scour. The finding of research workers, leave one in no doubt practical benefits of vitamin supplements fed to the young calf in preventing enteritis especially when colostrum has been fed although they do suggest that in some instances vitamin deficiency may be of importance. It is certain that a vitamin supplement is not effective in many instances in preventing enteritis.

(C) Physiological characters of E.coli.

Escherich (1885) noted the occurrence of two biochemical types: one E.coli (Bact.coli), formed fairly long rods,was motile and clotted milk slowly; the other, Bact.lactis aerogenes, formed shorter, plumper rods, was non-motile, and clotted milk more actively. Kruse (1894) emphasized the heterogeneity of the group covered by the term B.coli and pointed out that it included

a variety of related species, widely distributed as intestinal parasites and in water and soil. The use of a small series of fermentation tests, including especially glucose, lactose, sucrose, starch, inulin, action on litmus milk and indole formation, resulted in the recognition of certain primary divisions within this group. One of the groups, B. lactis-aerogenes type of Escherich organism fermented starch and inulin and failed to form indole, the lactis being usually omitted from the name. The second and third groups differed from Bact. aerogenes in failing to ferment starch and inulin and in forming indole in peptone water. They differed from each other in their action on sucrose. The sucrose (-) strain corresponded with the existing strains of Escherich's E. coli commune. The sucrose (+) was later on named as Bact. coli communius (E. coli communius) by Durham (1901).

Smith (1895) observed that gas was produced more rapidly and in greater amount by Bact. aerogenes than by E. coli (B. coli); and a rough estimation of the ratio of Co2:H2 in the gas evolved showed that this was higher with the former organism than with the latter. He noted also that the degree of final acidity was less with Bact. aerogenes than with E. coli (B. coli).

Voges and Proskauer (1898) described a colour reaction given by Bact. aerogenes (Aerobacter aerogenes) and not by E. coli by adding a few drops of a strong solution of Potassium hydrate to a culture grown in a dextrose medium. Russell and Bassett (1899) confirmed the differential value of a high or low Co2:H2 ratio observed by Smith (1895) and noted that the high ratio strains appeared to be normal soil forms rather than intestinal

Parasites. Eijkman (1904) found that coli and not aerogenes strains were able to form gas in a glucose broth medium when incubated at 46°C. Harden (1905) noted that the ratio between Co2:H2 was approximately 1:1 in E.coli and 2:1 in Aerobacter aerogenes. Harden (1906) confirmed the colour reaction of Voges and Proskauer (1898) and showed that it depends on the production of acetylmethylcarbinol, which in the presence of alkali and of atmospheric oxygen, is oxidized to diacetyl. The diacetyl reacts with the peptone of the broth to give the red colour. MacConkey (1909) observed the great preponderance of V.P.negative types among E.coli strains isolated from faeces; positive reactions were given by 11 of 178 strains isolated from human faeces, by 8 of 67 strains from the faeces of the horse, and by none of 87 strains from faeces of the calf,goat, pig or goose.

Clark and Lubs (1915) devised the methyl-red test for the differentiation of members of the coli-typhoid group. The E.coli is M.R.positive whereas A.aerogenes is M.R.negative. Levine (1916) found a very high negative correlation between the M.R.test and V.P.reaction and the lactose fermenting coliform bacilli could be divided into two primary divisions on the basis of the Co2:H2 ratio, the V.P. reaction and the M.R.test. The first of these containing strains giving a Co2:H2 ratio of about 2:1, V.P.positive and M.R.negative, comprised the majority of the strains isolated from plants,grains and unpolluted soil or water. Such strains were unknown in material obtained from the intestines of man or animals. This group could

be further divided on the basis of gelatine liquefaction, into the non-liquefying form - A.aerogenes and the much less common liquefying form Bact.cloacae. The second group containing strains giving a Co2:H2 ratio of approximately 1:1, V.P. negative and M.R.positive, included the majority of those strains isolated from the intestine of man or animals, as exemplified by E.coli commune. Further work revealed the occurrence of a third group of strains possessing properties intermediate between those of the two main groups and is most conveniently referred to as the intermediate group.

Brown (1921) drew attention to the usefulness of a medium containing citrate for distinguishing E.coli from A.aerogenes. Koser (1924,1926) devised a synthetic medium in which citrate was provided as the sole source of carbon. He found it possible to divide coliform bacilli into a M.R.+, V.P.-, citrate-, coli type; a M.R.-,V.P.+,citrate +,aerogenes type and a M.R.+,V.P.-,citrate +, intermediate type.

Koessler (1924) suggested that bacteria produce amines by simple decarboxylation of amino-acid in an acid environments as a protective mechanism.

Levine et al (1934) found that the production of hydrogen sulphide in a proteose peptone ferric citrate agar medium was the characteristic of intermediate strain.

Levine et al (1934) and Wilson et al (1935) confirmed the findings of Eijkman (1904) regarding the production of gas in glucose medium at 46°C and showed that its success depends on exact standardization of the incubating temperature, which

must be adjusted to 43-45°C in the medium itself. The satisfactory means of doing this is to incubate the tubes in a constant temperature water-bath. The differential value of the test is greatly enhanced by the replacement of glucose by lactose.

Gale (1946) described a number of decarboxylase enzyme in bacteria. Schafnitzl (1951) found that A.aerogenes and Friedlander's bacillus, differ from E.coli in the greater resistance of their respiratary system to KCN. Moller (1954) improved the technique of KCN test and employed liquid peptone medium with paraffinized corks to prevent the escape of KCN, thus such a medium could be stored at 4°C for 2 weeks. With this medium it was possible to differentiate between E.freundii, Ballerup-Bathesda, Klebsiella and Proteus which could grow in this medium and Salmonella, Arizona, Shigella and E.coli which do not grow. He further described a simplified technique for the detection of amino-acid decarboxylase activity.

Charter and Taylor (1952) carried out a series of biochemical tests of E.coli strains isolated from Nursery A and B. They found that the serologically identical strains often gave different fermentation reactions. The E.coli type canioni from the three different sources, gave three fermentation pattern though they were found by agglutination absorption test to have identical "O" and "B" antigens. The variations indicated that any attempt of classification on biochemical reactions alone would be unreliable, but these reactions might indicate the different origin of serologically identical strains.

In addition they found that a serologically unrelated E.coli might give reactions similar to one of the above patterns. All the serological types, so far, investigated by them had given the IMViC reactions ++-- and produced acid or acid and gas in MacConkey broth at 44°C, indicating that they were typical faecal strains of E.coli. They also described the anaerogenic type of E.coli type O111 from Nursery A and further stated that E.coli type O111:B4 could be subdivided into a sucrose (+) fermenting type with H2 antigens and non-sucrose fermenting type with H12 antigens.

Ewing et al (1955) described biochemical reaction of 58 E.coli cultures isolated from monkeys, which constituted a new biotype within O-group 111. Glucose,mannitol,sucrose and sorbitol were fermented within 24 hours incubation. Only two cultures produced gas from glucose, but the majority of strains formed small gas volumes (bubbles) from mannitol, sucrose and sorbitol. Lactose was fermented, without gas formation, after 3-5 days incubation. Salicin was fermented,usually with the formation of a small bubbles of gas, but in several instances the reaction was delayed. Acid was not produced from adonitol or inositol during 30 days incubation. There was no growth on simmons citrate agar; urea was not hydrolyzed, the V.P.reaction was negative, H_2S was not formed in triple sugar iron agar and the cultures were non-motile. The M.R.reaction was positive and indole was formed. Several cultures on MacConkey's agar gave rise either to a mixture of colonies,predominently colourless with a few pink colonies or to colourless colonies only which developed pink papillae upon further incubation. Lactose was

fermented within 24 hours upon transfer of pink colonies or papillae to lactose broth.

Wilson and Miles (1955) wrote that the E.coli produced acid and gas in glucose,maltose,mannitol,lactose,xylose, rhamnose, arabinose, but not in dextrin,starch,inositol or as a rule cellobiose. Sucrose,salicin,raffinose,glycerol and dulcitol were attacked by some strains,but not by others. E.coli acidified and clotted litmus milk, reduced nitrate, formed indole,produced gas in MacConkey's broth at 44°C and were M.R.+,V.P.-,Koser's citrate -, and H_2S negative.

Rees (1960) studied the biochemical reaction of O128:B12 E.coli strains isolated from 3 week old calf suffering from gastro-enteritis and compared the reactions with the type strain. The O128:B12 strain was motile,nonhaemolytic and produced indole. It was M.R.+, V.P.-,and Koser's citrate negative. It fermented glucose,maltose,mannitol,arabinose,trehalose,and rhamnose promptly with acid and gas; gave delayed fermentation of salicin and failed to ferment inositol in 15 days. Unlike the type strain, it fermented sucrose,sorbitol and sorbose promptly, xylose after several days and failed to ferment dulcitol in 15 days. The type strain fermented xylose promptly, sucrose, dulcitol and sorbitol after several days and failed to alter sorbose in 15 days.

Lovell et al (1960) studied four strains of E.coli for haemolysin production. The strains were grown in a medium consisting of infusion broth and lean beef that had been boiled in N/20 sodium hydroxide solution. Haemolysin was demonstrated

in filtrates from cultures 4-24 hours old with the highest titre in cultures 6-10 hours old. The haemolysin was destroyed by heating and could be removed from the filtrate by 30% ammonium sulphate solution.

Shirlaw (1960) studied 24 strains of E.coli isolated from scouring calves in Kenya; 19 fermented sucrose, 2 salicin and 3 both. Mansson (1962) studied the biochemical reactions of 171 haemolytic strains belonging to O138; O139 and O141. The O138 strains,unlike O141 strains were usually sorbose positive, sucrose positive and salicin negative; as a rule they fermented sugars either promptly or not at all. Many showed urease activity. About half of the strains decarboxylated lysine and a quarter could break down arginine.

Smith (1963) isolated, 76% in cattle,63% in pigs, 53% in sheep and 18% in men, haemolytic E.coli from faecal samples and studied the haemolysin production. At least two different haemolysin, designated alpha and beta were identified. The alpha haemolysin was probably identical with the filterable haemolysin described by Lovell et al(1960); the beta haemolysin was not filterable. The haemolysins were indistinguishable in their effect on blood agar. Preparation containing high concentration of alpha haemolysins were toxic for mice, rabbits and guineapigs when given I/v but not by stomach tube or injected into the rectum or duodenum. The principal lesion was intravascular haemolysis. Mice were the most resistant and usually had higher blood levels of antihaemolysins than rabbits and guineapigs.

(D) Serological characters of Escherichia coli and serotypes isolated from calves:

Escherich (1885) was the first who described E.coli commune. Jensen (1897) reported the presence of several different types of coli bacteria with fermentative main groups A and B. Joest (1903) found a lack of cross protection with sera prepared against different strains of E.coli, and said that the biochemical tests were of limited value. Christiansen (1917) continued the work of Jensen (1897) on typing of E.coli and found it impossible to distinguish between pathogenic and non-pathogenic strains of E.coli on the basis of morphology, cultural,biochemical or serological tests, as the biochemical and serological reactions of E.coli strains from normal and diseased calves were similar.

Smith (1927) worked with the E.coli isolated by Jensen (1897) from calves suffering from white scours. Those coli types grew as mucoid colonies; did not decompose saccharose, were non-motile and were able to form mutants which were non-mucoid, thin and translucent. The mucoid colonies had capsulated bacteria and were more toxic (pathogenic) than the non-mucoid ones having acapsular bacteria. Lovell (1927) was successful in preparing antisera and thus classified E.coli strains on the basis of serological tests. He inoculated rabbits with whole cultures and prepared antisera. He was able to divide the E.coli strains into specific types on the basis of precipitation tests and found the existence of pathogenic strains of E.coli for calves. A herd, of course, might have

contained more than one special strain of that organism at a time.

Smith (1928) expressed some doubt of the value of capsular material of E.coli for classification and hence he advocated serological methods for the same. He found two types of colonies in many strains of E.coli; one with entire edge, low convex and mucoid and the other as grey translucent one. The former being generally produced by capsulated and the latter by non-capsulated organism. Antiserum produced from the daughter non-capsulated form agglutinated the homologous acapsular forms to a high titre but had no influence on mucoid forms. Antiserum prepared from the mucoid form agglutinated the homologous capsular strain to a very low titre (1:40), but non-capsulated daughter forms to a high titre (1:40690). Theobald Smith (1928) demonstrated the presence of a capsular antigen in certain bovine strains.

Lovell (1937) established a land-mark in the serology of E.coli. He was able to place E.coli strains,mainly from scouring calves, into 8 specific groups and described the presence of two different antigens in E.coli; one - a soluble specific carbohydrate substance associated with the capsule("K"), and the other a somatic ("O") antigen. He,further,demonstrated that the mucoid bacteria produced two corresponding antibodies when injected into rabbits and also reported that the E.coli strains could not be classified on the basis of biochemical tests.

Kauffmann (1943), continuing on the serology of E.coli, became successful in using the agglutination test as a reliable

diagnostic aid in typing E.coli commune. He showed that the coli strains, earlier believed to be inagglutinable, were the bearers of "L" antigen - "L" from labile, which prevented "O" agglutination. This antigen was thermolabile. On immunisation of rabbits with an antigen prepared by boiling E.coli cultures at 100°C for 2½ hours, a serum was obtained in which living and formalin killed E.coli did not agglutinate if the organism was a carrier of the "L" antigen. On the other hand a serum was prepared through the injection of living culture, which immediately agglutinated the living bacteria as well as the coli antigen prepared by boiling at 100°C for 2½ hours. He inoculated rabbits with "O" inagglutinable cultures and obtained antisera against "O" and "L" antigens. On absorption of such antisera with boiled cultures the "O" antibodies were removed, while the "L" antibodies remained. Thus he obtained antisera against "O" and "L" antigens separately. The pathogenic effects of E.coli in the mouse were associated with the combined activity of "L" antigen and the heat stable somatic "O" antigen; the antibody which might be induced against the heat labile one had a greater protective action than the "O" antibody. Kauffmann, thus, described a close similarity between the "Vi" antigens of Felix and Pitt (1934) and Felix (1952) isolated from Salmonella typhi and other Salmonellae and the heat labile somatic antigens in E.coli.

Kauffmann (1944) examined 92 E.coli cultures and found 58 motile strains and demonstrated,initially, 3 flagellar (H) antigens. 19 more flagellar antigens were added by Kauffmann & Vahlne (1944) and Vahlne (1945). The flagellar antigens of E.coli

were similar to other flagellar antigens; they appeared to be monophasic with little or no overlapping. In the same year Kauffmann selected twenty "O" groups and established the first antigenic Schema for E.coli. He demonstrated in the same year that certain coli strains possessed an antigen which also prevented "O" agglutination even after heating at 100°C for 2½ hours. That antigen was latter regarded as "A" antigen. The colonies of strains of E.coli with "A" antigen were said to be whiter, denser and more opaque than those without such antigen; they were mucoid colonies and if left standing produced translucent out-growths.

Continuing the work of Kauffmann (1944) on E.coli, Knipschildt (1945) demonstrated the presence of heat resistant "O" agglutination inhibiting antigen, which was not destroyed by heating at 100°C , to which he designated "A" antigen, in the capsulated bacteria. Vahlne (1945) demonstrated that the inagglutinability of strains with "A" antigen could be abolished by autoclaving at 120°C for 2 hours. When the techniques for destroying "O" agglutination inhibiting factors were established, it was possible to type coliforms according to their heat resistant "O" antigens. He repeated and extended the work of Kauffmann (1944) and Knipschildt (1945) on the serology of E.coli group and typed E.coli strains isolated from men and animals,in normal and pathological conditions. It was tried, particularly, to find out whether certain types were more pathogenic than others. He,further,showed that the "O" antigens of E.coli remained qualitatively constant for a long period,

atleast 3 years in storage and typed the E.coli strains which were inagglutinable, by heating them at 120°C for 2 hours and thus rendering agglutinable.

Kauffmann and Vahlne (1945) suggested that the thermolabile as well as thermostable antigens in the capsule (envelope) were to be designated by the common term of capsular antigens "K" and by analogy all sera produced with antigens containing capsular factors to be termed as "K" sera. Kauffmann (1944), Knipschildt (1945) and Vahlne (1945) had demonstrated a total of 57 different "K" antigens.

Kauffmann (1944) had demonstrated 58 different "O" antigens and Knipschildt (1945) increased the number to 110, but in the antigenic schema of Kauffmann and Knipschildt (1945), they incorporated only 25 most common types. Their schema was further elaborated by Vahlne (1945) by adding more number of "K" and "H" antigens and established the serological classification of E.coli on a firm basis. This classification is used at present by those who are interested in the serology of E.coli. This schema divides the E.coli into groups,primarily according to their "O" antigens, with subdivisions according to their "K" and "H" antigens.

Knipschildt (1946) found an additional type of antigen called "B" antigen. "B" antigen differed from the "L" antigen by retaining its antibody binding properties inspite of heating at 100°C, while it differed from "A" antigen by loosing its "O" agglutination inhibiting properties on heating to 100°C.He concluded that L,A,and B antigens were localized within a capsule enveloping the soma (o). He also found that strains

possessing "A" antigen when treated with homologous serum showed a typical capsular reaction.

Kauffmann (1947) published an elaborate antigenic schema for the E.coli group which was based upon his investigation and those of his co-workers Knipschildt (1945,1946) and Vahlne (1945).

Since 1947, many investigators have studied E.coli serotypes, especially those associated with cases of infantile diarrhoea and calf scours, and as a result of their afforts, the E.coli schema has been extended. More than 145 "O" antigens, 100 "K" antigens and 50 "H" antigens have been recognised.

Wramby (1948) studied the serotypes of E.coli isolated from animals, especially from calf scours and named them as serotype 26W. He found that the strains of E.coli isolated from diseased animals showed a greater antigenic uniformity than those recovered from normal animals.

Orskov (1951) demonstrated the E.coli serotype 026:B6 in both sick calves and infants in Denmark. Bokhari and Orskov (1952) isolated E.coli strains 26W from calves at necropsy and stated that it was found in "one or two" of their 155 cases of coli septicaemia. Later Orskov (1952) reclassified those strains in the "O" group schema of Kauffmann as serotype O114. Bokhari and Orskov (1952) isolated a strain of O103 from one of 221 cases of white scours in calves, but did not attempt to determine the surface antigen.

Fey (1953) isolated E.coli strains of O86; O55 and O26 from cases of bovine mastitis in Switzerland, but his strain of O86 appeared to have an unusual surface antigen. Thus it would

appear that in some instances the vehicle of infection to children might be milk. Ulbrich (1954) isolated E.coli strain O55:B5 from calves with white scours.

Ewing et al (1954) found antigenic differences in "O" & "B" antigens of E.coli strains. He found O86a:B7 and O86a,86b:B9:H36 and O127a:B8 and O127a, 127b:B10:H4. The E.coli strains O86a:B7 and O127a:B8 were recovered from cases of diarrhoea of the new born, while O86a, 86b:B9:H36 and O127a, 127b:B10:H4 from the stools of adult or children who did not have symptoms of intestinal diseases. Orskov (1954a) demonstrated that the E.coli O86 cultures from bovine mastitis contained a "K" antigen which differed from the "K" antigens found in strains from cases of infantile diarrhoea. Kauffmann (1954) described that the strains of E.coli isolated from cases of disease show a greater antigenic uniformity than those recovered from normal animals.

Lochmann (1955) isolated seven strains of E.coli O114:B4 and two strains of E.coli O55:B5 from milk samples from a herd.

Ewing et al (1955) isolated E.coli O111a, 111c:B4,a new serotype from monkeys suffering from diarrhoea and the E.coli of infantile diarrhoea was labelled as O111a, 111b:B4. They commented that the described antigenic differences between E.coli group O111 culture isolated from cases of infantile diarrhoea and those recovered from monkeys at Okatis farms illustrated the importance of detailed analysis of strains which appeared to belong to the same serotype especially when such strains where from disease sources.

Wood (1955) reported that few serological types of E.coli appeared to be particularly pathogenic for the young calves.

Cultures antigenically the same as RVC95A and RVC95B had been recovered from cases of neonatal diarrhoea of children in Denmark, Finland and England and from white scours of calves in Sweden and Pakistan. Strains antigenically related to RVC118A and RVC330 had been recovered from dead calves in Sweden.

Fey (1956) isolated E.coli 078 with a new "B" antigen from septicaemia in calves. In his opinion it was a selective pathogen for calves and not merely a secondary invador. 35% of calf diarrhoea examined by him, were caused by that strains. Thomson (1956) isolated E.coli 026; 055; 086 and 0111 from 1% of milk sample, obtained directly from the farm. Those findings in themselves did not necessarily mean that strains recovered from milk were of direct bovine origin, as there were many possible sources of contamination. Charter (1956) studied 11 E.coli strains, two from calf scours received from Professor Lovell, 5 from cases of infantile diarrhoea received from Dr. Rogers and four locally isolated strains from babies. On serological examination all the 11 strains were proved to be 0114 and on "H" agglutination - H2, H32, H10 and H- unknown, were from human sources and H32 only from calf scours. There was no obvious relationship between the type of "H" antigen and the biochemical reactions.

Geurden et al (1957) isolated E.coli 08 from a calf. Uberko (1957) isolated E.coli strains 026:B6 and 0111:B4 from the liver,lymph glands and muscles of three of 445 calves examined.Muscles and organs of 586 adult cattle were free from infection with those strains.

Rees(1957) described that the E.coli strains most frequently found in natural cases of white-scours of calves in Great Britain were O9:A?; O35:L?; O78:B? and O137:L79. He, further,stated that O86:B7 had already been isolated from experimental calves which started to scour when deprived of colostrum. A survey was being made of the distribution of E.coli in the udder secretions from cases of bovine mastitis. The study was incomplete but at that moment no antigenic types associated with human disease had been found although several well known calf pathogens had been isolated and they included O9:A?; O78:B? and O117:K?. He,further, described the isolation of E.coli strains O86:B7 and O103:B? from naturally occurring cases of white-scours in calves.

Fey (1957) examined E.coli isolated from 105 calves suffering from coli-septicaemia and found 20, O-group and 49 serological or biochemical types. The predominant O-groups were O78; O113; O15 and O117 comprising 57% of the strains and 29% belonged to O-group O78 only. In another paper he described that out of 145 strains of E.coli septicaemia in calves, 37.5% belonged to a single group O78:B80.

Gould (1958) isolated O26 from the cases of white-scours. Rees (1958) studied the E.coli isolated from coli-bacillosis and found the serotypes O9:K? (type RVC118), O9:A (type RVC1798); O15:K?; O35:K?; O78:K80 (B); O86:B7; O103:K(B)?; O117:K? and O137:K79(L). He also isolated the serotype O9:A (type RVC1798); O24:K? and O78:K80(B) from the udder secretions of cows suffering from mastitis. Tatum et al (1958) described the occurrence of K61(B7) antigen from O-group 20 serotypes. This antigen had been

found only in E.coli 086:K61(B7) serotype. Davis et al(1958) described six new E.coli "H" antigens and numbered them from 41 to 46. They also described a new "O" antigen and recorded as 0140.

Glantz et al (1959) examined 152 E.coli strains isolated from scouring calves. They belonged to 16 different O-groups, strains belonging to O-groups 026; 08; 0119 and 03 were shown to be pathogenic; strains of O-groups 0101 and 0109 were not pathogenic for calves. Types 0119 and 08 were isolated more often from calves with spontaneous scours than the other types. Calves which received colostrum were susceptible to experimental infection with type 08 but not with 0119.

Kauffmann (1959) proposed that serological types of members of the family Enterobacteriaceae warranted the status of species; the concept that a species was a biochemically defined subdivision of the genus should be abandoned. A classification of the family was given. Mansson (1959) isolated E.coli strains 0138:K81:H14 from calves and mature cattle.

Kaechenbeek et al (1960) isolated 278 strains of E.coli from calves in Belgium and it was possible to classify 221 of them. 153 strains (69%) belonged to one of the following O-groups :- 078; 055; 086 and 015 of which the first two groups were the commonest.

Sahab (1960) described a method for titration of "B" antibodies in OB sera of E.coli, using the strained capsular reaction test, which demonstrated capsules on organisms with "B" surface antigen. Smith (1960) reported that the National Institute for Research in Dairying (N.I.R.D.) and Royal

Veterinary College (R.V.C.) London team used serological methods of classifying the E.coli in their epidemiological studies. They examined the serological types of E.coli isolated from 153 calves that died from scours in a calf house during the years 1950-1953, the calves having been brought there from neighbouring farms; many of them had received either no colostrum or only certain fractions of it. The death of each calf was usually associated with one type of E.coli that was isolated from all the tissues or from the intestinal contents and mesenteric lymph nodes only. Atleast 16 different serological types were involved during the whole experimental period, one type being dominant as a cause of death for a time, that type then being supplanted by others, and so on, as the season progressed. Many of those types were also found frequently in large numbers in the faeces of healthy calves in the pens.

Ingram and Lovell (1960) stated that the strains of E.coli had three important diagnostic antigens, somatic (O),surface(K) and flagellar (H). There were well over 100 known "O" antigens, nearly 100 known "K" antigens, some of which were labelled L,B, or A and about 50 known "H" antigens and the different serological types of E.coli were designated by numbers.

Rees (1960) described that there were strains of E.coli which were often isolated from outbreaks of epidemic infantile gastro-enteritis and of those O26:B6 (Orskov,1951); O55:B5 (Ulbrich,1954); O114:B? (Charter,1956); O86:B7 and O103:B? (Rees,1957) had occasionally been isolated from calves. Strains of a further serotypes O128:B12 had been isolated from babies by Taylor and Charter (1955). Rees (1960) also isolated O128:B12

from a three week old calf suffering from gastro-enteritis. Dam (1960) serologically typed E.coli strains isolated from 431 cases of coli-septicaemia in calves in Denmark and found that more than 40% belonged to O78 and 15% to O-groups 3 to 115. He also reported the work of Danish workers that O78 predominated in calf-scours. Linzenmeier (1962) stated that out of 30 strains of E.coli isolated from calf-scours, 21 were O78 type.

The difference between the L,A and B antigens of the E.coli strains, as published by Edwards and Ewing (1962) is reproduced below:-

The "K" antigens of E.coli

Variety of "K" antigen	Characteristics
L	1. Agglutinability of L antigen in L antiserum is inactivated by heat at 100°C in one hour.
	2. Suspensions rendered agglutinable in O antiserum by heat at 100°C in one hour.
	3. Antibody binding power inactivated by heat at 100°C in one hour.
	4. Antigenicity inactivated by heat at 100°C in 1 hr.
	5. Occur as envelope or sheath, occasionally as capsule.
A	1. Agglutinability of A antigen in A antiserum is inactivated by heat at 120°C in 2½ hours.
	2. Suspensions rendered agglutinable in O antiserum by heat at 120°C in 2½ hours.

Variety of "K" antigen	Characteristics
A	3. Antibody binding power not inactivated by heat at 100°C in 2½ hours or at 121°C in 2 hrs.
	4. Antigenicity inactivated by heat at 120°C in 2½ hours.
	5. Occur as capsule.
B	1. Agglutinability of B antigen in B antiserum is inactivated by heat at 100°C in one hour.
	2. Suspensions rendered agglutinable in O antiserum by heat at 100°C in one hour.
	3. Antibody binding power not inactivated by heat at 100°C in 2½ hours or at 121°C in 2 hours.
	4. Antigenicity inactivated by heat at 100°C in one hour.
	5. Occur as envelope or sheath or as capsule.

Ulendeev (1963) serologically typed 243 strains of E.coli isolated from 60 healthy and 181 sick calves suffering from acute gastro-intestinal disease. He employed 18 different O-group sera and found O-group:09; 0119; 0115; 0117; and 078 in decreasing frequency. Dam (1963) serologically typed the strains of E.coli isolated from cases of white-scours for over 5 years. He compared the occurrence of frequently isolated O-groups (078;0115 and 015) with the occurrence of same O-groups in earlier materials from Denmark and other countries. He concluded that a fairly constant predominance of strains belonging to those O-groups was to be expected.

Dam (1963) experimentally infected calves with serotypes O78:B80 and O115 which were commonly found in connexion with septicaemic calf-scours and with serotype O141, which was rare in calves but often associated with pigs. The virulence of those types for calves was found to decrease in order - O78 - O115 - O141. The virulence was so low that, to produce septicaemia in calves which had received colostrum, much larger doses were required than those to which calves would be exposed under practical conditions. Akhmedova et al (1963) studied 661 strains of E.coli isolated in Azenbaijan and Uzbekistan and found that 73% of calf strains and 74% lamb strains comprised the serotypes O86; O55 and O26. Those strains were not very pathogenic for mice, the most pathogenic was type O127, which constituted 11% of calf and 14% of lamb strains.

