STUDIES ON

The Incidence of *Salmonella* and *Escherichia coli*

in Sheep and Goats in Health and in Enteric Disorders.

Thesis
Submitted to the Rajendra Agricultural University
Bihar in Partial fulfilment of the
requirements for the Degree of
Master of Science (vet.) in
the Faculty of Veterinary
Science and Animal Husbandry.

By

Dr. Akhileshwar Prasad Verma

B. V. Sc & A. H. (Ran).

Post-graduate Department of Bacteriology and Pathology

BIHAR VETERINARY COLLEGE,

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Department of Bacteriology and Pathology,
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Prof-in-Charge.

This is to certify that the thesis entitled " STUDIES ON THE INCIDENCE OF SALMONELLA AND E. COLI IN SHEEP AND GOATS IN HEALTH AND IN ENTERIC DISORDERS" submitted in partial fulfilment for the award of the degree of Master of Science (Vet.) in Bacteriology to the Rajendra Agricultural University, Bihar, Patna by Dr. Akhileshwar Prasad Verma incorporates the results of his independent study which he has carried out under my guidance.

T. Sharma.
22.3.72.
(T. S. Sharma)

March, 1972.

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Dedicated to
my late Father
Shri Sahdeo Lal.

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INTRODUCTION

INTRODUCTION

In India goats and lambs are the Chief sources of protein diet for the majority of the non-vegetarian population. Poultry, pork or ham and beef are utilised only by a smaller percentage of the population. So for production of clean meat healthy flocks of sheep and goats are essential prerequisite. Salmonellosis is one of the zoonoses. Only sporadic attempts have been made to determine the prevalance of various Salmonella Serotypes in sheep and goats. Salmonella carriers among recovered animals and in those inapparently affected further add to the complexities. Affected animals which survive show retarted growth and become uneconomical. 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 people who come in contact with these animals, viz., meat handlers and butchers in the slaughter house and also among the consumers.

The object of Veterinary science is to preserve the life of animals only so long as this is human, economical and safe for the public health. The purpose for controlling Salmonella infections in animals are to facilitate the economic production of animals as a source of food for men and to ensure that this source of food is free from infection and safe for human consumption.

Salmonellosis of sheep has been most frequently reported from those countries where sheep farming is one of the major agricultural industries. Since last two decades the stress is given to the sheep husbandry. In this State also sheep husbandry and wool development are being encouraged by the State Government. Therefore, the control of zoonotic diseases which spread through sheep becomes paramount.

The common route of infection of Salmonella is by ingestion. Schermer and Ehrlich (1921) and many others have reported that foetal membranes and fluids from aborted foetuses were found heavily infected with S.abortus-ovis and comprised most dangerous source of infection.

Symptoms very widely from sudden death in the acutely affected animal to the symptomless carrier which excrets Salmonella in the faeces with no apparent sign of ill health. As in other animal species the young animal is more suceptible than the adult, and it is only in young lambs that the acute form of the disease commonly occurs. Death in lambs may take place within a few hours of the onset of symptoms, which usually consist of acute diarrhoea, dysentery and collapse. In less acute cases and in adult animals diarrhoea may be intermittent, there is loss of weight and large patches of the fleece may fall out. Pregnant ewes may abort and this applies especially to infection with S.abortus-ovis in which abortion is the classical symptom.

In cases of septicaemia the organism can be isolated from many sites in the body including the blood, bone

marrow and viseral organs. In less acute cases the organism may be found in the mesenteric lymphnodes and in the intestinal contents.

The importance of the symptomless carrier as a source of epidemics would not appear to be so great as it is among cattle yet it is not negligible. It should be remembered that under certain conditions of sheep farming and transport one carrier animal excreting Salmonella in the faeces could be responsible for initiating a severe out-break among animals in a low state of health (Selivanov, 1951).

Many serotypes have been recovered from sheep and goats but still there is derth of literature on the record of isolation of Salmonella serotypes from sheep and goats in India and particularly in Bihar. For these reasons an attempt has been made by this study to survey the problem in Bihar. While this is primarily a survey of salmonellosis in sheep and goats different aspects of cross infection from these two animals to man and vice-versa have necessarily been taken into account.

The knowledge about the association of Escherichia coli (E. coli) with various enteric disorders of man and animals can be dated back to last two decades of the ninteenth century.

In lambs and kids gastroenteritis, enteritis and diarrhoea are responsible for a heavy loss to the developing sheep and goat industry. At the same time the poor knowledge about the Coli strains associated with such syndromes in

sheep and goats leads to a confusion of its public health importance. As expressed by Orskov (1963) that little, if any thing, is known about the Coli strains found in goats in India and other countries and even less is known about the role of such strains in causing disease. E.coli, though mainly isolated from calves with scour or diarrhoea, enteritis, arthritis, meningitis and several other symptoms, has also been incriminated as the causative agent of infantile diarrhoea.

Strains of E.coli isolated from different animals point towards the need of search in to the extent of its existance causing enteric infection in sheep and goats. Thus to elucidate the extent of infection with such E.coli strains in sheep and goats an extensive work is needed.

An attempt has been made to aid in finding out the information regarding pathogenicity of E.coli strains, especially studied from sheep and goats.

CHAPTER II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Salmonella in Sheep and Goats

Levi (1949) recovered S. dublin from faeces of eighteen goats and from blood and organs of ten goats which died out of eighteen. It was further observed by him that young goats were more susceptible to artificial infection and to infection from close contact with infected animals.

Simmons and Sutterland (1950), and Smith and Buxton (1951) are of the opinion that the incidence of Salmonella infection in Sheep and goats is low and of sporadic nature. In their findings the occurrence was usually related to certain predisposing conditions.

Selivenov (1951) examined fifteen apparently healthy sheep for infection with Salmonella. He was able to isolate organisms only from two of the fifteen healthy sheep during their life. He infected twenty lambs and sheep artificially by subcutaneous route or per os and kept them apart. It was interesting to note that ten of the twenty artificially infected sheep and lambs sacrificed six days to six months later revealed Salmonella organisms in their mesenteric lymph-nodes, spleen, uteri, livers, gall-bladders and in duodenal contents.

Twelve blood samples from eleven rams in different flocks known to be infected from S. abortus-ovis were positive on rapid agglutination test (Mura et al 1952). They did not stress the possibility of transmission of infection through coitus with such rams but they emphasised their role as

carriers. In similar tests agglutinins were revealed from blood sera of twelve infected foetuses from sheep and from a goat.

In 1952, Simmons recorded ten out-breaks of salmonellosis in sheep within a period of one and half years.

An extensive study on epidemiology of S.typhimurium infection in sheep was done by Watts and Wall (1952) in Australia. Epidemics were on nine different properties and mortality rate was 3-4%. Birds were incriminated as transmitting agent of infection from one campus to another by fouling the water supply and were found to be infective 27 days after feeding on the infected material. Contaminated earth and water remained infective for 200 and 119 days respectively. Two experimentally infected sheep shed the organisms from 14 to 67 days.

S. abortus-ovis was first isolated in U.S.S.R. in 1930 by Nikolski. He gave a conventional account of the infection and described the results of his experiments on mice and guinea-pigs in 1952. In the same year a comparative study on infections of S.abortus-ovis and Br.melitensis causing abortion in sheep was summarized by Nardi and Depinto(1952).

Rae and Wall (1952) isolated Salmonella meliagridis from the uterus of an ewe. The affected horn contained a haemorrhagic, necrotic mass of placental material together with a macerated foetus.

