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Studies on Control of Fowl Cholera

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INTRODUCTION

Fowl cholera is a contagious disease of domestic fowls and many wild birds caused by a bacterium, *Pasteurella multocida.* It is one of the oldest known infectious diseases of poultry. Many reports on different phases of research on the disease have been published, dating to a period before Louis Pasteur, whose name is associated with early studies of fowl cholera. Although the disease has received much attention for over 100 years, its occurrence is still widespread and control measures have generally lacked effectiveness.

In South Dakota, fowl cholera has been a poultry disease of major importance for many years. Experimental work was undertaken at this station in 1944 to study fowl cholera as related to this area and to investigate the development or improvement of control measures to more effectively prevent losses from this disease. This bulletin is a report of that work.

NATURE OF THE DISEASE

Occurrence

Fowl cholera ranked with fowl leukosis and coccidiosis to constitute the three most frequently occurring diseases in poultry examined at the South Dakota State College veterinary diagnostic laboratory. It occurred at all seasons of the year but was most prevalent during late summer, fall, and early winter months from August through January (figure 1).

Most of the outbreaks of the disease in South Dakota poultry flocks were acute. In outbreaks of this nature, a heavy death loss occurred early in the outbreak; then less acute, and sometimes chronic, cases developed after the disease had been in progress for several days. A few outbreaks were more chronic

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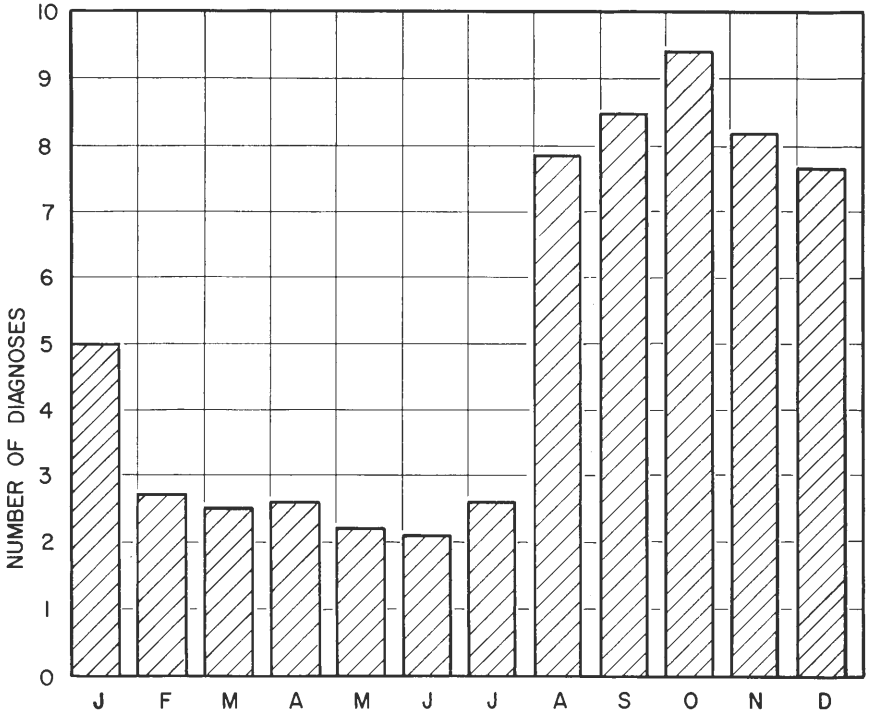


Figure 1. Average number of diagnoses of fowl cholera by month, 1949-58.

from the onset. With these, the death rate was not so rapid, although duration of the outbreak was more prolonged. It was not unusual in flocks affected with chronic cholera for the disease to suddenly become acute with a heavy death loss.

Symptoms and Lesions

Symptoms were lacking in most acute cases. Birds would be found dead with no earlier sign of disease. In the chronic form, birds were affected in different ways. Respiratory involvement was common and torticollis ("wry-neck") was observed occasionally (figure 2). Some birds had swollen eyes, wattles, or ear lobes. Some showed lameness resulting from swollen joints (figure 3).

Birds that died suddenly, showed few lesions on necropsy. A few petechiae were found on the heart and serous membranes, particularly over the gizzard fat. Small necrotic foci also were found in the liver in less acute cases (figure 4). Lesions associated with the chronic disease were characterized by accumulations of a yellowish, caseous exudate in affected parts such as the air sacs, ears, ear lobes, wattles, or joints.

Epizootiology

Histories which were obtained concerning individual outbreaks of fowl cholera often suggested that

Figure 2. Chronic infection in the ear causes torticollis.

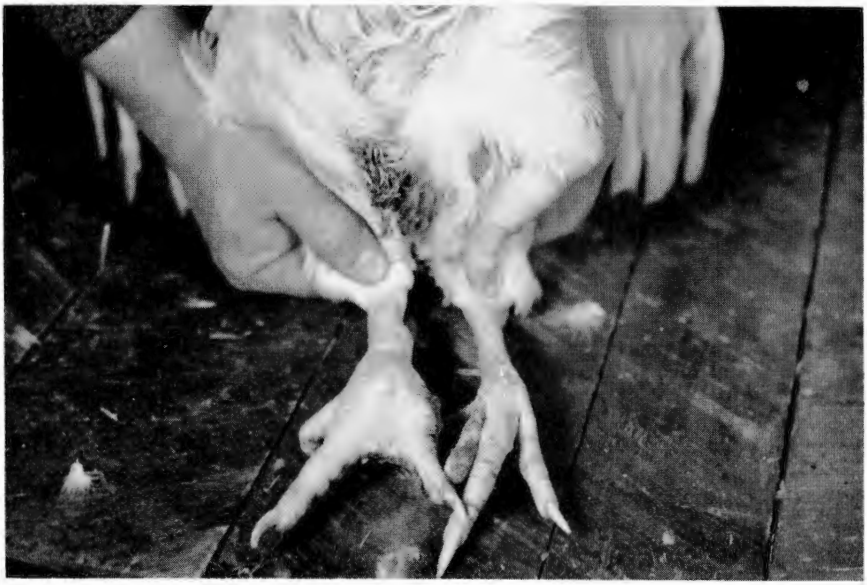
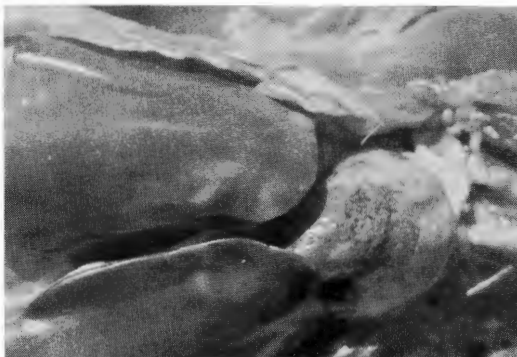


