# ESCHERICHIA COLI INFECTION IN CHICKENS

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# ESCHERICHIA COLI INFECTION

# IN CHICKENS

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# ESCHERICHIA COLI INFECTION

# IN CHICKENS

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# ESCHERICHIA COLI INFECTION IN CHICKENS

On July 4, 1947 it was reported by the Regional poultry Research Laboratory, United States Department of Agriculture, East Lansing, Michigan, that a few chickens had died suddenly from which a bipolar organism resembling Pasteurella avicida morphologically had been isolated. Very little was known about this case except that the birds appeared to be depressed; they showed no diarrhea or other intestinal disturbance. These birds died three days later and on autopsy, petechiae on the heart muscle and the gizzard were the only lesions observed.

Nine broth cultures containing the isolated bacteria were brought to the Department of Bacteriology, Michigan State College. Cultures I 266VTRWS, I409G liv and I409 HT were submitted first; and later six cultures F1111S, F468Q, F266B, F2SSW$_2$IV liv, H107SGIP and H113L$_2$ liv.

The purpose of this work was: 1. to identify the organism, 2. to determine its pathogenicity for chickens and 3. to ascertain whether it had any antigenic relationship to Salmonella pullorum.

# REVIEW OF LITERATURE

Klein (1889) described an infectious disease of grouse (Lagopus scoticus), caused by Bacterium coli, characterized by congestion of the lungs, necrotic areas in the liver and patchy redness of the intestines. These findings were verified by Smith. Ligniers (1894) examined a number of hens which died showing diarrhea but no other symptoms. At autopsy he observed generalized inflammation of the intestinal tract and a quantity of serous fluid in the body cavity together with enlargement of the spleen and liver. From the spleen he isolated a culture which appeared to be of the B. coli type. Sanfelice (1895) reported a disease among pigeons in Sardinia from which an organism resembling B. coli was isolated. In the dead birds the prominent pathological features included fibrinous exudate in the body cavity, and over the surface of the intestines, also enlargement of the spleen. However, he did not present data on the biochemical features of the organism. Fiorentini (1896) isolated from a swan affected with hemorrhagic septicemia an organism resembling P. avicida but differing from it. He stated that it grew like Bacterium coli communis on solid agar. His description suggested that it was a virulent colon bacillus. Martel (1897) isolated a virulent colon bacillus from hens and turkeys. On autopsy he found pericarditis, with an abundant false membrane without much liquid, congestion and friability of the spleen, inflammation of the small intestine and the ceca and suppurative conjunctivitis. From the blood, liver, false pericardial membrane and the conjunctival exudate he isolated a

bacillus, and upon studying it culturally and biochemically, proved it to be a colon bacillus. Dawson (1898) investigated a coli-like organism, isolated from sick birds in the vicinity of Washington, D. C., which produced acid and gas in lactose, dextrose and sucrose broth. The symptoms of the disease were: emaciation and voracious appetite lasting about three months before fetal issue. "Going light" or infectious asthenia was the name he gave to this disease. Mazza (1899) observed an outbreak of hemorrhagic enteritis among hens that was probably caused by B. coli. Joest (1902) isolated B. coli and B. intestinale gallinarum from the heart, blood, spleen and liver of dead hens. Morse (1906) studied a contagious disease of quail caused by B. coli. The post-mortem revealed slightly congested lungs, and a few areas of superficial necrosis in the liver, the intestines being studded with minute ulcers, the spleen was always congested. The organism was usually cultivated from the liver in which lesions existed. Claussen (1907) found pure cultures of colon bacilli in the blood of dead birds. This organism was very similar to Past. cholera gallinarum but appeared about twice its size. Upon dissection, the heart was found to be filled with black, partially clotted blood and the epicardium was covered with small petechiae. The intestinal mucosa appeared slightly inflamed and the parenchyma of the liver was slightly darkened. Hadley (1910) isolated B. coli from the spleen, liver and heart blood of sick birds affected with a cholera-like disease. High temperature and yellowish diarrhea were the symptoms observed. Upon examination, the liver was hyperemic and soft, the spleen was enlarged and the hemorrhagic inflammation extended through the entire intestine. Zeiss (1914)

