STUDIES ON

THE BACTERIAL SPECIES ASSOCIATED WITH GASTROENTERITIS IN GOATS

BY

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "STUDIES ON THE BACTERIAL SPECIES ASSOCIATED WITH GASTROENTERITIS IN GOATS" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or other similar title, of any other University or Society.

Mannuthy,
28-7-1979

Sebastian Joseph

CERTIFICATE

Certified that this thesis, entitled "STUDIES ON THE BACTERIAL SPECIES ASSOCIATED WITH GASTROENTERITIS IN GOATS" is a record of research work done independently by Sri. Sebastian Joseph under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to him.

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INTRODUCTION

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Gastroenteritis due to bacterial infections is often recorded at a high percentage in young animals. A common feature of all enteric disorders is diarrhoea. However, specific enteric disorders cause diarrhoea by varied and characteristic mechanisms. Thus, recognition of the cause and mechanism involved in the pathogenesis of diarrhoea in different enteric diseases is useful in understanding, diagnosing and controlling the disease.

Digestive disturbances of domestic animals are usually caused by a variety of infective agents. A review of literature on this disease syndrome reveals that the major bacterial species involved are Escherichia, Salmonella, Shigella, Vibrio and Enterobacter in the order of frequency of isolations. Detailed studies over the past thirty years clearly show the close association of certain serogroups of Escherichia coli (E.coli) in the causation of infantile enteritis, diarrhoea in neonatal domestic animals and so called traveller's-diarrhoea. However, many of them exhibit different degrees of pathogenicity and various patterns of sensitivity to antibiotic drugs (Heller and Drabkin, 1977; Ansari et al., 1978). E.coli is a normal inhabitant of the intestinal tract of different species of animals and man, besides being incriminated as the etiological agent of

several well defined disease entities. E.coli, once considered to be merely a commensal, has now been recognised to participate in the diarrhoeal syndrome due to enteropathogenicity, particularly in young subjects (Abdulrashid and Thapliyal, 1976; Johnson et al., 1978). Though E.coli is now known to be an important cause of the disease, the role of this organism as an etiological agent of diarrhoeal disease was confused for some time, in part because of failure to differentiate enterotoxigenic types from non-enterotoxigenic strains. The recent epidemiological studies of diarrhoea in human populations, employing different assays to determine the ability to synthesise enterotoxins by E.coli isolated from cases of enteritis, have helped better understanding of the pathogenicity of the organism.

Although E.coli has been recognised as an important agent causing enteric disorders, some diarrhoeal syndrome in goats are undoubtedly caused by microbial agents other than E.coli. The most important of them include some species of Salmonella, Shigella and Enterobacter.

Salmonella are considered to be ubiquitous in their occurrence. They have been identified in a wide variety of domestic and wild animals, in healthy and diseased conditions. The various sources and persistence of infection in the environment, the probable and possible modes of transmission from habitant and vector to animals are important in Salmonellosis.

Outbreaks of disease in lambs associated with Salmonella are less frequent than with the coliform group of organisms. Salmonella dublin, Salmonella abortus ovis and Salmonella typhimurium are some of the important serotypes involved in enteric disturbances in goats (Kapur et al., 1973; Bojka and Hudson, 1976). Enteritis due to Salmonella is not considered as a major livestock disease problem at present, but there are enough evidences of increased virulence of this organism in goats and other animals. Thus the disease may pose a threat to livestock industry in future.

In India, reports regarding the incidence, etiology and pathogenicity of E.coli and Salmonella in goat are very few. Most of the work in this regard has been done in pigs, calves and fowls. Therefore the present work is aimed to record the isolation, identification, pathogenicity and drug sensitivity of organisms causing gastroenteritis in goats, giving more emphasis to Escherichia, Salmonella and Enterobacter.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Incidence and etiology

Literature on the occurrence and etiology of enteritis in goats *per.se.* is scant. The problems of enteritis in young and adult stock of pigs, calves and birds are well studied and most of the reports are confined to these animals only. The bacterial agents of enteritis, their nature and pathogenicity were also studied in detail mainly from these species of animals. A short review of literature is attempted here giving particular emphasis on the subject of enteritis in goats, its causes and pathogenicity. The review is limited mostly to the records appeared after the year 1957.

Escherichia coli.

Roberts (1957) in his investigation on *E.coli* infection in lambs, observed that enteric infections due to *E.coli* are mainly seen in two to eight days old lambs and the septicaemic form in two to six weeks of age. Epizootics of colibacillosis are often found recorded in adverse environmental factors, such as wet, cold, windy weather, pronounced temperature changes, crowding and unsanitary lambing sheds. He also described an outbreak of Colibacillosis in young lambs in Australia, in which 16 lambs died of the disease and 30 to 50 were ill out of a flock of 250. In another study, Roberts (1958) found arthritis as a characteristic sign of infection

followed by death. In a flock of 280 lambs, which varied in age upto two months, 30 lambs died, the disease being confined to the higher age groups. Pure cultures of E.coli 078 were isolated from the dead animals and subcutaneous injection of these cultures to six week old lambs produced arthritic lesions similar to those found in natural outbreaks. Smith (1963) isolated different serotypes of E.coli from 63 per cent pigs which were showing clinical signs of gastroenteritis and according to him E.coli infection was the major cause of mortality in young piglets. From cases of diarrhoea in piglets, Stevens (1963) reported to have isolated E.coli from more than 75 per cent of specimens examined.

Pande and Acharya (1965) isolated E.coli from three kids died of enteritis and these isolates belonged to the serogroups 0146 and 04. These isolates when tested in guinea pigs were found to be potential pathogens. They also recovered several strains of E.coli belonging to the above serogroups from apparently healthy kids and goats indicating that these strains cannot be incriminated as specific pathogens for all types of enteritis in these species. In a report from Utter Pradesh, Punera (1968) concluded that gastrointestinal diseases caused 19.2 per cent of the mortality in Rambouillet lambs. Kaw and Khera (1970) also observed that gastroenteritis due to E.coli infection in crossbred lambs occurred at a higher frequency when compared to inbred lambs.

Bhagavan et al. (1974) examined 850 goats and 113 sheep slaughtered in abattoirs at Pantnagar, Bareilly and Haldwani for evidence of enteritis. Intestines of 75 goats (8.8 per cent) and 31 sheep (27.4 per cent) showed lesions of bacterial enteritis on histopathological examination. Kapur et al. (1974) observed gastroenteritis as the most important cause of mortality in lambs and kids. They isolated several E.coli strains from cases of gastroenteritis and the isolates belonged to serotypes 01, 03, 037, 039, 042, 027, 017, 048, 055 and 060. Pande et al. (1974) isolated E.coli from 27.5 per cent cases of acute infantile diarrhoea/gastroenteritis and from 20.6 per cent cases of chronic diarrhoea.

Studies were carried out by Pachalag et al. (1974) regarding the incidence, breed variation and etiological factors based on gross pathological lesions of lamb mortality at the Institute's farm, Rajasthan. Out of 275 and 659 lambs born in spring and autumn seasons respectively, 34.73 and 45.24 per cent died from birth to seven days. Pneumonia and other respiratory affections were the main causes which accounted for about 43.05 and 35.04 per cent respectively. Conditions like nephritis and enteritis of non-contagious type were observed in 6.63 per cent specimens. The authors in their studies could not point out the definite cause of deaths other than respiratory affections and low birth weight. Mittal (1976) reported an incidence of pneumonia, bacterial enteritis

and parasitic gastroenteritis as the main causes of kid mortality in the order of importance. Rajan et al. (1976) have reported that mortality was mainly due to coccidiosis in kids and Johne's disease in adult stock. Reid (1976) investigated the common diarrhoeal infections of sheep in Britain and found that Colibacillosis was the condition most commonly observed in young lambs, the majority of cases occurring in lambs upto ten days old.

Salmonella

The isolation of Salmonella was first reported by Salmon and Smith (1885) and demonstrated S.cholerae-suis which in those days was considered to be the etiological agent of hog cholera. The genus Salmonella now includes more than 1300 serotypes and new ones continue to be discovered at a rapid rate. Most of them are potential pathogens to man and animals, especially S.typhimurium which produces a fatal septicaemia (Galton et al., 1954). S.abortus ovis was identified as the serotype causing abortions and gastroenteritis in sheep in many parts of the world (Gibson, 1957). Watson (1960) considered S.dublin as the cause of abortion and gastroenteritis in both sheep and goat.

Khera (1962) reported Salmonellosis in various species of animals and birds in India. He has mentioned the prevalence of more than 52 serotypes of Salmonella distributed in

various species of animals, of which S.dublin and S.typhimurium being the most frequently occurring ones. Jayaraman et al. (1964) reported an outbreak of Salmonellosis in guinea pigs. S.typhimurium has been frequently isolated from normal pig at slaughter, but limited references are available on S.typhimurium as producing clinical disease in pigs (Khanna and Uppal, 1964). S.typhimurium as a cause of either an acute or chronic disease in pigs was reported by Heard et al. (1965). Jayaraman and John (1969) in their survey on the incidence of Salmonellosis in various species of animals showed that S.weltevreden has got a wide range of host specificity. They also found that S.typhimurium, S.bovismorbificans and S.anatum are the common serotypes causing gastroenteritis in goats. Simmons and Sutherland (1969) reported that ovine species are relatively resistant to Salmonella infection as it could be isolated from only one out of 400 specimens. Pandurangarao et al. (1970) observed heavy mortality in Rambouillet lambs due to S.typhimurium under semi-arid conditions of Rajasthan but no such mortality was encountered among the farm bred stock. Janakiraman et al. (1973) carried out an investigation on the cause of mortality in rabbits and guinea pigs, where they observed high percentage of death due to S.typhimurium, S.enteritidis, S.newport and S.weltevreden infection. Kapur et al. (1973) isolated

seven serotypes of Salmonella, viz. S.anatum, S.chester, S.kentucky, S.newport, S.enteritidis, S.richmond, and S.weltevreden from faecal samples of apparently healthy goats. Sulochana et al. (1973) isolated S.weltevreden from two pigs at Mannuthy, Kerala having gastroenteritis. However, experimental infection studies failed to reproduce the disease in healthy pigs.

