

A BACTERIOLOGICAL STUDY, WITH SPECIAL REFERENCE TO
SALMONELLA PULLORA, OF EGGS LAID BY HENS REACTING
POSITIVELY TO THE MACROSCOPIC AGGLUTINATION
TEST FOR SALMONELLA PULLORA.

Minor Thesis in Animal Pathology

prepared by
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June 1926

For the degree of Master of Science

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Introduction

Many investigators have made a bacteriological study of fresh eggs, but few have done so with special reference to *Salmonella pullora* infection.

This organism was first isolated by Rettger in 1899 and was given the name *Bacterium pullorum* in 1909. At this time the cycle of infection was fairly well known. The adult hen was recognized as the primary source of the infection, eliminating the organism through the yolk of the egg and thus to the chick. Since 1909, however, a few investigators have carried on work to determine the frequency of the presence of *Salmonella pullora* in eggs selected at random. A few also have made a bacteriological study, with special reference to *Salmonella pullora*, of eggs laid by hens reacting to the macroscopic agglutination test for *Salmonella pullora*. Therefore, a study with this aim in mind is important from two points of view,

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first to determine how frequently the organism is eliminated and whether all birds reacting to the agglutination test eliminate the organism.

Historical Review

Rettger and Stoneburn (1) in 1909 examined eighty six incubated eggs from different varieties of fowls to determine the presence of *Bacterium pullorum*. Eight were found to contain the organism and five were questionable. All the infected eggs came from two varieties of hens. Twenty six of these eggs came from one variety, seven of which contained the organism. Six eggs came from the other variety, one of which contained the organism.

In 1911, Rettger and Stoneburn (2) reported on a study of fresh eggs to determine the presence of *Bacterium pullorum*. A large number of eggs from different sources were examined where the disease had and had not been present. The organism was isolated from the yolks of eggs which came from the hens where the disease had been reported. The organism was not isolated from the eggs that came from the birds where the disease had not been reported. Eggs from badly infected

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birds hatched poorly and very few of the chicks survived, white diarrhea being apparent from the time of hatching. Not all of the eggs, produced by hens having infected ovaries, contain *Bacterium pullorum*. In cases where the ovary is badly infected a large number of eggs may be infected. As high as 75% of the eggs laid by certain hens were found to be infected. In other cases only a relatively small number were found to contain the organism. Eggs from a large number of hens were examined by both the fresh and incubated method with essentially the same results.

Rettger, Kirkpatrick, and Stoneburn (3) 1912 stated that one means of eliminating the infected stock was by bacteriological examination of incubated eggs from trap nested hens. A certain shipment of eggs, received at their laboratory, was examined and twenty per cent of the eggs tested were found to contain the organism.

Rettger (4) 1913 reported his findings on the bacteriology of the hen's egg for a period of three years. He made a bacteriological study of 3510 fresh eggs. The entire yolk was examined and 169 yolks of the 3510 eggs harbored

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Bacterium pullorum. A large number (1746) of eggs were artificially incubated for one week. Upon examination, twenty eggs of the 1746 contained Bacterium pullorum. A total number of 2166 were incubated for two weeks, twenty eight of which contained Bacterium pullorum. One hundred eight eggs out of 1984 which were incubated for three weeks contained Bacterium pullorum. He further states that the contents of normal fresh eggs are as a rule sterile. In case the ovary is infected with Bacterium pullorum the organism may invade the egg before it leaves the ovary.

In 1913, Jones (5) reported a case where a high mortality of chicks from bacillary white diarrhea was encountered and the eggs that failed to hatch were fed to the adult hens. A loss of fifty hens from bacillary white diarrhea was experienced.

Gage, Paige and Hyland (6) in 1914 found that the organism was more readily isolated from incubated eggs than from fresh eggs. It was observed that the organism was not found consistently in the eggs of infected hens and that as many as 21 eggs laid consecutively were examined before the organism was found. Hens whose eggs did not reveal the presence of the

infection likewise did not show evidences of infection upon autopsy. the yolk while held in the ovary or the oviduct.

These findings. In 1914, Rettger (7) reported that one of the most striking symptoms of the disease in baby chicks was the protrusion of the abdomen below the vent due to failure in absorption of the yolk. Bacteriological examination of the contents of the yolk sac invariably revealed *Bacterium pullorum*. It was assumed that the yolk played an important role in bringing about the disease in the chick while still in the egg. Eggs which were in various stages of incubation and also perfectly fresh eggs were found to harbor the organism in the yolk. This method would yield an infection of only 1.5

per cent. Birds which possess pathological ova may lay through an entire laying season or longer. It is probably that the most active layers are the most susceptible to ovarian infection. An inactive or dormant ovary is less apt to be disturbed. It was noted that only a comparatively small number of the eggs that are laid by fowls having abnormal ovaries contained the organism of bacillary white diarrhea. He found that the organism is present only in small numbers in eggs that are infected. Little or no reproduction of the organism takes

place. This method showed an infection of the ovary by this organism.

place in the yolk while held in the ovary or the oviduct. These inhibitive influences of the yolk are decreased after the egg is laid. Eggs from normal fowls were found, with rare exceptions, to be sterile.