Yadava (1965) studied serologically 34 E.coli strains isolated from calves and found the serotypes O26:B6(3); O119:B14 (7); O125:B15(1) and O126:B16(1). The rest 22 strains were untypable with the available sera.

(E) Serotypes of E.coli isolated from pigs,lambs,foals, poultry etc.:

There are many reports in the literature describing a disease very similar in appearance of calf-scours affecting piglets a few days old. An increasing volume of evidence has appeared suggesting an association between certain strains of E.coli and some of the more common enteritis syndromes. The coliform infections have always given rise to active controversy because of the ubiquitous nature of the organisms, the sporadic

onset of the troubles they produced, the difficulties encountered in making a specific diagnosis and our inability to reproduce disease experimentally in certain cases. However, a lot is now known about the role played by E.coli in piglets and this evidence may well help us to understand something about the possible mechanism of disease production in older pigs.

Schofield and Davis (1955) mentioned that a heavy growth of haemolytic E.coli has been noticed in faecal cultures made from cases of oedema disease in swine. This was the first indication that haemolytic E.coli might be associated with this condition and it received some support when Gregory (1955) reported that he had found haemolytic coliform organisms in 5 different outbreaks of oedema disease.

Geurden et al (1956) isolated 73 E.coli strains from diseased foals, calves, pigs, dogs, cats and fowls and studied the pathogenic role and antigenic structure. None belonged to O111:B4 group. Strains belonging to O-group O86:B7 and O26:B6 were found only in poultry and those belonging to group O55:B5 only in dogs, cats and pigs. Most of the strains recovered from dogs and cats were haemolytic. Same authors in the year 1957 studied 111 E.coli strains and found that type O8 was isolated from a calf, 2 sows and 2 fowls, type O25 from a sow with puerperal sepsis, type O86 and O26:B6 from 2 hens with granuloma and type O55:B5 from pigs and 3 dogs.

Timoney (1956) successfully protected pigs from the experimental reproduction of oedema disease by using hyperimmune serum prepared with haemolytic coliform organisms isolated from a case of oedema disease.

Gitter (1957) reported that the haemolytic E.coli could be recovered from pigs in conditions other than oedema disease. Sojka, Erskine and Lloyd (1957) found that two specific serotypes of haemolytic E.coli were associated with cases of oedema disease. They reported that these serotypes were not common in pigs except in association with oedema disease and concluded that the disease was an enterotoxaemia, the toxin being produced by these specific serotypes of haemolytic E.coli. In America, Ewing et al (1958) found that the strains of haemolytic E.coli isolated from swine with oedema disease fell into two serotypes. Quinchon et al (1958) in France also found three serotypes of haemolytic E.coli in cases of oedema disease. These authors considered that E.coli is an ordinary inhabitant of the intestinal tract and becomes pathogenic under the influence of external conditions.

Mansson (1959) isolated O138:K81:H14 and O141:H4 haemolytic E.coli strains from pigs suffering from enteritis. Rees (1959) studied serologically 259 strains of E.coli isolated from diseased pigs. Three serotypes : O138:K81(B); O139:K82(B) and RVC2909, normally haemolytic, occurred in a high percentage of cases of gastro-enteritis and oedema disease. Four serotypes O101:K?(A); O103:K?(B); O114:K?(B) and O117:K?, usually associated with coli-bacillosis in calves,occurred in a small number of diseased pigs. A serotype O119:B14, which has been isolated from cases of infantile gastro-enteritis, occurred in one of the pigs.

In Canada,Kelen et al (1959) reported that at least three serotypes of haemolytic E.coli were associated with oedema disease and of these two serotypes : O138:K81 and O139:K82 were found

in Great Britain, the United States and Canada. The third Canadian serotype and a third British serotype had an O-antigen in common but a different K-antigen; the former was thus tentatively designed as O141:Kx. Campbell (1959) also found serotype : O138:K81 in association with twelve outbreaks of acute gastro-enteritis and septicaemia in recently weaned piglets in the 8 to 14 weeks age group. In the same year he isolated 127 strains of haemolytic E.coli from 293 normal pigs. Out of 127 strains, 46 belonged to serotype : O138:K81; 9 to serotype O141:Kx and the remaining 72 could not be typed with the three sera (i.e. O138:K81; O139:K82 and O141:Kx) employed. Serotype O139:K82 was not isolated from any normal pigs.

Thal et al (1959) described numerous E.coli in the prepuce and semen of four bulls. All the four strains differed serologically. Glantz (1960) stated that at present 139 "O" antigens, 84 "K" antigens and 60 "H" antigens have been recognised amongst the E.coli group of bacteria. Various identical E.coli serotypes have been isolated from cases of calf-scours, lamb dysentery, oedema disease of swine, mastitis in cows and chronic respiratory disease in poultry and are probably the pathogenic agents.

D'Souza et al(1961) isolated E.coli, suspected to be the cause of relapsing type of fever, from the faeces and urine of 2 Alsatian dogs. The strains were not typed serologically, but its presence in the urine was correlated with the clinical relapses. Davis et al (1961) prepared an electrophoretically homogenous fraction from a sonic extract of a haemolytic strain of E.coli (strain 755) associated with oedema disease of pigs, using continuous paper electrophoresis. The fraction killed pigs

faeces of adult cattle and pigs and also from the faeces of 2 cats in houses where infants had diarrhoea.

Lofton et al (1962) studied the incidence of pathogenic E.coli in small wild mammals collected in nine counties of Colarado. They examined 589 animals. A total of 14 rodents yielded E.coli known to be pathogenic to man, including O86:[illegible], O127:B8; O55:B5 and O26:B6. Serotype O55:B5 was most frequ[illegible] isolated from Citellus lateralis and thus said that some ro[illegible] might,therefore, be significant reservoirs of pathogenic E.[illegible] in They also isolated O127:B8 from wild bird. Mansson (1962) [illegible]t isolated haemolytic E.coli of serotype; O138:K81(B):H14 fr[illegible] diseased and healthy pigs in a piggery during nine years. [illegible] in in the same year, isolated 272 strains from 108 pigs, 17 c[illegible]ned 45 dogs and 8 cats. 209 strains belonged to five O-groups:[illegible]s, O6; O138; O139 and O141. Serotypes O141:K85(B):H1 and O13[illegible]:B7 H14 were the most common types from pigs and were usually [illegible]n ated with acute enteritis. Serotype O141:K85(B):H1 was al[illegible] isolated from dogs and cats and O138:K81(B):H14 from catt[illegible]eritis serotype O139:K82(B):H1 was from cattle and pigs. Strains belonging to serotypes O2 and O6 were recovered from pigs[illegible] were and dogs, usually with local inflammatory lesions. [illegible]rtyone

Szabo (1962) studied, 161 haemolytic strains isola[illegible]coup: from cases of bowel oedema in pigs, 290 from cases of ga[illegible]:O1ab enteritis in pigs, 14 from pigs suffering from various o[illegible]ins conditions and 30 from healthy pigs, with 10 standard an[illegible] by the agglutination test. Five non-haemolytic strains f[illegible] isolated of bowel oedema were also examined. Out of the 500 strai[illegible]B13; gave positive results with one or the other of the stand[illegible] the antisera used. The standard antisera used were against th[illegible]

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Lofton et al (1962) studied the incidence of pathogenic E.coli in small wild mammals collected in nine counties of Colarado. They examined 589 animals. A total of 14 rodents yielded E.coli known to be pathogenic to man, including O86:B7; O127:B8; O55:B5 and O26:B6. Serotype O55:B5 was most frequently isolated from Citellus lateralis and thus said that some rodents might,therefore, be significant reservoirs of pathogenic E.coli. They also isolated O127:B8 from wild bird. Mansson (1962) isolated haemolytic E.coli of serotype; O138:K81(B):H14 from diseased and healthy pigs in a piggery during nine years. He, in the same year, isolated 272 strains from 108 pigs, 17 cattle, 45 dogs and 8 cats. 209 strains belonged to five O-groups: O2; O6; O138; O139 and O141. Serotypes O141:K85(B):H1 and O138:K81(B): H14 were the most common types from pigs and were usually associated with acute enteritis. Serotype O141:K85(B):H1 was also isolated from dogs and cats and O138:K81(B):H14 from cattle; serotype O139:K82(B):H1 was from cattle and pigs. Strains belonging to serotypes O2 and O6 were recovered from pigs,cattle and dogs, usually with local inflammatory lesions.

Szabo (1962) studied, 161 haemolytic strains isolated from cases of bowel oedema in pigs, 290 from cases of gastro-enteritis in pigs, 14 from pigs suffering from various other conditions and 30 from healthy pigs, with 10 standard antisera by the agglutination test. Five non-haemolytic strains from cases of bowel oedema were also examined. Out of the 500 strains, 416 gave positive results with one or the other of the standard antisera used. The standard antisera used were against the

serotypes: 0138; 0139; 0141; 08; 09; 021; 028; 041; 0101 and 0119. Out of 166 strains from bowel oedema cases, 142 belonged to the groups 0138; 0139; 0141 and 08. Out of 290 strains from gastro-enteritis cases, 247 also belonged to the above four groups of which the first three are generally known in the international literature as the "oedema coli" group. According to these findings, however, the various enteric conditions in pigs can not be associated with the specific prevalence of certain serological groups of E.coli. They may be regarded as aetiologically identical conditions which differ in clinical appearance from each other.

Bekajle et al (1963) examined 30,230 table poultry and found that 211 had lesions of serofibrinous pericarditis. In every disease poultry E.coli was isolated in pure culture. Three serotypes : 071; 08 and 03 were identified. Two of the isolated serotypes: 071 and 08 killed mice when injected by I/v with 0.1 ml of a 24 hours culture, but 1 ml dose given to 2 months old chicks proved harmless.

Dam et al (1963) examined 562 pigs and isolated haemolytic E.coli from 132 cases in Denmark. The serotypes: 08; 0141:K85ac; 0141:K85ab and 0130:K81 were equally represented. 0139:K82 was isolated three times.

Kater et al (1963) described two outbreaks of serotype: 078:K80 infection in lambs. One was characterised by sudden deaths of over 100 lambs, the other by lameness and signs of central nervous system involvement. The organism was pathogenic for lambs aged 5 to 8 weeks when given by parenteral route, and a polyvalent E.coli immune serum protected lambs against a lethal challange dose.

Witting (1963) isolated 654 haemolytic E.coli strain from 625 pigs suffering from enterotoxaemia. He found that the serotypes: O141:K85ab(B) was 39.8%, O139:K82B was 24.8%, O138: K81(B) was 12.4% and O141:K85ac(B) was 10% in 654 haemolytic E.coli strains. In pigs which had died of other disease, incidence of haemolytic E.coli was considerably lower.

Stevans (1963) described that the constant site of infection was the intestinal tract in piglets but the invasion of the other viscera might take place in the terminal stages of disease. The E.coli organisms were more frequently isolated from brain than from other visceral organs and the reason was difficult to explain. He,further, suggested that the disease arose when there was a lack of balance between passive immunity and certain strains of E.coli and recommendations were made for its control in the field.

Yadava (1965) in India, examined serologically 50 E.coli strains isolated from cow, buffalo and buffalo calves and found the serotypes: O55:B5(1); O86:B7(3); O102:B?(2); O112:B11(4); O119:B14(4); O125:B15(7) and O128:B12(1). The rest 28 strains were untypable with the available antisera.

(F) Serotypes of E.coli isolated from infants:

Bray (1945) first described a particular serotype of E.coli strain under the name Bacterium coli neopolitanum from cases of infantile gastro-enteritis which was later on designated as O111 by Kauffmann and Dupont (1950). Giles and Sangster (1948) isolated similar type of E.coli from infantile gastro-enteritis. Smith (1949) isolated O55:B5. Smith et al (1950) also isolated

the same serotype : O55:B5:H6. Orskov (1951) isolated E.coli O26 from cases of infantile diarrhoea and white-scours in calves and its biochemical and serological properties were studied. The strains could be divided into three fermentative types. The antigenic formula of the first two being O26:B6 and were non-motile, and the third was O26:B6:H11. Roger and Koegler (1951) isolated O111:B4 and O55:B5. Taylor and Charter (1952) isolated O55 from infantile gastro-enteritis and E.coli type canioni from cases of upper respiratory tract from Nursery A. They also isolated O111 from Nursery A, but it was not associated with intestinal symptoms. In a series of 255 healthy control babies, one was found to be carrying E.coli type canioni, one - E611 and two - O86. In Nursery B the canioni type was endemic for 5 months, the babies remaining healthy. Charter and Taylor (1952) studied six serological types, biochemically and serologically, which were isolated from Nursery A and B. They were O111:B4; O55:B5; O26:B6; O86; type canioni and type E611. No relationship was detected between O and B antigens of those six types. E.coli type E611 also possessed "H2" antigen. The sucrose fermenting O111:B4 had "H2" antigen whereas non-sucrose fermenting O111:B4 had "H12".

Smith (1953) described that O111 was predominant during the epidemic of 1947 and sucrose (+) strains with "H2" antigen were more common than sucrose(-) with "H12" antigen. Sucrose (-) with "H12" antigen strains were common during 1948 and 1949. During 1949-51, cases infected with O55:B5 were much more common. He described a new type O119:B14:H6 E.coli strain associated with the disease.

Le Minor et al (1953) isolated O55:B5:H21; O55:B5:H7 & O111:B4:H21 types of E.coli from cases of infantile diarrhoea. Ewing et al (1953) isolated O124:B17 and O12:B17:H32 from infantile diarrhoea. Grouroos (1954) described the association of O55:B5:H4 with the diarrhoeal disease in infants. Orskov & Fey (1954) isolated O55:B5:H10 and O55:B5:H8 of E.coli from infantile diarrhoea. Orskov (1954a) described several serotypes within O25 strains, two of which had been associated with diarrhoeal disease. Orskov (1954b,c) mentioned the association of O111:B4:H11 and O86a:B7:H34 respectively with infantile gastro-enteritis. Ewing et al (1955) and Merlin et al (1955) isolated O127:B8. Charter (1955) isolated O114 and Roger et al (1955) isolated O128. Ewing et al (1956) described E.coli O18a, 18c:K77 (B121):H7. Taylor and Charter (1955) isolated O128:B12. Charter (1956) isolated O114 from infantile diarrhoea and calf-scours. He said that the E.coli O111:B4; O55:B5 and O26:B6 were the main serological types responsible for infantile gastro-enteritis and less common serotypes were O119:B14; O86:B7; O125:B15 (type canioni) and O126:B16 (type E611).

Rees (1957) reported that the principal types of E.coli strains associated with infantile gastro-enteritis were O26:B6; O55:B5 and O111:B4. In addition O86:B7 and O103:B? were also reported. Davis et al (1958) isolated O26:B6:H46 and Ujvary (1958) isolated streptomycin resistant strain O4.

Linzenmeier (1962) described the changes in the incidence of E.coli types. He found in Munich infants clinic that the type O55 predominated from 1956 to 1958; type O128 in 1959 and type O114 in 1960. The incidence of O78 and O119 had increased and

many new types had been isolated in the recent years. Type 0111 and 026 were then infrequent and 055 was less common than formerly isolated.

(G) Bacteriophage typing:

Twort (1915) reported an interesting phenomenon, while working with staphylococcus isolated from calf-lymph vaccine. He observed a visible degenerative change in some of the colonies which was found to be transmissible and he suggested a "disease of bacteria". Furthermore, it was found that filtrates of the changed cultures would also initiate the "disease" of the staphylococcus. d'Herelle (1917), while searching for an evidence of a mixed aetiology for bacillary dysentery in man, demonstrated the occurrence of rapid and generalised lysis of Shiga's bacillus in broth, to which some of the filtrate from the original mixed culture had been added. He also transmitted the lytic agent to the cultures of susceptible organisms, in a prolonged series. The name - "Bacteriophage", that he gave to the lytic agent, came in general use and familiarly shortened to "phage" which he considered as filtrable virus. This view has won increasing support particularly within recent years.

Volman and Volman (1925) adopted a Shiga bacteriophage on Colon bacilli, resistant to it, by giving serial passages on it. d'Herelle (1930) found that it was difficult to get bacteria free from phage and referred to the phenomenon as "Symbiose-bacterial-bacteriophage" a name that reflects his interpretation of lysogeny. He hypothesized that the bacterial population was composed of individuals covering a considerable spectrum of

susceptibility to phage action, while the phage population covered a considerable range of virulence. The most susceptible bacteria and phage particles, in this way, would be perpetuated in mixed cultures.

Nylurgo (1931) described a method for isolating bacteriophages for various types of Colon bacilli, by adding, some of the material likely to contain bacteriophage, to the broth culture of the organism against which the phage was to be prepared, and then filtering after 24 hours incubation at 37°C. Verge and Vallie (1931) demonstrated the presence of a specific bacteriophage in the intestines of convalescent calves, whose virulence was exalted by passing in series in Vitro and its lytic properties were extended by adaptation to numerous strains of E.coli isolated from excreta of infected calves.

Lesbouynis and Renauldon (1935) succeeded in the treatment of acute coliform paraplegic mastitis in cows by injecting a vaccine composed of a killed culture of the organism which was followed by an administration of anti-coliform bacteriophage. Ellis and Delbruck (1935) described "one step" growth cycle in bacteriophages.

Luria et al(1943) described three phages of an E.coli strain B which had an opaque head about 80 mμ in diameter consisting of a pattern of granules and a less opaque tail about 120 mμ long. Demerec and Fano (1945) studied a collection of seven coli-phages and designated them as T1,T2,T3,T4,T5,T6 and T7. Anderson (1946) found that all the seven coli-phages except T3 & T7 were having tail. Delbruck (1946) described the latent period

of T2,T4 and T6 varying from 20-25 minutes; for T1, T3 and T7 - 13 minutes and for T5 - 40 minutes and the average burst size varied from 120-300 particles.

Toft (1946) described the "Capsulo-phages" or "K-phages" acting on the capsular forms of E.coli and with the help of nine phages, demonstrated three phage groups, viz. one that acted only on capsular forms, another that acted only on acapsular forms and the third that acted on both forms. He also found that out of these three phage groups, the first was the most commonest and the second the most infrequent. Anderson (1951) found by the electron-micrographic studies that T2 phage attached itself to the bacterial host by the tail end.

Nicolle et al (1952) concluded that the E.coli strains of the infantile gastro-enteritis could be classified by means of bacteriophage typing as some of the isolated phages were found to be showing lytic action over the strains of E.coli belonging to the serotypes : O111:B4 & O55:B5.

Fraser and Williams (1953) described short tail on T3 and T7 phages. Williams and Fraser (1953) studied the morphology of all the seven T-phages by electron-microscopy of specimens prepared by normal air-drying from aqueous suspensions and by freeze-drying. The air-dried materials showed evidence of considerable distortion of form in accord with previous observations and exhibited dimensions which were considered to be wholly unreliable. The heads of frozen-dried phages frequently appeared hexagonal in contour and had a height -to- width ratio near unity. They reported the dimensions of all the seven T-phages as follows:- T1 - head, 65 mμ and tail,150 x 10 mμ ; T2,T4 &

T6 - head, 85 x 70 m μ and tail,110 x 15 m μ ; T3 & T7 - head, 50 m μ and tail,doubtful and T5 - head,80 m μ and tail,180x10 m μ. They said that the dimensions listed above are believed to be the most reliable,obtainable at present. They,further, stated that two types of three dimensional shape for the phage heads are considered to be likely, a rhombic dodecahedron, and a hexagonal prism with bipyramidal ends. No choice can be made at this time between these two likely forms.

Nicolle et al (1954) classified E.coli belonging to serological groups : O111:B4; O55:B5 and O26:B6 into different phage types.

Smith and Crabb (1956) described a bacteriophage method for classifying E.coli strains of animal origin from the phages isolated from animals. With the help of 16 phages, viz. A,B,C,D, E,G,H,L,M,O,T,X, Z, Z2, Z3 & Z4, they found 70 different phage types in the faeces of healthy calves and 32 different phage types in the faeces of scouring calves. All the phage types found in the scouring calves were also found in the healthy calves except one phage type which was not found in the healthy calves. They also found the similarity in the flora of calves kept in the same pen, then between the calves kept in different pens, but given the same bulk milk. They concluded that the mother did not appear to be a potential source of origin of the E.coli population of the alimentary tract of her calf, but the pens themselves might be the most likely source of origin of the E.coli found in the calves.

Nicolle (1957) emphasized the great importance of phage typing in the epidemiology of infantile gastro-enteritis. It is

possible to know whether the contagion is occurring or denying any relationship of cases in a community and in assessing the role of carriers to establish several sources responsible for infection.

Smith (1958) combined the phage typing and drug sensitivity results of E.coli isolated from calves and found that the emergence of resistant E.coli during chemotherapy was probably due to in some instances to mutation of sensitive E.coli. The dominant E.coli in the faeces of all the calves that developed scours during an observation period of one year, belonged to one phage type, type 2. This type was also found in the faeces of some of the healthy calves, but never in the faeces of the cows of that herd.

Smith (1960) described the complexities of phage types that occur in animals especially in calves and stated that the bacteriophage method of classification would only yield information on the predominant types of E.coli present in the calves. Many more types might be present in quite considerable numbers, their presence being masked by the overwhelming numbers of the dominant types.

Kasatiya and Singh (1961) carried out the phage typing of 200 strains of animal and human origin with 43 phages isolated locally and 21 obtained from other places. They indicated that the strains of E.coli of animal origin belonged to different phage types than the strains of human origin.

Yadava (1965) studied 34 strains of E.coli isolated from calves with T-series of phages. Six strains were typable which belonged to three phage types.

(H) Sensitivity test of E.coli:

Various antibiotics are used either as a prophylactic or curative agent against the white scours. Handerson and McKay (1949) described that on farms which had previously a high mortality rate, the incidence of disease was considerably reduced following the administration, at birth and for the next 4 to 6 days, of daily doses of 0.25 gm.of streptomycin orally. Gardner et al (1952) described that penicillin had no effect on the incidence of calf diarrhoea.

Fox (1952) stated that calves with diarrhoea when given 0.5 gm per os or 0.5 gm by injection of streptomycin usually responded well to a single treatment. He,further,stated that streptomycin appeared to be more successful than treatment with sulphathalidine. Bortree et al (1952) conducted some field trials with chlortetracycline. In one outbreak of 58 cases of diarrhoea, which were treated with oral administration of chlortetracycline, 51 responded immediately to treatment and 5 gradually. Two of the calves did not respond to treatment and eventually died. In another investigation, 67 calves were given one tablet (500 mgs.) of chlortetracycline at birth. Forty of these calves remained healthy and 27 developed diarrhoea of varying intensity. 21 of the affected calves responded to a single treatment of 500 mgs. of chlortetracycline, but 6 required further treatment, one of those dying.

Kon et al (1953) reported that penicillin reduced the incidence of calf diarrhoea; on the other hand Knodt and Ross (1953) found that penicillin increased the incidence. Voelker and Jacobson (1953) observed that penicillin had no effect on

the incidence of calf diarrhoea.

Pearson (1954) described the therapeutic and prophylactic use of oxytetracycline in the control of field cases of calf diarrhoea in Northern Ireland. 41 clinical cases of calf diarrhoea were treated by giving 2 daily doses of 0.5 gm. of oxytetracycline and 40 completely recovered within 36 hours of the administration of antibiotics. When 21 calves on 6 other farms were given two doses of 0.5 gm. of oxytetracycline within the first 48 hours of life, none developed diarrhoea, and the other untreated animals on the same farms became affected with diarrhoea. Taylor and Gordon (1954) and Roy et al (1955) illustrated well the prophylactic value of chlortetracycline under conditions favouring the development of calf scours.

Lassiter (1955) concluded that the growth rate of calves may be stimulated by 10-30% during the first 4 months of life by feeding a supplement of either oxytetracycline or chlortetracycline, most of the increased growth occurred during the first two months of life. When the antibiotic was fed as a supplement they also reduced the incidence of calf diarrhoea, increased the feed consumption and efficiency and improved the overall condition of the animal. He recommended feeding these antibiotics at a level of 15-20 mgs. per 100 lbs. body weight per day, approximately 1/25th of the normal therapeutic dose.

Merlin et al (1955) performed the sensitivity test with E.coli strains 0127:B8 isolated from acute cases of infantile gastro-enteritis. The test was performed with antibiotics disks containing 10 and 30 µgs except sodium sulfadiazine which contained 10 and 50 µgs. All 0127:B8 E.coli strains were sensitive

to neomycin, aureomycin, chloramphenicol and terramycin and resistant to streptomycin and sodium sulfadiazine.

Smith and Crabb (1956) carried out rapid plate test to determine the sensitivity of E.coli isolated from white scours, with dried paper disks containing each of 20 mcgs. of dihydrostreptomycin sulphate, chlortetracycline hydrochloride (Aureomycin), oxytetracycline hydrochloride (Terramycin), chloramphenicol, and with 130 mcgs. of sodium sulphadimidine (sulphamezathine). The plates were incubated at 28°C for 18 hours and the zone of inhibition around the disk noted. Alternatively, if a more rapid result was required, incubation was carried out at 37°C for 7-8 hours. Out of 58 strains tested, 15 were resistant in Vitro to sulphadimidine, 11 to streptomycin and 2 to chlortetracycline and oxytetracycline; all were sensitive to chloramphenicol. Further, studies revealed that resistant strains of E.coli were commonly associated with cases of white scours on some farms, but not on others. On the two farms where chlortetracycline and oxytetracycline resistant strains were found, it had been the practice for several years to feed a chlortetracycline supplement to all calves for the first month of life.

Gould (1958) performed sensitivity test of the organisms isolated from white scours and found them resistant to tetracycline. He concluded that the resistant strains developed after the introduction of oxytetracycline prophylaxis, at first successfully, to prevent infectious white scours. Smith (1958) studied the sensitivity test on the E.coli isolated from white scours. He described many more drug resistant E.coli in the faeces of

calves in herds where the calves were given drugs therapeutically or prophylactically than in calves in herd in which chemotherapy had never been employed. The value of basing the drug treatment of white scours on sensitivity test was confirmed. E.coli with multiple resistance to streptomycin, tetracycline, chloramphenicol (each 50 mcgs.) and sulphadimidine (130 mcgs.) eventually formed the predominant faecal flora in cases of white scours in some of those herds. It was possible in Vitro to make drug sensitive culture of E.coli resistant to streptomycin, chloramphenicol and sulphadimidine,both singly and multiply, but not to tetracycline or furamazone.

Ingram et al(1958) reported the reduction in mortality of their experimental calves deprived of colostrum when chlortetracycline was given; there was a coincidental improvement in growth rate. They,further,stated that there were disadvantages in the use of antibiotics because of the increase resistance exhibited by strains. They demonstrated an increased resistance to penicillin and chlortetracycline in E.coli strains isolated from the small intestine of calves that had received supplements of these antibiotics in their milk during life, compared with strains from calves that had been fed milk without antibiotics. Osborne et al (1959) reported the observation of three groups, each of 21 calves only and said that the incidence of diarrhoea was reduced by both neomycin and furamazone but the death rate was reduced by the latter drug only.

Smith and Crabb (1960) carried out the effect of chemotherapy on the number of E.coli count in the faeces of healthy

calves. They found that 0.5 gm of streptomycin by mouth reduced the number of E.coli per gramme of faeces from 100,000,000 to 10 in all the treated calves. The depressant effect was observed for as long as 5 days after the administration of drug. The effect of giving a dose 4 times greater by injection was much less pronounced, a point of some importance, when the chemotherapy of calf scours is considered. The depressant effect of neomycin was equal to that of streptomycin. Treatment with polymyxin consistently resulted in a marked reduction; oxytetracycline, chlortetracycline, sulphadimidine and phthalylsulphathiazole had a considerable depressing effect on the E.coli faecal count, although in a small proportion of calves no decrease was found. The administration of chloramphenicol to 6 calves had no effect on the coliform count and similar results were found when calves were given furazolidone and furamazone. They also showed that the continued daily administration of streptomycin, neomycin and polymyxin had a very marked depressant effect on the growth of faecal E.coli. A much greater depressant effect was noted when the tetracycline and sulphonamides were given daily, than given in a single dose. Only a slight depressant effect was noticed when chloramphenicol, furamazone and furazolidone were given twice daily.

The use of 32 mgs. chlortetracycline hydrochloride twice daily in milk resulted in the development of 84% resistant E.coli against tetracycline in the faeces of group-B calves, compared with only 6% in the group-A calves which never received these or other chemotherapeutic agents.