Sojka et al. (1975) conducted a survey regarding the incidence of Salmonella infection in animals in England and Wales for the period from 1968 to 1973. Their investigation has shown the involvement of 137 different serotypes in nearly 2100 cases. Four serotypes, viz. S.dublin, S.typhimurium, S.cholerae-suis and S.abortus ovis accounted for 91.6 per cent of total isolates. The most common serotype - S.dublin was found to be associated with diseases of all animals including goats. S.dublin was obtained from 46.3 per cent of disease conditions in sheep, whereas in cattle from 78.9 per cent of cases. Infection due to S.typhimurium accounted for 19 per cent of the total incidence, ranging in different degrees in various animal hosts. S.cholerae-suis was predominantly isolated from diseases of pigs, whereas S.abortus ovis was entirely confined to sheep (1.3 per cent). Palade (1976) isolated several strains of S.poona from diarrhoeic Nigerian goats which had close contact with human beings in Nigeria, which points to the zoonotic problems involved in Salmonellosis. Two new serotypes, viz. S.denver and S.avonmouth were isolated

by Falade (Loc.cit) from the faeces of healthy Nigerian goats which indicated the existence of clinical and latent Salmonella infection in goats.

Haemolysin production by E.coli

Hamforth and Dudgeon (1952) reported that the haemolytic activity of E.coli was definitely influenced by the presence of calcium salts in the medium whereas any physical or chemical treatment of the organism reduced the haemolytic power. The haemolytic activity of the strains did not appear to have any relation to their pathogenicity when tested in rabbit ileum (Bhattacharya and Sarkar, 1956). The occurrence and role of haemolytic E.coli in gastroenteritis were more, compared to non-haemolytic E.coli, when studies on gastro-enteritis in cattle, sheep and man were made (Smith, 1963). According to him, haemolysin production by E.coli need not always be considered as a factor augmenting its pathogenicity. Haemolysin produced by E.coli was characterised into alpha and beta types based on its physico-chemical and serological characters (Smith, 1963). Alpha haemolysin is obtained free from the bacterial cell by centrifugation and filtration and its biological activity is inhibited by antiserum, whereas beta haemolysin is cell bound and is not inactivated by antiserum. The genetic factor which controls production of haemolysin present in E.coli strains freshly isolated from animals is shown to transfer this factor to non-haemolytic

recipient bacteria (Cogaoz, 1972). Hasiballa and Zubier (1978) conducted experimental pathogenicity studies in pregnant guinea pigs using a haemolytic culture of E.coli isolated from a case of bovine abortion and found that guinea pigs inoculated parenterally, aborted after seven days and those infected by oral route delivered normally.

Necrotoxin production by E.coli

Cooke (1968) studied the necrotoxin producing ability of E.coli isolated from disease conditions as well as from normal persons. He made isolations of E.coli from 47 ulcerative colitis, 44 acute diarrhoea and 49 normal cases. Out of these isolates, fourteen, three and one were necrotoxin producers respectively, when tested on rabbit skin intradermally. Pande et al. (1974) observed occurrence of 9.2 per cent of necrotoxin producing E.coli when tested 120 strains of organisms isolated from acute diarrhoea in human cases. Bisht et al. (1977) reported a higher incidence of haemolysin and necrotoxin production in E.coli isolated from extraintestinal origin. They came to the conclusion that haemolysin and necrotoxin properties of E.coli, though indicative of pathogenicity, cannot finally label a strain pathogenic, as a strain possessing both these properties may be entirely non-pathogenic.

Enterotoxin production by E.coli

The strain of E.coli that causes gastroenteritis is distinguished from others by its ability to form an enterotoxin, demonstrable by the secretion of fluid and distention of ligated loops of intestine in rabbits (McNaught and Roberts, 1958; Smith and Halls 1967 a; 1967 b; Larivire et al., 1972; Smith and Lingood, 1972; Stately et al., 1974; Cantely and Blake, 1977; Echeverria et al., 1977; Ansari et al., 1978). However, Smith and Halls (1967 b) have observed that for testing the enterotoxin production by E.coli isolated from a particular species of animal, the gut loops of the individuals of the same host are most suitable. Cooke (1968) used lamb ligated intestinal segment (LIS) for detection of enterotoxin production and classified the reactions in LIS as severe (large amount of exudate composed mainly of red blood cells), moderate (less amount of fluid with red blood cells) and mild (small amount of fluid with no distention of villi). Smith and Sack (1973) showed that the enterotoxins produced by different serotypes of E.coli are immunologically cross reactive, related to cell wall antigens, and antigenically related to Vibrio cholerae enterotoxin. Ansari et al. (1978) have reported that the posterior portion of the ileum may react poorly to enterotoxigenic E.coli in the lamb ligated intestinal segment.

Johnson et al. (1978) studied two types of enterotoxins. The most well studied enterotoxin, designated as heat labile enterotoxin (LT), is of high molecular weight, proteinaceous material exhibiting many similarities to Vibrio cholerae toxin. Heat stable enterotoxin (ST) on the other hand, is a low molecular weight compound which is non-immunogenic. Enterotoxins of E.coli contain LT alone or both LT and ST. They further showed that ST elaboration was detectable in the early logarithmic phase of growth and appeared to be related to the disappearence of glucose in the growth medium. Kanowalchauk et al. (1978) studied a heat labile cytotoxin (VT) affecting vero cells. The VT differed from the known LT in that VT did not affect cells commonly used for the quantitation of E.coli LT, nor infant mice used for the detection of ST and little or no response was noted in rabbit ileum.

Keusch et al. (1972) inoculated Shigella enterotoxin into ligated ileal loops of rabbits and found that by sixth hour, epithelial cells were shortened, with a decreased villus-to-crypt ratio and with many intact or degenerating transmigrating lymphocytes. Flores et al. (1974) demonstrated the enterotoxin produced by Shigella dysentriae type 1 which was able to elicit secretion of fluid in ligated segments of rabbit ileum and this secretion was characteristic in that it was rich in bicarbonate and low in protein content. However, the role of this organism in the pathogenesis of

diarrhoea has not been universally accepted. In animals, Shigella enterotoxins produce two cardinal signs of Shigellosis, viz. secretion of water and electrolytes which could account for the characteristic early diarrhoeic phase of the disease and cytotoxic epithelial cell damage which could account for the later colitis phase (Keusch and Jacewicz, 1977).

Pathogenicity to experimental animals

E.coli.

In the establishment of infection with pathogenic bacteria, it has been considered that the attachment of the bacteria to the surface of the mucosal epithelium is the essential step. Certain strains of E.coli were shown to be capable of adhering to the mammalian erythrocytes, intestinal epithelial cells and others by the aid of fimbriae in vitro (Duguid et al., 1955; Duguid et al., 1966; Tanaka and Katsube, 1978). Abdulla and Sulochana (1965) studied experimental pathogenicity of two strains of E.coli isolated from chicks and showed that the haemolytic and non-haemolytic strains tested were non-lethal to rabbits and guinea pigs. The failure to reproduce the disease in experimental hosts suggests that the resistance of the animal is an important factor in the dissemination of infection. Gorril (1965) produced pyelonephritis in mice, following intravenous injection of E.coli

and found considerable persistence of E.coli in the kidneys. Dam (1967) found that intraperitoneal infection of mice with the three common O groups of E.coli producing colisepticaemia in calves, had a decreasing virulence for mice in the order 078, 0115 and 015, which corresponded to the virulence in calves. The strain of E.coli 015 isolated from cases of diarrhoea in rabbits was administered orally to healthy rabbits in doses ranging from 1.5×10^2 to 4×10^{10} bacteria in order to assess the possible enteropathogenicity. The inoculated rabbits did not show any signs of illness or systemic disturbances (Cantely and Blake, 1977). Habiballa and Zubier (1978) conducted experimental pathogenicity studies in guinea pigs, using a haemolytic culture of E.coli isolated from a case of bovine abortion. They found that guinea pigs inoculated parenterally, aborted after seven days and those infected by oral route delivered normally.

Salmonella.

Ghosh and Chatterjee (1960) induced experimental infection with an 18 hour old broth culture of S.dublin, which caused death in guinea pigs and rabbits within a period of four to six days. S.typhimurium was also found to be pathogenic to rabbits both by oral and parenteral routes. Khanna and Uppal (1964) produced experimental infection in mice using one ml dilutions of a 48 hour old nutrient broth culture of S.typhimurium containing 10^6 organisms administered

intraperitoneally. All the inoculated mice succumbed to disease, and death occurred within four weeks. Kampelmacher et al. (1969) reported that persistence of S.typhimurium infection in rats was directly correlated with age of the rats and dosage. According to them, mortality after intraperitoneal infection in mice was not related to persistence of organisms in the system. The results were greatly influenced by differences between individuals and groups. Sharma and Singh (1970) conducted pathogenicity studies of five new Salmonella serotypes, viz. S.brijbhumi, S.vrindaban, S.gokul, S.goverdhan and S.mathura in mice. Mice inoculated with 10^8 viables of S.goverdhan died 24 hours post-inoculation and organisms were recovered from internal organs. None of the mice inoculated with other four Salmonella could kill mice. Makela et al. (1973) reported that intraperitoneal infection with as few as 100 - organisms of virulent S.typhimurium produced generalised infection and death in mice within five to ten days time.

Pathogenicity to natural host

E.coli.