reported two cases of coli septicemia in hens. The organism isolated from this case exhibited oval, elliptical and polar-stained rods which were very pathogenic for canary birds. Baudet (1922) isolated from the carcass of a hen a virulent strain of B. coli which killed mice and pigeons in two days after subcutaneous injection of 1 loopful of the cultures and chickens in a few days after intramuscular injection. Palmer and Baker (1922) found that Bacterium coli communis was the causative organism of an infectious enteritis of chickens, ducks and turkeys in Northern and Central Delaware. May and Tibbetts (1923) reported two bacterial cultures isolated from diseased poultry in Rhode Island. One of them originally came from Rahn of the Michigan Agricultural College. No further information accompanied the culture as to the symptom of the disease and the extent of the epidemic. They suggested that this organism might be a strain of B. coli. In 1928, an acute generalized E. coli infection in chicks was reported by Kansas Agricultural Experiment Station. The symptoms were sudden onset of pulmonary dyspnea, accompanied by marked depression. Lewis and Hitchner (1936) described a slow lactose fermenting organism pathogenic for chickens. This organism was proved distinctly pathogenic for young chicks when given orally or subcutaneously. Davis (1938) encountered colibacillosis in chickens in Maryland. Upon autopsy, the livers were about 50% larger than normal, mottled and contained numerous small white spots. The heart was slightly enlarged and the pericardial sac was markedly distended with amber colored fluid. He attributed the etiology of this disease to faulty incubation, insufficient moisture and improper ventilation. Twisselmann (1939)

reported that seventeen birds of a well isolated group of two hundred and fifty pullets were attacked by a rapidly fatal disease of which he stated, E. coli was the etiological agent. Autopsy showed the crops of several birds to be partially filled with grain. The pectoral muscles had a reddish congested appearance. The myocardium was degenerated and in some there was a fibrinous pericarditis. The liver showed congestion with small areas of degeneration and, in some cases, a slight greenish cast. Bunyea and MacDonald (1941) studied the pathogenicity of E. coli acidi lactici for turkeys, and considered it responsible for young poult mortality. Osborne, Witter and Hitchner (1947) studied chronic colibacillosis of fowls. The organism which they studied was proved to be very pathogenic for guinea pigs, rabbits and chicks. Durant and MacDougle (1947) found E. coli in the blood of adult fowl affected with the occular form of fowl paralysis. When 1 ml. amounts of the blood taken from the affected birds were injected into day old chicks, 84 of 85 chicks died.

# EXPERIMENTAL WORK

The organism submitted by the Regional Poultry Research Laboratory was identified by its morphological characteristics and biochemical properties.

For the determination of pathogenicity, chicks were fed a suspension of the living organism. Normal chicks were placed in the same cage with the exposed birds as controls.

A normal rooster was immunized with a killed suspension of the isolated organism. Cross agglutination tests were made using the immune serum from the rooster against S. pullorum antigen, and S. pullorum immune serum against the organism being tested.

For convenience, the procedure and results of each experiment will be presented separately.

## Identification of the Organism

Morphology and cultural characters. Smears were made from the original cultures. By using Burke's modification of Gram's stain, the organism was found to be a gram negative, slender, pleomorphic, short rod. It stained unevenly, but was not bipolar. No spores or capsules were found. The size of this organism was about 0.5 $\mu$ by 2 $\mu$.

Single, isolated colonies for further identification were obtained by plating the original cultures on blood agar and S S agar plates. At the same time the three original cultures (I 266VTRWS, I 409G liv. and I 409 HT) were injected into three chickens (three months old) designated

by leg bands No. 27778, 13715 and the third without band. The routes used for injection were intraperitoneal, subcutaneous and intravenous respectively. Two days after injection all these birds appeared dull, however there was no other symptoms to be observed. Five days later the birds were destroyed and autopsied. Cultures were made from the heart and liver of each bird.

The changes observed in these birds were: pericarditis, focal necrosis in the liver, congested kidneys and peritonitis. In addition bird No. 27778 showed petechiae in the intestines and bird No. 13715 showed diffuse enteritis.

