A CRITIQUE OF METHODS AND MEDIUMS FOR THE ISOLATION OF THE SALMONELLA GROUP FROM THE INTESTINES OF CHICKENS

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Introduction

According to the literature a fairly large variety of types of Salmonella have been found to occur in the common fowl; however, few of the reports on avian paratyphoid have been concerned with cultural methods or with the recovery of the organism suspected from the intestinal tract of the animal. Actually very little study has been given to the bacterial content of the intestines of chickens in diseased states, although in recent years a more systematic search for Salmonella pullorum has been made in connection with special work on this disease.

The object of this study was twofold; first, to evaluate some of the present methods and mediums used for the isolation of members of the paratyphoid group as well as their applicability to the isolation of this group from avian sources; and second, to ascertain whether Salmonella, other than Salmonella pullorum are present in the intestinal tract of normal and diseased chickens.

Historical

Diseases of birds due to organisms of the Salmonella group have been recorded from time to time.

Hadley (1) in 1918 made a detailed study of paratyphoid in birds. In this work he presents an excellent historical resumé of reports of epidemics in fowls which were typhoid-like in nature. Such reports date back as far as 1798. Within recent years the study of Salmonella infections in the common fowl has received an increasing amount of attention. The earlier studies deal only with pullorum disease and fowl typhoid, but since the division of organisms of the Salmonella group on a serological basis, the literature has reported

with increasing frequency the findings of new types other than Salmonella pullorum and Salmonella gallinarum. Mulson (2) in 1919 stated that Salmonella gallinarum (Bacillus sanguinarium) was first isolated in the United States in 1894 by Smith from an epidemic among fowls in Rhode Island while Salmonella pullorum (Bacterium pullorum) was first isolated by Rettger in 1899 from young chicks.

Spray and Doyle (3) in 1921 described an organism of the paratyphoid group isolated from baby chicks which closely resembled Bacterium paratyphosus B type; however, it was not definitely identified.

Doyle (4) in 1927 isolated an organism from the livers of chicks which he identified as Bacterium aertrycke.

Edwards (5) in 1929 reported a fatal infection of chicks due to bacilli of the paratyphoid B. group which he identified as B. aertrycke and Bacterium anatum.

Schalm (6) in 1937 studied a disease which had caused extensive losses in chicks and named a paratyphoid organism similar to Salmonella typhimurium as the agent.

Van Roekel (7) in 1937 stated that salmonellosis in chickens had been found to involve the following organisms: S. pullorum, S. gallinarum and S. anatum.

Edwards (8) in May, 1938, reported a new type, Salmonella kentucky which had been isolated from the intestinal tract of a chick affected with coccidiosis and ulcerative enteritis.

Bruner and Edwards (9) in November, 1938, reported two new types from fowls. These were S. worthington and S. minnesota.

According to the literature cited by Jungherr and Clancy (10) in 1939 the following had been reported: S. typhimurium, S. pullorum, S. gallinarum,

S. enteritidis, S. anatum, S. paratyphi A, S. paratyphi B, S. suispestifer, S. amersfoort and S. anatum var new brunswick. They also quote Lerche as having credited the common domestic fowl with occasionally harboring S. typhimurium, S. enteritidis var dublin and again S. pullorum. In their own serological investigation of twelve cases of paratyphoid infection in chicks, Jungherr and Clancy (11) established the presence of seven different types as follows: S. typhimurium, S. typhimurium var binnes, S. bareilly, S. oranienburg, S. montivideo, S. london and S. anatum.

Ryff (12) in an unpublished work was able to isolate S. give and S. aberdeen from the intestinal tract. He failed to corroborate Emmel's published findings with respect to the isolation of Eberthella, Shigella and the frequency of certain Salmonella species. The technic of both of these workers was similar.

Emmel (13) reported the isolation of S. paratyphosus A, S. paratyphi B, S. suispestifer, S. aertrycke and S. enteritidis from the intestinal tract. Of these Edwards confirmed the finding of the last four named.