Taylor (1966) studied the toxic effects of the extracts of somatic antigens from E.coli and several serotypes of Salmonella in human beings and had shown that there was little difference between them; extracts of E.coli were similar in effect to extracts of S.typhi. Smith and Halls (1967 b)

recorded the importance of enterotoxins in the pathogenesis of diarrhoea in natural hosts (pigs) and suggested that enterotoxin was important only in the pathogenesis of diarrhoea and the significant feature of individual strains that caused diarrhoea depended on their ability to proliferate in the anterior dpart of small intestine. Wedearis et al. (1968) demonstrated the role of cell wall lipopolysaccharide of E.coli for its antiphagocytic activity and virulence. Stately and Anderson (1970) observed acute inflammatory changes in the mucosa of domestic animals after exposure to invasive strains of E.coli. Arbuckle (1971) demonstrated the ability of some of the intestinal bacteria to produce an enzyme mucinase, and that this mucinase reduced the mechanical protective and lubricating properties of mucus in the intestinal tract. Thus this factor plays an important role in the pathogenesis of intestinal disorders. He also showed that the non-enteropathogenic E.coli were also capable of producing as much mucinase as enteropathogenic strains, but they were not able to colonise the mucopolysaccharide layer, to the extent of the enteropathogenic strains. Menshikova and Boltaev(1975) infected day old gnotobiotic lambs orally with E.coli serogroup 078 and found degenerative changes in the epithelial cells of the duodenum and fragmentation of microvilli with complex destruction and lysis. Moon (1978) reported that enterotoxins contribute to the accelerated transit of intestinal contents in enterotoxic Colibacillosis. Carson et al.(1978)

could recognise septicaemic and enteric forms of colibacillosis in neonatal calves, but they could not reproduce the enteric form experimentally.

Salmonella.

Pathogenicity of Salmonella is more or less localised to the intestinal wall. The presence of fimbriae increased the ability of bacterium to adhere to cells which would contribute to the virulence of the organism as demonstrated by Duguid et al. (1966). The importance of O antigens of Salmonella for the increased virulence of the organism to natural as well as experimental hosts was studied by Makela et al. (1973). Brown et al. (1976) infected several lambs with S.typhimurium via oral route. The dose of the infective agent ranged from 2×10^3 to 1×10^9. The infected lambs were slaughtered at definite intervals to determine the pathogenesis of the organism. The cultural examination of the faeces from live infected lambs was done to evaluate the efficiency of this diagnostic procedure. They have shown that 2×10^{11} organisms are always lethal to lambs under test. It was also recorded that there was no correlation between the immune response and cultural recovery of organisms from the gut within one or two weeks after an experimental dose of 10^3 to 10^4 organisms. The intranasal inoculation of a similar dose resulted in carriage of S.typhimurium for six weeks in the system. After trying to infect calves via different

routes with Salmonella, Nazer and Osborne (1977) concluded that alimentary infection was the most common route by which calves could be infected and thus common foci for natural spread. Spence and Westwood (1978) described an experimental infection of sheep with S.agona, which was isolated from an outbreak of Salmonellosis in sheep. None of the experimentally infected sheep died, even after months. At necropsy, S.agona could not be isolated from any of the visceral organs but cultural recovery of the organism was possible from faeces, even after 69 days of incubation.

Antibiotic sensitivity

E.coli.

The WHO expert committee (1961) had recommended standards of quality for commercial antibiotic sensitivity testing discs and they grouped the in vitro antibiotic sensitivity tests into two main catagories, viz. diffusion methods and dilution methods. Patersdorf and Cherris (1965) standardised an interpretation chart to designate the organisms as resistant/sensitive/intermediary, which helped in interpreting the results of antibiotic sensitivity tests. Blair et al. (1970) adopted antibiotic impregnated filter paper disc and compressed tablets for testing the ability and suitability of diffusion method of antibiotic sensitivity of bacteria. According to him, the use of filter paper disc diffusion method was most suitable, reliable and convenient.

Davis et al. (1973) studied the nature and functions of plasmid mediated drug resistance in E.coli. They showed that the R-factor consisted of Resistance transfer factor (RTF) and Resistant determinant (R-determinant) genes for drug resistance. They also showed that in E.coli, these two parts are found as one unit. However, in Salmonella and Proteus, they were often found as seperate plasmids. R-determinant plasmids were not transferred unless they fused with a transfer factor. Pandhy (1975) reported that if a population of bacteria were grown in the presence of tetracycline, most of them would be killed, but those carrying the genes for antibiotic resistance on their plasmids would continue to grow in the presence of antibiotics. Choudhary et al. (1976) in their investigation on the occurrence of antibiotic resistant strains of E.coli from goats have observed that maximum number were sensitive to kanamycin (94 per cent) at 10 mcg/ml. The isolates were least sensitive to erythromycin and chlortetracycline. Heller and Drabkin (1977) in their study showed that resistance of E.coli to sulfonamides and oxytetracycline was by far the most common in chicken and turkey isolates.

Salmonella.

McWhorter et al. (1963) isolated 652 strains of Salmonella and found that S.typhimurium had a much higher incidence of resistance to chlortetracycline than other

serotypes. Triskina (1968) screened 1530 strains of Salmonella for their antibiotic resistance. During the study he had obtained two strains of S.cholerae-suis and one strain of S.typhimurium with resistance to higher concentration of chlortetracycline (37 to 75 mcg/ml) and oxytetracycline (100 to 300 mcg/ml). Hooper and Hirsh (1975) demonstrated the possible transfer of resistance from the resident flora to the non-invasive, rough, avirulent variant in animals with Salmonella infection. Falade (Loc.cit.) isolated strains of S.poona from diarrhoeic Nigerian goats and on antibiotic sensitivity studies, they were found sensitive to nitrofurantoin (200 mcg), chloramphenicol (10 mcg), polymyxin B (100 mcg), streptomycin (10 mcg) and tetracycline (10 mcg) in descending order. Sojka and Hudson (1976) isolated several strains of Salmonella from cattle, sheep, pig and poultry and tested their susceptibility to antimicrobial agents. The percentage of total isolates resistant to sulfonamides (50 mcg), neomycin (10 mcg), tetracycline (10 mcg), chloramphenicol (10 mcg), furazolidone (15 mcg) and ampicillin (10 mcg) were 34.1, 4.3, 3.0, 1.3, 0.8 and 0.5 per cent respectively. All strains were sensitive to trimethoprim.

The antibiotic susceptibility of Enterobacter hafniae and Enterobacter liquefaciens was tested by Washington et al. (1971) and found that these organisms were most susceptible to gentamicin, kanamycin and chloramphenicol.

MATERIALS AND METHODS

MATERIALS AND METHODS

Collection of materials

Materials for this study were collected from the following sources.

a) All India Co-ordinated Research Project on Goat for Milk Production, Mannuthy.

b) Veterinary hospitals in and around Trichur town.

Ailing animals.

Rectal swabs were collected from 60 goats of different age groups manifesting clinical signs of gastroenteritis. Sterile cotton swabs moistened in sterile nutrient broth were used for collection of the material.

Dead animals.

Specimens collected aseptically from dead animals having history of gastroenteritis included intestinal contents (40), small intestine (30), large intestine (22), mesenteric lymph nodes (38), heart blood (8) and lung tissues (34). The details of the specimens are shown in table I.

Laboratory procedures for Isolation and Identification

Escherichia coli.

Rectal swabs and faecal materials.

Rectal swabs and faecal materials moistened in sterile nutrient broth were incubated aerobically for 18 hours at 37°C as described by Pande and Acharya (1965). These were

later plated on MacConkey Lactose Bile Agar (MLBA) and five per cent bovine blood agar and were incubated at 37°C for 24 hours. Typical lactose fermenting colonies on LMBA and smooth, colourless, circular, convex colonies on blood agar, were subcultured on nutrient agar slant for further confirmative tests. In addition to the usual Gram's staining method, the Gram reaction of the organisms was confirmed by the Potassium hydroxide test (Gregerson, 1978), by emulsifying few colonies with one or two drops of three per cent potassium hydroxide on a glass slide. After five to ten stirring, the inoculation loop was raised from the drop. If the potassium hydroxide solution have become viscous, a thread of slime follows the loop 1½ to 2 cm or more which indicates a Gram-negative reaction. The lactose fermenting colonies were further plated on Eosin Methylene Blue Agar (EMB). After incubation at 37°C for 24 hours, dark bluish green colonies with a metallic sheen around them were subjected to various biochemical tests as described by Edwards and Ewing (1972). The details of biochemical reactions are shown in table II.

Tissues.

The tissues were flamed following immersion in methyl alcohol in order to avoid surface contamination. Two to three grams of the tissues were emulsified in sterile mortar and pestle with aliquot normal saline making approximately ten per cent suspension. A loop full of this material was inoculated on MLBA and five per cent bovine blood agar.

Colonies developed in the medium were identified in the same lines as described above.

Haemolysin production

Tests for haemolysis were done both in solid and liquid media.

Solid medium. (Smith, 1963).

Petri dishes containing bovine blood agar (five per cent blood) were inoculated with the culture identified as E.coli and were incubated aerobically at 37°C for 24 hours. Haemolytic colonies observed were selected for further experimental studies.

Liquid medium.

E.coli were inoculated in a medium containing peptone water (one per cent bacteriological peptone and 0.85 per cent sodium chloride in distilled water) and two per cent bovine blood. The inoculated tubes were incubated at 36°C for 48 hours. Haemolysin production was assessed as per the guide lines described by Cooke (1968).

Salmonella.

The specimens (Rectal swabs, faeces and tissues) were inoculated directly on MLBA and Brilliant Green Agar (BGA) containing one per cent sodium citrate, in order to suppress swarming Proteus and were incubated at 37°C. The inoculated plates were examined at 24 hour intervals for a period of

72 hours. Typical non-lactose fermenting colonies of MLBA or pink colonies on BGA were picked up and transferred to Triple Sugar Iron Agar (TSI) for detection of hydrogen sulphide production. Those which produced hydrogen sulphide were tentatively identified as Salmonella. The identity of the isolates was further confirmed by biochemical reactions as detailed in table III.

Enrichment procedure.

In order to obtain better percentage of isolation of Salmonella the following procedures were also employed. The specimens were emulsified in sterile mortar and pestle with normal saline. Twenty ml test tubes half filled with enrichment media were used for cultural studies. The emulsified materials were inoculated at a ratio of two ml to ten ml of enrichment media which included Selenite F broth/Tetrathionate broth. The inoculated tubes were incubated at 37°C for 18 hours. After incubation, they were subcultured on BGA and MLBA. The final identification of the isolates was done in the line as described before.