The original nine cultures and the cultures isolated from the three inoculated chickens produced pale, moist, medium size, convex, round, regular, non-hemolytic colonies on blood agar plates. Culture I 409 HT did not grow. On S S agar plates, small, scanty, pink convex colonies were occasionally found from cultures F1111S, F468Q, H107SQIP liv. and I 266 V TRWS.

Fourteen separate colonies were picked from the blood agar plates and inoculated into lactose motility agar tubes. Each one showed motility and fermented lactose after 24 hours incubation except Nos. 5 and 11 which showed beaded growth along the stab line.

Table I shows the fermentation reactions of these organisms.

TABLE I

Fermentation Reactions in five common Sugars

| Culture number | S | l | ml | mn | s |
|---|---|---|---|---|---|
| 1 | AG | AG | AG | AG | - |
| 2 | AG | AG | AG | AG | - |
| 3 | AG | AG | AG | AG | - |
| 4 | AG | AG | AG | AG | - |
| 5 | A | A | A | -- | A |
| 6 | AG | AG | AG | AG | - |
| 7 | AG | AG | AG | AG | - |
| 8 | AG | AG | AG | AG | - |
| 9 | AG | AG | AG | AG | - |
| 10 | AG | AG | AG | AG | - |
| 11 | A | ± | -- | -- | A |
| 12 | AG | AG | AG | AG | - |
| 13 | AG | AG | AG | AG | - |
| 14 | AG | AG | AG | AG | - |

S = dextrose
l = lactose
ml = Maltose
mn = mannitol
s = sucrose

- = no fermentation
A = acid
AG = acid and gas
± = slight acid with insignificant pink color

These results show that the reactions of twelve of the fourteen cultures were identical. The twelve identical cultures were stained and found to be morphologically similar to the original cultures. Nos. 5 and 11 were gram positive streptococci and diplococci respectively, possibly contaminants and were discarded. For more exact identification, four cultures were selected for further studies, namely, 1, 3, 8 and 13 which were isolated from birds 13715, the bird without band, 27778 and original culture F 468 Q.

Additional cultural and biochemical characteristics of the four selected cultures were studied using eosin-methylene-blue agar plates, Kligler's iron agar slants, methyl-red tests. Voges - Proskauer tests, indol tests, nitrate reduction tests, gelatin liquefaction tests, citrate utilization tests, litmus milk coagulation tests and fermentation tests.

The eosin-methylene-blue agar, Kligler iron agar and Simmon citrate agar were Difco Bacto dehydrated media.

The results of these tests are summarized in Table II.

# TABLE II

## Biochemical Characteristics

| | | Culture Number 1 | 3 | 8 | 13 | |
|---|---|---|---|---|---|---|
| Kligler's iron agar | Slant | A | A | A | A | |
| | Butt | A.G. | A.G. | A.G. | A.G. | |
| | $H_2S$ production | - | - | - | - | |
| E.M.B. Agar | | M.S. | M.S. | M.S. | M.S. | |
| Citrate Agar | | - | - | - | - | |
| Litmus milk | | A.C.P. | A.C.P. | A.C.P. | A.C.P. | |
| M.R. test | | + | + | + | + | |
| V.P. test | | - | - | - | - | |
| Indol test | | + | + | + | + | |
| Nitrate Reduction test | | + | + | + | + | |
| Gelatin liquefaction test | | - | - | - | - | |
| Adonite | | - | - | - | - | |
| Arabinose | | A.G. | A.G. | A.G. | A.G. | |
| Cellubiose | | - | - | - | - | |
| Cellulose | | - | - | - | - | |
| Dextrin | | A | A | A | A | very slight acid reaction |
| Dextrose | | A.G. | A.G. | A.G. | A.G. | |
| Dulcitol | | A.G. | A.G. | A.G. | A.G. | a little gas; produced alkali 3 days after acid formation |

TABLE II (continued)