Figure 3. Localized chronic infection in the foot.

the source of infection could have been birds which were apparently normal, but were carriers of the specific organism. Some outbreaks occurred after birds of a healthy flock had been exposed to another flock in which there may have been birds carrying infection acquired during a previous outbreak. Others fol-

Figure 4. Acute fowl cholera showing petechiae on the heart and small, necrotic foci on the liver.



lowed the introduction of cockerels or pullets from another flock. Many of the outbreaks occurred in late summer or fall months after the association of susceptible pullets raised on the farm with the older flock, on range or in the poultry house. In some instances, a history of an earlier outbreak in the older birds was provided but this was not always the case.

A source of infection could not always be determined, nor could it be explained why a latent infection in a flock would suddenly develop into an explosive outbreak with a heavy death loss. It was not determined whether such occurrences resulted from a sudden increase in the virulence of *P. multocida* in the carrier birds, from lowered resistance of the birds, or perhaps a combination of these and other fatcors.

EXPERIMENTAL STUDIES

Studies of Pasteurella Multocida Strains

Most of the strains of *P. multocida* isolated from fowls submitted for diagnosis during the past 10-year period were retained. Isolations were made from blood, liver, or suggestive lesions on blood agar and identification was made by their biochemical characteristics and morphology. Subcultures from each new isolation were made on Difco stock culture agar slants and incubated 24 hours at 37°C. The tubes were sealed with waxed corks and then stored at room temperature. Many strains were held in this manner for 3 years or longer without transfer. Only a few strains became nonviable by this procedure.

Rosenbusch and Merchant (24) made a study of a number of *P. multocida* strains of mammalian and avian origin and divided them into three groups according to their ability to ferment xylose, arabinose, and dulcitol. This grouping was correlated by tube agglutination tests.

A similar study was made at this laboratory with 364 *P. multocida* strains of fowl origin. Fermentation of xylose, arabinose, and dulcitol was determined by color change in Difco purple broth base to which 0.5% of each sugar that was filter sterilized had been added. Three tubes, each containing 2.5 milliliters of purple broth and the respective sugar, were inoculated with each *P. multocida* strain and incubated at 37°C. The final reaction was recorded on the seventh day. Further incubation was not carried out because of evaporation.

Table 1 shows the basis for grouping these strains according to fermentation reactions, and the number and percent of strains for each group.

A few more xylose - negative strains could have been included in group I but were omitted because

Table 1. Distribution of Avian *P. multocida* Strains According to Fermentation Reaction

Group	Fermentation Reaction			Number of Strains	Percent
	Xylose	Arabinose	Dulcitol		
I	—	+	+	306	84.06
II	+	+ or —	—	53	14.57
III	+	+	+	5	1.37

of their inability to ferment arabinose and/or dulcitol.

Reciprocal agglutination tests were made to determine whether agglutination reactions might be correlated with biochemical reactions in classifying these *P. multocida* strains. Rapid, somatic antigens were prepared from group I, II, and III *P. multocida* strains. The method for preparing these antigens is described under the section, "Detection of Carriers by Agglutination Test."

The same strains of *P. multocida* that were used to prepare the rapid antigens were used to inoculate chickens to produce agglutinins. Difco tryptose phosphate broth was inoculated with the different strains and incubated 24 hours at 37°C. Incubation was then continued for 96 hours at 56°C. to remove the capsular substance and kill the cultures. Chickens were then inoculated intravenously with two 1 milliliter doses of each culture given 5 days apart. These birds were negative to agglutination tests with the different antigens prior to inoculation. About 1 week after the last inoculation the birds were bled from the heart. The serum was separated from the blood and preserved with merthiolate. Agglutination tests were conducted by mixing one drop of antigen with .02 milliliter of serum. Antisera of strains of each group were tested with each antigen. The results of these tests are shown in table 2.

These tests showed that the somatic antigens of strains of group I and group III were very similar. They were not closely related to those of group II. Group II strains, 33 and 338, fermented xylose but not arabinose and dulcitol. The other group II strain, 63, fermented xylose and arabinose. However, on

Table 2. Results of Reciprocal Agglutination Tests Between *P. multocida* Groups

	Antigen							
	Group I			Group II			Group III	
Antiserum	1	5	99	33	338	63	97	283
Group I								
1	+	+	+	—	—	—	+	I*
5	+	+	+	—	—	—	+	+
99	+	+	+	—	—	—	I	+
Group II								
33	—	—	I	+	+	+	—	—
338	—	—	—	+	+	+	—	I
63	—	—	—	I	+	+	—	—
Group III								
97	+	+	+	—	—	—	+	+
283	+	+	+	—	—	—	+	+

*Incomplete.

the basis of agglutination tests, their somatic antigens could not be distinguished. A slight relationship of strains of different groups for one another was shown, e.g. strain 33 to 99 and strain 338 to 283. This would indicate that some avian *P. multocida* strains of different groups might have related somatic antigens.

