STUDIES ON THE BACTERIAL SPECIES ASSOCIATED WITH DIGESTIVE DISTURBANCES IN PIGS

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CERTIFICATE

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

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K. P. Balakrishna Pillai.

INTRODUCTION

INTRODUCTION

Eversince the domestication of swine began, a few diseases were also recognised, particularly in early life. Of these, digestive disturbances are very important and this contagious malady has been found to affect swine of all ages, but most frequently the young ones as a primary infection or secondary to some other infectious diseases. Goodwin (1957) considered enteritis as one of the commonest diseases of pigs and that it is responsible for death, unthriftness and stunted growth. According to the survey conducted by Veterinary Investigation Service in U.S.A. (1959) nearly 26% of the piglets born, failed to survive until they were eight week old. Upto three weeks of age 13.8% of the mortality was due to gastroenteritis. The disease condition acquires greater importance in swine because it causes serious economic losses in the form of piglet mortality, stunted growth and increased time to market.

Gastroenteritis can occur in various pathological forms. For instance the inflammatory process can be serous, fibrinous or haemorrhagic in character. Necrotising gastroenteritis also may be encountered. The type of gastroenteritis also depends on its localisation in alimentary

tract; the inflammation may involve the stomach and intestine or solely to the large intestine.

Numerous discrepancies in research findings with regard to gastroenteritis appear to indicate the complexity of its etiology. An interplay of a number of factors may be responsible for this condition in many cases.

Over the past decade a close association between certain gastroenteric syndromes in pigs and specific serotypes of Escherichia coli has been established (Gossling & Rhoades, 1966; Djurise, 1967; Vacheev, 1968; Ahuja & Khera, 1971; Gupta & Singh, 1972). The infection is related to special serological types of E.coli which produce their harmful effects by proliferating in the anterior small intestine. According to Stevens et al. (1963) the E.coli produce gastroenteritis mainly in three different age groups; (a) piglets a few days old (b) unweaned piglets about three weeks old and (c) pigs about 10 to 12 weeks old (2 to 3 weeks after weaning).

The major manifestations of porcine Salmonellosis are gastroenteritis and septicaemia, and some times combined occurance of these two syndromes. Jubb & Kennedy (1963) stated that "Salmonella choleraesuis and the disease it produces are both poorly understood and there is ample opportunity to investigate the disease in areas where hog cholera does not occur".

Piglets form the main animal reservoir for the Salmonella organisms apart from poultry. Usually these organisms exsist in this host without showing any clinical symptoms. The nutrition and husbandry practices or primary infection such as hog cholera which have altered the innate immunity causes "breaking down" to a fluorid diarrhoeic process, which also provides generalised environmental contamination.

Of the hundred of known Salmonella serotypes only two, S.typhisuis and S.choleraesuis, are host adapted to swine. The other organisms rarely encountered in porcine epizootics include S.typhimurium and S.derby (Sorensen, 1970). The U.S. Public Health Service reported over 50 serotypes of Salmonella from swine during the year 1967. The disease is one of the young pigs, usually 2-4 months age especially in sucklings.

There had been reports of gastrointestinal troubles associated with some species of Salmonella among young pigs from different parts of India (Khera, 1962; Krishnamurthy & Kaushik, 1964).

At the Pig Breeding Farm, Mannuthy, a large number of pigs are raised under good managemental and hygenic conditions, but heavy losses due to gastroenteritis have been encountered. Although detailed studies on the importance of E.coli and Salmonella have been carried out in other parts of the world, a systematic investigation in this regard has

not been taken up in our country. Limited studies conducted in the Department of Bacteriology have revealed the predominance of E.coli and Salmonella from digestive diaturbances of pigs (Sulochana et al., 1973). Hence this thesis is directed into detailed studies of E.coli and Salmonella strains isolated from enteric disorders in piglings with special reference to their characters, pathogenicity and sensitivity to common therapeutic agents.

LITERATURE REVIEW

LITERATURE REVIEW

ESCHERICHIA COLI

Introduction

The term "Enteric colibacillosis" refers to a malady of young pigs caused by the bacterium Escherichia coli (E.coli). In neonatal pigs the disease is most commonly manifested as acute enteritis, bacteraemia and colibacillary diarrhoea. In weanling pigs enteric colibacillosis assumes two distinctive forms- diarrhoea or enterotoxaemia.

History and Incidence

Sojka (1965) provided the following history of colibacillosis. In 1885, Theobald Escherich isolated and described an organism from the faeces of new born baby and is now called Escherichia coli. Laruella suggested in 1889, that E.coli may be pathogenic. In later years the importance of E.coli as a pathogen has been established from calf diarrhoea (Smith and Orcutt, 1925); infantile gastroenteritis (Adams, 1927) and fatal enteritis in young lambs (Heller, 1933; Marsh & Tunnicliff, 1938).

The Veterinary Investigation Centre in England and Wales surveyed the incidence and death of pigs upto 8 weeks of age for a period of two years (1956 to 1958). This survey indicated that E.coli and Streptococci were the most important organisms contributing generalised infection and E.coli contributed half of the enteritis cases in pigs between three days and one week old (Sojka, 1965). Stevens (1963), estimated that nearly 75% of all baby pig diarrhoea cases are associated with E.coli infection.

Bacteriology

Escherichia coli is a normal inhabitant of the intestine of man and other vertebrates. E.coli is a member of the genus Escherichia, composed of motile or nonmotile bacteria.

In Bergey's Manual (Breed et al., 1957) E.coli is

described as possessing the following characteristics; Rods, usually 0.5 by 1.0 to 3 microns varying from almost coccoid forms to long rods occuring singly, in pairs and in short chains. Usually non capsulated, nonsporeforming gram-negative rods. In broth turbid, heavy, greyish sediment; no pellicle is produced. On blood agar plates different strains vary in their action. Some are haemolytic. Hydrogen-sulphide is not produced, Methyl red positive, Voges-Proskauer test negative. Acid and gas from glucose, fructose, galactose, lactose, maltose, arabinose, xylose, rhamnose and mannitol. Sucrose, raffinose, salicin, esculin, dulcitol and glycerol may or may not be fermented. Citric acid and salts of citric acid not utilised as a sole source of carbon. Nitrites are produced from nitrates and urea not decomposed.

Haemolysins

William Smith (1963) states that about 63% of strains isolated from pigs produced haemolysis on 5% ox blood agar. E.coli strains belonging to O groups 138, 139, 141 and 8 are haemolytic (Sojka et al., 1960). Two types of haemolysins had been described by William Smith (1963), the alpha and beta. The alpha haemolysin is filterable while the beta is not filterable. The types are indistinguishable on their effect on blood agar. Alpha haemolysin obtained by growing cultures in special alkaline medium extract for 2¼ to 2½ hours at 37°C. It was antigenic and produced neutralising antibodies when

injected in rabbits. High concentration of alpha haemolysin were toxic to mice, rabbits and guinea pigs when injected intra venously. All the strains of E.coli that dilated pig intestine except 08: K87; K88a,b; 045:K? K88a,c produced alpha haemolysin and also some dilatation negative strains produced beta haemolysin (Smith, 1963). Smith concluded that the haemolysin by pathogenic strains are not responsible for pathogenicity, but this is useful as an aid to recognition of potentially enteropathogenic strains.

Serotypes

Parnas et al. (1950) were among the first to report on serological examination of E.coli strains isolated from healthy and diseased pigs. Sakazaki and Namioka (1956) tested 50 isolates from mesentric lymphnodes of pigs. The strains identified belonged to the sero group 08,018,02,021,025,0111, 048,0113,0115 and 021. Eight of the 75 strains isolated from faeces of healthy pigs were found to be in the Ogroup 01,08, 05,028,076 and 0116. Ewing et al. (1957) in their serological studies with E.coli cultures, isolated in Ireland and United States from cases of Oedema disease found that serotypes frequently encountered belonged to sero groups 0138: K8(B) and 0139: K82 (B). Other serotypes which were of less common occurrence includes 05a, 5c, 046, OX12 and 75a, 75c.

More than 147 O groups with 89 K antigens and 49 H antigens have been typed from pigs with digestive disturbances (Dunne & Bennet, 1970). The most important serological groups

associated with gastroenteritis were 08, 0138, 0139, 0141 and 0149 (Gossling & Rhoades, 1966; Vacheev, 1968; Elder et al., 1969; Sweeny, 1970; Beh, 1971, Sweemy, 1972; Yalicin, 1972).

Three O groups have shown to be specifically associated with oedema disease are 0139, 0141 and 0138. A number of other O groups have been identified with oedema disease including 02, 05, 018, 020, 045, 075, 0111, 0115, 0117, 0121, 0133, 0145, 0147, OX11 and OX12 (Dunne and Bennet, 1970).

In India several workers have reported the isolation of different serotypes from both healthy and diseased pigs. Ahuja & Khera (1971) on their investigation on aetiologic agents associated with diarrhoeal disease of pigs aged one week and three weeks found that the E.coli serotype belongs to sero group 0142, 02, 0111 and 0133. Gupta and Singh (1972) isolated sero groups 08, 0101, 020, 015, 041 and 01 from pigs suffering from gastroenteritis and some of the isolates were untypable. A single strain isolated from adult pig with gastroenteritis possessed 045 antigen. The strain isolated from carrier pig belonged to 035 & 08.

Pathogenisis

The pathogenisis of enteric colibacillosis was described as a sequence of four events by Nielsen (1968): 1) Infection with pathogenic E.coli 2) Proliferation of the

pathogen 3) Production of enterotoxin and 4) Production of lesion of colibacillosis.

Pig acquires infection from many sources by ingestion. The organisms have got wide distribution in the environment. Adult swine (Gupta & Singh, 1972) as well as cats, dogs and other animals and human beings may be important carriers of E.coli strains that are pathogenic for swine. Karmer and Nedertio (1967) demonstrated experimentally that establishment of colibacillosis requires an adequate infective dose. Greater the number of organisms ingested, greater the possibility of infection.

The bacteria that cling to the surface of the mucosa or those able to propagate in crypts away from the streaming effect would be more pathogenic (Nielson, 1968). Arbuckle (1970) observed that the serotypes of E.coli that usually produce K88 antigen attach to the intestinal mucosa of piglets and Smith & Linggwood (1971) concluded that the virulence of E.coli for early weaned piglets increased after the bacteria had acquired the ability to produce K88 antigen. Jones & Rutter (1974) demonstrated that K88 antigen was synthesised by a K88 positive enteropathogenic strain of E.coli in small intestine of both gnotobiotic and conventional neonatal piglets, where it act as adhesin enabling the bacteria to adhere and colonise the mucosa. This adhesive ability can be neutralized by homologus antiserum. The adherence is not an essential mechanism for pathogenesis, but rather should be considered as an

enhancement of pathogenecity of certain strains (Wilson & Hobmann, 1974). The fimbriae of E.coli act as a mechanism for the attachment of the epithelium of small intestine (Duguid & Smith, 1955).

Dunpont et al. (1971) reported that the strain of E.coli cause disease at least by two mechanisms: the elaboration of cholera like toxin and Shigella like epithelial penetration. In either case the proliferation of enteropathogen is necessary for the production of clinical colibacillosis (Kenworthy & Crabb, 1963; Smith & Jones, 1968).

Production of enterotoxin

Acute inflammatory changes occurred in the mucosa of rabbit intestinal loop exposed to some strains of entero-pathogenic E.coli (Taylor et al., 1958; McNaught & Roberts, 1958) whereas other strains dilated the intestinal loop without inflammatory response (Taylor et al., 1961). These authors concluded that the gut dilatory effects were due to an active liable substance, enterotoxin produced by the organism. Smith & Halls (1967) reported that bacteria free filtrates prepared from soft agar cultures of E.coli produced the same dilatation in ligated intestinal loops as live bacterial suspension, whereas no dilatation reaction with bacteria free fluids prepared from strains of E.coli that did not produce dilatation when tested as live suspensions.

Two forms of enterotoxins have been described: one heat liable and the other heat stable (Smith & Gyles, 1970a).

The heat liable enterotoxin was mainly an extra cellular product and its pig intestinal dialatation properties were neutralized by specific antisera. The heat liable form is more consistently associated with bacterial cell lysates and were also neutralizable (Smith & Halls, 1967). No difference were noted in pig intestinal loop responses or in diarrhoeal syndromes in piglets following administration of either type of enterotoxin (Smith & Gyles, 1970a). Smith & Gyles (1970b) and Kholer (1971) observed that the heat liable enterotoxin prepared from porcine enteropathogens exhibits a stronger rabbit gut dialatory effect than that with heat stable forms. All the strains of E.coli enteropathogenic for pigs produced heat stable toxin but only those naturally possessing K88 antigen produced heat liable enterotoxin (Smith & Gyles, 1970a).

The enterotoxin production was controlled by a transmissible plasmid (Ent)(Smith & Halls, 1968; Smith & Linggwood, 1972). and are transmissible to other strains of E.coli and to S.typhimurium and S.choleraesuis by conjugation in mixed cultures. The transmission of "Ent" to two strains of Salmonella species did not affect their pathogenicity (Smith & Halls, 1968).