Biochemical Characteristics

| | Culture Number 1 | 3 | 8 | 13 | |
|---|---|---|---|---|---|
| Galactose | A.G. | A.G. | A.G. | A.G. | |
| Glycerol | A.G. | A.G. | A.G. | A.G. | slow gas formation on the 3rd day after incubation |
| Inosite | - | - | - | - | |
| Inulin | - | - | - | - | |
| Lactose | A.G. | A.G. | A.G. | A.G. | |
| Levulose | A.G. | A.G. | A.G. | A.G. | |
| Maltose | A.G. | A.G. | A.G. | A.G. | |
| Mannitol | A.G. | A.G. | A.G. | A.G. | |
| Mannose | A.G. | A.G. | A.G. | A.G. | |
| Raffinose | - | - | - | - | |
| Rhamnose | A.G. | A.G. | A.G. | A.G. | a little gas |
| Salicin | - | - | - | - | |
| Sorbitol | A.G. | A.G. | A.G. | A.G. | a little alkali produced 2 days after the acid formation |
| Starch | - | - | - | - | |
| Sucrose | - | - | - | - | |
| Trahalose | A.G. | A.G. | A.G. | A.G. | |
| Xylose | A.G. | A.G. | A.G. | A.G. | |

A = acid
G = gas
A.G. = acid and gas
M.S. = growth with metallic sheen
A.C.P. = acid curd and peptonization
+ = positive reaction
- = negative reaction

The fermentation reactions in dulcitol, salicin and sorbitol were variable when the cultures were kept for a longer time. Culture 3 produced a prominent quantity of alkali in three days. Culture 8 produced a rather abundant quantity of acid and gas in three days. All these four cultures reversed their action on dulcitol on the fourth day and at last neutralized the acid produced by the organism. Repetitions of the fermentation tests on dulcitol, salicin and sorbitol have been made, all showed the same results as before.

In litmus milk they produced acid on the third day, and peptonization on the fifth day.

The results when compared with the description of E. coli in Bergy's manual (5th edition) would indicate that the organism studied can be identified as Escherichia coli.

## Pathogenicity Tests on Chicks

Cultures 1, 3, 7, 8, 10 and 14 were chosen as the test organisms. Among these, Nos. 1, 3, 7 and 8 were reisolated from laboratory birds. Culture 1 was reisolated after subcutaneous injection. Culture 3 was reisolated after being given intravenously. Culture 7 was obtained from the pericardial sac of an intraperitoneally injected bird. Culture 8 was reisolated from the peritoneal fluid of an intraperitoneally injected bird. Twelve, normal two-day old chicks were selected as experimental birds. A saline suspension of each of the above mentioned cultures, standardized to a turbidity equal to that of Tube No. 1 MacFarland's nephelometer, was prepared from a 24 hours tryptose agar slant culture. One ml. of each

suspension was fed to each chick as shown in table III. These chicks were kept with 25 untreated chicks as controls.

After observation for two months; four chicks were dead as shown in table III. As a rule, one or two days before death, the birds refused to eat and drink, and walked with staggering gaits. Gram negative rods were isolated from venous blood of one bird before his death. The organism was identified as E. coli. Symptoms were always observed for two days except in the case of No. 3216 which showed sickness for two days and then seemed to have recovered. However, it died suddenly five days later.

Autopsies were made on each dead bird. Congestion of the lungs, peritonitis and cloudy swelling of the liver were the common pathological changes, except for slight inflammation of the jejunum in bird No. 3227. Cultures were made from lungs and livers. Through microscopic examination, EMB agar plates cultivation, M.R. tests, V. P. tests and indol tests these organisms were found to be identical with E. coli.

No control birds died, except one which died from ruptured liver. Cultures from the liver of the chick yielded no organism.

