A STUDY OF THE EFFECT OF BACILLARY WHITE DIARRHEA ON MATURE FOWLS AND THEIR PROGENY

THESIS FOR DEGREE OF M. S.

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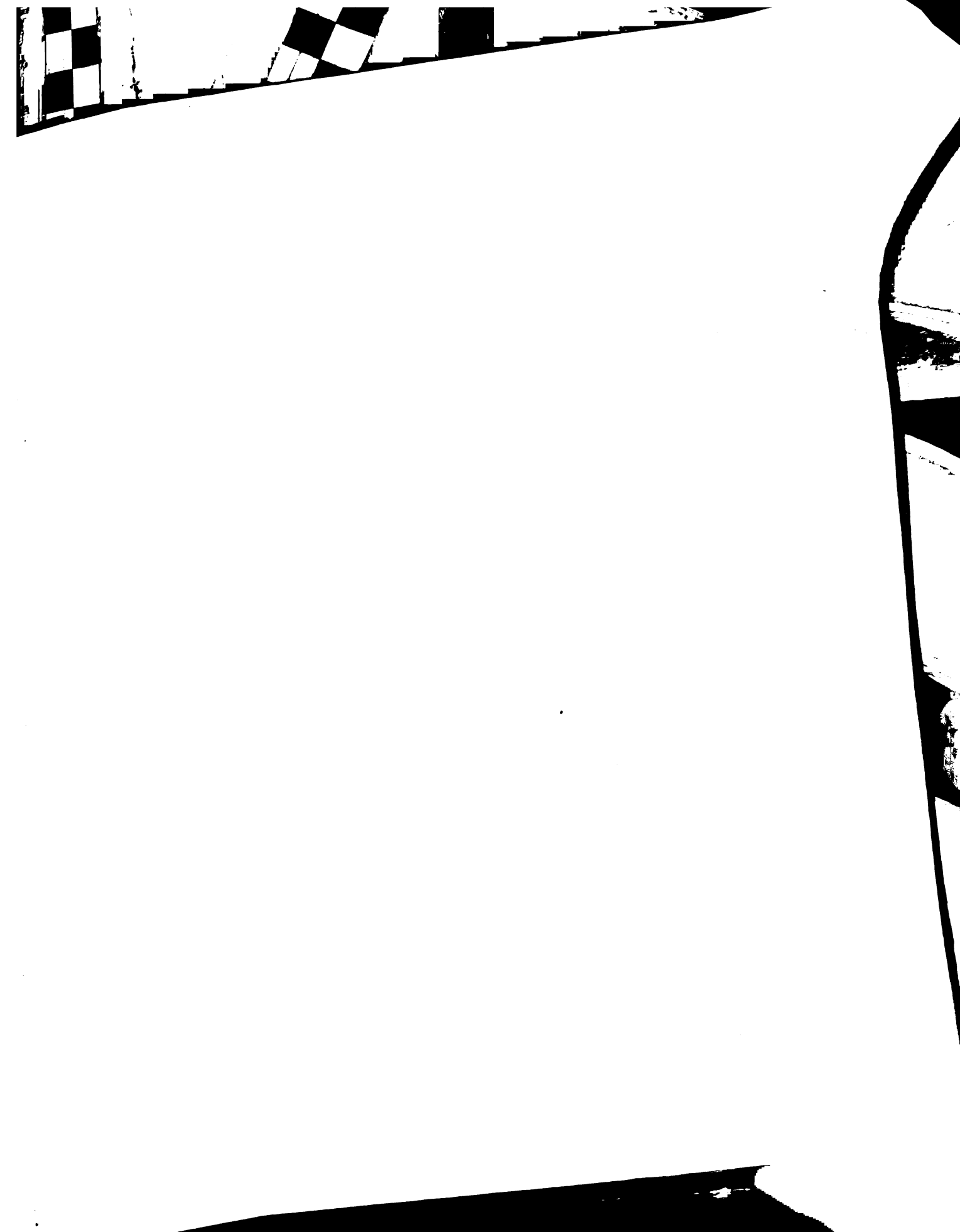

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THESIS

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M.S.

by

Harold Canfield

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THESIS

TABLE OF CONTENTS

INTRODUCTION

Bacillary white diarrhea is probably the cause of greater loss and more discouragement to the poultry raiser and hatcheryman than any other poultry disease.

A study of this disease is of particular interest because a very large proportion of the chicks reared are purchased from hatcheries. Some idea of the magnitude of the industry can be gained when we consider the fact that there are sixty-six hatcheries in Ottawa County, Michigan, with incubator capacity in excess of twenty thousand eggs each. More than six million chicks are sold from these hatcheries each year. Since a very great number of chicks are produced by hatcherymen, it seems very reasonable to suppose that the control or spread of this disease is dependent largely upon the effort made by hatcherymen to produce clean stock.

It was hoped that it would be possible to demonstrate the losses that may be expected in a flock of poultry from which the reacting birds have been eliminated only once, compared with the losses sustained in flocks where the infection is very heavy.