A comparison of death losses, from different fowl cholera outbreaks in South Dakota, showed no appreciable difference in the virulence of the causative strains of *P. multocida,* whether they belonged to group I or II. There were not enough outbreaks caused by group III strains to be considered in making this comparison. Greater variation in virulence could be found among the strains within one group.

Detection of Carriers by Agglutination Test

Several investigators (5, 11, 15, 18, 21, 22, 23, 26, 28) working on fowl cholera found that apparently healthy birds may carry *P. multocida.* Most commonly the organism was harbored in the upper respiratory tract. Because the source of infection in many fowl cholera outbreaks can be traced to exposure to such carriers, the detection and removal of carriers would be of value as a control measure.

Shook and Bunyea (26) prepared a *P. multocida* antigen for a rapid, whole-blood agglutination test and used it to test a flock in which a carrier problem had existed over several years. The antigen consisted of 24-hour agar growths of the autogenous strain suspended in physiological saline solution containing 1% formalin. The suspension was adjusted to a turbidity of 75x1 by the McFarland nephelometer scale and stained with 0.1% of crystal violet. The authors reported control of the fowl cholera problem in this flock by removal of the reactors.

Antigens prepared here by the Shook and Bunyea procedure sometimes proved unstable because of autoagglutination. A different procedure of preparation was finally evolved which resulted in an antigen that was stable and maintained its sensitiveness for several months when stored at 45°F. This procedure was as follows: Three serial passages of the antigen culture of *P. multocida* were made in 10-day-old chicken embryos. One liter of tryptose phosphate broth was inoculated with 1 milliliter of allantoic fluid from a third passage embryo and incubated 24 hours at 37°C. Then, 10 milliliters of 0.1% blue tetrazolium, an intravital dye, was added to the antigen culture. This was incubated at 37°C. for an additional 72 hours. The bacteria were stained dark blue shortly after adding the blue tetrazolium. Further incubation for 24 hours at 56°C. was used to insure that the organisms were killed and that the capsular substance had been removed. The bacteria were sedimented in an angle centrifuge and the broth was discarded. The organisms were resuspended and washed in phenolized (0.25%) saline solution, after which they were centrifuged again and the

saline wash was discarded. The bacteria were resuspended in 20 milliliters of phenolized, buffered saline which was prepared from Sorenson's standard solutions as follows: M/15 KH_2PO_4—90 milliliter, M/15 Na_2HPO_4—10 milliliters, 0.85% NaCl, and 0.25% phenol. The pH of the antigen was 5.7. Tests conducted in developing this method of preparation showed that as higher pH values were used the antigen became less sensitive and inagglutinable with weak *P. multocida* antiserum. The density of the antigen was about the same as the stained, rapid pullorum antigen (figure 5).

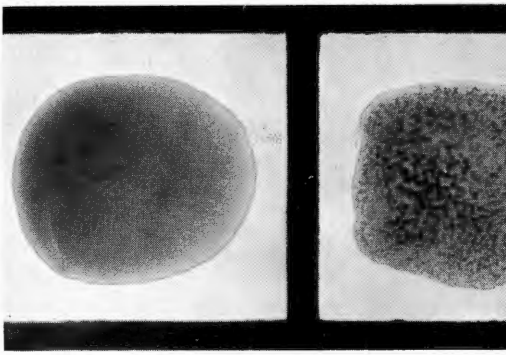
A natural outbreak of fowl cholera of low virulence occurred in a small group of hens in a breeding experiment. Most of the cases were chronic but occasionally a bird would die of acute cholera. The 29 surviving birds composing this group were tested with antigen, followed by necropsy and bacteriological examinations. Fifteen of the birds reacted, with 11 (73%) yielding cultures of *P. multocida*. Isolations were made from lesions which were generally characterized by accumulations of a yellowish, caseous exudate. None of the 14 negative birds were positive on culture.

Two farm flocks were blood tested when fowl cholera recurred after oxytetracycline (Terramycin) treatment. Facilities on the farms did not permit detailed study of the effectiveness of removing reactors in the control of the disease. Thirty-one reactors were found in the 421 birds comprising the two flocks. Eight of the reactors were cultured and *P. multocida* was isolated from seven. No attempt was made to culture the negative birds. After removal of reactor birds, a second course of oxytetracycline was given. When the antibiotic was discontinued, losses recurred in one flock. Poor management and insanitation were probably responsible for continued loss.

A more critical evaluation of the antigen to detect carriers was made on an adult flock of nearly 100 hens. Fowl cholera had been a problem for several years on the farm where this flock was raised. Losses from cholera occurred in pullets which had been in association with the older birds prior to the acquisition of the mature birds for this study. They were removed from the farm to the laboratory where they were housed as a group and given average care.

The hens were tested with two antigens, one prepared from a group I strain and the other from a group II strain. Earlier studies (9, 10) had shown that most cases of

Figure 5. Negative and positive tests with a rapid, whole-blood *P. multocida* antigen.



fowl cholera in South Dakota were caused by strains of these two groups of *P. multocida*. There were 13 reactors, seven reacting only to the group I, five only to the group II, and one to both antigens. These reactors were left in the flock.

During the next 6 months, swabs were made from the palatine cleft of each living bird. These were cultured in broth and the culture inoculated into mice. The mice that died were subcultured to attempt the isolation of *P. multocida*. By this procedure isolations were made from two of the reactors and from five of the negative birds.

Seventeen hens negative to the test died during the interval. *P. multocida* was isolated on culture from seven of these, four having died of acute cholera. Positive isolation was made from one of three reactors that died.