Sehmid and Valaudapillai (1953) recorded the isolation of Salmonella paratyphi-B,S. saintpaul, S.typhimurium, S.

virchow, S. newport, S. dublin, S. gallinarum, S. pomona and S. urbana from the faeces of apparently healthy domestic animals in Cylon. Positive cultures were obtained from one out of sixtyone cattle; one of thirty three dogs; one of sixty two fowls; three of fourty eight goats in one lot and seven of fifty eight goats in another lot imported from India and one out of sixty pigs.

An extensive study on abortion in Sheep and goats caused by Salmonella was carried out by Mura et al (1953). They confirmed the presence of specific agglutinins (in high titre) to S.abortus-ovis in blood sera of adult males and females of infected flocks and in the sera of aborted foetuses. They failed to demonstrate the presence of agglutinins in the sera of lambs and kids or of sexually immature females. They were unsuccessful in isolating the organism in culture from the blood of eight infected males and thirty three infected females. Preputial washings and semen also gave negative results.

A paracolon organism that possessed Salmonella 'H' antigens 1,3,5,(7) was isolated from the duodenum of a diseased ewe by Bruner et al (1955). Although the organism possessed 'H' antigens in common with Salmonella the isolate failed to ferment dulcitol and fermented lactose. Their pathogenic role for sheep could not be established.

Endrejai (1955) isolated S. abortus-ovis from 160 of 257 aborted foetuses of sheep. Ovine abortion virus together with S. abortus-ovis was present in six flocks. In one flock

there was mixed infection of Brucella and S.abortus-ovis. In the same year Khachatryan isolated several strains of Salmonella from cases of septicaemia in goats.

S.dublin was isolated from two out of 178 pneumonic lungs of sheep and ten out of 111 pneumonic lungs of goats and S.typhimurium was isolated from ten out of 178 pneumonic lungs of sheep at the Indian Veterinary Research Institute (I.V.R.I.), Mukteshwar-Kumaon during the year 1956-57.

Buxton (1957) in his review on salmonellosis in animals has recorded the presence of seventeen serotypes of Salmonella from sheep and goats.

Bartmann (1957) examined 400 uteri, 250 ovaries and 200 udders from slaughtered ewes and 220 testicles from rams for Salmonella and was able to isolate S.abortus-ovis from testicles of seven rams, only four of which were sexually immature. This finding demonstrated the importance of rams in the transmission of S.abortus-ovis infection.

Salisbury (1958) observed that salmonellosis of sheep is primarily a disease of adults especially of fat ewes. A sudden change from good to poor feed or confinement in yards or transport and transporting for long distances caused carrier ewes to scour and to infect others in close contact.

Smith (1959) examined mesenteric lymph nodes and faeces of apparently healthy pigs, cattle, sheep, dogs and cats for Salmonella. Salmonella was isolated from the mesenteric lymph nodes of 60 out of 500 pigs; 9 of 200 dogs; 5 of 200 cats and from none of 200 cattle and 100 sheep.

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

On the 10th day of Vaccination against sheep pox an out-break of salmonellosis occurred in a flock of 700 sheep in Turkey. S. reading was incriminated as the causative agent for this large out-break which was isolated from liver and spleen of sheep. The symptoms mainly comprised of fever, diarrhoea, dyspnoea, conjunctival congestion, ulceration and keratitis. It was noteworthy that 223 out of 700 sheep aborted (Yucel, 1961). None of the 500 sheep examined at an abattoir yielded S.reading. Neither cattle on this farm were positive for this organism.

Zwart (1962) conducted a survey on salmonellosis in sheep and goats and found thirty-seven percent of sheep and five percent of goats in Ghana infected with Salmonella.

In 1962, Agarwal isolated eighty three strains of Salmonella from buffaloes, cattle, sheep, goats and poultry. The serotypes isolated from sheep and goats constituted S.aberdeen, S. bovis morbificans and S.colombo; and S.motapani S.typhimurium and S.Weltevreden respectively.

In Sudan, Qudduskhan isolated S.salford from sheep and goats; S.amajer, S. derby and S.Kaudle from goats only in 1962.

S.mara and S.meskin were isolated from mesenteric lymphnodes of goats at Fort-lamy (Chad) by Minor et al (1963)

Vallette (1965) examined faeces and lymph nodes from 417 sheep slaughtered at Geneva and demonstrated S.Java in the

mesenteric lymph nodes of one sheep and S.typhimurium in the faeces of two sheep.

Dennis (1965) investigated the incidence of Salmonella in domestic animals from 1939-1964 and found that out of 247 isolates 59 were S.typhimurium from sheep. He further observed that more out-breaks were occuring in sheep than in other species and the majority of those occurred at pasture during drying condition.

Quesada and Izzi (1966) recorded ovine abortion in a flock of 115 ewes. Out of these 16 aborted about the third month of pregnency. S.typhimurium (phage type I) was isolated from an aborted foetus.

It was of epidemiological interest to note the observations of Robinson (1967). He found transient excretion of Salmonella in faeces of six adult sheep out of eight placed in contaminated yard for 24 hours. The sheep were without water and were not in contact with other sheep. He further discussed the role of contaminated sheep yard in disease transmission.

Stanoev and Milev (1967) studied three infected flocks of sheep at Bulgaria and found S.abortus-ovis present in 13 of 355 faecal samples and in 27 of 361 samples of vaginal secretion. It was also isolated from the preputial secretion of a ram. Agglutination test was positive in 151 of 587 blood samples. The carrier state lasted for three to eighteen months. Other serotypes were not isolated.

Bacteriological examination of faeces and mesenteric lymph nodes of 1243 sheep done by Gotze (1968) at Berline revealed latent Salmonella infection in 0.16 percent of the sheep.

An extensive study was carried out by Iannuzzi and Piragino (1968) on healthy carriers of Salmonella among slaughtered animals in Messina. S.typhimurium was found in four sheep at the public abattoir during their investigation.

Jayraman and John (1969) while surveying salmonellosis in domesticated animals in the State of Madras recorded for the first time S.newport and S.Virchow from the cases of enteritis of Indian sheep. In addition, they isolated S.newport and S.bareiley from the same flock.

Gran et al (1969) inoculated S.anatum or S.typhimurium in dilution of 10^{-7} to 10^{-8} into the rumen of sheep which were fed 1.3 kg. of lucern chaff daily; Salmonellae were eliminated from the rumen in two days and could not be detected in the faeces after about one week. During starvation both Escherichia coli (E. coli) and Salmonella grew in the rumen. Resumption of feeding after starvation for 3 days caused further multiplication of E.coli and Salmonella in the rumen but these were subsequently eliminated with further feeding. Inoculation of a starved sheep with as few as 400 Salmonella cells led to large numbers of Salmonella appearing in the faeces and being excreted in varying numbers, atleast, five weeks after resumption of feeding.

An extensive study on epidemiology of S.Senftenberg in sheep in Ryhope-hospital, Sunderland was done by Hugh-Jones (1969) who isolated the same organism from poultry, sheep and cattle on eight farms in England and Scottland within a period of one and half years. He further observed that S.Senftenberg was usually found by chance on bacteriological examination of animals with other diseased conditions.

Baker (1969) recorded an out-break of S.typhimurium (phage type 4162) infection in man. The course of events was probably man to cattle, to horse, to sheep and back to man. All the infected animals were adults and five of them had no clinical symptoms.