HISTORICAL RESUME

It is quite definitely understood that the disease, bacillary white diarrhea has existed for a long time. As early as 1899, Rettger (1) described the organism found in chicks suffering from white diarrhea. Later he named this organism Bacterium pullorum. In 1908 Rettger (2) discovered that a white diarrhea, fatal to young chicks, was caused by an organism having some characteristics common to typhoid and colon bacilli. He also noted that this disease was very hard to eradicate and occurred year after year among the young chicks. As a preventative measure, he recommended cleaning and disinfecting incubators, brooders, and brooder houses. The following year, 1909, Rettger (3) came to the conclusion that fatal septicemia and white diarrhea of young chicks were the same disease and the cause of this disease was an organism, which he called Bacterium pullorum. During the same year, Rettger and Stoneburn (4) published a bulletin explaining that Bacterium pullorum had been isolated from the yolks of fresh eggs. The conclusions were: "That the mother hen is the original source of infection of the chick. That a few chicks from infected hens have the disease when hatched. That the mortality depends upon the number and virulence of the organisms, mode and time of infection, and the vitality of the chicks. That most of the infected chicks die under four weeks of age. That a few may survive the attack, but remain weak through life and are very susceptible to other disorders."

In 1911, Rettger (5) reported the finding of Bacterium pullorum in the ovary of an eight months old pullet, a survivor of an attack of white diarrhea. This showed conclusively that the laying hen is the carrier of Bacterium pullorum.

Rettger and Stoneburn (6) reported in 1911 that a varying percentage of eggs from infected hens contained Bacterium pullorum. "Very few or as many as 70 per cent of the eggs from infected hens contain Bacterium pullorum." He also states that this disease may be spread through the medium of infected food and water; and normal chicks may acquire the disease by picking up infected droppings or food contaminated thereby. "Infection from chick to chick cannot, apparently, take place after they are three or four days of age."

Female chicks, which survive this disease, often harbor the infection and may become carriers of the organism, infection in the breeding pens being perpetuated in this manner.

He also observes that infected hens are apparently poor layers, especially in their second and subsequent laying season.

In 1912 Rettger (7) reported that the greatest danger from infection with bacillary white diarrhea lies within the first forty-eight hours after hatching, but that chicks may acquire the disease up to the time they are four days old. He also found that hens may become carriers after they have reached maturity. He believes that the infection is acquired through the mouth.

The feeding of sour milk to young chicks was recommended as a means of preventing or at least holding in check outbreaks of bacillary white diarrhea.

He concludes that the disease can be eliminated from the farm only be rejecting all birds that harbor the disease and by obtaining eggs or stock from sources where white diarrhea has not been known to exist.

Jones (8) reported in 1913 that he had found the macroscopic agglutination test to be of great value in detecting fowls that harbor Bacterium pullorum in their ovaries. Serum dilutions of 1-50, 1-100, and 1-200 were recommended as being the most practical. He states that the ovaries of fowls, harboring Bacterium pullorum, are not always pathological, but that seventy-five per cent of the carriers' ovaries are cystic.

In 1914 Rettger (9) stated that ovarian infection and germinal transmission of bacillary white diarrhea had been conclusively demonstrated. The cycle of the disease was outlined as follows: "The disease, primarily, infects young chicks which frequently survive and become permanent bacillus carriers, the ovary being the important seat of infection. The eggs from such carriers often harbor the organism of the disease in the yolk. Chicks that develop in infected eggs become in turn infected and have the disease at the time of hatching. The disease is transmitted to normal chicks through the infected droppings."

He concludes that there is no evidence to indicate that germinal transmission through the male takes place.

Horton (10) found that sulphocarbolates have very little, if any, value in the treatment of bacillary white diarrhea.

Rettger, Kirkpatrick, and Jones (11) reported that sour milk has a very beneficial influence on the growth of young chicks.

However, they did not regard the sour milk as an important agent in the prevention and suppression of white diarrhea.

Gage and Hyland (12) found that the testing of eggs from infected hens was too slow and unreliable to be used as a practical method for detecting fowls carrying Bacterium pullorum.

The macroscopic agglutination test was recommended as a practical method.

Kaup (13) advised the feeding of sour milk to young chicks as soon as they were taken from the incubator or nest. He believed that it would ward off attacks of white diarrhea and also produce a greater gain.

Rettger, Kirkpatrick, and Jones (14) reported in 1916 that a single set of agglutination tests and the elimination of the reactors was not an absolute guaranty that the flock was rid of ovarian infection of white diarrhea but that the percentage of carriers was greatly reduced.

Page (15) claimed that bacillary white diarrhea was completely stamped out in the flock that was tested for two years, when the directions were carried out.