Production of lesions

Not all enteropathogenic strains of E.coli produce their pathogenicity by the formation of enterotoxins. Some E.coli organisms produce their effects as a result of their

invasive capabilities (Salazaki et al., 1967; Ogawa et al., 1968; Dunpont et al., 1971). Organisms which have got invasive capabilities produce a Shigella like disease, cross react with Shigellae (Edward & Ewing, 1962) and have biochemical reaction to Shigella (Dunpont et al., 1971). Acute inflammatory changes are noticed in the mucosa of domestic animals after exposure to invasive strains of E.coli (Kenworthy, 1970; Stately et al., 1970). Intestinal epithelial penetration by certain strains of E.coli has been demonstrated in germfree and gnotobiotic pigs (Dress & Waxler, 1970; Menworthy, 1970 and in conventional pigs (Stately et al., 1973).

The entero pathogenic strains of E.coli have a greater tendency to be associated with epithelium of the small intestine than nonenteropathogenic strains (Berschinger et al., 1972). Invasion occur mainly in the upper one third of the villi of the jejunum and ileum and in large intestine (Dress & Waxler, 1970).

Tests for pathogenicity

Serotyping

Serotyping is a useful tool in helping to establish pathogenicity. But pigs without signs of the disease have been found to harbour E.coli that are enteropathogenic.

Ligated Intestinal Loop technique

The technique was formerly used to elucidate the etiology of neonatal diarrhoea in human patients (De Bhattacharya & Sarkar, 1956, McNaught & Roberts, 1958). It has been used

also for the detection of enteropathogens from domestic animals (Smith & Halls, 1967). These authors concluded that the anterior segment of the small intestine was more suitable than the posterior segments.

Cooke (1968) classified the loop reaction in rabbits into severe, moderate and mild type by their microscopical appearance. Severe type I reaction is characterised by large amount of exudate composed of mainly of erythrocytes. The intestinal wall is oedematous, and the blood vessels dilated. The villi are grossly distented. The columnar cells broken. In severe type II reaction is characterised by exudate of polymorphonuclear cells with some RBCs and debris. The columnar cells erroded and submucosa congested and oedematous. In moderate type I reaction there is less exudate but still composed of erythrocytes, villi distended with RBCs but no loss of continuity also. Some oedema and congestion of submucosa may be noticed. Moderate type II reaction, exudate is composed mainly of polymorphs with RBCs and debris. No loss of continuity of epithelium, oedema and congestion of mucosa. In severe reactions, bacilli are seen deeply staining gram-negative in intestinal lumen sometimes in layers of columnar cells. No bacilli are seen in tissue spaces. In moderate and mild reaction the bacilli stain pale and large numbers are not present on columnar epithelial cells.

A positive association between haemolysin production and necrotoxin production was observed in strains isolated from gastroenteritis and diarrhoea. These properties are beneficial in labelling a strain pathogenic (Cooke, 1968; Pandae et al., 1974). The strain produced both haemolysin and necrotoxin are pathogens where as a strain with either of these two properties are potential pathogens (Pandae et al., 1974).

White swiss albino mice was extensively used as the experimental animal for E.coli. But the mouse pathogenecity tests are not reliable. According to Jacks & Glantz (1967) the strain of E.coli from nonenteric, systemic sources were more pathogenic for mice. Shestakov et al., 1972 reported that necrotoxin production and pathogenecity for mice would be valuable for differentiating enteropathogens.

Antibiotic sensitivity

Richard & Frasier (1961) pointed out that since drug resistant strains of E.coli may be frequently encountered, sensitivity test should be done whenever possible. Saunders et al. (1960) observed that resistance of E.coli to tetracyclines which have been used as a feed additive on farms. Roberts & Valley (1969) found that significant haemolytic E.coli strains isolated from pigs, 17% completely and 13% were comparatively resistant to streptomycin and 1% to sulphathiazole. None of these strains were resistant to chloramphenicol in concentration of 100 micro grams.

Willinger & Genis (1969) on their investigation to the occurrence of antibiotic resistant strains of E.coli in pigs observed that out of total 128 strains, all the strains were sensitive to neomycin, colistine and nitrofurazolidone while 3% were resistant to chloramphenicol and 9% to dihydrostreptomycin. Resistance to streptomycin was most commonly observed in strains isolated from faeces.

Yadev & Gupta (1971) compared the antibiotic sensitivity patterns of E.coli isolated from gastroenteritis of domestic animals with that of sensitive standard strains of E.coli B on minimum inhibitory concentration values. Depending upon the resistant patterns, they grouped E.coli into four types, 1) those which were sensitive like E.coli B, 2) those which were showing only streptomycin resistance, 3) strains showing multiple resistance to pencillin, streptomycin and tetracyclines and, 4) those showing high "Mic" values for all the antibiotics tested.

Terkado et al. (1972) studied the antibiotic sensitivity of 937 strains of E.coli isolated from faecal samples of healthy domestic animals and man. They found that population of human strains were resistant to one antibiotic and multiresistance to tetracyclines, streptomycin, chloramphenicol, sulphadimethoxine, kanamycin and benzyl pencillin.

SALMONELLA

Introduction

Salmonellosis can occur in swine of all ages, occasional cases occur in young pigs and few cases occur in adult swine. However, majority of cases occur in pigs aged several weeks to several months. The character of the syndrome may vary from peracute septicaemia to chronic enterocaecocolitis. Young pigs are prone to septicaemic form, where as older ones to enterocaecocolitis (Sorensen, 1970). The clinical outbreak is characterised by acute diarrhoea and rapid death in store pigs. Other symptoms include anorexia, fever, nervous symptoms and discolourations of the extremities (Lawson and Dow, 1966).

Salmonella typhimurium constituted 20% of the recoveries from livestock and poultry (Council report of American Veterinary Medical Association, Council of Public Health and regulatory service, 1966). S.typhimurium has been frequently isolated from normal pigs at slaughter (Vanhoof, 1966; Chung and Froster, 1969) and also from severe clinical disease (Heard et al., 1965; Burner, 1973).

History and incidence

The first report on Salmonella isolation was in 1885, and it was described as "Hogcholera bacillus" found in pigs (Hagen and Bruner, 1961). Sorensen (1970) reviewed the history of porcine salmonellosis as follows. It was considered to be a casual agent of cholera until Schwenitz & Dorset (1903) reproduced the cholera with a bacteria free filtrate of body fluids taken from infected swine. Dorset et al. (1905) reported that Salmonella infection may be present as either secondary or primary active pathogens. Salmonella typhisuis was described by Glasser (1909) and has been reported from swine in various parts of the world. Salmonella typhisuis has little pathogenicity for animals other than swine, produces a more chronic syndrome and possesses greater potential for primary pathogenicity (Barnes & Bergeland, 1968).

Currently there are in excess of thousand known Salmonella serotypes, and new ones continue to be discovered at a rapid rate. These Serotypes often with remarkably low host specificity, may participate as secondary invaders with a tendency to produce fatal septicemia of which Salmonella typhimurium is of considerable importance. S. typhimurium has been frequently isolated from normal pig at slaughter (Scott, 1940; Galton et al., 1954; Newal et al., 1959). but limited references have been found reporting S.typhimurium as producing severe clinical disease in a herd of pigs.

But several workers have found that the organism can cause either an acute or chronic disease (Heard & Linton, 1965; Hoorens & Thoonen, 1968).

Heard & Linton (1965), reported an outbreak of S.typhimurium infection in a hysterectomised pig herd with approximately 30% morbidity. S.paratyphiB, S.typhimurium, S.gatuni, S.saintpaul, S.anatum and S.westerstade have been reported by Pateraki et al. (1966) from Athens and Greece. Barnes & Bergeland (1968) found that S.typhisuis was the most common species found in Minnesota premises. Mathisen (1968) states that S.typhimurium was the most common isolate in Norway during the period of 1964 to 1967. Acute or chronic form of the disease in pigs due to S.typhimurium, S.panama, S.brandenberg has been reported by Hoorens & Thoonen (1968). Grover et al. (1970) found that in Ontario, S.typhimurium was the commonest serotype accounting for about 20% cases and the other Salmonella includes S.heidelberg. Bruner (1973) reported that, of the Salmonella cultures typed during 1950 to 1971 in Cornell University from diseased animals, 46% of the isolates accounted for S.typhimurium. Guineae & Valkenberg (1974) reported in their study on the occurrence of Salmonella in Netherland between 1956 and 1973 that the proportion of Salmonella typhimurium isolates remain constant at about 61% between 1963 to 1973 from man, animals and feed stuff.

Incidence in India

Khera (1962), who summarised the reports on Salmonellosis in various species of animals and birds in India cited the incidence of 52 serotypes, out of which S.dublin and S.typhimurium were of common occurrance. Makholia & Singh (1963) isolated a new serotype S.brindavan from apparently healthy pigs. Datta & Singh (1964) reported the isolation of S.gokul from pigs.

Krishnamurthy & Kaushik (1964) reported some outbreaks of pigs with S.choleraesuis var kunzendrof from some parts of India. Jayaraman et al. (1964) reported an outbreak of Salmonellosis due to S.weltevreden in guinea pigs. Jayaraman & John (1969) suggested that S.weltevreden has got a wide range of host specificity. Nath et al. (1970) gave an account of the Salmonella serotypes identified from pigs during the period of 1965 to 1969 at National Salmonella & Escherchia Centre Kausauli. The most common serotype identified were S. anatum S.choleraesuis, S.dublin, S.enteritidis, S.kentucky, S.nassenve, S.newport, S.paratyphi B, S.stanely, S.virginia and S.weltevreden.

Goel & Malik (1971) reported the incidence of Salmonella in pigs in Uttar Pradesh. The serotypes isolated in the order of prevalance were S.stanely, S.choleraesuis var Kunzendrof and S.enteritidis. Bhatia & Pathak (1971) from U.P. isolated S.choleraesuis var kunzendrof, S.colombo, S.paratyphi B and S.hyttingfossa from pigs with diarrhoea and fever and S.ohio from apparently healthy pigs. Sulochana et al.(1973) isolated

S.weltevreden from Mannuthy, Kerala from two ailing pigs with the history of gastroenteritis. Experimental infection proved that pigs were refractory. Sashdhar (1974) isolated S.typhimurium var copenhagen, S.virchow, S.choleraesuis var kunzendrof, S.dublin, S.enteritidis and Salmonella D group with rough flagellar antigens from pigs in Bangalore.

Bacteriology

In Bergey's Manual (Breed et al., 1957) the genus Salmonella is described as possessing the following characteristics: Rods which are usually motile by means of peritrichous flagella, although non-motile forms may occur. Gram-negative, Gelatin not liquefied, indole not produced. Hydrogen sulphide production is variable. Acid is produced from glucose, mannitol, maltose and sorbitol. Gas production is usually observed. Lactose, sucrose salicin and adonitol are not attacked. The fermentation of other carbohydrates is variable. Acetylmethylcarbinol is not produced. Methyl red test is positive. Nitrites are produced from nitrates. Ammonium citrate is usually utilised. Urea not hydrolysed. KCN sensitivity is negative.

Pathogenisis

The disease caused by Salmonella species is in the form of enteric fever, pyemia and gastro enteritis. In gastroenteritis the infection is more or less localised in the intestinal wall (Wilson & Miles, 1964). Kastube &

Tanaka (1973) suggested that the organisms propagate slightly between the middle part of the large intestine. The caecum was found to be the most important site for Salmonella in swine under experimental conditions (Smith & Jones, 1967). The pathogenesis of Salmonella depends upon the penetration of the epithelium of gastrointestinal tracts and multiplication in the mucosa leading to systemic invasion (Takeuchi, 1971). The organism multiplies in the phagocytes principally in macrophages of the reticuloendothelial system (Wilson & Miles, 1964; Takeuchi, 1967). The most likely anatomical site of penetration is the intestine but the pharynx and tonsils are possible sites (Lawson & Dow, 1966; Smith & Jones, 1967; Barnes & Bergeland, 1968).

After penetration the organisms multiplies in the lymphoid follicles of the intestine and mesentric lymphodes. From these sites they are spilled into the thoracic duct and then into the general circulation. (Guinea et al., 1968). The fibriae increase bacterial adhesiveness to cell which would contribute to virulence of the organism (Darekar & Dugid, 1972).

The ecologic interaction between bacteria within the pigs intestine influence the population of Salmonella (Nielson, 1968). Bohnhoof et al. (1964) found that the bacteria antagonistic to Salmonella in intestinal tracts of mice were Bacteriodes species, which produces sufficient

acetic and butyric acid to inhibit the growth of Salmonella. Calves et al. (1973) demonstrated the retardation of growth of Candida albicans in broth in presence of actively growing Salmonella enteritidis and also in mice injected with the organism simultaneously. Koltan (1956); Davenport et al. (1964) suggested the production of paratyphus in pigs due to activation of the intestinal pathogens by Oesophagostomum larvae. Beh (1971) reported the occurrence of E.coli and S.typhimurium in two out breaks of haemorrhagic enteritis in weaned pigs.