Minev and Mineva (1969) recorded an out-break of S. typhimurium infection in sheep in Bulgaria, and observed that out of 417 lambs aged about five to seven months, 27 died from gastero-enteritis. S.typhimurium phage type -I and Ia isolated from the dead lambs and type Ia was isolated from the water supply. Some of the lambs were treated with streptomycin, Chloroamphenicol and furazolidin but three percent were still carrying the organism 90 days later. He also observed that sheep and goats that drank water contaminated with S.typhimurium showed the absence of serum agglutinins and S.typhimurium was recovered from only one sample out of 1353 faecal samples examined.

Tiwary (1969) recorded isolation of Salmonella from two out of 68 goats examined. The organism was isolated from (i) the intestinal content of one goat died of fibrinous enteritis

and (ii) from the mesenteric lymph node of an apparently healthy goat slaughtered for meat.

A new Salmonella serotype causing disease in sheep in Tanzania was studied by Croocock et al (1970) during an investigation of an out-break leading to severe loses in newly born lambs in 1968. Salmonellae isolated from intestines, livers and lungs were identified as a new serotype, S.muguga. Although 37.5 percent of the lambs died during the period between April to September deaths in June and early August were attributed to the adverse weather while those in late August and September might have been due to "Nairobi Sheep" disease.

In an investigation by Yalcin and Gane (1970) Salmonella was found to be responsible for goat abortion in three and Chalamydia in two out of twelve investigated cases.

Escherichia coli (E.coli) in Sheep and Goats

Loligar (1956) isolated E.coli from the seminal ejaculate of a goat. He inoculated broth culture of this organism in doses of 0.5 ml. into testicles of two goats and in the epididymis of other two goats. One from each group died; the Semen samples of the survivors revealed the presence of polymorphonuclear (P.M.N.) 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 intraepididymically.

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

E.coli was isolated from 27 of 98 ejaculates from affected goats but only from two of 43 healthy goats. He suggested that spermatic granuloma might occur by local infection with enteric bacteria, such as, E.coli. Housing may be predisposing.

Muller (1960) reported cases of sterility in female goats caused due to association with male goats which had

infection of haemolytic E.coli. In a herd of 26 goats with one buck, the conception rate was very low and haemolytic E.coli was isolated from cervical and veginal mucus of 8 out of 10 sterile goats and also from the ejaculates of the male which had bilateral testicular hypoplasia.

Panda et al (1965) isolaated 38 strains of E.coli from healthy and diseased kids and goats. All except serotype 0133 were pathogenic to guinea-pigs. The strains belonged to groups 08, 021, 026, 078, 0115, 0133 and 0140. One strain was tentatively considered as 0146. The strains haemolysed horse blood but not the ox blood. All including a new serotype 0146 which failed to agglutinate any of the known 'O' test sera such as 04, 08, 021, 026, 078, 0115, 0133 and 0140 were recovered from rectal swabs and heart blood from healthy goats and kids. In their study Panda et al (1965) also examined 2-8 days old kids which had died of enteritis and septicaemia during an out-break. 15 strains were isolated from diseased cases. Two were typed as 04 and one as 0146. This new serotype 0146 was widely distributed. All serotypes of E.coli exclussive of 0133 produced lesions in guinea-pigs (G.pigs) similar to those of kids dying of enteritis.

Smith and William(1966) recorded strains of E.coli which were drug resistant. His isolates of E.coli comprised of strains from calves, lambs and babies with neonatal diarrhoea or bacteraemia, pigs with neonatal or post Weaning diarrhoea or oedema disease and from fowl with coli septicaemia. The strains from calves and lambs with bacteraemia

and those from babies, pigs and fowls belonged to serotypes generally found pathogenic to these species. Complex drug resistance was the common feature-only two drugs polymyxin-B and nalidixic were active on all the strains.

CHAPTER III

MATERIALS AND METHODS

MATERIAL AND METHODS

(1) Animals :

(A) Goats: Goat materials used in this study were collected from the local slaughter house. These goats were brought from the adjoining villages. Another source was from the slaughter house of the Live-stock Research Station (L.R.S.), Bihar, Patna. These goats were infected with Rinder pest virus for the production of goat-tissue Virus (G.T.V.) vaccine. The particulars of these goats are given below :-

Sl.No.	Source of animals	No.of animals	R e m a r k s.
1	Local Slaughter house	40	Animals (Goats) are brought on truck or on foot from adjoining villages.
2	Slaughter house of the L.R.S., Bihar, Patna.	31	Used for manufacture of G.T.V.Vaccine. Animals are supplied by the contractor.
3	Clinical cases taken from surroundings villages.	134	

(B) Sheep : Rectal swabs were collected from the flocks which were available in neighbouring grazing fields. These were from different areas of Bihar.

(2) Collection of materials:

(A) From Living animals:

(a) Rectal swabs: A copper wire was cut to eight inches in length. Cotton swab was fixed at one end and was placed in a clean test tube and was sterilized at 180°C for 2 hours in the hot air oven.

Two rectal swabs from each clinical and subclinical case were collected with aseptic precautions. The anus was cleaned with water and sterlized with cotton wool soaked in 70% alcohol. The swab was introduced into rectum and material collected by rotating it for eight to ten times.

236 duplicate swabs were collected from 134 goats and 102 sheep.

(B) From slaughtered animals : Materials collected from slaughtered cases comprised of intestinal contents and internal organs.

(a) Intestinal content : Pooled intestinal contents of large and small intestines were collected in sterile petridishes.

(b) Internal organs : Materials from internal organs such as livers, kidneys, lungs, spleen, heart blood and mesenteric lymph nodes were collected with usual sterile precautions. Spleen was not collected from the goats slaughtered for G.T.V. vaccine.

(c) Bile from the gall bladder and blood from the heart were collected in sterile pasteur pipettes.

Particulars of materials collected have been summarised in Table-I.

TABLE I

Animals.	Living/Slaughtered	Rectal swab.	Slaughtered										Grand total
			Intestinal content	M.L.node	Liver	Lung	Kidneys	Heart blood	Spleen	Bile from Gall bladder	Total		
Sheep	Living	102	-	-	-	-	-	-	-	-	-		102
Goat	-Do-	134	-	-	-	-	-	-	-	-	-		134
-Do-	Slaughtered	-	39	39	71	71	71	71	39	71	472		472

3. Procedures for Isolation and Identification of Microorganism:

The material thus collected were inoculated into two different media. Nutrient broth was used for E. coli whereas tetrathionate broth was used for the isolation of Salmonella. Cultures were incubated overnight at 37°C for this purpose.

After overnight incubation a loopful of culture from both (nutrient broth and tetrathionate broth) media were separately streaked over MacConkey's agar plate and incubated at 37°C for 24 to 72 hours.

Salmonella : Moderately large, greyish-white, moist circular disks, dome shaped, smooth and non lactose fermenting colonies were further inoculated on brilliant green agar (B.G.A.) medium The medium was prepared by adding 0.4 gm. of brilliant green per 1000 ml. of MacConkey's agar (Cruickshank, 1965).

The colonies which were red, round, translucent, convex and about 1 mm. in diameter on B.G.A. were marked and transferred onto nutrient agar slants, incubated for 18 hours and kept in the refrigerater for further use. From agar slants smears were prepared and stained with the Gram's stain. Colony characters and morphology of organisms were studied.

Indole production, Methyl-Red (M.R.) reaction, Voges-Proskauer (V.P.) reaction, Citrate utilization and nitrate reduction tests were conducted by inoculating the test culture in peptone water, glucose phosphate peptone water, Koser's citrate and nitrate medium respectively for the primary identification of isolates.

The cultures which fermented dextrose with the production of acid or acid and gas, mannitol, dulcitol and sorbitol and failed to decompose urea, lactose, sucrose, adonitol and raffinose were primarily assigned to the genus Salmonella.