Gage and Martin (16) described the histo-pathology of the intestines of young chicks infected with Bacterium pullorum as follows: "From a study of sections made from the intestines there was revealed marked injury to the mucosa associated with hyperemia, hemorrhagic exudation, and leucocytic infiltration. In the individuals where the disease had run a longer course there were exhibited processes of regeneration. In many instances there was a thickening of the intestinal wall. There was a marked fi-

broblastic proliferation and wherever there was any of the columnar epithelium intact there was active secretion of mucous. Therefore, with these pathological conditions associated together and repeated observations confirming them, it is evident that the important histo-pathological conditions in the intestines of young chicks, dead of Bacterium pullorum infection, correspond to either an acute or beginning chronic condition of catarrhal inflammation."

In 1917, Hadley (17) reports: "We can no longer regard Bacterium pullorum infections as limited to young stock. We have found that Bacterium pullorum was the causative agent in an epidemic in adult fowls, indistinguishable in its clinical picture and pathological manifestations from fowl typhoid. The primary observations and the experimental features of the study lead to the conclusion that latent Bacterium pullorum infection was stimulated into active manifestations of fatal generalized infection or other pathological changes, following the feeding of a ration containing a large proportion of roughage in the form of oat hulls."

Ward and Gallagher (18) reported that very satisfactory results were obtained with an intradermal test for Bacterium pullorum. Their test fluid consisted of a killed and carbolized culture of Bacterium pullorum that had been grown for about a month. They reported that the results obtained checked very closely with the agglutination test.

Brown (19) reported the results of experiments conducted from 1912 to 1917 with a view to determining the influence of

natural and artificial incubation upon white diarrhea in chicks. "In 1912, six hundred Barred Plymouth Rock eggs laid by a flock infected with white diarrhea were secured. One-half of these eggs were hatched artificially and the other half were hatched with hens. Enough of the more vigorous pullets of each flock were saved to produce eggs for the following year, and each year the eggs used were from the last generation. The two flocks were always penned together and the males mated with them each year were hen hatched."

The percentage of loss each year from white diarrhea in the artificially hatched lot has shown no indication of diminishing, amounting to 23.75, 38.50, 26.75, 30.75, 44.44, and 69.48 per cent respectively, thus showing a material increase in 1917."

"Each season since 1913 one hundred fifty eggs from the naturally hatched line have been incubated artificially with the result that, with the exception of the first and second generations, no traces of white diarrhea have been found. The percentages of loss from white diarrhea, up to the age of twenty-five days, for the seasons 1913 to 1917 from eggs artificially hatched from the second, third, fourth, fifth, and sixth generations, respectively, of the naturally hatched line are as follows: 1913 chicks, 25 per cent; 1914 chicks, 4 per cent; and none for 1915, 1916, and 1917 chicks.

In 1919, Sherago and Benson (20) reported on a comparative study of the agglutination test for Bacterium pullorum infection and the intradermal test proposed by Ward and Gallagher.

"Both tests were used on 134 fowls and the most typical reactors to either or both tests were used in retests to check the results obtained at first and to observe the influence of previous injections with the intradermal test fluid on subsequent agglutination and intradermal tests."

From the results obtained, the authors conclude that the intradermal test is so inconsistent as to be worthless as a diagnostic agent for Bacterium pullorum infection in adult fowls. As possible reasons for the inconsistency of the test, it was suggested that edema is likely to develop and persist for some time after injection, even with sterile water. Any protein is likely to give the same results.

The experiments also indicated that a previous injection of the intradermal fluid caused at least 85 per cent of the birds retested to react to the agglutination test regardless of their reaction in the original test.

In 1919 Rettger, Kirkpatrick, and Card (21) reported that, due to the results obtained by experimentation, they had concluded that transmission of infection from hen to hen, through infected litter and by ordinary association, is rare.

No definite conclusions were drawn regarding the part played by the male in the transmission of the organism, but it was thought that this method of transmission could not be doubted. It was recommended that the males should not be allowed to run with the females except during the breeding season.

Gage (22) made the statement in 1919 that he firmly believed that Rettger and Stoneburn were justified in their conclusion that

white diarrhea, as poultrymen understand it, is a bacillary disease caused by Bacterium pullorum.

In 1922 Gage and Flint (23) report on the control of bacillary white diarrhea: "The number of cases of bacillary white diarrhea is being reduced each year."

"From review of the data obtained during the past year, there are indications of improvement among the breeding flocks in the State as a result of the testing work."

"Of the 110 flocks tested during 1921-1922, twenty-seven were found to be free from bacillary white diarrhea. It is not easy to rid flocks of this disease, especially if the original infection is great. It can be seen from the following table that the infection may be eliminated by testing the original birds, the progeny, and the progeny of the progeny; which means that elimination is possible only after a series of tests.

