THE EPIDEMIOLOGY OF FOWL CHOLERA

IV. Field Observations of the "Spontaneous" Disease

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The results of special studies on the bacteriology of fowl cholera, its mode of infection, and reaction of the host have been dealt with in the preceding papers (1 a, b). It is proposed now to present the data collected from field studies as the disease occurs spontaneously in poultry flocks.

Methods

A series of expeditions was made to the various poultry farms chosen for study. At each trip, a census was taken, the amount of clinical infection noted, and cultures made from the nasal passages of all or part of the flock. These cultures were then brought to the laboratory at New Brunswick or New York for study. In addition to this, birds dying within the period of observation were sent to New Brunswick if possible, and there autopsied and cultured.

To test for carriers, sterile cotton swabs of appropriate size were passed over the nasal mucosa and streaked on hemolyzed blood agar contained in 150 cm. Petri dishes. After 18–24 hours' incubation, the number of colonies of *P. avicida* on the plates was estimated and suspicious colonies transferred to appropriate media for identification and colony type determination. Cultures showing characteristic colony appearance and morphology, acid production in dextrose, saccharose, and mannite, and a positive indol test were considered *P. avicida*.

Individual birds in the flock were identified by numbered leg bands.

A Study of Epidemic P. avicida Infection

This investigation was initiated by the bacteriological examination of three dead birds sent to New Brunswick by their owner at Merrick, N. Y. He reported that the death rate at his farm had been consistently low, but that suddenly an increase had taken place.

The poultry plant was found to consist of three widely separated houses, well-kept and clean, large and airy. One entire side of each unit was enclosed by glass. The diet was carefully supervised and contained an abundance of sprouted oats and cod liver oil.

House I, 92 x 16 ft., contained 425 White Leghorn pullets, December, 1927. Cholera then appeared and gave rise to a relatively low constant death rate. By March, about 35 per cent of the population had succumbed. The entire population of 341 individuals was tested

TABLE I

Incidence of P. avicida Carriers and Occurrence of Localized Lesions at Merrick, Long Island

House	Date	Number of fowl examined	Per cent carriers	Nasal catarrh	Canker	Roup	Swollen wattles
II	Jan. 25 '28	22	45	0	0	0	0
I	Jan. 28	341	11	25	0	1	13
I	May 10	90	1	9	0	1	1

for carriers late in January, 1928. Thirty-seven or 11 per cent were positive, and note was made of a high incidence of local infection (Table I). Late in March, the survivors were combined with a similar group from House III. In May, 1928, a sample of 90 birds was tested for carriers. Only one was positive, although six of the sample were known to have been carriers at the previous testing in January. The physical condition of the birds also seemed much improved (Table I).

House II, 100 x 20 ft., housing 700 White Leghorn pullets, suffered the worst outbreak. The disease appeared here also in December, 1927, but in a more severe form. In four weeks 45 per cent of the population had succumbed. Losses then ceased abruptly. Late in January, when a "sample" of twenty-two birds was tested for carriers, 10 or 45 per cent were positive, but no local infections were noted.

House III was divided into three compartments. The first, 65 x 22 ft., housed 400 White Leghorn hens, the second, 50 x 22 ft., contained 300 similar hens. These two populations remained free of cholera. The remaining end compartment, 40 x 22 ft., housed 300 White Leghorn pullets, similar to those occupying Houses I and II. These birds began to die after mortality in Houses I and II had practically ceased, and by late spring the total deaths were estimated to be 37 per cent. No carrier determinations were made at the time of the epidemic, but the tested sample of survivors from Houses I and III showed a very low rate.

The total mortality in this flock can merely be estimated. The owner stated that 1325 pullets were added to his stock in the summer preceding the epidemic. Of these, 525 were known to have died during the winter of 1928. Forty-seven were received at the laboratory for autopsy; all but one yielded pure cultures of *P. avicida*. It was presumed, therefore, that the deaths were in the main due to *P. avicida* infection.