Enterobacter.

Attempts for the isolation of Enterobacter were made on the lines described by Edwards and Ewing (1972) The materials were directly inoculated into MLBA. After incubation at 37°C for 24 hours, typical lactose fermenting mucoid colonies were removed and tested for Enterobacter on the basis of their biochemical reactions shown in table IV.

Pathogenicity studies

Escherichia coli.

Mice.

Isolate which was haemolytic on five per cent bovine blood agar was tested for its pathogenicity to mice. White Swiss mice aged 30 to 45 days were used for the study. They were divided into two batches, each consisting of four animals. The first batch of mice was injected subcutaneously with 0.1 ml of the saline suspension of the organism containing approximately 15 x 10^4 organisms/ml. The second batch was also injected by intraperitoneal route with the same dose. The control mice were given 0.1 ml of normal saline. The animals were closely observed for signs of illness. Peripheral blood smears prepared from all the experimental mice at 24 hour intervals were stained and examined for the presence of bacteria. Materials collected from the dead as well as sacrificed animals were examined. Animals which withstood infection were sacrificed on seventh and eighth day and tissues were examined in detail (Table V).

Necrotoxin production in rabbits.

The ability of haemolytic E.coli to produce necrotoxin on rabbit skin was studied using the technique described by Cooke (1968). The fur on the back of the rabbit was removed and the area was sterilized and 0.1 ml of five hour old culture of E.coli in peptone water was given intradermally.

The site was examined daily for six days and the developments of induration and ulceration were recorded. Animals were destroyed on sixth day and skin sections prepared from the site of inoculation were examined for histopathological changes.

Enterotoxin production.
(Rabbit ligated gut loop reaction)

Two isolates of E.coli (one non-haemolytic, EC/15 and one haemolytic EC/11) were tested for dilatation reaction in rabbit ileal loop using three rabbits for each isolate (Group I and II). The test materials were prepared by the following methods.

Method 1. (Peptone water culture)

An 18 hour old peptone water culture of E.coli was prepared (Cooke, 1968).

Method 2. (Soft agar culture fluids)
(Smith and Halls, 1967 b).

The procedure consisted of growing the E.coli in 230 ml flat bottles each containing 40 ml of nutrient agar (agar content 0.36 per cent) with 0.2 per cent glucose. After incubation for 24 hours at 37°C the bottles were cooled to 5°C and the fluid was expressed from the medium by gentle squeezing through sterile muslin cloth and centrifuged at 12,000 rpm for 30 minutes to remove most of the bacteria present. The supernatant was collected and the remaining bacteria were killed by heating at 65°C for ten minutes. Neomycin 100 mcg/ml

was also added to the supernatant fluid to prevent the multiplication of any E.coli that might be present in the intestinal segments into which the fluid has to be injected. The test fluid was stored in 20 ml amounts at -20°C until required. The pH of the test material was adjusted to 6.5.

Method 3. (Acetone precipitated culture fluids)

To the supernatant fluid obtained from the previous method, eight volumes of acetone were added and the mixture was kept at -20°C for 18 hours for precipitation of the toxin. The supernatant fluid was discarded and the container was held at 37°C until the precipitate became dry. The precipitate was then dissolved in distilled water to a volume equivalent to that of the culture fluid from which the deposit was obtained. Neomycin 100 mcg/ml was added and pH adjusted to 6.5 (Smith and Halls, 1967 b).

The rabbits were anaesthetised with chloroform. The abdomen was opened and the small intestine was located. The ileum was then ligated at regular intervals so as to form segments of about four cm in length. Each rabbit of Group I was given peptone water culture and soft agar culture fluids of non-haemolytic E.coli in seperate ileal loops, in one ml volume, and the segment adjacent to the test segments was used as control. The same procedure was adopted for haemolytic strain also, using the peptone water culture and acetone precipitated culture fluids. The controls used for the peptone water culture and bacteria free extracts were peptone water

and nutrient broth respectively (Table VI).

Salmonella.

Mice.

One isolate was tested for its pathogenicity to mice. White swiss mice 30 to 45 days old were used for the study. They were divided into two batches, each batch consisting of four animals. The first batch of mice was injected subcutaneously with 0.1 ml of the saline suspension of the organisms containing approximately 15 x 10^4 organisms/ml and the second batch of mice with the same dose by intraperitoneal route. The control mice were injected with 0.1 ml of sterile normal saline. Since the inoculated animals did not show any signs of illness for a period of seven days, they were sacrificed on eighth, ninth and tenth day following inoculation and the internal organs and intestinal contents were cultured on enrichment media for isolation of Salmonella (Table V).

Antibiotic sensitivity

The following antimicrobial agents were employed for the study - ampicillin (10 mcg), bacitracin (10 mcg), chloramphenicol (30 mcg), erythromycin (15 mcg), gentamicin (10 mcg), kanamycin (30 mcg), furazolidone (300 mcg), penicillin (10 units), streptomycin (10 mcg), tetracycline (30 mcg) and sulfadiazine (300 mcg).

Disc preparation.

Discs of six mm diameter were punched from Whatman No.1 filter paper and were sterilized in hot air oven at 140°C

for one hour. Standard suspensions of antibiotics were made according to the technique described by Blair et al. (1970). One drop of the dilution which would provide optimum concentration of antibiotic for the experimental purpose was absorbed in each disc. These discs were stored in sterile vials at 4°C until used. Antibiotic incorporated discs were used within one month of their preparation.

Test medium.

The medium used for this study was nutrient agar, with an average of six mm thickness of agar medium when poured in petri dishes.

Preparation of inoculum.

The isolates to be tested were grown on blood agar medium for a period of 24 hours. Nutrient broth tubes were inoculated with five colonies removed from blood agar plates and incubated until there was moderate cloudiness indicating uniform growth of the organism.

Inoculation of plates.

Nutrient agar plates were inoculated by placing a few drops of the nutrient broth culture. The excess inoculum, if present, was removed by using sterile pipettes after spreading the inoculum uniformly by a sterile glass rod. The plates were then allowed to dry in the inverted position at room temperature for five minutes.

Application of discs.

The discs containing different antibiotics were plated on the medium, suitably spaced, and the plates were incubated at 37°C. Not more than six antibiotics were tested on a petri dish of standard diameter of four inches.

Interpretation of results.

The diameter of the zone around each disc in which no growth was macroscopically visible was measured with a pair of calipers after overnight incubation of the plates. The findings were recorded and interpreted based on the guide lines suggested by Balir et al. (1970). The details of the experiment are illustrated in appendix I.

RESULTS

RESULTS

A total of 190 specimens consisting of rectal swabs, intestinal contents, portions of large and small intestines and mesenteric lymph nodes collected from live/dead animals were examined for enteric pathogens. Eighty-six isolates of Escherichia coli, two isolates of Salmonella and 39 isolates of Enterobacter cloacae were obtained (Table I). Eight samples of heart blood and 38 specimens of lung tissues collected from goats that showed gastroenteritis on post-mortem examination were also examined. From these specimens, seven isolates of Streptococcus pyogenes (from heart blood), 15 isolates of Klebsiella pneumoniae (lung tissues) and one isolate of Corynebacterium pyogenes (lung tissues) were isolated.

Isolation and Identification

Escherichia coli.

Eighty-six E.coli isolates (45.26 per cent) were obtained from a total of 190 specimens examined. Out of these 86 isolates, 33 (55 per cent) were from 60 rectal swabs, 41 (44.56 per cent) from 92 specimens of intestinal contents/small intestine/large intestine and 12 (31.58 per cent) from 38 mesenteric lymph nodes. Pink colonies of MLDA and dark bluish green colonies with a metallic sheen on EMB agar were observed, which are typical for E.coli. All the isolates

have shown more or less the same reactions in artificial media. Typical Gram negative reaction was observed on Potassium hydroxide test. All the isolates gave positive biochemical reactions suggestive of E.coli. However, the fermentation reactions of a few isolates were found to be varied in sucrose, salicin, raffinose and rhamnose. Further, six isolates were found to be non-motile (EC/7, EC/28, EC/34, EC/39, EC/70, EC/78). The results of the biochemical reactions of 20 representative isolates including some of the isolates which gave varied biochemical reactions are shown in table II.

Haemolysin production

Among 86 isolates of E.coli tested, only one (EC/11) was found to produce haemolysin (alpha haemolysin) in solid and liquid media which contained bovine blood.

Salmonella.

Two isolates of Salmonella were obtained from 190 specimens examined and these were from the rectal swabs of apparently healthy goats. The isolates showed typical reactions on MLBA (colourless colonies), EGA (pink colonies) and triple sugar iron agar (acid butt and alkaline slant with blackening of the medium). Both the isolates were motile and they showed biochemical reactions typical of Salmonella. The results of the biochemical reactions are shown in table III.

Enterobacter.

A total of 39 isolations of Enterobacter were made during the course of the study. Out of these, ten isolates were from the rectal swabs of living animals and 29 from tissues of dead animals. All the isolates were identified as Enterobacter cloacae, based on their morphological, cultural and biochemical reactions. On primary isolation, the colonies were mucoid and lactose fermenting on MLSA. Five isolates (EN/8, EN/12, EN/13, EN/20, EN/32) were shown to have capsule on primary culture. On subsequent culturing in artificial media, these organisms were found to lose the property of capsulation. All the isolates were motile and gave a positive Voges-Proskauer reaction. All exhibited identical biochemical reactions except four (EN/3, EN/21, EN/32, EB/36), which failed to produce a positive test in salicin. The results of the biochemical reactions of 20 representative isolates of Enterobacter cloacae including those that gave varied cultural and biochemical reactions are summarised in table IV.

Pathogenicity studies

Escherichia coli.

Mice.