The isolates were subsequently tested for production of hydrogen sulphide (H_2S) in triple-sugar iron (T.S.I.) agar medium. The test for Indole, M.R., V.P., Citrate, nitrate reduction, Urea decomposition, H_2S and gelatine liquifaction were done using the procedures prescribed in the "Medical Microbiology" eleventh edition, edited by Robert Cruickshank(1965).

The isolates, primarily recognised as Salmonella, were further tested by rapid agglutination test using Poly 'O' antisera which were procured from the I.V.R.I.,Izatnagar(U.P.) For rapid agglutination test an eighteen hour growth on nutrient agar was used to make the antigen. The antigen was made by dissolving the alcohol treated culture in sterlized normal saline solution (0.85% Nacl) which was boiled for an hour at 100C in waterbath to destroy "H" and "VI" antigens. (Edwards and Ewing, 1962)

A drop of poly 'O' sera was taken on a clean glass slide by sterilised pasture pipette and to this a drop of prepared antigen was added by using another sterilised pasture pipette. A control test on the same slide with antigen and saline solution only to check autoagglutination was always performed. The agglutination was observed by naked eye and under low power of light microscope.

E.coli : On an average, four lactose fermenting round translucent and convex colonies of about 2 mm. diameter that developed on MacConkey's agar plate were individually picked up and streaked separately on the eosine methylene blue (E.M.B.) agar plate and incubated at 37C for 24 hours. Colony of a moderate size with dark (almost black) centre and showing metallic sheen in reflected light was further transferred on to the nutrient agar plats for the study of colony charecteristics. Similarly, another half of the same colony was also inoculated into the nutrient broth for examining the motility and morphology. Staining was done by the Gram's method (Jensen's modification using basic fuchsin as counterstain).

After this the isolate was maintained in nutrient agar slants and stored in refrigirator for biochemical and other studies.

All the isolates thus obtained were studied in detail for their biochemical reactions, such as, production of Indole, M.R., V.P., and reduction of nitrate to nitrites, utilisation of citrate, gelatinase and urease activity, and decomposition of dextrose, lactose, sucrose, arbinose, raffinose, adonitol, dulcitol, mannitol, srobitol, maltose and salicin. Indole positive and Lactose fermenting gram negative rods were considered to be of coli form group.

All the E.coli strains identified on biochemical tests were examined for the determination of their 'O' antigens. Available antisera used against 'O' groups were 026, 055, 086, 0114, 0119, 0126 and 0127 which were procured from the Haffikine Institute, Bombay.

With the above available antisera the serological typing of E.coli isolates were conducted by slide agglutination test. The modified method of Kauffmann (1947) and Barua et al (1956) was used for determination of specific 'O' antigen. For the preparation of 'O' antigen the overnight growth from one nutrient agar slant culture was washed with one ml. physiological saline. Growth was pooled from several tubes in this manner and was steamed at 100^{C} for one hour followed by two hours autoclaving at 121^{C}.

Slide agglutination test was done using each of the seven available antisera against each antigen. One drop of each of the seven available antisera was thoroughly mixed with one drop of each of the prepared antigen at a clean microscopic slide. A control with saline and antigen was also set up at another place of the same slide. To prevent drying, the slide was put over a wet blotting paper. The results were read upto the 15th minute under low power microscope or Fisher kahn viewer for the presence of agglutination reaction. The strain which gave positive agglutination with any one of the specific 'O' antiserum was considered to belong to that 'O' group.

Study of other organisms which were dissimilar from Escherichia and Salmonella was not included in this study.

CHAPTER IV

RESULTS

R E S U L T S

In the present study, a total of 307 domesticated animals comprising 102 Sheep and 205 Goats were examined for determining the incidence of *Salmonella* and *E.coli* as presented in Table-II.

Out of 205 goats examined for the incidence of *E.coli* and *Salmonella*, *Salmonella* was isolated from only five of them. No sheep was found harbouring *Salmonella*.

T A B L E II

Incidence of *Salmonella* and *E.coli* in Sheep and Goats.

Animals examined		Animals infected with			
Species	No.of animals	*Salmonella*		*E.coli*	
		No.of animals	Percentage infected	No.of animals	Percentage infected
Sheep	102	0	-	16	15.7
Goats	205	5	2.4	66	32.2
Total	307	5	-	82	-

The percentage of isolation of *Salmonella* and *E.Coli* obtained from the living and slaughtered animals is shown in Table-III.

TABLE III

Animals examined			Animals infected with			
Species	Living/Slaughtered	No. of animals examined	Salmonella		E.coli	
			No. of animals	Percentage infected.	No. of animals.	Percentage infected.
Sheep	Living	102	-	-	16	15.7
Goats	-Do-	134	-	-	51	38.06
-Do-	Slaughtered	71	5	7.04	15	21.12
Total		307	5	-	82	-

Salmonella:

In order to investigate the incidence of Salmonella infection in sheep and goats, 102 sheep and 205 goats were examined. Out of 205 goats, five goats (2.4%) proved positive for Salmonella organisms. Faecal samples from 134 of the goats which were having acute diarrhoea were bacteriologically examined. None of them revealed Salmonella organisms.

Intestinal contents, mesenteric lymph nodes and spleens from 39 goats were examined. Salmonella organisms were isolated from intestinal content of two and mesenteric lymph node of one of the goats examined.

71 specimens from livers, kidneys, heart blood, bile and lungs were cultured of which only one specimen of bile and one of heart blood yielded Salmonella.

On primary isolation the typical morphology of the genus-Salmonella was observed as follows : they were gram-negative non-spore forming, motile rods, about 2 to 3 μ in length and 0.6 to 0.8 μ in width.

The important physiological and biochemical characteristics alongwith serological reactions are summarised in Table-IV. All isolates were positive for M.R. reaction, utilised citrate, reduced nitrate to nitrites and produced H_2S whereas they were negative for V.P. reaction, urease and gelatinase activities and indole was not produced. They fermented dextrose, mannitol, dulcitol, sorbitol, maltose, ~~raffinose~~ and arbinose with production of acid and gas and were non-fermenters of lactose, sucrose, adonitol, ~~and~~ salicine& raffinose.

Recovered Salmonellae were tested with poly 'O' antisera of groups 1,2,12; 4, 5, 12; 6, 7, 8; 9, 12; and 3, 10, 15. They proved positive for these groups. For detailed serology the isolates have been referred to the Indian Veterinary Research Institute, Izatnagar (U.P.) because of non-availability of specific antisera in our laboratory.

E.Coli

E. coli was recovered from 16 out of 102 sheep and 66 out of 205 goats.

Morphology of E.coli was studied on primary isolation. They were gram negative short rods, non-sporeforming, motile, averaging 1 to 3 μ in length and 0.5 to 1 μ in width.

All isolates produced indole, reduced nitrate to nitrites and were V.P. negative and M.R.positive. They were

negative for urease and gelatinase activities and neither they produced H_2S nor utilized citrate.

The details of the biochemical patterns are recorded in Table-V. Thirteen different biochemical patterns were exhibited by 62 isolates of E.coli from sheep and goats.