Of the strains of *P. avicida* recovered, three were of the "fluorescent" and 64 the "intermediate" colony type. No "blue" colony forms were obtained. The three "fluorescent" colony strains came from the blood of fatal cases in House II at the height of the epidemic. The range of acid agglutination was tested for 48 of the strains plus three "blue" colony variants obtained from the "fluorescent" forms. The results are given in Table II. The three "fluorescent" strains showed the typical narrow zone of flocculation, pH 2.4–3.0; the "blue" variants, the usual wide zone, pH 2.4–5.4, and the "intermediates," a scattered range between the limits, tending toward the more acid range. No distinction between "autopsy" and "carrier" strains was apparent.

Agglutination tests were made with "Pa" antisera (1 a) diluted 1:100. All save the three "fluorescent" colony forms showed flocculation. The agglutination titre of twenty-eight "intermediate" plus the three "blue" variants from the "fluorescent" strains was then determined (Table III). One "blue" variant agglutinated to 1:640; the others approximately to titre. Agglutination of the intermediates ranged from 1:160 to 1:2500; the greater number fell in the range 1:640–1:1280.

The virulence of fourteen autopsy strains and nineteen carrier strains was tested according to the method described in the second paper of this series (1 b). Twenty birds were used for the tests above, for each strain. The results are recorded in Tables IV and V. Several of the autopsy strains were titrated repeatedly. Hence the data on these strains are considered to be the more accurate index of virulence. The average mortality of all autopsy strains was 33 per cent.

TABLE II

Zone of Acid Agglutination. P. avicida Strains from Merrick, N. Y.

Colony type	Zone of agglutination pH range	Number of strains
Fluorescent	2.4 only	1
	2.4–3.0	1
	3.0 only	1
Blue variant	2.4–5.0	2
	2.4–5.4	1
Intermediate	2.4–4.2	2
	2.4–5.4	4
	2.5–3.4	2
	2.5–3.6	3
	2.5–4.1	3
	2.5–4.4	4
	2.5–4.7	3
	2.5–5.1	5
	3.0–3.5	1
	3.0–4.2	1
	3.0–5.0	9
	3.0–5.2	2
	3.0–5.4	1
	3.2–3.5	1
	3.4–4.7	3
	Spontaneous	1

TABLE III

Reaction with "Pa" Type Serum. P. avicida Strains from Merrick, N. Y.

Colony form	Agglutination Maximum serum dilution	Number reacting
Fluorescent	None	3
Blue variant	1:640	1
	1:1280	1
	1:2500	1
Intermediate	1:160	1
	1:320	2
	1:640	7
	1:1280	10
	1:2500	8

TABLE IV

Virulence of Merrick "Autopsy" Strains of P. avicida

Culture	Type	Isolated	Tested	Per cent dead	
629	Fl.	Jan. 22	Jan. 23	20	
			Feb. 15	20	
			Mar. 6	10	av. 17
630	Int.	Jan. 22	Jan. 23	15	
			Mar. 8	20	av. 17
631	Fl.	Jan. 22	Jan. 23	35	
			Feb. 8	40	
			Mar. 8	40	
			Apr. 11	60	av. 44
635	Int.	Jan. 30	Mar. 8	30	
638	Fl.	Jan. 30	Feb. 15 ('28)	60	
			Mar. 8	50	
			Mar. 21	40	
			Apr. 18	30	
			Apr. 25	40	
			Mar. 6 ('29)	30	av. 40
641	Int.	Jan. 30	Mar. 6	20	
642	Int.	Jan. 30	Mar. 6	60	
			Apr. 5	30	av. 45
644	Int.	Jan. 30	Mar. 6	20	
645	Int.	Feb. 1	Feb. 15	50	
			Feb. 20	60	av. 55
647	Int.	Feb. 1	Mar. 6	20	
648	Int.	Feb. 1	Mar. 6	40	
650	Int.	Feb. 1	Feb. 15	30	
			Feb. 20	50	av. 40
651	Int.	Feb. 1	Mar. 8	50	
702	Int.	Feb. 6	Mar. 6	20	
Average.....				33%	

In these titrations no significant difference in the killing power of "fluorescent" and "intermediate" colony strains was apparent. The average mortality of all "carrier" strain titrations was 21 per cent. This figure is lower than that of the "autopsy" strains, but since only one titration of each was made, the difference is of questionable significance.