The efficiency of chemotherapeutic agents in eliminating sensitive strains of E.coli is matched by the extreme spread at which resistant strains may replace them during chemotherapy. They, in another paper, had shown that the changes from a predominantly sensitive to a predominantly resistant E.coli faecal flora often took place within 24-48 hours of administrating a chemotherapeutic agent.

Dalton et al (1960) carried out the experiment with nine different preparations of antibiotics i.e. streptomycin, neomycin, oxytetracycline, chlortetracycline, chloromycetin, penicillin and phthalylsulphathiazol, on the incidence of calf diarrhoea but they did not get significant reduction in the incidence of diarrhoea and death in young calves. The treatments were given twice daily on 14 successive days and at full therapeutic dose levels and the calves were housed in separate pens. In the end they concluded that the further evidence had been obtained to show that antibiotics had a part to play in the control of diarrhoea and the reasons for the irregular effect of the antibiotics remained to be elucidated.

Inglis (1960) reported that the prophylactic effects of including antibiotic supplements in the calf's food had been the subject of many studies. A number of workers have reported beneficial results both on the incidence and severity of scours by feeding a chlortetracycline supplement specially under conditions favouring the development of calf scours. Others have reported that under the conditions of their experiments chlortetracycline had no effect on the incidence of scours particularly under conditions of "low infection". These

experimental findings fit in well with field observation. He believed the finding was that chlortetracycline was frequently effective as a preventive of calf enteritis and some time it was not. He,further, stated that, although oxytetracycline had not been so extensively studied, a number of workers had observed that this substance had also prophylactic effect on the incidence of scours. It has been,indeed, suggested its effect is similar to that of chlortetracycline.

Glantz (1962) tested with antibiotics a total of 287 strains of E.coli isolated from animals and poultry diseases. The most effective compounds were colistin, chloramphenicol,furazolidone, N-(5-nitro-2-furfurylidene)-1-amino-2-pyrrolidene, thiofuradene and polymyxin. Intermediate in activity were dihydro-streptomycin, chlortetracycline, tetracycline and oxytetracycline. The least effective were penicillin, oleandomycin, furaltadone and nidroxyzone.

MATERIALS AND METHODS

(E.COLI)

MATERIALS AND METHODS

MATERIALS

The materials for the present investigation were mainly collected from healthy calves as well as calves showing diarrhoea at Government Cattle Farm, Patna. A total of 246 calves were examined. Out of these 46 calves were having diarrhoea of varying intensity at the time of collection of materials. Materials from 7 dead calves of Government Cattle Farm, Patna, were also collected from the post-mortem room at Bihar Veterinary College, Patna. Altogether 253 calves were examined for the prevalence of Escherichia coli. The age of the calves varied from zero day to one year or slightly more.

The feeding and management of the Government Cattle Farm, Patna, were according to standard schedules. The calves are weaned at birth and are pail-fed. They are kept in a separate calf-pen where there is ample space to roam about. After weaning they are given colostrum from the mother and subsequently mother's milk for one week at the rate of 3 litres a day. They are also given terramycin soluble powder one teaspoonful daily for seven days along with mother's milk. The feeding of terramycin soluble powder has been introduced from the beginning of the current year. After one week they are fed according to ration-sheet given below:-

Periods	Whole milk	Concentrate mixture	Rouphages (green grass)
First month	4 litres	nil	nil
Second month	4 litres	½ kg	ad lib.

Periods	Whole milk	Concentrate mixture	Rouphages (green grass)
Third month	3 litres	1 kg	ad lib.
4 to 6 months	2 litres	1½ kg	ad lib.
7 to 12 months	Nil	1½ kg	ad lib.

The concentrate mixture contains gaurmeal, bran and groundnut cakes equal parts plus 2% mineral mixture and 2% common salts.

Calves up to two months of age are not allowed to go out for grazing. After two months they are taken out for grazing and are regularly drenched for parasitic infestations. Calves above six months are also vaccinated against Haemorrhagic septicaemia, Black-quarters and Anthrax.

The antibiotics most commonly used in this farm as a curative measures are Pronapen, Cambiotics and Terramycin.

METHODS

The techniques adopted are described in detail under the following headings:-

(A) Collection of materials

(B) Isolation of organisms

(C) Test for purity of the isolated strains

(D) Preliminary identification

(E) Studies of biochemical characters (Sugar fermentation)

(F) Bacteriophage typing

(G) Serological typing

(H) Antibiotic sensitivity tests.

(A) Collection of materials:

Faecal samples from the young calves were collected with the help of swabs, whereas from the older calves, faeces were collected with the help of hand and kept in sterile containers. Intestinal ingesta were collected from the post-mortem cases. Sterile precautions were taken during the collection of materials.

(B) Isolation of organisms:

The most commonly employed MacConkey agar medium was used throughout the present investigation which facilitates the isolation of lactose fermenting organisms. The medium was prepared and about 20 ml. quantity was poured into sterile petridishes and dried at 37°C for 24 hours. Faecal samples were directly streaked on the dried MacConkey agar medium and incubated at 37°C for 24 hours. A single colony which was red, round, translucent, convex and about 2 mm. in diameter was picked up from each sample and transferred on plain agar slant which was incubated for 24 hours at 37°C.

(C) Test for purity of the isolated strains:

This was done by replating the cultures on MacConkey agar medium, observing the growth on agar slant and studying morphological characters after Gram's staining.

(D) Preliminary identification:

The cultures were tested for indole production, M.R. & V.P. reactions, nitrate reduction and growth in Koser citrate medium. These tests were done by adopting usual methods after four days incubation. The cultures which were positive for

indole, M.R. and nitrate and negative for V.P. and citrate were taken for further studies and the rest of the cultures were discarded.

(E) Studies of biochemical characters(Sugar fermentation):

Fermentation of carbohydrates and alcohols such as arabinose, glucose, lactose, raffinose, sucrose,adonitol, dulcitol, mannitol and sorbitol were done as described by Mackie and McCartney (1953). Liquid paraffin was used for the detection of gas production. 24 hours peptone water culture was used for inoculation of sugar media. Incubation at 37°C was prolonged atleast for 10 days and reading was taken at every 24 hours intervals. Care was taken to notice the gas production, if any. Tubes showing clear positive reaction, acid or acid and gas, were removed and others incubated further.

(F) Bacteriophage typing:

The T.series of bacteriophage from T1 to T7 along with propagating strain,E.coli strain B, were obtained from the kind courtsey of Dr. Neville Symonds, Medical Research Council, Microbial Genetics Research Units,Hammersmith Hospital, 150,Du Cane Road,London,W-12. Phages were received in one ml quantity, which were not sufficient to carry out the present work and as such they were propagated in this laboratory. The method employed for the propagation and titration of phages, was the same,with slight modification, as described by Smith & Crabb (1956).

Two loopful (4 mm loop) of phages were added separately to 3 ml of 18 hours broth culture of propagating strain and

incubated at 37°C for 24 hours and then left at room temperature for another 24 hours. Complete lysis was observed after this period. 3 ml of 18 hours broth culture of the propagating strain was added to all the phage tubes and incubated at 37°C for 24 hours and then left at room temperature for another 24 hours. The room temperature varied from 22.5°C to 36°C during the experimental periods. This process was repeated for four times to get a high titre phage preparation. Side by side the titre of the phages was tested.

The phage preparation were centrifuged at high speed for 30 minutes and the supernatants were collected with sterile precautions. 0.1 ml of chloroform was added to every 10 ml of the phage preparations and shaken well. This was done to kill bacteria, if any, present in the phage preparations. These preparations were tested for sterility and stored in the refrigerator.

Ten-fold dilutions of the phage preparations were made in phosphate buffer (KH_2PO_4-3.4g;Na_2HPO_4-6g; distilled water-1000 ml) to determine the critical test dilution. Six hours broth culture of the propagating strain was spread evenly over the surface of an agar plate, allowed to dry and then spotted with one loopful (4 mm loop) of each dilutions of the phage preparations. Plates were incubated at 37°C for six hours, left at room temperature for over night and then read. The highest dilution which produced confluent lysis was chosen as the critical test dilution(CTD). T1, T2, T4 & T7 gave one CTD in

10^{-7} dilution whereas T3, T5 and T6 gave one CTD in 10^{-6} dilution. 100 CTD was used as routine test dilution (RTD) in the present work.

Method of setting up test:- The method employed for the phage typing of the E.coli strains is the same, with slight modification, as devised by Wilson and Atkinson (1945) and modified by Smith and Crabb (1956). Nutrient agar plates were prepared and incubated at 37°C for 24 hours. The contaminated plates were discarded. Six hours broth culture of the strain of E.coli to be typed was spread evenly over the surface of one of the nutrient agar plates by means of a bent glass spreader. The plate was dried on the working bench for one hour and then divided into 7 sections equal to the number of phages to be spotted, with the help of greese pencil. A loopful(4 mm loop) of phages in their routine test dilution (i.e. 100 critical test dose) were spotted after proper numbering. Care was taken to avoid mixing of the drops together. After phage spotting, the plate was left on the working bench for half an hour for the drops to be absorbed over the plates and then incubated at 37°C for six hours. After this period it was kept at room temperature (22.5 - 36°C) for overnight. Reading was taken after 18 hours of phage spotting. All the strains of E.coli isolated from calves were tested similarly. Side by side a control test was setup with propagating strain.

(G) Serological typing:

The OB coli antisera against the serotypes; (1) O26:B6; (2) O55:B5; (3) O86:B7; (4) O111:B4; (5) O127:B8; (6) Polyvalent (Pooled) antisera of the above five; (7) O18a,18c:B21; (8) O119:B14;

(9) O126:B16 and (10) O128:B12 were received from Dr.S.N.Sinha, Deputy Bacteriologist, Public Health Institute, Patna. He had received the first six antisera from Dr.F.Orskov, Director, International Escherichia Centre, Copenhagen, Denmark and he was kind enough to spare some amount for my work. The last four antisera, which he supplied, were raised by him in his laboratory. The E.coli strains to raise these antisera were also received from Dr. F.Orskov. Polyvalent (pooled) antisera of the last four serotypes was made by adding equal amounts of the four antisera.

The method adopted for the determination of specific serotype was the same, with modification, as advocated by Kauffmann (1947) and Barua et al(1956). 24 hours broth culture of the E.coli strain to be typed, was heated at 100°C for one hour. Three loopful (4 mm loop) of the heated culture was thoroughly mixed separately with two loopful of the two polyvalent coli antisera, on the clean microscopic slide and kept in the petridish with moist filter paper at the bottom to prevant drying up. They were examined after 15 minutes under low power microscope for the presence of agglutination. If positive with any of the two polyvalent antisera, the heated culture was similarly examined with different monovalent specific sera corresponding to the polyvalent group to determine the specific "O" serotype. The strain was taken to belong to the particular O-group with whose serum it gave positive slide agglutination. After determining the specific "O" serotype, the living culture was examined for the presence of "B" antigen.

A loopful of the 18-20 hours culture, grown on agar slant, was suspended in normal saline solution and a loopful of the correspnding OB antisera was mixed and examined as above. In case of positive slide agglutination with living culture, it was taken that the culture is having the corresponding "B" antigen of the specific "O" serotype. The possibilities of acapsular form (without surface antigen) of E.coli strain giving positive slide agglutination with this method cannot be ruled out.

Side by side control test with normal saline solution (in place of serum) was set up to check autoagglutination.

(H) Antibiotic sensitivity tests:

Tetracycline hydrochloride (Achromycin),chlortetracycline hydrochloride (Aureomycin), Demethylchlortetracycline hydrochloride (Ledermycin), Dihydro-streptomycin sulfate, Mysteclin-v (each capsule contained 250 mgs.of tetracycline) and Penicillin G-sodium have been used in the present work to test the sensitivity of E.coli strains, isolated from calves. The Achromycin, Aureomycin and Ledermycin were received from M/S Cyanamid India Ltd., Agricultural Division,16,Queen's Road,Post-box no.1994, Bombay-1. The Dihydro-streptomycin sufate, Mysteclin-v, and penicillin were received from M/S Sarabhai Chemicals, S.P.Verma Road,Post-box no.44, Patna-1.

The method adopted for the sensitivity tests by disk method is the same, with slight modification, as described by Mackie and McCartney (1953). Whatman filter paper no.1 has been used to prepare the disks. The disks were cut out of the sheet

with a paper hole-puncher having a diameter of 6.5 mm. The disks so prepared were fractionally less than 6.5 mm. in diameter. They were counted accurately into lots of 100 and put into small screw capped bottles. These bottles were then sterilized in the hot air oven at 150°C for one hour.

The antibiotic solutions were prepared quantitatively in sterile distilled water. One ml of the required solution was then added to each bottle of 100 disks, and, as the entire volume was absorbed, we might assume that each disk contained approximately 0.01 ml. Thus the dilutions of the antibiotics were so prepared that 1 ml contained 100 times the quantity required in each disk. The disks were prepared in five different concentrations of 5 mcgms.,10 mcgms.,25 mcgms., 50 mcgms. and 100 mcgms. per disk of all the different antibiotics used except penicillin in which 50 units,100 units, 200 units, 500 units and 1000 units were used. These disks were used wet. The bottles containing disks of different antibiotics were stored in the refrigerator at 4°C for 10 days to complete the antibiotic sensitivity tests of the entire E.coli strains.

Agar plates were prepared and incubated at 37°C for 24 hours and contaminated plates were discarded. 18 hours broth culture was used to inoculate the agar plates. These were inoculated uniformly from the broth culture by flooding the surface and then removing the excess with the help of a fine capillary pipette. The plates were then allowed to dry in the inverted position in the incubator for 45 minutes. The incubated plates were marked and numbered at five different points, suitably

spaced apart, corresponding to the five different concentration of antibiotics used. Finally, the antibiotics impregnated filter paper disks were placed on the surface of the medium with a sterile fine pointed forceps. The forceps were kept with their tips immersed in 70% alcohol, which was flamed off every time before use. These plates were incubated at 37°C for overnight and next day the readings were taken. For each antibiotic the diameter of the circular areas of inhibition were measured, the areas of inhibition measured included the diameter of the disk as well as the surrounding zone of inhibition.

R E S U L T S

(E.COLI)

RESULTS

Faecal samples from 246 living calves and intestinal contents from 7 dead calves of Government Cattle Farm, Patna, were collected for the isolation of *E.coli*. Out of 246 living calves, 46 calves were having diarrhoea of varying intensity, at the time of collection of materials. The samples were streaked on MacConkey agar media and after 24 hours incubation at 37°C, they were examined. A single colony, which was red, round, translucent, convex and about 2 mm. in diameter and representing the majority group, was picked up from each sample and transferred on plain agar slant. Faecal samples from three new-born calves of zero-day old were sterile. Thus a total of 250 cultures were isolated and tested for purity. All the 250 pure cultures were tested for indole production, M.R. and V.P. reactions, growth in Koser's citrate medium and for nitrate reduction. This was done for preliminary identification and screening. The cultures, which gave IMViC reactions ++-- and nitrate positive were selected for further studies. Thus, out of 250 cultures, 174 cultures were selected for further studies and the rest 76 cultures were discarded. The 174 cultures, which were selected for further studies, belonged to healthy calves (131), diarrhoeal calves (37) and dead calves (6). The 76 cultures, which were discarded, also belonged to healthy calves (66), diarrhoeal calves (9) and dead calf (1). The three calves of zero-day old were from healthy group.

All the 174 selected cultures were tested for sugar fermentation, susceptibility to T-series of bacteriophage, determination of serotypes and sensitivity to six antibiotics in Vitro. The age, sex and the clinical conditions of 174 calves (including six dead calves), from which the 174 E.coli strains were isolated, and the results of serological typing, phage typing and sugar fermentation have been precisely presented in Table I.

Mortality in calves (Male and Female combined):

The mortality rate in the Government Cattle Farm, Patna, varies from year to year and is considerably high inspite of satisfactory managements. This data has been collected from the Government Cattle Farm, Patna, from the beginning of the year 1960 up to 28th September, 1965. The year and monthwise mortality in calves is given in Table II below.

TABLE-II

Mortality in calves

Period	1960 (165 calving	1961 (135 calving	1962 (141 calving	1963 (158 calving	1964 (194 calving	1965 (118 calving	Total (911 calving
1	2	3	4	5	6	7	8
January	7	nil	18	3	2	2	32
February	7	6	5	1	2	3	24
March	5	1	6	3	2	4	21
April	8	1	5	1	4	6	25
May	1	1	1	5	2	2	12
June	3	2	nil	1	2	2	10

TABLE-II (continued)

1	2	3	4	5	6	7	8
July	1	2	2	1	3	1	10
August	5	3	1	5	1	1	16
September	3	1	2	2	nil	nil	8
October	nil	1	2	nil	1	-	4
November	3	4	1	1	1	-	10
December	1	15	4	1	4	-	25
Total	44	37	47	24	24	21	197
Percentage of mortality	26.66	27.4	33.33	15.19	12.37	17.8	-

The highest mortality was 33.33% in 1962 and lowest was 12.37% in 1964. On an average the mortality rate varies from month to month and is comparatively high from December to April and is low in the rest of the months. It is,thus, evident that the winter and spring seasons are the dangerous periods and during that periods the mortality is comparatively high.

Sugar fermentation tests (reactions):

The results of sugar fermentation reactions have been given in Table I. All the 174 E.coli strains fermented arabinose, lactose and mannitol promptly within 24 hours except one strain which fermented arabinose in four days. All failed to ferment adonitol in 10 days - the period of observation. All the 174 strains attacked glucose promptly within 24 hours

TABLE - I

Table showing the age, sex and clinical conditions of calves examined, and the serological typing, phage typing and biochemical characters of Escherichia coli isolated from them.

Strain number	Age and sex of the calf examined.	Clinical condition of the calf at the time of collection of material.	Serotype of E.coli isolated	Bacteriophage typing							Biochemical characters.				
				T_1	T_2	T_3	T_4	T_5	T_6	T_7	Glucose	Sucrose	Raffinose	Dulcitol	Sorbitol.
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	F. 183 days	Apparently healthy	Untypable	-	-	-	4+	-	-	-	A	+	+	-	+
2	F. 180 do	-do-	-do-	-	-	-	4+	-	-	-	Ag	+	+	-	+
3	M. 177 do	-do-	E.coli:O86:B7	-	3+	-	4+	-	2+	-	A	-	-	+	+
4	F. 171 do	-do-	Untypable	+	4+	+	2+	+	+	+	Ag	-	-	-	-
5	M. 166 do	-do-	-do-	+	4+	+	2+	+	+	+	A	-	-	-	-
6	F. 165 do	-do-	-do-	-	-	-	4+	-	-	-	Ag	+	+	+	+
7	F. 161 do	-do-	-do-	-	-	-	4+	-	-	-	Ag	+	+	+	+
8	F. 159 do	-do-	E.coli:O55:B5	-	-	-	4+	-	-	-	A	-	-	-	-
9	F. 155 do	-do-	Untypable	-	-	-	4+	-	-	-	Ag	+	+	+	+
10	M. 154 do	-do-	-do-	-	-	-	4+	-	-	-	A	-	-	-	+
11	F. 152 do	-do-	-do-	-	-	-	4+	-	-	-	A	-	-	-	-
12	F. 152 do	-do-	-do-	-	2+	+	3+	+	-	-	A	-	-	-	-
14	M. 156 do	-do-	-do-	-	-	-	4+	-	-	-	Ag	+	+	+	+
17	M. 150 do	-do-	-do-	-	-	-	4+	-	-	-	A	+	+	+	+
18	F. 150 do	-do-	E.coli:O119:B14	+	+	+	+	3+	+	+	A	-	-	-	-
19	F. 148 do	Dia. with loose stool	Untypable	-	-	-	4+	-	-	-	Ag	+	+	+	+
20	M. 146 do	Apparently healthy	-do-	+	+	+	4+	+	+	+	A	-	-	-	-
21	M. 145 do	-do-	-do-	-	-	-	4+	-	-	-	A	-	-	+	+
22	M. 149 do	-do-	-do-	-	-	-	4+	-	-	-	Ag	-	-	+	+
23	F. 142 do	-do-	-do-	+	+	+	2+	+	+	+	A	-	+	-	+
24	F. 137 do	dia. with loose stool	E.coli:O126:B16	+	+	+	3+	+	+	+	A	-	-	-	-

TABLE- I (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
26	M. 135 days	Apparently healthy but weak.	Untypable	-	-	-	-	-	-	-	A	-	-	-	+
27	F. 128 do	Apparently healthy	-do-	+	+	+	+	+	+	+	A	+	-	-	-
28	F. 128 do	-do-	-do-	-	2+	-	4+	-	+	-	A	-	-	+	+
31	F. 112 do	-do-	-do-	-	-	-	-	-	-	-	Ag	+	+	-	+
32	M. 117 do	-do-	-do-	-	-	-	2+	-	-	-	Ag	+	+	-	+
33	M. 109 do	-do-	-do-	-	-	-	3+	-	-	-	A	-	-	-	+
34	F. 101 do	Dia.with loose stool	E.coli:086:B7	-	3+	-	3+	3+	4+	-	Ag	+	+	+	+
37	F. 98 do	Apparently healthy	Untypable	-	-	-	4+	-	-	-	A	-	-	-	-
40	M. 96 do	-do-	-do-	-	-	-	-	-	-	-	Ag	+	+	+	+
41	F. 101 do	-do-	-do-	-	-	-	-	-	-	-	A	+	+	-	+
42	F. 92 do	-do-	-do-	-	-	-	3+	-	-	-	A	-	-	-	-
43	F. 90 do	Dia.with loose stool and mucous.	-do-	-	-	-	4+	-	-	-	A	+	+	-	+
44	F. 90 do	Apparently healthy	-do-	2+	3+	3+	3+	3+	-	2+	A	-	-	-	-
46	F. 91 do	-do-	-do-	-	-	-	2+	-	-	-	Ag	+	+	-	+
47	M. 90 do	-do-	-do-	-	-	-	2+	-	-	-	A	-	-	+	+
48	F. 84 do	Dia. with loose stool	E.coli:0119:B14	-	-	-	4+	-	-	-	A	-	-	-	+
50	F. 83 do	Apparently healthy	E.coli:026:B6	-	-	-	-	-	-	-	A	+	+	-	-
53	M. 84 do	Dia. with loose stool	E.coli:086:B7	-	-	-	4+	-	-	-	Ag	-	-	+	+
54	F. 72 do	Apparently healthy	Untypable	-	-	-	3+	-	-	-	A	+	+	+	+
55	F. 76 do	-do-	-do-	-	-	-	4+	-	-	-	A	-	-	-	+
57	M. 64 do	-do-	-do-	-	-	-	-	-	-	-	A	-	-	-	+
58	F. 63 do	Dia. with loose stool	E.coli:055:B5	-	-	-	-	-	-	-	A	-	-	-	+
59	F. 57 do	Apparently healthy	Untypable	-	-	-	4+	-	-	-	A	+	+	+	+
62	F. 49 do	-do-	E.coli:0126:B16	-	2+	-	-	-	-	-	A	-	-	-	-

TABLE- I (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
92	M. 62 days	Apparently healthy	Untypable	-	-	-	-	-	-	-	A	+	+	+	+
93	F. 60 do	-do-	E.coli:055:B5	-	-	-	3+	-	-	-	Ag	+	-	-	+
94	F. 59 do	Dia.with loose stool	Untypable	-	-	-	3+	-	-	-	A	+	+	-	+
96	M. 57 do	Apparently healthy	E.coli:086:B7	-	-	-	-	-	-	-	A	+	-	-	-
97	M. 57 do	-do-	Untypable	-	-	-	-	-	-	-	A	+	+	+	+
99	M. 53 do	-do-	E.coli:086:B7	2+	-	+	3+	-	+	+	A	+	+	+	+
102	F.446 do	-do-	Untypable	-	-	-	4+	-	-	-	A	+	+	+	+
103	F.463 do	-do-	-do-	-	2+	-	3+	-	2+	-	Ag	-	-	-	+
104	F.463 do	-do-	E.coli:026:B6	+	3+	+	3+	+	+	+	A	-	-	+	-
105	M.459 do	-do-	Untypable	+	3+	+	3+	+	+	+	A	-	-	+	-
107	M.429 do	Dia.with loose stool & mucous.	E.coli:0126:B16	-	-	-	3+	-	-	-	A	+	+	-	+
108	F.451 do	Apparently healthy	Untypable	-	-	-	-	-	-	-	A	+	+	+	-
109	F.448 do	-do-	E.coli:086:B7	-	2+	-	-	-	2+	2+	A	-	-	+	-
113	M.415 do	-do-	Untypable	-	-	-	-	-	-	-	A	+	+	+	+
114	M.436 do	-do-	-do-	-	-	-	4+	-	-	-	A	-	-	+	-
115	M.417 do	Dia.with loose stool	E.coli:0119:B14	-	3+	-	2+	-	-	-	Ag	-	+	-	+
116	F.411 do	Apparently healthy	Untypable	-	2+	-	2+	-	2+	-	Ag	-	+	-	-
119	M.375 do	-do-	E.coli:0119:B14	-	2+	-	2+	-	2+	-	Ag	+	+	+	+
120	M.378 do	Apparently healthy but weak.	E.coli:055:B5	+	3+	-	3+	-	-	+	Ag	-	-	+	+
121	F.373 do	Apparently healthy	Untypable	+	2+	2+	2+	-	2+	-	A	+	+	-	+
124	M.395 do	-do-	E.coli:0126:B16	-	-	-	-	-	-	-	A	+	+	-	+
126	F.371 do	-do-	E.coli:0119:B14	+	+	+	+	+	+	+	A	+	+	-	+
127	F.371 do	Dia.with loose stool	Untypable	-	2+	-	-	-	-	-	Ag	+	+	+	+
128	F.366 do	Apparently healthy	-do-	-	-	-	3+	-	-	-	Ag	-	-	-	-

TABLE - I (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
63	F. 49 days	Apparently healthy	Untypable	-	-	-	-	-	-	-	Ag	-	-	-	-
65	M. 39 do	-do-	-do-	-	-	-	-	-	-	-	Ag	+	+	-	+
66	F. 39 do	Dia. with loose stool & mucous.Condition bad.	-do-	-	-	-	4+	-	-	-	Ag	+	+	-	+
67	F. 38 do	Apparently healthy	E.coli:055:B5	-	-	-	3+	-	-	-	Ag	+	+	-	+
68	M. 35 do	Dia.with bloody stool. Condition bad.	Untypable	-	-	-	-	-	-	-	Ag	+	+	-	+
69	F. 27 do	Apparently healthy	E.coli:086:B7	2+	2+	2+	2+	2+	2+	2+	Ag	+	+	-	+
70	F. 21 do	-do-	Untypable	-	-	-	-	-	-	-	A	+	+	-	+
72	F. 18 do	Dia.with loose stool & mucous.	-do-	-	-	-	4+	-	-	-	Ag	-	-	-	+
73	F. 17 do	Apparently healthy	E.coli:0119:B14	-	-	-	4+	-	-	-	Ag	+	+	-	+
74	M. 15 do	-do-	Untypable	-	-	-	3+	-	-	-	A	+	+	-	+
75	M. 15 do	Dia. with loose stool	E.coli:0119:B14	-	-	-	-	-	-	-	Ag	+	+	-	+
76	F. 14 do	-do-	E.coli:0126:B16	-	-	-	-	-	-	-	Ag	+	+	-	+
77	F. 12 do	Dia.with loose stool & mucous.Condition bad.	E.coli:026:B6	-	-	-	-	-	-	-	Ag	+	+	-	+
78	M. 11 do	-do-	E.coli:0111:B4	-	-	-	3+	-	-	-	A	+	+	-	+
79	M. 10 do	Apparently healthy	Untypable	-	-	-	-	-	-	-	A	+	+	-	+
80	F. 8 do	-do-	-do-	-	-	-	-	-	-	-	Ag	+	-	-	-
81	F. 5 do	-do-	-do-	-	-	-	2+	-	-	-	A	+	+	-	+
83	F. 48 do	Dia.with bloody stool & mucous.Condition bad.	E.coli:026:B6	2+	2+	2+	2+	2+	2+	2+	A	+	+	-	+
84	F. 40 do	-do-	Untypable	-	+	2+	3+	2+	2+	-	Ag	+	+	+	+
86	F. 74 do	Apparently healthy	E.coli:0111:B4	+	+	2+	+	+	+	+	Ag	+	+	+	+
87	M. 72 do	-do-	Untypable	-	-	+	3+	-	+	+	Ag	+	+	+	+
89	F. 69 do	Dia.with loose stool	-do-	-	+	2+	3+	2+	2+	-	A	+	+	-	+
90	M. 69 do	Apparently healthy	-do-	-	-	+	2+	-	2+	2+	A	+	+	+	+