One haemolytic E.coli (EC/11) obtained from the intestine of a goat died of gastroenteritis was tested for its pathogenicity to mice. The peripheral blood smears prepared at

24 hour intervals from inoculated animals did not reveal the presence of bacteria during a period of three days. All the four mice inoculated intraperitoneally died on the third day following injection. No gross lesions could be observed in the internal organs in any of these animals. However, E.coli could be recovered in pure culture from the heart blood and intestines of dead animals. On histopathological examination, tissues collected from the dead animals did not show any change except in myocardium where haemorrhagic areas were observed.

Two mice which were inoculated with E.coli subcutaneously, died on the fifth day of inoculation and the other two were sacrificed on seventh and eighth day following injection. Organisms could be isolated from the heart blood and intestines of these animals. There was no pathological lesion in the internal organs.

Necrotoxin production in rabbits.

The haemolytic strain of E.coli (EC/11) was tested for its ability to produce necrotoxin on rabbit skin. No gross lesions could be observed on the skin within four days following intradermal inoculation of the culture. However, on fifth day, the inoculated site appeared slightly necrotic and the thickness of the skin was found to be increased to four millimeters as against a normal skin thickness of one millimeter (Fig.1). On histopathological examination of skin on sixth day, the following lesions suggestive of

necrotoxin production could be observed. A focal encapsulated necrotic area was in the dermis which consisted of disintegrating inflammatory cells with other cellular debris. Reparitive fibrous tissue formation was seen extending into the lesion from the periphery (Fig. 2).

Enterotoxin production.

(Rabbit ligated gut loop reaction)

One non-haemolytic (EC/15) and one haemolytic (EC/11) E.coli were tested for their dilatation reaction in rabbit ileum. The three rabbits which received peptone water culture and soft agar culture fluids prepared from non-haemolytic E.coli were found dead at 20, 23 and 24 hours respectively, following administration of the test materials into ileal loops. The carcases were opened immediately and the changes in the ileal loop were observed. Ileal loops of all the three rabbits which received the test materials showed dilatation reaction while the control segments did not (fig. 3). In all the three cases the fluid collected in the ileal loop was approximately 15 ml in quantity. Further, the fluid was sanguineous and contained shreds of fibrin. On histopathological examination, sections from the ileal segments which received soft agar culture fluid showed lesions of enteritis. There was extensive damage to the intestinal mucosa and submucosa with infiltration of large number of inflammatory cells, mostly mononuclear and lymphoid types. A few neutrophils

were also observed (Fig.4). The lesions seen in ileal segments which received peptone water culture showed necrotic change in the mucosa. Infiltration of inflammatory cells was also noticed.

The three rabbits which received the peptone water culture and acetone precipitated culture fluids prepared from haemolytic E.coli died at 21, 23 and 24 hours respectively, following administration of the test fluids. On postmortem examination, no dilatation reaction was observed in the intestine in any of these rabbits. Both experimental and control segments were apparently normal on macroscopical examination. However, on histopathological examination, the inoculated segments revealed moderate necrotic changes, whereas, no abnormality could be observed in control segments. The results of the experiment are shown in table VI.

Salmonella.

Mice.

One isolate (S/1) was tested for its pathogenicity to mice. All the mice injected subcutaneously and intraperitoneally, resisted infection and they were sacrificed on eighth, ninth and tenth day of inoculation. No pathological change could be seen in any of the internal organs, although Salmonella could be recovered from the intestines.

Antibiotic sensitivity

One hundred and twenty-seven bacterial organisms isolated during this study (86 E.coli, 39 Enterobacter cloaca and two Salmonella) were tested for their sensitivity to various chemotherapeutic agents. The cultures which were considered sensitive to a chemotherapeutic agent included those which showed intermediate sensitivity also (Fig. 5). The results are illustrated in table VII.

Escherichia coli.

Sensitivity of 86 isolates of E.coli to 11 chemotherapeutic agents was studied. All the isolates were sensitive to gentamicin. Eighty-two isolates (95.35 per cent) were sensitive to nitrofuran, 76 (88.37 per cent) to chloramphenicol, 52 (60.47 per cent) to kanamycin, 35 (40.70 per cent) to streptomycin, 7 (8.14 per cent) to tetracycline and two (2.33 per cent) to erythromycin (Fig. 6). However, all the isolates tested were found to be resistant to ampicillin, bacitracin, penicillin and sulfonamide.

Salmonella.

Sensitivity pattern of two isolates of Salmonella to 11 chemotherapeutic agents had shown that both the isolates were sensitive to chloramphenicol, gentamicin, nitrofuran and streptomycin. But they were found to be resistant to ampicillin, bacitracin, erythromycin, kanamycin, penicillin, sulfonamide and tetracycline.

Enterobacter.

All the 39 isolates of Enterobacter cloacae were sensitive to gentamicin and kanamycin, whereas 30 isolates (76.92 per cent) were sensitive to chloramphenicol and nitrofuran and 15 isolates (38.46 per cent) to streptomycin (Fig. 7). However, all the isolates were resistant to ampicillin, bacitracin, erythromycin, penicillin, tetracycline and sulfonamide.

DISCUSSION

DISCUSSION

During the present study, a total of 190 specimens were screened for enteric pathogens and 86 isolates of E.coli, 39 Enterobacter cloacae and two Salmonella were obtained. The results of this study indicate E.coli as the major etiological agent in the causation of gastroenteritis, since majority of the bacterial isolates were E.coli (67.72 per cent). This higher percentage of isolation of E.coli establishes the definite role of this organism in producing enteritis in goats. This finding is in close agreement with the observations made by Pande and Acharya (1965), who identified E.coli as the major etiological agent in the causation of gastroenteritis in goats. E.coli was not isolated from any of the specimens other than gastrointestinal tract and mesenteric lymph nodes. Further, the heart blood from animals with signs of enteritis was also found negative for the presence of E.coli. Based on the present observation, it may be appropriate to consider that bacteraemia/septicaemia may not be a common feature in Colibacillosis. However, Pearson et al. (1977) have recorded distinct septicaemic and enteric forms of Colibacillosis in neonatal calves.

Majority of the isolates of E.coli (41 isolates) recovered during this investigation were from intestines of dead animals that showed lesions of gastroenteritis, while only 12 isolates were obtained from mesenteric lymph nodes.

The rest of the isolates (33) were from the rectal swabs of living animals. In support to this observation, Sojka (1965) reports to have made only few isolations of E.coli from mesenteric lymph nodes while majority of recoveries were from the rectal swabs of ailing animals.

The biochemical reactions of most of the isolates were in complete agreement with those described for the genus Escherichia by Edwards and Ewing (1972). However, a few of them exhibited varied biochemical reactions to salicin, raffinose and rhamnose which can be considered as a common feature to the members of the family Enterobacteriaceae especially to the coliforms as suggested by Greaves (1970). Out of the 86 E.coli isolates studied, six were found to be non-motile. Occourrence of non-motile species of E.coli has been reported by several workers in the past (Edwards and Ewing, 1972; Wilson and Miles, 1975).

Among the E.coli isolates, only one, (EC/11) was haemolytic when tested on solid and liquid media. This particular strain was isolated from the intestine of a goat which died of enteritis. Since only one haemolytic strain of E.coli was isolated from gastroenteritis during the study, no conclusion can be drawn about the participation of haemolytic strain of E.coli in causing gastroenteritis in goats. Bisht et al. (1977) also reported higher incidence of haemolysin production in E.coli isolated from extra-intestinal

sources. According to Smith (1963), haemolysin production by E.coli need not be considered as an indication for its pathogenicity.

Pathogenicity studies in mice using the haemolytic E.coli have shown that all the four animals that received the organism intraperitoneally and the two inoculated subcutaneously died on third and fifth day respectively following inoculation. Although organisms could be recovered from heart blood and intestines of these animals, no apparent lesions could be observed in any of the internal organs. The mice which were inoculated intraperitoneally did not survive more than three days while those inoculated subcutaneously could survive for a longer period. Moreover, two animals which were inoculated subcutaneously resisted infection, till they were sacrificed on seventh and eighth day. Hence, the route of injection seems to have a bearing on the time of onset of the disease syndrome in experimentally infected animals.

The strain of E.coli (EC/11), which was haemolytic and pathogenic to mice has produced lesions on the skin of rabbits when tested for its ability to produce necrotoxin. The lesions observed were similar to those described by other workers (Cooke, 1968; Pande et al., 1974) in their studies on the effect of necrotoxin on tissues. It is interesting to note that, haemolysin and necrotoxin production, and

pathogenicity to mice are interlinked properties of the organism. Pande et al. (1974) reported that, the strains possessing the property of producing haemolysin and necrotoxin are definite pathogens whereas, those possessing either of these are potential pathogens.

The six rabbits used for demonstration of enterotoxin production died at varying intervals of 20 to 24 hours. The reasons for the early death of rabbits may be attributed to the absorption of relatively larger quantities of bacterial polysaccharides or toxins from the intestines or due to an anaphylactic shock as suggested by Stevens (1963). The present study has shown that, only the non-haemolytic strain produced dilatation reaction and the haemolytic E.coli though proved to be a necrotoxin producer did not induce any change in the ileal loop. This observation is in agreement with the views of Bhattacharya and Sarkar (1956) in that, the haemolytic activity of the strains did not appear to have any relation to their pathogenicity as far as rabbit ileum is concerned. The failure of producing dilatation reaction by haemolytic E.coli in rabbit ileal loop cannot be considered as an indication of its inability to produce enterotoxin by itself. According to Smith and Halls (1967 b), rabbits are less reliable for ligated loop tests with some strains, and the tests will be of great value, when tested in the gut loops of the individuals of the same host from which the organisms are isolated.

On histopathological examination, ileal segments which received soft agar culture fluids of non-haemolytic E.coli showed extensive damage to the intestinal mucosa and submucosa with infiltration of large number of inflammatory cells suggestive of enteritis. On the other hand, the peptone water culture caused lesions suggestive of necrotic changes in the mucosa with infiltration of a few inflammatory cells. Similar observations were also made by Cooke (1968) and Smith and Halls (1967 b), in their studies using bacteria free extracts of the culture and peptone water culture. The rabbit ileum, in which haemolytic E.coli produced no macroscopic changes revealed only mild necrotic changes on histopathological examination.