Reduction of Percentage of Infection as Determined by Testing Work on Eighteen Different Flocks

1919-1921

Flock No.	Infection in original flock per cent	Infection in progeny per cent	Infection in progeny of progeny per cent
1	20	11	6
2	2	0	0
3	0	0	0
4	16	7	1
5	5	0	-
6	4	3	0
7	12	4	4
8	4	-	2
9	32	21	6.4
10	0	-	0
11	3	-	0
12	31	125	-
13			
14	25	18	-
15	0	0	-
16	4.9	0.3	-
17	7.6	5	4
18	6.3	1.7	-

In 1922 Gage (24) made a general report concerning the diagnosis of Bacterium pullorum infection in the domestic fowl: "Eighty-three specimens showing leg weakness or paralysis, were examined for Bacterium pullorum. The organism was isolated from only five of the eighty-three. This evidence does not indicate that leg weakness is due to Bacterium pullorum."

"In 1917 and 1918 several sets of experiments were carried out under the best known conditions for poultry husbandry. Eggs from sixty hens, known to have reacted positively to the agglutination test, were set in an electrobator. When tested at the end of the first seven days of incubation, thirty were found to be infertile and two were found dead in the shell. Of the twenty-eight left, ten were hatched. Three died at the end of the first day and Bacterium pullorum was isolated from the unabsorbed yolk. All eggs containing fully developed chicks were examined especially for Bacterium pullorum with the following results. The egg number in each case represents the number of the hen laying the egg."

Table Showing Results of Tests for Bacterium pullorum in Dead Chicks from Eggs Produced by Positively Reacting Birds.

Egg Number	Bacterium pullorum	Egg Number	Bacterium pullorum
8001	+	7925	-
8384	+	7998	-
8388	-	8430	+
8002	-	8430	-
8002	-	8565	+
8430	+	8388	+
7925	-	7998	+
8565	-	8430	-
8001	+	8384	-

"From this table it will be seen that with the methods used it was not possible to detect Bacterium pullorum in all the dead chicks, although adult hens were all positively reacting to the agglutination test. From eight, Bacterium pullorum was isolated without difficulty and the cultures were negative from the other ten.

After three months, following out three sets of incubation, the author was able to obtain from the three sets of eggs set, sixty in each lot, all from positively reacting hens, seven livable chicks in the first set, nine in the second set, and nine in the third set." These chicks were all given the number of the

parent stock from which they came: 7811, 7895, 7925, 7997, 7998, 8001, 8002, 8020, 8082, 8084, 8094, 8139, 8171, 8180, 8202, 8204, 8204, 8294, 8384, 8388, 8389, 8430, 8431, 8544, 8565, 8810.

"These twenty-five birds, all reared from positively agglutinating hens, were yarded together and blood taken at various intervals to determine whether their blood would show any agglutinative power."

Records of Agglutination Tests on Chicks Hatched from Eggs Laid by Positively Reacting Hens.

Chicks Number	July 17, 1917 Dilution of serum					July 21, 1917 Dilution of serum					Aug. 3, 1917. Dilution of serum					Aug. 26, 1917 Dilution of serum					Nov. 7, 1917 Dilution of serum				
	1-100	1-200	1-500	1-1000	1-2000	1-100	1-200	1-500	1-1000	1-2000	1-100	1-200	1-500	1-1000	1-2000	1-100	1-200	1-500	1-1000	1-2000	1-100	1-200	1-500	1-1000	1-2000
7811	?	?	?	?	?	c	?	?	?	?	c	c	c	cc		c	c	cc	c		c	c	c	c	c
7895	?	?	?	?	?	0	0	0	0	0	c	c	c	00		?	?	??	?						
7925	0	0	0	0	0	c	c	c	c	c	c	c	c	cc		c	c	c	c	c	c	c	c	c	c
7997	c	c	0	0	0	0	0	0	0		c	c	c	c		c	c	c	c	c	c	c	c	c	c
7998																									
8001	0	0	0	0	0	0	0	0	0	0	c	c	0	00		?	?	?	?	?	?	?	?	?	?
8002																									
8020	0	0	0	0	0	c	c	c	c	c	c	c	c	cc		c	c	c	c	c	c	c	c	c	c
8082	c	?	?	?	?	0	0	0	0		c	c	0	0	0	c	c	0	0						
8084	?	?	?	?	?	0	c	0	0	0	c	c	c	c	c	c	c	c	c	c	c	c	0	0	0
8094	0	0	0	0	0	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
8139	?	?	?	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8171	?	?	?	?	?	?	?	?	?	?	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
8180		c	c	c	c	c	c	c	c	c	c	0	0	0	0	c	c	c	0	0	c	c	c	c	c
8202	0	0	0	0	0	0	0	0	0	0						c	c	c	c	c	c	c	c	c	c
8204	?	?	?	0	0	c	c	c	c	?	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
8294						0	0	0	0	0	c	c	c	c	c	c	c	0	0	0	c	c	c	c	c
8384	c	c	c	c	c	0	0	0	0	0	0	0	0	0	0	c	c	0	0	0	c	c	c	c	c
8388	?	?	?	?	?	c	c	c	c	c	?	?	?	?	?	c	c	0	0	0	c	c	0	0	0
8430	?	?	?	0	0	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
8565	?	?	?	?	?	c	c	c	c	c	c	c	c	c	0	c	c	c	c	c	c	c	c	c	c
8810	?	0	0	0	0	0	0	0	0	0	?	?	?	?	?										

c - complete agglutination ? - doubtful agglutination 0 - no agglutination

"These experiments indicate that in chicks, hatched from eggs laid by positively reacting hens, at least six months' time should elapse before the normal agglutinative power of such sera would be sufficiently definite to furnish indication of past or present infection. The birds reared from hens 8001, 8139, and 8810 never showed an agglutinative power in their blood sera. The length of time a serum maintains its agglutinative power has not yet been determined."