The interpretation of these studies is necessarily limited. However, the following summary includes the salient facts. An epidemic of fowl

TABLE V

Virulence of Merrick "Carrier" Strains of P. avicida

Culture	Type	Isolated	Tested	Per cent dead
687	Intermediate	January 28	February 20	20
660	"	" 28	April 5	0
690	"	" 28	" 5	0
654	"	" 22	" 11	50
655	"	" 22	" 11	0
657	"	" 22	" 11	20
658	"	" 22	" 11	50
653	"	" 22	" 18	20
659	"	" 22	" 18	20
661	"	" 22	" 18	30
663	"	" 22	" 18	20
664	"	" 22	" 18	10
665	"	" 22	May 2	0
666	"	" 22	" 2	30
667	"	" 22	" 2	40
668	"	" 22	" 2	30
669	"	" 22	" 2	30
670	"	" 22	" 2	10
672	"	" 22	" 2	40
Average.....				21

cholera arose during the winter in a healthy flock of birds. Its mode of onset was not determined, and the owner stated that his birds had previously been free of the disease. The epidemic ran different courses in three population units and failed to appear in a fourth group of hens. In one infected group, the epidemic took an explosive form, with high, wave-like mortality over a brief period. Few cases of localized infection developed. In two other groups, the epidemic smoldered with low, relatively constant mortality rate over a period of

months. Strains of *P. avicida* from the two populations examined were indistinguishable. Following the epidemics, carriers were very few in number.

From these facts, it seems probable that "epidemic" strains of *P. avicida* were introduced into the flock from some outside source. Apparently, the birds possessed no specific immunity to the infection, but those in House I may have been more resistant than the population of House II. The crowding in House II, by decreasing the resistance of the inhabitants and increasing the risk of infection, may have been responsible for the explosive type of attack. Finally, the fact that few post-epidemic carriers were encountered indicates that, as in similar "D type" rabbit infections (1 c), "epidemic" strains of *P. avicida* are relatively non-vegetative.

B. Study of Endemic P. avicida Infection

A similar investigation was made at a small farm in Deans, N. J., consisting of 45 White Leghorn hens, cared for entirely by the owner. The pen was very dirty and neglected, dark, and in an exposed location. Some sort of epidemic of fowl cholera was known to have occurred in previous years, and the birds under observation were known to be survivors.

Five trips were made to the farm, November, 1927 to March, 1928. A census was taken, the clinical condition of the birds noted, and cultures obtained from the nasal passages of each. The birds were tagged, so that a four months' record of each was obtained. The results of these investigations are given in Table VI.

No birds died within the period. Nineteen showed local upper respiratory infections—roup, canker, mucous discharge, wattle disease —at one or more examinations. Thirty-one proved to be carriers of *P. avicida* on at least one test. The carrier rates at each examination were 9.7, 40.5, 16.2, 31.5, and 29.0 per cent.

Fifty-two strains of *P. avicida* were recovered from this flock. Apparently, all formed "blue" colonies (1 a) on the first transfer. Twenty-seven were titrated in "Pa" antiserum (1 a). The results are shown in Table VII. Two did not agglutinate; two showed spontaneous agglutination; three reacted within the range 1:32–1:128; four agglutinated at a dilution of 1:256; fourteen to 1:152, and two to 1:1024 and 1:2048 respectively.

TABLE VI

Results of Serial Examinations for P. avicida Carriers, at Deans, N. J.