A second test of the remaining birds was made 4½ months after the first at which time there were seven reactors. Three reacted only with group I antigen, two only with group II antigen, and two showed reactions with both. *P. multocida* isolations were not made from any of these birds. Three birds that were negative on the first test reacted on the second. Six birds showing reactions on the first test were negative on the second. An isolation of *P. multocida* had been made from the nasal cleft of one of these earlier, but it was negative on cultures of the nasal cleft and organs at necropsy following the last test (No. 44). Table 3 shows the results of agglutination tests and cultural examinations of the individual birds, excluding those birds which were negative both to the agglutination test and bacteriological examination.

In all, 30 cultures of *P. multocida* were recovered from swabs and organ cultures from this flock. On the basis of their ability to ferment xylose, arabinose, and dulcitol, all belonged to group I. One of the cultures, inoculated into a normal bird, resulted in an antiserum which agglutinated both the group I and group II antigens used in tests of the flock.

Although the correlation between reactions with *P. multocida* antigen and recovery of the organism was good in the earlier trials, results were not encouraging with this flock. Not only were isolations from reactor birds at a low level, but positive isolations were made from the palatine clefts of five living birds which did not react. Inability of the test to detect all carriers would reduce the value of the test as a control measure. From the experience in the small group of 29 birds undergoing the outbreak of chronic cholera, the antigen would appear to have some value as a diagnostic test to detect birds with chronic lesions.

Immunization

Although Pasteur (19) in 1880 demonstrated immunity in fowls inoculated with attenuated cultures of *P. multocida*, workers since that time have had irregular results with various vaccines and bacterins. Generally, no protection was provided in the vaccinated fowls, or

the resulting immunity was of a low level and of short duration. Immunization has never been accepted as a dependable control measure for fowl cholera.

More recently, Carter (4) found a chicken-embryo vaccine effective against experimental pasteurellosis in mice, and Daugherty (6) compared a duck-embryo vaccine with four commercial bacterins, finding the duck-embryo vaccine superior for immunizing White Pekin ducks against cholera.

A chicken embryo (CE) vaccine similar to that used by Carter was prepared to compare with a formalized broth culture bacterin as an immunizing agent in chickens. A group I strain (No. 21) was carried through three passages in 10-day chicken embryos to enhance the virulence of the organism. The entire contents of two last-passage

Table 3. Test and Cultural Record of Individuals from Adult Flock, Excluding the Negative Birds

Bird No.	1st Test, 11-5-57		2nd Test, 3-19-58		*P. multocida* Isolation	
	Group I	Group II	Group I	Group II	Nasal Swab*	Necropsy†
10	—	—	—	+	—	—
15	—	+	Died			—
17	+	—	Died		+	—
26	—	+	—	—	—	—
28	—	—	—	—	+	
32	—	—	Died			+
34	—	—	Died		—	+
37	—	—	+	—	—	—
40	—	+	Died			+
42	—	+	+	+	—	—
44	+	—	—	—	+	—
47	—	—	Died		+	+
51	—	—	Died		—	+
53	—	—	—	—	+	
57	+	—	—	—	—	—
67	—	+	—	—	—	—
75	+	—	+	+	—	—
80	—	—	—	+	—	—
81	—	—	Died			+
82	+	—	+	—	—	—
84	+	+	—	—	—	—
86	+	—	—	—	—	—
89	+	—	+	—	—	—
91	—	—	Died		+	+
93	—	—	—	—	+	
96	—	—	Died			+

*Cultures made when birds were alive.

†Cultures made from affected organs and/or palatine clefts of birds that died and surviving reactors which were killed at the termination of the experiment.

eggs were added to 100 milliliters of sterile physiological saline solution and mixed in a Waring blendor. Formalin was added to a 0.25% concentration. The broth culture (BC) bacterin was prepared from the same strain by 24-hour incubation of tryptose phosphate broth inoculated with allantoic fluid from the third passage embryo. This also was formalized. Both preparations were held for 72 hours in the refrigerator and sterility tested in mice.

Three pens of 5-week-old chickens were used in this trial. Pens 1 and 2, each with 35 birds, were given two intramuscular injections in 1.0 milliliter amounts a week apart of CE vaccine and BC bacterin, respectively. The third pen of 36 birds was not vaccinated and served as a control. Two weeks following the second injection, all the birds were challenged with *P. multocida* of the same strain. The organism for challenge was grown in chicken embryos. Dilutions of embryo suspension in peptone water of 1:1,000 or 1:10,000 were inoculated intramuscularly in 1.0 milliliter amounts.

As shown in figure 6, the mortality was 46% in pen 1, 6% in pen 2, and 83% in pen 3, the control group. Although mortality was reduced by both preparations, the BC bacterin was superior to the CE vaccine in this trial.

In a second immunization trial, a formalized BC bacterin prepared as above from strain No. 1 was compared with a commercial *P. multocida* bacterin. Three pens, each with 26 four-week-old White Rock

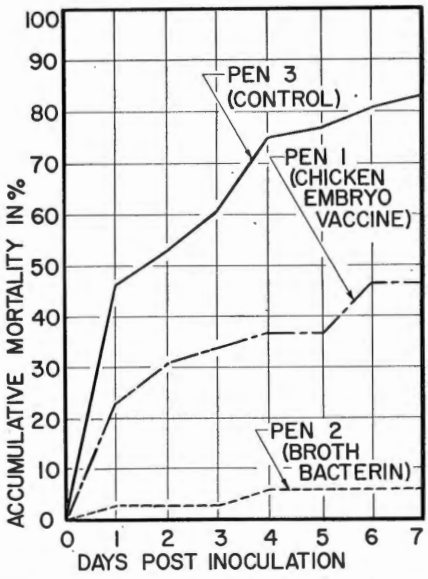


Figure 6. Immunity from broth culture bacterin and chicken embryo vaccines.

