SEROLOGICAL AND BIOCHEMICAL BEHAVIOUR, AND DRUG SENSITIVITY OF ENTEROPATHOGENS FROM CATTLE, GOATS, DOGS, POULTRY, AND PIGS .

THESIS

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Serological and Biochemical Behaviour, and Drug sensitivity of Enteropathogens from Cattle, Goats, Dogs, Poultry, and Pigs.

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C E R T I F I C A T E

This is to certify that the Thesis entitled " SEROLOGICAL AND BIOCHEMICAL BEHAVIOURS AND DRUG SENSITIVITY OF ENTEROPATHOGENS FROM CATTLE GOATS DOGS POULTRY AND PIGS submitted for the degree of Master of Science (Vet.) in Bacteriology to the Magadh University by Sri Birendra Kumar Tiwary embodies the result of his independent study which he has carried out under my supervision and guidance .

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Birendra
5/12/69

(B. K. Tiwary) .

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

The knowledge about the association of enteropathogens with various enteric disorders of man and animals can be dated back to the last two decades of the 19th century .

E. coli , though mainly recognised as the causative agent of calf scour or diarrhoea neonatorum has also been incriminated in cases of enteritis, arthritis, naval infection, meningitis, and septicaemia in calves. Many serotypes recovered from calves possess 'O' antigens which are common with the serotypes involved in infantile gastro-enteritis in man .

Orskov(1963) stated that little if anything is known about the coli strains found in goats in India and other countries and even less is know about the role of such strains in causing disease.

Strains of E. coli possessing 'O' antigens and common with those found in infantile gastro-enteritis are not infrequently isolated from faeces of family dogs. This constitutes a potential source of infection to children and those in contact with them. Deaths have also been encountered in dogs due to " fading puppy syndrome", an acute gastro-enteritis in young ones, and due to acute illness in adults attributable to a haemolytic strain of E. coli 'O' group-042 (Mansi, 1962) .

In small piglets, colisepticaemia, gastroenteritis, enteritis, diarrhoea and gut oedama are responsible for a heavy loss to swine industry. The predominant serotypes causing gut oedema belong to particular O groups such as (O139 : K82), (O141 : K85a,b(B) and (O138 : K81(B), while serotypes most frequently recovered from cases of enteritis, gastro-enteritis, and diarrhoea have (O141 : K85a, b(B), K88a, c(L) (O138 : K81(B), K88a,c(1)) ,(O147 : K89(B),

K89(B), K88a,c(L), (O8 : K87(B), K88a,c(L) .

Besides coligranuloma in poultry, other clinical entites such as haemorrhagic enteritis, infectious arthritis, bumblefoot, yolk sac infection, egg-peritonitis are common disease manifestations. Colisepticaemia alone takes a toll of about 50% among broiler chicks. Thus, it is a major profit killer of poultry farmers.

E. coli. possessing 'O' antigens such as 026,086, 09,08,0111, 078, harboured by poultry have been frequently incriminated as the cause of scour in calves, of which 026, 086, 0111, 078 are commonly recovered also from cases of infantile gastroenteritis.

Resistance of E.coli to various antibiotics and nitrofurans has been studied by many workers . Level of resistance to a particular drug is increasing gradually. Besides, some strains show multiple drug resistance which has been found to be transmissible. The variation in extent and pattern of drug resistance of the same serotype in clinical and subsclinical infection has also been recognised. Works reported in India are not comparable due to different methods employed by different workers .

Severe diarrhoea caused by salmonella or E. coli is responsible for heavy mortality among animals in first few weeks of life. Affected animals which survive show retarded growth and become uneconomical .

In India, only sporadic attempts have been made to determine the prevalence of various salmonella serotypes in livestock and poultry. Salmonella carriers among recovered animals and in those affected inapparently further add to the complexities of salmonellosis.

Thus, an extensive study is needed to elucidate the extent

of salmonella serotypes in all classes of livestock and poultry in different regions of the country. Only after such a study should it be possible to appreciate the size of the problem . This is necessary from the standpoint of public health.

Although infection in man by dysentry group of bacilli has been reported to be frequent in most countries, shigella infection has rarely been reported in domesticated animals. They have been recovered from dogs in China (Dold, 1916; and Dold and Fischer, 1927), Mexico(Verela, Perez Rebelo and Olbarte, 1951) and Egypt (Floyd, 1955). Shigella has also been reported from beavers in Italy(Padova, 1952) and calves in Belgian Congo (Deom and Mortelmans, 1954) .

Shigellosis being the common affection in man in India and else where it is imperative that an intensive study on the occurrence of the Shigella infection in animals should be made in order to elucidate the epidemiology and pathogenosis of the diseases in man. The diseases being of a great zoonotic importance, it is worth investigating its incidence in pet animals particularly in dogs and cats and their role as carriers of infection .

From the brief account given above, it will appear that much is still to be learnt of about the different enterpothegens particularly E. coli., Salmonella and Shigella organisms which are ascribed to cause clinical or inapparent infections in different species of livestock and poultry. In the present study, an attempt has been made to determine the extent of infection by these organisms in livestock and poultry. The isolates recovered from clinical and healthy carriers among different species of animals and poultry studied for their serological status and drug sensitivity in

order to get an insight into the epidemiology and pathogenesis of the disease in livestock and poultry .

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REVIEW OF LITERATURE

E. coli :

Escherichia coli was isolated from the faeces of a breast-fed infant by T. Escherich in the year 1885. He considered it to be a saprophyte .

The organism was then described under group Bacterium by Lehmann and Neumann(1896) with type species as Bacterium coli , whreas, Castellani and Chalmers (1919) included it under group Escherichia with the type species as Escherichia coli .

Rahn(1937) put it under the family Enterobacteriaceae.

(a) E. coli in cattle :

Though, Obich(1865) had already pointed out the association of E. coli with 'white scours' in calves, the credit went to Jensen (1893) who first confirmed it to be associated with this condition in calves, in Denmark and regularly isolated an oval shaped bacterium from the intestines, mesenteric lymphnodoes, heart blood, spleen, kidneys and liver of different cases. He reproduced the disease in calves by feeding faeces as well as culture of E. coli isolated from them. He concluded that the special races of E. coli were responsible for causing scouring in calves.

Jensen(1896) concluded it to be responsible for peritonitis and mastitis in cattle, and endometritis, pyelonephritis and abscess of the prostrate gland in dogs .

Christiansen(1915,1917,1923) associated it with heavy mortality in only very young calves, while Smith and Little(1922) thought it to be due to some particular biochemical types .

An interesting finding by Gram at al(1956) disclosed the protective activity of colostral antibody against white scour

in calves. Using colostral whey for slide agglutination test, he examined 150 strains of E.coli recovered from calves which died of white scour. He found that death of 45 out of 59 colostrum fed calves had occurred due to serotype whose agglutinin was not present in colostrum supplied to them . On the other hand, 66 deaths out of 94 colostrum deprived calves took place due to strains whose agglutinin was present in the surviving calves.

In Fey's (1956) opinion the 'Strain of group 078 of E.coli' was a selective pathogen for calves . He isolated this group from 35% cases of calf diarrhoea .

Fey(1957) studied serological and biochemical behaviour of strains of E.coli isolated from 105 calves. They belonged to 20 'O' groups and 49 serological or biochemical types . There were 5 dominant 'O' groups among them viz. 078,0115, 086, 015 and 0117 which comprised 57% of the total strains; 29% belonged to group 078, 99% of the strains possessed 'K' antigen. Of 145 strains of E.coli causing septicaemia in calves, 37.5% belonged to a single OB group 078 : 80B .

Glantz (1958) studied biochemical and antigenic characters of E.coli isolated from scouring calves. While he failed to differentiate the pathogenic from the nonpathogenic strains on the basis of biochemical behaviour, the former were readily identified on their O, K, and H antigens they possessed. This was confirmed on pathogenicity test in colostrum fed and colostrum deprived calves.

Osborne et al(1959) classified diseases associated with E. coli under three forms an acute rapidly fatal diarrhoea; a subacute debilitating diarrhoea and a chronic, localized joint, or middle ear infection .

Thal et al(1959) isolated 4 different serological types of E. coli from the prepuce and semen of 4 bulls. These bulls caused through mating, a transient vestibulovaginitis within 4-6 hours in 7 maiden heifers. The condition was also reproduced in heifers when inoculated in their vestibulum and vagina with E. coli cultures, but not when the heifers received the corresponding antiserum in the inoculum mixed with cultures inoculated .

Dam(1960) typed 431 strains of E. coli from calves suffering from Colisepticaemia in Denmark. Of these, over 40% belonged to group 078, and 15% to group 3-115 .

Fey and Margadant (1961) examined 37 calves that had died of colisepticaemia . From 12 of these calves, pathogenic serotypes of E. coli were isolated from all parts of body .

Ibragimov(1961) isolated Coli bacilli from mesenteric lymph nodes of 22 new born calves that had died of toxic dyspepsia. The strains fell into types 0111 : B4, 055: B5,026: B6; 086, 0180, and 0145 .

Burki and Fey(1962) encountered 61 cases of coli septicaemia in 5-7 days old calves when 88 calves had died of this infection.

Walser(1962) noticed cerebral involvement in 12 calves 3-6 months of age, which died of coli-septicaemia. Nervous symptoms included somnolence, peresis, opisthotonus, ataxia, spasms, and mydriasis. Enteritis was absent but rhinitis, sinusitis, and irititis were observed .

Ulendeev(1963) typed 249 strains, 68 from healthy and 181 from sick calves with the aid of 18 different O groupsera, In order of decreasing frequency, they fell into O groups 9, 119, 115, 117, and 78 .

Dam(1963) noted that 078, 0115 and 015 were the most frequently encountered types among the strains of E.coli isolated from cases of calf scours studied during the period over 5 years. He further compared the materials received from Denmark and other countries and a fairly constant predominance of strains belonging to these O groups was obtained .

Among 661 strains of E.coli isolated by Akhmedova and Agdami(1963) in Azerbaizan and Uzbekistan,73% of strains from colibacillosis in calves and 74% from lambs revealed the serotypes of O groups 086, 055 and 026. These strains were not pathogenic for mice. The most pathogenic type was 0127, which constituted 11% of calf and 14% of lamb strains .

Of the 320 strains typed by Ewing et al(1963), 85% were isolated from human beings and 15% from animals. Among which serotype 055: K59(B5): H19 was isolated from only one human being. They also insisted on point that E.coli OB group 055: K59(B5) had been widely associated with and frequently reported from human infants with gastroenteritis, their incidence in diseased animals were rare .

Glossing et al(1964) found certain serotypes of Escherichis coli O group 078:K80(B) and 0137:K79(L) to be responsible for severe septicaemia in young calves. The former was reported to be widespread and was found mainly in calves with colisepticaemia

(Dam 1960, Fey 1957, Kaeckenbeeck 1960). The latter was recovered from cases of septicaemia in calves in Britain, and was successfully used by Schoenaers and Kaeckenbeeck (958) to reproduce the disease. On the whole, Glossing et al(loc.cit) isolated E.coli from 265 cases of colibacillosis and showed that eight of the nine most common serotypes were more likely to be associated with this fatal disease in young calves. Two of these serotypes appeared to be most virulent serotypes while, a K(A) antigen were found most often in "problem" herd .

Glantz and Rothenbacher (1965) isolated E.coli 055:K59(B5):H 19 from calves with meningitis and septicaemia. This serotype had been recovered from cases of gastroenteritis in human, though, calves from which this serotype was recovered did not reveal any gastroenteritic leison . This serotype was probably reported for the first time from calves with meningitis and septicaemia .

Lysenko and Andreeva (1966) found in order of frequency 078, 041, 0101, and 08 from affected young calves. Only 5 strains were isolated belonging to serotypes pathogenic for human beings. They were 026, 0128, 0127 and 044 .

E.coli in goats :

Loliger(1956) isolated E.coli from the seminal ejaculate of a goat. He inoculated the broth culture of this organism in doses of 0.5 ml into the testicle, of two and in the epididymis of two other goats. One from each group died, the semen samples of the survivors revealed the presence of polymorphonuclear leucocytes, plasma cells and giant spermatid cells. He also observed inhibition of motility, malformation and agglutination

of spermatozoa. E. coli was recovered only from the ejaculate of the goat injected intra-epididymically. On postmortem examination after 21 days necrotising purrulent orchitis in the goat injected intratesticularly and epididymitis with abscess formation in the right accompanied with granulomate in epididymitis of the left (infected) uninoculated testicle was seen .

Loliger(1957a), while discussing the pathogenecity of the so called seminal retention in the epididymis of goat, reported that, of 13 kids aged 5 months in which epididymitis were produced experimentally, 7 developed granulomas with giant cells in the epididymis .4 kids had diffuse purrulent orchitis(with necrosis and diffuse epididymitis resulted in testicular abscess). He suggested the term testicular granuloma or epididymitis, in view of its histological structure and pathogenesis.

Loliger(1957b) gave an account of his survey on "spermatic granuloma" (blockage of seminal discharges) and testicular hypoplasia in young male goats. The incidence in different breeds of goats varied between 15.3% and 24.3% . The incidence was confined among those kept and fed indoor. In those goats, left at pasture all the year round, no testicular hypoplasia was found and incidence of "Spermatic granuloma" in them was only 2.4% .

E. coli was isolated from 27 of 98 ejaculates from affected goats, but from only 2 of 43 healthy goats. He suggested that spermatic granuloma might occur by local infection with enteric bacteria, such as E. coli and that this might be encouraged by housing .

Muller(1960) reported cases of sterility in female goats associated with genital infection of the male with haemolytic

E.coli. In a herd of 25 female and one male goats, the conception rate was very low and haemolytic E.coli was isolated from cervical and vaginal mucus of 8 out of 10 sterile goats and also from the ejaculates of the male which had bilateraly testicular hypoplasia. Very marked improvement was noticed after vaginal lavage of all females with 20% of Supronal solution and when new male was introduced in the herd .

Panda et al(1965) isolated 38 strains of E.coli from healthy and diseased kids and goat. All were pathogenic, except 0133 to guineapigs. The strains belonged to the 08,021,026,078, 0115,0133 and 0140 groups. One strain was tentatively considered as 0146 since, it failed to agglutinate with any of the known 145, O type sera. The strains haemolysed horse blood but not the ox blood .

E.coli in dogs :

Sears and his associates(1956) reported on the persistence of individual strains of E.coli in man and dog under varying conditions. One E.coli strains always predominated while others persisted in small numbers. Thereafter, without any apparent reasons, the dominant(resident) strain started declining and the other(non-resident) strain, which were previously in small numbers, became dominant. They tried to change this pattern by feeding dogs with known cultures of E.coli but without success .