Pattern (Strain)-1 fermented all the carbohydrates used except adonitol. Pattern (Strain)-2 did not decompose raffinose, dulcitol, adonitol and sorbitol. Pattern (Strain)-3 differed from pattern-2 by fermenting raffinose and non-fermenting sucrose. Pattern (Strain)-4 differed from strain-1 by non-fermenting dulcitol. Pattern (Strain)-5 differed from pattern-1 by non-fermenting mannitol. Pattern (Strain)-6 did not ferment sucrose, raffinose, mannitol and adonitol whereas Pattern (Strain)-7 did not ferment maltose and adonitol. Pattern (Strain)-8 differed from strain one by non-fermenting dulcitol and mannitol. Pattern (Strain)-9 was similar to strain-6 except that it did not ferment maltose. Likewise Pattern (Strain)-10 and two were alike except strain-10 fermented sorbitol which was not decomposed by strain-2. Pattern (Strain)-11 was like pattern-5 biochemically except it did not ferment raffinose. Pattern (Strain)-12 and strain-2 differed in fermentation of dulcitol and sorbitol as dulcitol was fermented by the former and not by the latter ~~as also~~ sorbitol was fermented by the former and not by the latter. Pattern (Strain)-13 did not ferment raffinose, dulcitol, mannitol and adonitol.

Cont No.

TABLE IV

Important Physiological and Biochemical Charecters of Salmonella isolated from Goats.

Goat No.	Isolate No.	Material collected from	Physiological and Biochemical reaction																				Sero-type with poly 'O' anti-sera *
			Indole production	M.R. reaction	V.P. reaction	Citrate utilisation	Nitrate reduction	H_2S production	Urease activity	Gelatinate activity	Dextrose	Lactose	Sucrose	Raffinose	Arabinose	Maltose	Dulcitol	Mannitol	Adonitol	Sorbitol	Salicin		
8	57	Bile	-	+	-	+	+	+	-	-	AG	-	-	-	AG	AG	AG	AG	-	AG	-		+
13	99	Intestinal content	-	+	-	+	+	+	-	-	AG	-	-	-	AG	AG	AG	AG	-	AG	-		+
14	107	-Do-	-	+	-	+	+	+	-	-	AG	-	-	-	AG	AG	AG	AG	-	AG	-		+
15	118	Heart blood	-	+	-	+	+	+	-	-	AG	-	-	-	AG	AG	AG	AG	-	AG	-		+
53	305	Mesenteric Lymph node.	-	+	-	+	+	+	-	-	AG	-	-	-	AG	AG	AG	AG	-	AG	-		+

* Serotype with Poly valent 'O' antisera comprising groups 1,2,12; 4,5,12; 6,7,8; 9,12; and 3,10,15;.

+ = Positive for the test.

- = Negative for the test or non-fermenter.

AG = Acid and gas.

TABLE V

Important Biochemical Properties of *E. coli* and Division of Patterns.

Biochemical pattern	Indole production	M.R.Reaction	V.P.Reaction	Citrate utilisation	Nitrate reduction	H_2S production	Urease activity	Gelatinase activity	Dextrose	Lactose	Sucrose	Arabinose	Raffinose	Maltose	Dulcitol	Mannitol	Adonitol	Sorbitol	Salicin
1	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	AG	-	AG	A
2	+	+	-	-	+	-	-	-	AG	AG	A	AG	-	A	-	AG	-	-	A
3	+	+	-	-	+	-	-	-	AG	AG	-	AG	A	A	-	AG	-	-	A
4	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	-	AG	-	AG	A
5	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	-	-	AG	A
6	+	+	-	-	+	-	-	-	AG	AG	-	AG	-	A	A	-	-	AG	A
7	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	-	A	AG	-	AG	A
8	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	-	-	-	AG	A
9	+	+	-	-	+	-	-	-	AG	AG	-	AG	A	-	A	-	-	AG	A
10	+	+	-	-	+	-	-	-	AG	AG	A	AG	-	A	-	AG	-	AG	A
11	+	+	-	-	+	-	-	-	AG	AG	A	AG	-	A	A	-	-	AG	A
12	+	+	-	-	+	-	-	-	AG	AG	A	AG	-	A	A	AG	-	AG	A
13	+	+	-	-	+	-	-	-	AG	AG	A	AG	-	A	-	-	-	AG	A

A = Acid.
AG = Acid and gas.
\+ = Positive for the test.
\- = Negative for the test or no fermentation.

E.coli strains were put to slide agglutination test with antisera against serological types 026, 055, 086, 0114, 0119, 0126 and 0127. Out of 82 isolates of E.Coli from sheep and goats, 44 (53.6%) cross reacted with the above seven available 'O' antisera whereas 38 isolates could not be agglutinated by these antisera.

The results of agglutination tests are recorded in Table-VI.

TABLE VI

Incidence of Different 'O' serogroups of E.coli in Sheep and Goats.

Animals	Serotypes								Untypable with seven available antisera	Total
	026	055	086	0114	0119	0126	0127	Total		
Sheep	4	0	0	0	0	2	0	6	10	16
Goats	5	0	6	1	5	10	11	38	28	66
Total	9	0	6	1	5	12	11	44	38	82

It is evident from the Table-VI that the frequency of serotypes of 0126 is the highest. 0127, 026 and 086 are next in order of frequency. The percentage recovery of 0119 is intermediate and 0114 is the least encountered. E.coli of group 055 is not recorded with the available antisera.

Sheep :

The isolation of *E.coli* from sheep was further studied in relation to the type of infection. The results are presented in Table-VII.

T A B L E VII

Incidence of *E.coli* in Sheep in Relation to the Type of Infection.

Types of Infection	No.of sheep examined.	No.of isolates	Percentage
Healthy or Subclinical cases.	62	9	14.5
Clinically sick *	40	7	17.5
Total	102	16	-

Different biochemical patterns (strains) exhibited by *E.coli* isolates recovered from sheep are presented in Table-VIII.

* Rectal swabs were taken from the cases of acute diarrhoea.

T A B L E VIII

Biochemical Patterns (Strains) of E.coli from Sheep.

Types of infection	Biochemical pattern					Total
	5	10	11	12	13	
Subclinical	4	3	0	0	2	9
Clinical	2	0	2	3	0	7
Total	6	3	2	3	2	16

From the Table-VIII it is clear that only three biochemical patterns (strains) based on sugar fermentation reactions were recorded among isolates recovered from apparently healthy animals. Clinical cases had three different strains one of which was common with pattern 5 of subclinical cases. Organisms of pattern number five were fermenters of sucrose, raffinose, maltose, dulcitol and salicin with production of acid and dextrose, lactose, arabinose and sorbitol with production of acid and gas and they were non-fermenters of mannitol and adonitol. Patterns 10 and 13 and 11 and 12 were noticed in subclinical and clinical cases respectively.

The results of the agglutination test are recorded in Table-IX and Table-X.

Details of serological typing of E.coli isolated from sick and apparently healthy sheep are presented in Table-X

and the corelation between strains and serotypes is recorded in Table-XI.

TABLE IX

Incidence of Different 'O' Groups of E.coli in Sheep.

Condition of animals	Serotypes									
	026	055	086	0114	0119	0126	0127	Total	Untypable with seven available antisera	Total
Subclinical	4	0	0	0	0	2	0	6	3	9
Clinical	0	0	0	0	0	0	0	0	7	7
Total	4	0	0	0	0	2	0	6	10	16

TABLE X

Serology of E.coli Isolated from Sheep

Condition of animals	Antigen	Available antisera						
		026	055	086	0114	0119	0126	0127
1	2	3	4	5	6	7	8	9
Clinically sick	6	-	-	-	-	-	-	-
	9	-	-	-	-	-	-	-
	16	-	-	-	-	-	-	-
	39	-	-	-	-	-	-	-

1	2	3	4	5	6	7	8	9
	40	-	-	-	-	-	-	-
	58	-	-	-	-	-	-	-
	78	-	-	-	-	-	-	-
Apparently healthy.	26	+	-	-	-	-	-	-
	49	-	-	-	-	-	+	-
	52	-	-	-	-	-	-	-
	56	+	-	-	-	-	-	-
	61	+	-	-	-	-	-	-
	64	-	-	-	-	-	+	-
	66	+	-	-	-	-	-	-
	84	-	-	-	-	-	-	-
	102	-	-	-	-	-	-	-

T A B L E XI

Corelation between Strains and Serotypes of E.coli Recovered From Sheep

Serotype	Biochemical pattern (stain)
026	5(4) *
0126	13(2)

* The number in parenthesis indicates the total number of E.coli isoltes for that pattern (strain).