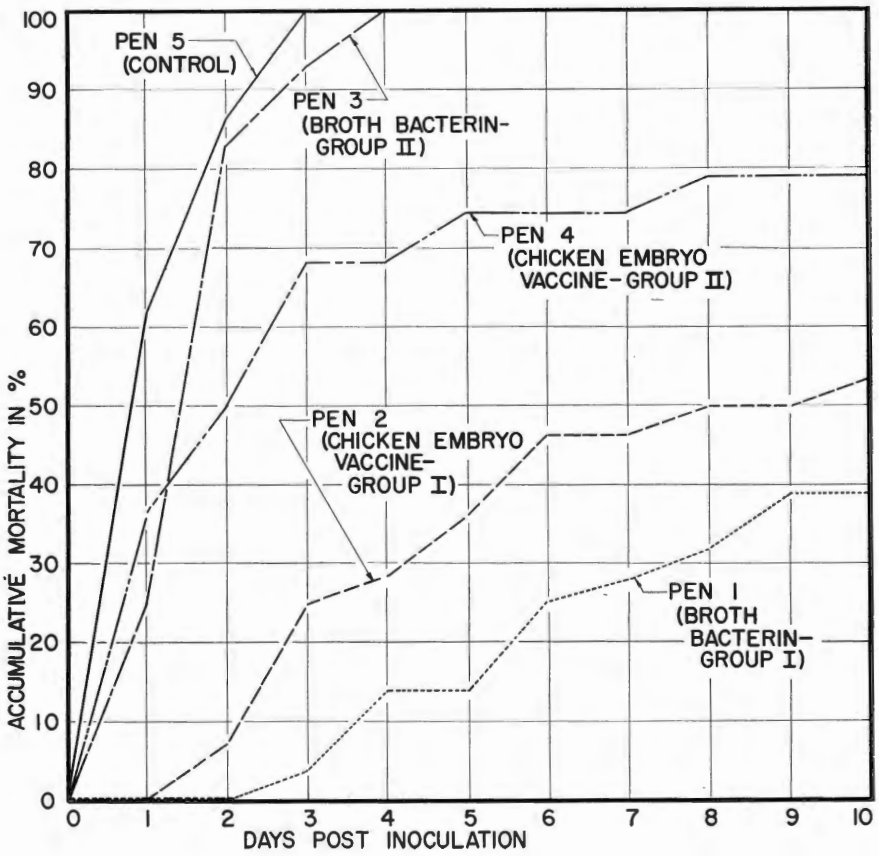
chickens were used. Pens 1 and 2 received two weekly 1.0 milliliter intramuscular injections of the BC and commercial bacterins, respectively, and pen 3 served as the control. A 1:10,000 dilution of a 24-hour broth culture of the organism in a 1.0 milliliter dose served as the challenge 16 days after the second immunizing dose. After 9 days, the mortality was 27% in pen 1, and 64% in both pens 2 and 3. No benefit was shown with the commercial bacterin and the prepared BC bacterin did not provide immunity of sufficient level for practical purposes.

The third immunization trial was designed not only to compare CE vaccines and BC bacterins but also to determine whether vaccination with group II organisms would provide cross protection for the more

prevalent group I *P. multocida* infections. Group I (Strain No. 4) and group II (Strain No. 10) CE vaccines and BC bacterins were prepared separately and vaccination was carried out by two weekly injections as before. Five pens of 4-week-old New Hampshire chickens were used, pen 5 serving as the control group. Pens 1, 2, 3, and 4, each with 28 birds, were vaccinated with group I BC bacterin, group I CE vaccine, group II BC bacterin, and group II CE vaccine, respectively.

The birds were challenged 16 days after the second vaccination with a 1:10,000 dilution of a 24-hour broth culture of group I *P. multocida* (strain T). As shown in figure 7, the challenge dose resulted in 100% mortality in the control group and in pen 3, vaccinated with group II BC bacterin. The death loss was complete in those two pens by the fourth day. A high mortality also occurred in the other three

Figure 7. Immunity from broth culture bacterins and chicken embryo vaccines prepared from group I and II strains.



pens over a 10-day period, but the greater protection was provided in pens 1 and 2 which received the group I preparations. The survival again was slightly better in pen 1, which received the group I BC bacterin. A similar trial using a group II *P. multocida* challenge was not made. Yaw, Briefman, and Kakavas (29) have suggested that the immunogenic specificity of types of *Pasteurella* should be considered when immunizing agents are prepared. The results in this trial tend to support that belief.

In each of the three trials, there was a high incidence of crippled and sick birds among the survivors at the termination of the experiments, which further reflects the low level of protection provided by these immunizing agents.

The fourth trial dealing with immunization involved a group of 72 5-month old pullets. Thirty-eight of them received two weekly injections of BC bacterin. Seven weeks following the second injection, a simulated fowl cholera outbreak was initiated in the pullets by placing with them six birds which were swabbed in the palatine cleft with a virulent culture of *P. multocida* (Strain S). All six of the swabbed birds had died of fowl cholera within 48 hours. Losses began to occur in the exposed birds 24 hours later and continued through the following 30 days. Thirty of the 34 (88%) nonvaccinated and 24 of the 38 (63%) vaccinated birds died. Again, the slight protection which appeared to have been provided was not of practical value.

Since these trials were completed, Heddleston and Hall (13) reported more favorable results in limited trials on fowl cholera immunization with a bacterin containing an adjuvant. Not only was the immunity stronger but also of longer duration than that provided by a chicken embryo vaccine.

