THE EPIDEMIOLOGY OF FOWL CHOLERA

III. Portal of Entry of P. avicida; Reaction of the Host

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Plate 7

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The part of these studies dealing with the identification and characteristics of *P. avicida* has been reported in previous papers. It is proposed now to describe a number or tests made to determine: a) the normal portal of entry of these organisms into the chicken host, and b) the forms of infection so established.

Various portals of entry for the spontaneous infection have been suggested, but the gastrointestinal route has been considered as the most likely (Kolle u. Wassermann, III Aufl.). However, experimental infection by feeding methods is known to be difficult. Hertel (1) and Muller (2), for example, reported that when the inoculum is instilled directly into the esophagus of fowls and pigeons, in such a way as to avoid contact with the nasal and pharyngeal mucosae, or given in the relatively dry form of a pellet, infection does not take place. Nevertheless, the opinion of field workers that the disease is gastrointestinal in nature is generally accepted. The clinical course of acute fowl cholera and the pathological findings at autopsy are fully described in the literature (Kolle u. Wassermann, III Aufl.). But references to chronic cases are exceptional and the existence of localized infections is recognized only in occasional reports (3–9).

In contrast to this incomplete knowledge of *P. avicida* infection are the facts relative to infection by a similar organism, *P. lepiseptica* (10). These bacilli are known to enter the rabbit host by way of the upper respiratory tract and to induce various forms of localized and general disease.

Accordingly, tests were planned to determine whether the natural portal of entry of *P. avicida* into fowls was by way of the mouth or nasal passages, and whether in fowl cholera as in rabbit snuffles-pneumonia, infection appeared in various generalized and local forms.

Normal Portal of Entry

Preliminary tests showed that cultures of *P. avicida* administered *per os* to chicks and adult fowl did not infect, whereas when dropped into the nasal cleft, they brought about the typical disease (Table I–IV). The details of these tests are as follows:

Two fluorescent strains, "Pa" and "Kansas," described in the previous paper (11), were employed. The dose 0.2 cc., and the method for its control have been discussed (11). The birds were either five

TABLE I

Comparative per Os and Intranasal Injection of P. avicida into Fowl

Test	Number of birds injected	Inoculum	Number dead	Per cent dead
I	15—adults	0.2 cc. "Pa" *per os*	0	0
	" "	0.2 cc. "Pa" intranasal	6	40
II	25—adults	0.2 cc. "Pa" *per os*	0	0
	" "	0.2 cc. "Pa" intranasal	7	28
III	12—pullets	0.5 cc. "Pa" *per os*	0	0
	" "	1 drop "Pa" intranasal	4	33
IV	20—adults	0.2 cc. "Kan" *per os*	0	0
	30— "	0.2 cc. "Kan" intranasal	15	50
V	25—chicks	0.2 cc. "Kan" *per os*	0	0
	50— "	0.2 cc. "Kan" intranasal	22	44
VI	20— "	0.2 cc. "Kan" *per os*	0	0
	" "	0.2 cc. "Kan" intranasal	11	55

weeks chicks, similar to those already described, or adult pullets from the same source (11). It is important to mention again that these fowls had had no previous exposure to *P. avicida*. Intranasal inoculations were made by holding the bird head downward and instilling the desired amount of inoculum from a syringe through a blunt needle over the surface of the mucosa on the roof of the nasal cleft. The *per os* instillations were made by placing the inoculum in a small gelatin capsule and introducing it at once into the esophagus of the chicken with a pair of forceps. After injection each bird was placed

in an individual cage and observed for ten days. Autopsies were performed on all fatal cases and cultures taken for identification.

Tests V and VI (Table I) were subjected to the following additional control, to demonstrate that cultures introduced in capsules were liberated and had retained their virulence. A number of additional birds were given the capsules, and every fifteen minutes thereafter one bird was killed, autopsied, and cultured. The specific strains of *P. avicida* were recovered from crop and upper intestinal tract of the autopsied bird, and then reinoculated intranasally in the usual manner into another series of normal chicks. These reinoculated strains were found to possess the same virulence as the control cultures. To prove that birds inoculated *per os* were as susceptible as those inoculated intranasally, they were reinoculated after ten days by the intranasal route. The mortality following the introduction of organisms by the nasal route was similar to that of the control intranasal series. It was concluded from the tests that *P. avicida* does not infect by way of the gastrointestinal tract. On the other hand, the chickens which succumbed to the intranasal instillations presented the usual clinical and pathological lesions typical of fowl cholera. This was regarded as evidence that the natural portal of entry of *P. avicida* is by way of the upper respiratory tract.

Reaction of the Host

To gain more information on the mode and route of infection of *P. avicida*, and especially to study the varieties of host response, series of birds were given intranasal doses of the organisms.