In 1916, Hadley and Caldwell (8) reported that no correlation was observed between percentage of infection and hatchability. Of the 2,520 fresh eggs examined, 8.7 per cent showed bacterial infection in the yolk. From these infected eggs, 40 bacterial types were obtained.

In 1924, May (9) found that eggs examined under strict aseptic methods, would yield an infection of only 1.6 per cent. The source of these eggs was from hens infected with or immunized against *Bacterium pullorum*, *Bacterium gallinarum* and *Pastuerella aviseptica*. The author did not find an increase in egg infection due to inoculation or infection of the hens. The inoculated organism was in no case transmitted through the yolks of the eggs. Three birds revealed *Bacterium pullorum* upon autopsy but it was never detected in their eggs. All the hens either fed or inoculated with living cultures of typical *Bacterium pullorum* and autopsied showed an infection of the ovary by this organism.

Source of the Eggs

Fifteen white leghorns were secured for this experiment, all of which reacted to the macroscopic agglutination test. These same hens were used for another project in conjunction with this one. The eggs from these hens were used for both projects. The eggs cultured in this experiment included fresh eggs and those that were removed from the incubator on the 7th, 14th and 21st days of incubation, due to their infertility and their failure to hatch. The hens were trap-nested and the identity of each egg was kept as regards date laid and leg band number of hen producing egg. The hens were housed and fed according to modern methods of poultry husbandry.

Procedure

Method of Egg Examination. The eggs in this experiment were examined by a method which is a modification of two or three methods that were employed by Rettger, Hadley, and May. The fresh eggs were incubated at 37° centigrade for 72 hours. The incubated eggs, which were candled out at the various stages of incubation, were cultured immediately after being removed from the incubator. Each egg was given a culture number. The eggs

were cleaned, if dirty, and placed in 5% phenol solution for ten minutes. The culture work was carried on in a transferring chamber to eliminate contamination as much as possible. The eggs were transferred from the phenol solution to a clean sterile dish covered with gauze moistened with phenol. The hands of the technician were thoroughly cleaned before each culturing period. The egg was grasped between the thumb and index finger. One end of the egg was sterilized in a gas flame. The sterilized portion of the shell was punctured with a sterile sharp probe and the opening made larger with a sterile forceps. The albumen of the fresh eggs was poured out of the shell into a container. The albumen of the incubated eggs was added to the broth along with the yolk on account of the difficulty in separating the two. The egg was placed on the mouth of the culture bottle with the open end down. By applying the flame to the side of the egg, the yolk was forced into the bottle. Sometimes the vitelline membrane did not rupture when the heat was applied. The whole yolk was then punctured in the bottle with a sterile probe. The culture bottles contained from 30 to 40 c. c. of sterile broth and 2 c. c. of Schardingus' reagent which is a very dilute solution (1/20,000) of methylene blue. The contents

was thoroughly mixed and incubated for seven days at 37 degrees centigrade. If growth was present the reagent would be reduced and the contents would appear yellow or greenish in color, whereas before the reduction it had a bluish tint. At the end of 48 hours of incubation transfers were made from bottles which showed reduction. This step eliminates the possibility to some extent, of loosing *S. pullora* due to rapid growth of other organisms when present. The transfers were made to sterile tubes of broth. On the seventh and last day of incubation transfers were made from all of the bottles. The broth tubes were incubated for 48 hours and those that showed growth were plated on agar plates. Colonies of growth on these plates that resembled *S. pullora*, were transferred to agar slants. All cultures that resembled *S. pullora* were tested in four carbohydrates, which are of diagnostic value in identifying *S. pullora*. The carbohydrates were dextrose, maltose, dextrin and dulcitol. Meat extract broth containing one per cent carbohydrate was employed in the different carbohydrate solutions. Andrade's indicator was used. A culture was further tested in nine carbohydrates, if a characteristic reaction was observed in the four carbohydrates. The nine carbohydrates employed to confirm the identification of *S. pullora* are

as follows: dextrose, maltose, arabinose, dextrin, dulcitol, saccharose, salicin, lactose and xylose. The inoculated carbohydrates were incubated for 96 hours at 37° centigrade. All cultures, when a characteristic reaction of *S. pullora* in the nine carbohydrates was observed, their antigenic affinities were then determined by the agglutination test using known immune sera of a reacting hen.