In spite of the wide use of antibiotics in treating and controlling enteritis, the disease still continues to cause serious havoc to goat husbandry, particularly in young subjects. In vitro drug sensitivity study of bacterial isolates can serve as a useful tool in the hands of clinicians for combating the disease in field conditions to a greater extent. Sensitivity study of 86 isolates of E.coli to 11 chemotherapeutic agents has revealed cent per cent sensitivity to gentamicin. On the other hand, cent per cent resistance was noticed to ampicillin, bacitracin, penicillin, sulfonamide and most of the isolates were also found to be resistant to tetracycline. Resistance to sulfonamide and tetracycline has also been reported by Heller and Drabkin (1977) during a

detailed investigation on sensitivity pattern of E.coli. Moreover, they have also found that, E.coli is one organism which exhibit different drug sensitivity pattern. Chopra and Howe (1978) showed the acquisition of plasmids by the organism as the major cause of tetracycline resistance in the majority of clinical isolates. The present result shows that 95.35 per cent of the isolates are sensitive to nitrofuran, 80.37 per cent to chloramphenicol, 60.47 per cent to kanamycin, 40.70 per cent to streptomycin, 8.14 per cent to tetracycline and 2.33 per cent to erythromycin. These results are closely comparable to the findings of Choudhary et al. (1976) on the antibiotic resistant strains of E.coli from goats. They have observed marked sensitivity to kanamycin (94 per cent) and nitrofurazolidone (93 per cent) and least sensitivity to streptomycin, erythromycin and tetracycline.

Salmonella

Only two isolates of Salmonella were obtained from 190 specimens examined for enteric pathogens. For isolation of Salmonella, direct culture and enrichment culture procedures were adopted. Those two isolates of Salmonella were from specimens collected from apparently healthy goats and cultured by enrichment procedures. This observation points to the rare occurrence of Salmonella in goats, or to the limited role played by the organism in causing gastroenteritis. It is also worthwhile to note that direct culture procedure may be

inadequate to detect small number of Salmonella present in the gastrointestinal tract, since only enrichment procedure proved effective for isolation of Salmonella. Similar findings were also reported by Simmons and Sutherland (1969), where they considered ovine species as more resistant to Salmonella infection, when compared to other species of animals.

The results of the biochemical reactions of both the isolates of Salmonella were in complete agreement with those described by Edwards and Ewing (1972).

One isolate of Salmonella was tested for its pathogenicity to mice. All the mice injected subcutaneously and intraperitoneally resisted infection. No gross pathological lesions in the internal organs could be observed at necropsy even on the tenth day. However, organisms could be isolated from the intestine which indicate that, Salmonella could survive in the internal organs without producing apparent clinical signs. Isolation of Salmonella from apparently healthy goats, though their prevalence is much less, explains how animals can act as carriers, facilitating dissemination of infection to other animals.

Results of antibiotic sensitivity studies on the isolates of Salmonella have shown the sensitivity of the organism to chloramphenicol, gentamicin, nitrofuran and streptomycin. However, resistance to ampicillin, bacitracin, erythromycin, kanamycin, penicillin, sulfonamide and tetracycline was observed.

The resistance of Salmonella to tetracycline has been reported by several workers (Mc'horter et al., 1963; Triskina, 1968) and they found that S.typhimurium has got a much higher resistance to tetracycline than other antibiotics. The resistance of Salmonella to other chemotherapeutic agents like ampicillin, penicillin, neomycin and sulfonamide have also been reported by Sojka and Hudson (1976).

Enterobacter

Thirty-nine isolations of E.cloacae were made from a total of 190 specimens examined, which formed the second major group of isolate in this study. Some of them were recovered along with E.coli, from cases of gastroenteritis. It has been reported that E.cloacae is a normal inhabitant of the intestinal tract of man and animals under natural conditions (Wilson and Miles, 1975). The role of this species of organism in disease conditions in animals is yet to be determined. The possible role of this organism along with E.coli or other enteric pathogens in the pathogenisis of enteritis in goats need to be studied in detail.

Antibiotic sensitivity studies of 39 isolates of E.cloacae have revealed, cent per cent sensitivity to gentamicin, kanamycin and 76.92 per cent to chloramphenicol. Similarly, Washington et al. (1971) in their studies on Enterobacter species have also reported a higher percentage sensitivity of E.cloacae to gentamicin, chloramphenicol and

kanamycin. But cent per cent resistance was also noticed to ampicillin, bacitracin, erythromycin, penicillin, sulfonamide and tetracycline during the present study.

SUMMARY

SUMMARY

A total of 190 specimens were collected and processed from living as well as dead animals with the history of enteritis. Specimens included faeces, mesenteric lymph nodes and portions of intestine.

The enrichment and selective media used included Selenite F broth, Tetrathionate broth, Brilliant green agar and MacConkey lactose bile agar, in addition to the common media used in routine isolation of enteric pathogens.

A total of 86 isolates of E.coli, two Salmonella and 39 Enterobacter cloacae were isolated during this investigation. The identity of the isolates was confirmed by biochemical and biological tests described in standard text books. One isolate of E.coli was found to be haemolytic and this particular strain was found to be pathogenic to mice and able to produce necrotoxin on rabbit skin. Enterotoxin production in rabbit ileal segments was tested using different test materials prepared from haemolytic and non-haemolytic E.coli, the latter proved positive.

One isolate of Salmonella tested, was found to be non-pathogenic to mice, on experimental infection.

Seven isolates of Streptococcus pyogenes, 15 Klebsiella pneumoniae and one Corynebacterium pyogenes were also isolated and identified from heart/blood/lung tissues of animals having history of gastroenteritis.

According to the results obtained in the drug sensitivity study, the drugs of choice for E.coli are gentamicin, nitrofuran and chloramphenicol; for Enterobacter cloacae - gentamicin, kanamycin, nitrofuran and chloramphenicol and for Salmonella - gentamicin, chloramphenicol, nitrofuran and streptomycin.

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APPENDIX

Appendix I.

Zone size interpretative chart used for antibiotic sensitivity test (Blair et al., 1970)

Antibiotic/ chemotherapeutic agent	Disc.potency	Inhibition zone. Diamter in millimeters		
		Resistant	Intermediate	Sensitive
1. Ampicillin	10 mcg	20 or less	21-28	29 or less
2. Bacitracin	10 ,,	8 ,,	9-12	13 ,,
3. Chloramphenicol	30 ,,	12 ,,	13-17	18 ,,
4. Erythromycin	15 ,,	13 ,,	14-17	18 ,,
5. Gentamicin	10 ,,	--	--	13 ,,
6. Kanamycin	30 ,,	13 or less	14-17	18 ,,
7. Nitrofuran	300 ,,	8 ,,	9-12	13 ,,
8. Penicillin	10 units	20 ,,	21-28	29 ,,
9. Streptomycin	10 mcg	11 ,,	12-14	15 ,,
10. Sulfonamide	300 ,,	12 ,,	13-16	17 ,,
11. Tetracycline	30 ,,	14 ,,	15-18	19 ,,

(concl.)

TABLES

Table I.

Results of specimens examined for isolation of *E.coli*, *Salmonella* and *Enterobacter cloacae*

Type of specimens	No.of specimens examined		No.of *E.coli* isolated	Percentage	No.of *Salmonella* isolated	Percentage	No.of *Enterobacter* isolated	Percentage
Rectal swabs	60		33	55	2	3.33	10	16.67
Intestinal contents	40							
Small intestine	30	92	41	44.56	Nil	0.00	23	25
Large intestine	22							
Mesenteric lymph nodes	38		12	31.58	Nil	0.00	6	15.79
Total	190		86	45.26	2	1.05	39	20.53

(concl.)

Table II.

Results of biochemical reactions of E.coli isolated from goats

Tests	Identity of cultures and source of isolation									
	EC/4 (IN)	EC/7 (IN)	EC/9 (RS)	EC/11 (IN)	EC/15 (IN)	EC/28 (RS)	EC/34 (ML)	EC/36 (IN)	EC/39 (RS)	EC/42 (ML)
Motility	+	−	+	+	+	−	−	+	−	+
Haemolysis	−	−	−	+	−	−	−	−	−	−
Growth in air	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+
Indole	+	+	+	+	+	+	+	+	+	+
Methyl red	+	+	+	+	+	+	+	+	+	+
Voges-Proskauer	−	−	−	−	−	−	−	−	−	−
Simmons citrate	−	−	−	−	−	−	−	−	−	−
Hydrogen sulphide (TSI)	−	−	−	−	−	−	−	−	−	−
Phenylalanine deaminase	−	−	−	−	−	−	−	−	−	−
Urease	−	−	−	−	−	−	−	−	−	−
Gelatin	−	−	−	−	−	−	−	−	−	−
Nitrate	+	+	+	+	+	+	+	+	+	+
Gas from glucose	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	−	+	+	+	−
Lactose	+	+	+	+		+	+	+	+	+
Sorbitol	+	+	+	+	+	+	+	+	+	+
Salicin	−	−	−	−	−	+	−	−	−	+
Raffinose	+	+	−	+	+	−	+	+	−	+
Rhamnose	+	+	+	+	+	+	+	−	+	−
Inositol	−	−	−	−	−	−	−	−	−	−
Adonitol	−	−	−	−	−	−	−	−	−	−
Mannitol	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+	+	+

Note: + = positive
- = negative

IN = intestinal contents/small intestine/large intestine
RS = rectal swabs; ML = mesenteric lymph nodes

Table II.