Guerden et al(1956) isolated and studied 73 pathogenic strains of E.coli from diseased foals, calves, pigs, dogs, cats and fowls and found that type 055 55 belonged to dogs only. They also found that most of the strains isolated from dog and cats were haemolytic . Guerden et al(1957) further isolated types

086 and 026 B6 from 3 dogs and 2 pigs .

Davies(1958) made experimental studies on the possibility of cross-infection between animals and infants. She isolated 10 strains of E.coli from dogs of which had antigenic structure similar to those found in calf scour. She did not find any relationship between this strain and those found in cases of infantile gastroenteritis .

Mian(1959) isolated 34 strains from the faeces of the apparently healthy dogs, examined serologically, and found that 12% of these strains possessed 'O' antigens commonly associated with cases of infantile gastroenteritis in babies(0111, 055, 026, 0127, and 0125). He also found a case of gastroenteritis in a baby with antigenic structure 055, B5 and pointed out that this serotype was also recovered in almost pure culture from the faeces of the family dog .

Werselinoff and Dinow(1961) reported E.coli serotypes commonly associated with infantile gastroenteritis in babies (025,026, 055,0111,018,044,0119,0125,0126 and 0128). Of the 41 isolates recovered from dogs they found only one strain with an antigenic structure similar to those serotypes (026: B6; H11) which affected infants .

Mansi(1962) stressed theimportance of haemolytic strains of E.coli in infections of canines . He found that 70% of the cases of the so called"fading puppy" syndrome, 43% of cases of acute gastroenteritis in young dogs and 16% of total in adult dogs which were caused by haemolytic strains of E.coli which belonged to 'O' group 042. Based on clinical syndrome, Mansi(loc-cit) divided the E.coli infection of dogs into 3 forms,(i) the first

affecting puppies from birth to 4 weeks of age,(ii) the second affecting dogs from 5 weeks to 9 months of age and (iii) third in adult dogs of over 9 months of age . The Strains isolated from the carcases of newborn puppies were all haemolytic and belonged to O group 042. In the second group of animals (5weeks to 9 months old), he isolated haemolytic E.coli from all the 3 dogs, one of which died of acute gatroenteritis. The strain was recovered from all the internal organs including heart blood of two dogs while from the third it was found only in the intestine and mesenteric lymph nodes. He also attributed haemolytic E.coli of O group 042 from 35 out of 80 dogs in this age group . Of a total of 80 carcasses examined in this age group, the death in 35 dogs was attributed to 042, haemolytic E.coli strain. In 10 cases this serotype could be recovered from all internal organs and in 25 cases only from intestines and mesenteric lymph nodes.

In dogs over 9 months of age, the common symptoms were persistent diarrhoea causing about 16% mortality in them. Of 96 deaths in this age group only 16 were associated with strain of O group 042 .

Mansson and Lindblad(1963) studied 46 hemolytic strains of E.coli isolated from dogs, which died either of septic infections or gastrointestinal disorders. The strains possessed the O antigens(06,02, and 014) .

Malik(1963) isolated about 140 strains from sheep, cattle, horses and dogs and found that most effective antibioties against their isolates were neomycin, tetracycline, oxytetracycline and chloramphenicol; both streptomycin and chlortetracycline were only moderately effective and penicillin and erythromycin had no

action .

E. coli in poultry :

The association of E. coli with certain pathological conditions in poultry was recognised in the latter part of the 19th century. In 1889, Klein described an infectious disease of goose (Lagopus scoticus) caused by an organism which was later recognised by Theobald Smith as E. coli (Dawson, 1898) . Lighieres(1894) described a disease in fowls caused by E. coli and demonstrated the pathogenicity of the organism by experimental inoculation in fowls and pigeons. Mazza(1899) described epidemics in fowls characterised by haemorrhagic enteritis, which he considered to be probably associated with E. coli infection. The literature concerning E. coli infection in birds was adequately reviewed upto 1922 by Palmer and Baker (1923). Since then a number of reports on the subject have been published (Pomeroy and Fenstermacher, 1937; Davis,1938; Twisselman,1939; Hjarre and Wramby, 1945; Wramby, 1948; Ulbrich 1951, 1954; Edwards and Ewing 1954;) .

E. coli was reported to cause fatal enteritis in two flocks of birds in West Pakistan by Quereshi (1957). The birds were vaccinated against new castle disease. He also reproduced the disease by inoculating the suspension of organisms from the affected birds. The organisms were recovered again from 207 out of the 378 chicks with enteritis .

Gurumurthi and Panduranga Rao(1962) observed colibacillosis causing heavy mortality in brooder house chicks under 1-4 weeks of age. Death occurred in a few hours after the rist symptom, White pasty diarrhoea, blocking of the vent with dried faeces and wetting of the surrounding area was noticed in about 30%

of the affected chicks. Nonhaemolytic strains of E.coli were isolated from the affected animals .

Ciosek(1965) typed 396 E.coli strains isolated from healthy and 110 strains from diseased fowls with 10 'O' and 'OK' antisera. He could type 44 strains of which 19 fell into O2:K1:H4. The other belonged to group O8, O1, O3, O22, O78 and OF42 . The birds infected with E.coli of O group O2 : K1: H4 had syndrome characterised by pneumonia, pericarditis, air sacculitis, hepatitis, enteritis and oedema of the spleen and kidney . Strains obtained from healthy birds belonged to O group O2, O8, O78, O3 and O22 .

Hjarre and Wramby(1945) described the "coligranuloma" which was characterised by tuberculoid granulomatous leisons in the intestine and liver and from which they consistently isolated mucoid E.coli strains. The condition was reproduced by experimental inoculation of cultures into fowls. They found its incidence to be 0.37% of 8,000 birds examined by them, though Kohler(1951) found in higher percentage .

Wramby (1948) investigated 50 mucoid E.coli strains isolated from cases of coligranuloma in fowls, and found that these organisms fell into 3 fermentative and serological groups .

Ulbrich(1951,1954) carried out similar investigation on the coligranuloma strains. He (1951) found that most of his 36 mucoid "Coligranuloma" strains belonged to two O groups, O8 and O16. The commonest K antigens in these strains, were 6,7,9 and 12 (some possessed K 4 and K8 antigens too) .

Several reports had been from France (Lissot, 1949), from Canada (Wickware, 1948), from America (Kohler,1951) and from

Ceylon(Bhandarnayke, 1954) .

Iyer *et al* (1965) reported a granulomatous condition in fowl caused by mucoid phase of *E.coli*. This strain affected internal organs of the fowl particularly the intestine (caeca) and liver . They could reproduce the nodules .While Kulkarni (1964) could isolate *E.coli* possessing 08,078, and 0111 from coligranuloma(Hjarre's disease)cases .

Narula and Kuppuswamy (1966) reported from Bihar a case of coligranuloma. They found tuberculous like leisons over all surfaces of liver .

Nayak et al(1967) could obtain *E.coli* from affected organs of a case of avian coligranulomatosis .

Clausen(1907) investigated a cholera like syndrome in fowls dying during shipment and concluded that *E.coli* may under certain conditions leave the intestine, become virulent and cause a septicaemia in hens, especially if their resistance has been weakened by hunger, thirst, cold or lack of good ventilation .

Davis(1938) described, under the name"colibacillosis" a disease of chicks from which pure culture of *E.coli* was obtained. He considered the typical leisons to be those of pericarditis. On the other hand, he considered colisepticaemia to be a disease of broiler chicks 6-10 weeks old, characterised by fibrinous pericarditis and hepatitis .

Gross(1957a) recorded an outbreak of panophthalmia in chickens due to E.coli and experimentally produced severe colibacterimia .

Harry(1957) reported that *E.coli* might be one of the causative agents for death of embryos and chicks resulting from infection

of yolk sac .

Gross and Siegel(1959) described egg peritonitis in fowls caused by E. coli .

Pathak, et al (1960) reported frequent isolations of E. coli while examining yolk flora of 100 dead chicks .

Sojka and Carnaghan(1961) reported that E. coli was constantly isolated in profuse culture from the internal organs from cases of colisepticaemia and that a remarkable degree of serological uniformity was found among those isolates .

Reid et al(1961) reported a strain of E. coli pathogenic for chicks. This strain infected the hatching egg by shell penetration on the 1st day. The eggs which were infected on 1st day had low hatchability as compared to the 83% of the controls and a total mortality upto 9th day of incubation was 8.2% as compared to 1.8% . After hatching difference was noticed in the mortality rate after 10 days and reduction in weight between 9 and 80 days was also noted .

According to Gordon(1961 d) "colisepticaemia" proved a problem of major concern to the broiler industry and economic losses were not dut to mortality but also due to carcasse condemnation and inferior meat quality .

Bekajle and Bost(1963) examined 30,230 table, of which 211 had leisons of serofibrinous pericarditis. In every case E. coli were isolated in pure culture. The serotypes identified were 071, 08,03. The first two killed mice when injected intravenously with 0.1 ml of 24 hours broth culture, but one ml dose given to 2 months old chicks proved harmless .

Gupta(1963) recorded the isolations of E.coli with antigenic characteristics of 078: K80, 01:K1, 08, 023, 026, and 0111 from cases of coli septicaemia of poultry in India. This was the first record of E.coli serotypes causing colisepticaemia in poultry in India .

In India, Gupta(1963) for the first time recorded the isolation of E.coli of O groups 08,09,018,088 and 0111 from cases of egg peritonitis of poultry . Subsequently Kulkarni (1964) isolated E.coli 08,078 and 0111 from this condition in India .

Varma(964) also reported colibacillosis in Bihar which took a toll of about 958 chicks between the age of 2-9 weeks .

Sarkar(1966) isolated E.coli from chicks in one of the farms in Bihar which had high mortality .

Yadava(1966) attributed colibacillosis to be the cause of mortality in chicks in one of the poultry farms in U.P.(India).

Gallo(1964) described an "infectious arthritis" in fowls, which could be reproduced experimentally by inoculation of the causal organism referred to as "Escherichia venezuelensis .

Gross(1964) reported a disease of chicken characterised by reduced weight gain and caseous yolk sac which was set up by dipping the incubating eggs in a culture suspension of E.coli of the group 'O' 103 obtained from a field flock affected with a similar condition.

Gupta and Singh(1969) isolated 17 strains of E.coli from 21 cases of egg peritonitis in hens. Of these 15 proved to be pathogenic for mice. Four of 15 strains had 08 antigen and one each possessed 09,017,018,023,051,083,0101 and 0111. Three 'O' antigens could not be identified .

E. coli in pigs :

Wittig(1963) isolated 654 haemolytic strains from 625 pigs suffering from enterotoxaemia. In order of prevalence, they were 0144 : K85 a,b(B) 39.8%, 0139: K82(B)-24.8%, 0138:K81(B) 10%. He found a low incidence of haemolytic strains in pigs died of other diseases than enterotoxaemia .

Glossing and Rhodes(1966) isolated 52 E. coli 'O' groups from pigs suffering from a number of enteric disorders. Among these 020, 0101, 0138 were most prevalent. They recognised 08 : K85 : H19 and 0138. K81 : N.M. strains as the specific infectious aetiologic agent of enteritis of baby pigs .

E. coli affects pigs even to the age of 10-12 weeks, unlike other animals. The oedema disease affects mostly the pigs between 8-12 weeks of age . The organism has been increminated with Gastroenteritis mainly in 3 age groups (a) piglets-few days old (b) unweaned piglets, about 3 weeks old and(c) pigs-about 10-12 weeks age (Stevens, 1963 a,b) .

Hudson et al(1951), Buntain(1951) and Gitter(1953) recorded haemolytic strains of E. coli from intestinal contents of pigs suffering from Oedema disease. Schofield and Davi(1955) isolated these organisms from similarly affected pigs. Gregorj(1955) reported the prevalence of haemolytic strains in the intestinal content of the oedema disease cases and recovered almost a pure culture out of E. coli isolates. Timoney(1956) also associated the haemolytic strains with the oedema disease in pigs. An extensive study was done by Lloyd on the prevalence of haemolytic E. coli in association with oedema diseases in British counties. In addition many workers from different countries have also

confirmed the isolation of haemolytic E.coli from pig intestine (Sojka 1965) .

Sojka et al(1957) isolated haemolytic E.coli strains from the contents of large intestine and mesenteric lymph nodes . Of 77 strains, 61 could be typed with a single "Oedema disease" E.coli antiserum, 7 gave agglutination reaction with the antiserum to other strains, and 9 remained untyped. In a survey of healthy pigs from only 5 plates prepared out of 106 rectal swabs .

Sojka et al(1960) examined 411 strains(380 haemolytic), and typed 82% of the isolates with only 3 sera. They reported the predominance of strains in series- "E 4"(0139: K82)-38.9%, E68 (0141:k85 a,b)- 30.2% and E 57(0138:K81)- 12.9%, 7.8% strains could not be typed with any one of the 44 antisera used by them.

Tadd and Hurst (1961) recovered haemolytic E.coli more commonly 0139:K82 and 0138:K81 from healthy pigs 10-12 days old. However, they did not find these types when the pigs were 30-40 days old .

Drug sensitivity :

E.coli : Numerous chemotherapeutic agents such as various antibiotics, sulponamides, nitrofurans have been used against E.coli , Salmonella and Shigella and other enteric infections. A rich stock of literature on the sensitivity of an organism to different antimicrobial compounds has accumulated .

Smith and Crab(1960) studied the effect of chemotherapeutic agents on the E.coli count in the faeces of healthy calves. 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. This

depression effect continued upto 5th day after administration of drug. An interesting observation recorded by them was that when a dose 4 times greater than that given orally, administered by inoculation, the effect was not as pronounced . Neomycin also had similar effect . Plymyxin reduced the count consistently. Oxytetracycline, chlortetracycline, sulphadimidine and phthalylsulphathiazole had a considerable depresing effect on the E.coli count in faeces. Chloramphenicol, furazolidone and furamazone given to 6 calves had no effect on coliform count . They also noticed that administration of 32 mgm chlortetracycline twice daily in milk resulted in development of 84% coli strains resistant to tetracycline as against only 6% in control group.

Dalton et al(1960) used different antibiotics viz.streptomycin, neomycin, oxytetracycline, chlortetracycline, chloromycetin, penicillin and phthalylsulphathiazole, therapeutically in cases of calf diarrhoea. They kept the calves in separate pens and treated them for 14 successive days at full therapeutic dose levels. They could not explain the failure of these antibiotics used in their study .

Inglis(1960) studied the effect of chlortetracycline supplement in feed on the icidence and severity of the calf scour. He concluded that this antibiotic supplement was frequently, though not always, effective as preventive against calf enteritis, similar to that of oxytetracycline .