The first tests were made to determine the extent of variation in per cent mortality of groups of similar birds given similar doses of the same strain of *P. avicida*.

"Fluorescent" colony strains, "Pa," "638," and "Kansas," and "intermediate" colony strains "629" and "631" were prepared as usual (11) and instilled intranasally in standard doses, 0.2 cc., 20,000,000 bacilli. The birds employed were 4–5 weeks old, 100–150 gms. chicks, raised under uniform conditions and free of previous exposure to *P. avicida*. At least twenty were used for each titration. The results of these tests are given in Table II.

Nine titrations of the "Pa" strain showed an average mortality of 31.9 ± 2.6 per cent. This average figure does not include the two

low values of May 16 and 23. Three titrations of "638," 40 ± 4 per cent; nine of "Kansas," 50.5 ± 5 per cent; three of "629," 16 ±

TABLE II

Comparison of Results of Repeated Virulence Tests on Same Strains
(5 weeks chicks; dose 0.2 cc. culture, intranasally)

Date	Strain	Number injected	Per cent mortality	
March 29, '27	"Pa"	63	35	
April 4		21	29	
11		21	33	
18		27	29	Average* 31.9
May 3		56	31	Deviation =
5		50	30	2.6
9		66	29	
16		22	14	
23		30	13	
Oct. 27, '28		10	40	
Dec. 8		13	31	
Feb. 6	629	10	10	Average 16.6
March 10		10	20	
25		10	20	
March 8	631	10	40	Average 42.5
21		20	35	
April 11		10	60	
May 2		20	35	
Jan. 29	"Kansas"	10	60	
Feb. 4		50	44	
6		30	50	
8		20	65	Average 50.5
14		35	46	Deviation =
April 24		10	40	5.0
27		10	50	
28		10	50	
May 7		20	50	

* This average does not include the low determinations of May 16 and 23, 1927.

4.5 per cent, and four of "631," 42.5 ± 8.7 per cent. The small variations in per cent mortality between tests indicate that the technique was adequate, and that under these conditions the virulence of

each strain was constant and the average resistance of the groups of chicks the same. Tests with adult birds gave similar results.

The possible effect of season is shown in the mortality figures of the "Pa" series. The drop in death rate in May from a relatively constant level of 30 ± to 15 per cent, and the subsequent rise to this level again in October is a phenomenon similar to those occurring in mouse typhoid, mouse pneumonia, and rabbit pneumonia infections (12).

The effect of dosage on mortality is shown in Table III. For this test, each of six groups of ten birds were given, in uniform volume, a definite dose of *P. avicida*, "Kansas," ranging from ten times the standard amount of 20,000,000 bacilli to 1/10,000 of this amount. The resulting mortality indicates that groups of chicks react similarly

TABLE III

Effect of Varying Dosage on Mortality

(0.2 cc. "Kansas" strain, intranasally, into groups of 10 chicks)

Dose	Per cent mortality
Standard (equal to 18 hr. broth culture)	40
10 times standard	80
Standard diluted 1:10	50
" " 1:100	40
" " 1:1000	40
" " 1:10,000	40

to doses between 20,000,000 and 20,000 bacilli, but are more susceptible to greater amounts and less susceptible to smaller numbers. Two additional tests of this sort gave similar results.

The response of young birds to the intranasal instillations of *P. avicida* took two forms,—either they died abruptly of typical fowl cholera, or remained quite healthy. None showed chronic or local forms of infection. Nevertheless, evidence was obtained indicating that *P. avicida* infection in nature and under proper experimental conditions takes the form not only of acute septicemic fowl cholera, but also of chronic and localized disturbances.

Indirect evidence of local and of chronic *P. avicida* infection came in part through the kindness of Dr. Arnold Branch and William Steenken, Jr., and is presented with their permission at this time.

A small flock of nineteen fowl maintained at Trudeau for studies on avian tuberculosis became infected "spontaneously" with *P. avicida*. Within a few

TABLE IV

P. avicida Infection in a Flock of Chickens at Trudeau, N. Y.

No.	Course of disease	Autopsy findings		Occurrence of *P. avicida*				
		External	Viscera	Blood	Wattle	Roup	Eye	Pericardium
A 854	Acute		Pericarditis Liver degeneration	+				+
C 33	Acute		Liver degeneration	+				
A 861	Acute		Liver degeneration	+				
A 853	Acute	Caseous wattle	Pericarditis Liver degeneration	+				+
C 34	Acute	Wattle disease	Pericarditis	+	+			+
A 858	Acute	" "	Pericarditis Pneumonia	+	+			+
A 852	Acute	Wattle disease; roup	Focal hemorrhage in lung	+				
C 31	Acute		Pericarditis	+				
A 863	Acute	Wattle disease	Pericarditis Perihepatitis	+				+
C 32	Acute	" "	Caseous lung focus	+	+			
B 219	Chronic	Rhinitis; roup		+		+		
B 218	Chronic	Roup		0		+		
E 31	Chronic	"		+		+		
B 222	Chronic	Roup; intraorbital abscess		0		+	+	
E 32	Chronic	Roup		0				
B 217	Chronic	Roup; rhinitis	Caseous nodules in liver	0		+		
B 220	Chronic	Roup		0		+		
B 223	Chronic	"		0				
B 221	Chronic	"		0				