Results of biochemical reactions of *E.coli* isolated from goats

Tests	Identity of cultures and source of isolation									
	EC/48 (RS)	EC/51 (RS)	EC/56 (RS)	EC/62 (RS)	EC/70 (ML)	EC/72 (IN)	EC/75 (IV)	EC/77 (IV)	EC/78 (RS)	EC/80 (RS)
Motility	+	+	+	+	−	+	+	+	−	+
Haemolysis	−	−	−	−	−	−	−	−	−	−
Growth in air	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+		+	+	+	+	+
Indole	+	+	+	+	+	+	+	+	+	+
Methyl red	+	+	+	+	+	+	+	+	+	+
Voges-Proskauer	−	−	−	−	−	−	−	−	−	−
Simmons citrate	−	−	−	−	−	−	−	−	−	−
Hydrogen sulphide (TSI)	−	−	−	−	−	−	−	−	−	−
Phenylalanine deaminase	−	−	−	−	−	−	−	−	−	−
Urease	−	−	−	−	−	−	−	−	−	−
Gelatin	−	−	−	−	−	−	−	−	−	−
Nitrate	+	+	+	+	+	+	+	+	+	+
Gas from glucose	+	+	+	+	+	+	+	+	+	+
Sucrose	+	−	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+
Sorbitol	+	+	+	+	+	+	+	+	+	+
Salicin	−	+	−	−	+	−	−	+	−	−
Raffinose	+	+	+	+	−	+	+	+	+	+
Rhamnose	+	+	+	+	+	+	+	+	+	+
Inositol	−	−	−	−	−	−	−	−	−	−
Adonitol	−	−	−	−	−	−	−	−	−	−
Mannitol	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+	+	+

Note: + = positive
− = negative

IN = intestinal contents/small intestine/large intestine
RS = rectal swabs; ML = mesenteric lymph nodes

(concl.)

Table III.

Results of biochemical reactions of Salmonella cultures isolated from goats

Tests	Identity of cultures and source of isolation	
	S/1 (RS)	S/2 (RS)
Motility	+	+
Haemolysis	-	-
Growth in air	+	+
Catalase	+	+
Indole	-	-
Methyl red	+	+
Voges-Proskauer	-	-
Simmons citrate	+	+
Hydrogen sulphide (TSI)	+	+
Phenylalanine deaminase	-	-
Urease	-	-
Gelatin	-	-
Nitrate	+	+
Gas from glucose	+	+
Lactose	-	-
Sucrose	-	-
Sorbitol	+	+
Salicin	-	-
Rhamnose	+	+
Raffinose	+	+
Inositol	-	-
Adonitol	-	-
Arabinose		-
Mannitol	+	+
Maltose	+	-

Note: + = positive
- = negative
RS = rectal swabs.

(concl.)

Table IV.

Results of biochemical reactions of *Enterobacter cloacae* isolated from goats

Tests	Identity of cultures and sources of isolation									
	EN/3 (IN)	EN/8 (IN)	EN/12 (RS)	EN/13 (IN)	EN/15 (IN)	EN/17 (IN)	EN/20 (ML)	EN/21 (IN)	EN/23 (RS)	EN/24 (IN)
Motility	+	+	+	+	+	+	+	+	+	+
Capsule	-	+	+	+	-	-	+	-	-	-
Growth in air	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+
Indole	-	-	-	-	-	-	-	-	-	-
Methyl red	-	-	-	-	-	-	-	-	-	-
Voges-Proskauer	+	+	+	+	+	+	+	+	+	+
Simmons citrate	+	+	+	+	+	+	+	+	+	+
Gelatin	+	+	+	+	+	+	+	+	+	+
Potassium cyanide	+	+	+	+	+	+	+	+	+	+
Hydrogen sulphide (TSI)	-	-	-	-	-	-	-	-	-	-
Phenylalanine deaminase	-	-	-	-	-	-	-	-	-	-
Urease	+	+	+	+	+	+	+		+	+
Nitrate	+	+	+	+	+	+	+	+	+	+
Gas from glucose	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	-	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+	+
Glycerol	-	-	-	-	-	-	-	-	-	-
Salicin	-	+	+	+	+	+	+	-	+	+
Adonitol	-	-	-	-	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	-	-	-	-
Sorbitol	+	+	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+	+	+
Rhamnose	+	+	+	+	+	+	+	+	+	+
Raffinose	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+

Note: + = positive, - = negative. IN = intestinal contents/small intestine/large intestine; ML = mesenteric lymph nodes; RS = rectal swabs.

(contd.)

Table IV.

Results of biochemical reactions of *Enterobacter cloacae* isolated from goats.

Tests	Identity of cultures and source of isolation									
	EN/26 (RS)	EN/29 (IN)	EN/32 (IN)	EN/33 (IN)	EN/34 (IN)	EN/35 (ML)	EN/36 (RS)	EN/37 (IN)	EN/38 (IN)	EN/39 (IN)
Motility	+	+	+	+	+	+	+	+	+	+
Capsule	−	−	+	−	−	−	−	−	−	−
Growth in air	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+
Indole	−	−	−	−	−	−	−	−	−	−
Methyl red	−	−	−	−	−	−	−	−	−	−
Voges-Proskauer	+	+	+	+	+	+	+	+	+	+
Simmons citrate	+	+	+	+	+	+	+	+	+	+
Gelatin	+	+	+	+	+	+	+	+	+	+
Potassium cyanide	+	+	+	+	+	+	+	+	+	+
Hydrogen sulphide (TSI)	−	−	−	−	−	−	−	−	−	−
Phenylalanine deaminase	−	−	−	−	−	−	−	−	−	−
Urease	+	+	+	+	+	+	+	+	+	+
Nitrate	+	+	+	+	+	+	+	+	+	+
Gas from glucose	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+	+
Glycerol	−	−	−	−	−	−	−	−	−	−
Salicin	+	+	−	+	+	+	−	+	+	+
Adonitol	−	−	−	−	−	−	−	−	−	−
Inositol	−	−	−	−	−	−	−	−	−	−
Sorbitol	+	+	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+	+	+
Rhamnose	+	+	+	+	+	+	+	+	+	+
Raffinose	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+

Note: + = positive; − = negative

IN = intestinal contents/small intestine/large intestine

ML = mesenteric lymph nodes; RS = rectal swabs.

(contd.)

Table V.

Results of experimental infection studies of E.coli and Salmonella in mice.

Species of organism	Dose and route of injection	No.of mice used	Period of observation in days	Number died	Number killed	Lesions on histopathology	Site of isolation
E.coli (EC/11) Haemolytic	0.1 ml intraperitoneal	4	3	4	Nil	Haemorrhagic areas on myocardium	Intestine and heart blood
E.coli (EC/11) Haemolytic	0.1 ml subcutaneous	4	8	2*	2	No lesions	Intestine and heart blood
Salmonella (S/1)	0.1 ml intraperitoneal	4	10	Nil	4	No lesions	Intestine
Salmonella (S/1)	0.1 ml subcutaneous	4	10	Nil	4	No lesions	Intestine

Note: * Died on the fifth day.

(concl.)

Table VI.

Results of rabbit ileal loop inoculation with E.coli.

Organism	Nature of test materials given	No. of rabbits used	Death in hours	Reaction in ileal loop	Lesions
E. coli (EC/15) Non-haemolytic	Peptone water culture and soft agar culture fluids	1	20	Dilatation (+)	Extensive damage to the intestinal mucosa with infiltration of mononuclear and lymphoid cells seen in segments received the soft agar culture fluid. Mild necrosis observed in segments received the peptone water culture.
		2	23	Dilatation (+)	
		3	24	Dilatation (+)	
E.coli (EC/11) Haemolytic	Peptone water culture and acetone precipitated culture fluids	1	21	No dilatation (-)	Necrotic changes
		2	23	No dilatation (-)	
		3	24	No dilatation (-)	

(concl.)

Table VII.

Results of drug sensitivity of E.coli, Salmonella and Enterobacter cloacae to various chemotherapeutic agents

Chemotherapeutic agents	Disc potency	Organisms	No. of strains tested	Number sensitive	Percentage sensitivity
Ampicillin	10 mcg	E.coli	86	Nil	00.00
		Salmonella	2	Nil	00.00
		E.cloacae	39	Nil	00.00
Bacitracin	10 mcg	E.coli	86	Nil	00.00
		Salmonella	2	Nil	00.00
		E.cloacae	39	Nil	00.00
Chloramphenicol	30 mcg	E.coli	86	76	88.77
		Salmonella	2	2	100
		E.cloacae	39	30	76.92
Erythromycin	15 mcg	E.coli	86	2	2.33
		Salmonella	2	Nil	00.00
		E.cloacae	39	Nil	00.00
Gentamicin	10 mcg	E.coli	86	86	100
		Salmonella	2	2	100
		E.cloacae	39	39	100

Table VII.

Results of drug sensitivity of *E.coli*, *Salmonella* and *Interobacter cloacae* to various chemotherapeutic agents

Chemotherapeutic agents	Disc potency	Organism	No. of strains tested	No. sensitive	Percentage sensitivity
Kanamycin	30 mcg	*E.coli*	86	52	60.47
		Salmonella	2	Nil	00.00
		E.cloacae	39	39	100
Nitrofuran	300 mcg	*E.coli*	86	82	95.35
		Salmonella	2	2	100
		E.cloacae	39	30	76.92
Penicillin	10 units	*E.coli*	86	Nil	00.00
		Salmonella	2	Nil	00.00
		E.cloacae	39	Nil	00.00
Streptomycin	10 mcg	*E.coli*	86	35	40.70
		Salmonella	2	2	100
		E.cloacae	39	15	38.46
Sulfonamide	300 mcg	*E.coli*	86	Nil	00.00
		Salmonella	2	Nil	00.00
		E.cloacae	39	Nil	00.00
Tetracycline	30 mcg	*E.coli*	86	7	8.14
		Salmonella	2	Nil	00.00
		E.cloacae	39	Nil	00.00

(concl.)