An examination of these studies shows that 21 different types of Salmonella have been reported which have the chicken as a source. These are as follows:

S. pullorum, S. gallinarum, S. aertrycke, S. anatum, S. kentucky, S. worthington, S. minnesota, S. paratyphi A, S. paratyphi B, S. suispestifer, S. enteritidis, S. amersfoort, S. anatum var new brunswick, S. enteritidis var dublin, S. typhimurium var binnes, S. bareilly, S. oranienburg, S. montvideo, S. london, S. give and S. aberdeen.

In addition to these types in chickens other types have been found in turkeys, ducks, quails, pigeons, and canaries. Fitch and Pomeroy(14) in a review of Salmonella infections in poultry name 17 different organisms of the

paratyphoid group. These are S. typhimurium, S. anatum, S. newington, S. senftenberg, S. derby, S. bareilly, S. newport, S. oranienburg, S. kentucky, S. montevideo, S. bredeney, S. worthington, S. london, S. muenchen, S. minnesota, S. new brunswick and S. enteritidis.

Exclusive of S. pullorum, few of the above named organisms designated as paratyphoids have been demonstrated as inhabitants of the intestinal tract. Most of the original isolations, where such information is given, have been made from the visceral organs, that is, the heart blood and liver or from the unabsorbed egg yolk. Of the reports given Emmel's (15) is concerned chiefly with studies of the bacterial flora of the intestines. In these studies he attempted to determine the percentage occurrence of the various microorganisms present in each group of birds examined. Attention here will be given only to his results in regard to members of the paratyphoid group. The first study was on the bacterial flora of the feces of twenty healthy mature birds. Here Salmonella icteroides (now listed in Bergey's 5th edition as Bacillus icteroides) was credited as constituting 25.4% of the organisms present in one bird and 30.2% of the organisms present in another. A second study concerned the bacterial flora of the intestinal contents of twenty adult birds affected with enteritis. He gave an average of 20.9 per cent of the organisms present in the intestinal contents of this group of birds as belonging to the Salmonella group. Later in connection with studies on intestinal parasitism, Emmel again demonstrated the presence of paratyphoids in chicken feces. Six species of the genus Salmonella -- S. aertrycke, S. schottmülleri, S. enteritidis, S. suipestifer, S. typhimurium, and S. pullorum as well as one species of Eberthella, E. typhosa, were isolated from the intestinal contents of thirteen out of eighteen birds affected with enteritis associated with coccidiosis. In eleven out of eighteen birds affected with enteritis associated with helminths he listed four species o

the genus Salmonella, one species of Shigella and two of Eberthella. As a result of these findings more birds so affected were examined and he found that in 137 of 230 samples of intestinal contents there were eight species of the genus Salmonella, one of Eberthella and one of Shigella. Salmonella aertrycke was given as the predominating organism and occurred in 77 out of the 137 positive cases.

The evidence presented in Emmel's studies with regard, not only to the presence of numbers of the Salmonella genus, but also of those of the Eberthella and Shigella genera in the intestinal contents of chickens is particularly interesting from a public health standpoint. If organisms of such pathological significance are being harbored in the intestinal tract of diseased chickens, further and more systematic cultural methods should be used in the routine examination of such birds. It was thought in this investigation that the use of a regular routine method such as that used in the examination of human stools for members of the typhoid-paratyphoid group might be applicable to avian material and might also reveal the presence of members of the Salmonella group which have not hitherto been found.

For the routine cultivation of members of the typhoid-paratyphoid group in humans and lowers animals from fecal material the general procedure usually consists of plating out portions of the specimen directly on a differential plating medium and of planting another portion of the sample into what is called a selective enrichment broth. After a period of from 24 to 48 hours incubation, the plates are examined for the presence of these organisms and both streak and pour plates are made from the enrichment on various types of differential mediums.

Topley and Wilson (16) in a review of methods state "that since the feces contain a rich and varied bacterial flora, the isolation of the

particular organism depends in a large part on the use of selective and differential mediums."

The choice of an enrichment broth often varies, but it is customary in routine work to select some fluid medium which contains some inhibitory substance that will hold back the growth of bacteria other than typhoid and paratyphoid bacilli. Most of the methods used include a combination of enrichment and selective mediums.