In 1923, Gage and Flint (25) made further report on the progress of the work on control of bacillary white diarrhea.

"The hatchability of the eggs has greatly improved. Previous to testing and before control measures were followed by locating and eliminating disease carriers, great losses were sustained in the twenty-nine poultry plants now free from disease."

"After a series of tests had indicated that there were no reactors in these twenty-nine flocks, this department sought to find where eggs had been sold for hatching, so that a record could be made of the value of the test as a means of producing disease free flocks, from which clean stock could be produced. Data have been obtained by personal visits and by correspondence. The reports thus far received have been most striking. On one of the large farms where the breeding birds were infected with Bacterium pullorum, it became almost impossible to rear even a small percentage of the chicks hatched. Testing was begun in

1919 and the agglutination test revealed 27 per cent of the birds in the breeding pen infected."

"In 1920 another test was run and the percentage of infection had dropped to 20 per cent. The progeny of this flock was tested in 1921 and only 6.5 per cent were found infected. During the present season, 1922-1923, the breeding flock was found to be free from birds which could be classed as carriers. This flock being established as a disease-free flock, records were obtained from all persons receiving eggs for hatching from this flock."

"The results were gratifying. From 1110 tested breeding birds 11,600 eggs were incubated and 8,700 chicks were hatched, 92.9 per cent of which were reared. This is considerably better than in 1919 when less than 15 per cent matured, the deaths in the first few days after hatching being due to infection with Bacterium pullorum."

"The experience of the last five years has indicated that poultrymen must test their breeding birds consecutively until no reactors are found in the breeding flock."

"When close cooperation does not exist between the poultryman and the scientific worker, the disease condition is not improved and may be even worse than when work was started."

In 1923 Beaudette, Bushnell, and Payne (26) reported on work which they had done on the relation of Bacterium pullorum to the hatchability of eggs.

"During four years (1919-1922 inclusive) 1,462 fertile eggs were obtained from the infected hens and 553 or 37.8 per cent hatched. Twenty-five eggs were examined which contained dead embryos. Twelve or 48 per cent yielded a culture of Bacterium pullorum."

A Comparison of the Hatchability of Eggs from Infected and Non-infected Hens. (1922)

	Number of Hens	Eggs Incubated	Per cent Fertile	Per cent Hatched
Infected	41	861	69.57	53.59
Non-infected	218	6,387	77.10	65.10
Total	259	7,248	76.21	63.86

Summary of Data on Hatching Record of Hens Known to be Infected with Bacterium Pullorum.

Number of Hens	Eggs Incubated	Fertile Eggs	Per cent of Fertile Eggs Hatched	Eggs Examined	Bacterium pullorum Found
34	2,073	1,462	37.8	25	12

Comparison of Hatchability of Fertile Eggs from Infected Hens with the Flock Average.

Year	Infected Hens			Per cent Hatched by Entire Flock
	Number of Eggs	Fertile Eggs	Per cent Hatched	
1919	3	123	18.9	30.3
1920	13	569	23.3	32.0
1921	15	278	46.4	53.9
1922	28	492	54.4	63.9

"The percentage of fertile eggs hatched from the entire flock for the four years was 43.3 per cent as compared with 37.8 per cent hatchability of fertile eggs from the infected hens during this period."

"There was considerable variation in the hatchability of eggs from the different hens. No definite explanation based on known facts can be given for this variation. In those cases in which the hatchability of fertile eggs from infected hens was below the average hatched, it might be assumed that the organism was present in a large percentage of the eggs used for hatching purposes. Yet this does not seem to be an adequate explanation, because it is known positively that the organism may be present, the egg may hatch, and the chick suffer from white diarrhea after hatching."

"The correlation between infection and low hatchability as indicated by the records for 1922 seemed to justify the application of the tests the following year. In 1923, one hundred eighty-three fowls were tested and nineteen or 10.3 per cent of the flock were found to be infected. The percentage of fertile eggs that were hatched from infected and non-infected hens is given in the table below, which shows that 18.2 per cent more of the fertile eggs from the non-infected hens hatched than from the infected hens. It will also be noted that the fertility of eggs from non-infected hens was 33.4 per cent more than from infected hens. The difference in hatchability of fertile eggs from infected and non-infected hens was greater in 1923 than in 1922, yet the removal of infected birds from this flock would only have increased the hatchability by 0.8 per cent."