Glantz(1962) performed the in vitro sensitivity test of 287 strains of Escherichia coli isolated from animals and poultry to antibiotics and nitrofurans. The most effective compounds

were colistin, chloramphenicol, furazolidone, N-(5-nitro-2 furfiz cidene)-1 amino-2 pyrolidene, thiofuradene and polymyxin. The activity of dihydrostreptomycin, chlortetracycline, tetracycline and oxytetracycline were intermediate. Penicillin, Oleandomycin furaltadone and nidroxyzone were least effective .

Glantz(1965) tested 530 strains of E.coli for their in vitro sensitivity to 13 sulfonamides by the broth tube and disc methods. The majority of the strains were sensitive to 0.25 to 1mg/ml. levels of all compounds tested. The results obtained by broth tube methods were more consistent than those when discs were used. There appeared to be some relationship between sensitivity and the O group to which the E.coli strains belonged .

McKay, Ruhnke and Barnum(1965) listed the results of sensitivity tests on animal pathogens conducted during the period 1956-63 . The strains of E.coli and Salmonella species showed evidence of marked increase in resistance. They tested a total of 3,898 E.coli strains. The results disclosed that while in 1957 the percentage of resistant strains of E.coli isolated from clinical pathological specimens was 60%, it rose to 90% by 1963. The tetracycline resistant strains increased from 0% to 29% during these years. The corresponding increase in percentages of neomycin and chloramphanicol resistant strains during these years were 10% to 50% and 8% to 22% respectively. The nitrofurazone and chlorhexidine had been very effective. Only less than 10% of the strains were found to be resistant to these compounds. Treatment of cases of enteritis (complicated with septicaemia) with these drugs alone proved ineffective.

Anan'ev et al(1966) found that the most frequent strains in enteritis of unweaned piglets were those possessing 04,08,09, 08,078 and 0127. Of 'O' groups 46 enteropathogenic strains tested in vitro against seven antibiotics, 63% were resistant to chlortetracycline, 54% to polymyxin, and 35%-20%, in decreasing order of resistance to chloramphenicol, munomycin, neomycin, mycerin and colimycin . The most effective combination of antibiotics against 29 strain in vitro was mycerin with furazolidone, mycerine with chlortetracycline, polymyxin plus chloramphenicol and polymyxin plus chlortetracycline .

Smith(1966) found a high incidence of drug resistance, mostly of infectious type, amongst strains of E. coli isolated from babies with neonatal diarrhoea, calves and lambs with neonatal diarrhoea or bacterimia, pigs with neonatal or post-weaning diarrhoea or oedema disease, and from fowls with colisepticaemia. The strains from the babies, pigs and fowls, and those from calves and lambs with bacterimia, belonged to serotypes generally accepted as being pathogenic for these species. Complex drugresistance patterns were the common features, particularly amongst the human and calf strains, some being resistant to six drugs. Only two drugs, polymycin and nalidixic acid were active on all strains .

Hemsley et al(1967) tested 213 avian E. coli strains against 24 drugs. Chlorhexidine, polymyxin B, colistin, and nitrofurazone exhibited the greatest inhibitory activity. Various potencies of antibiotics were compared. Sulphachlorpyridiazine on various types of media failed to show clear zones of inhibition. Group 078 exhibited an antibiotic sensitivity pattern different

from those of the other O groups .

Truszczyn'ski et al(1966) conducted in vitro sensitivity tests of 200 E. coli strain isolated from oedema disease or enterotoxaemia in pigs. Among them all were resistant to penicillin and erythromycin, moderate resistance was observed to chlortetracycline and tetracyclive while least number of strains were resistant to streptomycin and chloramphenicol.

Salmonella :

As early as 1880, the causative agent of enteric fever typhoid fever) was discovered by Eberth, and the organism was later named as Eberthella typhosum by Gaffky(1884) who, for the first time succeeded in isolating the organism in pure culture .

Salmon and Smith(1885-86) reported the isolation of Salmonella cholerae-suis(Bacillus suipestifer) from pigs .

In Germany, Gaertner(1888) first reported the isolation of Salmonella enteritidis(the then called Bacillus enteritidis) from a person who had consumed beef and died. By the end of the 19th century, two French workers(Archard and Besande, 1896) isolated S. paratyphoid B from urine and pus collected from patients with clinical syndromes of typhoid fever. This organism was later studied in detail by H.schottmueller in 1900, hence the name Salmonella schottmuelleri .

The organism responsible for paratyphoid A infection in man(Salmonella paratyphi A) was studied in detail by the German bacteriologists A.Brion and H.Kayser in 1902 .

Bosworth and Lovell(1931) reported two outbreaks of S.dublin infection in calves. Prodominant clinical signs were diarrhoea,

and pneumonia in some of them. In another outbreak the prodominant clinical signs were those of pneumonia, diarrhoea was not observed in any of them .

Shirlaw(1935) reported isolation of Salmonella from septicaemic diseases of calves and regarded them to be S.enteritidis which were later on reexamined by Rajgopalan(1938) and reported to be as S.dublin .

In Britain, Gibson(1961) listed the isolations of Salmonellae obtained from calf materials at veterinary investigation centres in England and wales from 1958 to 1960 and confirmed that S.dublin was the dominant serotype in them. Of 918 strains isolated during this period, 573(62%) were S.dublin and 313(34%) , S.typhimurium . The other 32 strains comprised 12 different serotypes. In contrast to this, Anderson, Galbraith and Taylor(1961) found S.typhimurium to be the predominant serotype when they examined the British calves at slaughter. Thus these two reports agreed to what was pointed out by Buxton earlier in 1960 that young calves were more susceptible to Salmonella infection than the adult and S.dublin and S.typhimurium caused the majority of outbreaks .

In Belgium, Thomas(1961) found that S.dublin and S.typhimurium were equally prevalent in cattle. Of 39 isolates, 18 were S.dublin, 17 S.typhimurium, 3 S.enteritidis and 1 S.bareilly.

From Russia, Zagaevskii(1962) found that 70(56%) of 124 strains isolated from cattle were Salmonella dublin, 24(19%) were S.typhimurium and 8(6.3%) S.enteritidis.

Khan(1962a,b) reported isolation of S.dublin from 1 of 161 specimens examined during an abattoir survey .

Ellis(1962) did not encounter S. dublin infection among the 40 outbreaks of salmonellosis in calves, he observed in Florida during a 2 years period .

Similarly, Bigland(1962) listed salmonellae isolated from animals and birds in Alberta during the period 1949-1960 . 24 isolates were from cattle but none of these, and none of 164 isolates from other host species was S. dublin .

In North America, Moran(1962) reviewed 6216 strains of salmonellae isolated during a period of 4½ years. She found 185(42%) of the bovine strains to be S. typhimurium and 50 strains S. dublin. Other isolates from cattle comprised 33 different salmonella serotypes .

LeNoc(1963) reported that upto 1962 S. typhimurium was the only serotype obtained from cattle in Madagasear. Similarly, Sakazaki(1961) listed the isolates obtained in Japan between 1949 and 1957 and found that cattle had yielded S. nagoya and S. enteritidis but not S. dublin .

Rude(1963) described 46 cases of Salmonellosis in cattle caused by S. typhimurium of which 9 were isolated from mature cattle and 37 from calves .

Prasad and Ahmad(1965) reported a fatal outbreak of salmonellosis in buffalocalves caused by mixed infection with S. typhimurium (Phage type 1 a ?) and S. dublin. The outbreak occurred following a vaccination against R.P. During the year 1967, while investigating the aetiology of calf mortality and pig mortality at certain farms of U.P. and West Bengal a total 58 salmonella strains were isolated (Mitra and Khera, 1958). The serotypes isolated from calves were S. dublin-8, and S. rostock-1 .

Prasad (1965) isolated S. Weltevreden in pure culture from

a pulmonary abscess in a calf. The calf was suffering from skin disease. There was no gastroenteristis .

Jayaraman and John(1969) examined the rectal swabs from cases of diarrhoea,postmortem materials, the intestinal contents and the viscera from the abattoirs. They recorded S.bournemouth, S.lanka, and S.sladun(monophasic) for the first time in Indian cattle

Salmonella in goats : As pointed out by Simmons and Sutterland((1950), and Smith and Buxton(1951) the incidence of salmonella infection in sheep and goats was low and of sporadic nature. The occurrence was usually related to certain predisposing conditions.

An investigation carried out at I.V.R.I., 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 and 10 out of 111 pneumonic lungs of goats . S.typhimurium was recorded from 10 out of 178 pneumonic lungs of sheep(Report, 1956-57) .

Buxton(1957) reviewed salmonellosis in animals and indicated 17 serotypes from sheep and goats .

Sharma and Singh(1960) reported S.magma, S.motapani and S.weltevreden from goats .

Jayaraman and John(1969) while surveying the salmonellae of domisticated animals in the state of Madras, recorded for the first time S.newport and S.virchow from the cases of enteritis in Indian sheep. In addition they reported a simultaneous isolation of S.newport and S.bareilly from the same sheep .

Butler and Basbee(1967) in their 2nd survey of salmonella isolated serotype or related organisms isolated from 13 out of

132 dogs, most of which were exhibiting signs of intestinal diseases. There was a much higher incidence of isolations from housepets than from other groups, and that was related to freedom of movement and access to garbage .

Khera(1967) reported the isolation S.dublin, S.cholerae smis and S.stanley from pigs .

Sahai and Nilkanthan(1947) isolated five strains of salmonella organisms from the materials, collected from dead sick and apparently healthy pigs . Out of these five strains, 2 were S.bovismorficaus, 2 S.derby and one S.anatum .

Sharma and Singh(1960) reported the isolation of S.anatum and S.newpost from pigs. The materials examined were pieces of liver spleen, gallbladder and intestinal scrapings from the dead and rectal swabs from live animals.

Makholia and Singh(1963) isolated an atypical strain of salmonella from pigs possessing "O" and "H" antigens common for salmonella and Arizona . However, the biochemical characters were not typical either of Salmonella or Arizona. The authors proposed the name Salmonella brindaban for this atypical strain .

Datta and Singh(1964) isolated 29 Salmonella strains comprising 9 serotypes, including a new one, S.gokul, from apparently healthy pigs. The antigenic formula of S.gokul was 1,51: d:- and the organisms was placed in subgenus 1 on the basis of their biochemical behaviour .

Salmonellosis in poultry : Since the early isolation of host specific salmonella serotypes such as S.gallinarum from an outbreak of fowl typhoid by Klein in 1889 and Salmonella pullorum

from bacillery white diarrhoea by Rettger in 1909, very large number of literatures have accumulated on the occurrence of different Salmonella serotypes in poultry . Some of them reported during the last decades are renamed briefly below .

Walker(1960) stressed that salmonella serotypes other than S.gallinarum and S.pullorum were the important source of infection in men and birds . Further he stated the emergence of antibiotic resistant strains of salmonella due to wider use of antibiotics in broilers ration which might cause an increase in carrier role .

Hammer(1960) made an extensive study of salmonella serotypes in domestic animals, animal products and the feed stuffs. He placed 1,203 strains isolated by him under 37 serotypes excluding S.pullorum and S.gallinerum . Amongst them, S.typhimurium, S.Cholerae suis Var kunzendorf and S.blockley were the three commonest serotypes. The important sources of isolation were pigs and fowls . The principal origin of infection as stated by the authors were the imported feeding stuffs, which in turn passed to livestock, and thence to butchers and food handlers. Finally, through uncovered kitchen waste and sewage, animals become infected .

Kulkarni and his associates(1960) recorded S.gallinarum infection in a flock of 700 R.I.R. and W.L.H. birds .

Klatt(1961) reported isolation of S.montevideo from 72 organs samples from 48 fowls in Helsinki .

Rao and Gupta(1961), reporting the isolation S.weltevreden from 6-21 day old chicks observed that chicks were imported from U.S.A. and were healthy in their first generation but the losses were experienced in the second generation. S.dublin caused only

sporadic deaths in adults .

Sharma and Singh(1961) examined faecal samples and internal organs of 1332 birds inorder to determine the incidence of salmonella carriers in India. They isolated 10 salmonella serotypes in which Sweltevredens predominated (41.6%), Salmonella gallinarum pullorum was not recovered from any of them .

Bigland(1962) isolated S.typhimurium, S.thompson, S.heidelburg, S.bareilly and S.oranienburg from the turkey and fowls at the Alberta laboratory between 1949 to 1960 .

Consgrove and Lindenmaier(1962) reported isolation of S.pullorum from the swollen hocks of 3 to 4 weeks old chicken. In nine flocks, birds had shown acute lameness and the mortality rate in them was high .

Das and Panda(1962) in Orissa reported infection in adult young fowls due to a variant strains of S.pullorum and S.gallinarum.

Ghosh(1962) reported the isolation of S.typhi from carrier fowls . It was also reported that the viscera of an apparently healthy hen was found to be a source of infection for other healthy fowls .

Sharma et al (1962) reported 2.32% salmonella infection in 600 and isolated 6 strains of S.typhimurium which were untypable by phage .

Khera(1962) in his review of animal salmonellosis in India reported that salmonella infection in fowls and other domestic birds was much more varied and widespread than in the mammals and the losses sustained through this group of organisms were probably far greater than those resulting from other single cause. The infection was most common and serious in very young birds ,

though the outbreaks involved both adult and young birds. Adults usually suffered from a chronic form of disease with leisons in ovaries, testes, joints, liver, spleen, whereas in young birds, septicaemia and enteritis with changes in the internal organs were most frequently observed. From the public health point of view, the authors felt that the salmonella infection in poultry had assumed a great importance because numerous serotypes recovered from birds were potentially pathogenic for man .

Working at National Salmonella and Escherichia Centre, Kasauli during 1958-1960, Agarwal(1962) identified S.champaign, S.matopani, S.san-diego S.typhimurium and S.weltevreden from poultry. He also gave a consolidated account of salmonella strains examined during this period and found that out of 110 strains isolated from animals, 41 were from poultry alone. The common types were S.typhimurium, S.richmond S.weltevreden and S.chester and other less common serotypes were S.anatum, S.champaign, S.matopani, and S.san-diego .

Kulasegram(1963) reported S.bareilly and S.typhimurium infection in poultry and observed that the imported birds were the most probable source .

Kulkarni et al(1963) reported an outbreak of fowl typhoid in a farm and compared the mortility and mortality rates between R.I.R. and W.L.H. birds in the same flock. The latter was found to be less affected than the former one .

Sharma and Singh(1963) isolated S.chester from 5, S.richmond from 2, S.typhimurium from 6 and S.weltevreden from one of 600 faecal samples of poultry .

Sharma and Singh(1963) isolated S. bareilly and S. chester from 15 day old chicken in a flock which had suffered a heavy mortality. The recovery of S. chester from the heart blood, liver, spleen and other visceral organs suggested that it was the cause of death. In a study extended over a period of 3 year.