## TABLE III

### Results of Pathogenicity Tests by Feeding Cultures to Chicks

| Chick Number | Culture Number being fed | Date of feeding | Date of death | Survived days |
|---|---|---|---|---|
| 3209 | 1 | Oct. 24 '47 | | 60 |
| 3214 | " | " | | 60 |
| 3215 | 3 | " | | 60 |
| 3216 | " | " | Nov. 2 '47 | 8 |
| 3226 | 7 | " | Nov. 15 '47 | 21 |
| 3227 | " | " | Oct. 28 '47 | 3 |
| 3228 | 8 | " | | 60 |
| 3251 | " | " | | 60 |
| 3252 | 10 | " | | 60 |
| 3253 | " | " | | 60 |
| 3254 | 14 | " | | 60 |
| 3255 | " | " | Oct. 26 '47 | 1 |

## Cross Agglutination Tests

Preparation of E. Coli Antiserum. Two strains of S. pullorum, No. 1-137-5 isolated from a turkey and No. 119-3 from a baby chick, were obtained from Mrs. Ruth Gunn, Instructor of Bacteriology, Michigan State College. One rooster and samples of pullorum positive serum and pullorum negative serum were obtained from Dr. Saul Narotsky, Instructor of Bacteriology, Michigan State College. Before carrying out the immunization, 8 ml of blood was drawn, the serum separated and placed in a refrigerator. Agglutination tests performed with these sera proved the bird to be negative to the pullorum test. E. coli No. 1 S. pullorum No. 1-137-5 and No. 119-3 were seeded on tryptose agar slants and incubated for 24 hours. The antigens were prepared by washing off the growth with saline (pH 8.25), heating in a water bath at 56° C for 1 hour and washing three times. The turbidity of all antigens used for the agglutination test was adjusted to equal that of Tube No. 1 of MacFarland's nephelometer.

In order to identify cultures No. 1-137-5 and 119-3 as S. pullorum and to prove that the serum of the rooster was free from pullorum antibodies and anticoli antibodies agglutination tests were performed with three kinds of sera and three kinds of antigens as shown in table IV.

## TABLE IV

### Preliminary Antigenic and Serological Testing

| Sera | Antigens | Dilutions 20X | 40X | 80X | 160X | 320X | 640X | Control |
|---|---|---|---|---|---|---|---|---|
| Serum of rooster which was to be used for immunization | S. pullorum No. 1-137-5 | - | - | - | - | - | - | - |
| | S. pullorum No. 119-3 | - | - | - | - | - | - | - |
| | E. coli | - | - | - | - | - | - | - |
| Pullorum positive serum | S. pullorum No. 1-137-5 | ++++ | ++++ | ++++ | +++ | +++ | +++ | - |
| | S. pullorum No. 119-3 | ++++ | ++++ | ++++ | +++ | +++ | +++ | - |
| | E. coli | ± | - | - | - | - | - | - |
| Pullorum negative serum | S. pullorum No. 1-137-5 | - | - | - | - | - | - | - |
| | S. pullorum No. 119-3 | - | - | - | - | - | - | - |
| | E. coli | ± | - | - | - | - | - | - |

++++ = complete agglutination

+++ = fairly strong agglutination

± = doubtful reaction

- = no reaction

The preliminary tests proved the rooster to be satisfactory for the purpose of immunization. Antigen of E. coli was prepared as described above. The rooster was injected intravenously four times at one-day intervals starting with 1 ml of the suspension, followed by two injections of 2 ml at one-day intervals.

The rooster was bled seven days after the last injection and the serum separated. Agglutination tests were made with E. coli antigen. This antigen was prepared by seeding E. coli No. 1 on tryptose agar slants and incubating for 24 hours. The growth was washed off with physiological saline solution (pH 8.25) containing 0.5% phenol and adjusted with physiological saline solution (pH 8.25) containing 0.3% phenol to the turbidity of Tube No. 1 MacFarland's nephelometer. The results are shown in Table V.

TABLE V

Titration of Immune Serum

| Serum | Antigen | 20X | 40X | 80X | 160X | 320X | 640X | 1280X | 2560X | Cont. |
|---|---|---|---|---|---|---|---|---|---|---|
| E. coli immune serum of rooster | E. coli | ++++ | ++++ | +++ | +++ | ++ | + | + | + | - |

It was shown that the agglutinating titer of the rooster's serum was 4 + up to 160X and fairly strong in 320X etc. The E. coli antiserum was therefore satisfactory for agglutination tests.

Antigens of S. pullorum No. 1-137-5, 119-3 and E. coli No. 1 were then prepared by the same method as described before and cross agglutination tests were made with E. coli antiserum and pullorum positive serum. The results are shown in table VI.