To attempt a description of all the numerous methods which have been recommended for the isolation of this group from fecal material would take up more time and space than is necessary, but some of the more widely used technics will be briefly reviewed.

Brilliant green has proved successful for both fluid and solid mediums.

Browning, Gilmour and Mackie (17) adopted a procedure of planting a series of tubes of peptone water medium for enrichment which contained varying amounts of brilliant green, incubating for 20-24 hours, and then planting on a suitable solid medium from each tube.

Krumwiede and Pratt (18) found that in addition to the enrichment broth a brilliant green agar gave better results.

Rakieten and Rettger (19) recommended a buffered brilliant green enrichment medium. In conjunction with this they used a modified Endo agar. This combination gave more satisfactory results than direct plating on Endo's, eosin-methylene-blue, or on brilliant green agar alone.

Torrey (20) found that by the use of brilliant green he could stimulate the para-typhoid group and in a later work (21) suggested the use of brom-cresol purple-lactose agar as a plating medium.

Mallmann (22) recommended a brilliant green liver infusion agar for the isolation of members of the para-typhoid group from avian tissues. In this medium a concentration of 1:75,000 brilliant green was found to give

the best results.

Kerr (23) found that routine plating of feces on brilliant green agar was a valuable aid to the diagnosis of bacillary white diarrhea. He also used a brilliant green fluid medium as an enrichment medium.

Teague and Clurman (24) developed a brilliant green and eosin combination in agar.

Sodium tetrathionate broth is a comparatively new enrichment medium. Kauffmann (25) suggested a medium which contained tetrathionate, brilliant green and ox gall. Following 20 hours incubation at 37° a loopful from the enrichment medium was streaked out on Drigalski agar plates.

Jones (26) used a selective brilliant green eosin lactose agar with sodium tetrathionate broth as an enrichment medium for the isolation of the typhoid group.

Khalil (27) in a study of the incidence of organisms of the Salmonella group in wild rats attributed the success in isolation from the intestinal contents to the use of tetrathionate broth and brilliant green eosin agar.

Gauger, Greaves and Cook (28) made an extensive serological and bacteriological study of paratyphoid in pigeons. To determine whether the causative organisms could be isolated from feces they carried out a series of experiments using in the bacteriological examination a modification of the brilliant green technique of Rakieten and Rettger, and sodium tetrathionate broth. They were able to recover the organism from the birds "suffering from acute and subacute infection, and from the feces and mouth fluids of apparently healthy birds that were serologically positive."

The salts of selenium have been used in both fluid and solid mediums. Guth (29) in 1916 described a selenium agar (0.15% Na_2SeO_3) as a culture medium for the selective growth of typhoid bacilli. Leifson (30) suggested its use for enrichment purposes.

Gray (31) found that certain members of the Salmonella group were resistant to salts of lithium. He recommended for an enrichment medium the addition of 1,2, and 3 ml. of the chemical to 10 ml. of peptone water (pH 7.6) with the use of MacConkey's agar as a subsequent plating medium.

A fluid enrichment medium consisting of boullion, glucose, sodium sulphite, and liquid bismuth has proved useful in the isolation of E. typhosa from enteric stools. This combination with the addition of brilliant green and agar has been used for a number of years. (Willson and Blaie) (32)

English workers seem to prefer the bile salt medium of MacConkey and the bismuth sulphite medium in routine work.

A rapid survey of these numerous methods revealed conclusively that a combination of enrichment and differential mediums was necessary especially where a study of the intestinal material was desired. For this reason experiments were set up to determine which method might be successful in this study.

Experimental

As a preliminary to the actual routine examination of fecal material from chickens, experiments were set up utilizing some of the mediums now available and recommended for the cultivation of members of the typhoid-paratyphoid group. These experiments were carried out not only as a means of testing their selectivity but also to become familiar with the growth of the representative organisms on the mediums.