Treatment

Effective treatment of poultry flocks for fowl cholera was not possible until sulfonamide drugs became available. A number of workers (1, 2, 7, 8, 16, 25) found that different sulfonamides were effective in reducing mortality. They were of particular value in acute outbreaks if treatment was started early. However, considerable death loss usually occurred before the drug became effective. Prolonged medication produced toxic reactions and it was found necessary to stop treatment every few days to avoid this. During the period when the flock was not treated, the disease often recurred, making it necessary to start treatment again. Some flocks do not respond well to sulfonamide treatment, particularly after one or two courses fail to check the disease. Outbreaks of a chronic nature seldom respond. Other workers (3, 14) reported that certain sulfonamides cause a marked drop in egg production. Despite these disadvantages, the sulfonamides have been the drugs chiefly used against cholera.

Antibiotics have not been used extensively to treat flocks affected with the disease because of their

relatively high cost. Antibiotics are used widely in poultry production as growth stimulants and for a favorable effect on egg production. The cost is not high for this purpose, as only small amounts of antibiotics are needed and generally the more economical residues are used. Several trials using penicillin, streptomycin, and chlortetracycline (Aureomycin) at growth promoting levels were run at this station to determine whether they would protect against experimental fowl cholera. Results were irregular when trials were compared but it was concluded that such low levels of these antibiotics were of no value in preventing or controlling outbreaks of the disease.

McNeil and Hinshaw (17) reported that streptomycin completely inhibited the growth of *P. multocida in vitro*. The antibiotic also prevented mortality in experimental fowl cholera in turkeys, but the protection was not complete. Prier (20) found that chlortetracycline was more effective against *P. multocida in vitro* than penicillin or streptomycin. He also found that chlortetracycline, given at the rate of 250 and 500 milligrams per kilogram of mash, materially reduced the mortality rate in chickens to experimental infection. Gualandi (12) used oxytetracycline in the mash successfully against experimental *Pasteurella* infection in birds.

Trials were conducted at this station to determine the efficacy and the level at which some common antibiotics would be effective against fowl cholera. The first trial compared penicillin, streptomycin, oxytetracycline, sulfamerazine, and sulfaquinoxaline. The two sulfonamides, shown to be effective against cholera, were included as a basis for comparison. The antibiotics were mixed in the mash at a level of 1.0 gram per kilogram (1:1,000). Sulfamerazine was used at a 0.5% and sulfaquinoxaline at 0.05% level in the mash.

Treatment was started about 48 hours before the birds were challenged. Each treatment was used on approximately 65 seven-week-old chickens. A pen with a similar number of untreated birds was the control. The birds were challenged by intramuscular inoculation with 1.0 milliliter of a 1:10,000 dilution of a 24-hour tryptose phosphate broth culture of *P. multocida* (strain 18). An LD50 test, using brooder mates, indicated that each bird received approximately 100 LD50 doses.

As shown in figure 8, oxytetracycline and both sulfonamides afforded complete protection against the challenge. Penicillin and streptomycin were not effective; the birds receiving these antibiotics died about the same rate as the controls.

Similar trials have been run with chlortetracycline, tetracycline, and a furazolidone concentrate (NF-180). Chlortetracycline was compared to oxytetracycline at 1 gram and 0.5 gram per kilogram of mash. The 1:2,000 level of oxytetracycline provided practically as much protection as the 1:1,000 level of chlortetracycline against experimental

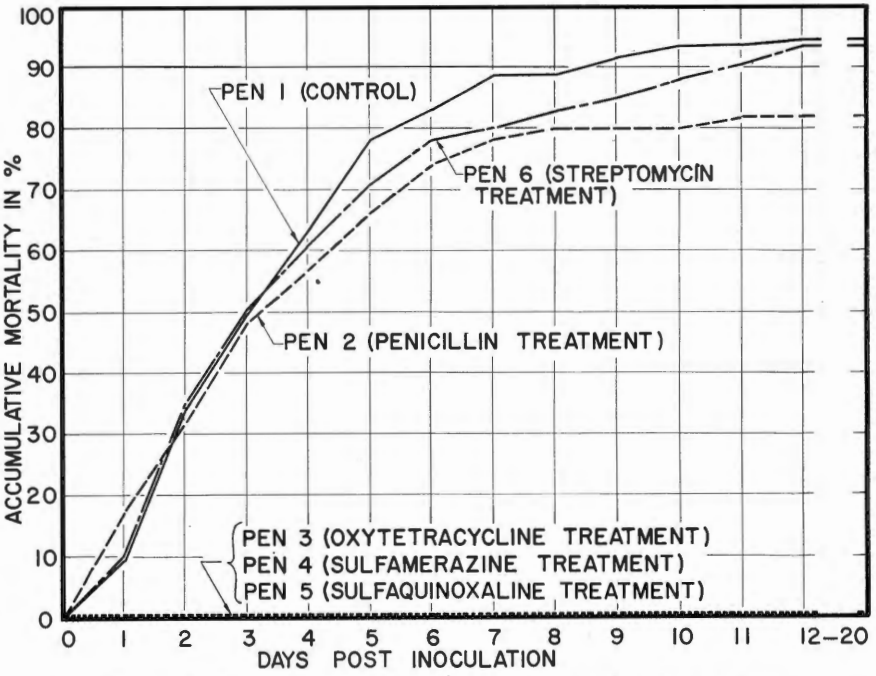


Figure 8. The efficacy of different treatments against fowl cholera.

infection (figure 9). One-half gram of pure tetracycline per kilogram of mash was ineffective against experimental fowl cholera. This may have been due to faulty mixing as a very small amount was used, compared to the relatively large amount of residues of oxytetracycline and chlortetracycline that was needed to prepare a similar concentration.

A soluble oxytetracycline preparation used in the drinking water at 0.5 gram per liter was more effective against the experimental disease than 0.033% furazolidone in the mash. The challenge in this trial was so severe that 100% of the control and furazolidone-treated birds died, compared to 22% of those treated with oxytetracycline.