Clinical signs

The clinical signs in field cases are acute diarrhoea and rapid death. Majority of cases occur in pigs aged twelve to thirteen weeks and has got a seasonal occurrence. Clinical signs includes reduced feed intake, fever and discoluration of the extremeties. Diarrhoea was not a consistent feature and was found approximately in 40% of outbreaks. (Rockburgh et al., 1964; Lawson & Dow, 1966). In some cases of Salmonella typhisuis infection massive enlargement of cervical region were noticed (Branes & Bergeland, 1968). The important clinical signs observed in experimental Salmonellosis with S.choleraesuis were listlessness, recumbency, vomition, anorexia and bluish discolouration of the abdomen. Diarrhoea may be noticed on third or fourth day after infection and persisted for 12-14 days. Increase in body temperature was noted seven

days after infection ranging from 105-107°F. After five days the temperature declines. Several days after loss of body weight about 7-20% were noticed (Smith & Jones, 1967).

Pathological lesions

Grossly the carcass emaciated dry and reddened. In some animals the surface of the liver shows white foci. Spleen and regional lymphnodes were enlarged. Extensive areas of reddening, inflammation of the small intestine particularly terminal ileum and in some cases mucous membrane of the cloon were necrotic. Caecum and colon inflammed and walls thickened and mucous membrane necrotic. The mesentric lymphnodes were oedematous and often haemorrhagic (Smith & Jones, 1967). In some cases fibrocaseous cervical lymph adnitis and partoid sialoadenitis were observed (Barner & Bergeland, 1968; Singh, 1968).

In experimental cases irregular fibropurlent membranes were noticed in crainal end of caecum. In some cases shallow ulcers were noticed over the entire surface of the mucosa. Greater curvature of the stomach shows congestion on the diaphramatic surface. In some cases congestion of the kidney and spleen were observed. Marbling of the mesentric lymphnode and haemorrage in the gastrointestinal tract noticed in few cases (Lawson & Dow 1966; Sofrenovic and Jovanovic, 1968).

Antibiotic sensitivity

Edward & Ewing (1972) reviewed the subject on drug

resistance in Salmonella. In S.typhimurium several antibiotics either to individual antibiotics or more frequently for a combination of resistance determinants. R-factor determines the resistance to ampicillin, streptomycin, tetracyclines, and sulphonamides. Rfactor transfer from E.coli to Salmonella has been demonstrated (Anderson & Lewis, 1965).

Rfactor occur only in gram-negative bacteria and these episomal factors are responsible for development of multiple resistance of micro organisms to antimicrobial agents. Transfer of R-factor takes place primarily through conjugation. Physical transfer of R-factor material from cell to cell apparently takes place by means of sexual fimbriae, the presence of which is genitically controlled. The R-factor consists of RNA and resumble other bacterial episomes and plasmids. They are of two kinds or consists of two parts, the resistance transfer factor (R.T.F.) and the genetic determinants for drug resistance. The transfer of genitic material is responsible for resistance mediated by RTF (Anderson, 1968). When a drug is introduced into a hetrogenous population of gramnegative bacteria; selective pressure (called antibiotic pressure) is exerted upon the population. This pressure facilitate the development of multiple resistant strains of bacteria, which then may become predominent. This occurs in animals under analogous circumstances, as shown by Guinea (1965) and Anderson (1968).

Forhishnyi & Schmidov (1967) observed that the organisms isolated from pigs treated with tetracycline, chloramphenacol and streptomycin exhibits the following resistance pattern. The isolates resistant to chlor and oxy tetracycline and streptomycin with unchanged sensitivity to chloramphenacol. The strain with induced resistance to streptomycin remained susceptible to chlor and oxytetracycline and chloromycetin. Bugae (1969) studied the antibiotic sensitivity of S.choleraesuis var kunzendrof, S.paratyphi, S.typhimurium, S.bovismorbificans, S.heidelberg and S.enteritidis isolated from pigs. The results observed were the following. All are sensitive to polymyxin, neomycin, chloramphenicol, furazolidone and resistant to pencillin, streptomycin, tetracycline and erythromycin.

Bulling and Stephen (1972) experimentally infected pigs with chloramphenicol or tetracycline sensitive strains of S.typhimurium and S.choleraesuis suggested that the resistance is controlled by Rfactor and transfer of Rfactor from resistant coliform population to sensitive Salmonella occurs invivo. Ulsen (1972) reported that the sensitivity of the isolates of Salmonella to streptomycin tetracycline, furazolidone, chloramphenicol, neomycin, Kanamycin and ampicillin varied considerably.

MATERIALS AND METHODS

MATERIAL AND METHODS

Materials examined in this study were collected from pigs of the University Pig Breeding Farm, Mannuthy. Rectal swabs were collected from piglets under 8 weeks of age, suffering from diarrhoea with temperature reaction. Intestinal contents, pieces of ileum, jejunum, colon, mesentric lymphnodes, liver, spleen, stomach and bile were collected aseptically from piglets which have died of gastroenteritis. A total of 274 specimens were examined. Details of specimens collected for isolation of Salmonella and E.coli are shown in Table 1.

Rectal swabs and faecal material

Rectal swabs and faecal materials were inoculated directly into 5% sheep blood agar, MacConkey lactose bile agar (M.L.B.A.), Dihydrostreptomycin broth (D.H.S.) and Dihydrostreptomycin agar (Ramirez & McCleskey, 1968) (Appendix Ia & b) and incubated at 37°C. Subcultures were made from D.H.S. broth after 18-24 hrs. of inoculation to blood agar and DHS agar in order to isolate enteropathogens in pure cultures.

Tissues

The tissues were flamed following immersion in methyl alcohol in order to minimise surface contamination. The tissues were then emulsified in sterile mortar and

pestle with normal saline approximately making 10% suspension. A loop full of emulsified material was inoculated on MLBA, DHS agar and 5% sheep blood agar. The lactose fermenting colonies on MLBA, DHS agar and haemolytic colonies on blood agar were identified as E.coli on the basis of their biochemical reactions produced in appropriate media as described by Edward and Ewing (1972). The details of the biochemical reactions are shown in Table 2.

Attempts for the isolation of Salmonella organisms were made in the lines described by Edward & Ewing (1972). The materials (faecal materials, rectal swabs and tissues) were inoculated directly as well as through enrichment media. A loop full of the emulsified material was inoculated on Brilliant Green Agar (B.G.A.) and MacConkeys agar (with Brilliant green in the ratio 1:10,000). The plates were examined after 24-72 hours of incubation at 37°C. Sodium citrate was incorporated in B.G.A. in the ratio of 1% to supress swaming proteus.

The enrichment media used were tetrathionate broth and selenite broth. The specimens processed were inoculated in the ratio of 2 grams (2ml. of bile) to 10 ml. quantities of enrichment media. These inoculated media were then incubated at 37°C for 24-48 hours and subcultures were made on B.G.A., MacConkey's agar and modified MacConkey's medium as advocated by Sharma (1973). (Appendix IIa).

Pink colonies on B.G.A., non-lactose fermenting colonies on MLBA and colourless colonies on modified medium with hydrogen sulphide production were suspected to be Salmonella. To eliminate Proteus organisms, suspected colonies were inoculated into modified MacConkey's agar as advocated by Sharma (1961). (Appendix IIb). Mannitol was used instead of lactose in this medium.

For preliminary identification of Salmonella, suspected colonies were inoculated into triple sugar iron agar simultaneously with composite medium I & II devised by Chitin et al., (1972). (Appendix IIIa). Part I was used for detection of glucose and lactose fermentation, hydrogen sulphide production and phenylpyruvic acid and part II for fermentation of mannitol, sucrose, motility and indol production. The cultures exhibiting typical reactions were further tested for biochemical reactions for differentiation of the species of Salmonella from other members of Enterobacteriaceae as described by Edward and Ewing (1972). Details of the biochemical reactions are described in Table 3.

The cultures tentatively identified as E.coli were further tested for production of haemolysin, necrotoxin and pathogenicity as detailed below.

The cultures identified as Salmonella on the basis of their cultural and biochemical reactions were sent for serological typing to Dr. Sharma, Haryana Agricultural

University, Hissar. Two strains were tested for their pathogenecity for laboratory animals as well as to the primary hosts.

Pathogenicity tests

E.coli

Haemolysin production:- The E.coli strains which produce haemolysis on 5% sheep blood agar were further subjected for haemolysin production on solid as well as liquid media. The E.coli strains were inoculated into sheep erythrocyte blood agar and incubated at 37°C for 18 hours, one plate aerobically and one under 10% carbondioxide tension. Haemolysin production was also tested on liquid media containing 1% sheep erythrocytes as described by Cooke (1968) and examined after 6-48 hours.

Mice

25 strains which were capable of growing on D.H.S. agar or haemolytic on 5% sheep blood agar were tested for pathogenicity to mice. White swiss mice aged 1-1½ months were used for the study. Two mice were used for each strain. E.coli grown on nutrient agar containing 1% glucose was emulsified in normal saline and the opacity was adjusted between No.5 and No.6 of the Brown's opacity tubes. This contained approximately 10^3 micro organisms per ml. and 0.2 ml. of the emulsion was given intra peritoneally. The control mice were given 0.2 ml. of normal saline. The injected mice were

observed for 96 hours for evidence of infection. The mice which died at various intervals were examined for pathological changes in the internal organs. Materials collected from them were cultured in MacConkey's lactose agar.

Necrotoxin production

Necrotoxin production was tested by the method described by Cooke (1968). The strain pathogenic for mice or producing haemolysins were studied for their ability to produce necrotoxin. The fur on the back of rabbits ~~were~~ was removed with clippers. ~~Shaved~~ The area was shaved and marked into 2.5 ~~x 2.5~~ cm. squares. 0.1 ml. of 5 hour old peptone water culture of the organism was given intradermally. The skin on the inoculated area was examined daily for 5 days, for the possible development of ulceration (Table 5).

Rabbit ligated gut loop reaction

The strains producing haemolysin, necrotoxin or those that were pathogenic for mice were subjected for dilatation reaction in rabbit ileal loop. Fifteen strains were tested. The dilatation reaction in ligated rabbit intestinal loops was tested using the method described by Cooke (1968). The animals were anaesthetised with ether. After induction of anaesthesia the ileum was ligated at regular intervals so as to form segments of about 7.5 cm. in length. Injections of 1 ml. of 18 hour old peptone water culture of E.coli strains containing approximately 10^6 organisms per ml. were made into alternate

segments. The segments adjacent to the test segment were considered as control and were given 1 ml. of normal saline. After 24 hours the animals were killed. The abdomen was opened immediately and the small intestine was examined for the presence or absence of dilatation of the segments. The volume and character of the fluid contents were also recorded. The segments were removed and placed in 4% formaldehyde saline for histologic study. The details of the experiments are summarised in Table 5.

Based on the tests described above five cultures were identified as enteropathogens. They were sent to National Salmonella and Escheriehia Centre, Kassuli, Punjab for serotyping.

Pathogenicity studies

Salmonella

Saline suspension of organisms from 18 hr. old nutrient agar culture plates with 1% glucose were used for the pathogenicity study. The density of the culture suspension was adjusted between 5 & 6 of the Brown's opacity tube so as to contain 10^3 micro organisms per ml.

Mice

Swiss albino mice aged 1-1½ months were infected intra peritonially with 0.2 ml. of saline suspension of S.typhimurium and S.weltivreden. Two animals were used for each strain tested. All the animals were observed

for evidence of illness for 96 hours. The mice which died at various intervals were examined in detail for pathological changes. The materials collected from them were cultured in various enrichment and selective media for detection of Salmonella organisms. The details of the experiments are given in Table 6.

Guinea pigs

Four guinea pigs in groups of two were used. One group was infected with S.typhimurium var copenhagen and the other group with S.weltevreden. One ml. of the saline suspension of the cultures was administered subcutaneously and 0.5 ml. intraperitoneally. The animals were watched for 96 hours. The guinea pigs which succumbed to infection within 96 hours of inoculation were examined in detail for pathological changes. The materials collected were processed for recovery of organisms. The details of the experiments are given in Table 6.

Rabbits

One ml. of a saline suspension prepared as described above was introduced subcutaneously into rabbits. Two animals were used for each strain. All the animals infected were examined for 96 hours. The animals which died at various stages of observation were examined for pathological changes in the internal organs. The materials collected from various internal organs and intestinal contents were processed in

suitable media for recovery of the organisms. The details are tabulated in Table 6.