In the first experiment sterile fecal material was used. Into three large test tubes containing 10 ml. of sterile saline was placed about 1 gram of fecal material. To the first tube was added a standard seeding from a 24 hour agar slant culture of S. typhimurium. To the second tube was added E. coli, and to the third both organisms were added. The contents of these

tubes were thoroughly emulsified and left standing until the solid material had settled to the bottom of each tube. From each tube there was streaked one bismuth sulphite (Difco) agar plate and two pour plates of this same medium using a 5 ml. amount and a 1 ml. amount. Also from each tube there was streaked out two plates of MacConkey's agar (Difco), two of brilliant green liver infusion and two each of tryptose and plain nutrient agar.

For enrichment, samples of the fecal material were placed in each of three large tubes containing tetrathionate broth (Difco), three of tryptose broth and three of plain nutrient broth. To the first tube of each series was added a seeding from a 24 hour culture of S. typhimurium, to the second was added E. coli and to the third of each series both cultures were added. These tubes were allowed to incubate for 24 hours at 37° C. Following incubation, plates of bismuth sulphite agar, MacConkey's agar, brilliant green liver infusion agar, tryptose agar and plain nutrient agar were streaked and poured in the same manner as for the direct plating. All of the bismuth sulphite agar plates were allowed to incubate for 48 hours.

The plates which were seeded directly from the feces containing pure cultures yielded a good representative growth of these organisms. Where a mixture of both Esch. coli and S. typhimurium were present, however, the former organism outgrew overwhelmingly the other organism on both the tryptose and plain nutrient agar medium. On MacConkey's agar very few of the paratyphoid organisms were able to survive. Esch. coli was completely suppressed on the brilliant green liver infusion agar plates and on the bismuth sulphite medium only typical paratyphoid colonies grew.

From the enrichment broths the results were similar. Typical colonies of each organism in pure culture showed up on the plates. Where the mixture of the two organisms was present, however, on the tryptose and plain nutrient agar plates, colonies of Esch. coli persisted in crowding out those of S.

typhimurium. This fact was true also of the MacConkey's agar but Esch. coli was inhibited on the brilliant green liver infusion agar and on the bismuth sulphite agar.

In order to simulate natural conditions, another experiment was set up using the same procedure as above with the exception that unsterilized fecal material was used. Here again the results were similar. Wherever E. coli was present in combination with the other organism on a medium containing no inhibitory agent, it overgrew that organism.

The results of these experiments clearly demonstrated that an inhibitory medium was necessary not only to suppress the growth of Esch. coli but also to facilitate recognition of the paratyphoid organisms. In Emmel's studies of the intestinal organisms of chickens it was rather interesting to note, in his procedures for isolation of the paratyphoids, he used no selective or enrichment mediums. His procedure consisted in simply making dilutions in plain nutrient agar. Nowhere in these preliminary experiments was it possible to recover the paratyphoid organisms because of the abundant overgrowth of the coliform organism.

It was also interesting to note that sodium tetrathionate did not completely inhibit the growth of Esch. coli in pure culture where used in combination with tryptose and plain agar; however, the growth of the organism on these two mediums was very slight. Esch. coli was completely suppressed on all of the differential mediums streaked except MacConkey's agar from the tetrathionate enrichment broth. Apparently the combination was highly toxic for the organism. On MacConkey's agar, Esch. coli persisted but it was inhibitory enough to allow some of the paratyphoid colonies to grow and they were easily distinguishable.

It was found that pour plates of bismuth sulphite agar were more satisfactory than the streak plates.

In this study the following mediums were employed for routine cultivation.

for enrichment-----Tetrathionate broth (Difco)

-----Brilliant green liver infusion broth

for direct smears and plating from enrichment

-----Bismuth sulphite agar (Difco)

-----MacConkey's agar (Difco)

-----Brilliant green liver infusion agar

for differential medium

-----Kligler's iron agar (Difco)

The brilliant green enrichment broth was discontinued early in the study.

A section of the duodenum with its contents was selected from each bird and placed in a sterile petri dish. The date of collection, source and case history, where obtainable were recorded at the autopsy. No preservative was used on these specimens because they were cultured immediately after they were collected. The duodenum was selected because this region has been found to harbor few organisms of the coliform group.