TABLE- I (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
129	F. 369 days	Apparently healthy	Untypable	-	2+	-	-	-	-	-	A	+	+	+	+
131	F. 365 do	-do-	E.coli:O126:B16	-	-	-	-	-	-	-	A	+	+	+	+
132	F. 360 do	-do-	E.coli:O86:B7	-	-	-	-	-	-	-	A	+	+	+	+
133	F. 363 do	Apparently healthy but weak	Untypable	-	-	-	4+	-	-	+	A	+	+	-	+
135	F. 363 do	Apparently healthy	-do-	-	-	-	3+	-	-	-	A	+	+	+	+
137	F. 359 do	Dia. with loose stool	E.coli:O26:B6	-	-	-	-	-	-	-	A	+	+	+	+
139	M. 380 do	Apparently healthy	Untypable	-	3+	-	2+	2+	2+	-	Ag	-	+	-	+
140	M. 352 do	-do-	-do-	-	2+	2+	2+	2+	2+	2+	A	+	+	-	+
141	M. 378 do	Apparently healthy but weak	E.coli:O119:B14	-	3+	-	3+	-	-	-	A	-	+	+	+
142	F. 356 do	Dia. with loose stool	E.coli:O126:B16	-	-	-	-	-	-	-	A	+	+	+	+
143	F. 350 do	Apparently healthy	Untypable	-	2+	-	-	-	-	-	Ag	-	-	-	+
144	M. 376 do	-do-	E. coli:O55:B5	-	2+	-	-	-	2+	-	Ag	-	-	-	+
147	M. 352 do	-do-	Untypable	-	-	-	4+	-	2+	-	A	-	+	-	+
148	F. 351 do	Apparently healthy but weak	-do-	-	-	-	3+	-	2+	2+	Ag	-	+	-	+
149	F. 347 do	Apparently healthy	-do-	-	2+	-	2+	-	-	2+	Ag	+	-	-	+
151	M. 349 do	-do-	E.coli:O119:B14	-	3+	-	3+	3+	4+	-	A	-	+	+	+
152	M. 349 do	-do-	Untypable	-	-	-	-	-	4+	-	A	-	+	-	-
153	F. 347 do	-do-	-do-	-	3+	-	3+	3+	3+	-	A	+	+	+	+
154	F. 345 do	-do-	E.coli:O26:B6	-	2+	-	2+	-	-	-	Ag	+	+	-	+
155	M. 340 do	-do-	Untypable	2+	2+	2+	2+	-	-	2+	Ag	-	-	-	+
156	M. 342 do	Apparently healthy but weak	-do-	2+	2+	2+	2+	-	+	2+	A	-	+	+	+
158	F. 363 do	Apparently healthy	-do-	-	-	2+	-	2+	2+	-	A	-	-	-	-
161	F. 362 do	Dia. with loose stool	E.coli:O126:B16	-	2+	2+	-	2+	2+	2+	A	-	+	-	+
164	M. 332 do	Apparently healthy	E.coli:O86:B7	-	-	-	-	-	-	-	Ag	+	+	+	+

TABLE - I (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
165	F. 357 days	Apparently healthy but weak	Untypable	-	-	-	3+	-	-	-	Ag	-	-	-	+
166	M. 329 do	-do-	-do-	-	-	-	3+	-	-	-	A	-	-	+	+
167	F. 332 do	Apparently healthy	-do-	-	2+	-	2+	-	-	-	A	+	+	+	+
169	F. 328 do	-do-	-do-	-	3+	2+	2+	-	-	-	A	-	+	-	+
170	F. 223 do	Dia. with loose stool	-do-	-	3+	3+	2+	+	+	+	A	-	-	-	-
172	M. 326 do	Apparently healthy	-do-	+	+	2+	+	+	+	+	A	-	+	-	+
173	M. 324 do	-do-	-do-	-	-	-	-	-	-	-	A	-	+	+	+
175	F. 312 do	Dia. with loose stool	-do-	-	-	-	-	-	-	-	Ag	+	+	-	+
177	M. 309 do	Apparently healthy	-do-	-	2+	-	2+	-	-	-	Ag	-	-	-	-
178	F. 303 do	-do-	E.coli:055:B5	-	2+	-	3+	-	-	-	A	-	+	-	-
183	M. 288 do	-do-	E.coli:0126:B16	-	-	-	-	-	-	-	A	-	+	+	-
184	M. 287 do	-do-	Untypable	+	+	2+	2+	-	-	2+	A	-	+	-	+
186	M. 303 do	Apparently healthy but weak	-do-	-	-	3+	-	-	-	2+	Ag	+	+	-	+
188	M. 272 do	Dia. with loose stool	-do-	-	-	-	4+	-	-	-	A	-	-	-	-
189	F. 272 do	Apparently healthy	E.coli:086:B7	+	+	+	+	-	2+	-	A	-	-	-	+
191	F. 261 do	-do-	Untypable	+	2+	2+	2+	-	2+	-	A	-	+	-	+
192	M. 260 do	-do-	E.coli:0126:B16	-	-	-	-	-	-	-	A	+	+	-	+
195	M. 245 do	Apparently healthy but weak	Untypable	-	-	-	-	-	-	-	Ag	+	+	-	+
196	F. 244 do	Apparently healthy	-do-	-	2+	-	2+	-	-	2+	A	-	+	-	+
198	M. 238 do	-do-	-do-	-	2+	-	-	2+	2+	2+	A	-	+	+	+
199	F. 233 do	-do-	E.coli:026:B6	-	2+	-	2+	-	2+	-	A	-	+	-	+
201	M. 228 do	-do-	E.coli:0119:B14	-	3+	-	3+	-	2+	-	A	-	-	-	+
202	M. 227 do	-do-	Untypable	-	3+	2+	3+	2+	-	-	Ag	-	+	-	+
203	M. 225 do	Dia. with loose stool	-do-	2+	2+	2+	2+	2+	2+	2+	Ag	+	+	+	+

TABLE-I (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
204	F. 223 days	Apparently healthy but weak	Untypable	2+	2+	-	2+	-	2+	-	Ag	-	-	+	+
206	M. 221 do	Apparently healthy	E.coli:O86:B7	-	2+	-	2+	-	-	-	A	-	-	-	-
207	F. 225 do	-do-	E.coli:O126:B16	-	-	-	-	-	-	-	A	-	-	-	-
208	M. 216 do	-do-	Untypable	-	-	-	-	-	-	-	A	+	-	-	+
209	F. 216 do	Apparently healthy but weak	-do-	2+	2+	2+	2+	-	2+	-	A	-	+	+	+
210	F. 219 do	Apparently healthy	-do-	2+	2+	2+	2+	-	2+	-	A	-	+	-	+
212	M. 214 do	-do-	-do-	-	2+	-	2+	-	-	2+	Ag	-	+	-	+
213	M. 215 do	-do-	E.coli:O119:B14	-	3+	-	3+	-	-	-	A	-	+	-	+
214	M. 207 do	-do-	E.coli:O119:B14	-	3+	-	3+	-	-	-	Ag	-	+	-	+
215	F.207 do	-do-	Untypable	-	-	-	-	-	-	-	Ag	-	+	+	+
217	F. 51 do	-do-	-do-	-	-	-	4+	-	-	-	Ag	+	+	+	+
218	M. 51 do	-do-	E.coli:O119:B14	-	-	-	-	-	-	-	Ag	+	+	-	+
219	F. 48 do	Apparently healthy but weak	E.coli:O111:B4	-	2+	-	2+	-	-	-	A	+	+	-	+
220	F. 48 do	Dia. with loose stool	E.coli:O86:B7	2+	2+	2+	2+	2+	2+	2+	Ag	+	+	-	+
221	F. 46 do	-do-	Untypable	-	-	-	-	-	-	-	Ag	+	+	+	+
222	F. 44 do	Apparently healthy	-do-	-	-	-	3+	-	-	-	A	+	+	-	+
224	M. 43 do	Dia. with loose stool. Condition extremely bad.	E.coli:O126:B16	+	+	2+	2+	2+	2+	-	A	-	-	-	+
225	F. 40 do	Apparently healthy	Untypable	-	-	2+	2+	2+	2+	-	A	-	-	-	+
228	F. 37 do	-do-	E.coli:O26:B6	-	-	-	3+	-	-	-	A	+	-	-	+
229	M. 36 do	-do-	E.coli:O55:B5	-	-	-	4+	-	-	-	A	+	-	-	+
234	F. 23 do	Dia. with loose stool	E.coli:O55:B5	-	2+	-	2+	-	-	-	A	-	+	-	-
236	F. 20 do	Apparently healthy but weak	Untypable	-	-	-	-	-	-	-	A	-	-	-	+
237	F. 19 do	Dia. with bloody stool	-do-	-	2+	-	3+	-	2+	-	A	-	-	-	-

TABLE- I (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
238	F. 12 days	Dia.with loose stool and mucous.	Untypable	-	-	-	4+	-	-	-	A	-	-	-	+
239	F. 10 do	Apparently healthy	-do-	-	-	-	3+	-	2+	-	A	-	+	-	+
240	F. 9 do	Dia. with loose stool	-do-	-	2+	-	2+	-	-	-	A	-	-	-	+
242	F. 6 do	Dia. with loose stool & mucous.Odour offensive.	-do-	+	2+	+	+	+	+	+	A	-	-	-	+
243	F. 2 do	Dia.with loose stool and mucous.	-do-	-	-	-	-	-	-	-	A	-	-	-	+
Dead calves															
247	M. 39 do	Gastro-enteritis	-do-	-	3+	-	4+	-	2+	-	Ag	-	+	+	+
248	F. 56 do	-do-	E.coli:086:B7	-	3+	2+	2+	-	-	-	Ag	-	+	-	+
249	F. 40 do	Enteritis	Untypable	-	3+	2+	3+	-	2+	-	A	-	+	-	+
251	M. 5 do	Gastro-enteritis	E.coli:055:B5	+	2+	2+	2+	2+	+	+	Ag	+	+	-	+
252	M. 46 do	Enteritis	Untypable	-	-	-	-	-	-	-	Ag	-	+	+	+
253	F. 60 do	-do-	-do-	-	2+	-	4+	-	-	-	A	+	+	-	+

Note:- 1. All the E.coli strains were positive for indole, M.R., nitrate, lactose, mannitol, and arabinose, and negative for V.P.,citrate and adonitol.

2. F = Female; M = Male; A = Acid; Ag = Acid and gas; + = Positive; - = negative

producing acid or acid and gas. 64 strains produced acid and gas and the rest 110 strains produced acid only. 84 strains fermented sucrose ($64^{1d(day)}$, 6^{2d}, 3^{3d}, 5^{5d}, 2^{6d} 3^{9d} and 1^{10d}) and 90 failed to ferment. 110 strains fermented raffinose (107^{1d}, 1^{2d} and 2^{4d}) and 64 failed to ferment. 57 strains fermented dulcitol (19^{1d}, 13^{2d}, 5^{3d}, 6^{4d}, 7^{5d}, 2^{6d}, 3^{8d}, 1^{9d} and 1^{10d}) and 117 failed to ferment. 139 strains fermented sorbitol (128^{1d}, 1^{2d}, 3^{3d}, 5^{4d} and 2^{5d}) and 35 failed to do so.

Altogether 24 biochemical patterns of sugar fermentation, representing 24 biochemical types have been found in 174 E.coli strains. The biochemical types of E.coli, their number and their correlation with age groups in healthy, diarrhoeal and dead calves have been given in Table III. The majority of E.coli belongs to the biochemical types 1, 2,3, 4, 12, 19 and 24. All the 24 biochemical types of E.coli have been encountered in the healthy calves, whereas only few biochemical types were found in diarrhoeal and dead calves. Out of 24 biochemical types, 10 and 5 biochemical types were found in diarrhoeal and dead calves respectively. All the biochemical types found in diarrhoeal and dead calves, were also found in healthy calves.

Bacteriophage typing:

All the 174 E.coli strains were tested,for susceptibility, with T-series of bacteriophage from T_1 to T_7 and the results of bacteriophage typing have been precisely given in Table I. The confluent lysis (clear plaque) was given ++++ and the

TABLE - III

Table showing the various biochemical types of *E.coli* prevalent in the calves examined, and their correlation with age in healthy, diarrhoeal, and dead calves.

Biochemical types	Sugar fermentation pattern					Number of calves examined															
						Healthy calves						Diarrhoeal calves						Dead calves			
	Glucose	Sucrose	Raffinose	Dulcitol	Sorbitol	Zero to 15d.	16 to 30d.	31 to 60d.	61 to 180d	181d. and above	Total	Zero to 15d.	16 to 30d	31 to 60d	61 to 180d	181d. and above	Total	Zero to 15d.	31 to 60d	Total	Grand total
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
						(10)	(9)	(21)	(53)	(107)	(200)	(9)	(2)	(11)	(13)	(11)	(46)	(2)	(5)	(7)	(253)
1	Ag	+	+	+	+	nil	nil	1	7	2	10	nil	nil	2	2	2	6	nil	nil	nil	16
2	A	+	+	+	+	nil	nil	3	4	8	15	nil	nil	nil	nil	2	2	nil	nil	nil	17
3	Ag	+	+	-	+	nil	2	3	4	3	12	3	nil	3	nil	1	7	1	nil	1	20
4	A	+	+	-	+	3	1	2	1	7	14	1	nil	2	2	1	6	nil	1	1	21
5	A	+	+	+	-	nil	nil	nil	nil	1	1	nil	nil	nil	nil	nil	nil	nil	nil	nil	1
6	Ag	-	+	+	+	nil	nil	nil	nil	1	1	nil	nil	nil	nil	nil	nil	nil	2	2	3
7	A	-	+	+	+	nil	nil	nil	nil	6	6	nil	nil	nil	nil	nil	nil	nil	nil	nil	6
8	Ag	+	-	-	+	nil	nil	1	nil	1	2	nil	nil	nil	nil	nil	nil	nil	nil	nil	2
9	Ag	-	-	+	+	nil	nil	nil	1	2	3	nil	nil	nil	1	nil	1	nil	nil	nil	4
10	Ag	-	+	-	+	nil	nil	nil	nil	5	5	nil	nil	nil	nil	1	1	nil	1	1	7
11	A	+	-	-	+	nil	nil	2	nil	1	3	nil	nil	nil	nil	nil	nil	nil	nil	nil	3
12	A	-	+	-	+	1	nil	nil	1	9	11	nil	nil	nil	nil	1	1	nil	1	1	13
13	A	-	-	+	+	nil	nil	nil	4	1	5	nil	nil	nil	nil	nil	nil	nil	nil	nil	5
14	A	+	+	-	-	nil	nil	nil	1	nil	1	nil	nil	nil	nil	nil	nil	nil	nil	nil	1
15	A	-	+	+	-	nil	nil	nil	nil	1	1	nil	nil	nil	nil	nil	nil	nil	nil	nil	1
16	Ag	+	-	-	-	1	nil	nil	nil	nil	1	nil	nil	nil	nil	nil	nil	nil	nil	nil	1
17	Ag	-	-	-	+	nil	nil	nil	nil	5	5	nil	1	nil	nil	nil	1	nil	nil	nil	6
18	Ag	-	+	-	-	nil	nil	nil	nil	1	1	nil	nil	nil	nil	nil	nil	nil	nil	nil	1

TABLE-III (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
19	A	-	-	-	+	nil	1	1	5	2	9	4	nil	1	2	nil	7	nil	nil	nil	16
20	A	+	-	-	-	nil	nil	1	1	nil	2	nil	nil	nil	nil	nil	nil	nil	nil	nil	2
21	A	-	-	+	-	nil	nil	nil	nil	4	4	nil	nil	nil	nil	nil	nil	nil	nil	nil	4
22	A	-	+	-	-	nil	nil	nil	nil	2	2	nil	1	nil	nil	nil	1	nil	nil	nil	3
23	Ag	-	-	-	-	nil	nil	1	1	2	4	nil	nil	nil	nil	nil	nil	nil	nil	nil	4
24	A	-	-	-	-	nil	nil	1	9	3	13	nil	1	nil	1	2	4	nil	nil	nil	17
	Total :-					5	4	16	39	67	131	8	3	8	8	10	37	1	5	6	174

d = days; + = positive; - = negative.

semi-confluent lysis were given + to +++ according to the proportion of lytic area and bacterial growth on the spot where the phages were deposited. If the lytic area covered more space than the bacterial growth, the sign +++ was given and in reverse case +. The sign ++ was given when the lytic area and bacterial growth were equally represented in the specified area.

Figures 1 and 2 indicate the action of T_4 phage on the E.coli strains number 21 and 22 respectively, producing confluent lysis (clear plaque) on the spot where the phage was deposited and the sign ++++ has been awarded in both the cases. Figure 3 indicates the action of T_2, T_4 and T_6 phages on E.coli strain number 3. T_4 has produced confluent lysis (++++)& T_2 has produced semi-confluent lysis and the lytic area is more than the bacterial growth and the sign +++ has been awarded. T_6 has produced semi-confluent lysis, the clear and lytic areas were equally represented and the sign ++ has been awarded, but unfortunately this semi-confluent lytic area is not very clear in the photograph. Figure 4 indicates the action of all the 7 phages. T_2 has produced confluent lysis (++++), T_4, semi-confluent lysis (++) and the rest of the five phages have produced semi-confluent lysis of low grade where the bacterial growth is more than the lytic area and the sign + has been given to each. Figure 5 indicates the semi-confluent lysis of various grades, T_4 (+++), T_2 (++) and T_3 & T_5 (+) on E.coli strain number 12. Figure 6 indicates the semi-confluent lysis of two

Fig- 1

Fig- 2.

Fig.no.1

Showing the action of T_4 phage on *E.coli* strain no.21 producing confluent lysis (clear plaque on the spot where the phage was deposited and the sign ++++ has been given.

Fig.no.2

Showing the action of T_4 phage on *E.coli* strain no.22 producing confluent lysis and the sign ++++ has been given.

Fig.no.3

Showing the action of T_2 , T_4 & T_6 phages on E.coli strain no.3. T_4 produced confluent lysis (++++), T_2 produced semi-confluent lysis (+++) & T_6 produced semi-confluent lysis (++).

Fig.no.4

Showing the action of all the seven phages on E.coli strain no.5. T_2 produced confluent lysis (++++), T_4 produced semi-confluent lysis (++) & the rest 5 phages produced semi-confluent lysis (+).

Fig - 3

Fig - 4

Fig.no.5

Showing the action of T_2 , T_3 , T_4 & T_5 on E.coli strain no.12. T_4 produced semi-confluent lysis (+++), T_2 Produced semi-confluent lysis (++) & T_3 and T_5 produced semi-confluent lysis (+).

Fig.no.6

Showing the action of T_1 , T_2 , T_3 , T_4 , T_5 & T_7 on E.coli strain no.44. T_2 , T_3 , T_4 & T_5 produced semi-confluent lysis (+++) and T_1 & T_7 produced semi-confluent lysis (++).

Fig – 6

Fig – 5

grades on E.coli strain number 44, +++ with T_2 , T_3 , T_4 and T_5 and ++ with T_1 & T_7 .

Out of 174 E.coli strains, 134 could be typed with the T-series of phages and 40 were untypable. The E.coli strains behaved differently with regard to the susceptibility to bacteriophage. Among 134 typable E.coli strains, 32 phage types have been found. The various phage types of E.coli, their number and their correlation with age groups in healthy, diarrhoeal and dead calves have been given in Table IV. The majority of E.coli belonged to the phage types, 1, 20, 25 and 31. Altogether 28,10 & 5 phage types have been found in healthy, diarrhoeal and dead calves respectively. The phage types,which were not found in the healthy calves, were 2, 9, 10 and 16. All the 10 phage types found in diarrhoeal calves, were also found in healthy calves except the phage types 2, 9 and 10, which were exclusively confined to the diarrhoeal calves. The E.coli from dead calves belonged to the phage types 1,16,19,20 and 25. All the phage types found in dead calves were also found in healthy calves except type 16, and diarrhoeal calves except types 16 and 19. The phage type 16 was found in one dead calf or

Serological typing:

All the 174 E.coli strains were tested with nine available standard antisera to determine the serotypes. The results of serological typing have been given in Table I. 59 E.coli strains could be typed with the nine standard antisera and the rest 115 strains could not be typed serologically, they

TABLE - IV

Table showing the various phage types of E.coli prevalent in the calves examined, and their correlation with age in healthy, diarrhoeal and dead calves.

Phage type	Susceptibility to bacteriophage							Number of calves examined												
								Healthy calves						Diarrhoeal calves						Dead calves			Grand total
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	Zero to 15d.	16 to 30d.	31 to 60d.	61 to 180d	181d. and above	Total	Zero to 15d.	16 to 30d.	31 to 60d.	61 to 180d	181d. and above	Total	Zero to 15d.	31 to 60d.	Total	
								(10)	(9)	(21)	(53)	(107)	(200)	(9)	(2)	(11)	(13)	(11)	(46)	(2)	(5)	(7)	(253)
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	+	+	+	+	+	+	+	nil	1	nil	7	4	12	1	nil	2	1	1	5	1	nil	1	18
2	+	+	+	+	+	+	-	nil	nil	nil	nil	nil	nil	nil	nil	1	nil	nil	1	nil	nil	nil	1
3	+	+	+	+	+	-	+	nil	nil	nil	1	nil	1	nil	nil	nil	nil	nil	nil	nil	nil	nil	1
4	+	+	+	+	-	+	+	nil	nil	nil	nil	1	1	nil	nil	nil	nil	nil	nil	nil	nil	nil	1
5	-	+	+	+	+	+	+	nil	nil	nil	nil	1	1	nil	nil	nil	nil	1	1	nil	nil	nil	2
6	+	+	+	+	-	+	-	nil	nil	nil	nil	5	5	nil	nil	nil	nil	nil	nil	nil	nil	nil	5
7	+	+	+	+	-	-	+	nil	nil	nil	nil	2	2	nil	nil	nil	nil	nil	nil	nil	nil	nil	2
8	+	-	+	+	-	+	+	nil	nil	1	nil	nil	1	nil	nil	nil	nil	nil	nil	nil	nil	nil	1
9	-	+	+	+	+	+	-	nil	nil	nil	nil	nil	nil	nil	nil	1	1	nil	2	nil	nil	nil	2
10	-	+	+	-	+	+	+	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	1	1	nil	nil	nil	1
11	+	+	-	+	-	-	+	nil	nil	nil	nil	1	1	nil	nil	nil	nil	nil	nil	nil	nil	nil	1
12	+	+	-	+	-	+	-	nil	nil	nil	nil	1	1	nil	nil	nil	nil	nil	nil	nil	nil	nil	1
13	-	+	+	+	+	-	-	nil	nil	nil	1	1	2	nil	nil	nil	nil	nil	nil	nil	nil	nil	2
14	-	+	-	+	+	+	-	nil	nil	nil	nil	3	3	nil	nil	nil	1	nil	1	nil	nil	nil	4
15	-	+	-	-	+	+	+	nil	nil	nil	nil	1	1	nil	nil	nil	nil	nil	nil	nil	nil	nil	1
16	-	+	+	+	-	+	-	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	1	1	1	1
17	-	-	+	+	-	+	+	nil	nil	nil	2	nil	2	nil	nil	nil	nil	nil	nil	nil	nil	nil	2
18	-	-	+	+	+	+	-	nil	nil	1	nil	nil	1	nil	nil	nil	nil	nil	nil	nil	nil	nil	1
19	-	+	+	+	-	-	-	nil	nil	nil	nil	1	1	nil	nil	nil	nil	nil	nil	1	1	2	2

TABLE- IV (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
20	-	+	-	+	-	+	-	nil	nil	nil	2	5	7	nil	1	nil	nil	nil	1	nil	1	1	9
21	-	+	-	+	-	-	+	nil	nil	nil	nil	3	3	nil	nil	nil	nil	nil	nil	nil	nil	nil	3
22	-	+	-	-	-	+	+	nil	nil	nil	nil	1	1	nil	nil	nil	nil	nil	nil	nil	nil	nil	1
23	-	-	+	-	+	+	-	nil	nil	nil	nil	1	1	nil	nil	nil	nil	nil	nil	nil	nil	nil	1
24	-	-	-	+	-	+	+	nil	nil	nil	nil	1	1	nil	nil	nil	nil	nil	nil	nil	nil	nil	1
25	-	+	-	+	-	-	-	nil	nil	1	nil	8	9	1	1	nil	nil	1	3	nil	1	1	13
26	-	+	-	-	-	+	-	nil	nil	nil	nil	1	1	nil	nil	nil	nil	nil	nil	nil	nil	nil	1
27	-	-	+	-	-	-	+	nil	nil	nil	nil	1	1	nil	nil	nil	nil	nil	nil	nil	nil	nil	1
28	-	-	-	+	-	+	-	1	nil	nil	nil	1	2	nil	nil	nil	nil	nil	nil	nil	nil	nil	2
29	-	-	-	+	-	-	+	nil	nil	nil	nil	1	1	nil	nil	nil	nil	nil	nil	nil	nil	nil	1
30	-	+	-	-	-	-	-	nil	nil	1	nil	2	3	nil	nil	nil	nil	1	1	nil	nil	nil	4
31	-	-	-	+	-	-	-	2	1	7	19	7	36	2	1	2	4	2	11	nil	nil	nil	47
32	-	-	-	-	-	+	-	nil	nil	nil	nil	1	1	nil	nil	nil	nil	nil	nil	nil	nil	nil	1
33	*E. coli* untypable							2	2	5	7	13	29	4	nil	2	1	3	10	nil	1	1	40
TOTAL								5	4	16	39	66	131	8	3	8	8	10	37	1	5	6	174

d = days; + = lysis (plaque); - = no lysis

were untypable with the available antisera. The serologically typable E.coli strains belonged to the serotypes: O26:B6 (8); O55:B5 (10); O86:B7 (13); O111:B4 (3); O119:B14 (13) and O126:B16 (12). All the six serotypes were found in healthy calves as well as calves showing enteric disorders. Only two serotypes: O55:B5 and O86:B7 were found, one each, in dead calves. Various serotypes of E.coli, their number and their correlation with age groups in healthy,diarrhoeal and dead calves have been presented in Table V. The majority of typable E.coli strains belonged to the serotypes: O86:B7 (13); O119:B14 (13) and O126:B16 (12). Next in order were the serotypes: O55:B5 (10) and O26:B6 (8).The serotype: O111:B4 was from three cases only.

Altogether 39 E.coli strains belonging to the serotypes: O26:B6 (5); O55:B5 (7); O86:B7 (9); O111:B4 (2); O119:B14 (10) and O126:B16 (6) were found in healthy calves. 23, 9, 5 and 2 were found in the age groups, 181 days and above, 31 to 60 days, 61 to 180 days and 16 to 30 days respectively. None of the serologically typable E.coli strains were detected in the healthy calves, age group zero to 15 days.

Eighteen E.coli strains belonging to the serotypes: O26:B6 (3); O55:B5 (2); O86:B7 (3); O111:B4 (1); O119:B14 (3) and O126:B16 (6) were found in calves showing enteric disorders. Four strains representing four different serotypes were found in the age group, zero to 15 days; one serotype: O55:B5 was found in the age group, 16 to 30 days; three strains representing three different serotypes were found in the age group, 31 to 60

TABLE - V

Table showing the various serotypes of E.coli prevalent in the calves examined, and their correlation with age in healthy, diarrhoeal and dead calves.

Serotypes of E.coli	Number of calves examined															
	Healthy calves						Diarrhoeal calves						Dead calves			Grand total
	Zero to 15d (10)	16 to 30 d. (9)	31 to 60 d. (21)	61 to 180d. (53)	180d. & above (107)	Total (200)	Zero to 15d. (9)	16 to 30d. (2)	31 to 60d. (11)	61 to 180d (13)	181d and above (11)	Total (46)	Zero to 15d. (2)	31 to 60d. (5)	Total (7)	(253)
026:B6	nil	nil	1	1	3	5	1	nil	1	nil	1	3	nil	nil	nil	8
055:B5	nil	nil	3	1	3	7	nil	1	nil	1	nil	2	1	nil	1	10
086:B7	nil	1	2	1	5	9	nil	nil	1	2	nil	3	nil	1	1	13
0111:B4	nil	nil	1	1	nil	2	1	nil	nil	nil	nil	1	nil	nil	nil	3
0119:B14	nil	1	1	1	7	10	1	nil	nil	1	1	3	nil	nil	nil	13
0126:B16	nil	nil	1	nil	5	6	1	nil	1	1	3	6	nil	nil	nil	12
Untypable	5	2	7	34	44	92	4	2	5	3	5	19	nil	4	4	115
Total:-	5	4	16	39	67	131	8	3	8	8	10	37	1	5	6	174

d = days.

days; five strains representing four different serotypes were found in the age group, 61 to 180 days and five strains representing three different serotypes were found in the age group, 181 days and above.