Bird	November 23	December 17	January 17	February 7	March 23
1	0	0 Canker	0	−	+
2	0	0 Mucus	0	0	0
3	−	0	−	0	+
4	+ Canker	0	0	0	+
5	0	+ Mucus	0	0	0
6	0	+	0	0	+
7	0 Membrane	−	0	+ Canker	0
8	0	0 Canker	0	+	−
9	0	0 Mucus	0	0 Roup	−
10	0	0	0	0	0
11	0	0 Mucus	0	+	0
12	+	+ Mucus	0	0	−
13	0	+ Wattle	0	0 Mucus	0
14	0	+ Canker	0	+	+
15	0	0	0	0	0
16	0	0	0	0	0
17	0	+	0	−	0
18	0	+ Mucus	0	+ Mucus	0 Roup
19	+	+	+	0	+
20	−	0 Mucus	0	+	0
21	0	+	0	0	0
22	0	0	0	0 Mucus	0
23	0	+ Canker	0	0 Roup	0
24	0	−	0	0 Roup	−
25	0	0	+	0	0
26	0	0	0	0	0
27	+	0	0	+	0
28	0	+	+	+	+
29	0	0	0	0	0
30	0	0	0	0	0
31	0	0	0	0 Canker	0
32	0	+	0	0	−
33	0	+	0	0	0
34	0	+	+	0	0
35	0	0	0	+	0
36	0	0 Mucus	0	0 Mucus	0
37	0	0	+	+	0
38	0	0	0	+	+
39	0	0	0	0	−
40	0	+ Mucus	0	−	0
41	0	0	0	0	0
42	0	0	0	0	+
43	−	+	0	−	+
44	0	+	+	+ Roup	+
45	−	−	−	+	−
Positive	4 = 9.7%	17 = 40.5%	6 = 16.2%	13 = 31.5%	11 = 29%
Negative	37	25	37	28	27
Not done	4	3	2	4	7

0 = Negative; + = *P. avicida* recovered; − = No examination.

Acid agglutination tests, made on twenty-nine strains, are given in Table VIII. Twenty-four showed the wide zone characteristic of "blue" colony forms, but five came down well toward the acid side, pH 2.4–4.0

This study may be summarized as follows: in a small flock, known to have survived cholera infection the previous year, the endemic manifestation of the disease was studied. No deaths occurred, but the per cent of local lesions and nasal carriers increased during the winter

TABLE VII

Reaction with "Pa" Serum. P. avicida Strains from Deans, N. J.

Serum dilution	Number of strains reacting	Serum dilution	Number of strains reacting
No reaction	2	1:256	4
Spontaneous	2	1:512	14
1:32	1	1:1024	1
1:64	1	1:2048	1
1:128	1		

TABLE VIII

Zone of Acid Agglutination. P. avicida Strains from Deans, N. J.

Zone of agglutination pH range	Number of strains	Zone of agglutination pH range	Number of strains
2.4 only	1	2.4–4.6	4
2.5–3.0	1	2.4–5.0	1
2.4–3.2	1	2.4–5.4	6
2.4–3.5	1	3.0–4.2	1
2.4–4.0	1	3.0–4.6	3
2.4–4.2	5	3.0–5.4	4

months. Apparently the birds differed in their ability to resist infection, for in spite of the evidence of spread of the organisms, some birds remained free throughout. All, however, were probably more than normally resistant, for they were survivors of a previous epidemic. The organisms recovered possessed the general characteristics of the "blue" colony form, although a few resembled the "intermediates" in their behavior. Unlike the "epidemic" fluorescent and intermediate colony forms, they exhibited the power of vegetation and spreading in the community. In this respect they were similar to the "mucoid" strains of *P. lepiseptica* (1 c).

SUMMARY

Field studies of fowl cholera on two commercial poultry farms are described. One farm, previously free of cholera, was studied during an active epidemic, which occurred during the winter months. The strains of *P. avicida* recovered, both from "autopsy" and from "healthy carriers" proved generally similar, and to be of the "fluorescent" or "intermediate" colony type, which is of relatively high virulence. After the subsidence of the epidemic, these strains tended to disappear.

The second flock consisted of a small group of birds which had survived an epidemic of cholera the previous year, and in which the infection was prevailing in endemic form. No deaths occurred during the period of observation, but the number of birds with localized lesions and the number of carriers increased to a high level during the winter months. The strains of *P. avicida* were apparently of the "blue" colony form, although some, as shown by their acid and serum agglutination reactions, resembled the "intermediates." These strains appeared to be spreading rather than dying out. The individual fowls differed in their response to the presence of infection; some showed localized lesions, others were carriers, while still others seemed entirely refractory.

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