Piglets

Healthy piglets aged 4-6 weeks were used for the experiment. The piglets were divided into 3 groups, each group consisting of 4 numbers. They were kept under close observation for one week before experimental infection. Rectal temperature was recorded and rectal swabs were examined to confirm that they were free from Salmonella infection. At the end of the observation period, the ration for the experimental animals was cut down to one third, the quantity of the normal ration in order to induce stress. The experiments were conducted on similar lines described by Smith & Jones (1967). Three piglets in each group were infected orally while the fourth one in each group was kept as controls. The dosage employed was 30 ml. of 18 hour broth culture containing approximately 10^6 to 10^8 organisms per ml. The first group was given S.typhimurium var Copenhagen, second group S.weltevreden and the third group a mixture of S.typhimurium var Copenhagen and S.weltevreden. A rubber tube was used for oral dosing so as to ensure deposition of the inoculam on the back of the tongue. The animals were closely observed for any clinical signs of illness and the symptoms exhibited were recorded during the experimental period. Rectal temperature was recorded in the

morning and evening till they were sacrificed. Rectal swabs were taken daily and examined for the presence of Salmonella organisms. In order to detect septicaemic stage of infection, blood collected from anterior venacava was inoculated daily for 5 days into dextrose broth for evidence of bacterial growth. The animals were sacrificed at 5, 10 and 15 days after oral feeding.

Pieces of tissues from stomach, intestine, caecum and proximal and middle sections of colon, mesenteric lymphnodes, liver, kidney, spleen, lung and intestinal contents were collected under aseptic conditions and were cultured to detect the presence of Salmonella organisms.

Antibiotic sensitivity

Preparation of inoculum

Saline suspensions were made from 18 hour old nutrient agar culture plates. The density of the culture suspension was adjusted so as to get approximately 10^3 micro-organisms per ml. This suspension was used for drug sensitivity test.

Disc diffusion test

Five E.coli strains and 15 Salmonella strains were tested against the antibiotics. The organisms isolated both E.coli and Salmonella were tested for their sensitivity against the following common therapeutic agents available in the market. Pencillin, Streptomycin, Tetracycline,

Erythromycin, Ampicillin, Gentamycin and Chloramphenicol were tested.

Discs of 6 m.m. diameter were punched from Whatman No.1 filter paper and sterilised at 140°C for one hour in lots of 100, in petri dishes. Standard suspensions of antibiotics were prepared. The antibiotic discs were prepared as per the technique described by Cruickshank (1965) and were stored at 4°C until use.

Nutrient agar plates were inoculated with the culture suspension by flooding the surface and then removing the excess. The plates were then allowed to dry in the inverted position in the incubator at 37°C for 30 minutes. The discs containing different antibiotics mentioned above with appropriate concentration were placed on the medium suitably spaced and the plates were incubated at 37°C overnight. The sensitivity to antibiotics were assessed by the zone of inhibition around the discs. The diameter of the zone of inhibition was recorded and interpreted as described by Petersdrof & Sherris (1965).(Appendix IV).

RESULTS

RESULTS

The 274 samples examined during this study included specimens collected from both living as well as from dead animals. All the sick animals examined had a history of altered temperature reactions varying from 104°-106°F in the initial stages followed by diarrhoea, stunted growth and unthriftiness. The carcass of the dead piglets were emaciated and in few instances fluid was noticed in the thoracic and abdominal cavities. Some of them showed greyish leathery necrotic ulcers on the mucosa of the colon with adhered fibroid purulent material. In all cases mesenteric lymphnodes showed oedema or haemorrhage.

Isolation

E.coli

A total of 75 strains of E.coli were isolated during this investigation, out of which 50 strains were from rectal swabs of living animals suffering from enteritis and the rest from small intestine, faeces and large intestine of dead piglets. None of the strains were isolated from heart blood, liver, lung, bile and spleen (Table 1). Five strains were considered as pathogenic based on haemolysin, necrotoxin and enterotoxin production tests and by pathogenicity to mice. It is also interesting to note that all these five isolations were made from piglets aged

3-8 weeks. Serological identification of the five pathogenic strains has revealed that they belong to three sero groups: 05, 039 and 017. All of them were recovered from the rectal swabs collected from animals showing enteric disorders. It is also worthwhile to mention that dihydrostreptomycin broth and dihydrostreptomycin agar were found to be very useful for primary isolation of the strains mentioned above from faecal materials, although many nonpathogenic strains failed to come up in these two media. The results of the biochemical reactions of the 25 strains which were either haemolytic, pathogenic for mice or capable of growing on D.H.S. agar are detailed in Table 2.

Haemolysin production

Of the 75 strains isolated, 15 strains were found haemolytic on 5% sheep blood agar. Further testing of these strains for their capacity to produce haemolysins on solid as well as liquid media, also confirmed their haemolytic property. These cultures incubated at 10% carbon-dioxide tension were found to produce more wider zone and degree of haemolysis than when incubated at 37°C. Of the five strains considered to be pathogenic, four were found to be haemolytic.

Pathogenicity to mice

Out of the 25 strains tested for their pathogenicity

in mice only 5 strains were found to be pathogenic, producing death within 96 hrs. following inoculation. However the three antigenic types (05, 017 and 039) identified by National Salmonella and Escherichia centre were found to be fatal to mice. The strain E6/74 (05) and E15/75 (05) killed mice within 24 hrs. after intraperitoneal injection. The cultures E7/74 (017) and E3/74 induced death within 48 hrs. and the culture E10/74 (036) killed mice within 96 hrs. The rest of the strains tested were found to be nonpathogenic for mice. Gross lesions were absent in the internal organs of the dead animals although reisolation of E.coli was made on all occasions from tissues. Pure cultures of the organisms could be isolated from heartblood, liver, spleen and lungs even in the absence of any apparent lesions.

Necrotoxin production

The strains of E.coli that produced haemolysis on 5% sheep blood agar and pathogenic for mice were found to produce lesions in the skin of rabbits tested. Ulcers started developing at the site of inoculation 5 days following intradermal injection of the cultures. They were about 2 to 2.5 cm. (Fig.1) in diameter with moist surface and occasionally fluid was oozing from these ulcers. They persisted for a period of 4-6 days and there was no tendency for extension to neighbouring area. They were completely healed up within

a period of two weeks with scars left at the site of injection. There was no thermal reaction in any of the animals throughout this experimental period. The results are summarised in Table 5.

Rabbit ileal loop reaction

The strains producing either haemolysin or necrotoxin and pathogenic for mice were tested for enterotoxin production. Of the 15 strains tested only one culture (E7/74) produced dilatation reaction on rabbit intestinal loop 24 hrs after the injection of the culture (Fig 2). About 15 ml. of fluid was noticed in loops injected with culture E7/74 which belonged to sero group 017. The fluid produced was haemorrhagic in nature and contained shreds of fibrin. However no gross lesions could be observed on the mucosa of the loops. Control loops and the loops infected with other strains did not ~~show~~ show any dilatation or accumulation of fluid.

Histopathological examination of the segments which showed positive results for dilatation showed the following changes. There was destruction of the columnar epithelial cells and oedema of the submucosa. Most of the glandular structures were intact. But in certain areas destruction and attempted hyperplasia of glandular epithelium were noticed. Desquamation of epithelium and few inflammatory cells predominantly mononuclear and polymorphonuclear types were observed. (Fig 3)

In Gram-stained sections bacilli resembling E.coli could be observed in large numbers in the lumen. They were found only in the lumen and none of them were seen in tissue spaces. Segments which did not show dilatation reaction revealed changes similar to that of a toxic reaction.

Sensitivity studies

Sensitivity studies conducted on 5 strains of E.coli presumably to be pathogenic showed the following pattern of reaction. All the strains were sensitive to chloramphenicol and gentamycin at a concentration of 30 µg. and 10 µg. per disc respectively. Four were found to be sensitive to nitrofurantoin and 3 for Erythromycin. Two strains showed resistance to streptomycin and three for tetracycline. The results of the sensitivity tests were communicated to the Superintendent, Pig Breeding Farm for adopting suitable remedial measures. The results of the drug sensitivity conducted during the course of this work are summarised in Table 8.

Salmonella

A total of 24 strains of Salmonella were isolated from 274 specimens (Table 1) showing incidence in 8.7% of the samples examined. These isolates were identified as Salmonella based on their morphological, cultural and biochemical characters. The source of isolation, symptoms or lesions exhibited and the results of the antigenic

typing are detailed in Table 4.

Out of the 24 strains isolated 18 were identified as Salmonella weltevreden and 6 as S.typhimurium var Copenhagen. The identity of the isolates were confirmed serologically by Dr. V. K. Sharma of Haryana Agricultural University. The isolates produced colourless lactose negative colonies with a blackish centre on modified medium (Sharma, 1973)(Fig.) due to the production of iron sulphide. On MacConkey's medium containing mannitol instead of lactose the organisms formed pink colonies due to fermentation of mannitol. The composite medium was found equally good as Triple sugar iron agar with production of acid butt, alkaline slant and blackening of the slant as a result of Hydrogen sulphide production. The composite medium II also gave good results for fermentation of mannitol forming a blue ring at the top and also 'fan' shaped growth as a result of motility.

Pathogenicity

Mice

The mice which were given S.typhimurium var copenhagen (3/74 and 13/74) succumbed to infection at periods varying from 24-72 hrs. The mice that died before 48 hours of infection revealed no apparent lesion in the visceral organs. However one animal which died after 48 hrs showed scattered pneumonic patches on both lungs and areas of necrosis in the liver. The organisms could be recovered from heart blood,

spleen, liver and lungs of the animals ~~those~~ that died at various stages of infection.

The mice which were infected with S.weltevreden (1/74 & 25/74) died at periods varying from 48-96 hrs. No gross lesions could be observed in the internal organs in any of the animals infected. However organisms could be isolated from liver, spleen, heart blood and lungs. The details of the experiments and their results are summarised in Table 6.

Guinea pigs

S.typhimurium var copenhagen (3/74) killed the guinea pigs varying from 48-96 hrs after intraperitoneal and subcutaneous infections. Acute peritonitis was noticed in the guinea pig injected by intraperitoneal route. The other animal which was infected subcutaneously showed patchy congestion of lungs and small white necrotic foci on the surface of spleen along with congestion. The organisms could be recovered from heart blood, lungs, liver and spleen from both animals.

Cultures of S.weltevreden killed the guinea pig within 48 hrs and 96 hrs after intraperitoneal and subcutaneous infection. The animal that died on 48 hrs of intraperitoneal infection showed acute peritonitis and enlargement of spleen. The other that died after 48 hrs showed enlargement of spleen, liver and mesentric lymphnodes.

Lung showed pneumonic areas. The organisms could be isolated in pure cultures from heart blood, spleen, Mesenteric lymph nodes and lungs from the animal inoculated by i/p route and from liver, spleen, lungs and mesenteric lymph nodes from the animal injected by s/c route.

Rabbits

All the four rabbits injected by S.typhimurium var copenhagen and S.weltevreden succumbed to infection within a period of 48 hours. Pulmonary oedema and enlargement of spleen were noticed in one of the animals from each group. None of the animals showed enteric disorders. The organisms were isolated in pure cultures from the heart blood and lungs from all the inoculated animals. The results are summarised in Table 6.

Piglets

There was no temperature reaction in any of the animals before the experimental inoculation. Examination of the faecal samples also proved to be negative for salmonella. Within 1-2days after inoculation all the piglings appeared ill. Appetite of the animals was poor but intake of water was found increased. By the second day after infection temperature of the animals was elevated and varied from 104.6-106.8°F. There was no incidence of any enteric disorders for a period of 3 days. However cultural examination of blood collected at various stages

of thermal reaction proved to be negative for salmonella. Diarrhoea started on the 3rd or 4th day following infection and persisted for the rest of the observation period. With the commencement of diarrhoea temperature became normal. Cultural examination of faeces from all the inoculated animals proved to be positive for Salmonella from third day of inoculation.

Group I (infected with Salmonella typhimurium var copenhagen)

The animals sacrificed on the 5th day of infection showed congestion of the mucosa of the terminal portion of the ileum and of the colon. The mesenteric lymphnodes, especially at the portion of the ileum and caecum, were greatly enlarged and oedematous.

The animal sacrificed on the 10th day after infection showed reddening of the skin on the abdomen and dependent parts. Carcass was emaciated. Congestion of mucosa at the greater curvature of the stomach was noticed. Catarrhal enteritis and petechiae in the mucous membrane of ileum, jejunum and colon were observed. The lungs were diffusely congested. The trachea and bronchi contained blood tinged frothy fluid.

Animals killed on the 15th day of observation showed the following changes. The skin on the inner part of the thigh showed necrosis. Haemorrhagic streaks were observed on the mucosa of the ileum. Thickening of the walls of the ileum was noticed as a result of proliferation

of lymphoid tissues. The mucous surface of the colon showed small foci of necrosis. The mesentric lymphnodes were greatly enlarged.

Group II (Infected with S.weltevreden)

Piglets sacrificed after the fifth day of experimental infection showed no gross lesions on the gastrointestinal tract, liver, spleen and lungs. Only the mesentric lymphnodes were slightly enlarged. Organisms could be recovered from mesentric lymphnodes and ileum.

The animal sacrificed after the 10th day of infection showed congestion of the ileum and colon and enlargement of mesentric lymphnodes. No other gross lesions could be observed on the visceral organs. Organisms could be recovered from faeces, ileum and mesentric lymphnodes.

Animal killed on the 15th day of observation showed cyanosis of the skin, catarrhal enteritis, hyperemia and haemorrhage of the jejunum and ileum. The wall of the ileum was thickened with proliferation of lymphoid tissues. Mesentric lymphnodes were enlarged and haemorrhagic. No gross lesions could be observed on liver, spleen and lungs. Organisms could be isolated from faeces, jejunum, ileum, mesentric lymphnodes and spleen.