Isnonev, Mateev, Slavkov and Bozhilov(1963) isolated 302 strains of salmonella belonging to 8 serotypes. Amongst these, S. gallinarum constituted 65.4% and Salmonella typhimurium 255% which was next in order of frequency .

Luthgen(1964) examined 625 fowls over 4 months old 18 yielded Salmonella. The serotypes were S. gallinarum S. pullorum (9) S. brandenberg(3), S. enteritidis(2) S. blockley(2) S. thompson(1) and one had a mixed infection with S. newport .

S. enteritidis var-jena, a serotype said to be responsible for mortality in brooder house chicks was isolated by Mallick and Rao(1965) .

Gordon and Tucker(1965) observed a cycle of trans-ovarian infection of S. menston in poultry, similar to that of S. thompson, S. menston was present, in adult fowls and their eggs. Chicks hatched from these eggs and eggs laid by their progeny carried the infection to maturity .

Datta and Singh(1965) on examination of faecal samples of 20 fowls and heart blood and intestinal organs of 208 dead chicken for the presence of salmonellae, isolated 16 strains of salmonella comprising S. bareilly(1), S. stanley(8), S. typhimurium(1), S. weltevreden (1) .

Khera et al(1965) reporting an outbreak of S. stanley in laying hens observed that the lesions were localised in ovaries and oviducts .

Kulkarni and Narsimham(1966) isolated S. typhi from a dead R.I.R. hen from chempur(Bombay) .

Kashiwazaki, and Coworkers (1966) reported the isolation S. typhimurium from the reactor birds .

Talbot and Rampling(1966) reported S. newington infection in weak old chicks. Further, they mentioned of heavy loss due to infection, the death rate reaching upto 95% .

Vasenius, et al(1967) collected intestinal samples from chicken from different slaughter houses situated in different parts of Finland. Salmonella could be detected only in four samples. The serotypes were S. typhimurium .

Godchild and Tucker(1968) examined on 382 autopsy birds for salmonella and isolated S. indiana, S. menston and S. typhimurium. All these serotypes were pathogenic to 50-60 days old chicks on inoculation .

Resistance of enteropathogens to antimicrobial compounds :

Salmonella : Several workers have reported a significant increase in the proportion of salmonella that exhibit resistance to tetracyclines and to chloramphenicol .

Ramsey and Edwards(1961) reported that whereas only 5% of 100 strains of S. typhimurium isolated from man in 1956-57 were resistant to tetracyclines, 14% of 158 strains isolated in 1959-60 were resistant .

Manten et al(1961) reported a slight decrease between 1958-60, but a significant increase between then and 1961 (Manten, Kampelmacher and Guinee (1963) noticed that 6 (1%) of 511 strains of S. dublic and 33(18%) of 183 strains of S. typhimurium isolated from cattle were resistant to tetracyclines.

McDhorter et al(1963) also found that S. typhimurium showed a higher incidence of resistance to chlortetracycline than did other serotypes. Of 48 strains from cattle, 45(94%) were resistant. They stated that the incidence of resistance shown by strains isolated from man had increased from 14% in 1959/60 to 38% in 1962 . Strains from fowls did not show this increase .

McKey et al(1965) reported the results of sensitivity tests on animal pathogens conducted over the period 1956-1963.They reported that in 1957 strains of salmonella typhimurium infection in the bovine was quite sensitive to streptomycin. Since then, a high incidence(90% in 1963) to this drug was observed . In 1957, one-half of the strains tested were resistant to tetracyclines but this figure rose to 90% in 1963. They tested a total of 128 strains of salmonella cholerae suis . Over 90% of the strains of Salmonella cholerae-suis were resistant to streptomycin in 1963, but during the same period only 20% showed resistance to tetracycline . In most cases, a good response to treatment were obtained by the the use of neomycin, chloramphenical, nitrofurazone, chlorhexidine, or a combination of these drugs in salmonellosis in all animal species. The carrier stage was the most difficult to treat and control .

Stuart et al(1967) reported the emergence of Furazolidone resistant strains of Salmonella gallinarum .

Thus, it will appear that with the lapse of years and large scale use of antibiotics salmonella organisms have been showing greater resistance,some serotypes exhibiting more resistance to a particular antibiotic than the other .

Shigella :

The causative agent of human dysentry was discovered by A. Grigoryev in 1891. In 1898 this organism was studied in detail by K. Shiga in Japan and in 1900-1901 by W. Kruse in Germany. Thus, the name Shiga bacillus was assigned to it .

In the Phillipines, S. flexner and R. Strong and in Manila W.E. Musagrave isolated dysentry organisms (Flexner bacillus) in the year 1900, which possed properties distinct from those mentioned earlier .

Shigella schmitzi was isolated by Schmitz(1917) in Romania. Other non-mannitol fermenters were also described by Large and Sankaran(1934) and by Sachs(1943) in India .

Shigella organisms have seldom been isolated from animals other than captive primates .

Dold and Fischer(1921) recovered Shigella dysentrie and Shigella flexneri from dogs in China. Earlier Shigella flexneri was recovered from rabbits in Germany by Messerschmidt (Lentz, 1902).

Scott(1927) reported isolation Shigella flexneri from a beaver in England .

Varela et al(1951) found Shigella sonnei in the faeces of a dog in Maxico .

Sette(1952) isolated Sh. dysentriae 1 from beavers in Italy .

✓ Deom and Mortelmans(1954) isolated Sh. sonnei from the blood of a calf in Belgian Congo .

Floyd(1955) during his survey of Salmonellosis in Egyptian domestic animals in Cairo between April and October, 1954 isolated isolated Sh. flexneri 3, Sh. flexneri 6 and Sh. boydii 3 and Sh. sonnei-2.

Thus, a total of five strains(0.5%) were obtained from 1000 dogs .

Gonzalez-Licea and Yardley(1966) isolated Sh. flexneri from the stool of a 23 year old woman suffering from diarrhoea and a typical case of acute ulcerative colitis in England . The patient was reported to be cured rapidly by the Sulpha diazine therapy .

Singh and Singh(1967) examined 205 pooled samples of raw buffalo milk from villages for salmonella and shigella. Two salmonella and and two shigella strains were isolated. Salmonella strains were typed as S. paratyphi A (1,2,12:a:-) and S. newport (6,8: e,h :1,2) . One of the shigella strains was identified as Sh. flexneri and the other remained untyped because of the roughness of colonies . They discussed the possible source of these bacteria in relation to public health .

M A T E R I A L S A N D M E T H O D S

1. ANIMAL :

(A) Calves: Calves used in this study belonged to the Government Cattle Farm, Patna. They were between 0 day to 8 months of age except two, which were 18 to 24 months old. The majority of calves fell between the age groups of 1 week to 1 month. The age distribution of calves included in the study is shown below :

Sl. no.	Age group.	Apparently normal calves.	Clinical cases.	Autopsy materials.
1.	Within 18 hours.	4	Nil	Nil
2.	0 day to 1 week.	10	6	4
3.	1 week to one month.	27	18	Nil
4.	1 month to six months.	15	9	Nil
5.	Six months to one year.	6	5	Nil
6.	Over one year.	Nil	Nil	2
	Total	62	38	6

The calves were housed in separate pens. They were immediately weaned after birth with colostrum. Subsequently they were maintained on ration schedule given below :

Period	Whole Milk	Concentrate Mixture	Roughages
First month	4 litres	Nil	Nil
Second month	4 litres	½ kg.	ad.lib(Grazing)
Third month	3 litres	1 kg.	ad.lib (do)
Fourth to sixth month	2 litres	1½ kg.	ad.lib (do)
Seventh to twelveth month	Nil	1½ kg.	ad.lib (do)

The concentrate mixture constituted of wheat bran 33%, ground nut cakes 40%, guar meal 20% plus 2% mineral mixture (Supermindiff)* and 2% common salt .

No antibiotic feed supplement was incorporated in the ration during the period of study. However, TM5(Terramycin)** was supplied to the livestock regularly until a year back.

Narrow and broad spectrum antibiotics inclusive of sulpha drugs were used quite frequently according to the nature of illness involved. Nitrofurans were never used for therapeutic purposes.

Calves above six months of age were vaccinated against Haemorrhagic septicaemia , Black Quarter and Anthrax .

A severe outbreak of foot and mouth disease in cattle due to Asian type I virus occured at the farm, between the period from 23rd January 1969 to 15th February 1969. This took a toll of 14 calves. At the initial stage of outbreak, milk was supplied to them after heating .

(B) Goats : Goats used in this study were mainly from slaughter house; Livestock Research Station, Patna. These were infected with Rinderpest Virus for the production of G. T. V. caccine. At the peak of temperature the spleen were harvested . Another source of goat were the local meat shop where the goats are collected from the adjoining villages. The particulars of them are given below :

Sl. No.	Source of animals	No. of animals	Remarks.
1.	Slaughter house of Livestock Research Station, Patna .	36	Used for G. T. V. vaccine production .

* Boots Pure Drug House .

** Pfizer .

2.	Local meatshops	26	Animals are brought on truck of onfoot from adjoining villages.
3.	Bihar veterinary college hospital	5	Clinical cases brought for treatment .
4.	Post-mortem room Bihar veterinary college	1	Hospital cases referred to Pathology Section .

(C) Dogs : Clinical cases of dogs come to the Bihar veterinary college from adjoining villages and Patna town .

Mostly the dogs were the household pets, though some of them were stray village dogs .

(D) Pigs : The pigs studied in this period were only two. One of the sample was from post-mortem room of Bihar veterinary College . The other was from Ranchi Bacon Factory. From the later the isolate was sent by Principal, Ranchi veterinary college to this laboratory for identification and bacteriological investigation.

(E) Poultry : The birds used in this study belonged to Central Poultry Farm, Patna. The breeds maintained at this farm are Rhode Iland Red and White Leghorn. The dead birds are sent to the Pathology laboratory of this college for post-mortem examination .

The birds at the farm were given the feed mixture comprising of maize, wheat bran, groundnut cake, fishmeal, bone meal, limestone, common salt and the mineral mixture. In addition, vitamin A, B_2 and D_3 were supplemented. T.M-5(Pfizer) and Bifuran(S.K.F.) were provided to the brooder chicks from time to time. Piperazine adipate(I.C.I.) was used for deworming the fowls at regular intervals.

Postmortem lesions recorded in the birds were as follows: Enteritis(12 cases), salpingoperitonitis(6), bronchopneumonia (9),

coligranuloma(1), protozoan and other parasitic infestations (19), heat stroke (9), cachectic(2) and avian leukosis complex(1).

The age distribution of the birds examined were as under :

	Hen	Cock	Pullet	Cockrel	Chicks
R.I.R.	8	4	2	Nil	5
W.L.H.	14	2	7	6	11
	22	6	9	6	16

2. COLLECTION OF MATERIALS :

(A) From living animals :

(a) Rectal Swabs : A copper wire was cut to 8 inches long . Cotton swab was fixed at one end and the plug at the other. It was put in a clean test tube and was sterilized at 180°C for 1 hour .

Two rectal swabs each from clinical and sub-clinical cases were collected with routine aseptic precautions. The anus was cleaned with water and sterilised with cotton soaked in 70% alcohol. The swab was introduced into rectum and material collected by rotating it for 8-10 times .

A total of 100 duplicate swabs were collected from 100 calves and 5 duplicate swabs from 5 goats . Similarly, 68 duplicate swabs were collected from dogs.

(b) Urine samples : Urine samples were collected from dogs only. First, the prepuce was cleaned and then samples collected in sterile test tube after inducing micturition by physical manipulation . Fourteen urine samples were collected from these dogs .

(B) From dead animals :

Materials collected from autopsy cases comprised faecal samples of intestinal contents and internal organs .

(a) Faecal samples: The faecal samples collected were the pooled content of large and small intestines. They were collected in sterile petridishes .

(b) Internal organs : Materials from internal organs such as liver, kidney, heart, lungs, spleen and mesenteric lymph nodes etc. were collected with usual sterile precautions.

(c) The specimens of bile from the gall bladder and heart blood were collected in sterile pasteur pipettes.

Particulars of materials collected have been summarised in Table I .

Table I

Animal/ age	LIVING		DEAD									
	Rectal Swabs	Urine	Intest. content	Liver	Bile	Mes. lym. node	Lungs	Heart	Kidney	Spleen	Others	Total
Calves :												
0 - 18 hours	4	-	-	-	-	-	-	-	-	-	-	4
0 day to 1 wk.	16	-	-	-	-	-	-	-	-	-	-	16
1 wk. to 1 mo.	45	-	-	-	-	-	-	-	-	-	-	45
1 mo. to 6 mo.	24	-	-	-	-	-	-	-	-	-	-	24
6 mo. to 1 yr.	11	-	-	-	-	-	-	-	-	-	-	11
Over 1 yr.	-	-	2	2	2	2	2	2	2	2	-	16
0 day to 1 wk.	-	-	4	4	4	4	4	4	4	4	2	34
												150
Goats :												
About 1 yr.	5	-	42	42	38	35	34	37	36	31	-	300
												300
Dog :												
Pup	11	4	-	-	-	-	-	-	-	-	-	15
Adult	51	10	2	2	1	1	2	2	1	2	-	74
												89
Pig :												
Piglet	-	-	2	-	-	1	-	-	-	-	-	3
												3

Poultry : Chick	-	-	16	16	16	-	16	16	16	16	-	110
Cockrel and pullet	-	-	14	14	14	-	15	14	13	13	-	94
Cock and hen	-	-	24	19	19	-	23	22	20	15	4	146
												353

3. METHODS FOR ISOLATION AND IDENTIFICATION .

The materials thus collected were enriched by inoculating into two different media . Nutrient broth was used for E. coli whereas Selenite F Broth was used for the isolation of Salmonellae and Shigellae. Cultures were incubated overnight at 37°C for this purpose.

After overnight incubation, a loopful of the nutrient broth culture was streaked over MacConkey's agar plate. The Selenite F broth culture was inoculated on Leifson's Desoxycholate citrate Agar(D.C.A.) plate as modified by Hynes (Cruickshank,1965).

E. coli : On an average, 4 lactose fermenting round, translucent and convex colonies of about 2 m.m. diameter that developed on MacConkey's agar plate were picked up and streaked on the Eosine Methylene Blue agar(E.M.B.) plate and incubated at 37°C for 24 hours. Colonies of a moderate size with dark(almost black) centres and showing metalic sheen in reflected light were further transferred on to the Nutrient Agar plate for the study of colony characteristics. Similarly another half of the same colony was also inoculated into Nutrient Broth for testing the motility and morphology of the organism. The morphology was studied in smear from broth culture stained by Gram's method.

After this the isolate was maintained in agar slants and stored in refrigirator for further study .