Oxytetracycline checked losses in a simulated natural outbreak of cholera in a pullet flock. The outbreak was started in the 103-bird flock by swabbing seven birds in the palatine cleft with a virulent *P. multocida* culture (Strain G). When 20 birds had died the remainder of the flock was divided into two nearly equal groups by partitioning the pen. The birds on one side of the pen were treated with oxytetracycline in the mash at the 1:1,000 level for 9 days and at 1:2,000 for 11 succeeding days. Losses stopped in the treated group the first day after treatment was begun and over a period of 50 days, five (12%) of the treated birds had died, compared to 33 (80%) of

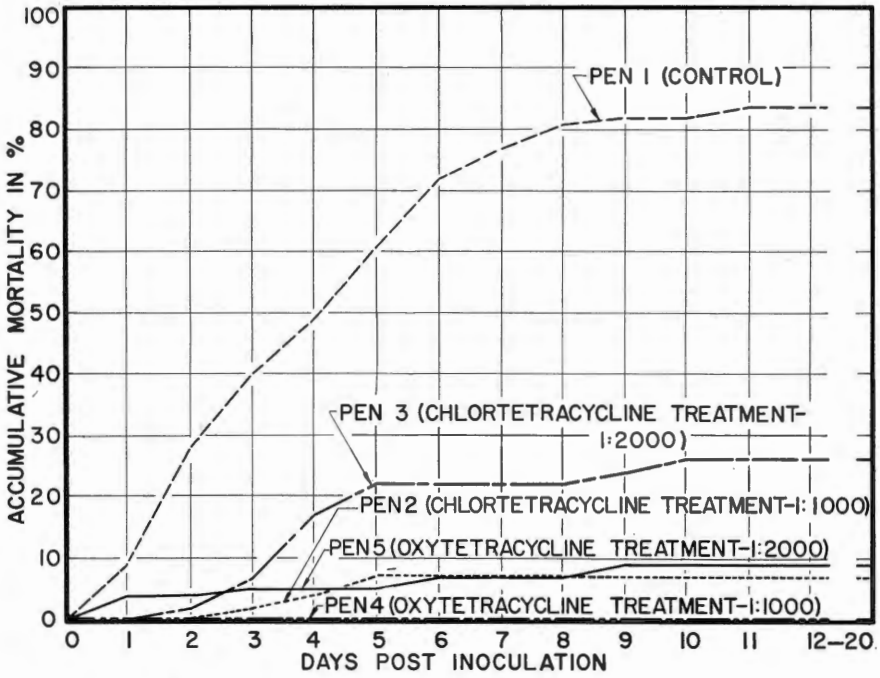


Figure 9. The efficacy of two levels of chlortetracycline and oxytetracycline against fowl cholera.

the untreated group. No toxic effects were observed during the 20 days of treatment and egg production was not depressed.

Oxytetracycline was used to treat six farm flocks affected with cholera. Four of these flocks had been treated unsuccessfully with sulfonamides. Most of the flocks were treated with oxytetracycline in the mash at the 1:1,000 level for 3 days, then at the 1:2,000 level for 3 days.

Losses stopped in the first flock the first day after treatment was started and did not recur. In the second flock losses dropped from 18 birds the first day of treatment to three the second day and continued about one per day for 5 days and stopped. The disease recurred about 2½ months after treatment was stopped. Losses stopped in the third flock in 2 days and did not recur. The fourth flock was blood tested with a *P. multocida* antigen when the disease recurred after a course of treatment. The reactors were removed and the flock was given a second course of treatment. Losses stopped without recurrence. Losses in the fifth flock were reduced but continued at about one per day during a course of treatment. A check revealed that the birds were getting grain in addition to the treated mash and that

some old hens had been kept from the previous flock. These were culled and losses stopped after the first day when they received only treated mash. The outbreak did not recur. The sixth flock was handled in about the same manner as the fourth flock. Although treatment stopped losses, the disease recurred even after a blood test was conducted and the reactors were removed. These results will have to be considered with reservation as the farm facilities did not provide for establishing control groups.

Smith (27) reported that one intramuscular injection of 25 milligrams of oxytetracycline per kilogram of body weight was usually effective against experimental fowl cholera in chicks and chickens. A limited trial was conducted at this laboratory with an experimental injectable form of oxytetracycline for poultry. None of 20 birds treated with this preparation was affected by a challenge inoculation of *P. multocida* that killed 70% of a similar control group. This type of treatment might prove of value for checking early losses in acute fowl cholera outbreaks.

SUMMARY AND CONCLUSIONS

Most outbreaks of fowl cholera in South Dakota poultry flocks are acute and cause heavy death loss. Chronic outbreaks are less common.

The disease is caused by strains of *P. multocida* that belong to at least two different serological groups. These groups can be identified roughly by biochemical reactions. Over 80% of the avian strains isolated in South Dakota belong to group I. The strains of both groups are equally virulent.

A whole-blood agglutination test was developed and used to detect chronic carriers of fowl cholera. Although studies showed that some of the reactors were carriers, it also was found that some nonreactors carried the infection in the upper respiratory tract. It is doubtful that removing reactor carriers would halt cholera outbreaks as long as nonreactor carriers remain sources of infection.

A satisfactory immunizing agent for fowl cholera was not developed. Results tended to support the proposal that consideration should be given to immunogenic specificity of the two main groups of *P. multocida* when immunizing agents are prepared.

Oxytetracycline (Terramycin) was the most effective antibiotic tested against experimental fowl cholera. It was as effective as the sulfonamides experimentally and also was effective in field trials where sulfonamides had failed. Although oxytetracycline offers additional advantages, it is not used extensively against fowl cholera because of the cost.