Group III (Infected with S.typhimurium var copenhagen & S.weltevreden)

In the third group the animal sacrificed on the

5th day of infection revealed mild congestion of the mucosa of the ileum and colon and enlargement of the mesentric lymph-nodes.

One animal that died on the 9th day of inoculation with mixed culture of Salmonella typhimurium var copenhagen and Salmonella weltevreden showed the following changes. The fundic portion of the stomach showed severe diffuse congestion. The jejunum and ileum had yellowish fluid contents. Streaks of haemorrhage were noticed on the mucosa of ileum, jejunum and colon. The liver showed congestion. The bronchial lymphnodes were greatly enlarged. Organisms could be recovered from bronchial lymphnodes, liver, bile, mesentric lymphnodes, ileum and colon.

The animal sacrificed on the 15th day of infection in group III showed the following changes. The carcass was emaciated. The entire mucosa of the colon was studded with greyish white nodules with erosions (Fig5). Terminal portion of the ileum was haemorrhagic and thickened. The mesentric lymphnodes were enlarged and oedematous. Salmonella organisms could be recovered from faeces, mesentric lymphnodes, colon, ileum and bile.

Antibiotic sensitivity

Antibiotic sensitivity studies have revealed that all the 15 strains tested were sensitive to chloramphenicol at the concentration of 30 µg/disc. 11 of them were

sensitive to gentamycin and streptomycin at the concentration of 10 and 20 µg. per disc respectively. Only two strains were found sensitive to tetracycline and nitrofurantoin (Fig6). All the strains were found resistant to Pencillin, ampicillin and erythromycin. The results of the sensitivity studies at various concentrations of different antibiotics are summarised in Table 8.

DISCUSSION

DISCUSSION

A number of workers have reported the association of certain serotypes of E.coli and some species of Salmonella with gastrointestinal troubles in pigs (Heard and Linton, 1965.; Brane and Bergeland, 1968.; Nath et al., 1970; Goel & Mallik, 1971). In the present study 75 strains of E.coli and 24 strains of Salmonella were isolated and identified. However only 5 strains of E.coli were found to be pathogenic as evidenced by various tests. It is interesting to note that these five strains were isolated from the faecal materials of piglings aged 3-8 weeks and suffering from gastroenteritis. This evidently shows that gastroenteritis due to E.coli is more common in piglings from three weeks to weaning period. Stevens (1963) in a detailed study of enteritis in pigs also observed similar findings. He found that three age groups are more prone to E.coli infection. 1) Neonatal pigs aged 1-4 days old 2) three weeks old and 3) weanling piglets. He also suggested that the three forms of enteritis are not commonly observed in one farm. The piglets affected previously show some resistance to subsequent infection and hence it may be suggested that the piglets which fail to contact infection at three weeks of age may get infected at weaning. None of the animals examined

during the course of this investigation have revealed E.coli in heartblood or other internal organs. The failure of isolation of enteropathogenic E.coli from heartblood or other viseral organs of piglets died of gastroenteritis suggests the absence of septicaemic form of the disease. Dunne & Bennet (1970) reported that colibacillosis is manifested in three distinctive clinical entities, septicaemia, diarrhoea and oedema disease. All the three forms can commonly occur in preweaning pigs and the oedema disease is observed more commonly in pigs 8-16 weeks of age. In neonatal pigs infection occurs shortly after birth and may die of bacteraemia. Stevens (1963a & 1963b) put forward the following theory for the occurance of colibacillosis. In weanling pigs ingestion of potentially pathogenic serotype changes the balance between organism and immunity. Change in food and environment in weaning facilitate the rapid multiplication of the pathogenic serotypes and diarrhoea or death results from toxic action of E.coli. Initial sensitization followed by further contact with the same strain of E.coli develops hypersensitive stage resulting in oedema disease or enteritis. The hypersensitivity theory for the development of colibacillosis has been supported by various research workers like Kashiwazaki et al. (1969) and Dunne & Bennet (1970). Serological identification of the pathogenic strains isolated in this study have revealed that two out of the five strains belonged

to sero group 05. Sero group 05a ~~and 05a~~, 5c: K?:H19 have been reported to be the cause of oedema disease in pigs by Gregory (1957) and Ewing et al. (1958). This sero group has also been reported from the internal organs of dead piglets showing lesions of acute gastroenteritis. (Parnas et al., 1950), mesenteric lymphnodes (Namioka, 1956) and also from healthy pigs. But the results obtained in this study were contradictory to Parnas et al. (1950) in that no organism could be isolated from internal organs of piglets died of gastroenteritis.

Haemolytic reactions studied on solid as well as liquid media were closely comparable to the findings of Cooke (1969) and Pandae et al. (1974). Four out of the five pathogenic strains studied in this investigation were found to produce haemolysin. The haemolysis on sheep erythrocyte agar at 10% carbondioxide tension produced wider zone of haemolysis than those incubated aerobically at 37°C. William Smith (1963) in a more detailed study have observed that about 63% of the strains isolated from pigs produced haemolysis on 5% ox blood agar. The occurrence of haemolytic E.coli as the etiologic agent of gastroenteritis in pigs have also been reported by several other workers (Sojka et al., 1965; Ahuja and Khera, 1971; Goel & Mallick, 1973).

Pathogenicity tests conducted on mice have shown that five strains were pathogenic to this species,

death occuring in 24-96 hrs. Although organisms could be recovered from dead animals, no apparent lesions could be observed in any of the animals artificially injected. These findings were in agreement with Harris (1901); Fidesjostede (1946) and Gupta and Singh (1971) who likewise found that E.coli strains isolated from pathological conditions to be for more toxic than normal intestinal strains. Jacks and Glentz (1967) observed that E.coli strains isolated from nonenteric systemic sources were more pathogenic to mice.

The results of the reaction for necrotoxin production of the strains which were haemolytic and pathogenic for mice were inagreement with the findings of Cooke (1969) and Pandae (1974). The strains of E.coli that produced haemolysis on 5% sheep blood agar and pathogenic for mice were found to produce lesions in the skin of rabbits tested. The lesions produced were typical and were similar to the lesions described by other workers. Ewerstsen (1947) and Kauffman (1954) found that 26 of the 160 strains of E.coli isolated from various kinds of material haemolysed horse erythrocyte and 19 of the 26 caused necrosis, when injected into the skin of rabbits. Thus capacity for haemolysis appeared to be associated with the ability to cause necrosis, and with toxicity. Pandae et al. (1974) reported that the strain possessing the property of producing haemolysin and necrotoxin are definite pathogens whereas a strain possessing either of these two properties are potential pathogens.

Experiments on rabbit ileal loop have shown that only one of the strain tested have produced dialatation reaction although five were found to be pathogenic for mice and positive for haemolysin and necrotoxin production. The failure of producing dialatation reaction in 7 others may be due to their inability to produce enterotoxin in rabbits. Smith and Halls (1967) have suggested that rabbits are less reliable for the ligated loop tests. The test is of great value in its application to the animals of the same species from which the test organisms were isolated. Moon et al. (1970) have also observed that the rabbit ileum is more sensitive to the action of enterotoxin where as the living organisms need not consistently produce positive reactions. It has also been found enterotoxin production is not true with all enteropathogenic strains of E.coli (Salazaki et al., 1967).

Histopathological studies of the segment which showed positive reactions revealed that the changes were similar to that of severe type II reactions described by Cooke (1968). The severe type II reactions were characterised by exudate of polymorphomuclear cells with some erythrocytes and debris. The columnar cells were eroded and submucosa was congested and oedematous. Segments which showed negative reactions had histopathological changes similar to those of toxic reactions. This may be due

to excessive absorption of endotoxins as a result of the multiplication of the enteropathogens and hence the changes may be similar to those obtained in hypersensitivity reactions. Stevens (1963) suggested that the absorption of relatively larger quantities of bacterial polysaccharides from the intestine resulting from sudden multiplication of the E.coli may leads to an anaphylactic shock. Serological typing of the isolates have revealed that the strains belonged to O groups 05, 039 and 017. The occurrence of 05 serotype gave further evidence for the presence of hypersensitivity reactions.

Antibiotic sensitivity studies have revealed that all the strains were sensitive to chloramphenicol and gentamycin. Four were found to be sensitive to nitrofurantoin and 3 for erythromycin. Two strains showed resistance to streptomycin and three for tetracycline. All the strains were resistant to penicillin and ampicillin. Resistance of certain strains of E.coli isolated from faeces of pigs to streptomycin, tetracycline have been observed by other workers (Roberts & Valley, 1969; Willinger & Genis, 1969; and Yadev and Gupta, 1971). Terkado et al. (1972) found that high proportions of strains of E.coli isolated from faeces were resistant to antibiotics like streptomycin, tetracycline, chloramphenicol, sulphadimethoxine, kanamycin and benzylpenicillin. However in this study all the strains tested were found to be sensitive to chloramphenicol.

Application of Dihydrostreptomycin broth and Dihydrostreptomycin agar for isolation of pathogenic strains was found very useful during this study. All the pathogenic strains were isolated through D.H.S. broth and D.H.S. agar although many nonpathogenic strains were inhibited by these media. These findings were in agreement with the observation made by Ramirez & MacClesky (1968). Arbuckle (1968) also reported that inclusion of certain antibiotics in the enrichment media permitted the detection of small number of pathogenic E.coli organisms.

The results of the biochemical reactions of 25 strains tested were in complete agreement with those given by Edward and Ewing (1972).

Salmonella

From 274 specimens examined, 24 strains of Salmonella were isolated showing the incidence of Salmonellosis in 8.7% of samples examined. Of the 24 isolates, 18 were S.weltevreden and 6 were S.typhimurium var copenhagen. Sixteen of the Salmonella strains isolated were from faeces collected from sick and dead animals out of which 4 were from rectal swabs of pigs suffering from gastroenteritis and eight from mesenteric lymphnodes. All the strains isolated were from piglets aged 3-8 weeks.

Nath et al. (1970) in their report on Salmonella pattern in India, cited the occurance of S.weltevreden in

pigs. Jayaraman & John (1969) suggested that Salmonella weltevreden has a wide range of host specificity. S.typhimurium has also been reported to be causing aevere clinical disease in pigs in the farm of acute or chronic gastroenteritis (Heard and Linton, 1965; Hooren & Thoonen, 1968; and Beh, 1971). The isolation of Salmonella typhimurium var copenhagen has been made from pigs by Sasidhar (1974) during his investigation in the incidence of various Salmonella serotypes among percine population in and around Bangalore City.

The two species isolated, S.weltevreden and S.typhimurium var copenhagen are also potential human pathogens. The incidence of these two species of Salmonella have been reported from divergent sources either from clinical cases or from healthy carriers by many research workers (Jayaraman et al., 1964; Datta & Singh, 1961; Jayaraman & John, 1969; Bhatia & Agarwal, 1968; Radhava & Kalara, 1970; Nath et al., 1970). The disease in the present study was found to be endogenous in the farm. There had been previous report on the isolation of Salmonella weltevreden from baby pigs of the same farm with the history of gastroenteritis (Sulochana et al., 1973). The frequent isolation of Salmonella species from porcine populations of the farm reveals the higher incidence of Salmonella infection. The isolation of these two species of Salmonella from necrotic

enteritis of pigs suggest that they may probably be associated in clinical syndromes of enteritis either alone or in association with other enteric pathogens. Experimental infection studies in pigs with mixed cultures of S.weltevreden and S.typhimurium also revealed that severity of the clinical condition was more intense than with individual cultures. Similar observations has been put forward by Goel & Mallik (1971). Beh (1971) reported the occurance of Salmonella typhimurium and enteropathogenic E.coli in pigs with gastro-enteritis.

All the strains of organisms were isolated from pigs aged 3-8 weeks. This shows that the occurance of the disease is more common in weaners because of their high susceptibility due to decreased resistance and may partially be also due to the change in the environment. John Myooch & Haddick (1969) reported that the incidence of Salmonellosis is more common in pigs aged one and two days and 4-12 weeks. Guinee et al. 1965 on their investigation on the incidence of Salmonellosis in pigs have found that the disease is more commonly observed in pigs after the 2nd week of life and in pigs aged 6-8 weeks old.

Experimental infection of laboratory animals such as mice, guinea pigs and rabbits with Salmonella typhimurium var copenhagen showed no apparent lesions on visceral organs of animals died at earlier period of injection. The animals that died at later periods showed lesions on the lungs, liver and spleen. Similar observations had been made by Ghosh & Anina Chatterjee (1960) with

S.typhimurium and its variants S.typhimurium var copenhagen in rabbits. The reisolation of the organism from various internal organs of experimental animals that died at various periods suggests that the organisms have got definite invasive power for the tissues of the experimental hosts like mice, guinea pigs and rabbits. Wilson & Miles (1967) also suggested that S.typhimurium given parenterally have very definite invasive power for the tissues of mice and other laboratory rodents.