A suspension was prepared from the specimen by emulsifying a portion of the sample in 10 ml. of saline. The coarse particles were forced to the bottom of the tube by pushing a loose cotton plug through the liquid. Poured plates of bismuth sulphite agar were made from this suspension using 1 ml. and 5 ml. amounts of the suspension as a seeding: however, it was found that better results could be obtained by using a 1 ml. and a 1 drop amount. At first direct smears were made from the fecal material and the amount adhering

to the needle used as an inoculum for the MacConkey's agar, brilliant green agar and bismuth sulphite agar streak plates. Later it was discovered that direct smears made from the saline suspension were successful on MacConkey's agar.

The contents of a portion of the intestine was emulsified in about 15 ml. of tetrathionate broth. The coarse particles were forced to the bottom of the tubes by means of a loose cotton plug and a wooden applicator. A small portion of the intestinal contents was placed in a flask containing 50 ml. of the brilliant green broth. The cultures were incubated at 37° C for 24 hours.

Following the 24 hour incubation period all plates were examined for growth and platings to the various differential mediums already mentioned were made from the primary enrichment. All bismuth sulphite agar plates were allowed to incubate for 48 hours.

Only those colonies which answered to the description for the typhoid-paratyphoid group were picked to tryptose agar slants, from which after 18-24 hours incubation they were transferred to Kligler's iron agar medium. Tubes of this medium showing typical changes for paratyphoids were kept for further identification.

Previous to any further identification procedures, cultures were restreaked from tryptose broth on MacConkey's agar for purification. Smooth representative colonies were picked from these plates to tryptose agar slants. These cultures were kept as stock.

This investigation extended over a period of approximately six months. The birds represented in this routine examination were not normal but were sent in for autopsy either from the Michigan State College Poultry plant or from private sources. Included within the list of disorders were peritonitis, lymphomatosis, abscesses, roup, epicarditis, internal hemorrhagic conditions,

necrosis of the liver, acute toxemia, fowl paralysis and avitaminosis. Altogether 294 birds were cultured from 48 of which 72 strains of organisms were isolated which according to their biochemical reactions belonged to the Salmonella group. These strains were sent to Dr. P.R. Edwards of the Kentucky Agricultural Experimental Station to whom we are indebted for their identification on the basis of their serological reactions.

The cultures were placed in the Salmonella group on the following bases: negative Gram staining property, failure to produce indol, production of hydrogen sulfide, production of acid and gas in dextrose, and their inability to ferment lactose and sucrose. In order to eliminate slow lactose fermenters, each culture was grown in tryptose lactose broth (34) for a period of two weeks. Other sugars and alcohols run were arabinose, maltose, xylose, mannitol, inositol and sorbitol. The basis for the carbohydrate broths was a one per cent (Bacto) peptone water containing .5 per cent salt. This medium was adjusted to pH 7.2 and 1 per cent of Andrade's indicator was added. Throughout this work on all mediums, known pure cultures of S. pullorum, S. typhimurium and Esch. coli were run as controls. The antigens of those organisms which did not ferment maltose but attacked the other sugars variably were tested with a known pullorum serum. Of these 34 were positive.

On the basis of their antigenic properties an interesting variety of organisms according to type were isolated. Of the 72 organisms sent to Dr. Edwards 35 were S. pullorum, one of which produced acid but no gas in maltose. The others were as follows:

Salmonella	california	9 strains
"	worthington	9 strains
"	oranienburg	2 strains
"	paratyphi B	1 strain
"	give	1 strain
"	new brunswick	1 strain

Salmonella	urbana	1 strain
"	aberdeen	3 strains
"	hvittingfoss	5 strains
"	muenchen	2 strains

The fermentation reactions of these types are charted. Two of the organisms sent to Dr. Edwards were found to be rough and as a result could not be classified. Of this group, four we believe have not hitherto been reported as being found in chickens. These are S. california, S. urbana, S. aberdeen and S. hvittingfoss. Whereas the remaining types have been reported none so far as we are able to find, with the exception of S. pullorum, have been recovered from the intestinal tract of the animal.