TABLE VI

Cross Agglutination Tests

| Sera | Antigens | Dilution 20X | 40X | 80X | 160X | 320X | 640X | Control |
|---|---|---|---|---|---|---|---|---|
| E. coli imm. serum | S. pullorum 1-137-5 | + | ± | ± | - | - | - | - |
| E. coli imm. serum | S. pullorum 119-3 | + | + | - | - | - | - | - |
| Pullorum positive serum | E. coli No. 1 | + | + | - | - | - | - | - |

- = negative reaction

± = doubtful reaction

+ = weak positive reaction

This procedure was repeated with greater care, because the results obtained were so indefinite. The antigens were centrifuged and washed four times. Three clear, satisfactory suspensions of antigens were obtained and cross agglutination tests were made. The results are shown in Table VII.

TABLE VII

Final Cross Agglutination Tests

| Sera | Antigens | Dilution 20X | 40X | 80X | 160X | 320X | 640X | Control |
|---|---|---|---|---|---|---|---|---|
| E. coli imm. serum | S. pullorum 1-137-5 | + | - | - | - | - | - | - |
| E. coli imm. serum | S. pullorum 119-3 | ± | - | - | - | - | - | - |
| Pullorum Positive serum | E. coli No. 1 | + | + | - | - | - | - | - |

- = negative reaction

± = doubtful reaction

+ = faint positive reaction

These results show that there was no significant antigenic relationship between this strain of E. coli and S. Pullorum.

# DISCUSSION

Strictly speaking, E. coli is but an opportunist. It can cause dysentery and navel infection in calves, pyelonephritis, cervicitis and mastitis in cows and wound infections in various animals when the environment favors its growth. Also it has been recovered from eggs of hens affected with fowl paratyphoid. It was found in the liver of quail suffering from paratyphoid.

Summing up the data from all the cases cited in the review of literature, concerning the nature of the disease caused by E. coli, it seems reasonable to assume that this organism has a predilection for the mucous membranes of the digestive tract and the parenchymatous organs, such as the liver, spleen and heart. This organism also causes septicemia. The case encountered in our laboratory was very similar to that described in Maryland.

According to the pathogenicity tests performed in this work, it has been shown that culture No. 7 will kill chicks when given orally or injected intraperitoneally.

Considering the history of the case, this organism might be assumed to be very virulent, but it did not prove to be so by virulence tests. The reasons for this disagreement might be: 1. bacterial variation 2. natural field infection may differ from experimental infection 3. lack of necessary contributory factors 4. different susceptibility of individual birds.

It is noteworthy that there has been no outbreak of bacterial disease in the stock of the Regional Poultry Research Laboratory in

East Lansing during its existence until this one occurred. Therefore some support is given to the thought that the strain of E. coli isolated from the affected birds may have been the cause.

In regard to the serological relationship between E. coli and S. pullorum, some information is available. According to the Kauffmann-White schema "Bact. coli 3" possesses a part of the O antigen found in S. pullorum, and numerous other coliform bacilli have the same antigen.

Stamp and Stone[1] have described the presence of a common antigen in many members of the coliform and paracolon group. It is more heat-stable than the H antigen and less stable than O antigen. It is inactivated at 100° C in 15 minutes but withstands a temperature of 75° C for 1 hour. It is associated with recently isolated strains, and tends to be lost on subculture. Thus it is doubtful whether this antigen was retained by the organism under investigation. The antigenic composition of the coliform bacilli has not been completely determined.

The cross agglutination tests revealed no significant antigenic relationship between E. coli No. 1 and S. pullorum.

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[1] Wilson, G. S. and Miles, A. A. 1946 Topley and Wilson's Principles of Bacteriology and Immunity. 3rd. ed. The Williams & Wilkins Co., Baltimore, p. 666.

## SUMMARY

Some strains of an organism which proved to be E. coli were isolated from chickens affected with a cholera-like disease.

This organism proved to be moderately pathogenic for chicks when administered orally.

Cross agglutination tests with S. pullorum revealed no significant antigenic relationship between these two organisms.

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