Experimental infection studies with S.weltevreden showed no gross lesion on the visceral organs in mice although definite lesions on the visceral organs were observed in other species of hosts. Some showed enlargement of liver, spleen, mesenteric lymphnodes and pulmonary congestion. Similar observations have also been made by Jayaraman et al. (1964) in natural and experimental studies in guinea pigs.

Studies on experimental infection on primary hosts have revealed the following observations. Gross lesions were observed in animals inoculated by Salmonella typhimurium var copenhagen and also in animals infected by a mixture of S.typhimurium and S.weltevreden when destroyed 5 days after infection. No lesion was seen in piglings infected with Salmonella weltevreden alone, eventhough isolation of the

organisms could be made from the faecal material and mesentric lymphnodes. This definitely shows that S.typhimurium var copenhagen or a mixture of two organisms could produce definite pathological changes in tissues even within a short period of 5 days. These lesions were more pronounced in animals sacrificed 10 and 15 days following oral infection with S.typhimurium. The organisms might have penitrated through the intestinal wall and multiplied in adjacent drainage lymphnodes. Lawson and Dow (1966) observed in their experimental infection studies with S.choleraesuis var kunzendrof that initial infection of the body tissues occured through the walls of the small intestine and colon and penetration of the drainage lymphnodes within 24 hours of infection.

The organisms could not be recovered from blood cultured at the peak of temperature reactions. Lawson & Dow (1966) found that blood cultured at various stages of infection gave only few positive results and the failure for the recovery of organisms was explained on the basis that bacterimia may be intermittent. But in septicemic form of infection other lesions such as exudation of fluid in the abdominal cavities, thoracic cavities and lesions on the visceral organs were noticed (Smith and Jones, 1967). In this study no fluid accumulation was noticed in the body cavities and marked gross lesions were also absent in internal organs.

Animals sacrificed at various stages of infection in all the three groups yielded position results for isolation of the organisms from intestinal tract and mesentric lymphnodes. These results prove that the infection confines to the intestinal tract and neighbouring lymphnodes.

The high frequency of isolation of Salmonella organisms from faecal materials and mesentric lymphnodes from both natural as well as experimental cases also gave further evidence for the enteric nature of the infection. Various authors have also reported the isolation of Salmonella from faeces and mesentric lymphnodes from animals showing enteritis or from healthy carrier animals (Scott, 1940; Vanhoof, 1966; Ghung & Froster, 1969; Guinee, 1974).

The pig which died after the 9th day of oral dosing with the mixed cultures of S.typhimurium var copenhagen and S.weltevreden organisms could be recovered from bronchial lymphnodes. This may be due to the pharyngeal dosing procedure employed. Lawson & Dow (1965) suggested that the higher percentage of bronchial lymphnodes giving positive results early in the experimental periods could be caused by small numbers of organisms gaining entry to the upper trachea during dosing. In two cases the organisms could be recovered from bile. Lawson & Dow (1966) stated that the recovery of the organisms from liver and bile may be due to an ascending infection of the bile duct and may be an

artifact resulting from the killing process. Isolation of organisms from the faeces and internal organs with definite indications of pathological changes indicate that S.weltevreden and S.typhimurium could produce disease condition in piglings atleast when resistance is lowered due to adverse conditions.

Antibiotic sensitivity studies have revealed that all the strains of S.weltevreden and S.typhimurium var copenhagen tested were sensitive to chloramphenicol at the concentration of 30 µg./disc. Eleven of them were sensitive to gentamycin and streptomycin at the concentration of 10 and 20 µg./disc. respectively. Two strains, one from each group were found to be sensitive to tetracycline and Nitrofurantoin. Ulsen (1972) reported that the sensitivity of the Salmonella isolates to streptomycin, tetracycline, furazolidone, chloramphenicol, neomycin, kanamycin and ampicillin varied considerably. The organisms isolated in this study have also showed considerable variation in their sensitivity to tetracycline, furazolidone and streptomycin. However, none of them were found resistant to chloramphenicol. This variation may be due to the transfer of R-factor from the same species of organisms or from other organisms like E.coli. It is clear from Table 8 that majority of S.weltevreden and S.typhimurium

tested against tetracycline showed resistance to this antibiotic. In the farm where this investigation was carried out, animals were fed TM5 (which contains tetracycline hydrochloride) as a feed supplement for better growth and disease resistance. Thus the isolation of tetracycline resistant Salmonellae may be attributable to the practice of giving tetracycline as a feed aditive.

The modified medium devised by Sharma (1973) was found to be useful for preliminary identification of strains of Salmonella which produce hydrogen sulphide. MacConkey's medium in which mannitol was incorporated instead of lactose was found to be more useful in eliminating proteus organisms. The composite medium divided by Chitin et al. (1972) was found to be equally good as triple sugar iron agar. The medium has got the additional advantage in that it can be used for testing deamination of phenylalanine to phenylpyruvic acid. The test for deamination of phenylalanine was usually done in routine procedures for elimination of proteus species. Some Proteus species produce characteristic results in triple sugar iron agar by forming acid butt, alkaline slant with production of hydrogen sulphide (Edward and Ewing, 1972). Hence it may lead to false positive results. The composite medium I helps for detection of the reaction in T.S.I. along with phenylpyruvic acid production and thus saving much time and labour with

excellent results. The composite medium II also was found to be useful in testing formentation of mannitol and also for testing motility. The motile organisms produced 'fan' shaped growth in semisolid medium. The motility testing by hanging drop methods may lead to false positive results (Edward & Ewing, 1972).

The results of the biochemical reactions obtained were in agreement with the reaction for Salmonella described by Edward and Ewing (1972).

SUMMARY AND CONCLUSION

SUMMARY & ~~CONCLUSION~~

A bacteriological study was conducted to assess the incidence of enteropathogenic E.coli and Salmonella in piglets with digestive disturbances.

2. A total of 274 samples were collected and processed from living as well as dead animals with the history of enteritis. Samples included faeces, mesenteric lymphnodes, heartblood, liver and spleen.

3. The enrichment and selective media used, included selinite broth, tetrathionate broth with brilliant green, Dihydrostreptomycin broth and Dihydrostreptomycin agar, in addition to the common media used in routine isolation. Modified MacConkey's medium I & II was found to be useful for identification of Salmonella organisms by their characteristic appearance as a result of hydrogen sulphide production. The composite medium was also found useful for detection of the reaction in T.S.I. along with pehnylpyruvic acid production. The D.H.S. agar was found to be of great service for isolation of enteropathogenic E.coli organisms from faeces.

4. A total of 24 strains of Salmonella and 75 strains of E.coli were isolated during this investigation. The identity of the isolates was confirmed by various biochemical and biological tests described by standard

textbooks. The antigenic typing of Salmonella and E.coli strains was conducted by Dr. V. K. Sharma, of Haryana Agricultural University and by National Salmonella and Escherichia centre, Kasauli.

5. Salmonella strains isolated and studied belonged to two main sero groups, S.weltevreden and S.typhimurium var copenhagen.

6. Various tests conducted to determine the pathogenicity of E.coli isolates have revealed that five strains belonged to pathogenic group. The tests included haemolysin, necrotoxin and enterotoxin production and pathogenicity to mice. These five strains belonged to sero groups 05, 017 and 039.

7. Pathogenicity studies on two strains of S.weltevreden and 2 strains of S.typhimurium var copenhagen have revealed that they are pathogenic to mice, guinea pigs and rabbits.

8. Pathogenicity studies on primary hosts have shown that they can produce an enteric form of disease, lesions mainly confining to the gastrointestinal tract.

9. Antibiotic sensitivity studies of the enteropathogenic E.coli serotypes by single disc diffusion technique showed that all strains were sensitive to chloramphenicol and gentamycin. The strains showed multiple resistance to streptomycin, tetracycline, erythromycin and ampicillin.

10. Antibiotic sensitivity studies of 15 strains of Salmonella have revealed that they were sensitive to chloramphenicol. The organisms exhibited multiple resistance to nitrofurantoin, tetracycline, ampicillin, erythromycin and penicillin. The results of bacteriological and drug sensitivity studies were communicated for appropriate action.

11. The possible role of E.coli and Salmonella in enteric disorders of piglings is discussed in detail.

APPENDICES

APPENDICES

Appendix Ia.

Dihydrostreptomycin broth (Ramirez & McClesky, 1968)

Peptone	10gms.
Sodium chloride	5gms.
Beef extract	10gms.

Distilled water 1000 ml.

Adjust pH 7.2

The ingredients are mixed together and heated until dissolved. The medium is then sterilized at 15 lb. pressure/Sq. inch. Allowed to cool and added streptomycin at the concentration of 4 ug./ml. and tubed in 10 ml. amounts under sterile precautions.

Ib.

Dihydrostreptomycin agar

Peptone	20g.
Lactose	10g.
Bile salts	1.5g.
Sodium chloride	5g.
Neutral red	0.03g.
Crystal violet	0.001g.
Dihydrostreptomycin	4 ug./ml.

added before pouring plates

IIa.

MacConkeys medium I (Modified Sharma, 1973)

Peptone	10g.
Yeast extract	3g.
Sodium chloride	5g.
Ferric Ammonium citrate	1g.
Sodium thiosulphate	8.5g.
Sodium citrate	8.5g.
Lactose	10g.
Bile salts	1g.
Neutral red	0.03g.
Distilled water	1000ml.
pH	7.4

IIb.

Modified MacConkey medium II(Sharma, 1961)

Peptone	20g.
Mannitol	10g.
Bile salts	1.5g.
Sodium chloride	5g.
Neutral red	0.03g.
Crystal violet	0.001g.
Distilled water	1000ml.

pH 7.4

IIIa.

Composite medium (Part I) (Chitin et al., 1972)

Peptone (Proteose peptone)	20g.
Sodium chloride	5g.
Lactose	10g.
Glucose	10g.
Ferrous ammonium sulphate	0.2g.
Sodium thiosulphate	0.2g.
L.phenylalanine	4g.
Agar (Oxoid)	13g.
Distilled water	1000 ml.
Phenol red (2.4%)	3 ml.

pH was adjusted to 7.5. The medium was steamed for 1 hour and filtered through cotton pads. Dispensed in 3ml. amounts, plugged loosely and autoclaved at 10 lbs/ sq.inch pressure for 15 minutes. It was allowed to solidify in slant position so as to give a generous butt (2.5cm) and short slant.

IIIb.

Part II

Peptone	10g.
Tryptone	10g.
Sodium chloride	5g.
Mannitol	2g.
Sucrose	10g.

$Na_2HPO_4, 12H_2O$	5g.
Bromthymol blue	(0.4%) 3ml.
Distilled water	1000ml.
Agar	4g.

pH was adjusted to 7.4. The medium was steamed for 1 hour and filter through cotton wool pads. Dispensed in 10 ml. amounts plugged loosely and autoclaved at 10 lbs./sq.inch for 15 minutes. Allowed to soldify in vertical position.

Phenyl pyruvic acid test was performed after noting down the other results. 0.5ml. of ferric chloride 10% aquous solution was added and noted the reaction after 2 minutes.

Appendix IV

Interpretation of disc sensitivity tests (Peters drof, R.G & Sherris, J.C. Amer. J.Med. 39:766-69, 1965).

Antibiotic or chemotherapeutic agent	Disc potency	Inhibition zone. Diameter in mm. Resistant.	Intermediate.	Sensitive
Ampicillin	10ug.	20 or less	21-28	29 or more
Bacitracin	10units	8 or less	9-12	13 or more
Cephalothin	30ug.	.	.	18 or more
Chloramphenacol	30ug.	12 or less	13-17	18 or more
Colistin	10ug.	8 or less	9-10	11 or more
Erythromycin	15 ug.	13 or less	14-17	18 or more
Kanamycin	30 ug.	13 or less	14-17	18 or more
Methacillin	5ug.	9 or less	10-13	14 or more
Neomycin	30 ug.	12 or less	13-16	17 or more
Nitrofurantoin	300ug.	8 or less	9-12	13 or more
Pencillin G.	10 units	20 or less	21-28	29 or more
Streptomycin	10 ug.	11 or less	12-14	15 or more
Sulpha	300ug.	12 or less	13-16	17 or more
Tetracycline	30 ug.	14 or less	15-18	19 or more

TABLES

DETAILS OF SPECIMENS COLLECTED FOR ISOLATION OF ESCHERICHIA COLI & SALMONELLA

Table 1

Type of specimen	Age group				No.of specimens examined	No. of E.coli isolated	No. of Salmonella isolated
	Birth to one week	one to three weeks	3 to 6 weeks	6 to 8 weeks			
Animals with enteritis							
Rectal swabs	25	20	25	50	120	50	4
Dead piglets							
Intestinal contents			5	17	22		
Small intestine			5	20	25	25	12
Large intestine			5	21	26		
Mesenteric lymphnodes			5	20	25		8
Liver			5	8	13		
Bile			5	8	13		
Spleen			4	6	10		
Lung			4	6	10		
Heart blood			4	6	10		
Total	25	20	67	162	274	75	24

BIOCHEMICAL REACTIONS OF CULTURES OF ESCHERICHIA COLI ISOLATED FROM PIGS

Table 2

Culture Numbers	E1/74	E2/74	E3/74	E4/74	E5/74	E6/74	E7/74	E8/74	E9/74	E10/74	E11/75	E12/75
Test Substrate												
Eijkman's test	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
Growth on D.H.S.	+	+	+	+	+	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+	+	+	+	+	+
Haemolysis	-	-	+	-	+	-	+	-	+	+	-	+
Indol	+	+	+	+	+	+	+	+	+	+	+	+
Methyl red	+	+	+	+	+	+	+	+	+	+	+	+
Voges-Proskauer	-	-	-	-	-	-	-	-	-	-	-	-
Simmons's citrate	-	-	-	-	-	-	-	-	-	-	-	-
Hydrogen sulfide (TSI)	-	-	-	-	-	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-	-	-	-	-	-
KCN	-	-	-	-	-	-	-	-	-	-	-	-
Lysine decarboxylase	+	+	+	+	+	+	+	+	+	-	+	+
Phenylalanine deaminase	-	-	-	-	-	-	-	-	-	-	-	-
Malonate	-	-	-	-	-	-	-	-	-	-	-	-
Gas from glucose	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	-	-	+	-	-	-	-	-	-	-	-	-
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+
Dulcitol	+	+	-	+	+	+	-	-	+	+	-	-
Salicin	-	-	-	+	-	+	+	+	-	+	-	-
Adonitol	-	-	-	-	-	-	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	-	-	-	-	-	-
Sorbitol	+	+	+	+	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+	+	+	+	+
Raffinose	+	+	+	+	+	+	+	+	+	+	+	+
Rhamnose	-	+	+	+	-	+	-	+	+	+	+	-
Nitrate	+	+	+	+	+	+	+	+	+	+	+	+

contd..