Cells of four isolates belonging to biochemical pattern 5 from subclinical cases were agglutinated by the 026 antiserum and the other two from the clinical cases showing the same sugar fermentation reaction could not react with seven available antisera.

Cells of two isolates exhibiting biochemical pattern 13 that is, fermenter of sucrose, maltose and salicin with production of acid, and of dextrose, lactose, arabinose and sorbitol with production of acid and gas and nonfermenter of raffinose, dulcitol, mannitol and adonitol were agglutinated by the 0126 antisera.

Goats :

Percentage of isolation of E.coli from subclinical and clinical cases of goats have been summarised in Table-XII.

T A B L E XII

Incidence of E.coli in Goats.

Types of infection	No. of goats examined	No. of isolates	Percentage
Subclinical	71	15	21.12
*Clinical	134	51	38.06
Total	205	66	-

* All cases of acute diarrhoea. Only rectal swabs were taken.

Out of 71 apparently healthy goats examined 15 (21.12%) yielded E.coli whereas E.coli was isolated from 51 (38.06%) of 134 goats suffering from acute diarrhoea.

Nine different biochemical patterns (strain) were exhibited by 66 isolates of E.coli isolated from goats. Results are presented in Table-XIII.

T A B L E XIII

Biochemical Patterns of E.coli Isolated from Goats.

Type of infections	Biochemical patterns									Total
	1	2	3	4	5	6	7	8	9	
Subclinical	3	1	1	0	6	2	0	1	1	15
Clinical	21	0	3	1	9	0	6	4	7	51
Total	24	1	4	1	15	2	6	5	8	66

21 E.coli isolates out of 51 from clinical cases exhibited the fermentation pattern 1, that is, fermenter of sucrose, raffinose, maltose, dulcitol and salicine with production of acid and of dextrose, lactose, arabinose, mannitol and sorbitol with production of acid and gas and nonfermenter of adonitol. Biochemical pattern 5 was the next in order, that is, nine out of 51 isolates belonged to this pattern. Biochemical pattern 5 was more prevalent (6 out of 15) in subclinical cases. Other patterns comprised of only a few isolates in each group.

Serological groupings of E.coli isolated from goats are presented in Tables XIV-XVI.

TABLE XIV

Serological Groupings of E.coli Isolated from Goats.

Types of infection	Serotypes									
	026	055	086	0114	0119	0126	0127	Total	Untypable with seven available antisera	TOTAL
Subclinial	1	0	3	1	3	2	4	14	1	15
Clinical	4	0	3	0	2	8	7	24	27	51
Total	5	0	6	1	5	10	11	38	28	66

With seven available antisera of 'O' groups, 38 isolates reacted with different antisera whereas 28 were non-reactors. Typable strains comprised of 14 isolates from subclinical cases and 24 from clinical cases of diarrhoea. The following groups of E.coli were recorded in order of frequency: 0127, 0126, 086, 026 and 0119, and 0114.

Four isolates out of 14 from subclinical cases and seven out of 24 from clinical cases were grouped in type 0127 whereas eight isolates from clinical cases were of 0126 type. The other serological groups comprised of 086, 026, 0119 and 0114 in order of frequency.

TABLE XV

Serological Typing of *E. coli* Recovered from Healthy Goats.

Antigen No.	Seven available antisera						
	026	055	086	0114	0119	0126	0127
81	+	-	-	-	-	-	-
108	-	-	-	-	+	-	-
115B	-	-	-	-	-	-	-
120	-	-	+	-	-	-	-
128	-	-	-	-	-	-	+
223	-	-	-	-	+	-	-
234	-	-	-	-	-	-	+
261	-	-	-	-	-	+	-
269	-	-	-	-	-	-	+
421	-	-	-	-	-	-	+
433	-	-	-	-	-	+	-
457	-	-	+	-	-	-	-
465	-	-	-	-	+	-	-
471	-	-	-	+	-	-	-
482	-	-	+	-	-	-	-

+ = Positive for the agglutination reaction.

- = Negative for the agglutination reaction.

T A B L E XVI

Serological Typing of *E.coli* Recovered from Sick Goats.

Antigen No.	Seven available antisera						
	026	055	086	0114	0119	0126	0127
1	2	3	4	5	6	7	8
123	-	-	+	-	-	-	-
124	-	-	-	-	-	+	-
125	+	-	-	-	-	-	-
130	-	-	-	-	-	-	-
131	-	-	-	-	-	-	-
132	-	-	-	-	-	-	+
134	-	-	-	-	-	+	-
412	+	-	-	-	-	-	-
413	-	-	+	-	-	-	-
414	-	-	-	-	-	-	+
415	+	-	-	-	-	-	-
416	-	-	+	-	-	-	-
417	-	-	-	-	+	-	-
498	-	-	-	-	-	+	-
502	-	-	-	-	-	-	-
505	-	-	-	-	+	-	-
521	-	-	-	-	-	-	-
522	-	-	-	-	-	-	-
523	-	-	-	-	-	-	-
524	-	-	-	-	-	-	-

1	2	3	4	5	6	7	8
538	-	-	-	-	-	-	-
539	-	-	-	-	-	+	-
540	-	-	-	-	-	+	-
543	-	-	-	-	-	-	-
545	-	-	-	-	-	+	-
546	-	-	-	-	-	-	-
547	-	-	-	-	-	-	+
548	-	-	-	-	-	-	-
550	-	-	-	-	-	-	-
551	-	-	-	-	-	-	+
552	-	-	-	-	-	-	-
553	-	-	-	-	-	-	-
554	-	-	-	-	-	-	-
555	-	-	-	-	-	-	-
556	-	-	-	-	-	-	-
558	-	-	-	-	-	-	-
559	-	-	-	-	-	-	-
560	-	-	-	-	-	+	-
561	-	-	-	-	-	-	-
567	-	-	-	-	-	-	+
569	-	-	-	-	-	-	+
570	-	-	-	-	-	-	-
571	-	-	-	-	-	-	-
575	-	-	-	-	-	-	-

1	2	3	4	5	6	7	8
576	-	-	-	-	-	-	-
579	-	-	-	-	-	+	-
585	+	-	-	-	-	-	-
586	-	-	-	-	-	-	-
587	-	-	-	-	-	-	-
590	-	-	-	-	-	-	+
591	-	-	-	-	-	-	-

+ = Positive for the agglutination reaction.

- = Negative for the agglutination reaction.

Corelation of Serotypes with biochemical patterns (strains) of E.coli isolates recovered from goats are presented in Table-XVII.

T A B L E XVII

Serotypes	Biochemical types
026	5(1), 7(4) *
055	nil
086	1(1), 3(4), 5(1)
0114	5(1)
0119	1(1), 5(4)
0126	5(5), 8(5)
0127	1(1), 6(2), 9(8)

The typable E.coli isolates belonged to the biochemical patterns 5, 9, 8, 3, 7, 1 and 6 in order of frequency.