All the isolates thus obtained were studied for fermentation reaction of carbohydrates and alcohols, such as lactose, glucose, sucrose, arabinose, raffinose, adonitol, dulcitol, mannitol, sorbitol and salicin . Lactose fermenting gram negative bacilli were considered to be of coligroup.

Salmonella and Shigella : On D.C.A. plate, the different colonies were provisionally assigned to the different group of micro-organisms based on the following cultural characteristics (Dyke, 1951) .

(i) Salmonella- 0.5 to 1.0 m.m. diameter; colourless and opaque with black dot in the centre .

(ii) Shigella - (a) Shigella sonnei : colonies one to two m.m. in diameter, colourless on the 1st day later becoming pale, pink, but much paler and less opaque than E. coli .

(b) Shigella flexneri : Colonies round and colourless with narrow plane periphery and a central dot .

Colonies were transferred onto agar slant and kept in the refrigerator after overnight incubation . From another half of the same colony, smears were prepared and stained with Gram's stain. Colony character and morphology of the organisms were studied under microscope .

IMViC test, Nitrate reduction test and growth in Koser's Citrae medium were conducted for the preliminary identification of the isolates .

The 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 were mainly assigned to the genus salmonella .

The isolates were subsequently tested for H_2S production in Triple Sugar Iron Agar(T.S.I) medium and for haemolysis on ox blood agar plate. The tests for Indol, M.R.V.P., gelatin liquifaction etc. were done using conventional procedures .

The isolates suspected for salmonella, as judged by the cultural characteristics on selective media were further checked with salmonela genus O-1 specific phage, kindly supplied by Dr.S.C. Adalakha, Professor of Bacteriology, U.P.College of Veterinary Science and Animal Husbandry, Mathura, U.P.

Propagation of Salmonella genus O-1 specific phage :

The phage supplied was freez dried. This was propagated in this laboratory. The procedure followed is given briefly below :

(i) The phage broth was prepared by adding 2 gms of lablemco and 0.7 gm. of sodium chloride to 100 ml. of distilled water. The reaction was adjusted to 7.0 and was sterilized at 15 lbs.pressure for 15 minutes .

(ii) This lot of broth was inoculated with the indicator Strain Salmonella paratyphi - B phage type 1(76) and was incubated at 37°C for 5 hours .

(iii) One ml. of original phage giving confluent lysis at 10^{-5} dilution was added to this 5 hours broth culture and again incubated for nine hours .

(iv) It was then kept in refrigerator at 4°C for 10 hours .

(v) The culture was centrifuged and supernatant was stocked in sterile flasks .

(vi) Chloroforms was then added to a final concentration of 1% and the phage preparation was stored at 4°C .

(vii) After 3-4 days the preparation was tested for sterility and titered .

(viii) The phage titre was estimated by serial tube dilutions made from 10^{-1} to 10^{-6} . By this method, the phage titre was obtained and was stored . This was used for identifying the strains tentatively assigned to salmonella genus on the basis of biochemical and morphological studies.

The isolate was inoculated into nutrient broth and incubated at 37°C for 6hours . A blob culture was made on fresh nutrient agar plate . It was allowed to dry on bench. One drop of the phage preparation was put in the centre of the culture . It was incubated overnight and lysis was observed. The cultures showing semi confluent or confluent lysis were identified as salmonellae and were kept in nutrient agar slants for further studies .

The strains recognised as salmonellae were further tested by slide agglutination using poly 'O' and poly 'H' antisera received from Haffkin Institute, Bombay .

Slide agglutination was conducted with each of the two poly 'O' and poly 'H' sera . For this a culture suspension was made in normal saline on a clean slide . To this was added two loopful of poly 'O' antisera . A control test was run on the same slide with the culture suspension and normal saline (no serum) to check autoagglutination. The agglutination was observed under low power microscope or Fisher Kahn viewer . The culture was similarly tested with poly 'H' serum .

4. SEROLOGICAL TYPING :

E.coli : All the E.coli strains identified on biochemical tests were examined for the determination of their 'O' Antigens . Antisera available were against O groups 026, 055, 086, 0114,0119, and 0126 which were procured for the Haffkine Institute, Bombay.

With the above available antisera, two Polyvalent antisera were prepared. Polyvalent I antiserum comprised 026, 055 and 086, and Polyvalent II antiserum was prepared by pooling 0114,0119,0126 .

The method adopted for determination of specific 'O' antigen was the same as advanced by Kauffmann(1947) and Barua et al(1956). For the preparation of 'O' antigen, the growth from overnight agar slant culture was washed with 0.5 to 1 ml physiological saline and autoclaved at 121°C for one hour .

Slide agglutination test was done using each of the two (Poly I and Poly II) Polyvalent sera. Three loopful of the prepared antigen were thoroughly mixed with two loopful of each of the polyvalent sera at two different places of a clean microscopic slide. A control with saline and antigen was also set up at the third place. To present drying, the slide was put over a wet blotting paper. The results were read up to the 15th minute under low power microscope or with the Fisher Kahn viewer for the presence of agglutination reaction . The strain which gave positive agglutination with any one of the polyvalent serum was further tested in the same way with the specific 'O' antiserum, and the strain giving agglutination reaction with the specific serum was considered to belong to that 'O' group .

Salmonella : Since specific factor sera were not available the strains were tested by slide agglutination test against

polyvalent 'O' and 'H' antiserum only in order to confirm the genus.

Shigella : Due to non-availability of Shigella antisera , they could not be typed serologically .

5. DRUG SENSITIVITY TESTS :

The strains isolated during the study were tested against seven chemotherapuntic agents . The particulars of drugs used are summarised in the following table II

Table II

Sl. no.	Trade name	Compound	Form of supply	Content
1.	Ambistryn (Squibb)	Dihydro Streptomycin sulphate	Vials	1 Gm.
2.	Steclin (Squibb)	Tetracycline hydrochloride	Vials	500 mg.
3.	Ledermycin (Cynamid)	Demethylchlor-tetracycline hydrochloride	Capsules	250 mg.
4.	Chloromycetin (Parke-Davis)	Chloramphenicol	Capsules	250 mg.
5.	Ampicillin (Juggat Pharma Pvt. Ltd.)	Ampicillin trihydrate	Capsules	250 mg.
6.	Erythromycin (Abbot Lab. Pvt. Ltd.)	Erythromycin stearate	Tablet	250 mg.
7.	Furadentine (S.K.F. Pharmaceuticals)	Nitrofurans	Tablet	250 mg.

The method adopted for the sensitivity test was basically that advocated by Glantz(1962) with little modications . Since the tube dilution method is supposed to be a better and more accurate procedure than plate method, the former was adopted in the present study .

All cultures of E.coli, Salmonella and Shigella which had been isolated from healthy and diseased animals and poultry were put to test .

Stock solutions of the antibiotics were prepared by dissolving the dry powder in nutrient broth to give a concentration of 1000 μg/ml .

The nitrofuran was dissolved in sterile demineralized water and made up to the concentrations of 100 μg/ml. The stock solutions of antibiotics and nitrofuran were suspended into sterile screwcapped tubes and stored in refrigerator . Each day one of the tubes of stock was removed from the refrigerator and kept at room temperature for sometime (about ½ to 1 hr), and desired dilutions were prepared for the day's test. The protocol of dilutions is shown in table III .

Table III

Preparation of antimicrobial dilutions

Compound	Conc. of stock (mcg./ml.)	Dilution Stock (ml.)	Dilution Broth (ml.)	Tube Concentration Initial (mcg./ml.)	Tube Concentration Final* (mcg./ml.)
Antibiotics	1,000	1	24	40	20
	1,000	1	49	20	10
	1,000	1	99	10	5
Nitrofuran	100	5	20	20	10
	100	2	18	10	5
	100	1	19	5	2.5

* After the addition of 0.5 ml. broth culture to 0.5 ml. initial antibiotic dilution .

The results were recorded as positive (+) for tubes in which growth of the bacteria was evident(resistant) and negative(-) in the absence of visible growth (sensitive). The overall results were then reported as very sensitive(VS), sensitive(S) and not sensitive(NS) .

The following were the minimum inhibitory concentration for each rating :

VS = No growth at 5/μg/ml. or less .

S = No growth at 10/μg/ml. or less .

NS = Growth at 10 /μg/ml. or less .

For furadentin-e

VS = No growth at 2.5 /μg/ml. or less .

S = No growth at 5 /μg/ml. or less .

NS = Growth at 5 /μg/ml. or less .

RESULTS

In the present study, a total of 303 domesticated animals and poultry comprising 106 calves, 68 goats, 68 dogs, 2 pigs and 59 poultry were examined for determining the incidence of enteropathogens. Among them the incidence of *E. coli* was highest in calves(52.8%) and lowest in dogs(29.4%) . In goats and poultry the percentage recovery of E. coli was approximately 33%. Both of the two pigs which had died during a severe outbreak of enteritis yielded *E. coli* .

The incidence of Salmonella in various classes of livestock and poultry examined ranged between 1.5 in dogs to 3.4 % in poultry . The percentage in calves and goats were 1.9 and 2.9% respectively. On of the two dead pigs examined yielded salmonella .

Shigellae were isolated from only two out of 68 dogs examined giving the rate of recovery at 3.2% .

Table

Incidence of enteropathogens in domesticated animals and poultry.

Animals examined		Animals found infected with					
		E. coli		Salmonella		Shigella	
Species	No. animals	No.	Percentage	No.	Percentage	No.	Percentage
Calves	106	56	52.8	2	1.9	0	0
Goats	68	22	32.3	2	2.9	0	0
Dogs	68	20	29.4	1	1.5	2	2.9
Pigs	2	2	100	1	50	0	0
Poultry	59	20	33.8	2	3.4	0	0

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Pigs	2	2	100	1	50	0	0
Poultry	59	20	33.8	2	3.4	0	0

The percentage of isolations of different enterpathogens obtained from living and autopsy cases in further shown in Table

Table VI

Incidence of enteropathogens in living and autopsy cases of domesticated animals and poultry .

No. of animals examined			Animals found infected with					
			E. coli		Salmonella		Shigella	
			No.	Percentage	No.	Percentage	No.	Percentage
Calves 106	Living	100	52	52	1	1	0	0
	Dead	6	4	66.6	1	16.6	0	0
Goats 68	Living	5	4	80	0	0	0	0
	Dead	63	18	28.5	2	3.1	0	0
Dogs 68	Living	66	19	28.7	1	1.6	2	3.2
	Dead	2	1	50	0	0	0	0
Pigs* 2	Living	0	0	0	0	0	0	0
	Dead	2	2	100	1	50	0	0
Poultry 59	Living	0	0	0	0	0	0	0
	Dead	59	20	33.8	2	3.3	0	0
Total		303	120	39.6	8	2.6	2	0.66

All the isolates recovered from different species of animals were studied for their biochemical reactions, serotypes with six available antisera and for their drug resistance. The results obtained are reported briefly below .

* These were autopsy materials from cases of enteritis .

Escherichia coli :

As mentioned earlier, 56 out of 106 calves examined were found to be infected with E.coli . From these 56 calves, 61 isolates were recovered since two calves aged 4 and 5 days which had died of acute illness showing fever, enteritis and bronchopneumonia yielded E.coli from various internal organs . These calves were considered obviously to have died of colisepticaemia. As will appear from the data shown in the appendix, the strain belonged to 026 and 0119 respectively .

Out of 134 strains of E.coli recovered from different species of animals and poultry, 68(50.8 %) were typable with six available 'O' antisera. The result are presented in table VII .

Table VII

Incidence of E.coli 'O' groups in domesticated animals and poultry .

Animals	Serotypes							Unty-pable	Total
	026	055	086	0114	0119	0126	Total typable		
Calves	6	6	8	0	11	6	37	24	61
Goats	3	3	1	0	2	0	9	13	22
Dogs	2	4	0	0	2	2	10	10	20
Pigs	0	0	0	0	0	0	0	2	2
Poultry	6	0	4	2	0	0	12	17	29
Total	17	13	13	2	15	8	68	66	134

It will appear that among the 68 typable strains the frequency of isolation of 026 ranked highest (25%) 0119 was the next in order of frequency(22%). The percentage recovery each of 055 and 086 was intermediated (19.1%). 0126 was less frequently encountered (11.8%). E.coli of O group 0114 were only 2(3%) .

Calves: The isolation of E.coli from calves was further studied in relation to their age groups vis-a-vis the type of infection. It was found that the highest frequency of isolation both in clinical and subclinical cases was in calves within the age group of 0 day to 1 week. The percentage did not seem to be significantly different in subchlinical and clinical infections.

Table VIII

Table VIII

Incidence of E.coli in calves in relation to the age group and type of infection .

Age	Subclinical			Clinical		
	No. of calves examined	No. of isolates	Percent	No. of calves examined	No. of isolates	Per-cent.
Within 18 hours	4	0	0	0	0	0
0 day to 1 week	10	7	70	10	6	60
1 week to one month	27	12	44.4	18	10	55.5
1 month to six month	15	9	60	9	5	55.5
6 months to one year	6	3	50	5	3	60
Over one year	0	0	0	2	1	50
Total	62	31	50	44	25	56.8

Incidence of E.coli serotypes was further examined in relation to the type of infection and the sex. It was found that in subclinical cases there was no association between the

Table IX

Incidence of E.coli serotypes from calves.

	Age	Serotypes						Total
		026	055	086	0114	0119	026	
Sub cli ni cal	0 day to 1 week	0	0	1	0	1	0	2
	1 week to 1 month	0	0	3	0	2	1	6
	1 month to 6 month	0	1	0	0	1	1	3
	6 month to 1 year	0	0	1	0	0	0	1
	Over(one) year	0	0	0	0	0	0	0
	Total	0	1	5	0	4	2	12
Cli-ni cal	0 day to 1 week	2	1	1	0	5	0	9
	1 week to 1 mont	1	1	2	0	1	3	8
	1 month to 6 month	1	2	0	0	0	1	4
	6 month to 1 year	2	0	0	0	1	0	3
	Over one year	0	1	0	0	0	0	1
	Total	6	5	3	0	7	4	25
	Grand Total	6	6	8	0	11	6	37

susceptibility and sex. On the other hand, amongst the clinical and autopsy cases the female calves were more susceptible than the male ones. Further, the degree of susceptibility between male and female calves and the serotypes of E. coli recovered from them could not be established table IX.

Biochemical reactions :

The 14 biochemical patterns shown by 134 strains are given in table X.

Table X

Biochemical patterns shown by E. coli from domesticated animals and poultry .