Culture Numbers	E13/75	E14/75	E15/75	E16/75	E17/75	E18/75	E19/75	E20/75	E21/75	E22/75	E23/75	E24/75	E25/75
Test Substrate													
Eijkman's test	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
Growth on D.H.S.	+	+	+	+	-	-	+	+	-	-	+	+	-
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+
Haemolysis	-	+	+	+	+	+	-	+	+	+	-	-	+
Indol	+	+	+	+	+	+	+	+	+	+	+	+	+
Methyl red	+	+	+	+	+	+	+	+	+	+	+	+	+
Voges-proskauer	-	-	-	-	-	-	-	-	-	-	-	-	-
Citrate	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydrogen sulfide	-	-	-	-	-	-	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-	-	-	-	-	-	-
KCN	-	-	-	-	-	-	-	-	-	-	-	-	-
Lysine	-	+	+	+	+	-	+	+	+	-	+	-	+
Phenylalanine	-	-	-	-	-	-	-	-	-	-	-	-	-
Malonate	-	-	-	-	-	-	-	-	-	-	-	-	-
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	-	-	-	-	-	-	-	-	-	-	-	-	-
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+
Dulcitol	+	+	+	-	+	-	-	+	+	-	-	+	-
Salicin	-	-	-	-	+	-	-	-	-	-	-	-	-
Adonitol	-	-	-	-	-	-	-	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	-	-	-	-	-	-	-
Sorbitol	+	-	+	-	+	+	+	+	+	-	+	+	+
Arabinose	+	+	+	+	+	+	+	+	+	+	+	+	+
Raffinose	+	+	+	-	+	+	-	+	+	-	-	+	+
Rhamnose	+	+	+	+	+	+	+	+	+	+	-	+	+
Nitrate	+	+	+	+	+	+	+	+	+	+	+	+	+

Note: A.G. = Acid and gas production
+ = Positive
- = Negative

BIOCHEMICAL REACTIONS OF CULTURES OF SALMONELLA ISOLATED FROM PIGS

Table 3

Culture Numbers	1/74	2/74	3/74	5/74	6/74	7/74	9/74	10/74	12/74	13/74	14/74	19/74	20/74
Test Substrate													
Indol	-	-	-	-	-	-	-	-	-	-	-	-	-
Methyl red	+	+	+	+	+	+	+	+	+	+	+	+	+
Voges-proskauer	-	-	-	-	-	-	-	-	-	-	-	-	-
Simmons's citrate	+	+	+	+	+	+	+	+	+	+	+	+	+
Hydrogen sulfide (TSI)	+	+	+	+	+	+	+	+	+	+	+	+	+
Urease	-	-	-	-	-	-	-	-	-	-	-	-	-
KCN	-	-	-	-	-	-	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+
Lysine decarboxylase	-	+	+	+	+	+	-	+	+	+	+	+	+
Phenylalanine deaminase	-	-	-	-	-	-	-	-	-	-	-	-	-
Malonate	-	-	-	-	-	-	-	-	-	-	-	-	-
Gas from glucose	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-	-	-	-	-	-	-	-
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+
Dulcitol	+	+	+	+	+	+	-	+	+	+	+	+	+
Salicin	-	-	-	-	-	-	-	-	-	-	-	-	-
Adonitol	-	-	-	-	-	-	+	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	-	-	+	-	-	-	-
Sorbitol	+	-	+	+	-	-	-	+	+	+	+	-	-
Arabinose	-	+	+	+	+	+	+	+	+	-	+	+	+
Raffinose	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhamnose	+	+	+	+	+	+	+	+	+	+	+	-	-
Nitrate	+	+	+	+	+	+	+	+	+	+	+	+	+

contd..

Culture Numbers	21/75	22/75	23/75	25/75	26/75	27/75	28/75	30/75	32/75	33/75	34/75
Test Substrate											
Indol	-	-	-	-	-	-	-	-	-	-	-
Methyl red	+	+	+	+	+	+	+	+	+	+	+
Voges-proskauer	-	-	-	-	-	-	-	-	-	-	-
Simmons's citrate	+	+	+	+	+	+	+	+	+	+	+
Hydrogen sulfide (TSI)	+	+	+	+	+	+	+	+	+	+	+
Urease	-	-	-	-	-	-	-	-	-	-	-
KCN	-	-	-	-	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+	+	+	+	+
Lysine decarboxylase	-	+	+	+	+	+	+	-	+	+	+
Phenylalanine deaminase	-	-	-	-	-	-	-	-	-	-	-
Malonate	-	-	-	-	-	-	-	-	-	-	-
Gas from glucose	+	+	+	+	+	+	+	+	+	+	+
Lactose	-	-	-	-	-	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-	-	-	-	-	-
Mannitol	+	+	+	+	+	+	+	+	+	+	+
Dulcitol	-	+	+	+	+	-	+	+	+	+	+
Salicin	-	-	-	-	-	-	-	-	-	-	-
Adonitol	-	-	-	-	-	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	-	-	-	-	+
Sorbitol	-	-	-	-	-	-	-	-	-	-	-
Arabinose	+	+	+	+	+	+	+	+	+	+	-
Raffinose	-	-	-	-	-	-	-	-	-	-	-
Rhamnose	+	-	-	+	+	+	+	+	+	+	+
Nitrate	+	+	+	+	+	+	+	+	+	+	+

Note: + = positive
- = negative

Details of the Salmonella species isolated from pigs and the results of their serological typing

Table 4

Age group	Cultural No	Source of isolation	Symptoms/lesions	Antigenic formula	Serotype
6-8 weeks	1/74	Faeces	Necrotic enteritis	3,10:r:z6	S.weltevreden
,,	2/74	Mesentric lymphnode	Necrotic enteritis	3,10:r:z6	S.weltevreden
,,	3/74	Mesentric lymphnode	Necrotic enteritis	4,12:i:1,2	S.typhimurium-05
,,	5/74	Mesentric lymphnode	Necrotic enteritis	3,10:r:z6	S.weltevreden
,,	6/74	-do-	-do-	4,12:i:1,2	S.typhimurium-05
,,	7/74	Faeces	-do-	4,12:i:1,2	S.typhimurium-05
,,	9/74	Mesentric lymphnodes	-do-	3:10:r:z6	S.weltevreden
,,	11/74	Faeces	-do-	4,12:i:1,2	S.typhimurium-05
,,	12/74	Faeces	-do-	3:10:r:z6	S.weltevreden
,,	13/74	Faeces	-do-	4,12:i:1,2	S.typhimurium-05
,,	14/74	Mesentric lymphnodes	Enteritis	3,10:r:z6	S.weltevreden
3-5 weeks	19/74	Rectal swab	-do-	3,10:r:z6	S.weltevreden
,,	20/74	,,	-do-	3,10:r:z6	S.weltevreden
,,	21/74	,,	-do-	3,10:r:z6	S.weltevreden
,,	22/74	,,	-do-	3,10:r:z6	S.weltevreden
,,	23/75	Mesentric lymphnode	Necrotic enteritis	3,10:r:z6	S.weltevreden
,,	25/75	Faeces	Gastroenteritis	3,10:r:z6	S.weltevreden
,,	26/75	,,	Gastroenteritis	3,10:r:z6	S.weltevreden
,,	27/75	Mesentric lymphnodes	Gastroenteritis	3,10:r:z6	S.weltevreden
,,	28/75	Intestinal contents	Gastroenteritis	3,10:r:z6	S.weltevreden
6-8 weeks	30/75	,,	Enteritis	3,10:r:z6	S.weltevreden
,,	32/75	,,	,,	3,10:r:z6	S.weltevreden
,,	33/75	,,	Gastroenteritis	3,10:r:z6	S.weltevreden
,,	34/75	Faeces	Enteritis	4,12:i:1,2	S.typhimurium-05

Details of Haemolysin production, necrotoxin production, pathogenicity to mice and rabbit ileal loop reaction

Table 5

Culture No	Haemolysin production				Necrotoxin production	Pathogenicity to mice	Rabbit gut loop reaction
	Solid media		Liquid media				
	Aerobic 37°C	10%CO_2 37°C	Aerobic 37°C	10%CO_2 37°C			
E1/74	NT	NT	NT	NT	NT	-	-
E2/74	NT	NT	NT	NT	NT	-	-
E3/74	+	+	+	+	+	(++)	-
E4/74	NT	NT	NT	NT	NT	-	-
E5/74	+	+	+	+	-	-	-
E6/74	NT	NT	NT	NT	+	(+++)	-
E7/74	+	+	+	+	+	(++)	+
E8/74	NT	NT	NT	NT	NT	-	-
E9/74	+	+	+	+	-	-	-
E10/74	+	+	+	+	+	(+)	-
E11/75	NT	NT	NT	NT	NT	-	-
E12/75	+	+	+	+	-	-	-
E13/75	NT	NT	NT	NT	NT	-	-
E14/75	+	+	+	+	-	-	-
E15/75	+	+	+	+	+	(+++)	-
E16/75	+	+	+	+	-	-	-
E17/75	+	+	+	+	-	-	-
E18/75	+	+	+	+	-	-	-
E19/75	NT	NT	NT	NT	NT	-	-
E20/75	+	+	+	+	-	-	-
E21/75	+	+	+	+	-	-	-
E22/75	+	+	+	+	-	-	-
E23/75	NT	NT	NT	NT	NT	-	-
E24/75	NT	NT	NT	NT	NT	-	-
E25/75	+	+	+	+	-	-	-

Note: + =Positive reaction
- =Negative reaction
NT =Not tested

(+++) = death within 24 hours
(++) = death within 48 hours
(+) = death within 96 hours

Details of Experimental infection studies of S.typhimurium var copenhagen and S.weltevreden in laboratory animals

Table 6

Culture No	Experimental animal	Sps. of Salmonella tested	Route of infection	Period of observation	Gross lesions	Site of isolation
	Mice	S.typhimurium var copenhagen				
3/74	1		i/p.0.2ml.	48hrs.D	No gross lesions	Heart blood,spleen, liver & lungs.
	2		i/p.0.2ml.	72hrs.D	Scattered pneumonic lesions on both lungs and areas of necrosis on the surface of liver	Heart blood,spleen, liver & lungs.
13/74	1		i/p.0.2ml.	24hrs.D	No gross lesions	Heart blood,lungs, liver and spleen
	2		i/p.0.2ml.	48hrs.D	No gross lesions	Heart blood,lungs, liver and spleen
		S.weltevreden				
1/74	1		i/p.0.2ml.	48hrs.D	No gross lesions	Heart blood,lungs, liver and spleen
	2		i/p.0.2ml.	72hrs.D	No gross lesions	Liver,heartblood, lungs and spleen
25/74	1		i/p.0.2ml.	96hrs.D	No gross lesions	Liver,heartblood, lungs and spleen
	2		i/p.0.2ml.	48hrs.D	No gross lesions	Heart blood,lungs liver and spleen

contd....