Hatchability of Fertile Eggs from Infected and Non-infected Hens. (1923)

	Number of Hens	Eggs Incubated	Per cent Fertile	Per cent Fertile Hatched	Per cent Total Incubated Hatched
Infected	19	387	57.0	45.2	25.8
Non-infected	164	5,066	90.4	63.4	57.4
Total	183	5,453	88.0	62.6	55.1

"That low hatchability due to infection by this organism exists in flocks other than the one reported, was shown by the large number of inquiries received regarding losses in the shell."

"For illustration, we mention the hatching record of a flock of twenty-six fowls of which eleven or 43.3 per cent were found to be infected in 1922. The infected fowls were eliminated and the flock replenished with new hens, and prior to 1923 hatching season the flock was again tested and found to be free from the infection. A comparison of the hatching record shows that the percentage of fertile eggs hatched was increased from 35.18 per cent to 97.14 per cent or a difference of 61.96 per cent. This difference cannot be attributed to better management, because both hatches received the same attention. It might be added that hens were always used for hatching in this flock. Two dead embryos were produced during this year, due to chilling. Of the sixty-eight chicks produced none died of white diarrhea."

"From our study of Bacterium pullorum in relation to poor hatchability it appears that this organism is, at least, one factor to be taken into consideration. While the eggs from all infected hens do not show a uniformly low hatchability, the average hatchability of eggs from infected hens is below the average for non-infected hens."

"The fertility of eggs from infected hens seems to be considerably lower than the fertility of eggs from non-infected hens. From this it might be supposed that the organism may exert its influence at different stages of the incubation period. The egg may be rendered infertile or death of the embryo may take place at any stage of the incubation period. Usually the largest mor-

tality takes place about the nineteenth day of incubation. Bacterium pullorum infection of chicks from the eggs has long been known, but, in view of our experiments, it appears that we must recognize another loss due to this organism."

In 1923, Hitchner (27) reported that blood sera from hens in heavy laying condition gives very unsatisfactory results in the macroscopic agglutination test for bacillary white diarrhea. He claimed that sera has a high fat content that causes cloudy reactions. He recommends starving the birds for at least thirty-six hours before bleeding in order to get clear sera.

It is generally understood that this cloudiness of reaction is due to a protein in the sera and not to fat.

Gage and Flint (28) made a third report on the control of bacillary white diarrhea in 1924. They stated that the livability of chicks at nearly all plants which buy from bacillary white diarrhea free flocks, had been excellent and that the hatchability of eggs had been normal. Further claim was made that the presence bacillary white diarrhea in the breeding birds seriously influenced the hatchability. On the other hand, if bacillary white diarrhea is not present, it is no assurance that the hatch will be above normal. Low hatchability of eggs is not altogether a disease problem.

"However, when chicks are once procured free from Salmonella pullora infection the chances of their maturing are enhanced tre-

mendously. White diarrhea control has advanced to day in Massachusetts to a point where the original source of infection is being eliminated and chicks hatched have a fair show to live."

"The lowest percentages of livability, reported by customers, of day old chicks from bacillary white diarrhea free poultry plants were 71.80 per cent, 71.87 per cent, and 76.4 per cent. These low livability percentages can readily be explained. In the first instance, the loss was due to poor breeding methods; in the second, the chicks were taken care of by an inexperienced poultryman who was not fully trained in brooder management; and in the third, the low percentage of livability was due to lack of attention to the details of brooder management."

"Of the 20,665 day old chicks sold from representative bacillary white diarrhea free breeding flocks, aside from the three mentioned above, the livability averaged nearly 90 per cent. Many individual plants have reported that nearly 100 per cent of all chicks received from bacillary white diarrhea free flocks were living and in good health."

EXPERIMENTAL WORK.

Object of the Experiment.

Past experience has shown that bacillary white diarrhea is very common in the poultry flocks of Michigan. Where this infection exists in the flock, the mortality of chicks seems to increase each year unless some control measure is practiced. In many cases the mortality of young chicks increases to such an extent that it is impossible to keep more than twenty to thirty per cent of the chicks alive for six weeks. However, most of the infected chicks die between four and fourteen days of age.

At present the most practical control of the disease is accomplished by locating the infected birds in the breeding flock with the macroscopic agglutination test and eliminating these infected individuals sometime during the winter before eggs are saved for hatching purposes.

It is quite generally known that a single agglutination test does not eliminate all infected fowls.

This experiment was planned to make a comparative study of the egg production of both the hens giving a positive reaction and those giving a negative reaction to the macroscopic agglutination test for Bacterium pullorum, using two tubes of antigen with serum dilutions of 1-40 and 1-100. It was also planned to compare the fertility and the hatchability of eggs from non-reacting birds.

Lastly, it was planned to compare the quality and livability of chicks from reacting hens with the quality and livability of chicks from negatively reacting hens.

From this study it was hoped to determine the efficiency of a single agglutination test in eliminating the reacting fowls from a poultry flock and the extent of the losses sustained by infection with this disease.