Pattern numbers	Lactose	Glucose	Sucrose	Salicin	Mannitol	Dulcitol	Adonitol	Arabinose	Sorbitol	Raffinose
1	A	A	-	-	A	-	-	A	-	-
2	A	A	A	A	A	A	-	A	A	A
3	A	A	-	-	A	A	-	A	A	-
4	A	A	A	-	A	-	-	A	A	A
5	A	A	A	-	A	A	-	A	A	A
6	A	A	-	A	A	-	-	A	-	-
7	A	A	-	A	A	-	-	A	A	A
8	A	A	A	A	A	-	-	A	A	A
9	A	A	-	-	A	-	-	A	A	A
10	A	A	-	-	A	-	-	A	A	-
11	A	A	-	A	A	A	-	A	A	A
12	A	A	A	-	A	A	-	A	-	-
13	A	A	-	A	A	-	-	A	-	A
14	A	A	A	-	A	-	-	A	A	A

A = Acid .

- = Non fermenter .

Table XI

Biochemical patterns of
E.coli from calves .

	Age	Biochemical pattern numbers														Total
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Sub cli-ni-cal	0 Day to 1 week	1	1	1	2	0	0	1	0	0	0	1	0	0	0	7
	1 Week to 1 month	1	1	2	1	0	3	1	0	1	0	0	0	1	1	12
	1 Month to 6 month	1	0	1	0	0	2	0	0	1	2	0	1	1	0	8
	6 Month to 1 year	0	0	0	0	0	1	2	0	0	0	0	0	0	0	3
	Over 1 year	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total	3	2	4	3	0	6	4	0	2	2	1	1	2	1	31
Cli-ni-cal	0 Day to 1 week	1	2	2	2	1	1	0	1	0	0	0	0	0	0	10
	1 Week to 1 month	1	0	2	1	1	2	1	0	2	0	0	0	0	0	10
	1 Month to 6 month	0	0	2	0	0	0	0	0	1	1	0	1	0	0	5
	6 Month to 1 year	0	0	0	1	0	0	2	0	0	0	0	0	0	0	3
	Over 1 year	0	0	1	0	1	0	0	0	0	0	0	0	0	0	2
	Total	2	2	7	4	3	3	3	1	3	1	0	1	0	0	30
	Grand Total	5	4	11	7	3	9	7	1	5	3	1	2	2	1	61

Table XII

Biochemical patterns of different serotypes of E.coli from calves .

	Serotypes	Biochemical pattern number														Total
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Subclinical	026	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	055	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
	086	0	1	0	1	1	2	0	0	0	0	0	0	1	0	5
	0119	1	0	0	0	0	1	1	0	0	0	1	0	0	0	4
	0126	1	0	0	0	0	1	0	0	0	0	0	0	0	0	2
	Total	2	1	0	1	1	2	1	0	0	0	1	0	2	0	11
Clinical	026	2	0	1	1	0	1	1	0	0	0	0	0	0	0	6
	055	0	0	1	0	1	0	1	1	1	0	0	0	0	0	5
	086	0	0	0	1	0	1	0	0	1	0	0	0	0	0	3
	0114	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0119	5	0	0	0	0	0	1	0	1	0	0	0	0	0	7
	0126	0	0	2	0	1	0	0	0	0	0	0	1	0	0	4
	Total	7	0	4	2	2	2	3	1	3	0	0	1	0	0	25
Grand Total		9	1	4	3	3	4	4	1	3	0	1	1	2	0	37

Based on sugar fermentation reactions, 61 calf strains were assigned to 14 biochemical patterns (table XI).

Six out of 31 strains isolated from the subclinical materials belonged to the biochemical pattern number 6. On the other hand, 7 out of 30 strains from clinical cases showed pattern number 3. It was interesting to find that 31 subclinical strains fell to 12 different patterns while 30 clinical strains showed 11 patterns.

Thirty seven strains of E. coli tested by six 'O' group (Table X) antisera were further examined for their biochemical patterns. The relationship between serotypes and biochemical patterns are presented in (table XII) .

It would appear that there was no definite pattern amongst the serotypes isolated from subclinical cases. On the other hand, these recovered from clinical and autopsy materials showed preponderance of certain biochemical patterns. This may be illustrated from the observation that 5 out of 7 strains of O119 belonged to biochemical pattern number 1 .

Drug resistance :

One hundred and thirty four strains of E. coli recovered from different animals and poultry were subjected to sensitivity test against 7 antimicrobial drugs. Of these, 70 were resistant to ledermycin, 66 to steclin, 58 to streptomycin, and 30 to erythromycin. The numbers of strains resistant to ampicillin chloromycetin , and furadentin were 5, 2 and 2 respectively (table XII) .

A correlation study between the serotypes and drug resistance of different animal strains were also examined table XIII.

Table XIII

Incidence of E. coli resistant strains to antimicrobial drugs .

Animals	No. of strains	Number of resistant strains						
		Streptomycin	Erythromycin	Ampicillin	Chloromycetin	Ledermycin	Steclin	Furadentine
Calf	61	27	14	2	1	32	31	0
Goat	22	11	3	20	0	12	9	0
Dog	20	6	7	0	1	10	10	2
Pig	2	0	0	0	0	0	2	0
Poultry	29	14	6	0	0	16	14	0
Total	134	58	30	5	2	70	66	2

The incidence of 61 E. coli strains obtained from calves were also examined in relation to type of infection and drug resistance .

Amongst 31 subclinical strains 19,16, and 16 were found to be resistant to steclin, ledermycin and streptomycin respectively. 5 were resistant to erythromycin , 1 each to ampicillin and chloromycetin and none to furadentine . On the other hand, out of 30 clinical strains 16 were resistant to ledermycin, 12 to steclin , 11 to streptomycin and 9 to erythromycin. Only two were resistant to ampicillin and none to chloromycetin and furadentine (table XIV) .

Table XIV

Resistance of E. coli to drugs and type of infection .

Type of infection	No. of strains examin-	Antimicrobial drugs						
		Streptomycin	Erythromycin	Ampicillin	Chloromycetin	Ledermycin	Steclin	Furadentine
Subclinical	31	24	16	5	1	16	19	0
Clinical	30	11	9	2	0	16	12	0

Table XV

Resistance patterns shown by strains of *E. coli* from domesticated animals and poultry

Pattern number	Ambistryn	Erythromycin	Ampicillin	Chloromycetin	Ledermycin	Steclin	Furadentine
1	S	S	S	S	R	R	S
2	R	R	S	S	R	S	S
3	S	S	R	S	S	S	S
4	S	S	S	S	S	R	S
5	R	S	S	S	R	S	S
6	S	R	S	S	R	S	S
7	S	R	S	S	S	R	S
8	R	S	S	S	S	R	S
9	R	S	R	S	S	S	S
10	S	S	S	R	S	S	S
11	R	S	S	S	S	S	S
12	R	S	S	S	R	R	S
13	R	R	S	S	S	R	S
14	S	R	S	S	S	S	S
15	S	S	S	S	R	S	S
16	S	S	S	S	S	S	S
17	S	R	S	S	R	R	R

S = Sensitive .

R = Resistant .

All 134 strains were subjected to *in vitro* sensitivity test to seven antimicrobial drugs and their resistance patterns were recorded i.e. the resistance of the organism to the particular drug in seriatum was recorded to designate its pattern e.g. pattern no. 1 denotes organisms resistant to ledermycin and steclin . Table XV .

Table XVI

Resistance patterns of E.coli from calves to seven antimicrobial drugs.

	Age	Resistance pattern numbers																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Total
Sub cli ni cal	0 Day to 1 week	0	0	0	1	4	0	0	1	0	1	0	0	0	0	0	0	0	7
	1 Week to 1 month	2	0	0	2	0	1	1	1	0	0	0	3	0	1	0	1	0	12
	1 Month to 6 month	1	1	0	0	2	0	0	2	1	0	0	0	1	0	1	0	0	9
	6 Month to 1 year	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	0	0	3
	Over 1 year	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total	3	1	0	3	6	1	1	4	1	1	0	5	1	1	2	1	0	31
Cli ni cal	0 Day to 1 week	1	3	0	1	1	0	2	0	0	0	0	0	0	0	2	0	0	10
	1 Week to 1 month	1	0	0	1	1	0	0	0	1	0	0	1	1	0	2	2	0	10
	1 month to 6 month	0	0	1	1	1	0	0	0	0	0	1	1	0	0	0	0	0	5
	6 month to 1 year	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	3
	Over 1 year	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	2
	Total	2	3	1	3	3	1	3	0	1	0	2	2	1	0	4	4	0	30
	Grand Total	5	4	1	6	9	2	4	4	2	1	2	7	2	1	6	5	0	61

Table XVII

Resistance patterns of different serotypes of *E. coli* from calves to seven antimicrobial drugs.

	Serotypes	Resistance pattern numbers																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Total
Subclinical	026	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	055	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	086	0	0	0	1	1	1	0	0	0	0	0	1	0	0	0	0	0	5
	0119	0	0	0	0	0	0	1	0	0	1	0	0	1	1	0	0	0	4
	0126	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	2
	Total	0	0	0	2	1	1	1	2	1	0	1	1	1	0	0	0	0	12
Clinical	026	1	1	0	0	0	2	0	0	0	0	0	0	0	0	0	2	0	6
	055	1	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	5
	086	0	1	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	3
	0114	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0119	5	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	7
	0126	1	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	4
	Total	8	2	1	1	1	2	1	0	1	1	1	1	1	0	3	3	0	25

The resistance patterns of the 37 typable strains from calves were examined in relation to type of infection. It was fouund that the subclinical strains did not show any particular resistance patterns , while of 26 clinical strains 8(30.7%) fell in pattern No.1 (Steclin and Ledermycin) . It may be mentioned here that out of 7 strains of O119, 5 showed resistance to this pattern. Pattern No.14 and 15 came next in order of frequency (table XVII).

Goats : A total of 68 goats were examined of which 22 (32.4%) yielded E.coli. Nine of them could only be typed with six available sera. Three of them belonged each to O55 and O26 and 2 and 1 to O group O119 and O86 respectively .

E.coli of group O26 were recovered from the clinical cases only while O55 were recovered from the subclinical cases . None belonged either to O126 or O114 .

As against 14 biochemical patterns shown by E.coli from calves, the goat strains showed only 10 patterns. Seventeen subclinical strains of E.coli recovered from goats showed 10 different biochemical patterns while 6 clinical strains fell in 6 patterns only. It may be mentioned here that among the calf strains too, the number of biochemical patterns shown by clinical strains were less than those recovered from subclinical cases (table XI) .

Table XVIII

Biochemical patterns of E.coli from goats .

Type of infection	Strains examined no.	Biochemical pattern numbers										Total
		2	3	4	5	7	8	10	11	12	13	
Subclinical	16	1	1	2	1	2	2	2	3	1	1	16
Clinical	6	0	1	0	1	1	1	0	1	0	0	6

There was no association between the serotypes and drug resistance pattern .

Dog : Rectal swabs from 66 dogs admitted at Bihar Veterinary College Hospital for various ailments such as diarrhoea, dysentry, fever including those brought for vaccination were examined. Urines samples from 14 of these cases were also examined. In addition, internal organs from 2 autopsy cases were available. Thus, materials from 68 dogs were examined. E. coli were isolated from 13 rectal swabs, 6 urin samples and one autopsy materials. Of these 68 dogs, 20(29.4%) yielded E. coli .

Forteen dogs from whom both rectal swabs and urin samples were examined, E. coli were isolated only from 2 of the urine samples and none from the rectal swab. Thus, the percentages of isolation from different specimens were as follows :

Specimens	Number examined	Number positive for E. coli	Percentages of infection
Rectal swabs	66	13	19.6
Urin	14	6	42.8%
Autopsy materials	13	1	7.7%

All but one strains were non-haemolytic . One haemolytic strain was recovered from a case of gastroenteritis which could not be typed with six available sera (Figure No. 1) .

Two of 6 strains recovered from urin samples of 14 dogs were serologically untypable and were sensitive to only Ambistryn and chloromycetin and were resistant to chloromycetin, ledermycin steclin, and furadentine. It may be mentioned here that both of them belonged to the same private owner and died after an illness of one and half month showing intermittent high temperature (103°F 105°F

and 103 to 105.4°F) Prior to collection of urine samples made at the height of temperature, they were treated with a course each of terramycin and furadentine but was ineffective .

Out of 20 strains from 68 dogs ,3(42.8%) of 7 strains from subclinical and 7(53.8%) of 13 strains from clinical cases were typable with available sera. There was no relationship between serotype and subclinical infection but there was some association between the serotype and clinical infection in that 4 out of 7(57.1%) possesed 055 antigen table XVI.

Table XVI

E. coli serotypes from dogs and types of infection .

Type of infection	Serotypes				Total	
	026	055	0119	0126	Typable	Untypable
Subclinical	1	0	1	1	3	3
Clinical	1	4	1	1	7	7
Total	2	4	2	2	10	10

The incidence of E. coli was further studied in relation to age group and type of infection. The carrier rate in Dogs and Bitches was more them in pups whereas the rate of isolation of E. coli from clinical cases was higher in pups than in adults .

Table XVII

Incidence of E. coli in relation to age group and type of infection.

Age		Number of animals examined	Number found infected with	Percentage
Subclinical	Pup	14	2	14.3
	Dog and Bitch	9	5	55.5
	Total	23	7	30.4
Clinical	Pup	3	1	33.3
	Dog and Bitch	42	12	28.6
	Total	45	13	28.8

Poultry : Autopsy materials from 59 birds(R.I.R. and W.L.H.) were available for study .

Amongst them the numbers of chicken, cockrels and pullets , and cocks and hens were 16, 15 and 28 respectively. The percentages of isolation of E.coli from these groups of poultry were 43% , 33.3% 28.5% respectively. The frequency of isolation from these birds were studied in relation to the causes found responsible for their death at necropsy(Table XVII).

Table XIX

Incidence of E.coli in poultry in relation to age group and type of infection .

Age	Enteritis* No. infected / No. examined	Per-cent	Parasitic enteritis No. infected / No. examined	Per-cent	Others** No. infected / No. examined	Per-cent	Non specific death No. inf. / No. exm	Per-cent
Chick	4/6	66.6	1/2	50	0/3	0	2/5	40
Cockrel and pullet	0/4	0	3/5	60	2/3	66.6	0/3	0
Cock and Hen	1/4	25	2/10	20	5/10	50	0/4	0
Total	5/14	35.7	6/17	35.3	7/16	43.7	2/12	16.6

* Non-parasitic enteritis .

** Bronchopneumonia, Salpingo-peritonitis and coligranuloma .

\+ Heat stroke .