The biochemical pattern 5 has been recorded the most prevalent in both sheep and goats.

* The number in parenthesis indicates the total number of E.coli isolates in that pattern.

CHAPTER V

DISCUSSION

D I S C U S S I O N

For this study materials were collected from living sheep, goats and slaughtered goats. Altogether 102 living sheep, 134 living goats and 71 slaughtered goats were examined for the incidence of Salmonella and Escherichia coli. Out of 102 living sheep, 40 sheep were having diarrhoea and all the 134 living goats were suffering from diarrhoea. Salmonella from five healthy goats were isolated and subjected to biochemical tests and serological typing with polyvalent 'O' antisera.

An attempt was made to determine the incidence, pathogenesis and serotypes of Salmonella and E.coli in sheep and goats.

Several workers from different parts of the world have reported the role of animal enteropathogens in diseases of man. In India, the informations regarding the ecology, prevalence, epidemiology and intrinsic and extrinsic factors which contribute to either establishment or failure of infection in an animal are inadequate. As rightly pointed out by Orskov (1963) very little is known about E.coli infection in livestocks, particularly in sheep and goats.

As ascertained in this study the percentage of infection by Salmonella and Escherichia in sheep is 0%, 15.7% respectively whereas in goats it is 2.44% and 32.2% respectively.

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 true pathogens for animals or man or for both and are therefore of zoonotic importance. Organisms, such as, Pasteurella multocida, E.coli, Staphylococcus aureus, Salmonellae and others possessing disease producing potentiality are frequently harboured by the animal hosts with no 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 not fully known.

During the course of this study, five strains (2.4%) of the genus Salmonella were isolated from 5 out of 205 goats examined.

It was pertinent observation that none of the strains was isolated from more than one sites. None of the rectal swabs obtained from 102 sheep yielded Salmonella organisms.

Out of five isolates of Salmonella, two were isolated from intestinal contents, one from mesenteric lymph node, one from bile and one from heart blood of apparently healthy goats which were killed in the local slaughter house for meat purpose.

The low incidence of Salmonellosis in goats has been previously recorded by Buxton (1957). Findings recorded in this study are similar to those of Buxton so far the severity of incidence is concerned.

However, in view of the fact that almost all Salmonella

serotypes have been incriminated in human infection their occurrence either in cases of clinical salmonellosis or in symptomless carriers is a potent hazard to human health. Human infection may occur among meat handlers and butchers in the slaughter house and among consumers through the contaminated meat. Further more, healthy carriers are particularly important to preventive veterinary medicine since the prophylactic vaccine against rinderpest is prepared from goats spleen.

Investigations carried out so far revealed that every animal species is found to harbour these organisms in their body, especially in their intestinal tract. For example, S.typhimurium which causes typhoid like disease in mouse and other rodents, septicaemia and death in young animals and birds, occasional gasteroenteritis in adult animals, is the most prevalent cause of meat born salmonellosis in man. It displays the capacity to pass from animal to man and vice-versa by the oral route. Microorganisms of the genus Salmonella can get adapted to any kind of multicellular animal species and thus, the control of salmonellosis in man and animals is, indeed, a difficult problem.

As the pathogenesis of salmonellosis is complex phenomenon and as in most instances Salmonella may establish themselves in the tissue of the host to produce a more or less permanent carrier state even after recovery from an acute case and as these carriers may not exhibit any clinical symptoms, it is of prime importance to investigate

in details the prevalence of salmonellosis in goats and sheep which are the common source of meat in the State of Bihar.

One way to control the transmission of Salmonella from animals to man would be adequate inspection of animals slaughtered for meat. However, if the disease is not active, the detection of infected animals may be very difficult but can be achieved by the help of bacteriological laboratory.

E.coli is widely distributed in nature, among man, animals and birds as recorded by Dubos and Hirsch (1965), Merchant and Packer (1967) and others. Out of 102 sheep examined in course of the present study, 16 (15.7%) showed infection with E.coli . Five strains of E.coli were isolated from nine subclinical and seven clinical cases. Out of nine isolates recovered from subclinical cases, six could be typed with seven available antisera among which four belonged to 026 and two to 0126. None of strains isolated from clinical cases could be serologically typed with the available antisera.

From Tables II and XII, it would appear that out of 205 goats examined, 66 (32.20%) harboured E.coli; out of 71 healthy cases, 15 (21.12%) yielded E.coli and E.coli was isolated from 51 (38.06%) out of 134 clinical cases in goats. This suggests that carrier rate of E.coli in goats (21.12%) is higher than that in healthy sheep (14.5%).

Out of 38 typable isolates, 11 belonged to type 0127;

10 belonged to 0126; six belonged to 086; five belonged each to 026 and 0119 and one to 0114 respectively. Information is meagre for different serotypes of E. coli occuring in goats in India. Panda et al (1965) while examining 38 goat strains reported nine serotypes including 026. The present studies have revealed that apart from 026 serotype, 086, 0114, 0119, 0126 and 0127 are also found in goats. Further serological examination of 28 untyped isolates may reveal other serotypes. It is worthy to note that all the serotypes isolated from goats have been incriminated as human pathogens (Dubos and Hirsch, 1965). Chances of transmission of infection from goats and sheep to man are there indeed due to habitation of these animal in and around human dwellings in villages in India as well as inhygienic situations present in Indian slaughter houses. Therefore its zoonotic importance is in no way less then that of Salmonella. An extensive study is needed to determine the prevalence of E. coli in clinical infections of sheep and goats as well as their various serotypes causing such infections.

From the public health point of view emphasis has to be laid on serotypes which are incriminated in causing infantile diarrhoea as well as in invading organs anatomically related to intestinal tract, such as the appendix, gallbladder, peritoneal cavity, kidney and bladder, especially in these days when use of penicillin to kill gram positive organisms is indiscriminate in this country.

CHAPTER VI

SUMMARY

SUMMARY

1. Prevalence of Salmonella and E.coli was studied in relation to diseases caused by them in sheep and goats in the State of Bihar.

2. The isolates recovered from the clinical and apparently healthy cases were studied for their biochemical and Serological characteristics.

3. The percentage of infection with Salmonella in goats was 2.4. No Salmonella was isolated from sheep.

4. The percentage of infection caused by E.coli in sheep and goats was 15.7 and 32.2 respectively.

5. The percentage of isolation from 62 apparently healthy sheep was 14.5 whereas it was 17.5 in clinical cases of diarrhoea.

6. Similarly, the frequency of isolation from 71 apparently healthy goats was 15 against 51 from 134 cases of diarrhoea. The percentage comes to 21.2 and 38.1 respectively.

7. It was also established that carrier rate of E.coli in healthy sheep was less than that in healthy goats.

8. Biochemically all E.coli isolates were grouped in nine patterns which could be taken as strains whereas isolates from sheep were grouped in five strains on the basis of their physiological and biochemical characteristics.

There was a common biochemical pattern for 15 isolates from goats and six isolates from sheep.

9. Of the six isolates that could be typed with available antisera four belonged to O26 and two belonged to O126 serotypes. Among 38 typable goat isolates O127 accounted for 11; O126 for 10; O86 for six; O26 and O119 for five each; and O114 for one isolates.

10. Fourteen typable isolates were recovered from apparently healthy animals whereas others came from clinical cases of diarrhoea.

11. The pathogenesis of these enteropathogens was discussed in relation to the public health.

CHAPTER VII

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Gran. F.H., L.E. Brownlie and M.G.Smith, 1969. J Sc.Vet. intake on numbers of Salmonella and Escherichia cteriology and faeces of sheep. J.appl.Bact. 32 : 112-117 ersity (U.S.