A brief discussion of each type follows:

1. Salmonella california--Edwards (34) reported the incidence of this type from an outbreak of paratyphoid infection in turkey poults.

This organism was found to be present in eight different birds. Case histories were not available on seven of these birds, but one of the cultures (C2852) was isolated from a baby chick (BPR) which was suffering from a vitamin deficiency. S. pullorum was isolated from the same chick from the heart and liver by Ryff.

2. Salmonella worthington--Bruner and Edwards (35) reported the identification of this type.

Four of the strains came from the same lot of baby chicks and one came from a second lot belonging to the same owner. S. oranienburg was also isolated from the first lot as well as S. pullorum. Two of the strains were from experimental birds which had been subjected to S. pullorum.

3. Salmonella oranienburg-- This organism was recently identified by Edwards (36) as the cause of a septicemic disease in baby quail.

Both strains of this organism came from baby chicks belonging to the

same lot. S. worthington and S. pullorum were also isolated from this lot. These strains were characterized by their inability to ferment inositol which agrees with former findings.

4. Salmonella paratyphi B--This organism is particularly interesting, because its occurence in lower animals is comparatively rare. Spray and Doyle (37) encountered an organism which closely resembled this type but did not definitely identify it. It has been considered of little importance as a causative agent of disease amongst animals. Edwards (38) confirmed Emmel's finding of this organism in the intestinal tract of a chicken.

This strain did not attack arabinose.

5. Salmonella give--This organism was reported isolated by Ryff (12) from the intestinal tract of a chicken.

This strain came from an experimental bird sent in from the M.S.C. poultry plant.

6. Salmonella new brunswick--This organism was isolated from an M.S.C. poultry plant bird.

7. Salmonella urbana--This organism was isolated from an M.S.C. poultry plant bird.

8. Salmonella aberdeen--Found in two different poultry plant birds, one of which apparently died of peritonitis, the other of which had a lymphomatosis. This organism was first reported by J. Smith (39) who isolated it from a case of acute enteritis in a child. It did not attack inositol. Ryff (12) also isolated this organism from the intestines of a chicken. In this study two of the strains came from the same bird. All three strains failed to attack inositol.

9. Salmonella hvittingfoss--Five strains of this organism were isolated--four came from the same bird, a white pullet, which exhibited nervous symptoms, had fowl paralysis and in addition had ocular roup, and the

right kidney was absent. The remaining strain was isolated from a poultry plant bird which had roup and an abscessed left thoracic air sac. Acid only was produced in inositol by one of the strains but the others produced acid and gas in this sugar.

10. Salmonella muenchen--Both of the strains isolated came from separate outside private owners. Culture number C 1102 was isolated from a bird showing upon autopsy a necrotic liver, enlarged spleen, acute toxemia and some peritonitis. Culture number C 1367 came from a single comb White Leghorn cockerel on which there was no case history.

11. Salmonella pullorum--On the basis of fermentation reactions thirteen different groups were, according to their antigenic properties, designated as S. pullorum. Few, if any of these strains follow the standard biochemical conception of this species of organism except that all of them except one did not ferment maltose and this exception produced only acid.

Discussion

During the past few years an increasing number of organisms belonging to the paratyphoid group have been isolated from the visceral organs of chickens. This fact is probably due more to the fact that more attention is being directed toward diseases of this type in fowls, rather than to the fact that these diseases are actually increasing in occurrence. Very few of the reports seem concerned with the actual method of isolation or with the presence of these organisms in the intestinal tract of the animal. From the standpoint of economic losses suffered from paratyphoid infections as well as their hazard in regard to public health, it would seem that the solution of this problem depends a great deal upon the isolation and identification of the causal agent of each outbreak. A method has been presented

here for the isolation of members of this group from infected fecal material. It is an adaptation from some of those used in the routine isolation of members of the typhoid group from humans. It was found that, as an enrichment, tetrathionate broth offered the best possibilities, and that a combination of differential mediums to be used with the enrichment broth would aid in the recovery of organisms which may have been overlooked or overgrown by the coliform organisms which are present in such abundance in all fecal material. As yet no perfect medium has been devised for the isolation of these organisms from fecal material. Brilliant green has perhaps been used most widely and with greatest success thus far. Tetrathionate broth offers a great many advantages over this former medium because of the technical simplicity with which it can be prepared. It was found in this study that this medium as well as the bismuth sulphite agar could be kept in stock in the refrigerator for several days without apparently lessening the selectivity of either medium.