The serotypes: 055:B5 and 086:B7 were isolated from dead calves showing gastro-enteritis belonging to the age groups, zero to 15 days and 31 to 60 days respectively.

Each serotype of E.coli belonged to various phage types and biochemical types. The correlation of serotypes with phage types and biochemical types have been given in Table VI below.

TABLE - VI

Correlation of serotypes with phage types and biochemical types

Serotypes	Phage types	Biochemical types
1	2	3
026:B6 (8)	1(2), 20(1), 25(1), 31(1),untypable(3).	2(1), 3(2),4(1),11(1), 12(1), 14(1),& 21(1).
055:B5 (10)	1(1),11(1),25(2), 26(1),31(4), untypable(1).	3(2), 8(1), 9(1),11(1), 17(1),19(1),22(2) & 24(1).
086:B7 (13)	1(2),6(1),8(1),14(1) 19(1),20(1),22(1), 25(1),31(1), untypable (3).	1(2),2(2),3(2),9(1),10(1), 13(1),19(1),20(1),21(1) & 24(1).
0111:B4 (3)	1(1),25(1), untypable (1).	1(1), 3(1) & 4(1).

TABLE- VI (continued)

1	2	3
O119:B14 (13)	1(2),14(1),20(2), 25(4),31(2), untypable (2).	1(1), 3(3), 4(1),7(2), 10(1),12(2),19(2) and 24(1).
O126:B16(12)	1(1),2(1),10(1), 30(1),31(1), untypable (7).	2(2),3(1),4(3),12(1), 15(1),19(1) and 24(3).
Total -	1(9),2(1),6(1), 8(1),10(1),11(1), 14(2),19(1),20(4), 22(1),25(9),26(1), 30(1),31(9), untypable (17) = 14 phagetypes (42) & untypable (17).	1(4),2(5),3(11),4(6),7(2), 8(1),9(2),10(2),11(2),12(4), 13(1),14(1),15(1),17(1),19(5), 20(1),21(2),22(2),and 24(6) = 19 biochemical types (59).

N.B.- The number in parenthesis indicates the number of E.coli strain or strains in that type.

The majority of serologically typable E.coli strains belonged to the phage types 1,25 and 31. Then come phage types 20 and 14 respectively in order of frequency. The rest of the phage types were rare. The majority of serologically typable E.coli strains belonged to the biochemical types 11,4 & 24,2& 19, and 1& 12 in order of frequency. The rest of the biochemical types came once or twice.

Fig – 7

Fig – 8

Fig – 7

Fig – 8

Fig.no.7

Showing the behaviour of the sensitive E.coli strain no.72 with different concentrations of achromycin. The zone of inhibition produced were 9,10,12,14, & 15 mm.diameter with 5,10,25,50 & 100 mcgms. concentration respectively.

Fig.no.8

Showing the behaviour of the resistant E.coli strain no.81 where the zone of inhibition produced were 6.5, 7,7,8 & 9 mm.diameter with 5,10,25,50,& 100 mcgms. concentration of achromycin respectively.

Sensitivity tests:

All the 174 E.coli strains were tested with different concentrations of six antibiotics used. The results of the sensitivity tests in Vitro have been precisely given in Table VII, which indicates the action of antibiotics on individual strains separately. The diameter of the disks employed were near about 6.5 mm., and the zone of inhibition were produced around the disks. The diameter of the zone of inhibition was measured, which included the diameter of the disk in the centre. The E.coli strains behaved differently with different concentrations of six antibiotics used. Some of the concentrations inhibited the growth of the E.coli strains around the disks whereas others failed to do so.

Figure 7 indicates the behaviour of the sensitive E.coli strain number 72 with different concentrations of achromycin. The zone of inhibition produced were 9, 10, 12, 14 and 15 mm. diameter with 5, 10, 25, 50 and 100 mcgms. concentrations of achromycin respectively. Figure 8 indicates the behaviour of the resistant E.coli strain number 81 where the zone of inhibition produced were 6.5, 7, 7, 8 and 9 mm. diameter with 5, 10, 25, 50 and 100 mcgms. concentration of achromycin respectively. Figure 9 indicates the behaviour of sensitive E.coli strain number 89 with different concentrations of dihydrostreptomycin sulfate. The zone of inhibitions were 9, 10, 12, 13, & 15 mm. diameter with 5, 10, 25, 50 & 100 mcgms. concentration of dihydrostreptomycin sulfate respectively. Figure 10 indicates

TABLE - VII

Table showing the results of sensitivity tests of *E.coli* strains isolated from calves with different concentrations of six antibiotics. The zone of inhibition is given in mm.diameter.

Strain No.	Tetracycline hydrochloride (Achromycin)					Chlortetracycline hydrochloride (Aureomycin)					Demethylchlortetracycline hydrochloride (Ledermycin)					Dihydro-streptomycin sulfate					Mysteclin-V (Each capsule contained 250 mg.of tetracycline)					Penicillin G. sodium.				
	5 µg	10 µg	25 µg	50 µg	100 µg	5 µg	10 µg	25 µg	50 µg	100 µg	5 µg	10 µg	25 µg	50 µg	100 µg	5 µg	10 µg	25 µg	50 µg	100 µg	5 µg	10 µg	25 µg	50 µg	100 µg	50 ut	100 uts	200 uts	500 uts	1000 uts
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1	10	12	12	14	15	10	10	11	12	12	9	10	11	12	13	8	10	12	14	16	10	12	13	14	15	9	10	12	14	16
2	6.5	6.5	6.5	6.5	7	6.5	6.5	6.5	6.5	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	6.5	7	6.5	6.5	6.5	6.5	7	8	10	12	14	15
3	10	10	12	14	15	10	10	11	12	12	9	10	10	10	12	6.5	9	11	12	13	9	10	10	11	12	10	10	12	12	13
4	10	10	12	14	16	10	10	12	12	12	9	10	12	12	13	10	12	12	14	16	10	10	10	11	11	9	10	11	12	13
5	10	12	12	14	15	10	10	10	12	12	8	10	10	11	12	10	12	13	14	15	10	10	11	12	12	8	10	11	12	13
6	10	12	14	15	16	10	11	12	13	14	9	10	10	11	12	10	12	14	16	17	10	10	10	10	11	8	10	10	11	12
7	9	10	12	12	14	10	11	12	12	13	10	10	10	12	13	8	10	10	12	14	9	10	10	11	11	8	10	11	12	14
8	9	10	12	12	14	10	10	10	12	13	6.5	6.5	6.5	6.5	7	9	10	12	13	15	10	10	10	10	11	9	10	12	12	13
9	10	12	12	14	14	10	12	12	13	13	10	10	10	11	12	10	12	14	14	16	10	12	13	13	14	9	10	11	12	13
10	10	10	12	14	14	6.5	6.5	6.5	6.5	7	9	10	10	10	11	10	12	13	14	16	10	10	11	11	12	6.5	6.5	6.5	7	8
11	6.5	6.5	6.5	6.5	7	6.5	6.5	7	8	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	6.5	7	6.5	6.5	6.5	6.5	7	6.5	7	8	9	10
12	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	6.5	7	6.5	6.5	7	8	9
14	10	12	14	14	15	10	10	12	13	13	9	10	10	12	12	10	10	12	14	16	9	10	11	12	12	9	10	11	12	13
17	10	12	12	14	16	12	12	14	16	16	10	12	14	14	15	10	12	12	14	16	6.5	9	10	10	10	8	10	11	12	14
18	9	10	10	12	14	10	12	12	13	13	10	10	11	12	12	6.5	6.5	6.5	8	10	10	11	11	12	12	9	10	11	12	13
19	10	12	12	12	14	10	10	12	13	13	9	10	10	12	12	10	13	15	18	20	8	9	10	10	10	8	9	10	11	12
20	6.5	6.5	7	8	10	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8
21	8	10	13	15	16	10	11	12	13	14	9	10	10	11	12	10	10	13	15	18	10	11	11	12	13	9	10	11	12	13
22	9	10	12	15	17	10	10	10	12	13	10	11	12	13	14	10	12	13	15	18	10	11	12	13	15	10	10	11	12	13
23	10	10	12	12	14	10	10	11	12	13	10	10	11	12	13	10	10	12	12	14	10	10	11	11	12	8	9	10	11	12
24	10	12	14	14	14	11	11	11	12	13	10	10	11	12	12	10	12	14	16	18	10	10	10	11	12	9	10	11	12	

TABLE-VII (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
26	13	15	18	20	24	14	16	18	20	21	12	14	16	18	20	10	10	12	15	18	10	10	12	13	14	6.5	6.5	6.5	7	8
27	10	10	12	14	16	10	12	12	13	14	6.5	6.5	6.5	7	8	10	12	14	15	17	10	11	11	12	13	10	12	14	16	18
28	10	11	13	15	18	10	11	12	13	13	10	10	11	12	13	8	10	12	14	16	10	11	12	13	14	10	10	12	13	14
31	9	11	12	12	14	9	10	10	11	12	9	10	10	11	12	9	10	12	14	17	6.5	6.5	6.5	7	9	6.5	6.5	6.5	7	8
32	10	12	15	20	22	10	12	12	13	14	9	10	12	12	13	6.5	6.5	6.5	6.5	7	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8
33	12	14	16	18	19	10	10	10	11	11	9	10	10	11	11	10	12	14	16	20	9	10	10	10	11	9	10	10	11	11
34	10	12	13	14	16	10	10	11	12	12	9	10	10	11	11	8	10	12	12	14	10	10	11	12	12	10	10	12	13	13
37	6.5	7	8	9	10	6.5	6.5	7	9	10	6.5	6.5	6.5	7	9	6.5	6.5	6.5	7	8	6.5	6.5	7	8	9	6.5	7	9	10	11
40	9	12	12	14	16	10	10	12	12	14	10	10	11	12	12	10	12	14	15	18	10	11	12	14	14	10	11	12	13	13
41	6.5	6.5	6.5	7	9	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	7	6.5	6.5	6.5	7	8
42	12	12	14	15	17	12	12	13	15	15	11	12	12	13	14	12	13	15	17	20	10	12	13	13	15	10	11	12	15	18
43	10	10	12	12	14	10	11	12	13	14	10	10	11	12	12	6.5	6.5	8	10	12	9	10	10	11	11	8	9	10	10	11
44	14	16	18	18	18	12	14	15	16	18	12	13	14	15	16	6.5	6.5	10	12	14	12	12	13	15	16	6.5	6.5	7	9	10
46	10	10	12	12	13	10	12	13	14	14	10	10	11	12	13	6.5	6.5	6.5	6.5	7	10	10	12	12	12	6.5	6.5	6.5	7	8
47	10	12	12	14	16	10	10	10	11	11	10	10	10	10	11	10	10	12	12	14	9	10	10	11	12	9	10	11	12	13
48	10	12	13	14	15	10	10	11	12	12	10	10	10	11	11	10	12	13	14	16	9	10	11	12	12	10	12	13	13	14
50	10	12	14	15	16	10	10	11	12	13	10	10	11	12	13	10	13	14	15	18	10	10	12	12	13	10	10	12	13	14
53	6.5	6.5	10	12	13	10	10	11	12	13	10	10	11	12	12	10	12	14	16	18	6.5	6.5	6.5	7	8	8	10	10	12	13
54	8	10	10	12	13	10	10	11	12	12	9	10	10	11	12	10	12	13	14	15	9	10	10	10	10	9	10	12	12	13
55	7	10	12	12	14	10	10	12	13	13	10	10	11	12	12	10	12	14	15	15	6.5	6.5	7	7	8	10	10	12	13	14
57	10	12	14	16	18	12	13	13	14	15	9	11	12	13	14	10	12	14	16	18	10	11	12	14	15	10	10	12	13	13
58	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	9	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8
59	9	10	12	14	14	10	10	11	12	13	10	10	11	12	12	10	12	13	14	15	9	10	10	11	12	8	9	9	10	10
62	10	12	14	14	16	10	10	12	14	14	9	10	11	12	13	10	12	12	14	16	9	10	11	12	12	7	9	12	13	14

TABLE- VII(continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
63	10	12	14	15	15	10	12	13	15	16	10	10	12	14	15	10	12	14	16	18	10	11	11	12	12	10	12	13	14	15
65	12	12	12	14	16	10	10	12	14	14	9	10	11	12	13	10	12	14	15	16	10	10	11	12	12	8	9	10	11	12
66	8	10	12	12	15	10	10	11	12	13	9	10	11	11	12	8	10	12	14	14	10	11	12	13	13	9	9	10	12	13
67	10	12	14	14	16	10	11	12	13	13	9	10	10	11	11	6.5	6.5	6.5	7	7	10	11	11	12	12	9	10	11	12	13
68	8	10	12	14	16	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	7	6.5	6.5	6.5	7	8	8	9	10	11	12
69	6.5	6.5	6.5	6.5	7	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	6.5	7	6.5	6.5	6.5	7	8	6.5	6.5	7	8	9
70	10	12	13	15	16	10	10	11	12	12	9	10	12	12	12	6.5	6.5	6.5	7	8	9	10	10	11	11	8	9	10	11	12
72	9	10	12	14	15	9	10	11	12	13	8	10	10	10	11	8	10	12	14	16	8	9	10	10	11	8	9	10	10	11
73	10	12	14	14	15	10	10	11	12	12	9	10	11	12	13	10	12	14	16	18	10	11	11	12	12	8	9	10	11	12
74	6.5	6.5	6.5	7	8	6.5	6.5	6.5	6.5	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	7	6.5	6.5	6.5	7	8	6.5	6.5	8	9	10
75	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	6.5	7	6.5	6.5	6.5	7	8	6.5	6.5	6.5	8	9
76	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	6.5	7	6.5	6.5	6.5	7	8	6.5	6.5	6.5	6.5	7	6.5	6.5	6.5	7	8
77	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	6.5	7	6.5	6.5	6.5	7	8
78	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8
79	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8
80	8	9	10	11	11	10	10	10	11	12	10	10	10	11	12	8	10	10	11	12	6.5	7	7	8	9	8	9	10	11	12
81	6.5	7	7	8	9	6.5	7	8	9	10	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	7	8	9
83	6.5	6.5	7	8	9	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	7	8	9
84	6.5	6.5	7	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	8	9	10
86	10	12	14	14	16	10	11	12	13	13	9	10	11	12	12	9	10	10	12	14	10	10	10	11	11	8	9	10	11	12
87	10	10	12	14	16	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	9	10	10	10	11	8	9	10	11	12
89	9	10	12	13	15	10	12	13	14	15	10	10	12	13	13	9	10	12	14	15	10	10	11	12	13	8	9	12	13	14
90	10	12	14	14	16	10	10	11	12	13	10	10	10	11	12	10	10	12	13	15	9	10	10	11	11	7	9	10	12	13
92	6.5	6.5	6.5	7	8	10	10	11	12	13	9	10	10	12	12	6.5	6.5	6.5	7	8	7	8	9	10	10	7	8	9	10	12

TABLE- VII (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
93	6.5	6.5	6.5	8	9	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8
94	10	12	13	15	17	10	10	11	12	13	10	10	11	12	12	6.5	6.5	6.5	7	8	9	10	10	11	11	9	10	12	13	14
96	10	12	14	15	18	10	12	13	14	15	10	11	12	14	14	10	12	14	16	18	6.5	7	8	8	9	6.5	7	8	9	10
97	9	10	12	14	16	10	12	13	14	14	10	11	13	14	14	10	12	12	14	18	6.5	6.5	7	8	9	6.5	6.5	6.5	8	9
99	12	14	16	18	20	10	13	14	16	16	10	12	13	15	16	10	13	16	16	18	10	12	12	14	14	10	10	12	14	15
102	9	10	11	12	15	10	12	13	14	14	9	10	12	13	14	10	12	14	15	15	9	10	11	12	12	8	10	11	12	13
103	9	10	12	12	13	12	12	15	18	18	10	12	14	14	15	8	10	10	12	12	6.5	7	8	9	9	6.5	6.5	6.5	7	
104	6.5	7	8	9	10	11	12	13	15	17	10	11	12	13	13	7	9	10	12	13	6.5	6.5	7	8	9	6.5	6.5	8	9	1
105	9	10	12	13	14	10	10	11	12	12	9	10	10	11	12	10	12	13	14	14	8	9	10	10	10	7	8	9	10	
107	12	15	18	20	22	12	12	13	15	15	10	10	12	14	14	12	14	15	18	20	10	10	11	13	14	7	9	10	10	
108	10	10	12	15	16	11	12	12	14	15	11	12	12	13	14	10	12	15	16	18	10	10	11	12	12	9	10	12	14	
109	9	10	11	12	14	10	10	11	12	12	10	10	12	12	12	8	9	10	11	11	10	11	12	13	13	6.5	8	9	10	
113	10	10	12	15	16	10	11	12	13	14	10	11	12	13	13	10	10	12	14	16	9	9	10	10	11	8	10	10	12	
114	8	10	12	12	13	9	10	11	12	13	9	11	12	12	13	8	9	10	10	11	9	10	11	13	13	7	8	9	10	
115	10	12	13	14	15	11	12	12	13	14	10	11	13	13	14	10	12	14	16	18	9	10	12	13	14	8	10	12	13	
116	10	11	12	14	15	12	12	13	14	15	10	12	12	14	14	10	12	13	15	18	10	11	12	12	13	9	10	11	13	
119	9	11	12	13	14	10	10	12	13	14	9	10	12	12	13	10	11	12	15	16	8	10	10	11	12	10	13	15	15	
120	10	12	14	15	16	10	12	13	14	15	10	11	12	12	13	10	12	13	15	17	9	10	10	11	11	9	11	13	14	
121	10	12	13	14	15	10	10	11	12	12	9	10	12	12	13	12	13	15	18	20	7	9	10	10	10	10	10	12	14	
124	10	12	16	20	20	12	12	14	16	17	12	12	14	16	16	10	12	15	18	18	8	10	10	11	12	6.5	6.5	6.5	7	
126	10	10	12	14	16	10	12	12	13	13	10	11	12	12	13	10	10	13	15	16	6.5	8	10	11	12	7	8	10	12	
127	8	10	12	14	16	12	13	13	14	15	11	11	12	13	14	10	12	15	16	18	6.5	7	8	9	10	6.5	6.5	7	9	
128	10	12	14	16	18	8	10	10	11	12	10	11	12	13	13	10	12	16	18	20	10	12	13	13	14	10	12	14	16	
129	10	12	15	18	20	10	11	12	14	15	10	12	13	14	14	10	12	14	15	16	6.5	7	9	11	12	6.5	6.5	7	8	

TABLE-VII (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
131	10	12	14	16	18	11	12	13	13	14	11	11	12	13	13	10	12	12	16	20	9	10	10	11	12	9	10	12	13	15
132	8	10	12	12	14	10	10	11	12	13	6.5	6.5	6.5	7	8	10	12	14	18	20	9	10	10	11	12	8	9	10	12	13
133	10	12	12	14	16	10	10	11	12	12	9	10	10	11	12	10	12	14	16	18	9	10	11	12	12	9	10	12	13	14
135	10	10	12	13	15	10	10	12	12	13	9	10	11	12	12	10	12	14	16	19	10	11	11	12	13	8	9	10	12	12
137	10	12	14	16	16	10	11	12	13	13	10	11	12	12	13	10	12	16	20	20	10	10	11	12	13	9	10	10	12	13
139	9	10	12	12	13	10	12	12	13	14	10	12	13	13	14	8	10	12	13	15	8	9	10	11	12	9	11	12	13	15
140	9	10	11	12	14	10	11	11	12	12	10	11	11	12	13	10	14	15	18	20	9	10	10	11	12	9	10	12	14	16
141	10	11	12	14	15	10	12	13	15	15	10	12	12	13	14	10	11	13	14	15	8	10	10	11	12	8	10	12	14	15
142	12	14	16	16	18	12	12	12	13	13	10	11	12	12	13	12	14	16	18	20	10	10	11	12	13	8	9	10	12	14
143	10	11	12	12	15	9	10	10	12	12	9	10	10	12	13	11	14	16	16	18	8	9	10	10	11	7	8	9	10	12
144	8	10	12	13	14	10	11	12	12	13	10	10	11	12	13	8	10	12	12	13	8	9	10	10	11	8	10	11	12	15
147	10	12	14	16	18	10	10	12	12	14	9	10	11	12	12	10	12	16	18	20	10	10	11	12	12	10	12	13	14	16
148	10	12	14	16	18	9	10	11	11	13	9	10	10	11	12	10	13	15	18	20	10	12	12	13	14	9	12	14	15	18
149	10	10	14	15	16	9	10	12	14	15	9	10	11	12	12	10	12	16	16	18	10	10	11	12	12	10	12	13	14	16
151	10	12	14	14	16	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	12	14	15	16	18	9	10	10	11	12	6.5	6.5	6.5	7	8
152	10	10	12	12	14	10	11	12	12	13	9	10	11	12	13	10	12	14	16	18	10	11	12	12	13	9	10	10	12	13
153	10	12	13	14	15	9	10	10	12	12	10	10	10	11	12	10	14	15	16	18	10	10	12	13	13	8	9	10	12	13
154	10	10	14	14	16	11	12	12	13	14	10	11	11	12	13	10	12	14	16	18	10	10	11	12	13	9	10	12	14	16
155	10	10	12	13	15	10	11	12	14	14	10	10	11	13	13	10	12	13	16	18	10	11	11	12	12	10	12	12	14	15
156	10	11	12	14	15	10	10	11	12	13	10	10	11	12	13	10	12	13	14	16	10	10	10	11	12	9	10	12	13	13
158	10	12	15	15	16	10	10	12	13	13	9	10	11	11	12	10	12	15	15	16	10	11	12	14	15	10	11	12	14	15
161	10	10	13	15	16	10	12	13	14	14	10	11	12	13	13	6.5	6.5	7	9	10	10	12	12	13	13	10	11	12	14	14
164	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	7	6.5	6.5	6.5	7	7	6.5	6.5	7	8	10
165	10	12	12	13	13	10	10	10	11	12	9	10	10	11	11	10	12	12	12	14	10	10	11	12	12	8	9	10	12	14

TABLE- VII (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
166	12	14	15	16	18	12	12	13	14	15	11	13	14	14	15	10	12	15	18	20	10	12	12	13	14	10	12	14	17	19
167	11	12	13	13	14	12	12	13	13	13	10	11	12	12	13	10	11	12	13	15	8	10	11	12	12	8	10	12	14	15
169	10	12	12	13	14	10	11	11	12	13	9	10	11	11	11	10	12	13	16	18	10	10	11	12	13	6.5	7	9	10	12
170	10	12	15	15	15	10	11	12	13	13	10	10	11	12	13	10	10	12	15	18	9	10	10	11	12	8	10	10	12	13
172	10	12	14	15	18	10	11	13	13	13	10	10	10	12	13	10	12	13	15	18	10	10	12	13	14	9	10	12	16	17
173	10	10	12	12	14	10	11	12	12	13	10	11	11	12	12	10	11	12	12	14	9	10	11	11	11	8	9	10	12	13
175	10	11	11	12	13	10	11	11	11	12	8	9	10	10	11	8	10	12	13	14	10	11	12	13	13	9	10	12	13	14
177	8	10	10	11	12	9	10	10	11	12	9	10	11	11	11	9	10	13	14	15	9	10	10	10	11	6.5	7	8	9	10
178	10	12	14	14	14	10	10	11	12	13	10	11	11	11	11	10	12	13	13	15	10	10	11	11	12	6.5	7	8	9	12
183	10	10	10	12	13	10	11	11	11	12	9	10	11	11	12	10	10	12	13	15	10	10	10	11	12	8	9	10	11	11
184	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	7	6.5	6.5	6.5	7	8	6.5	6.5	7	8	9	6.5	6.5	6.5	7	7	6.5	6.5	7	8	9
186	10	10	12	12	14	10	10	11	11	12	10	10	11	12	12	10	12	12	14	16	10	11	12	12	13	9	10	12	14	15
188	10	12	12	12	13	10	10	11	12	13	10	11	12	13	13	10	12	12	13	15	10	11	11	12	12	10	12	14	16	18
189	10	12	12	13	14	10	11	11	11	12	10	11	11	12	12	10	12	14	15	16	9	10	11	12	13	8	10	12	14	16
191	10	11	12	13	14	10	11	12	12	12	9	10	10	11	12	10	11	12	14	16	10	10	11	12	12	10	10	10	12	13
192	10	10	11	11	11	10	11	11	11	12	10	11	11	11	11	9	10	11	12	12	10	11	12	12	12	9	10	12	14	15
195	12	13	14	15	15	12	12	14	14	15	11	12	13	13	13	12	13	13	14	15	11	12	13	13	13	10	12	14	16	16
196	10	10	12	13	15	11	12	13	14	14	11	12	12	13	13	10	12	14	14	18	9	10	10	11	12	8	9	10	12	14
198	10	11	12	13	14	10	10	11	12	12	10	10	10	11	12	10	11	14	14	16	10	10	10	10	11	9	10	10	12	14
199	10	10	12	12	13	11	12	12	13	13	11	12	12	12	12	10	10	13	14	15	10	10	11	12	12	10	11	11	12	14
201	10	10	12	12	13	10	10	10	11	11	9	10	10	11	11	10	12	13	14	18	9	10	11	12	12	8	9	10	12	14
202	10	10	12	12	14	10	10	10	11	11	10	10	11	11	11	10	13	14	15	16	10	10	11	11	12	10	12	14	16	16
203	11	12	12	13	14	10	12	12	13	14	10	10	11	12	13	12	13	13	14	15	10	10	10	11	11	8	10	12	13	15
204	11	12	13	13	14	10	10	10	11	12	10	10	11	12	13	12	12	14	14	16	6.5	6.5	7	8	9	10	12	14	16	16

TABLE -VII (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
240	8	10	12	13	15	12	12	12	13	13	9	11	11	12	13	9	10	12	14	15	8	9	10	12	12	8	10	12	13	15
242	9	10	13	14	15	10	12	13	15	15	10	11	13	13	14	8	10	11	13	14	8	10	10	11	12	8	10	11	13	16
243	12	14	16	17	19	10	13	14	14	16	11	12	14	14	14	10	10	12	13	15	10	10	12	12	14	9	12	14	15	17
247	6.5	6.5	6.5	7	8	6.5	6.5	7	8	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	7	6.5	6.5	7	8	9
248	6.5	6.5	6.5	7	8	6.5	6.5	7	8	9	6.5	6.5	6.5	8	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	7	8	9
249	10	10	12	13	15	10	10	11	12	13	10	10	10	11	12	10	12	13	15	17	8	10	10	11	12	9	10	12	13	14
251	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	9	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	7	6.5	6.5	8	9	10
252	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	6.5	7	6.5	6.5	7	9	10
253	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	9	6.5	6.5	6.5	7	8	6.5	6.5	6.5	7	7	6.5	6.5	7	9	10

ut or uts = units.

the zone of inhibition produced by the different concentrations of achromycin and dihydro-streptomycin sulfate on E.coli strain number 121. The zone of inhibitions were 10,12, 13, 14 & 15 mm. diameter with 5, 10, 25, 50 & 100 megms. concentration of achromycin respectively, whereas the zone of inhibitions were 12, 13, 15, 18 & 20 mm. diameter with 5, 10, 25, 50 & 100 megms. concentration of dihydrostreptomycin sulfate respectively.

The zone of inhibitions produced by the six antibiotics in different concentrations and the number of E.coli strains found in that zone have been given in Table VIII. This table has been deduced from Table VII and indicates the maximum, minimum and intermediate zone of inhibition produced by the antibiotics and the number of E.coli strains falling in that zone. From Table VIII it appears that the achromycin had no effect in 10 megms. concentration on 31 E.coli strains and in 25 megms. concentration on 27 E.coli strains. The E.coli strains which resisted the action of achromycin up to 25 megms. concentration were regarded as the resistant strains and as such the resistant strains against achromycin were 27 (15.51%). Similarly the resistant strains against aureomycin, ledermycin, dihydrostreptomycin sulfate and mysteclin-V were found to be 28(16.09%), 37 (21.26%), 39 (22.41%) and 35(20.11%) respectively. Penicillin G sodium in 100 units concentration had no effect on 41 (23.56%) E.coli strains and they were regarded as resistant strains.

