THE EPIDEMIOLOGY OF FOWL CHOLERA

VI. The Spread of Epidemic and Endemic Strains of Pasteurella avicida in Laboratory Populations of Normal Fowl

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In previous studies on spontaneous fowl cholera in commercial poultry flocks, it was noted that the epidemic form of infection was usually associated with strains of *Pasteurella avicida* which appeared to be relatively highly virulent and unable to survive in the tissues of the host, while the endemic infection was associated with strains of low virulence and high vegetative capacity (1). The present experiments were made to test these relationships under the more controlled conditions of the laboratory, and in addition to determine whether "laboratory" variants of the epidemic strains were similar to the endemic strains in their capacity to survive and spread in their native host.

Technique

The young birds used in these tests were White Leghorns from a single inbred flock. The chicks were received in the laboratory in batches of 100 to 200 when 1 day old and were raised in strictly isolated quarters out of contact with other birds. They were free of infectious disease and exposure to *P. avicida*. The older birds used in Experiment 3 were White Leghorns of an unselected farm stock.

The strains of *P. avicida* employed have been described previously (2). The "Kansas" strain was obtained from a spontaneous epidemic of fowl cholera; Strain 773 was obtained from a flock in which fowl cholera was endemic.

P. avicida was administered to the test birds in a manner calculated to simulate natural conditions and at the same time be relatively quantitative, by instilling into the nasal cleft of each individual a uniform number of the bacteria from an 18–24 hour blood broth culture. Carrier tests were made by passing a sterile cotton covered swab over the surface of the nasal cleft of the bird and subsequently streaking it over the surface of a freshly prepared blood agar plate. Birds dying in the course of the experiments were autopsied and cultured for the presence of

P. avicida. These various procedures have been fully described in earlier publications (1,2).

EXPERIMENTAL

Epidemic Strains

Experiment 1.—March 12, 1928. Eight chicks, aged 1 month, were each given intranasally 0.2 cc. broth culture of *P. avicida* "Kansas" and placed in a cage measuring 12 x 16 x 12 inches. Eight similar uninoculated birds were added to the group as "contacts." Examinations for carriers of *P. avicida* were made 1, 3, 5, 24, and 30 hours, and 2, 3, 4, and 7 days later.

TABLE I

Virulence and Spread of an Epidemic Strain of P. avicida

Test animals	Hrs.					Days				Condition of animal
	1	3	5	24	30	2	3	4	7	
Inoculated No. 1	+	+	+	+	+	+				Died
" " 2	+	+	+	+	+	0	0	0	0	Well
" " 3	+	+	+	+	+	0	0	0	0	"
" " 4	+	+	+	+						Died
" " 5	+	+	+	+	+	0	0	0	0	Well
" " 6	+	+	+	+	+	+	0	0	0	"
" " 7	+	+	+	+	+	+				Died
" " 8	+	+	+	+						"
"Contact" Nos. 1 to 8	0	0	0	0	0	0	0	0	0	Well

+ = *P. avicida* present. 0 = *P. avicida* absent.

The results of this experiment are given in Table I. 50 per cent of the inoculated birds were dead of fowl cholera within 3 days—a figure which agrees with previous data on the virulence of the "Kansas" strain (2). The survivors, however, showed no signs of infection and no evidence of carrying the organisms in the nasal cleft after the 2nd day. The contacts remained healthy and did not become carriers.

This test was repeated March, 1928, with similar results.

Experiment 2.—February 8, 1929. Twenty chicks, aged 1 month, were each given 0.2 cc. of *P. avicida* "Kansas" intranasally and placed in a floor pen 4 x 8 feet in dimension. Normal, uninoculated birds were added thereafter at the rate

of one per day for 20 days. Carrier tests were made on the 7th and 18th days of observation.

The results of this test are given in Table II. Seven of the twenty inoculated birds were dead of fowl cholera (35 per cent) within 7

TABLE II

Virulence and Spread of an Epidemic Strain of P. avicida

Date	Identification No. of dead chicks	Surviving population	Remarks
2/ 8/29		21	Birds numbered 1 to 20 inoculated. One normal bird added daily and numbered serially from 21 to 40
2/ 9/29	15	21	
2/10/29	5, 9, 11, 13	18	
2/13/29	19	20	
2/15/29	8	21	Carrier tests negative
2/26/29		32	" " "
2/28/29		33	Tests terminated

TABLE III

Virulence and Spread of an Epidemic Strain of P. avicida

Date	Identification No. of dead chicks	Surviving population	Remarks
2/ 8/29		21	Birds numbered 1 to 20 inoculated. One normal bird added daily and numbered serially from 21 to 40
2/10/29	8, 13, 14	19	
2/11/29	1, 9, 12, 15	16	
2/12/29	7	16	
2/13/29	4, 8	15	
2/14/29	10	15	
2/15/29	5	15	6, 11, 16, 17, and 20 found to be carriers
2/16/29	2	15	
2/17/29	11, 16, 17	13	
2/26/29		22	6 found to be carriers
2/28/29		22	Test terminated

days. The survivors and contacts remained healthy and free of *P. avicida* on the two occasions when tested.