It was found that the largest number of chicks(66.6%) had died of enteritis caused by E. coli . Again among the cockrels and pullets, the highest percentage of death due to broncho-pneumonia was associated with infection with E.coli. Further in this group birds 60%

Table XX

E. coli serotypes from poultry .

Age	Serotypes		
	026	086	0114
Chicks:			
Subclinical	2	2	0
Clinical	1	0	1
Cockrels and Pullets :			
Subclinical	0	1	0
Clinical	1	0	1
Cocks and Hens :			
Subclinical	0	0	0
Clinical	2	1	0
Total	6	4	2

Table XXI

Resistance of E. coli strains from poultry and type of infection .

Type of infection	No. of strains tested	Antimicrobial drugs						
		Streptomycin	Erythromycin	Ampicillin	Chloromycetin	Lédermycin	Stecolin	Furadentine
Subclinical	13	8	4	0	0	8	6	0
Clinical	16	6	2	0	0	8	8	0
Total	29	14	6	0	0	16	14	0

that were considered to have died due to parasitic enteritis also showed infection with E. coli . Similarly, E. coli was incriminated in other infection in cocks and hens. Thus, from the data shown above (Table IXX) it was clear that the largest number of deaths associated with E. coli in chicks resulted from enteritis but in cockrels and pullets as well as in cocks and hens there were other cases. Out of 29 strains isolated from 59 birds only 12 were typable with six available sera. The remaining 17 could not be typed. Among the typable strains, 50% belonged to 'O' group 026, 33.3% to 086 and 16.7% to 0114 (Table XX) . On examining the association between the serotypes and drug resistance pattern, it was revealed that out of 6 strains of 026 which were predominant over other serotypes, 50% showed resistance pattern No.6(Erythromycin and Ledermycin)/ (Table XXI) . No such relationship was found with other serotypes.

Further, there was no relationship between the serotypes and biochemical pattern .

Pigs : Only two strains from pigs were available for study. One of them was recovered in pure culture from severe outbreak of enteritis and the other was isolated from a case of enteritis received in the department for autopsical examination. The former was non-haemolytic while the latter was haemolytic on bovine blood agar plate. It may be mentioned that from the latter case, a strain of salmonella (to be described later) was also recovered. Both the strains of E. coli were non-typable serologically and were resistant to steclin only. A striking difference was noted between the fermentation reactions of the two strains in that while the former(non-haemolytic epidemic strain attacked sucrose, salicin and raffinose, the latter (haemolytic strain) failed to do so .

Table XXII

Incidence of Salmonella infection in different domesticated animals and poultry .

Specimens examined	Number of Salmonella strains isolated				
	Calves 106	Goats 68	Dogs 68	Pigs * 2	Poultry 59
Rectal (living)	0/100	0/5	1/62	0/0	0/0
Intestinal content	1/6	1/42	0/2	1/2	1/59
Liver	1/6	0/42	0/2	0/0	0/51
Bile	0/6	0/37	0/2	0/0	0/50
Mesenteric lymph node	0/6	1/35	0/1	0/1	0/0
Spleen	0/6	0/36	0/2	0/0	0/42
Lungs	0/6	0/41	0/2	0/0	1/50
Heart	0/6	0/37	0/1	0/0	0/51
Kidney	0/6	0/36	0/1	0/0	0/46
Others	0/2	0/0	0/0	0/0	0/4
Total	2/150	2/300	1/75	1/3	2/353
Percentage of isolation according to number of animals.	1.9	2.9	1.5	50	3.38

* Only two cases of severe enteritis were examined .

$\frac{\text{Number of Salmonella isolated}}{\text{Number of specimen examined}}$.

Salmonella :

In order to investigate the incidence of salmonella infection in livestock and poultry, 106 calves, 68 each of goats and dogs, 2 pigs and 59 poultry were examined. Out of them, 2 calves (1.9%), 2 goats (2.9%) 1 dog (1.5%), 1 pig (50%) and 2 poultry (3.4%) yielded salmonella organisms. The percentage of infection in different animals and frequency of isolation from different specimens are presented in table XXII . It will appear that in all the salmonella infected cases, whether clinical or subclinical the organisms were not recorded from more than one site. None of the rectal swabs from 100 living calves yielded salmonella organism . The serotypes of these salmonellae could not be identified due to non-availability of factor sera .

Salmonella strains from calves, poultry and dogs showed resistance patterns against 3 antibiotics only(streptomycin, lédermycin and steclin) . Against the remaining 4 drugs used in the test(Ampicillin, Erythromycin , Chloromycetin and Furadentine), all were found to be susceptible. Both strains isolated from goats were sensitive to all the seven drugs while the one isolated from pig was resistant to streptomycin only (Table XXIII) .

TableXXIII

Resistance patterns of salmonella organisms to antimicrobial drugs .

Species from which organisms isolated	Strain number	Antimicrobial drugs						
		Strep-tomy-cin	Leder-mycin	Stec-lin	Ampici-llin	Erythro-mycin	Chloro-mycetin	Furad-entine
Calves	1 CSL	S	R	R	S	S	S	S
	2 CSL	R	R	S	S	S	S	S
Poultry	5 PSLU	R	S	R	S	S	S	S
	6 PSI	S	R	R	S	S	S	S
Dog	7 DSI	S	R	R	S	S	S	S
Pig	8 SSWI	R	S	S	S	S	S	S

R = Resistant . S = Sensitive .

Shigella :

Two ( ) out of 68 faecal samples collected from living dogs yielded Shigella organisms. A gram negative, non-motile rod (Fig. 3) giving on detail systematic bacteriological examination characteristic features of Shigella flexneri was observed . The results of sugar fermentation and biochemical tests are shown below :

Table XXIV

Biochemical reactions and sugar fermentation tests of Shigella organism.

Indol production	Positive
M. R. reaction	Positive
V.P. reaction	Negative
Citrate utilization	Negative
Nitrate reduction	Positive
Hydrogen sulphide production on T. S. I. agar medium	Negative
Gelatin liquefaction	Negative
Fermentation reactions:	
Lactose	Negative
Glucose	Acid
Sucrose	Negative
Salicin	Negative
Mannitol	Acid
Dulcitol	Acid
Adonitol	Negative
Arabinose	Positive
Sorbitol	Negative
Raffinose	Acid

Both the strains isolated gave the above typical reactions .

One of the strains (SD 40) was isolated from a pup suffering from mild dyscentry and the other (SD 63) was recovered from a healthy pup. The serotypes of the above strains could not be determined due to non-availability of antisera .

On *in vitro* drug sensitivity test both of them were found to be resistant to Streptomycin and Erythromycin .

***

D I S C U S S I O N

An intensive study on enteropathogens occuring in livestock and poultry is sought for due to various considerations :

(i) The majority of enteropathogens occuring in livestock and poultry have pathogenic potentiality for man .

(ii) The susceptible hosts after recovery perpetuate infection since they carry the organisms for a long time as persistent carriers or silent excretors .

(iii) Animals without ever manifesting disease syndromes may harbour the organisms in their system which shed them intermittently .

(iv) The principal habitat of these organisms is alimentary tract which facilitates the transmission of infection to other animals and man by direct or indirect contact .

Several groups of workers all the world over have resorted to determining the role of animal enteropathogens in diseases of man. In India, the informations regarding the prevalence ,ecology, epidemiology and intrinsic and extrinsic factors which contribute to either establishment or failure of infection in an animal population are inadequate. As rightly pointed out by Ørskov(1963), very little is known about E. coli infection in livestock in general and goats in particular .

In the present investigation, an attempt was made to determine the incidence, pathogenesis, serotypes and drug resistance of enteropathogens viz. E. coli, Salmonella and Shigella in various classes of livestock and poultry .

The results have shown the following percentages of infection by the members of the above enteropathogenic organisms in domesticated

animals and poultry .

Animals	E. coli	Salmonella	Shigella
Calves	52.8%	1.9%	0%
Goats	32.3%	2.9%	0%
Dogs	29.4%	1.5%	2.9%
Pigs	100%	50%	0%
Poultry	33.8%	3.4%	0%

The observations made in respect to the above findings are discussed below :

Escherichia coli : Calves :

It can be seen from tableVIII that during the first 18 hours of life, E. coli could not be isolated from the faecal samples. From subclinical and clinical cases, E. coli were isolated with the highest frequency during 0 day to 1 week after birth . Later, in the subclinical group the infection rate gradually declined reaching 0 in calves over 1 year old. On the other hand, in clinical groups the infection rate remained almost the same throughout . It may be mentioned here that immediately before the collection of rectal swabs the new-born calves were deprived of colostrum as a measure to prevent transmission of Foot and Mouth Disease which was then raging in the herd .

The high infection rate during the first week of life may be attributed to the fact that the majority of cases of colibacillosis in calves occur in this age group(Sojka, 1965) .

However, it may as well be that withholding of colostrum may have further contributed to the highest infection rate during the first week of life .

Among 37 typable strains obtained from calves, the serogroup 0119 showed predominance (11 strains) over other types. Strains with 086 agglutinin ranked next in order of frequency(8); and 6 strains each belonged to 026, 055 and 0126 groups (table It can be seen that from the clinical cases the serogroups 0119, 026, 055, 0126 and 086 were isolated in decreasing order of frequency. On the other hand, from the subclinical cases the serogroups 086, 0119, 0126 and 055 were isolated in decreasing order . 026 was not isolated from the subclinical cases, and 0114 was not present in either clinical or subclinical case. The above data suggest that in calves 0119, 026 are most frequent serogroups encountered in calf scour and 086 is more frequently associated with subclinical than in clinical condition .

The above findings are in consonance with those of Glantz (1958) who also reported frequent occurrence of these serotypes in calf scour. This author too failed to find the serogroup 0114 among 164 strains of E.coli. The isolation of 0114 from calf has been recorded but its etiological role has not been proved (Sojka, 1965) .

The pathogenecity of these serotypes for man has been unequivocally shown (Taylor and Charter 1952 ; Smith 1953; Kauffmann 1954). Further , Taylor(1961) reported that in an epidemiological study of an outbreak of infantile diarrhoea, 0119 was the main source of infection. Apart from other pathogenic serotypes causing enteritis in calves, 086, 026, 055 and 078 have been isolated from mastitic milk(Fey, 1952, 1953, 1955; Rees,1958). The first three groups are frequently encountered in human diarrhoeic

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diseases(Edward and Ewing 1962) .

It hardly needs to be emphasized that under Indian conditions of cattle husbandry and dairy management, there is a great scope of transmission of infection from animals to man and vice versa. Therefore, the association of these serotypes with diseases in calves is of considerable public health importance .

In the present study one of the most frequently encountered pathogenic serogroup for calves O78 was not detected due to non-availability of sera. It may be that this O group may have been present in the 24 untypable strains .

Based on sugar fermentation reactions all the 61 strains from calves were allocated to 14 different biochemical types as shown in table XI. It will appear that among the 31 subclinical strains 6 belonged to pattern number 6, similarly among 30 clinical strains 7 fell into pattern number 3. Others did not show any definite pattern .

Metabolic activities have been studied in other micro-organisms in order to differentiate pathogenic from non-pathogenic strain. Some of the extracellular proteinaceous substances and enzymes elaborated by the organisms have been particularly investigated to differentiate the fermentative activity, respiration co-efficient and aminoacid transport in staphylococci (Ivler, 1965).

It has been shown that pathogenic strains are metabolically more active than the non-pathogenic ones. Since, in the present study the numbers of strains assigned to different biochemical patterns were small, it is difficult to appreciate the metabolic differences between the clinical and subclinical strains. Further studies aimed at elucidating the metabolic activities of a large

number of highly pathogenic and less pathogenic strains may resolve this problem .

All the 61 strains of E.coli isolated from calves were subjected to in vitro drug sensitivity test. As set out in table XIV it will appear that the most effective drugs were furadentine(100%), chloromycetin(98.3%) and ampicillin(95%). Erythromycin and streptomycin, were moderately effective, and steclin and ledermycin were less effective .

There was no significant difference between clinical and subclinical strains in regard to their resistance to antimicrobial drugs. It has been reported that with the increasing use of broad spectrum antibiotics a large number of resistant strains have developed with the lapse of time(McKay et al 1965). Therefore,increasing difficulty is being encountered by the practitioners of Veterinary as well as human medicine largely due to emergence of resistant strains. A large number of resistant strains found against ledermycin, steclin and streptomycin bear testimony to this fact. The introduction of nitrofurans in drug therapy against various infections has offered a great promise during the recent years .This is evident from the fact that animal strains in the present study have scarcely shown resistance to furadentine .Perhaps at the present time resistance to these drugs has not been developing with the same degree as with the antibiotics. A scruitiny on the relationship between serotypes and drug resistance pattern did not show significant association between them except that five out of seven 'O' group O119 recovered from the clinical cases showed resistance pattern No. 1(ledermycin and steclin).

E. coli in goats :

From table VI it would appear that out 68 goats examined 22(32.3%) showed infection with E.coli . Out of 60 healthy

cases, 17(26.6%) yielded E. coli against 5 (63.3%) out of 8 clinical cases. This suggests that E. coli carrier rate in goats is not as high in healthy goats as in healthy calves. In the latter, the carrier rate was 50%.

Of these 22 strains, only 9 were typable, 3 of them belonged each to 055, 026 and 2 and 1 to 0119 and 086 respectively. As remarked earlier, very little is known about E. coli serotypes occurring in goats in India. Panda et al(1965) while examining 38 goat strains reported nine serogroups including 026. The present results have revealed apart from 026, serogroups such 055, 0119 and 086. It may be that further examination of 13 untypable strains may reveal other serogroups as well. In this connection, it is worthy of note that all the sergroups are highly pathogenic for man. The chances of transmission of infection from goats to man are quite large indeed due to habitation of these animals in and around human dwellings villages as well as unhygienic methods adopted at slaughter houses. Besides, goat meat is one of the principal non-vegetarian diets in India. Therefore, its zoonotic importance can not over emphasized. An extensive study is needed to determine the prevalance of clinical and asymptomatic infection in goats as well as the various serotypes associated with them.

The in vitro tests for sensitivity of the strains to antimicrobial drugs used showed that 54.5% were resistant to ledermycin and 50% streptomycin. To steclin, it was moderate (40.5%), and to erythromycin and ampicillin their percentages were 13.6 and 9.1 respectively. None of them was resistant to furadentine and chloromycetin. There was no relationship between the serotype and drug resistance pattern.

Biochemically the strains from goat showed only 10 different patterns against 14 shown by calf strains. As discussed earlier, it is difficult to infer whether or to what extent such difference in biochemical behaviours could be responsible for low carrier rate in healthy goats .

E. coli in dogs :

Out of 68 dogs of all age groups, 29.4% showed E. coli . Amongst these 17 were pups which showed the infection rate at 17.6% . Among 51 adults(Dogs and Bitch) the infection rate was found to be 34% .