The maximum number of resistant strains were found in

the zone of inhibition produced by the different concentrations of achromycin and dihydro-streptomycin sulfate on E.coli strain number 121. The zone of inhibitions were 10,12, 13, 14 & 15 mm. diameter with 5, 10, 25, 50 & 100 mcgms. concentration of achromycin respectively, whereas the zone of inhibitions were 12, 13, 15, 18 & 20 mm. diameter with 5, 10, 25, 50 & 100 mcgms. concentration of dihydrostreptomycin sulfate respectively.

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The maximum number of resistant strains were found in

TABLE - VIII

Table showing the zone of inhibition produced by six antibiotics in different concentrations in 174 E.coli strains, isolated from calves.

Antibiotics	Concentration in /ug	Zone of inhibition produced (in mm. diameter) in E.coli strains																		
		6.5	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Tetracycline hydrochloride (Achromycin)	5	34	1	12	20	86	6	13	1	1	-	-	-	-	-	-	-	-	-	-
	10	31	3	-	2	58	10	58	3	6	2	1	-	-	-	-	-	-	-	-
	25	27	4	2	-	6	8	67	17	28	7	5	-	3	-	-	-	-	-	-
	50	3	24	4	2	-	3	30	22	45	22	10	1	4	-	4	-	-	-	-
	100	-	3	23	4	3	2	2	13	34	31	34	3	14	2	3	-	2	-	1
Chlortetracycline hydrochloride (Aureomycin)	5	35	-	1	9	102	9	17	-	1	-	-	-	-	-	-	-	-	-	-
	10	34	1	-	-	62	30	40	5	1	-	1	-	-	-	-	-	-	-	-
	25	28	6	1	-	17	43	44	26	6	2	-	-	1	-	-	-	-	-	-
	50	3	25	5	2	-	18	52	31	23	9	4	-	1	-	1	-	-	-	-
	100	-	2	25	6	2	4	35	47	25	19	4	2	2	-	-	1	-	-	-

TABLE-VIII (Continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Demethyl chlortetracycline hydrochloride (Ledermycin)	5	42	-	6	36	76	11	3	-	-	-	-	-	-	-	-	-	-	-	-
	10	37	-	-	1	82	33	18	2	1	-	-	-	-	-	-	-	-	-	-
	25	37	-	-	-	35	46	40	9	6	-	1	-	-	-	-	-	-	-	-
	50	2	33	2	-	6	32	60	22	13	2	1	-	1	-	-	-	-	-	-
	100	-	3	31	3	1	15	46	44	23	4	3	-	-	-	1	-	-	-	-
Dihydro-streptomycin sulfate.	5	44	2	18	10	89	3	8	-	-	-	-	-	-	-	-	-	-	-	-
	10	43	-	-	5	37	8	63	12	6	-	-	-	-	-	-	-	-	-	-
	25	39	2	1	-	9	5	38	26	30	16	7	-	-	-	-	-	-	-	-
	50	6	32	2	1	2	3	14	16	34	23	27	2	11	-	1	-	-	-	-
	100	-	11	27	1	2	2	5	4	15	25	27	6	33	2	14	-	-	-	-
Mysteclin-V	5	47	2	17	36	70	1	1	-	-	-	-	-	-	-	-	-	-	-	-
	10	40	5	2	12	81	24	10	-	-	-	-	-	-	-	-	-	-	-	-
	25	35	6	3	2	48	44	30	6	-	-	-	-	-	-	-	-	-	-	-
	50	7	30	6	2	16	38	45	20	8	2	-	-	-	-	-	-	-	-	-
	100	-	17	17	9	7	21	55	26	13	7	2	-	-	-	-	-	-	-	-
Penicillin G sodium	50uts	48	8	47	38	33	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	100 "	41	6	6	28	64	11	17	1	-	-	-	-	-	-	-	-	-	-	-
	200 "	19	17	9	8	34	19	48	8	10	2	-	-	-	-	-	-	-	-	-
	500 "	-	16	14	15	11	12	39	27	22	7	9	1	1	-	-	-	-	-	-
	1000 "	-	-	16	12	18	8	15	33	25	17	13	6	9	1	1	-	-	-	-

uts = units.

penicillin and the minimum in achromycin. Dihydrostreptomycin came next to penicillin, then ledermycin, mysteclin-V and aureomycin in that order. The percentage of resistant E.coli strains found, were quite considerable. 24 strains were resistant to all the six antibiotics; one to aureomycin, ledermycin, dihydrostreptomycin and mysteclin-V; one to aureomycin, ledermycin and dihydrostreptomycin; one to aureomycin, ledermycin and penicillin; one to dihydrostreptomycin, mysteclin-V and penicillin; two to dihydrostreptomycin and penicillin; and one to achromycin and dihydrostreptomycin. One strain was resistant to aureomycin; two to achromycin; nine to dihydrostreptomycin; ten to ledermycin; nine to mysteclin-V; and 13 to penicillin.

The correlation of serotypes with sensitivity tests have been given in Table IX below.

TABLE - IX

Correlation of serotypes with sensitivity tests

Serotypes	Results of sensitivity tests
026:B6 (8)	(1) Two strains were resistant to all the six antibiotics.
	(2) One strain was resistant to mysteclin-V.
	(3) Five strains were sensitive to all the six antibiotics.
055:B5 (10)	(1) Four strains were resistant to all the six antibiotics.
	(2) One was resistant to ledermycin.

TABLE-IX(continued)

Serotypes	Results of sensitivity tests
	(3) One was resistant to dihydrostreptomycin
	(4) Four strains were sensitive to all the six antibiotics.
O86:B7 (13)	(1) Four strains were resistant to all the six antibiotics.
	(2) One strain was resistant to achromycin and mysteclin-V.
	(3) One was resistant to ledermycin.
	(4) Seven strains were sensitive to all the six antibiotics.
O111:B4 (3)	(1) One strain was resistant to all the six antibiotics.
	(2) Two strains were sensitive to all the six antibiotics.
O119:B14 (13)	(1) Two strains were resistant to all the six antibiotics.
	(2) One was resistant to aureomycin, ledermycin and penicillin.
	(3) One was resistant to dihydrostreptomycin.
	(4) Nine were sensitive to all the six antibiotics.
O126:B16 (12)	(1) One strain was resistant to all the six antibiotics.

TABLE-IX (continued)

Serotypes	Results of sensitivity tests.
	(2) One was resistant to dihydrostreptomycin.
	(3) One was resistant to penicillin.
	(4) Nine were sensitive to all the six antibiotics.

N.B.- The number in the parenthesis indicates the number of E.coli strains in that type.

Two serotypes: 055:B5 and 086:B7 were resistant to all the six antibiotics used. These two serotypes were isolated from two dead calves.

D I S C U S S I O N AND C O N C L U S I O N S

(E.COLI)

D I S C U S S I O N AND C O N C L U S I O N S

General:

In the present investigation the materials were collected from March,1965 to July,1965 from the calves of Government Cattle Farm, Patna. Altogether 246 living calves and 7 dead calves were examined for the prevalence of Escherichia coli. Out of 246 living calves, 46 calves were having diarrhoea of varying intensity. Out of 7 dead calves, 3 were having gastro-enteritis and rest were having enteritis only. The clinical conditions along with age and sex of 174 calves which revealed the presence of E.coli have been given in Table I. The E.coli from 131, 37 and 6 healthy,diarrhoeal and dead calves respectively were isolated and subjected to biochemical tests, phage typing, serological typing and sensitivity tests in Vitro with 6 antibiotics.

E.coli is widely distributed among man, varieties of animals and birds. It is the predominant organism in the intestinal canal of man and animals. The organisms gain enterance to the intestinal canal shortly after birth and persist throughout the life. Most of them are intestinal parasites. Investigations carried out, so far, revealed that every animal species is found to harbour these organisms in their bodies especially in their intestinal tract . The serotypes isolated in the present investigation are potential pathogens for man and animals.

Calf mortality:

Information regarding the death of calves, from one week to one year old or slightly more, at Government Cattle Farm,Patna, was collected from 1960 to 28th September,1965. The mortality was 26.06%, 27.4%, 33.33%, 15.19%,12.37% and 17.8% in 1960, 1961, 1962, 1963, 1964 and 1965 respectively. It was also found that the mortality rate varies from one season to another. Out of a total of 197 deaths during the periods of nearly six years, 32 (16.24%) occurred in January, 24 (12.18%) in February, 21 (10.66%) in March, 25 (12.69%) in April, 12 (6.09%) in May, 10 (5.07%) in June & July, 16 (8.12%) in August, 8 (4.06%) in September, 4 (2.03%) in October,10(5.07%) in November and 25 (12.69%) in December. The mortality was higher in January,April and December than the rest of the months.

Lovell and Hill (1940), descussing the mortality incidence in Great Britain have concluded that "factors inimical to calf's life have a greater influence in the first half of the year, chiefly it seems in the first quarter, and that their effect is more pronounced in Scotland than in England and Walse". Withers (1952) in a statistical study of calf mortality in relation to management and environmental factors has suggested that the seasonal scouring mortality during the early months of the year, is related to wide temperature variations and absence of sun shine common at this time in Great Britain. Earlier, Jordon (1933) attributed this higher susceptibility of spring born

calves to infectious diseases, in Scotland, to vitamin A deficiency in colostrum milk of winter fed cows. Hansen et al (1946) obtained evidence which suggested that factors other than vitamins present in "summer skimmed milk" influenced the survival of young calves. McCandlish (1939) considers that these seasonal changes may be associated with the character of milk curd formed in the stomach of the calf and that abnormal curd formation leads to digestive disturbances and even death as observed by Sheehy (1939). Blaxter et al (1951) found that deliberate over loading of the abomasum by suddenly giving the whole daily allowance of food in one feed induced scours.

In India, Minnett (1946) in a statistical study of calf mortality, concluded that the extreme climatic conditions, the grouping together of young calves and their nutrition played a part in the comparatively high mortality observed during the year 1938-44. Dhanda et al (1957) found 15.46% and 6.9% mortality in dairy herd and in the livestock & breeding herds respectively in calves up to two months of age during 1950-53. The mortality was comparatively high in the third quarter of the year. They suggested that the dampness due to heavy rainfall, in that season, unhygienic conditions and grouping together of young calves might be having some influence on the heavy mortality in that season.

In the absence of any correct and authentic recorded information about the feeding management and environmental factors in India, particularly in Bihar, it is difficult to assign any reason for the observed rise in the mortality rate

in the winter and spring seasons (December to April) in calves. Keeping of calves together in calf-pens, close congregation of calves, especially during winter, nutrition, seasonal variation in resistance to infection and lack of personal attention are some of the factors favaourable for the spread of infection from one calf to another susceptible calf. But, in the presence of reported satisfactory managements in the farm, it is extremely difficult to assign the reason or reasons to the heavy mortality. It appears that a number of factors are involved in the calf mortality which need close observation to elucidate the possible factors.

Biochemical characters:

All the 174 E.coli strains, of the present observation, were positive for indole, M.R. and nitrate and negative for V.P., citrate and adonitol. They gave the IMViC reactions ++-- . All fermented glucose, lactose, arabinose and mannitol promptly within 24 hours except one strain which fermented arabinose in four days. The fermentations of sucrose, raffinose, dulcitol, sorbitol and production of gas from glucose were variable. 64 strains produced gas from glucose and 110 strains were anaerogenic. 84 strains fermented sucrose in 1 to 10 days, 110 raffinose in 1 to 4 days, 57 dulcitol in 1 to 10 days and 139 sorbitol in 1 to 5 days time. Altogether 24 biochemical patterns of sugar fermentations, representing 24 biochemical types have been detected in 174 E.coli strains. The biochemical types and their correlation with age groups in healthy,diarrhoeal and dead calves

have been shown in Table III. All the 24 biochemical types of E.coli were found in healthy calves and only 10 & 5 biochemical types were found in diarrhoeal and dead calves respectively. All the biochemical types found in diarrhoeal and dead calves were also found in healthy calves.

In the present study the IMViC reactions were ++-- which corresponded with the IMViC reactions reported by Charter and Taylor (1952), Ewing et al (1955), Wilson and Miles (1955), Breed et al (1957) and Rees (1960). The anaerogenic type of E.coli has also been reported by Charter and Taylor (1952) and Ewing et al(1955) in the E.coli type canioni and E.coli serotypes O111. In the present work, the aerogenic as well as anaerogenic types of E.coli have been found in the serotypes: O26:B6; O55:B5; O86:B7; O111:B4; O119:B14 and O126:B16 as well as in serologically untypable E.coli strains. Sucrose positive E.coli strains have been reported by Charter and Taylor(1952), and Ewing et al (1955) in the serotype: O111, Rees (1960) in the serotype: O128:B12, Mansson (1962) in O138 and also by Shirlaw (1960) in E.coli strains. In the present investigation the sucrose positive & sucrose negative, raffinose positive & negative, dulcitol positive & negative and sorbitol positive & negative strains were found in all the above mentioned serotypes as well as serologically untypable strains. Raffinose and dulcitol positive strains have been described by Wilson and Miles (1955) and Breed et al (1957) . Rees (1960) described dulcitol negative strain in the serotype O128:B12, but the type strain fermented dulcitol. Sorbitol positive strain has been

described by Ewing et al (1955) in the serotype O111 and Rees (1960) in O128:B12.

In the present investigation serologically identical E.coli strains gave different biochemical pattern of reactions and serologically unrelated strains gave identical biochemical reactions. The correlation of serotypes with biochemical pattern (types) have already been given in Table VI. Charter and Taylor (1952) found that the serologically identical strains gave different fermentation reactions. E.coli type canioni, from three different sources, gave three fermentation pattern. In addition a serologically unrelated E.coli may give reaction similar to one of the above pattern.

Fey (1957) found twenty O-groups and 49 biochemical types in 105 E.coli strains isolated from coli-septicaemia in calves. Sinha (1965) carried out the biochemical tests of 61 E.coli strains, isolated from infantile gastro-enteritis. The E.coli belonged to 9 different serotypes. He found that the serologically related strains often gave different fermentation reactions.

Bacteriophage typing:

All the 174 E.coli strains were tested for susceptibility with T-series of phages. Results of bacteriophage typing have been given precisely in Table I. Altogether 32 phage types have been found in the calves. Out of 32 phage types, 28,10 and 5 were found in healthy, diarrhoeal and dead calves, respectively. Various phage types of E.coli, their number and their correlation with age groups in healthy,diarrhoeal and dead calves have been shown in Table IV. All the 10 phage types

found in diarrhoeal calves were also found in healthy calves except the phage types 2, 9 & 10 which were only found in diarrhoeal calves and not in healthy or dead calves. All the five phage types observed in dead calves were also found in healthy calves except the phage type 16 and in diarrhoeal calves except the phage types 16 & 19. The phage type 16 was only found once in a dead calf.

The fact that many more phage types of E.coli were identified in the present survey by means of phage typing is not surprising. Nicolle et al (1952), working with strains of E.coli of human origin found that several different phage types could be found amongst strains of the same serological type. Nicolle et al (1954) classified E.coli strains belonging to the serotypes: O111:B4; O55:B5 and O26:B6 into different phage types. Smith and Crabb (1956) classified E.coli strains of animal origin into phage types. They used 16 phages isolated from animals and found 70 phage types of E.coli in healthy calves and 32 phage types in scouring calves. Kasatiya and Singh (1961) indicated that the E.coli strains of animal origin belonged to different phage types than the strains of human origin. Yadava (1965) found three phage types of E.coli in six typable E.coli strains, out of 34 strains isolated from calves. According to Smith and Crabb (1956) a possible explanation of this is that many of the phage types differ from each other by means of acquired phage resistance only, i.e. some strains through contact with different phages in the field have become resistant to them and as a result their susceptibility to the

test phages may have altered; such strains would then accordingly be differentiated from other-wise identical strains which had been exposed to other and different phages.

The E.coli from the healthy and diarrhoeal calves did not behave differently with regard to the phage typing. All the 10 phage types found in diarrhoeal calves were also found in healthy calves except the phage types 2, 9 & 10. This observation is not the new finding of the present investigation. Smith and Crabb (1956) found that all the 32 phage types found in scouring calves were also found in healthy calves except one phage type. Smith (1958) described that the dominant E.coli in the faeces of all the calves that developed scouring during an observation period of one year, belonged to one phage type, type 2, which was also found in the faeces of some of the healthy calves, but never in the faeces of the cows of that herd. One can wonder here what part the E.coli plays in the disease process of the scouring calves, when the same phage types are prevalent in the intestine of healthy calves. The association of E.coli with white scours has been so well established, that it does not need any further explanation.

The classification of E.coli on phage typing resulted in a complex problem. Serologically identical strains gave different phage types and serologically unrelated strains were identical in phage types. There is no correlation between serotypes and phage types, which have been shown in Table VI. Smith and Crabb (1956) found up to eight phage types from the same specimen of faeces in calves. Smith (1960) described the

complexities of phage types that occur in animals especially in calves. He further stated that the bacteriophage method of classification would only yield information on the predominant types of E.coli present in the calves.

Serological typing:

Out of 174 E.coli strains tested with nine standard antisera, 59 were typable and 115 were untypable. 39, 18 & 2 serologically typable E.coli strains were found in healthy, diarrhoeal and dead calves respectively. Various serotypes of E.coli found and their correlation with age groups in healthy, diarrhoeal and dead calves have been shown in Table V. All the 6 serotypes: O26:B6; O55:B5; O86:B7; O111:B4; O119:B14 and O126:B16 were found in healthy as well as in diarrhoeal calves and only serotypes: O55:B5 and O86:B7 have been found one each in two dead calves.

The serotypes in order of frequency in healthy calves were - O119:B14 (10); O86:B7(9); O55:B5(7); O126:B16(6);O26:B6 (5); and O111:B4(2). The serotypes in order of frequency in diarrhoeal calves were - O126:B16(6); O26:B6(3); O86:B7(3); O119:B14(3);O55:B5 (2) and O111:B4(1).

Each of the serotypes of E.coli belonged to various phage types and biochemical types. The correlation of serotypes with phage types and biochemical types have been shown in Table VI. The majority of serologically typable E.coli strains belonged to 1, 25 & 31 phage types and 11, 4 & 24 biochemical types.

The results of the present survey are quite comparable with the results obtained by others. Orskov (1951) isolated E.coli serotype: O26:B6 from sick calves. Ulbrich (1954) isolated E.coli serotype: O55:B5 from calves with white scours. Fey (1956) isolated E.coli serotype O78 with a new "B" antigen from septicaemic calves. In his opinion this serotype was the selective pathogen for calves. Geurden et al (1957) isolated E.coli serotype: O8 from a calf. Uberko (1957) isolated E.coli serotypes: O26:B6 and O111:B4 from calves. Rees (1957) reported that the E.coli strains most frequently found in natural cases of white scours in Great Britain were - O9:A?; O55:L?; O78:B? and O137:L79. He also reported the isolation of O86:B7 and O103:B? from naturally occurring cases of white scours in calves.

Fey (1957) examined E.coli isolated from 105 calves suffering from coli-septicaemia and found twenty O-groups. The predominant O-groups were - O78; O113; O15 and O117, comprising 57% of the strains and 29% were O78 only. In another paper he reported that O78:B80 represented 37.5%, out of 145 strains studied. Gould (1958) isolated O26 from the cases of white scours. Rees (1958) reported the E.coli from the coli-bacillosis in calves. They belonged to the serotypes: O9:K?; O9:A; O15:K?; O35:K?; O78:K80; O86:B7; O103:B?; O117:K? and O137:K79(L). Glantz et al (1959) found that the 152 E.coli strains isolated from scouring calves belonged to 16 different O-groups. Strains belonging to the serotypes: O26; O8; O119 and O3 were pathogenic. The serotypes:O119 and O8 were isolated more often from calves with spontaneous scours than the other

serotypes. Kaechenbeek et al (1960) found the serotypes: 078; 055; 086 and 015 in calves. The first two serotypes were commonest. Smith (1960) reported the work of N.I.R.D. and R.V.C. London, team. They found 16 different serotypes involved in the experimental calves. Only one type was isolated from each calf. Most of the serotypes were also found in the faeces of healthy calves in the pens.

Rees (1960) reported that the serotypes: 026:B6; 055:B5; 0114:B?; 086:B7; 0103:B? and 0128:B12 which were often isolated from outbreaks of epidemic infantile gastro-enteritis, were also occasionally been isolated from calves suffering from gastro-enteritis (white scours). Dam (1960) found that more than 40% E.coli, out of 431 cases of coli-septicaemia in calves in Denmark, belonged to the serotype: 078. Linzenmeier (1962) found that out of 30 E.coli strains, 21 belonged to the serotype: 078. Ulendeev (1963) typed 243 E.coli isolated from 60 healthy and 181 sick calves suffering from acute gastro-enteritis. He found the serotypes: 09; 0119; 0115; 0117 and 078 in decreasing frequency. Dam (1963) found that the serotypes: 078; 0115 and 015 were frequently isolated from cases of white scours in Denmark. Akhmedova et al (1963) found that 73% of calf strains and 74% of lamb strains, out of 661 strains, belonged to the serotypes: 086; 055 and 026. Yadava (1965) in India found the serotypes: 026:B6(3); 0119:B14(7); 0125:B15(1) and 0126:B16(1), out of 34 E.coli strains isolated from calves.

In the present survey, 026:B6; 055:B5; 086:B7; 0111:B4; 0119:B14 and 0126:B16 have been found in healthy as well as in

calves showing enteric disorders. From the literature reviewed, it appears that all the six serotypes have been reported by various workers. O26:B6 has been reported by Orskov(1951), Uberko (1957), Gould (1958), Glantz et al (1959), Rees(1960), Akhmedova et al (1963) and Yadava (1965). O55:B5 has been reported by Ulbrich (1954), Rees (1957,1960), Kaechenbeek et al (1960) and Akhmedova et al (1963). O86:B7 has been reported by Rees(1957, 1958,1960), Kaechenbeek et al (1960) and Akhmedova et al (1963). O111:B4 has been reported by Uberko (1957). O119:B14 has been reported by Glantz et al (1959), Ulendeev (1963) and Yadava (1965). O126:B16 has been reported by Yadava (1965).

With regard to the frequency of occurrence of these serotypes, it differs from workers to workers and also from one place to another and the results of the present investigation do not tally exactly with any of the workers.

Serotype: O128:B12 has been isolated by Rees (1960) but this serotype could not be detected in the present investigation. The isolation of the serotypes: O18a, 18c:B21 and O127:B8 from calves have not been reported so far in the literature reviewed. This finding is in complete agreement with the present findings.

The serotypes: O3; O8; O9; O15; O35; O78; O103; O113; O114; O115; O117 and O138 have been reported from calves by various workers. The serotypes: O8; O9; O15; O78 and O115 are potential pathogens for calves, and very often been reported in the literature but in the present investigation due to non-availability of the antisera against these serotypes, these serotypes could not be determined.

Sensitivity tests:

All the 174 E.coli strains were tested with six antibiotics. The results of the sensitivity tests on individual strains are given in Table VII. Another table (Table VIII) has been deduced from table VII which indicates the zone of inhibition produced by the antibiotics on the number of E.coli strains collectively. Altogether 15.51%, 16.09%, 21.26%, 22.41%, 20.11% and 23.56% resistant strains were found against achromycin, aureomycin, ledermycin, dihydrostreptomycin, mysteclin-V and penicillin respectively. 24 strains with multiple resistance to all the six antibiotics were found and the rest were resistant to either single antibiotic, two or three antibiotics. The correlation of different serotypes with sensitivity tests have been shown in Table IX. 2, 4, 4, 1, 2, & 1 strains were found resistant in the serotypes: O26:B6; O55:B5; O86:B7; O111:B4; O119:B14 and O126:B16 respectively. Two serotypes:O55:B5 and O86:B7 from the dead calves were resistant to all the six antibiotics used.

The results of the present survey are quite comparable with the results obtained by other workers on sensitivity tests. The fact that many more resistant strains, some with multiple resistance and the others with single or double resistance, have been found in the present survey, is not surprising. The possible explanation for this is that there is a practice of feeding terramycin soluble powder to young calves during the early life up to 7 days. This feeding practice might have resulted in the increased resistance of E.coli strains against the anti-

-biotics used in the present survey. Positive evidence exists that such changes take place in the E.coli strains. Smith and Crabb (1956) found 11 E.coli strains resistant to streptomycin and two to aureomycin and terramycin, out of 58 E.coli strains isolated from white scours, by using 20 mcgms. concentration dried disks of antibiotics. They stated that the resistant strains of E.coli were commonly associated with cases of white scours in farms where the aureomycin was used as feed supplement to calves. Gould (1958) found tetracycline resistant E.coli strains which were responsible for white scours in calves. The resistant strains developed after the introduction of terramycin prophylaxis. Smith (1958) found more resistant strains in herds where drugs were used either as prophylactic or curative measures, than the herds where no drugs were used. E.coli with multiple resistance to streptomycin, tetracycline and chloramphenicol eventually formed the predominant faecal flora in cases of white scours in calves. Ingram et al (1958) demonstrated an increased resistance to penicillin and aureomycin in E.coli strains where the calves were given these antibiotics in milk during early life.

Further support is also available regarding the changes taking place in E.coli strains. Smith (1958) found that it was possible in Vitro to make, drug sensitive cultures of E.coli, resistant to streptomycin, chloramphenicol and sulpha dimidine, both singly and multiply, but not to tetracycline or furamazone.

There was no correlation between the serotypes and sensitivity tests. Serologically identical strains behaved

differently with different antibiotics and serologically unrelated strains gave identical sensitivity reactions. Sinha (1965) carried out sensitivity tests with 61 E.coli strains belonging to 9 different serological types and found multiple resistant strains to streptomycin, chloromycetin, terramycin, neomycin and achromycin. Some of the strains exhibited multiple resistance to 2, 3 or 4 antibiotics. There was no correlation between the serotypes and resistant strains.

CONCLUSIONS

Altogether 174 E.coli strains, isolated from healthy, diarrhoeal and dead calves, were examined for sugar fermentation, susceptibility to T-series of phages, serological typing and sensitivity tests with six antibiotics. No diagnostic criteria or relationship, between serotype and biochemical type or serotype and phage type, was found in the present studies. Serologically identical strains belonged to various biochemical types and phage types, and serologically unrelated strains were similar in biochemical types and phage types. Similarly there was no relationship between serotype and sensitivity tests carried out.

The cause of heavy mortality in calves of Government Cattle Farm, Patna, as already discussed, could not be ascertained. The managements of the Government Cattle Farm, Patna, as per report, are satisfactory and according to standard schedules, but it needs close observation. The calves are given colostrum from the mothers, still they are suffering from enteric disorders.

It has already been established that the E.coli is one of the major causes of gastro-enteritis in calves producing a syndrome of white scours. The serotypes, which have been isolated from diarrhoeal calves, are also found in healthy calves. All the six serotypes isolated in the present survey are potential pathogens for man and animals, especially the calves and children. Thus to establish the correct epidemiology of the disease, white scours in calves, an extensive investigation, extending for a number of years, is necessary to elucidate the various factors associated with mortality and sources of infection, whether they are from calf pens themselves or from outside sources including human beings.

The increased resistant strains of E.coli found in the Government Cattle Farm, Patna, might be due to the feeding of terramycin soluble powder to young calves. They were found in healthy, diarrhoeal and dead calves. This necessitates the testing of E.coli strains in Vitro with antibiotics before adopting the curative measures. This procedure is the basis of modern therapy, especially when there is increased resistant E.coli strains prevalent.

In the present studies, it was found that the penicillin G sodium produced zone of inhibition varying from 7 mm.diameter to 20 mm. diameter with different concentrations of antibiotic, though the percentage of resistant strains, with 100 units of penicillin, were more than the other antibiotics used. It is the prevailing concept that penicillin has no effect on gram-negative organisms. The results of the present studies are quite

contradictory to the prevailing concept and it has been found that penicillin do have some effect on E.coli strains in Vitro tests.

The serotypes: O26:B6; O55:B5; O86:B7; O111:B4; O119:B14 and O126:B16, isolated in the present survey are potential pathogens for human beings, especially the children. From the literature reviewed, it appears that they, especially the first four, are very often been isolated from the cases of infantile gastro-enteritis. Thus they are of public health importance.

Though the findings of this small survey may not reveal the exact picture of the prevalence of pathogenic E.coli in the intestine of calves in health as well as in enteric disorders, these certainly indicate that our animals are not free from pathogenic E.coli of epidemiological importance and also public health point of view. To determine the actual state of affairs, an extensive research work will have to be undertaken by examining, not only a large number of animals in the farms and in the neighbouring rural and urban areas,but also possibly a good number of faecal samples of human origin, extending over a period of number of years for establishing the various predominant serotypes,phage types,biochemical types, etc.,of E.coli in animals & man and the epidemiological relationship of gastro-enteric disorders recorded in both animals and man.