Experiment 3.—February 8, 1929. Twenty 1 year old pullets from the demonstration farm of the New Jersey State Agricultural Experiment Station were each

given intranasally 0.2 cc. of *P. avicida* "Kansas" and placed in a suitable pen. Normal, uninoculated birds were added thereafter for 20 days at the rate of one per day. Carrier tests were made on the 7th and 18th days.

Table III shows the results of this test. Sixteen of the inoculated birds died of fowl cholera within 9 days. On the 7th day, five birds proved to be carriers. Three of these died of cholera 2 days later and

TABLE IV

Virulence and Spread of an Epidemic Strain of P. avicida

Date	Identification No. of dead chicks	Surviving population	Remarks
2/27/29		40	Birds numbered 1 to 20 inoculated. Birds numbered 21 to 40 added immediately. One normal bird added daily and numbered serially from 41 to 52
2/28/29	7	40	
3/ 1/29	5, 10, 11, 12, 15, 17, 18, 19	33	
3/ 2/29	14	33	
3/ 3/29	2, 20	32	
3/ 4/29		33	Tested for carriers. All negative
3/ 5/29	6	33	
3/ 8/29		36	" " " " "
3/11/29		38	Addition of birds discontinued
3/12/29		38	Seven survivors and 32 contacts given 0.2 cc. *P. avicida* intranasally
3/13/29	21, 23, 26, 31, 45, 46	32	
3/14/29	33, 39	30	
3/15/29	22	29	
3/16/29	26, 34	27	
3/17/29	32, 55	25	
3/18/29	16, 44	23	Experiment terminated

one 9 days later, after a second positive carrier test. The other survivors and all contacts showed no signs of infection and did not become carriers.

Experiment 4.—February 27, 1929. Twenty chicks, aged 1 month, were each given intranasally 0.2 cc. of the "Kansas" strain of *P. avicida* and placed in a floor pen. Immediately thereafter twenty normal birds were added and subsequently one bird per day for 12 days. Carrier tests were made on the 5th and 9th days.

On the 10th day, all survivors and contacts were given an intranasal dose of 0.2 cc. of *P. avicida.*

The results of this test are given in Table IV. Thirteen of the twenty originally inoculated birds (65 per cent) died of cholera within 6 days. The remainder, together with the thirty-two contacts, showed no signs of infection and no *P. avicida* when the two carrier tests were made. Subsequent intranasal inoculation of the contacts, however, was followed by a 44 per cent cholera mortality, indicating that they were susceptible to *P. avicida.*

Similar tests with two other epidemic strains of *P. avicida* from different sources gave similar results. It is concluded, therefore, that the epidemic strains of *P. avicida* tested are of relatively high virulence but do not survive in the tissues of the healthy host nor readily spread and survive in contact hosts.

Endemic Strains

Experiment 5.—March 25, 1929. Five chicks, aged 1 month, were each given 0.2 cc. of an 18 hour broth culture of the endemic Strain 773 and placed in a cage measuring 12 x 16 x 12 inches in dimension. Five normal, uninoculated chicks were then added. Carrier tests were made on the 1st, 4th, 5th, 6th, 9th, 10th, 13th, 15th, and 17th days of observation. On the 28th day, all were given intranasally 0.2 cc. of the epidemic "Kansas" strain of *P. avicida.*

The results of this test, given in Table V, differ sharply from those of the preceding tests. In the first place, none of the inoculated birds died, in the second place, all became carriers, and in the third place, all the normal contact birds remained healthy but became carriers. Finally, upon exposure to the epidemic strain, 50 per cent of these contacts succumbed to cholera.

A second similar test gave comparable results and showed that the endemic strain of *P. avicida* was capable of surviving in the nasal cleft for at least 65 days.

Experiment 6.—April 25, 1929. Twenty chicks, 1 month old, were inoculated intranasally with 0.2 cc. of the endemic strain of *P. avicida,* No. 773, and placed in a floor pen. Ten similar uninoculated chicks were added at once and one daily thereafter for 30 days. Carrier tests were made at frequent intervals for 60 days.