ILLUSTRATIONS

Fig-1

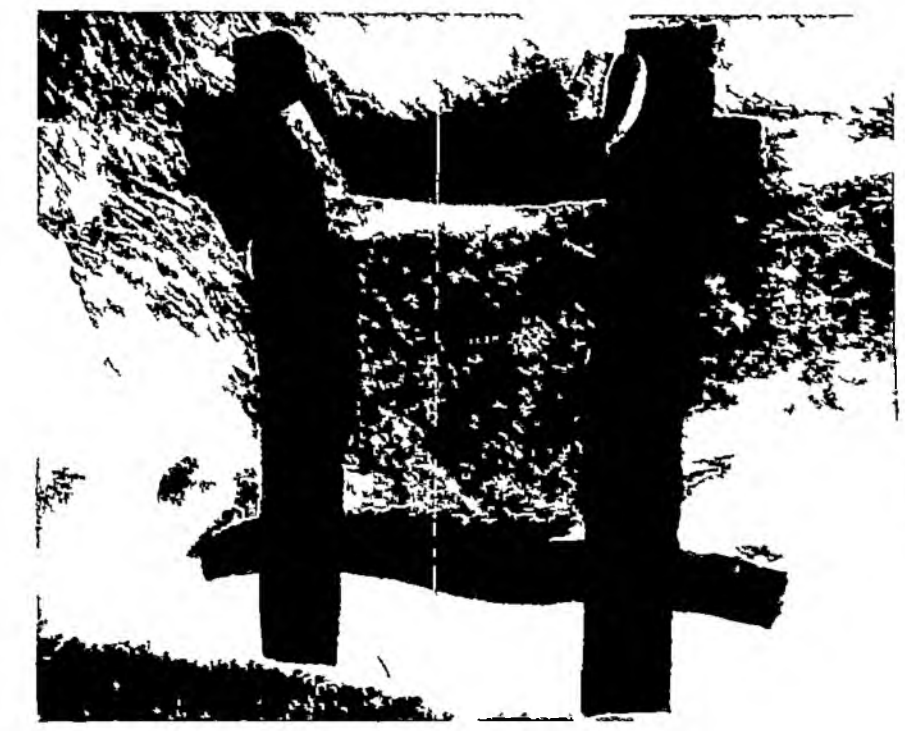


Fig 2

Fig. 3

Fig. 4

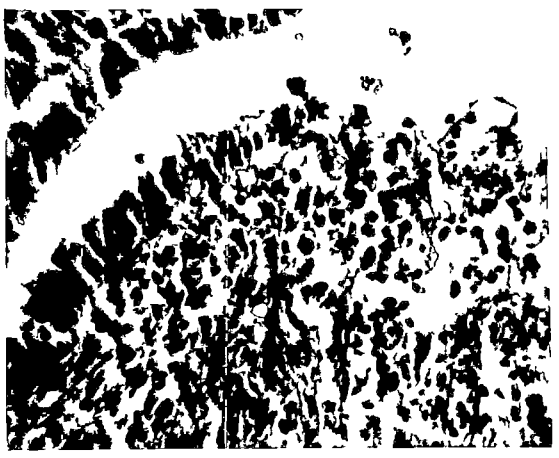
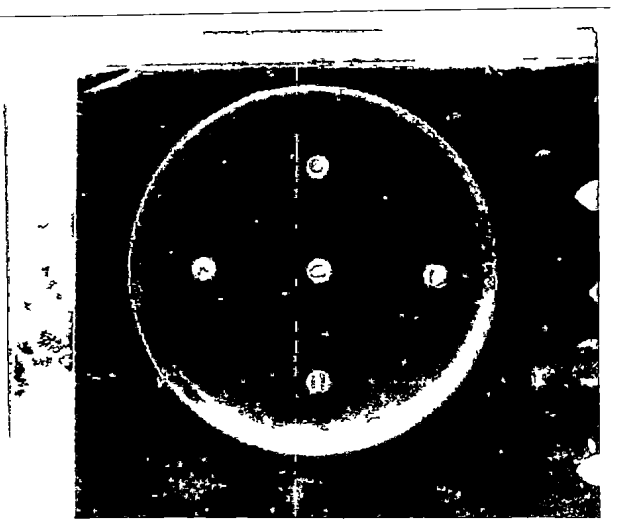


Fig. 5



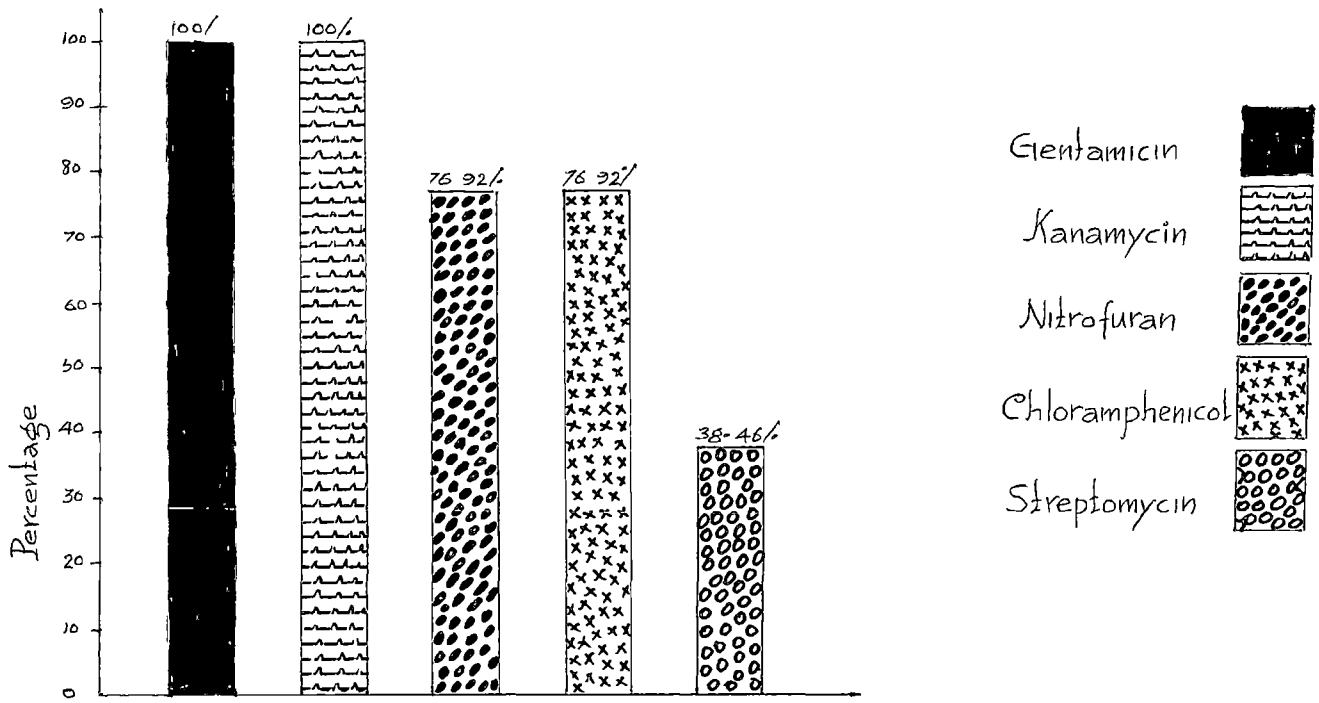


Fig. 7 Percentage sensitivity of Enterobacter cloacae to various chemotherapeutic agents.

STUDIES ON

THE BACTERIAL SPECIES ASSOCIATED WITH GASTROENTERITIS IN GOATS

BY

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ABSTRACT OF A THESIS

Submitted in partial fulfilment of the requirement for the degree

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ABSTRACT

The information regarding the incidence, etiology and pathogenicity of enteric pathogens in goats is very meagre in our country. The present study is aimed at the isolation, identification and characterisation of Enterobacterial organisms from cases of enteritis in goats. The study also included, determination of sensitivity pattern of the isolates to various chemotherapeutic agents.

A total of 190 specimens, which included rectal swabs (60), intestinal contents, portions of large and small intestines (92) and mesenteric lymph nodes (38) collected from live/dead animals were examined for enteric pathogens. From these specimens examined, 86 isolates of Escherichia coli (45.26 per cent), 39 Enterobacter cloacae (20.53 per cent) and two Salmonella (1.05 per cent) were obtained. Of all the E.coli isolates, only one (EC/11) was found to be haemolytic.

In addition to the above specimens, eight samples of heart blood and 34 specimens of lung tissues collected from cases of gastroenteritis were also examined for the presence of bacterial organisms. Seven isolates of Streptococcus pyogenes (from lung tissues only), 15 isolates of Klebsiella pneumoniae (from lung tissues only), and one isolate of Corynebacterium pyogenes (from lung tissues only) were obtained.

The ability of haemolytic E.coli (EC/11) to produce necrotoxin on rabbit skin was tested and the lesions produced were of necrotic changes. The strain was also found to be pathogenic to mice when tested.

One isolate of Salmonella (S/1) was also tested for its pathogenicity to mice, and found non-pathogenic.

Enterotoxin production in rabbit ileal loop was studied with haemolytic (EC/11) and non-haemolytic (EC/15) E.coli. The test materials included peptone water culture, soft agar culture fluid and acetone precipitated culture fluid. The results of the experiment have shown that, non-haemolytic E.coli produced dilatation reaction, while the haemolytic E.coli did not. The lesions noticed in the ileal segments of positive reaction were typical of enteritis.

Antibiotic sensitivity studies were conducted using 11 chemotherapeutic agents (Ampicillin, bacitracin, chloramphenicol, erythromycin, gentamicin, kanamycin, nitrofuran, penicillin, streptomycin, sulfonamide and tetracycline) on E.coli Salmonella and Enterobacter cloacae. The result showed that cent per cent isolates of E.coli were sensitive to gentamicin, 95.35 per cent to nitrofuran, 88.37 per cent to chloramphenicol, 60.47 per cent to kanamycin, 40.70 per cent to streptomycin, 8.14 per cent to tetracycline and 2.33 per cent to erythromycin. All the 39 isolates of Enterobacter

~~clones~~ tested were sensitive to gentamicin and kanamycin, whereas 30 (76.92 per cent) were sensitive to chloramphenicol and nitrofuran and 15 (38.46 per cent) to streptomycin. The drugs of choice for Salmonella were found to be gentamicin, chloramphenicol, nitrofuran and streptomycin.

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