Results

The results of this problem extend over a period of two months, February and March. Of the fifteen hens, thirteen were laying in both months. Some were just commencing to lay the latter part of February while others decreased in production in March. There is but a slight difference in production between the two months. In February 102 eggs were laid of which 89 were cultured. Fifty-two of the 98 eggs were cultured as fresh eggs, 6 that had been removed from the incubator on the 7th day, 4 on the 14th day and 27 on the 21st day. The total number infected with *Salmonella pullora* was 28. Sixteen of these cultures came from fresh eggs, 12 from eggs that were removed from the incubator. The total per cent infected with *Salmonella pullora* was 31.4. All the hens that were laying did not eliminate the

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organism thru egg. Seven hens eliminated the organism thru the egg. The total number of eggs laid by these hens was 64. Nearly 43.7% of this number was infected. Hen 5431 laid 15 eggs, of which 9 were infected. Hen 5409 laid 8 eggs of which 6 were infected. Other hens eliminating the organism through the egg were 5260, 5265, 5290, 5319 and 5430. Three eggs were laid on the floor therefore have no number. The heaviest layers were eliminating the organism more frequently than the low producers. Summarized data are given on Tables I and II.

In March 110 eggs were laid by thirteen hens. Total number of eggs cultured was 108, sixty eight were cultured as fresh eggs, 20 as candled out on 7th day, 15 as candled out on the 14th day and 5 on the 21st day. Twelve of the fresh eggs contained the organism and six of the incubated eggs contained the organism. The total number of eggs infected was 18 or 16.6% of the total number cultured. Only six hens were eliminating the organism in this month. The egg production is slightly increased but the number of eggs infected has decreased. Two hens eliminated the organism ^{in March.} this month that did not do so in February. Hen 5431 laid the same number of eggs as in the previous month with but a slight decrease in the number of infected. Some of the hens

The total number of eggs cultured per hen per month and
number infected with *Salmonella pullora*.

Hen No.	February		March	
	Total No. eggs cultured	Number containing <i>S. pullora</i>	Total No. eggs cultured	Number Containing <i>S. pullora</i>
5245	0	0	0	0
5260	12	5	12	3
5265	13	3	2	0
5274	3	0	7	0
5275	0	0	0	0
5286	1	0	2	0
5287	2	0	6	1
5289	4	0	3	0
5290	3	1	10	0
5312	8	0	15	1
5318	4	0	6	0
5319	9	3	17	2
5409	8	6	4	1
5430	4	1	4	0
5431	15	9	15	8
No number	3	0	5	2
TOTAL	89	28	108	18

TABLE NO. II - SUMMARIZED DATA OF CULTURED EGGS LAID DURING FEBRUARY AND MARCH

Month	Total No. Hens laying	Total No. Eggs laid	Number Eggs Cultured			Total No. Tested	Number eggs infected with Salmonella pullora			Total No. Infected	Per Cent Infected		
			Fresh	Incubated for 7 da.	Incubated for 14 da. 21 da.		Fresh	Incubated for 7 da.	Incubated for 14 da. 21 da.				
Feb.	13	102	52	6	4	27	89	16	2	1	9	28	31.4
March	13	110	68	20	15	5	108	12	2	4	0	18	16.6

decreased in production and also in the number of infected eggs. Five eggs were laid on the floor, two of which contained the organism. The foregoing data are presented in Tables I and II.

Thirty eggs were infected with some organism other than *Salmonella pullora*, which the author did not attempt to identify. Some of these organisms may not have come from the hen, but may have gained entrance during the culturing process.

Production of either acid or gas or both in the three carbohydrates, dextrose, arabinose and xylose, was considered a characteristic reaction of *Salmonella pullora*. All the cultures gave a characteristic reaction for *Salmonella pullora* except one which gave acid and gas in dextrose, maltose, arabinose, dextrin, lactose and xylose, and also acid in saccharose. This culture, however, resembled the cultural characteristics of *Salmonella pullora* and was agglutinated, by known serum immune to *Salmonella pullora*, up to 1-1280. The remaining cultures were also agglutinated at that dilution.

Conclusions

From the results the following conclusions are justified:

1. Hens that are infected with *Salmonella pullora* and react to the macroscopic agglutination test, may eliminate the organism through the egg.
2. The infected ovary of the mother hen is the original source of infection.
3. Results show that hens badly infected with *Salmonella pullora* are not always low producers.
4. With the present results at hand, one is not justified in stating that some of the reacting hens do not eliminate the organism through the egg. The number of eggs examined is not sufficient to draw this conclusion.

Bulletin No. 75, November, 1915.

Experiment Station.

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