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A P P E N D I X I

Important Biochemical Reactions and Serology of Escherichia coli Isolated from Sheep.

Sl. No.	Animal No.	Isolate No.	strain(biochemical pattern)	Material collected from.	Condition of animals	Biochemical and Sugar Fermentation Reaction.																				Serotypes
						Indole Production	M.R. Reaction	V.P. Reaction	Citrate Utilisation.	Nitrate reduced to Nitrite	H_2S Production	Urease activity	Gelatine lequifaction.	Dextrose	Lactose	Sucrose	Arabinose	Raffinose	Maltose	Dulcitol	Mannitol	Adonitol	Sorbitol	Salicin		
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25		26
1	6	6	12	Faecal material (Rectal swab)	Diarrhoea	+	+	-	-	+	-	-	-	AG	AG	A	AG	-	A	A	AG	-	AG	A		+
2	9	9	11	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	-	A	A	-	-	AG	A		*
3	16	16	5	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	-	-	AG	A		*
4	26	26	5	-do-	Healthy	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	-	-	AG	A		026
5	39	39	12	-do-	Diarrhoea	+	+	-	-	+	-	-	-	AG	AG	A	AG	-	A	A	AG	-	AG	A		*
6	40	40	5	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	-	-	AG	A		*
7	49	49	13	-do-	Healthy	+	+	-	-	+	-	-	-	AG	AG	A	AG	-	A	-	-	-	AG	A		0126
8	52	52	10	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	-	A	-	AG	-	AG	A		*
9	56	56	5	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	-	-	AG	A		026
10	58	58	12	-do-	Diarrhoea	+	+	-	-	+	-	-	-	AG	AG	A	AG	-	A	A	AG	-	AG	A		*
11	61	61	5	-do-	Healthy	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	-	-	AG	A		026
12	64	64	13	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	-	A	-	-	-	AG	A		0126
13	66	66	5	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	-	-	AG	A		026
14	78	78	11	-do-	Diarrhoea	+	+	-	-	+	-	-	-	AG	AG	A	AG	-	A	A	-	-	AG	A		*
15	84	84	10	-do-	Healthy	+	+	-	-	+	-	-	-	AG	AG	A	AG	-	A	-	AG	-	AG	A		*
16	102	102	10	-do-	-do-	+	+	-	-	+	-	+	-	AG	AG	A	AG	-	A	-	AG	-	AG	A		*

* They could not typed with available antisera, such as , 026, 055, 086, 0114, 0119, 0126 and 0127.

A P P E N D I X II

Important Biochemical Reactions and Serology of *E.Coli* Isolated from Goats.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
17	11	81	5	Intestinal content	Healthy	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	-	-	AG	A	026
18	14	108	1	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	-	A	-	AG	-	-	A	*
19	15	115B	2	Kidney	-do-	+	+	-	-	+	-	-	-	AG	AG	-	AG	A	A	-	AG	-	-	A	086
20	16	120	3	Bile	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	AG	-	AG	A	0127
21	17	128	1	Spleen	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	-	-	AG	A	0119
22	42	223	5	Kidney	-do-	+	+	-	-	+	-	-	-	AG	AG	-	AG	-	A	A	-	-	AG	A	0127
23	44	234	6	Int.cont.	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	-	-	AG	A	0126
24	47	261	5	Heart blood	-do-	+	+	-	-	+	-	-	-	AG	AG	-	AG	-	A	A	-	-	AG	A	0127
25	48	269	6	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	-	AG	A	-	A	-	-	AG	A	0127
26	86	421	9	Bile	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	-	-	-	AG	A	0126
27	88	433	8	Lung	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	AG	-	AG	A	086
28	93	457	1	Kidney	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	-	-	AG	A	0119
29	95	465	5	Liver	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	-	-	AG	A	0114
30	96	471	5	Bile	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	-	-	AG	A	086
31	98	482	5	Kidney	-do-	+	+	-	-	+	-	-	-	AG	AG	-	AG	A	A	-	AG	-	-	A	086
32	18	123	3	Rectal swab	Diarrhoea	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	-	-	-	AG	A	0126
33	19	124	8	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	-	A	AG	-	AG	A	026
34	20	125	7	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	-	A	AG	-	AG	A	*
35	25	130	7	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	AG	-	AG	A	*
36	26	131	1	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	-	AG	A	-	A	-	-	AG	A	0127
37	27	132	9	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	-	-	-	AG	A	0126
38	29	134	8	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	-	A	AG	-	AG	A	026
39	78	412	7	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	-	AG	A	A	-	AG	-	-	A	086
40	79	413	3	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	-	AG	A	-	A	-	-	AG	A	0127
41	30	414	9	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	-	A	AG	-	AG	A	026
42	81	415	7	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	-	AG	A	A	-	AG	-	-	A	086
43	82	416	3	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	-	-	AG	A	0119
44	83	417	5	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	-	-	AG	A	0126
45	110	498	5	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	AG	-	AG	A	*
46	114	502	1	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	-	-	AG	A	0119
47	117	505	5	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	-	AG	-	AG	A	*
48	133	521	4	-do-	-do-																				

Appendix II contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
49	134	522	1	Rectal swab	Diarrhoea	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	AG	-	AG	A	*
50	135	523	1	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	AG	-	AG	A	*
51	136	524	5	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	-	-	AG	A	*
52	150	538	1	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	AG	-	AG	A	*
53	151	539	8	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	-	-	-	AG	A	0126
54	152	540	8	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	-	-	-	AG	A	0126
55	155	543	5	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	-	-	AG	A	*
56	157	545	5	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	-	-	AG	A	0126
57	158	546	1	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	AG	-	AG	A	*
58	159	547	9	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	-	AG	A	-	A	-	-	AG	A	0127
59	160	548	1	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	AG	-	AG	A	*
60	162	550	1	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	AG	-	AG	A	*
61	163	551	9	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	-	AG	A	-	A	-	-	AG	A	0127
62	164	552	5	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	-	-	AG	A	*
63	165	553	1	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	AG	-	AG	A	*
64	166	554	1	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	AG	-	AG	A	*
65	167	555	1	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	AG	-	AG	A	*
66	168	556	7	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	-	A	AG	-	AG	A	*
67	170	558	1	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	AG	-	AG	A	*
68	171	559	1	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	-	-	AG	A	0126
69	172	560	5	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	AG	-	AG	A	*
70	173	561	1	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	-	AG	A	-	A	-	-	AG	A	0127
71	179	567	9	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	-	AG	A	-	A	-	-	AG	A	0127
72	181	569	9	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	AG	-	AG	A	*
73	182	570	1	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	AG	-	AG	A	*
74	183	571	1	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	AG	-	AG	A	*
75	187	575	1	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	AG	-	AG	A	*
76	188	576	1	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	-	-	AG	A	0126
77	191	579	5	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	-	A	AG	-	AG	A	026
78	197	585	7	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	AG	-	AG	A	*
79	198	586	1	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	AG	-	AG	A	*
80	199	587	1	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	AG	-	AG	A	0127
81	202	590	9	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	-	AG	A	-	A	-	-	AG	A	0127
82	203	591	1	-do-	-do-	+	+	-	-	+	-	-	-	AG	AG	A	AG	A	A	A	AG	-	AG	A	*

* They could not be typed with available antisera, such as, 026, 055, 086, 0114, 0119, 0126 and 0127.

A = Acid
AG = Acid and Gas
+ = Positive for the test.
- = Negative for the test or non-fermenter.