S U M M A R Y

(E.COLI)

SUMMARY

The materials for the present investigation were mainly collected from healthy calves as well as calves showing diarrhoea at Government Cattle Farm, Patna. A total of 246 living calves and 7 dead calves were examined. Out of 246 living calves, 46 calves were having diarrhoea of varying intensity at the time of collection of materials. The feeding and management of Government Cattle Farm, Patna, as reported, were satisfactory and according to standard schedules. The calves are given colostrum from the mothers and one tea-spoonful of terramycin soluble powder along with milk, during the early life up to 7 days. In spite of reported good management, the farm is experiencing heavy mortality in calves. The mortality rate from 1960 to 28th September, 1965 has been given, which varies from one year to another and from season to season. The heavy mortality has been recorded in the winter and spring seasons (from December to April). The reasons for the heavy mortality in calves could not be explored.

Out of 253 calves examined of the age group, zero day to one year or slightly more, 174 *E.coli* were isolated. All the 174 *E.coli* gave the IMViC reactions ++-- and were nitrate positive. They were subjected to biochemical tests, phage typing, serological typing and sensitivity tests. The methods used for the biochemical tests and sensitivity tests were the same as described by Mackie and McCartney (1953); the method adopted for phage typing was the

same, with slight modification, as described by Wilson and Atkinson (1945) and modified by Smith and Crabb (1956), and the method adopted for the serological typing was the same, with modification, as advocated by Kauffmann (1947) and Barua et al (1956).

The results of biochemical tests, phage typing and serological typing have been given in Table I and of sensitivity tests in Table VII. The faeces of three calves of zero-day old were sterile. All the 174 E.coli strains fermented glucose, lactose, arabinose and mannitol promptly within 24 hours, except one strain which fermented arabinose in four days. The results of gas formation from glucose, fermentation of sucrose, raffinose, dulcitol and sorbitol were variable. Altogether 24 fermentation patterns were found which constituted 24 biochemical types. All the 24 biochemical types were found in the healthy calves, and only 10 & 5 biochemical types were found in diarrhoeal and dead calves respectively. All the biochemical types found in diarrhoeal and dead calves were also found in healthy calves. The majority of the E.coli belonged to the biochemical types 1, 2, 3, 4, 12, 19 and 24. The correlation of biochemical types with age groups in healthy, diarrhoeal and dead calves have been shown in Table III, and the correlation of serotypes with biochemical types in Table VI.

Altogether 32 phage types were found in calves. Out of 32 phage types, 28, 10 and 5 phage types were found in healthy, diarrhoeal and dead calves respectively. All the phage types found in diarrhoeal calves, were also found in healthy calves

except the phage types 2, 9, & 10, which were exclusively confined to the diarrhoeal calves. All the five phage types found in dead calves were also found in healthy calves, except the phage type 16, and in diarrhoeal calves except the phage types 16 and 19. The phage type 16 was found once in a dead calf. The correlation of phage types of E.coli with age groups in healthy, diarrhoeal and dead calves as well as the correlation of serotypes with phage types have been shown in Table IV and Table VI, respectively.

Fiftynine E.coli strains were typable with the nine standard antisera available and the rest 115 strains could not be typed with the available antisera. Serologically typable E.coli strains belonged to the serotypes: O26:B6 (8); O55:B5(10); O86:B7 (13); O111:B4 (3); O119:B14 (13) and O126:B16(12). All the six serotypes were found in healthy calves as well as calves showing enteric disorders. Only two serotypes: O55:B5 and O86:B7 were found one each in two dead calves. The correlation of serotypes with age groups in healthy, diarrhoeal and dead calves as well as the correlation of serotypes with phage types and biochemical types have been shown in Table V and Table VI, respectively. Out of 59 serologically typable E.coli strains, 39, 18 and 2 strains were found in healthy, diarrhoeal and dead calves respectively. The correlation of serotypes with sensitivity tests have been shown in Table IX.

Out of 174 E.coli strains tested with different concentrations of six antibiotics in Vitro, 27 (15.15%) were found to be

resistant to 25 mcgms. of achromycin. Similarly 28 (16.09%) strains were resistant to aureomycin, 37 (21.26%) to ledermycin, 39 (22.41%) to dihydrostreptomycin and 35 (20.11%) to mysteclin-V. 41 (23.56%) strains were resistant to 100 units of penicillin. 24 strains were resistant to all the six antibiotics; one to aureomycin, ledermycin, dihydrostreptomycin and mysteclin-V; one to aureomycin, ledermycin and dihydrostreptomycin; one to aureomycin, ledermycin and penicillin; one to dihydrostreptomycin, mysteclin-V and penicillin; two to dihydrostreptomycin and penicillin and one to achromycin & dihydrostreptomycin. One strain was resistant to aureomycin, 10 to ledermycin, 9 to dihydrostreptomycin, 9 to mysteclin-V, 13 to penicillin and two to achromycin.

The serotypes: O26:B6; O55:B5; O86:B7; O111:B4; O119:B14 and O126:B16 are potential pathogens, particularly the first four serotypes, for animals and man, especially for the calves and children. They are very often been isolated from infantile gastr-enteritis and as such they are of public health importance.

An extensive research work on entero-pathogenic group of organisms has been suggested to study the epidemiology of the disease, white scours in calves, and its epidemiological relationship with infantile gastro-enteritis in children.

PART - II

INTRODUCTION

(SALMONELLA)

INTRODUCTION

Cattle of all ages may become infected with various Salmonella organisms which may give rise to either clinical or subclinical infection. In adults and yearlings, clinical salmonellosis is usually sporadic. Affected animals typically show signs of septicaemia followed by profuse diarrhoea or dysentery, and death commonly occurs within a few days of the onset of symptoms. In calves the disease tends to spread rapidly and often reaches epidemic proportions; symptoms are more variable and may include pneumonia or arthritis, and the course is often more prolonged (Gibson,1961).

The history of the bacteriology of salmonella did not commence until the end of the 19th century, but the clinical descriptions of fever in man were described and published long before. Eberth (1880,1881) observed "typhoid bacilli" in infected tissues and Gaffkey (1884) first succeeded in isolating the causal organism by cultural methods. At about this time Klein, in England, had studied the disease of hog cholera to which he gave the name "Pneumo-enteritis of pigs" and during 1885-86 Salmon et al in the U.S.A. isolated and described in detail Salmonella cholerae suis. Following this, many related organisms were isolated and given various names by different workers, including S.typhimurium (B.typhimurium, Loeffler, 1892; B.aertrycke, de Nobele,1898), S.abortus equi (Kilborne, 1893; Smith,1893), S.bovis-morbificans (Basenau,1894), S.gallinarum (B.sanguinarium, Klein,1889), S.pullorum

(B.pullorum, Rettger,1909) and S.enteritidis (Bacillus of Gaertner,1888).

Early in the 20th century there was great confusion as to the relationship between these various organisms because the properties and characteristics of some of these strains had been imperfectly described. In 1893, Theobald Smith noted a similarity between the so-called hog cholera bacillus, the bacilli of the Gaertner group and B.typhimurium. Following the lead of Lignieres (1900) that the hog cholera bacillus should be named Salmonella, a number of people suggested that other similar organisms should also be classified as Salmonella. Detailed studies of the antigenic structures of these organisms began to be made, but due to the S-R variations of the somatic antigens the results were confusing until the importance of White (1926) and the diphasic nature of the flagellar antigens on some serotypes, was understood (Andrewes,1922,1925). It was not until the publication of the report of the International Association of Microbiologists in 1934 that the position was clarified. A sub-committee of that Association recommended the adoption of the terminology introduced by White(1929a,b) and modified by Kauffmann (1931), which recognised the generic status of the Salmonella group and the specific rank of each of the antigenically distinguishable types(Buxton,1958).

The Salmonella group of organisms comprise over 600 serologically distinct types, all of which are believed to cause disease in human beings, in animals or in both (Taylor,1960). Moreover, new serotypes are being added annually to this

extensive list; and it is apparent that, with few exceptions, there is little host specificity - the majority of serotypes being potentially pathogenic to man and animals alike. This is the complex problem which faces the veterinary profession today, and it is of concern to those engaged in large and small animal practice, to those working in diagnostic and advisory service, and to those engaged in food inspection and public health.

Salmonellosis in animals occurs in all parts of the world. The occurrence of salmonella among poultry is relatively high, and is associated with a very wide variety of serotypes. It is significant that the majority of outbreaks are still caused by relatively few serotypes, being confined mostly to S.pullorum, S.gallinarum and S.typhimurium. From time to time, other serotypes may temporarily predominate in a particular district or country; as was the case of S.thompson infection in the United Kingdom and of S.niloese in Denmark. In the U.S.A. S.oranienburg, S.bareilly and S.montevideo are more common. Pigs are also known to be common carriers of Salmonella and many serotypes have been isolated from these animals. However, the majority of clinical infections are confined to S.cholerae suis and S.typhimurium. Similar circumstances occur in other animal species in which the burden of clinical infections is associated with few serotypes. Among cattle, the common infections are S.dublin, S.typhimurium and S.enteritidis; those in horses are S.abortus equi and S.typhimurium and in sheep are S.abortus-ovis and S.typhimurium. From this overall picture it is apparent that

the world problem of Salmonellosis in farm animals is associated with S.typhimurium and relatively few host-specific serotypes (Buxton, 1958).

The chief causes of Salmonellosis in calves are S.dublin and S.typhimurium. Secondary factors such as age, immunity, management, nutrition and concurrent infections with other micro-organisms have influence on the susceptibility of the individuals. Adult carriers are an important source of both S.dublin and S.typhimurium infection for the young calves. Calves may contract the disease through the medium of contaminated food, water or other utensils used in the farm or calf-pen. Calves may be infected in utero or at any time during calfhood. The clinical signs of infection are variable, and include still birth and neonatal losses, acute septicaemia in young calves, and more prolonged illness, with diarrhoea, pneumonia or arthritis or any combination of these in older calves. Other cases are subclinical. Very few calves remain carriers of the organism.

Galbraith (1961) has pointed out three main sources of infection to human beings. These are meat, meat products and bulk egg products. These materials are produced in bulk and this is probably related to their high rates of contamination with Salmonellae. Water and milk are also produced in bulk for the community but strict measures of hygiene are imposed to prevent water-borne and milk-borne infections. He further pointed out that Knacker's dog meat may be a source of human Salmonellosis.

In the present study, an attempt was made to examine various physiological characters, serological behaviour and action of antibiotics on Salmonellae isolated from calves. This small survey was undertaken with a view to find out the various serotypes of Salmonella prevalent in the normal as well as diseased calves at Government Cattle Farm, Patna, and to know whether they are of public health importance, so that, in future, an extensive survey may be undertaken to elucidate the epidemiology of the disease and their eradication.

REVIEW OF LITERATURE

(SALMONELLA)

REVIEW OF LITERATURE

(A) Salmonella serotypes isolated from calves:

Obich (1865) was the first to incriminate an infectious agent as the cause of enteritis in calves, but it was not studied bacteriologically. Jensen (1891,1893) investigated outbreaks in Denmark and isolated an oval coliform bacillus from the viscera of affected calves. He was unable to distinguish the organism morphologically and culturally from strains of *E.coli* recovered from the intestines of normal calves, but found that unlike the latter it was pathogenic for new born calves, reproducing the disease when fed to them in milk. Hutcheon (1893) reported a disease of calves in South Africa that can be regarded as calf-paratyphoid. This disease was apparently identical with the condition described as "Lewersiekte (liver disease)" or "yellow liver" by Otto Henning (1894). Hutcheon (1893) thought that infection was spread from farm to farm by means of the faeces of infected calves. Outbreaks in calves were also investigated in Holland by Thomassen(1897) and Poels (1899), who isolated "Gaertner -like" organism resembling *B.coli* and *S.typhi*, which they named "Pseudo-typhoid bacilli" and "Pseudo-coli-bacilli" respectively. Jensen (1903) used the term "Bacillus paracoli" or "Paracolon-bacillus" for the organism isolated from the Danish outbreaks. Langer (1904) studied cultures of bacteria obtained from calf livers disseminated with greyish-yellow necrotic nodules of paratyphoid and found

that these formed Hgs but failed to produce indole, and the suspensions of the organisms were agglutinated by S.typhi antiserum. Schmidt (1908) also isolated "Gaertner-like" bacteria from the organs of calves affected with septicaemia, diarrhoea and pneumonia. He stated that the disease was identical with "Pseudo-coli bacillosis" of Poel and "Para-coli-bacillosis" of Jensen. Titze and Weichsel (1908) examined 28 strains of the so-called "Paracolon-bacillus" and were unable to differentiate 23 of them from "Gaertner's bacillus". Savage (1909) concluded that Bacterium paratyphosus B and " bacilli of the aertryeke type" were serologically distinct, but this view was not accepted at that time.

Uhlenhuth and Hubener (1913) and Jensen (1913) reported that most of the strains labelled "Bacterium paracoli" were, infact, Salmonella enteritidis, but some resembled S.paratyphi B Further, outbreaks of disease in calves caused by "Gaertner's bacillus" or S.enteritidis were reported by Luxwolda (1913), Christiansen (1914), Warnecke (1914), Douma (1916), Meyer et al (1916) and several others.

White (1926) re-examined a number of strains isolated in Copenhagen from cases of Salmonellosis in calves and described by Christiansen (1914) as B.paratyphosus B, Schottmuller (i.e. S.paratyphi B). All were found to be typical strains of S.typhimurium.

White (1929) differentiated the strain S.dublin from S.enteritidis serologically and gave it the status of an independent species, which clarified most of the earlier confusion. Some old laboratory strains obtained from outbreaks of food

poisoning, septicaemia and meningitis and labelled as B.enteritidis,Gaertner, were re-examined by Smith and Scott (1930) and found to be serologically identical with S.dublin of Bruce White(1929). Six strains of "Bacterium paracoli" isolated from cases of calf paratyphoid in Denmark were also examined and all six were found to be identical with S.dublin. They concluded that this organism had a special association with cattle and cow's milk was the common vehicle of human infection.

Bosworth and Lovell (1931) reported two outbreaks of S.dublin infection of calves. One outbreak was characterised by acute diarrhoea with fluid offensive faeces. Some of these calves also developed pneumonia, but in the second outbreak the predominant clinical signs were those of pneumonia and diarrhoea was not observed.

When the Salmonella group had been more clearly defined, it became apparent that the organism most commonly associated with bovine Salmonellosis,in those parts of Europe where the disease was endemic, was S.dublin. S.typhimurium was next in order of frequency, being found in some cases in adult cattle and in a few cases in calves. Other Salmonella types were incriminated only occasionally. In other parts of Europe where bovine Salmonellosis was un-common,S.typhimurium tended to be the predominant organism (Bartel,1938; Lutje,1939). Henning (1939) showed that S.dublin was by far the most common cause of paratyphoid in calves. Craig et al (1941) reported the outbreak in calves with S.dublin infection and Field (1948) reported

that this organism was responsible for the vast majority of cases of paratyphoid in adult cattle.

In Newzealand, where, according to Josland (1950), S.dublin has not been recorded so far, all the 80 isolations made from bovine sources were found to be S.typhimurium. Similarly in Australia outbreaks of calf Salmonellosis due to S.typhimurium infection appear to be the commonest according to Simmons and Sutherland (1950). Out of five outbreaks recorded by these authors, four were due to this organism.

Henning (1953) in a comprehensive review of the literature on the subject has shown the world wide distribution of calf disease due to Salmonella infection and stated that the infection with S.dublin is by far the most common. He also isolated 507 strains of Salmonella from outbreaks of calf paratyphoid during the period 1939-1950 in South Africa. Of these 491 were identified by him as S.dublin, eleven as S.typhimurium, four as S.enteritidis and one as S.bovismorbificans. In addition, he also isolated 12 strains of S.dublin from adult cattle.

Collard et al (1956) examined mesenteric lymph glands and faeces of 200 Zebu cattle in Ibardan, and found a carrier rate of 5.5%. The types isolated were S.typhimurium, S.dublin, S.elizabethville, S.rubislav, S.oranienburg, S.monschaui and S. johannesburg. Muller (1956) examined 8016 dead calves in Copenhagen from July 1948 to April 1956 and found 2.17% incidence of Salmonella infection; S.dublin infection was 1.79%

and S.typhimurium 0.26%. Other serotypes isolated were S.enteritidis (2), S.enteritidis var danysz (3), S.bredenoy(2), and one each of S.duessoldorf, S.saint-paul and S.chester. Kampelmacher (1957) examined 1600 slaughtered cattle in Netherlands and found S.dublin in 4 cases and S.bareilly in 5 cases.

Salisbury (1958) reported the isolation of 1385 Salmonella strains of six serotypes from animals and birds in Newzealand from 1948 to June 1957. Out of 416 strains from cattle, 412 were S.typhimurium,two were S.bovis-morbificans & 2 S.cholerae-suis and S.newington cases were usually sporadic in cattle. Muller (1958) reported that S.dublin and S.typhimurium comprised 74% and 14% respectively of 229 isolates from calves, but he also found a high incidence of infection with exotic serotypes, in that 28 (12%) of the isolates belonged to 17 different serotypes. Khera et al (1958), citing the older literature, stated that the infection with S.typhimurium appeared to come second in the order of incidence, both as a cause of disease in calves and in adult bovines.

Rokey et al (1959) isolated S.dublin from mesenteric lymph nodes of a calf which died of uncontrollable foetid diarrhoea. There was a previous history of similar sickness and high mortality among calves and out of 20 calves in the pen of the present case, there was a morbidity of 85% and mortality of 33⅓%. Shirlaw (1959) described that out of 884,000 cattle population in Kenya settled areas, the average mortality amongst calves in the first 6 months of life was about 25%. The main

causes were white scours, pneumonia, anaplasmosis, a non-specific glomerulonephritis and most important calf paratyphoid due to S.dublin. Smith (1959) examined mesenteric lymph nodes and faeces of apparently healthy pigs, cattle, sheep, dogs and cats for Salmonella. Salmonella was isolated from the mesenteric lymph nodes of sixty (12%) of 500 pigs, nine (4.5%) of 200 dogs, five (2.5%) of 200 cats and none of 200 cattle and 100 sheep. Buxton (1960) reported that young calves are more susceptible to Salmonella infection than the adult and S.dublin and S.typhimurium causes the majority of outbreaks.

Gibson (1961),citing the older literature,reported that the S.dublin was found to be the predominant Salmonella infection of calves in Germany, Holland, Denmark, Italy, South Africa, Venezuela and Brazil. He, further, reported the Salmonella isolation work from calf, carried out at Veterinary investigation centre, England and South Wales, during 1958-1960, and listed 14 serotypes out of 969 isolates. Two serotypes, S.dublin and S.typhimurium, accounted for 96 to 97% of the isolations. Other serotypes were, S.agama, S.bovis-morbificans, S.brancaster, S.enteritidis, S.give, S.heidelberg, S.menston, S.pullorum, S.reading, S.saint-paul,S.tennissee & S.thompson.

Rude (1963) described 46 cases of Salmonellosis in cattle caused by S.typhimurium. Of these 9 occurred in mature cattle and 37 in calves.

(B) Salmonella serotypes isolated in India:

I. Cattle and buffalo:- Shirlaw (1935) isolated a Salmonella like organism from an outbreak of pyocepticaemia in calves in Lahore area but serological identity was not worked out. This organism was later on identified by Rajagopalan (1938) as S.enteritidis var dublin. After that though outbreaks of disease in calves were some time suspected to be due to Salmonella infection, these were not confirmed by bacteriological diagnosis.

Priestly and Artioli (1946) encountered heavy mortality among buffalo-calves in Military farms in Lahore area. Out of 282 calves born at various farms kept under their observation, as many as 204 (72%) died within 14 days of birth. Organisms resembling S.typhimurium were recovered from 76 of the 124 calves examined. S. dublin was not recovered from any case in this group. From six calves over one month of age, died of scouring, organisms resembling S.dublin were isolated from Mesenteric lymph glands of five.

Khera and Dhanda (1958) did an extensive investigation for finding the aetiology of diarrhoeal infection in young calves at the Aarey Milk Colony,Bombay, Government Dairy Farm, Chalkganjaria (Lucknow) and Indian Veterinary Research Institute, Dairy Farm, Mukteshwar. The serotypes of Salmonella recorded were S.dublin (45), S.typhimurium(11), S.enteritidis (4), S.weltevreden(3), Salmonella species unidentified (probably S.weltevreden)(2), S.newport(2), S.richmond(2) S.chester,

Salmonella pomona, S.uchanga, S.hvittingfoss and S.butantum one each. The last five serotypes were reported for the first time in India. Faecal samples from 100 she-buffalo of the Aarey Milk Colony were negative for Salmonella, but four cows and one buffalo, out of 100 cows and 100 buffaloes of Chalkganjaria Farm were found to excrete same Salmonella types isolated from calves, in their faeces. Ganguli (1958) isolated S.dublin and S.typhimurium from calves and S.enteritidis from cattle.

Sharma and Singh (1960) isolated S.aba, S.anatum, S.charity, S.matopeni, a new type of Salmonella with its antigenic structure as (9) 46:i:enz$_{15}$ for which the authors proposed the name Salmonella mathura, S.pomona, S.typhimurium and S.weltevreden from cattle and buffalo during the course of a survey work.

Prasad & Ahmad (1965) isolated S.typhimurium and S.dublin from a fatal outbreak of gastro-enteritis in buffalo-calves which was a mixed Salmonella infection and stated that vaccination was inciting factor for the Salmonellosis in buffalo-calves.

II. Pigs:- Sahai and Nilakantan (1947) isolated five strains of Salmonella organisms from the materials collected from dead, sick and apparently healthy pigs at Keventers piggery in Aligarh during an ivestigation of a disease, causing heavy mortality among pigs. Out of these five strains, 2 were S.bovis-morbificans, 2 S.derby and one S.anatum.

Sharma and Singh (1960) reported the isolation of S.anatum and S.newport from pigs. The materials examined were pieces of liver,spleen,gall-bladder and intestinal scrapings from dead and

rectal swabs from live animals.

Makholia and Singh (1963) isolated an atypical strain of Salmonella from pigs with Salmonella "O" and "H" antigens 45:a:e,n,x and "O" and "H" antigens of Arizona 11:35-28. The biochemical characters were not typical for either Salmonella or Arizona. The authors proposed the name Salmonella vrindaban for this atypical strain.

Datta and Singh (1964) isolated 29 Salmonella strains comprising nine serotypes, including a new one, S.gokul, from apparently healthy pigs. The antigenic formula of S.gokul is 1,51:d: - and the organism has been placed in sub-genus I on the basis of its biochemical behaviour. Murty et al(1964) isolated S.cholerae suis var kunzendorf from pigs. The strains were highly sensitive to aureomycin, sensitive to terramycin and chloromycetin, but resistant to Dihydro-streptomycin,Penicillin, Bacitracin and Triple sulfa (Sulfadiazine,sulfamethizine and Sulphamerazine).

III. Equines:- Probably, the first record of the isolation of Salmonella from animal paratyphoid in India was the isolation of S.abortus equi from mares and foetuses in an outbreak of contagious equine abortion at the Government Cattle Farm, Hissar (Ann.Report.G.C.F.,Hissar,1919-20).

Cordy and Davis (1946) reported an outbreak of severe gastro-enteritis among horses and mules in the American Quarter Master Pack Unit, stationed in India. Five,out of eight affected animals, died. The first case occurred within three

weeks of arrival, and from all of the dead animals and one destroyed "in extremes" cultures of Salmonella were isolated which were later identified as S.bovis-morbificans.

IV. Sheep and goats:- During an investigation carried out at Indian Veterinary Research Institute,Mukteshwar in 1956-57, on the bacterial aetiology of pneumonia in sheep and goats, S.dublin was isolated from 2 out of 178 pneumonic lungs of sheep, 10 out of 111 pneumonic lungs of goats and Salmonella typhimurium from 10 out of 178 pneumonic lungs of sheep (Ann. Report.I.V.R.I.1956-57).

Sharma and Singh (1960) isolated S.magna, S.matopani and S.weltevreden from goats.

V. Poultry:- In 1931, Cooper and Naik reported Salmonella gallinarum infection for the first time in India, during the course of an investigation of an outbreak of a disease among poultry at Bhowali(Kumaon Hills).

One outbreak of fowl typhoid occurred at Punjab and another at Mukteshwar in 1932. S.gallinarum was isolated from each outbreak (Ann.Report.I.V.R.I.1930-31 & 1931-32).

Organism resembling S.anatum was isolated from the dead chicks, 1-17 days old, which hatched out from eggs supplied from a village, neighbourhood of Mukteshwar,though the culture was not typed serologically. The organism was obtained almost from every dead chick (Ann.Report.I.V.R.I.1932-33).

Shirlaw & Iyer(1937)isolated S.enteritidis from an outbreak of Salmonellosis in pigeons.

Hayes and Freeman (1945) recorded S.litchfield from sick duck with non-specific disease. The same organism was also isolated by Rao (1956) from chicks suffering from diarrhoea resulting in 50% mortality and by Ganguli (1958) from fowls.

Salmonella bovismorbificans was isolated from chickens dying due to septicaemia with 60% mortality at a Poultry Farm in Delhi (Pande and Nilakantan,1953).

Iyer and Rao (1950) recorded S.typhimurium from chickens. The same organism and S.anatum were isolated by Dixit (1952) from chicks suffering from diarrhoea with high mortality, at Poona Poultry Farm. S.tyhpimurium was also recorded by Gupta and Rao (1960) from chickens in an outbreak of sub-acute disease and tibio-tarsal arthritis.

Das and Jayaraman (1955) isolated S.alachua from chickens showing gastro-enteritis and prostration.

Salmonella pullorum has been isolated from materials received from Allahabad (Ann.Report.I.V.R.I.Mukteshwar.1958-59), though Rao (1955) had reported the isolation of a varient strain of this organism.Das et al (1959) isolated S.pullorum from chickens in West Bengal,where they observed necrotic myocarditis, hyperaemia of lungs and mild enteritis. They also isolated S.gallinarum from the cases of peritonitis in fowl and S.typhimurium from pigeons showing haemorrhagic enteritis.

S.gallinarum has also been reported by Rao et al(1952) from the cases of fowl and also by Ganguli(1958). Gupta & Rao (1960) isolated S.concord and S.newport from an outbreak of

paratyphoid with heavy mortality in chickens.

Sharma and Singh (1960) recorded the presence of S.anatum, S.champaign, S.chester, S.hvittingfoss, S.matopeni, S.pomona, S.richmond, S.sandiego and S.weltevreden in fowls examined by them during a survey work. S.richmond and S.sandiego were isolated from dead embryos (in shell) and ducks respectively.

VI. Human beings:- Hayes and Freeman (1945) did a systematic survey and isolated S.typhi, S.paratyphi A,B,& C, S.enteritidis, S.dublin, S.typhimurium, S.anatum, S.virchow, S.cholerae-suis, and S.bovis-morbificans from typhoid and paratyphoid cases of human beings. Other serotypes isolated occasionally from the cases of human salmonellosis are S.bareilly (Bridge & Scott,1931), S.poona (Bridge & Scott,1935), S.kirkee (Bridge & Scott,1936), S.cubana (Chapekar et al,1957),S.bovis-morbificans(Dutta,1954), S.weltevreden (Freeman,1953 & Mathur, 1959), S.morehead (Ganguli et al, 1956),S.banana (Ganguli,1956) and S.chittagong (Taylor et al,1948).

Ganguli (1958) mentioned 502 S.typhi, 164 S.paratyphi A, 37 S.typhimurium, 14 S.enteritidis, 10 S.chester,7 S.bareilly, 4 S.paratyphi C, 2 S.anatum, 2 S.bovis-morbificans, 2 S.orion, 3 S.weltevreden, S.banana, S.cubana, S.london, S.morehead, S.reading and S.virchow one each. Materials examined were faeces, blood, pus and cerebro-spinal fluid.

MATERIALS AND METHODS

(SALMONELLA)

MATERIALS AND METHODS

MATERIALS

The materials for the present investigation were mainly collected from healthy calves as well as calves showing diarrhoea at Government Cattle Farm, Patna. A total of 246 calves were examined; out of these 46 calves were having diarrhoea at the time of collection of materials. Materials from 7 dead calves of Government Cattle Farm, Patna and 12 experimental pigs, which were killed, were also collected from the post-mortem room at Bihar Veterinary College, Patna. Altogether 253 calves and 12 pigs were examined for the presence of Salmonella. The age of the calves varied from zero-day to one year or slightly more.