All birds remained healthy. *P. avicida* spread to one contact within 6 days and to ten within 4 weeks. In 5 weeks 20 per cent of the inoculated and 31 per cent of the contacts were carriers, and in and 7 weeks 55 per cent and 40 per cent respectively. Other endemic strains tested gave similar results.

From these experiments it is concluded that the endemic strains under observation were of relatively low virulence and high vegetative capacity and ability to spread from host to host.

TABLE V

Virulence and Spread of an Endemic Strain of P. avicida

Date	Identification No. of dead birds	Identification No. of carriers	Remarks
3/25/29			Birds numbered 1 to 5 inoculated; birds numbered 6 to 10 added immediately
3/26/29		4, 5	
3/29/29		2	
3/30/29		5, 7	
4/ 1/29		1, 2, 3, 6, 10	
4/ 4/29		2, 3, 4, 5, 6, 7, 8, 10	
4/ 5/29		1, 4, 5, 6, 7, 8, 9, 10	
4/ 8/29		1, 4, 5, 6, 8, 9, 10	
4/10/29		4, 5, 6, 7, 8, 9, 10	
4/12/29		1, 2, 3, 4, 5, 6, 7, 8, 9, 10	
4/23/29			All inoculated intranasally with 0.2 cc. "Kansas"
4/25/29	3, 4, 7, 8, 9, 10		
4/29/29		1 (Endemic strain)	
4/30/29			Experiment terminated

"Laboratory" Variants from the Epidemic Strain.—Epidemic strains of *P. avicida* will, when cultivated in broth or on agar under aerobic conditions, undergo dissociation. Details of this process have been described previously (3). Certain of these variants bear a close resemblance to the endemic strains and insofar as can be determined by bacteriological and immunological tests, are identical. It seemed desirable, however, to submit them to an epidemiological test, that is, to determine whether or not they resembled the endemic strains in

being of low virulence and high capacity to survive and spread in fowl populations. Tests similar to those described above were run with four variants obtained from the "Kansas" strain. In no instance did the variants kill and, with a single exception in which two contacts were carriers for 1 day, in no instance did they spread to normal contacts. They survived in the nasal clefts of the inoculated birds for periods less than 2 weeks. Thus the laboratory variants differed fundamentally from the endemic strains in not surviving in the host or spreading to contacts.

DISCUSSION

The distinctive parasitic characteristics of epidemic and endemic strains of *P. avicida* brought out by these experiments are not limited to these organisms alone. The same relationships have been shown to exist in the case of epidemic and endemic strains of *P. lepiseptica* (4), and epidemic strains of mouse Friedländer and endemic strains of mouse typhoid (*B. enteritidis*) bacilli (5). Similar distinctions in parasitic behavior probably exist among the various types of pneumococci (6). It appears, therefore, that these inherent, relatively stable characteristics of parasites which are demonstrable experimentally and under natural conditions are common to many specific agents and that they determine largely the severity and spread of infection in a previously unexposed population.

The failure of epidemic strains of *P. avicida* to survive in nature and to spread among specially bred chickens in the experiments just described is not understood. Possibly they require hosts of abnormally low resistance; possibly they require the presence of some other agent which as yet has not been discovered.

Variants of epidemic strains occurring under laboratory conditions, although indistinguishable from endemic strains according to bacteriological and immunological tests, were shown to be quite different by the epidemiological test. Care should be exercised, therefore, in assuming that laboratory strains necessarily behave in a similar manner to parasites under natural conditions.

CONCLUSIONS

1. Strains of *P. avicida* from "spontaneous epidemics" of fowl cholera, when introduced intranasally in fixed doses into specially bred chickens,

induced fatal fowl cholera in about 40 per cent but did not survive in the nasal clefts of resistant birds nor spread to normal contacts.

2. Strains of *P. avicida* from "spontaneous endemics" of fowl cholera, when introduced similarly into chickens, failed to kill but did survive in the nasal clefts of inoculated birds and spread readily to normal contacts.

3. "Laboratory" variants of the epidemic strains of *P. avicida* failed to kill or survive in the test birds and did not spread to contacts.

REFERENCES

1. Pritchett, I. W., Beaudette, F. R., and Hughes, T. P., *J. Exp. Med.*, 1930, **51**, 249.
2. Hughes, T. P., and Pritchett, I. W., *J. Exp. Med.*, 1930, **51**, 239.
3. Hughes, T. P., *J. Exp. Med.*, 1930, **51**, 225.
4. Webster, L. T., *J. Exp. Med.*, 1926, **43**, 555, 573.
5. Webster, L. T., *J. Exp. Med.*, 1930, **52**, 901, 909, 931.
6. Webster, L. T., and Hughes, T. P., *J. Exp. Med.*, 1931, **53**, 535.