days 50 per cent died of typical fowl cholera and were autopsied. Positive blood cultures were obtained in all cases. Six of the number showed localized wattle

disease and one roup. Cultures taken from three of the infected wattles yielded the epidemic strain of *P. avicida*. Nine of the birds survived but not without sickness and loss of weight. After a few weeks they were killed and autopsied. Two had positive blood cultures. All showed severe upper respiratory lesions, manifested clinically as mucopurulent discharge, roup, rhinitis, and orbital abscess. From the lesions of six of these cases, *P. avicida* was recovered. One bird showed a chronic pulmonary lesion; another a similar focus in the liver. The strains sent to the Rockefeller Institute laboratory appeared identical; no distinction was possible between cultures from local lesions of chronic cases and blood cultures of acute "cholera" cases.

An additional study of "spontaneous" local lesions was made on birds from poultry farms in New Jersey. Lesions from twenty-seven

TABLE V

Bacteriological Examination of Local Infections Occurring in Chickens (a) "Spontaneously" and (b) Following Intranasal Inoculation with P. avicida

Lesion	From "spontaneous" lesions		From lesions occurring in experimentally inoculated birds	
	Positive for *P. avicida*	Negative for *P. avicida*	Positive for *P. avicida*	Negative for *P. avicida*
Ocular roup	7	3	7	0
Mixed ocular and sinus roup	0	1	0	0
Sinus roup	0	5	4	1
Edema of wattle	5	3	1	1
Otitis media	2	0	8	0
Submandibular abscess	1	0	0	0

birds on a number of different farms were cultured; fifteen yielded *P. avicida* of the "blue" colony type (Table V) (Figs. 1 to 3). Of these, nine were tested for virulence and, as usual, failed to kill.

Direct evidence of chronic and localized *P. avicida* infection was obtained by giving cultures intranasally to tested non-carrier, healthy fowl. In all, twenty-two local lesions occurred, classified as roup, edema of wattle, and abscess. Twenty of these proved positive (Table V). It is concluded, therefore, that roup, edema of the wattle, and sinusitis in fowl are frequently local forms of *P. avicida* infection.

One other form of *P. avicida* infection was studied,—the carrier

state. That a certain number of birds in infected flocks carry the organisms in the nasal cleft will be brought out in the following paper; at this time it will suffice to present the results of tests on birds given the organisms experimentally by the nasal route. Birds were tested before inoculation and at intervals thereafter (Table VI). The results show that the carrier state is a definite, though clinically

TABLE VI

Occurrence of Carriers among Injected Birds

Date	Strain	Number injected	Per cent mortality	Per cent carrier
April 4	642	10	30	0
February 7	642	10	60	0
	648	10	40	0
March 8	631	10	40	10
	635	10	30	10
	638	10	50	10
	651	10	50	0
	661	10	30	0
	667	10	40	0
	672	10	40	0
April 11	631	10	60	0
April 18	661	10	30	0
April 5	660	10	0	20
	690	10	0	30
	735	10	0	10
	793	10	0	10
	629 B	10	0	20
March 22	745	10	0	20
	749	10	0	10
	773	10	0	20
March 29	770	10	10	30
	779	10	0	20
	785	10	10	10

concealed form of *P. avicida* infection, and that it occurs most frequently with the "blue" type, so-called endemic forms, and rarely with the "fluorescent" colony "epidemic" types.

DISCUSSION

The data contained in this report indicate that fowl cholera is not primarily a gastrointestinal disease, but that, like rabbit pasteurellosis

(12), it is upper respiratory in origin. These observations show further that the etiological agent, *P. avicida*, gives rise not only to the well-known septicemic cholera, but also to various types of chronic pneumonia and liver abscesses, and local disturbances of the upper respiratory tract, such as roup, sinusitis, and inflammation of the wattle. Still another form of infection, clinically unrecognizable, is the "healthy" nasal-carrier state. Finally, it appears that of any group of healthy, selected chickens given a similar dose intranasally, some will resist infection entirely.

The fact that chickens under controlled conditions react differently to the same intranasal dose of *P. avicida* indicates that they differ in their ability to resist the infection and that, as in the case of rabbits exposed to *P. lepiseptica* (13), the most resistant individuals are not infected; those less so become carriers, others more susceptible contract local upper respiratory lesions, while the most susceptible die of the acute generalized infection.