Plan of the Experiment.

The plan of this experiment was:

(a) To apply the macroscopic agglutination test for Bacterium pullorum to all the hens in a given flock of poultry.

(b) To place in a pen, separate from the main flock, all birds giving a positive reaction to the test.

(c) To give the same care, feed, and attention to infected and non-infected birds.

(d) To keep trap nest records for at least twelve weeks of all birds in the entire flock.

(e) To incubate eggs from infected and non-infected hens and keep records of the fertility and hatchability of these eggs.

(f) To feed and care for the chicks hatched for at least fourteen days and keep records of the deaths during this period and also note, if possible, the causes of death.

Procedure.

Preparation of Antigen.

About 30 c.c. of liver infusion agar (2 per cent agar) was placed in each of twenty sterile Kolle flasks. These flasks,

containing the agar, were sterilized in the autoclave with fifteen pounds steam pressure for fifteen minutes. As soon as the agar was cool the flasks were then inoculated with one c.c. of Bacterium pullorum (strain No. 51) and then incubated for forty-eight hours at 37°C. The resulting growth was washed from the surface of the agar with phenolated physiological salt solution, care being taken to keep the volume of the suspension as small as possible. About 50 c.c. of a fairly heavy suspension of organisms was collected and filtered through cotton.

Trial dilutions were made to find out what proportion of phenolated physiological salt solution should be added to the suspension to make an antigen having the same turbidity as tube No. 1 of McFarland's Nephelometer.

Three blood samples were used in testing the new antigen. In a previous test, one sample had given a strong positive reaction, another had given a weak positive reaction and the third had given a negative reaction.

The antigen was found to be satisfactory and stored in the ice box for future use.

Collecting the Blood Samples.

About two c.c. blood were collected in small sterile glass vials from each hen, the blood being taken from the wing. As soon as the sample was drawn the vial was laid in slanting posi-

tion on a shelf until the blood coagulated, forming a blood slant. The blood samples were left at room temperature for about six hours in order to allow the serum to separate from the clot and then stored in the ice box until about two or three hundred samples had been collected.

Making the Tests.

When a sufficiently large number of blood samples had been collected, the macroscopic agglutination test was applied to each sample for Bacterium pullorum.

For making this test clean Wasserman tubes were placed in racks holding thirty-two tubes and numbered in pairs, one to sixteen inclusive. One c.c. of Bacterium pullorum antigen, diluted so that the turbidity was the same as No. 1 tube of McFarland's nephelometer was measured into each tube. Two tubes were used for each sample. In the first tube was placed 0.025 c.c. of blood serum and 0.01 c.c. in the second tube, giving dilution of 1-40 and 1-100 respectively. The serum and antigen were thoroughly mixed by shaking. After incubating at 37°C. for twenty-four hours the tubes were examined and results recorded.

Feeding and Management of the Hens.

Of the five hundred ninety-three hens tested, fifty-eight reacted to the agglutination test. The fifty-eight hens reacting to the test were placed in pen No. 13 with seven male birds,

two of which were infected. These birds were given the same feed and care as other birds at the poultry plant. The ration consisted of dry mash (20 parts bran, 20 parts ground corn, 20 parts ground oats, 20 parts middlings, and 10 parts dried beef scraps), scratch grain (equal parts of corn and wheat), sprouted oats, and semi-solid buttermilk.

The hens were given free access to the dry mash at all times and the scratch grain was fed twice daily, the caretaker using his own judgment as to the quantity to be fed. As much semi-solid buttermilk was smeared on the wall as the hens would clean up in fifteen minutes. Each morning about ten o'clock, sprouted oats were fed, allowing about one cubic inch of sprouted oats for each hen. Oyster shell, charcoal, grit, and water were before the hens at all times.

Trap nest records of the hens at the plant are kept throughout the year and during the hatching season each egg is marked with the number of the hen when it is taken from the trap nest.

Incubation of the Eggs.

The first lot of eggs from the infected hens was incubated in a Buckeye incubator and a Prairie State incubator was used for the second lot. The third lot of eggs from the infected hens was incubated for eighteen days in a Petersime electric incubator and removed to a Newtown Mammoth incubator to finish hatching. The

three lots of eggs from the hens showing a negative reaction were incubated in the Newtown Mammoth incubator.

Feeding and Brooding the Chicks.

All the chicks hatched were placed in brooder houses ten feet square, heated by hard coal burning brooder stoves. The temperature was kept about 95°F. under the canopy. When forty-eight hours old the chicks were given a small amount of Domino Chick Starter on a newspaper, some fine grit, and warm water. For the first week the chicks were fed five times daily as much Chick Starter as would be cleaned up in fifteen minutes. After the chicks were a week old the Chick Starter was fed in small feed dishes and the chicks were given free access to it at all times. A small amount of chick scratch feed and oat sprouts were added to the ration at this time.

Tables No. I and II.*

Table III.

Showing the Livability of Chicks from Hens not Reacting to the Agglutination Test.