Among these are included the strains isolated from 14 urine samples . Of 20 strains 4 belonged to 055, 2 each to 0119, 0126, and 026 . The remaining 10 strains remained untypable . These serogroups have been reported to occur in the faeces of healthy dogs by Wesselinoff and Dimow(1961) who attempted to associate these serotypes with infantile gastroenteritis in babies. Of 41 strains only one had an antigenic structure (026; B6 : H11) common between them. Besides Mian(1959) reported the isolation of E. coli 055 :B5, in pure culture from the faeces.of the family dog and incriminated the serotype in a case of gastro-enteritis of babies. Thus, it is clear that the healthy family dogs act as carriers of pathogenic serotypes affecting human babies .

Out of 20 strains the highest percentage(50%) showed resistance to Ledermycin and steclin . Streptomycin and erythromycin showed moderate activity while, chloromycetin and ampicillin were most effective. It was interesting to note that 2(10%) of 20 strains were also resistant to furadentine. As mentioned earlier, these two strains were isolated from the urine samples

of two family dogs belonging to the same owner which had died of high fever despite treatment with terramycin and furadentine. The 'O' agglutinin of these strains could not be ascertained with the available sera. It is not unlikely that resistant variants may have emerged due to frequent and prolonged treatment with the drugs. Had the antigenic structure of these strains been known, it would have revealed its possible role in causing gastro-enteritis in man .

It is worthy of note that of 103 E. coli strains isolated from cattle, goats, dogs only one was found to be haemolytic on bovine blood agar plate. Unfortunately, this strain remained untyped . However, Mansi(1962) reported that very large numbers of so called "fading puppy" syndrome and of acute gastro-enteritis in dogs were associated with haemolytic E. coli possessing 'O' group 042. The association of this serogroups with human infection has scarcely been reported. Therefore, a close search is necessary to exclude the possibility of such a strain being associated with human infection .

E. coli in pigs :

As stated before, survey on the incidence of E. coli infection in pigs was not under the purview of this study. However, two strains were obtained from pigs which died of severe gastro-enteritis. One of them was haemolytic but it was not associated with any clinical or autopsical signs of oedema disease. Both the strains remained untyped and were resistant to only steclin.

E. coli in poultry :

59 birds received in the department for necropsy were examined for the presence of E. coli out of which 20(33.8%) were f

to

be infected .

On perusal of the result shown in table XIX it will appear that frequency of isolation of E. coli from non-parasitic and parasitic enteritis was almost the same(35%), whereas, from the cases of non-specific deaths the infection rate was only half as much(16.6%). However, from the above table it can be seen that death rate in chicks due to enteritis caused by E. coli was the highest (66.6%). On the other hand, among the cockerels and pullets 60% yielded E. coli in association with parasitic enteritis . Deaths due to pneumonia caused by E. coli in cockerels and pullets was found to the extent of 66.6%. Similarly, in cocks and hens, other fatal infections comprising salpingoperitonitis, pneumonia and coligranuloma due to E. coli was to the tune of 50%. Such mortality patterns caused by E. coli are not unexpected . Chicks are known to be susceptible to colibacillosis. Similarly, E. coli has been largely incriminated as the etiological agent of coli-granuloima, salpingoperitonitis and pneumonia in poultry . The high isolation rate from cases of parasitic enteritis may be ascribed to imbalance of the intestinal microflora providing opportunities to E. coli to invade other system under debilitating conditions caused by the parasite .

Out of 12 typable strains, 6 belonged to group 026, 4 to 086 and 2 to group 0114 .

Six strains belonging to O group 026 were isolated from cases of salpingoperitonitis, coligranuloma and bronchopneumonia. While 086 were largely isolated from those dying of heatstroke. It was interesting to find that out of 134 strains isolated from different livestock and poultry only two poultry strains possessed

O114 agglutinin . Since, the association of these serotypes and particularly O114 in the diseases of poultry has not been widely reported , More work is necessary to determine the specificity of the various serotypes in poultry and the specific infections caused by them .

Salmonellae :

It has been very well recognised that a large varieties of organisms belonging to a number of genera are carried by animals. Many of them are pathogenic for animals and man or both and are therefore of zoonotic importance particularly in temperate zones . Organisms , viz. Pasteurella multocida, E.coli, Staph.aureus , Salmonellae and others possessing disease producing potentiality are frequently harboured by the natural hosts without any clinical evidence of infection . The factors, either extrinsic or intrinsic, which strike the balance of the host parasite relationship and activate latent infection into clinical disease are yet to be learnt of .

Salmonellosis is of considerable importance from the stand point of public health since the most, if not all, members of this Genus affect animals and man. As the pathogenesis of salmonellosis is complex and is yet only poorly understood, salmonellae are commonly used to study the basic mechanism of infection and resistance in experimental animals .

In the present investigation, six strains of salmonella organisms were isolated from 244 animals and 2 from 59 birds examined. Their percentages of isolation in different animals are shown in table

From the table XXIII it can be seen that none of the strains was isolated from more than one site. In calves, none of the rectal swabs obtained from 100 calves yielded salmonella organisms. Of 2 strains from calves, one was recovered from the intestinal content of a fatal case of gastro-enteritis complicated with pneumonia and the other from the liver of a 5 days old calf which died of high fever. In the latter, there was no evidence of infection of the umbilicus stump, but there were signs of peritonitis and increased in the amount of serous fluid in the abdominal cavity. It was noteworthy that E. coli were not isolated from any of these two calves. Thus, it seems reasonable to believe that the former died of acute salmonellosis and pulmonary involvement was the result of secondary infection. The latter was interesting particularly because the victim was only 5 days old and salmonella was not recovered from any other visceral organ. Salmonellosis in calves is more prevalent after 1 week of age than before(Gibson, 1961). Further, salmonellae are excreted in milk by cows in the febrile stage of clinical salmonellosis. However, while the possibility of milk borne infection cannot be altogether ruled out, it seems to be unlikely because clinical salmonellosis was not encountered in the dam. Further, failure to isolate the organism from viscera also eliminates the alimentary route to be the possible portal of entry of the organism. Thus, considering the liver as the only site of isolation in a 5 day-old calves, it is more than probable that infection was neonatal. It is supported by the observations of Avery and Niilo(1963) who reported the isolation of S. dublin from 1.2% bovine foetuses. More investigation is needed to ascertain the extent of congenital transmission of salmonella infection

in the bovine. One of the two salmonella strains showed resistance to ledermycin and steclin while the other was found to be resistant to streptomycin and ledermycin .

Two(2.9%) out of 68 goats examined yielded salmonellae. One was isolated from the intestinal content of a goat died of fibrinous enteritis and the other from the mesentric lymph node of a healthy goat at slaughter house . Both of them were sensitive to all the seven drugs tested . This suggests that incidence of salmonellosis in goats is low and of sporadic nature and agrees with the observations of other workers(Buxton,1961). The serotype of the organisms could not be determined. However, in view of the fact that almost all the salmonella serotypes have been incriminated in human infection their occurrence either in cases of clinical salmonellosis or in symptomless carriers or excretors is a big hazard to human health because human infection may occur among meat handlers and butchers in the slaughter house and among the consumers through the contaminated meat. Furthermore, healthy carriers are particularly important in preventive veterinary medicine since the prophylactic vaccine against Rinderpest is prepared from goats' spleen .

Salmonella organisms were isolated from only one diarrhoeic dog out of 68(1.5%) examined . The strain was resistant to ledermycin and steclin and sensitive to others. Despite low incidence recorded in the present survey, its role in the transmission of infection to man and children can not be disregarded because of close association between the family dogs and their masters .

Since no systematic survey for enteropathogens in pigs was made, autopsy materials from only two pigs were available for study. One of them which died of ~~sense~~ severe enteritis showed salmonella

organisms in their intestinal content which also yielded E. coli. The strain was resistant to only streptomycin .

Out of 59 fowls examined, 2(3.4%) yielded salmonellae. One was recovered from the intestinal content of chick died of heat stroke and the other from the pneumonic lung of a cockerel. One of them was resistant to streptomycin and steclin and the other to ledermycin and steclin .

Bihar State has been so far found to be free from the host specific serotype Salm. gallinarum-pullorum. However, several serotypes potentially pathogenic for man have been isolated from pultry by workers in India (Sharma et al,1962, Khera, 1962; Agarwal, 1962; Das and Panda, 1962). Moreover, Ghosh(1962) also reported the isolation of Salm. typhi from a carrier fowl. In view of the extensive rearing and development of poultry in India and its importance from the stand-point of public health, it is necessary that investigation on salmonellosis in poultry be organised at state and regional level in order to determine the extent of the problem.

Shigella :

Shigellae were isolated from only 2(2.9%) of 68 dogs examined. All other animals were found to be free of infection. One of them was recovered from the rectal swab of a family pup suffering from dysentry, and the other from a healthy pup brought to the College hospital for antirabic vaccination. On systematic bacteriological examination, the strains were identified as Shigella flexneri. Both were resistant to streptomycin and erythromycin only. Their serotype could not be ascertained .

Only a few sporadic reports are available in the literatures regarding the carrier status and clinical illness caused by shigella in dogs . Sh.flexneri, Sh.dysenteriae, Sh.sonnei (Doldand Fischer, 1921; Varela et al 1951)was found from faeces of dogs . Sh.flexneri has also been isolated from the raw buffalo-milk(Singh and Singh, 1967). Thus, it is apparent that shigellosis which is reported to be prevalent in a large proportion of human population in India may be transmitted not only between men, but also between men and dogs and vice-versa. It may as well be that the disease, being milk-borne, could be transmitted to man and dogs through the medium of infected milk. In view of the rareity of the reports in the literatures on the subject, more extensive work is needed to ascertain the role of dogs and other family pets in the transmission of shigella infection to man .

SUMMARY

This thesis deals with an investigation on the diseases of livestock and poultry caused by enteropathogens with particular reference to E.coli, Salmonellae and Shigellae. The isolates recovered from the clinical and apparently healthy cases were studied for their serologic and biochemical characteristics and for their in vitro sensitivity to antimicrobial drugs . The subject has been reviewed in retrospect, and the pathogenesis of these enteropathogenic infections has been discussed in relation to public health .

In the present study, the over-all percentage of infection caused by E.coli in different species of animals and poultry was as follows : Young calves- 52.8% , goats- 32.3%, dogs- 29.4% and poultry - 33.8%. Besides, E.coli were also isolated from two pigs died of severe enteritis .

Young calves within 18 hours after birth were not found to excrete E.coli in their faeces . The most susceptible age of calves for infection with E.coli was found to be between 0 day and 1 week .

Of 37 typable strains of E.coli isolated from calves, 11 (29.7) belonged to serogroup 0119 . The percentages of isolation of the remaining serogroups in order of decreasing frequency were 086(21.6%), 026 (16.6%), 055(16.6%), 0126(16.6%) . E.coli possessing 'O'agglutinin 0119 and 026 were most frequently encountered in clinical cases of Colibacillosis in calves. On the other hand 086 were more frequently associated with subclinical than clinical infection. Serogroup 026 was not isolated from the subclinical cases and 0114 was not encountered in either conditions .

Based on sugar fermentation reactions, 61 calf strains of E. coli were grouped under 14 biochemical types. Among 30 clinical strains, 7(23.3%) belonged to pattern 3 (Lactose, Glucose, Mannitol, Dulcitol , Arabinose and Sorbitol fermenters) while 6(19.3%) of 31 subclinical strains were allocated to pattern 6(Lactose, Glucose, Salicin, Mannitol and Arabinose fermenters). The difference in metabolic activities between the clinical and subclinical strains in relation to their pathogenecity has been discussed . The in vitro sensitivity test of 61 calf strains of E. coli to 7 antimicrobial drugs revealed that the most effective drugs were furadentine(100%), chloromycetin(98.3%) and ampicillin(95%). Erythromycin and Streptomycin were moderately effective (% and %), and steclin and ledermycin were less effective (% and %). Based on drugs sensitivity test, 61 calf strains were put to 17 resistant patterns . There was no significant relationship between the E. coli serotypes and drug resistance except that 5(71.4%) out of 7 strains of O119 showed resistant pattern 1 (resistant to ledermycin and steclin) .

The overall infection rate in 68 goats with E. coli was found to be 32.3%. The frequency of isolation from 60 healthy goats was 26.6% against 5 (63.3%) out of 8 diarrhoeal cases. It was also revealed that E. coli carrier rate in healthy goats was less than that in healthy calves. Of 9 typable goat strains 3 belonged each to serogroup 055 and 026, and 2 and 1 to O119, 086 respectively. The pathogenicity of E. coli serotypes for man has been discussed.

Of 22 strains of E. coli from goats 54.5% were resistant to ledermycin and 50% to streptomycin. To steclin the resistance was intermediate (40.5%) and to erythromycin and ampicillin these per-

percentages were 13.6% and 9.1% respectively . None was resistant to furadentive and chloromycetin .

Biochemically the goat strains showed only 10 different patterns against 14 shown by calf strains. Such a difference in biochemical behaviours was only conjenctured for the low carrier rates in goats but this could not be established .

The percentage of E.coli infection in dogs was found to be 17.6%. Of 20 dogs strains 4 belonged to 055, 2 each to 0119, 0126 and 026. The rest were untypable . Ten(50%) out of 20 strains were sensitive to ledermycin and steclin. Chloromycetin and ampicillin were most effective. Out of 134 strains of E.coli subjected to drug sensitivity test in this study, only 2 drug strains were found to be resistant to furadentine. These were isolated from urine of two dogs suffering from high fever.

20(33.8%) out of 59 birds were found to be infected with E.coli . The percentage of enteritis in chicks due to E.coli was the highest (66.6%), while 50% cases of other infections(salpingoperitonitis, pneumonia and coligranuloma) were associated with E.coli (026) .Out of 12 typable poultry strains 6 belonged to O group 026, 4 to 086 and 2 0114 . It was interesting that out of 134 total strains from different livestock and poultry only two poultry possessed O agglutinin 0114 .

The percentage of infection with Salmonellae in calves was 1.9 in goats 2.9, in dogs 1.5 and poultry 3.4 . One out of two pigs yielded salmonella organism . Evidence was broughtout to show that in one of the calves the infection was congenital .

In contrast to E.coli , Salmonellae were found to be less resistant. They showed resistance to only ledermycin,streptomycin and steclin in different combinations. To others (Ampicillin, Erythro

mycin, chloromycetin and furadentine) they were sensitive. The rôle of livestock and poultry in transmission of salmonella infection in man has been discussed . Only two (2.9%) of 68 dogs examined yielded Shigella organisms which on systematic bacteriological examination were identified as Sh. flexneri. Other species of animals were found to be free of Shigella infection .

Both the strains were resistant streptomycin and erythromycin only .

The role of dogs and family pets in transmission of Shigella infection in man has been discussed .

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