METHODS

Most of the organisms belonging to the family Enterobacteriaceae are closely related in respect of their morphological and cultural characters and one finds it difficult to choose a satisfactory method avoiding pitfalls. Some workers obtained excellent results with a particular method while others with other different methods. So one has to adopt a method or procedure which should not fall below certain minimum standards. The method adopted in the present work has been proved to be satisfactory and excellent in the hands of a number of bacteriologists, especially those working on enteric group of organisms for a long time (Kauffmann, 1930-31; Hormaeche et al, 1936; Scott, 1940; Robin et al, 1941;

Kauffmann & Edwards,1947; Smith & Buxton,1951; Edwards and Ewing,1955).

The techniques adopted are described in detail under the following headings:-

(A) Collection of materials

(B) Processing of materials

(C) Isolation of organisms

(D) Preliminary identification

(E) Identification of serotypes

(F) Sensitivity tests.

(A) Collection of materials:

Faecal samples were collected from the living calves. Caecal contents, mesenteric lymph glands, spleen,lung,heart blood and bile were collected from the post-mortem cases. The faecal samples from the young calves were collected with the help of swabs whereas from the older calves faeces were collected with the help of clean fingers and kept in sterile containers. Sterile precautions were taken during the collection of materials.

(B) Processing of materials:

(I) Mesenteric lymph glands, spleen and lungs:- The connective tissue and fats were removed aseptically from the above organs(tissues) and instead of burning the external surface after dipping in ethylalcohol (Smith,1959), the tissues were plunged into boiling water for 10 seconds (Scott,1940). The treated tissues were placed in a sterile mortar and with the

help of a sterile pestle ground into pulp in sufficient quantity of sterile sand. Two to three ml. of sterile normal saline solution was added, mixed well, allowed to sediment and one ml. of the supernatant was transferred into tubes of enrichment medium separately.

(II) Heart blood and bile:- About one ml. of heart blood or bile was added to the enrichment medium separately directly at the time of collection.

(III) Faeces:- Approximately 1 gm. of faeces was transferred into tube containing enrichment medium.

(IV) Swabs:- The swabs were cultured into tubes of enrichment medium by rubbing against the internal wall of the tube.

(C) Isolation of organisms:

(I) Enrichment:- The most commonly used enrichment media viz. tetrathionate broth (Muller,1925) modified by Kauffmann (1930-31) was prepared and used in the present investigation which facilitates the isolation of Salmonella group of organisms from contaminated materials like faecal matter. The medium was tubed about 10-12 ml. in each and after adding the inoculum i.e. tissue suspensions, blood, bile or faeces, incubated at 37°C. Regarding the period of incubation of cultures in enrichment medium at 37°C, opinion of workers varies. Smith (1952) in an experimental work to evaluate the effectivity of different enrichment media, found that maximum isolation of Salmonella organisms could be obtained after 30 hrs.

Hormaeche et al(1959) advocate that the culture in tetrathionate broth should be incubated for 24 hours and 3 days. In the present investigation, the inoculated tubes of tetrathionate broth were incubated at 37°C for 36 to 40 hours and then subcultured on selective media.

(II) Selective media:- For primary culture brilliant green agar (Kristensen, Lester and Jurgens, 1925) modified by Hormaeche et al (1959) was used throughout the present investigation. MacConkey agar was used occasionally. The medium was prepared and about 20 ml. quantity was poured into sterile petridishes, dried at 37°C for 24 hours and streaked from enrichment cultures and incubated at 37°C for 24 hours and then reading was taken. If no growth was observed on the selective medium after this period, the plates were reincubated for another 24 hours and examined. The brilliant green agar gives excellent results if used for primary isolation of Salmonella after enriching in combined Muller - Kauffmann's tetrathionate broth (Kauffmann, 1931; Hormaeche et al, 1959).

(III) Picking up of suspicious colonies:- The colonies which were red, round, translucent, convex and about 1 mm. in diameter on brilliant green agar were fished out and transferred on plain agar slants. In each case five colonies were picked up. Care was taken to pick a colony only by touching its centre and not to touch the surface of the medium as no selective medium is completely satisfactory and some organisms remain alive without growing on it (Hormaeche et al, 1959). The transplanted

agar slant was incubated for 24 hours.

(IV) Test for purity of isolated strains:- This was done by replating the cultures on modified MacConkey agar media containing mannitol in place of lactose and picking up mannitol and non-mannitol fermenters on agar slants, which were incubated for 24 hours at 37°C and then observing the characters of growth on agar slants and morphological characters after Gram's staining.

(D) Preliminary identification:

(I) Studies of biochemical characters:- Urease activity was observed in fluid medium prepared according to Kristensen (1946) and modified by Hormaeche and Munilla (1957). The inoculated medium was observed for four days. Tests for motility, indole production, M.R. and V.P. reactions, nitrate reduction, growth in Koser citrate medium and gelatine liquefaction were done by adopting usual methods. Tests for indole, M.R., V.P. and nitrate were done after four days incubation. Growth in Koser citrate and gelatine liquefaction were observed upto 10 days only. Test for Hydrogen sulphide production was done in 2% bacto-peptone water as advocated by Hormaeche et al (1959).

Fermentation of carbohydrates and alcohols such as i. arabinose, ii. glucose, iii. lactose, iv. raffinose, v. sucrose, vi. adonitol, vii. dulcitol, viii. mannitol and ix. sorbitol were done as described by Mackie and McCartney (1953). 24 hours peptone water culture was used for inoculation of sugar media. Incubation at 37°C was prolonged atleast for 10 days and reading was taken at every 24 hours intervals. Care was taken to notice gas production.

Tubes showing clear positive reaction, acid or acid and gas, were removed and others incubated further.

Cultures which fermented glucose, with the production of acid or acid and gas, mannitol, dulcitol and sorbitol and failed to ferment lactose, sucrose, adonitol and raffinose and were positive for citrate, H_2S, M.R. and nitrate and negative for gelatine, indole, urease and V.P. reaction were subjected to phage-lysis test and agglutination test with polyvalent Salmonella "O" serum.

(II) Bacteriophage-lysis test:- The Salmonella genus specific "O-1" bacteriophage was supplied by the bacteriology section, Bihar Veterinary College, Patna which in term had received from Dr. C.M.Singh, Head of the Department of Pathology and Bacteriology, College of Veterinary Science and Animal Husbandry, Mathura, U.P. Six hours broth cultures of the organisms were evenly spread on agar plate with the help of platinum loop on specified areas marked with greese pencil and the plate was allowed to dry for half an hour on the working bench. A loopful (4 mm) of the phage was deposited in the centre of the marked areas separately and incubated at 37°C for 8 hours and then left at room temperature for overnight. The room temperature varied, during the experimental period, from 20 to 31°C. Next day the lysis was noted on the spot where the phage was deposited.

(III) Agglutination test with polyvalent Salmonella "O" serum of group A-E :- The group sera A to E was obtained

from the National Reference Centre for Salmonella and Escherichia, Central Research Institute, Kasauli and the polyvalent "O" serum of group A-E was prepared by adding equal amounts of A to E group sera. Suspension of culture was made in normal saline solution on a clean microscopic slide and two loopful of Salmonella polyvalent "O" serum was added and mixed well. Positive reaction was observed within 30 seconds, the organisms forming into fairly large clumps, but the result was noted after 5 minutes. Control test with normal saline solution only was always included side by side to check the auto-agglutination.

(E) Identification of serotypes:

Twentyfour hours agar slant cultures were used to determine the serotypes by slide agglutination test. The cultures were first placed into different groups (A-E) of Kauffmann - white schema (1955) by mixing group sera with the suspensions of organisms in normal saline solusion as described above and then individual species specific sera were used to determine the serotype of Salmonella. The individual specific sera were received from the bacteriology section, Bihar Veterinary College, Patna which in term had received from Pathology Department, Armed Forces Medical College, Poona (India).

(F) Sensitivity tests:

The Salmonella cultures were also tested with impregnated filter-paper disks containing varying concentration of different antibiotics to determine their sensitivity in Vitro.

The antibiotics used were Tetracycline hydrochloride (Achromycin), Chlortetracycline hydrochloride (Aureomycin), Demethylchlortetracycline hydrochloride (Ledermycin), Dihydro-streptomycin sulfate, Mysteclin-V (which contained 250 mgms. of tetracycline per capsule) and Penicillin G.sodium. The concentration of antibiotics used were 5, 10, 25, 50 and 100 mcgms. of the active principles and 50, 100, 200, 500 and 1000 units of Penicillin. The method for the sensitivity tests and the preparation of antibiotics impregnated filter-paper disks has already been described under the heading E. coli.

R E S U L T S

(SALMONELLA)

R E S U L T S

(A) Salmonella isolated from living calves:

Faeces from 246 living calves of Government Cattle Farm, Patna were examined for Salmonella. Out of these, 46 calves were having diarrhoea of varying intensity. Suspected colonies from 18 cases were fished out and transferred on plain agar slants. Organisms from three cases produced pigments at room temperature indicating Pseudomonas spp. and were discarded. Rest of the organisms were replated on modified MacConkey medium. Red and white colonies were picked up separately from the plate. The idea of picking white colonies from the modified MacConkey medium was to look for Shigella organisms also. These organisms were tested for urease activity. Organisms from five cases were positive for urease and were discarded. The remaining organisms from 10 cases were tested for biochemical activities. Organisms from six cases were identified as late lactose fermenter belonging to coli-group and fermented lactose after 3-7 days. Organisms from four cases fermented glucose (with the production of acid and gas), mannitol, dulcitol and sorbitol and failed to ferment lactose, sucrose, adonitol and raffinose. They were motile and were also positive for citrate, H_2S, M.R. and nitrate and negative for gelatine, indole, urease and V.P. reaction. These were tentatively identified as Salmonella organisms.

None of the organisms were suspected for Shigella on biochemical characters.

The Salmonella identified organisms were subjected to phage-lysis test with Salmonella genus specific "O-1" bacteriophage and a clear confluent plaque was observed in all the cases. They also reacted with polyvalent Salmonella "O" serum of group A-E. The late lactose fermenters belonging to coli-group were also tested with bacteriophage and polyvalent Salmonella "O" serum. They neither showed lysis with bacteriophage nor reacted with polyvalent Salmonella "O" serum and thus proved negative for Salmonella and were discarded.

The remaining organisms were tested with A,B,C, D & E Salmonella group sera. One reacted with group B, two with group D and one with group E sera. These organisms were then tested with species specific sera. The organism of the group B reacted with Salmonella typhimurium and the organisms of the group D reacted with Salmonella dublin. The organism of the group E could not be identified with the available sera.

All the four cultures of Salmonella have been sent to Dr. Joan Taylor, Director, Salmonella Reference Laboratory, Colindale Avenue, London, N.W.9 for final typing and confirmation. The result is awaited.

S. typhimurium has been isolated from a female calf aged one month and 18 days. The calf was having diarrhoea and was passing bloody stool mixed with mucous. The condition was deteriorating at the time of collection of material.

Salmonella dublin has been isolated from two calves. One from healthy female calf aged 5 months and 2 days and another from male calf aged one month and 13 days. The male calf was having diarrhoea, passing bloody stool and the condition was deteriorating at the time of collection of material.

Salmonella belonging to group E was isolated from a male calf aged one year and 2 months. This calf was having diarrhoea with loose stool mixed with mucous at the time of collection of material.

(B) Salmonella isolated from dead calves:

Materials were examined from 7 dead calves. Suspected colonies from two cases were isolated but one was identified as Pseudomonas spp. and another as Proteus spp. No Salmonella and Shigella could be isolated from dead calves.

(C) Salmonella isolated from experimental pigs:

Materials from 12 slaughtered experimental pigs were examined and suspected colonies from two cases were isolated . One was identified as Proteus spp. and another late lactose fermenter belonging to coli-group. No Salmonella and Shigella could be isolated from the pigs.

(D) Sensitivity tests:

The results of the sensitivity tests carried out with six antibiotics are precisely presented in Table I. 10 mcgms. disks of tetracycline, chlortetracycline and demethylchlortetracycline produced the zone of inhibition 10 mm or more with all

the four Salmonella tested. Dihydrostreptomycin with 10 mcgms. concentration produced the zone of inhibition less than 10 mm. in S.typhimurium and S.dublin, but produced 10 mm. in Salmonella belonging to group E. Mysteclin-V with same concentration produced less than 10 mm. zone of inhibition in all the four Salmonella strains. Penicillin at 100 units concentration produced 10 mm. zone of inhibition in all the four Salmonella strains.

TABLE - I

Table showing sensitivity tests in Vitro of Salmonella strains with six antibiotics in different concentrations.

Antibiotics	Concentration	Zone of inhibition in S.typhimurium. (mm. dia.)	S.dublin (2) (mm.dia)	S.dublin (3) (mm.dia)	Salmonella of group E. (mm. dia.)
1	2	3	4	5	6
Tetracycline hydrochloride (Achromycin)	5 µg	10	10	10	10
	10 µg	10	12	12	12
	25 µg	12	14	14	14
	50 µg	14	15	15	16
	100 µg	16	16	16	18
Chlortetracycline hydrochloride (Aureomycin)	5 µg	10	10	10	10
	10 µg	10	12	12	11
	25 µg	11	12	12	12
	50 µg	12	13	12	12
	100 µg	12	13	13	13
Demethylchlortetracycline hydrochloride (Ledermycin)	5 µg	10	10	10	10
	10 µg	10	11	11	10
	25 µg	12	12	11	12
	50 µg	13	13	13	12
	100 µg	14	14	14	13

TABLE-I (continued)

1	2	3	4	5	6
Dihydro-strepto-mycin sulfate	5 µg	7	7	7	10
	10 µg	8	8	8	10
	25 µg	9	9	9	12
	50 µg	10	10	10	14
	100 µg	11	12	12	16
Mysteclin-V (Each capsule contained 250 mgs.of tetra-cycline)	5 µg	6.5	6.5	6.5	8
	10 µg	7	7	7	9
	25 µg	9	9	9	9
	50 µg	9	10	10	10
	100 µg	10	10	10	10
Penicillin G. sodium	50 uts.	7	9	8	9
	100 uts.	10	10	10	10
	200 uts.	10	12	12	12
	500 uts.	12	13	13	15
	1000 uts.	14	14	14	16

uts = units.

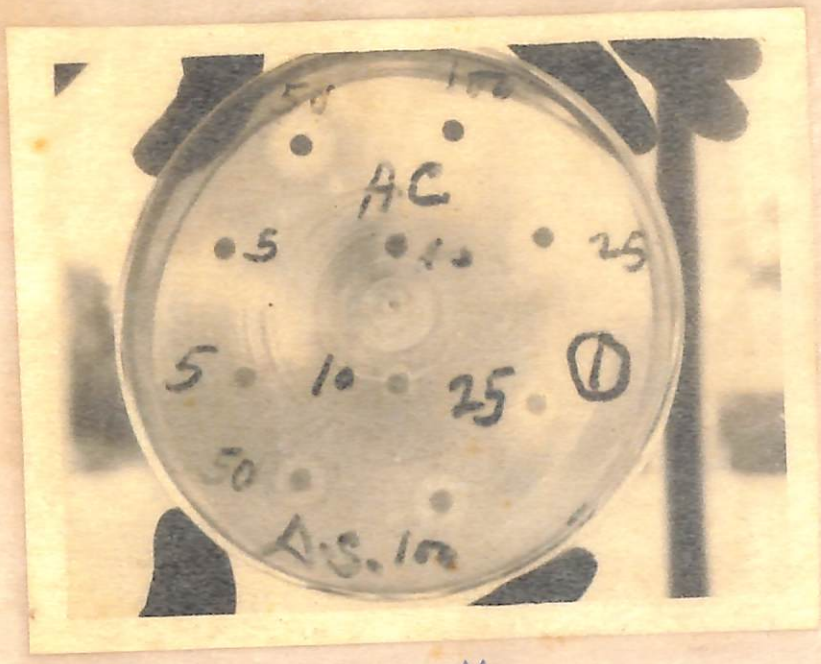

Fig.— 11

Fig.— 12.

DISCUSSION AND CONCLUSIONS

General:-

The Salmonella group of organisms comprise over 600 serologically distinct types (Taylor,1960). These are widely distributed among man and varieties of animals and birds. Most of them are intestinal parasites and they can not multiply and live in soil, water and other materials, except for a short period of time. Investigation carried out so far revealed that every animal species is found to harbour these organisms in their body, especially in their intestinal tract. For example S.typhimurium which causes typhoid like disease in mouse and other rodents; septicaemia and death in young animals and birds; occasional gastro-enteritis in adult animals, is the most prevalent cause of meat-borne Salmonellosis in man. It displays the capacity to pass from animal to man and back again by devious routes. The members of the genus Salmonella can get adapted to any kind of multicellular animal species and,thus, the control of Salmonellosis in man and animals is,indeed, a difficult problem. All the serotypes recorded are potential pathogens for man, animals and birds.

Technique:-

The method adopted has yielded positive isolation of Salmonella and negative for Shigella. The difficulty was encountered in the course of processing the material. A number of cultures which showed delayed fermentation of lactose or

hydrolysed urea or produced pigment and showed the reaction of Pseudomonas species grew well in the enrichment medium - combined Muller - Kauffmann's tetrathionate broth, used for preliminary culture. The enrichment media allowed the growth of lactose fermenting organisms to more than desirable extent. Though, it is stated that Proteus is highly inhibited in Muller - Kauffmann's tetrathionate medium, a number of cultures of these organisms were obtained after enriching the faecal matter in the above medium. Brilliant green agar was used throughout the present work and the result was found satisfactory.

Various workers on enteric bacteriology have encountered difficulties with Proteus and Pseudomonas while isolating Salmonella organisms by using enrichment and selective media. Lactose fermenting organisms, growing in enrichment media, can easily be eliminated when plated on selective media. But, Proteus, Pseudomonas and other late lactose fermenters can not be easily differentiated. Galton et al (1952) used combined tetrathionate broth with 0.125 mg of sodium sulphathiazole per 100 ml. to suppress Proteus. The same authors (1954) noted that in the examination of materials in which Pseudomonas were present in large numbers, the addition of 6-8 mgs.of sodium sulphadiazine per 100 ml. of enrichment medium gave excellent results. Jeffries (1959) used 40 megms. of novobiocin per ml. of tetrathionate broth to suppress the growth of Proteus organisms and found that it was superior to plain tetrathionate broth. Opinion of workers varies regarding the efficacy of

selective media. Hobbs et al (1945) and Thomas (1954) stated that desoxycholate citrate agar and bismuth sulfite agar gave better results if used after enriching the materials in selenite broth. Hormaeche et al (1959) advocated the use of brilliant green agar after enrichment in combined tetrathionate broth. Regarding the period of incubation of the inoculated enrichment media, Smith (1952) preferred 30 hours of incubation at 37°C while Hormaeche et al (1959) advocated incubation for 1 and 3 days in combined tetrathionate broth and 30 hours in selenite broth. In the present work 36-40 hours incubation was used and the result is satisfactory.

Results:-

A total of 253 calves (including 7 dead calves) and 12 pigs were examined for Salmonella and Shigella. None of the animals examined revealed the presence of Shigella organism. Perhaps this failure might be due to the fact that the method adopted was suitable for Salmonella and not for Shigella isolation and brilliant green agar probably inhibites the growth of Shigella organisms (Hormaeche et al,1959). The desoxycholate citrate agar - the best suited medium for the isolation of Shigella organism was not used due to some technical difficulties.

None of the dead calves and slaughtered pigs revealed the presence of Salmonella organisms. This failure might be due to the fact that smaller number of animals have been examined for the isolation of Salmonella.

Out of 246 living calves examined, 200 were apparently healthy and 46 were having diarrhoea of varying intensity. Four strains of Salmonella have been isolated, one from apparently healthy calves and three from calves showing diarrhoea. Correlation of age and sex with the type of Salmonella infection has been shown in Table II below:-

TABLE - II

Table showing correlation of age and sex with the type of Salmonella infection.

Salmonella type	Number of calves examined				Total
	0 day to 1 month	1 to 2 months	2 to 6 months	6 months & above	
	M. 9+1d	M. 11+2d	M. 22	M. 61	L. 246
	F.21+1d	F. 21+3d	F. 44	F. 57	d. 7
	= 32	= 37	= 66	= 118	= 253
S.typhimurium	Nil	1F	Nil	Nil	1
S.dublin	Nil	1M	1F	Nil	2
Salmonella group E	Nil	Nil	Nil	1M	1

M= male; F= female; L= living; d= dead.

It would be observed from the literature reviewed that principal serotypes of Salmonella related to Salmonellosis in calves are S.dublin, S.typhimurium and S.enteritidis. Other serotypes have occasionally been isolated from Salmonellosis in calves. Henning (1953) isolated 507 strains of Salmonella from outbreaks of calf paratyphoid in South Africa. Out of

these 491 were identified as S.dublin, 11 as S.typhimurium, 4 as S.enteritidis and one as S.bovis-morbificans.Muller(1956) examined 8016 calves in Copenhagen and found 2.17% incidence of Salmonella infection, S.dublin infection was 1.79% and S.typhimurium 0.26%. Other serotypes isolated were S.enteritidis (2), S.enteritidis var danysz (3), S.bredenoy (2) and one each of S.duessoldorf, S.saintpaul and S.chester. Muller (1958) reported S.dublin 74% and S.typhimurium 14% of 229 isolates from calves. Khera et al (1958) examined 191 calves and isolated S.dublin (45), S.typhimurium (11), S.enteritidis (4), S.weltevreden (3), Salmonella species unidentified (probably S.weltevreden) (2), S.newport (2), S.richmond (2),S.chester, S.pomona, S.uchanga, S.hvittingfoss and S.butantum one each. Smith (1959) examined mesenteric lymph nodes and faeces of 200 apparently healthy cattle and failed to isolate the Salmonella from them. Gibson (1961) listed 14 serotypes out of 969 isolates. Two serotypes, S.dublin and S.typhimurium, accounted for 96-97% of the isolation.

In Newzealand where S.dublin has not been recorded so far, all the 80 isolations made from bovine sources were found to be S.typhimurium (Josland,1950). Similarly, S.typhimurium appear to be the commonest in Australia (Simmons and Sutherland,1950).

The sensitivity tests carried out showed that the Salmonella strains isolated are sensitive to broad spectrum antibiotics like Tetracycline hydrochloride,Chlortetracycline hydrochloride and Demethylchlortetracycline hydrochloride. These antibiotics can be employed to exterminate the infection as well as

the carriers present in Government Cattle Farm, Patna. The feeding of Terramycin soluble powder to calves up to one week age might have eliminated the Salmonella infection in young stock and as such no Salmonella could be isolated from the young calves up to one month age group.

Public health importance:-

It is needless to stress the public health importance of the serotypes isolated. Man does not seem to act as an important reservoir of infection, except for one or two members like typhoid and paratyphoid bacillus, and the invading organisms are quickly thrown off and the chronic carrier rate is unusual (Wilson and Miles, 1955). Salmonella organisms are most commonly associated with human food poisoning. Taylor (1960) reported isolation of S.typhimurium, S.newport, S. enteritidis, S.derby and S.anatum from 3097, 118, 76, 58 and 55 cases of human food poisoning respectively during the year 1958. The other serotypes isolated can also produce enteric fever and gastro-enteritis. Anderson et al (1961) described an outbreak of 90 cases of infection with S.typhimurium phage type 20a in South-East England which were associated with infection in calves. A high proportion of the initial cases were in orthodox Jews suggesting Kosher meat as a possible vehicle of infection. In our country, Hayes and Freeman (1945) isolated S.typhi, S.paratyphi A,B & C, S.enteritidis, S.dublin, S.typhimuriun, S.anatum, S.virchow, S.cholerae suis and S.bovis-morbificans from typhoid and paratyphoid cases of human beings.

Mathur (1959) described an outbreak of food poisoning in a family due to S.weltevreden after consumption of contaminated milk.

Sources of infection in human being is consumption of contaminated foods and drinks. Contamination may take place from infected animals or through the agencies of flies,rodents, handlers and processing plants (Dolman,1957; Taylor,1960). Galbraith (1961) pointed out that meat, meat products and bulk egg products are three main sources of infection to human beings. Out of 645 outbreaks where vehicle of infections were identified with reasonable certainty, 47% were due to meat and meat products, 27% to eggs including egg products, 15% to sweetmeats and 11% to other vehicles. 41% of S.typhimurium outbreaks were caused by meat and 66% of outbreaks due to other serotypes were also caused by meat.

In our country, though, the meat is generally consumed after proper cooking, the indirect ways of contamination of other stuffs, water, milk and utensils from the contaminated meat can not be ruled out. Contaminated meat in the retail meat shop is also a danger to public, especially, in this country. In the market there are number of shops where prepared food and sweets are sold. If these are in the neighbourhood of the meat shop, flies can easily spread the Salmonella organisms to these.

In the abattoirs of our country, the bowels of slaughtered animals are emptied on the same floor where all the carcasses are dressed. Some time even the dressed carcasses are washed with

the water from the drain in which intestines of animals are emptied.

There is also close association of human beings and various animals in our country. The villagers are in the habit of living and sleeping in a place where the animals are kept (tied). So there is chance of infection to human beings due to close association.

Therefore, if control measures are to be adopted for Salmonellosis in human beings, the needs for a high standard of hygiene in dwelling places and in abattoirs can not be overlooked. Detection of carriers and improving the method of husbandry will surely lessen the number of infected animals and, thus, the danger to the public.

CONCLUSIONS

In our country, where sanitary measures are not adopted to the desirable extent and it seems from the available literature that no serious attempt has been made to determine the distribution of Salmonella types among different domestic animals, especially bovines, such an information will be highly desirable for the control of Salmonellosis both in livestock and man. The present investigation was, therefore, undertaken to study the prevalence of Salmonella in the intestine of calves in health as well as showing enteric disorders.

Though the finding of this investigation may not reveal an exact picture of the prevalence of Salmonella in the intestine of calves in health as well as in enteric disorders, these certainly indicate that our animals are not free from Salmonellae

as thought to be. For an accurate estimate, extensive research work will have to be carried out by examining a large number of animals extended over a number of years, not only from well managed Government Farms but also from rural and urban areas.

S U M M A R Y

(SALMONELLA)

SUMMARY

A total of 253 calves (including 7 dead calves) and 12 slaughtered pigs (experimental) were examined for the presence of Salmonella and Shigella organisms. Out of 246 living calves, 46 calves were having diarrhoea of varying intensity and the rest of the living calves were apparently healthy. Faeces were collected from the living calves. Caecal contents, mesenteric lymph glands, spleen, lung, heart blood and bile were collected from the dead calves and experimental pigs. Materials were inoculated in enrichment media and after 36-40 hours incubation at 37°C, the cultures were streaked on brilliant green agar medium. Suspected colonies were isolated and studied. Four Salmonella strains - one S.typhimurium, two S.dublin and one Salmonella belonging to group E were identified. No Shigella organism could be isolated. This failure might be due to the fact that the method employed was not suitable for the isolation of Shigella organism. One strain of S.dublin was from apparently healthy calf and the rest of the three Salmonella strains were isolated from the calves showing diarrhoea of varying intensity. No Salmonella could be isolated from dead calves and experimental pigs.

Microbial sensitivity tests were carried out in Vitro with six antibiotics. The concentration used were 5, 10, 25, 50 and 100 mcgms. with five antibiotics and 50, 100, 200, 500 and 1000 units of penicillin. All the four Salmonella strains were sensitive to Tetracycline hydrochloride, chlortetracycline hydrochloride and Demethylchlortetracycline hydrochloride.

Salmonella belonging to group E was also sensitive to Dihydrostreptomycin whereas the rest of the organisms were resistant to it. All the four strains were resistant to Mysteclin-V. Penicillin at 100 units concentration produced 10 mm. zone of inhibition in all the four Salmonella strains.

Difficulties were encountered in the isolation and identification of Salmonella organisms as Pseudomonas, Proteus, late lactose fermenter coli-group and E.coli also grew in enrichment media. It becomes difficult to identify and differentiate with certainty the Proteus colonies and some time late lactose fermenter and Pseudomonas colonies from the Salmonella colonies. This type of difficulty has also been encountered by the workers engaged in Salmonella works. Lactose fermenter coli are easily identified on brilliant green agar medium.

All the Salmonella organisms isolated are potential pathogens for man, animals and birds. They are of public health importance. Public health importance of Salmonellae has also been discussed.

R E F E R E N C E S

PART- I ESCHERICHIA COLI

PART-II SALMONELLA.

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