	First Lot Hatched March 2	Second Lot Hatched March 17	Third Lot Hatched March 26	Total of Three Lots
Number of Chicks	31	47	39	117
Number of Chicks Died	4	1	2	7
Per cent Livability	87.09	97.8	92.3	94.02

* These tables will be found in pocket on the cover.

Table IV.

Showing the Livability of Chicks from Hens Reacting to the Agglutination Test.

	First Lot Hatched March 31	Second Lot Hatched April 14	Third Lot Hatched May 22	Total of Two Lots
Number of Chicks	49	76	52	125
Number of Chicks Died in 14 days	46	51	-	97
Per cent Livability	6.12	32.89	-	22.4

Table V.

Showing the Death Rate of Chicks from Hens Reacting to the Agglutination Test.

Age of chicks in days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Chicks surviving 14th day
No. of chicks dead in Lot #1	0	0	1	2	3	12	11	7	3	4	3	0	0	0	3
Appearance of liver	-	-	x	x	x	x	x	x	rx	rx	rx				
Unabsorbed yolk	-	-	+	+	+	+	+	+	+		+	+			
No. of chicks dead in Lot #2	4	2	0	2	2	9	11	6	2	3	5	2	1	2	25
Appearance of liver	x	x	-	x	xp	xp	xp	xp	xr	xr	xr	r	nf	r	
Unabsorbed yolk	+	+	-	+	+	+	+	+	+	+	+	+	+	+	

Note:

x - ochre color

rx - ochre color with red lines

xp - ochre colored patches

r - red lines

nf - necrotic foci

\- - unabsorbed yolk present

During the feeding period, all chicks that died were examined for lesions. After fourteen days all the chicks still alive in the infected lots were killed and examined for lesions. The chicks from eggs laid by hens giving a negative reaction were not killed as they appeared very strong and sturdy, showing no symptoms of disease.

Discussion of Data.

It has been quite generally understood that birds infected with bacillary white diarrhea will produce practically as many eggs during the first year as uninfected hens, but during subsequent years they are very poor producers. The data obtained in this experiment do not show much evidence that the one and two year old infected hens produce less than could be expected of them normally. The numbers in Table No. I preceded by the letter c represent pullets, numbers preceded by b represent year old hens, and numbers preceded by a represent two year old hens.

However, the average egg production of the positively reacting hens was about three-fourths as great as the egg production of the hens in the check lot. The average production of the reacting hens was 37.37 per cent, while production of the hens in the check lot was 45.56 per cent.

The positively reacting hens apparently produced a higher percentage of infertile eggs and eggs having weak germs, than was produced by the hens giving a negative reaction. The re-

actors produced an average of 20.8 per cent of infertiles and 17.5 per cent dead germs, while birds in the check lot produced an average of only 8.4 per cent infertiles and 6.6 per cent dead germs. An average of 41.9 per cent of the eggs laid by the hens in the check lot produced chicks that died in the shell, while only 25.9 per cent of the reactor's eggs, which were incubated, produced fully formed chicks that failed to hatch. Chicks were hatched from 35.9 per cent of the reactors' eggs and 43.1 per cent of the check birds' eggs that were incubated.

Apparently the only explanation for the small percentage of fully formed chicks failing to hatch from eggs laid by infected hens is that many of the embryos die in the early stages of incubation.

The difference between the hatchability of eggs from infected hens and the hatchability of eggs from non-infected hens was far less marked than the difference in livability of chicks from the two lots.

Of the chicks hatched from eggs laid by hens in the check lot, an average of 94.02 per cent were living and apparently vigorous on the fourteenth day, while an average of only 22.4 per cent of the chicks from reacting hens were living at that time. Of all of the infected chicks living at the end of the fourteenth day only four showed any indications of recovery. The rest of the chicks appeared stunted and diseased. All of the infected chicks, remaining alive on the fourteenth day,

were killed and examined. In all cases the livers showed marked indications of the presence of Bacterium pullorum.

Cultures were made from three chicks hatched from eggs laid by hens in the check lot and eight chicks hatched from eggs laid by the reactors. Bacterium pullorum was found in each case. This shows that bacillary white diarrhea was not completely eradicated by one elimination of reactors.

The tables showing the death rate of chicks from infected hens indicate that the greatest mortality occurs during the sixth and seventh days after hatching.

The chicks from the infected hens appeared as healthy and strong as normal chicks when first hatched and did not show a particularly unhealthy appearance until they were five or six days old.

Conclusions.

From the data obtained the following conclusions could be drawn.

1. The egg production of hens harboring bacillary white diarrhea infection is usually lower than egg production of hens free from this disease.

2. The fertility and hatchability of eggs is materially lowered by the presence of bacillary white diarrhea infection in the flock.

3. The hatchability of eggs and the livability of chicks is usually improved by even one elimination of hens reacting to the macroscopic agglutination test.

4. A single elimination of hens reacting to the agglutination test does not remove all birds harboring Bacterium pullorum.

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