BIBLIOGRAPHY

1. Alberts, J. O. "The prophylactic and therapeutic properties of sulfamerazine in fowl cholera." *American Journal of Veterinary Research,* **XI**:414-420. 1950.
2. Armstrong, W. H. and R. A. Bankowski. "Sulfaquinoxaline treatment in an acute outbreak

of fowl cholera." *Veterinary Medicine,* 44:306-307. 1949.

3. Bankowski, R. A. "A decrease in egg production following sulfamerazine and sulfamethazine medication." *Journal of the American Veterinary Medical Association,* 113:49-50. 1948.

4. Carter, G. R. "Studies on a *Pasteurella multocida* chicken embryo vaccine I. The comparative immunizing value of broth bacterins and a chicken embryo vaccine in mice." *American Journal of Veterinary Research,* XI:252-255. 1950.

5. Cernaianu, C. "Ueber die Gelflugel cholera, sine durch Haltung, Ernahrung and Ausbentung bedingte Krankheit." *Zeitschr. Infektionskrankh. parasitaire Krank. u. Hyg. Haustiere,* 58:142-159. 1942.

6. Daugherty, E. 3rd. "The efficacy of several immunizing agents for the control of fowl cholera in the White Pekin duck." *Cornell Veterinarian,* XLIII : 421 - 426. 1953.

7. Delaplane, John P. "Sulfaquinoxaline in preventing upper respiratory infection of chickens inoculated with infective field material containing *Pasteurella avicida." American Journal of Veterinary Research,* VI:207-208. 1945.

8. Delaplane, J. P. and T. C. Higgins. "Sulfaquinoxaline in the prevention and control of chronic fowl cholera." *Cornell Veterinarian,* 38:267-272. 1948.

9. Dorsey, T. A. "Vaccination and blood tests for fowl cholera." *South Dakota Farm and Home Research,* III:23-24. 1951.

10. ——— "Fowl cholera." *South Dakota Farm and Home Research,* IX:10-13. 1958.

11. Gogoasa, V. "Frecventa purtatorilor de Pasteurella intr-o enzootie de holera aviara la rate in plina evolutie." *Probl. Epiz. Microbiol., Inst. Pat. Igien. Anim., Bucresti,* 5:13-18. 1956.

12. Gualandi, G. "La terramicina nello lotta contro il colera aviare." *Arch. Vet. Ital.,* 4:539-543. 1953.

13. Heddleston, K. L. and W. J. Hall. "Studies on pasteurellosis II. Comparative efficiency of killed vaccines against fowl cholera in chickens." *Avian Diseases,* 2:322-335. 1958.

14. Hinshaw, W. R. and E. McNeil. "Experiments with sulfanilamide for turkeys." *Poultry Science,* 22:291-294. 1943.

15. Hughes, Thomas P. and Ida W. Pritchett. "The epidemiology of fowl cholera. III. Portal of entry of *P. avicida;* reaction of host." *Journal of Experimental Medicine,* 51:239. 1930.

16. Kiser, J. S., J. Prier, C. A. Bottorff, and L. M. Greene. "Treatment of experimental and naturally occurring fowl cholera with sulfamethazine." *Poultry Science,* 27:257-262. 1948.

17. McNeil, Ethel and W. R. Hinshaw. "The effect of streptomycin on *Pasteurella multocida in vitro* and on fowl cholera in turkeys." *Cornell Veterinarian,* 38: 239-246. 1948.

18. Nobrega, R. and R. C. Bueno. (Inst. Biol., Sao Paulo.) "Sobre o reconhecimento de portodores na colera aviaria." *Arq. Inst. Biol.* (Sao Paulo.) **15**:339-342. 1944.

19. Pasteur, L. "De l'attenuation du virus du cholera des poules." *Compt. Rend. Acad. Sci.*, **91**: 673. 1880.

20. Prier, J. E. "The *in vivo* and *in vitro* effect of aureomycin upon *Pasteurella multocida*." *Veterinary Medicine*, **45** : 243 - 245. 1950.

21. Pritchett, Ida W., F. R. Beaudette, and Thomas R. Hughes. "The epidemiology of fowl cholera. IV. Field observations of the spontaneous disease." *Journal of Experimental Medicine*, **51**:249. 1930.

22. ——— "The Epidemiology of fowl cholera. V. Further field observations of the spontaneous disease." *Journal of Experimental Medicine*, **51**:259. 1930.

23. Reis, J. and T. C. Bueno. (Inst. Biol., Sao Paulo.) "Vacinacao e mortalidade na colera aviaria." *Arq. Inst. Biol.* (Sao Paulo.) 4:81-86. 1943.

24. Rosenbusch, Carlos T. and I. A. Merchant. "A study of the hemorrhagic septicemia *Pasteurellae*." *Journal of Bacteriology*, **37**:69-89. 1939.

25. Saurat, P. "De utilisation des sulfamides dans la pasteurellose aviaire. Leu association aves la vaccination." *Rev. Med. Vet., Lyon et Toulouse*, **95**:106-115. 1944.

26. Shook, Warren H. and Hubert Bunyea. "The detection of carriers of fowl cholera and its control by means of a stained-antigen, rapid, whole-blood test." *Poultry Science*, 18:146. 1939.

27. Smith, H. Williams. "The chemotherapy of experimental fowl cholera in fowls *(Gallus Domesticus)*." *Journal of Comparative Pathology and Therapeutics*, **65**:309-316. 1955.

28. Van Es, L. and J. F. Olney. "An inquiry into the influence of environment on the incidence of poultry diseases." *University of Nebraska Agricultural Experiment Station Research Bulletin*, **118**:17-21. 1940.

29. Yaw, K., L. Briefman, and J. C. Kakavas. "A comparison of virulence for mice and chickens of different colonial variants of the three serological types of *Pasteurella multocida*." *American Journal of Veterinary Research*, **XVII**:157-159. 1956.