Another observation of importance is that a given strain of *P. avicida* given intranasally to a number of groups of controlled and selected birds gives rise, in general, to a similar amount of mortality. From this the conclusions are drawn that the *technique* of titration is adequate for measuring virulence of organisms, and that under these conditions the *virulence* of a given strain and the *average resistance* of the chickens are constant. The summer decrease in mortality is at present considered due to an increase in the resistance of the birds.

The standard dose, estimated to be approximate to that available to flocks at epidemic times, may be increased ten times, or decreased 1000 times without apparent effect on mortality. Outside of those limits, however, dosage influences mortality. These observations are in accord with similar tests with mouse Friedländer (14) and typhoid (15) bacilli.

SUMMARY

1. In the experiments here presented, *P. avicida* proved incapable of inciting fowl cholera when introduced directly into the alimentary tract. On the other hand, when administered into the upper respiratory passages, it induced typical disease.

2. When *P. avicida* was introduced into the nasal passages of con-

trolled, selected chickens, some died of typical septicemic cholera, a few developed chronic pneumonias and other conditions and succumbed, a few developed localized upper respiratory inflammations, such as rhinitis, roup, and wattle involvement, while yet a few others became "healthy" nasal carriers. Usually, however, more than 50 per cent resisted infection. Repeated titrations of this sort gave, in general, uniform results, save that in spring and summer the per cent mortality decreased.

3. *P. avicida* was recovered from a number of cases of "spontaneous" roup, rhinitis, and wattle disease.

4. Groups of chickens reacted similarly to doses of virulent *P. avicida* varying from 20,000,000 to 20,000. Outside these limits, dosage exercised a marked influence on mortality.

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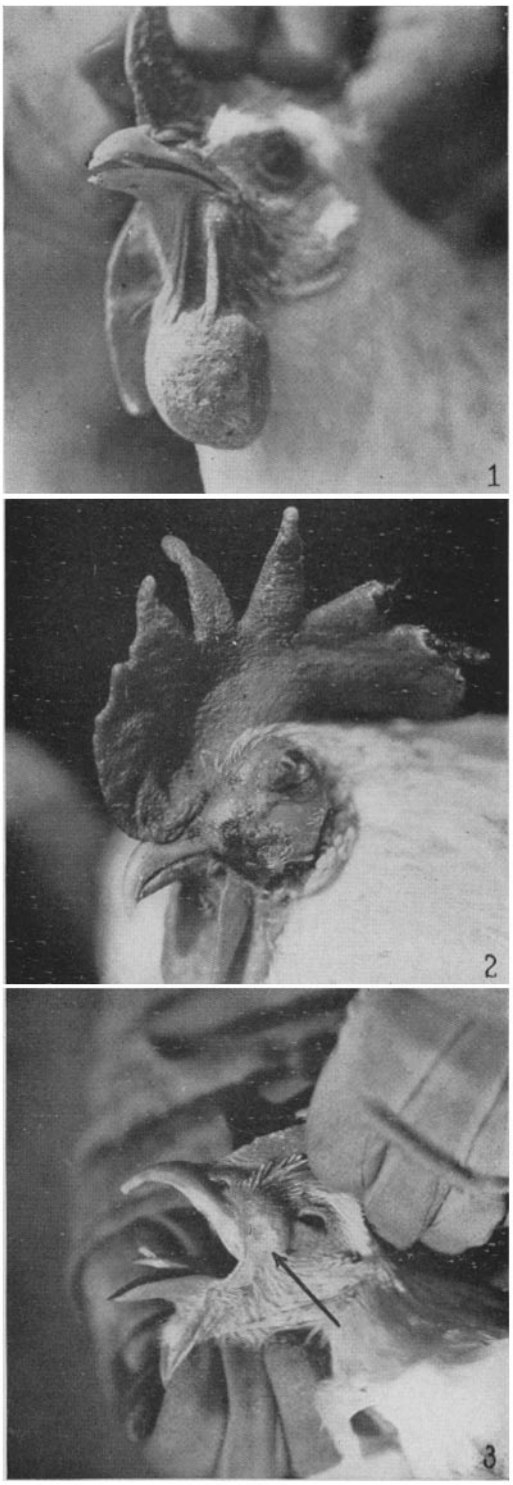
EXPLANATION OF PLATE 7

Spontaneously occurring lesions yielding *P. avicida* on culture.

FIG. 1. Wattle disease.

FIG. 2. Ocular roup. The lesions on the comb are due to fowl pox.

FIG. 3. Sinus roup.



(Hughes and Pritchett: Epidemiology of fowl cholera. III)