A discussion of diseases due to Salmonella infection is beyong the scope of this paper, but it seems appropriate to point out that practically all of the species named here have been found in human infections, and their presence in the intestinal contents of these unhealthy birds, is therefore, of interest from an epizoological standpoint. Much more work remains to be done yet in the investigation of natural reservoirs of infection for this group of organisms.

Summary and Conclusions

1. The methods used for the isolation of paratyphoids from humans were found applicable to avian tissues. A combined method using tetrathionate broth as an enrichment medium and either MacConkey's agar or bismuth sulphite agar as differential mediums is presented.

2. The need of a selective enrichment medium to allow the growth of the paratyphoids is demonstrated. This need is made apparent from the results obtained where both organisms of the paratyphoid group and Esch. coli group are mixed. The latter obscures or overgrows the paratyphoid organisms when no inhibitory agent is present.

3. A selective diagnostic medium for isolation is necessary because of the similarity in morphology of the two groups of organisms.

4. A total of 204 diseased birds were represented in this study. From 48 of these 72 strains of organisms were isolated from the intestinal tract which according to their biochemical and serological reactions belong to the Salmonella group. Salmonella pullorum was the predominating organism, 35 of the strains identified as this species.

5. Of the strains isolated S. california, S. urbana, S. aberdeen, and S. hvittingfoss have not, we believe, been reported from chickens.

LITERATURE CITED

1. Hadley, P., Elkins, M.W. and Caldwell, D.W., "The Colon Typhoid intermediates as causative agents of disease in birds. I The Paratyphoid bacteria," R.I.Agric. Exp. Sta. Bulletin 174, 1918.
2. Mulson, F.W., "The Differentiation and Distribution of the Paratyphoid-Enteritidis Group VI Avian paratyphoid: A comparative study of B. pullorum and B. sanguinarium" Jour. Inf. Dis., 25: 135, 1919.
3. Spray, Ribb S., Doyle, Leo P., "Paratyphoid bacilli from chicks," Jour. of Inf. Dis., 28: 43-47, 1921.
4. Doyle, T.M., "B. aertrycke infection of chicks," Jour. of Comp. Path. and Therapy, 40: 71, 1927.
5. Edwards, P.R., "A fatal infection of chicks due to Bacilli of the paratyphoid B group," Jour. Inf. Dis., 45: 191, 1929.
6. Schalm, O.W., "Study of paratyphoid infection in chicks," Jour. Inf. Dis., 61: 208, 1937.
7. Van Roekel, H, and Bullis, K.L., "Salmonella infections in chickens," Jour. Am. Vet. Med. Ass'n N.S., 44: 48, 1937.
8. Edwards, P.R., "A New Salmonella type: S. kentucky," Jour. Hyg. 38: 306, 1938.
9. Edwards, P.R., Bruner, D.W., "Two new Salmonella types isolated from fowls," Jour. Hyg., 38: 716, 1938.
10. Jungherr, E. and Clancy, D.F., "Serological types of Salmonella isolated from paratyphoid in chicks, " Jour. Inf. Dis., 64: 1, 1939.
11. Ibid.
12. Ryff, J. F., unpublished data.
13. Emmel, cited by Edwards, P.R., "Paratyphoid Infections of Fowls," Jour. Am. Vet. Med. Ass'n., 90: 403, 1937.