Culture No.	Experimental animal	Sps. of Salmonella tested	Route of infection	Period of observation	Gross lesions	Site of isolation
	Guinea pigs	S.typhimurium var copenhagen				
3/74	1		i/p.0.5ml.	48hrs.D	Acute peritonitis. No other gross lesions.	Heart blood,lungs, liver and spleen
	2		S/c.1ml.	96hrs.D	Patchy congestion of lungs small necrotic foci on surface of ~~kxxxxx~~ spleen along with congestion	Heart blood,lungs, liver and spleen
		S.weltivreden				
1/74	1		i/p.0.5ml.	48hrs.D.	Acute peritonitis. Spleen enlarged and congested	Heart blood, Liver,spleen,lungs and mesentric lymph-nodes
	2		S/c.1ml.	96hrs.D.	Enlargement of spleen,liver and mesentric lymphnodes. Lungs showed pneumonic areas	Liver,spleen,lungs & Mesentric lymphnodes
	Rabbit					
		S.typhimurium var copenhagen				
3/74	1		S/c.1ml.	48hrs.D.	Pulmonary oedema, enlarge-ment of spleen	Heart blood,lungs & spleen
	2		S/c.1ml.	36hrs.D.	No gross lesions.	Heart blood & lungs
		S.weltevreden				
1/74	1		S/c.1ml.	48hrs.D.	No gross lesions	Heart blood & lungs
	2		S/c.1ml.	48hrs.D.	Pulmonary oedema. Conges-tion and enlargement of spleen	Heart blood, lungs and spleen

Note: D = Death K = Killed

Details of experimental infection studies on S. typhimurium var copenhagen and Salmonella weltevreden in piglets

Table 7

Culture No.	No. & Age group	Sps. of Salmonella	Dose & route of infection	Period of observation	Symptoms/lesions	Site of isolation
		S. typhimurium var copenhagen				
3/74	1/107 5wks. 1		30ml. oral	5 daysK	Temperature 104°F. Congestion of the terminal portions of ileum & colon. The mesentric lymphnodes enlarged and oedematous.	Faeces, ileum and mesentric lymphnode.
3/74	2/107 5wks. 2		30ml. oral	10 daysK	Reddening of the skin on the abdomen and dependent parts. Carcass emaciated. Acute gastritis and congestion of the greater curvature of the stomach catarrhal enteritis, mild congestion and petichae of the mucous membrane of ileum jejunum and colon were observed. The bronchi contained frothy fluid.	Faeces, ileum, mesentric lymphnodes and colon
3/74	3/107 5wks. 3		30ml. oral	15 daysK	The skin on the inner aspect of thigh necrotic. Rice in temperature after 2nd day of oral dosing upto 105°F. Diarrhoea after the 6th day. Temperature declined with the on set of diarrhoea. Catarrhal enteritis haemorrhagic streaks on the mucosa of ileum. The walls of the ileum thickened. The mucous surface of colon necrotic. Mesentric lymphnodes enlarged.	Faeces, jejunum, ileum, mesentric lymphnodes and spleen.

Culture No.	No. & Age group	Sps. of Salmonella	Dose & route of infection	Period of observation	Symptoms/lesions	Site of isolation
		S. weltevreden				
1/74	3/108 4wks.		30ml. oral	5 daysK	No gross lesions on gastrointestinal tract, liver, spleen & lungs. The mesentric lymphnodes slightly enlarged.	Faeces mesentric lymphnodes and ileum
1/74	5/108 6wks.		30ml. oral	10daysK	Temperature 105°F. Diarrhoea & inappetence. Congestion of ileum and colon and enlargement of mesentric lymphnodes	Faeces, ileum and mesentric lymphnodes
1/74	4/108 5wks.		30ml. oral	15daysK	Temperature 104.8°F. Cyanosis of the skin, catarrhal enteritis, hyperemia of jejunum and ileum. The walls of the ileum thickened. Mesentric lymphnodes enlarged and haemorrhagic.	Faece, jejunum ileum mesentric lymphnodes & spleen
		S. typhimurium var copenhagen and S. weltevreden				
3/74) 1/74)	6weeks		15ml) 30ml. 15ml)	5daysK	The animal showed high rise in temperature varying from 105-106°F. Mild congestion of the mucosa of the ileum and colon was observed. The mesentric lymphnodes enlarged.	Intestinal contents, ileum, colon and mesentric lymphnodes.

contd..

Culture No.	No. & Age group	Sps. of Salmonella	Dose & route of infection	Period of observation	Symptoms/lesions	Site of isolation
3/74) 1/74)	6 weeks		15ml) 15ml)30ml.	9 daysD.	Temperature varied from 105-106.8°F. The jejunum and ileum contained yellowish fluids. The fundic portion of the stomach showed severe diffuse congestion. Streaks of haemorrhage noticed on mucosa of ileum, jejunum & colon. The bronchial lymphnodes were enlarged.	Bronchial lymphnodes, liver, bile, mesentric lymphnodes, ileum and colon
3/74) 1/74)	4 weeks		15ml) 15ml)30ml.	15 daysK.	Temperature varied from 104-104.8°F. Diarrhoea noticed after 5th day of dosing. Carcass emaciated. The entire surface of the mucosa of colon showed greyish white nodules with erosions. Terminal portion of ileum haemorrhagic and thickened. The mesentric lymphnodes greatly enlarged.	Faeces, mesentric lymphnodes, colon, ileum and bile

Note: K = killed
D = died

Table depecting the sensitivity of Salmonellae to various chemotherapeutic agents at different concentrations

Table 8

Organisms tested.	Chemotherapeutic agent tested and concentrations/disc.	No. of strains tested	No. of strains sensitive	No. of strains resistant
S. weltevreden	Penicillin 10 ug.	9	-	9
	Streptomycin 10 ug.	9	7	2
	Streptomycin 20 ug.	9	7	2
	Tetracycline 10 ug.	9	-	9
	Tetracycline 25 ug.	9	1	8
	Erythromycin 10 ug.	9	-	9
	Ampicillin 10 ug.	9	-	9
	Chloramphenicol 25 ug.	9	9	-
	Chloramphenicol 30 ug.	9	9	-
	Nitrofurantoin 100 ug.	9	1	8
	Gentamycin 10 ug.	9	7	2
S. typhimurium var copenhagen	Pencillin 10 ug.	6	-	6
	Streptomycin 10 ug.	6	4	2
	Streptomycin 20 ug.	6	4	2
	Tetracycline 10 ug.	6	-	6
	Tetracyclin 25 ug.	6	1	5
	Erythromycin 10 ug.	6	-	6
	Ambicillin 10 ug.	6	-	6
	Chloramphenicol 25 ug.	6	6	-
	Chloramphenicol 30 ug.	6	6	-
	Nitrofurantoin 100 ug.	6	1	5
	Gentamycin 10 ug.	6	4	2

contd..

Organisms tested.	Chemotherapeutic agent tested and concentrations/disc.	No.of strains tested	No.of strains sensitive	No.of strains resistant
E.coli.	Penicillin 10 ug.	5	-	5
	Streptomycin 10 ug.	5	3	2
	Streptomycin 20 ug.	5	3	2
	Tetracycline 10 ug.	5	2	3
	Tetracycline 25 ug.	5	2	3
	Erythromycin 10 ug.	5	3	2
	Ampicillin 10 ug.	5	-	5
	Chloramphenicol 25 ug.	5	5	-
	Chloramphenicol 30 ug.	5	5	-
	Nitrofurantoin 100 ug.	5	4	1
	Gentamycin 10 ug.	5	5	-

Figure 1

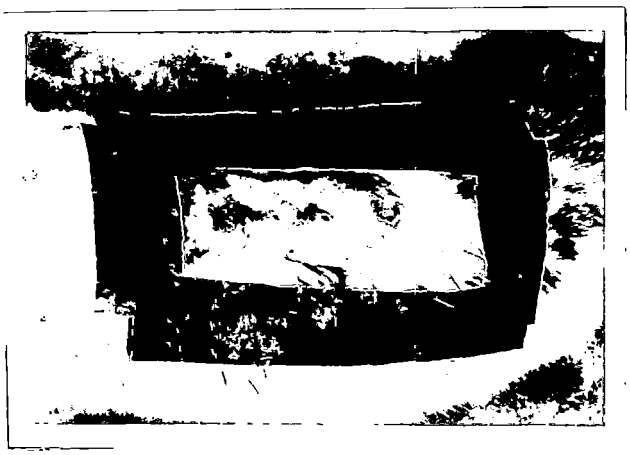


Figure 2

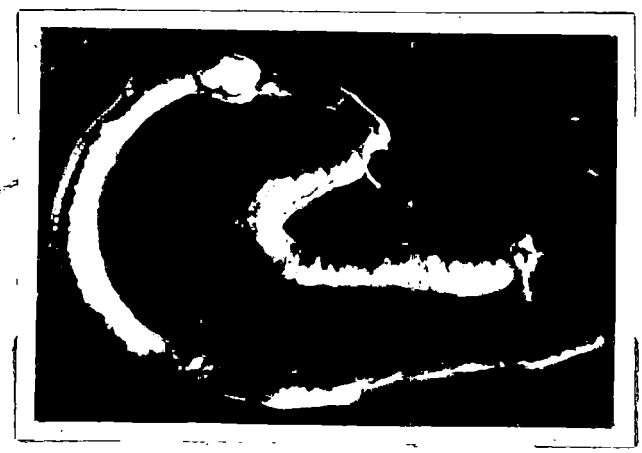


Figure 3

Figure 4

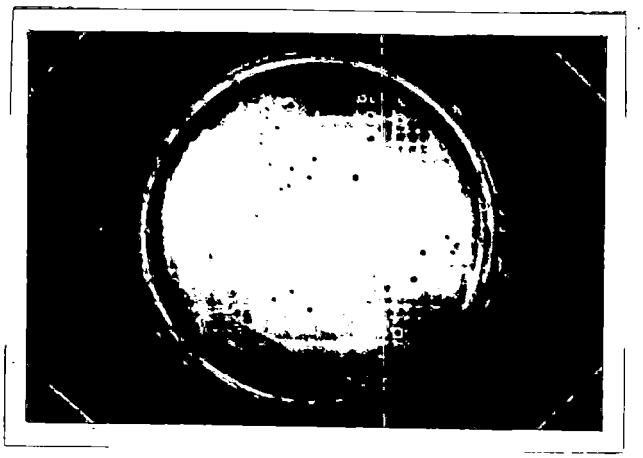
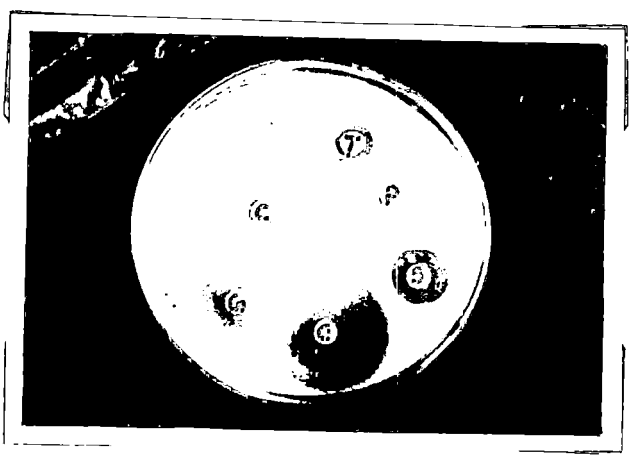


Figure 5

Figure 6



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STUDIES ON THE BACTERIAL SPECIES ASSOCIATED WITH DIGESTIVE DISTURBANCES IN PIGS

BY

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ABSTRACT OF A THESIS

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ABSTRACT

Prior to this investigation, limited studies conducted in the department of Bacteriology, College of Veterinary and Animal Sciences, Mannuthy, have revealed the association of pathogenic strains of E.coli and Salmonella with enteric disorders of pigs. Therefore, a detailed study of the incidence and magnitude of prevalence of those pathogens was carried out.

A total of 274 specimens collected from sick as well as dead animals were examined. Faecal materials collected from living as well as dead animals, mesentric lymphnode, spleen, liver, lungs and heart blood formed the materials for isolation studies. Both enrichment and selective media like selinite and tetrathionate broth, D.H.S. broth and D.H.S. agar, modified MacConkey medium I & II, and composite medium I & II were employed for isolation of pathogens. A total of 75 strains of E.coli and 24 strains of Salmonella were isolated and studied. Most of the isolations were made from piglings aged 3-8 weeks. Out of 75 strains of E.coli only 5 strains were found pathogenic based on various tests like haemolysin, necrotoxin and enterotoxin production and pathogenicity to mice. These isolates belonged to serogroup 05, 017 and 039. Salmonella strains belonged to two serotypes,

S.weltevreden and S.typhimurium var copenhagen. The identity of the isolates were confirmed biochemically and serologically.

Pathogenicity studies conducted with two strains of Salmonella weltevreden and Salmonella typhimurium var copenhagen have revealed that they were pathogenic to laboratory animals like mice, guinea pigs and rabbits. It has also been observed that these serotypes could produce enteric form of the disease in primary hosts.

Invitro drugsensitivity studies were carried out to determine the effectiveness of antibiotic to gastrointestinal disorders caused by these species. It has been observed that all E.coli and Salmonella strains tested were sensitive to chloramphenicol. However multiple resistance was observed to penicillin, streptomycin, tetracycline, erythromycin and nitrofurantoin.

The significance, possible role of infection by these species and their drug sensitivity reactions are discussed.

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