14. Fitch, C.P. and Pomeroy, B.S., "Value of Accurate diagnosis of poultry diseases," Vet. Med., 34: 693, 1939.

15. Emmel, M.W., Thesis: A Study of the Bacterial Flora of the Feces and Intestinal Contents of the Fowl, June 1930.

----------"The Etiology of Fowl Paralysis, Leukemia and allied conditions in animals. III The Intestinal Flora of chickens affected with Enteritis associated with Intestinal Parasitism, "Fla. Ag. Ex. Sta. Tech. Bull., 293: 1936.

16. Topley and Wilson.

17. Browning, C.H., Gilmour, W. and Mackie, T.J., "Isolation of typhoid bacilli from feces by means of brilliant green in fluid medium," Jour. Hyg., 13: 335, 1913-14.

18. Krumwiede, Chas. J., Pratt, Josephine S. and McWilliams, Helen, "Use of Brilliant Green for the Isolation of Typhoid and Paratyphoid bacilli from feces," Jour. Inf. Dis., 18: 1, 1916.

19. Rakieten, Morris L. and Rettger, Leo F. "Simple Modification of the Brilliant Green Methods of Isolating Bacterium Typhosum from Feces," Jour. Bact., 11: 103, 19-1926.

20. Torrey cited by Krumwiede: Jour. Exp. Med., 19, 501, 1914.

21. Torrey, J.C. "A comparatively simple technic for the bacteriological study of fecal material," Jour. Inf. Dis. 39: 351, 1926.

22. Mallmann, W.L., Thorp Frank and Semmes, Margaret, "A Medium for the Isolation of Salmonella pullorum and Other Members of the Paratyphoid Group from Avian Tissues," Jour. Am. Vet. Med. Ass'n., LXXIII, N.S., 26: 825, 1928.

23. Kerr, W.R., "Selective Media for cultivation of B. pullorum and B. sanguinarium," Jour. Comp. Path. and Ther., 43: 77, 1930.

24. Teague and Clurman: cited by Jones, E.R., Jour. of Path. and Bact., 42:455, 1936.

25. Kauffmann, F., "A combined enrichment method for typhoid and paratyphoid bacilli," Cent. Bakt. I. Abt. Orig., 113: 148, 1930-31.

26. Jones, E.R. "The Use of Brilliant Green Eosin Agar and Sodium Tetrathionate Broth for the Isolation of Organisms of the Typhoid Group," Jour. Path. and Bact., 42: 455, 1936.

27. Khahil, A.M., "Incidence of Organisms of the Salmonella Group in Wild Rats in Liverpool," Jour. Hyg., 38: 75, 1938.

28. Gauger, H.C., Greaves, R.E., Cook, F.W., "Paratyphoid in Pigeons."

29. Guth cited by Leifson.

30. Leifson, E., "New Selenite medium enrichment media for the isolation of typhoid and paratyphoid bacilli," Am. Jour. of Hyg., 24: 423, 19--1936.

31. Gray, J.D., "New Lithium Selective and Enrichment Methods for the isolation of Salmonella organisms," Jour. Path. and Bact., 34: 335, 1931.

32. Wilson, W.J. and Blair, E.M., "Combination of Bismuth and Sodium Sulphite affording an enrichment and selective medium for the Typhoid-paratyphoid groups of bacteria," Jour. Path. and Bact., 29: 310, 1926.

33. Emmel - see no. 15-16.

34. Edwards, P.R. and Bruner, D.W., "A New Salmonella type from turkeys: S. california," Jour. Inf. Dis., 66: 127, 1940.

35. Edwards, P.R. and Bruner, D.W., "Two new Salmonella types isolated from fowls," Jour. Hyg., 38: 716, 1938.

36. Edwards, P.R., "Occurrence of Salmonella, oranienburg type, in an infection in Quail," Jour. Bact., 32: 259, 1936.

37. Spray, R.S., and Doyle, L.P., "Paratyphoid bacilli from chicks," Jour. Inf. Dis., 28: 43, 1921.

38. Edwards, P.R., "Paratyphoid Infection of Fowls," Jour. Am. Vet. Med. Ass'n, 90: 403, 1937.

39. Smith, J., "Sporadic Salmonella Infections: A new Salmonella type," Jour. Hyg., 34: 353, 1934.