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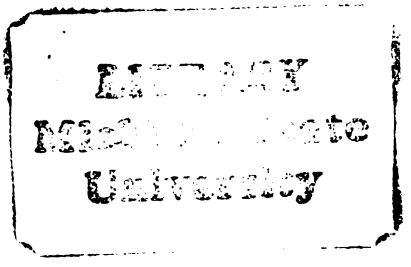


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THE USE OF A STREPTOMYCIN DEPENDENT STRAIN
OF PASTEURELLA MULTOCIDA AS A VACCINE
FOR THE PREVENTION OF FOWL CHOLERA

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Michael Alan McKinney

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# THE USE OF A STREPTOMYCIN DEPENDENT STRAIN OF PASTEURELLA MULTOCIDA AS A VACCINE FOR THE PREVENTION OF FOWL CHOLERA

By

Michael Alan McKinney

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ABSTRACT

THE USE OF A STREPTOMYCIN DEPENDENT STRAIN
OF PASTEURELLA MULTOCIDA AS A VACCINE
FOR THE PREVENTION OF FOWL CHOLERA

By

Michael Alan McKinney

Fowl cholera is presently a major disease problem in turkeys. The only live vaccine available for controlling this disease causes a slight mortality in the flocks that were vaccinated with this product. Experiments were conducted to develope a live non-pathogenic vaccine for fowl cholera using a streptomycin dependent mutant strain of *Pasteurella multocida*, P-1059. This experimental vaccine was administered through the drinking water when the turkeys were six and eight weeks old. The turkeys were challenged with three wild type strains of *Pasteurella multocida*, (P1059, X-73, and P-1662), when they were twelve weeks old.

The results indicated that if the antigenic characteristics of the streptomycin dependent mutant strain could be stabilized this experimental vaccine would be a viable alternative to the live vaccine that is now available.

*DEDICATION*

*To my loving parents,*
*Clarence and Fern McKinney*

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# INTRODUCTION

Fowl cholera has been recognized as a major problem in poultry for over 100 years. Pasteur (1880) recognized fowl cholera as a major disease in Europe while Salmon (1880) was the first to study this disease in the United States.

Between 1880 and the early 1900's most disease problems in poultry were attributed to fowl cholera. By 1910, enough disease outbreaks had been studied by bacteriologists in the United States to show that fowl cholera was widely distributed and of primary importance in the New England states (Hadley, 1910). Six years later outbreaks of fowl cholera were being reported in Nevada and Nebraska (Mack and Records, 1916; Van Es and Martin, 1920).

From 1941 to 1950 an estimated $8.5 million was lost annually due to fowl cholera; out of that $514,000 was lost in turkeys (Cockrill, 1971).

In the early 1960's fowl cholera was thought to be decreasing in incidence in poultry (Harshfield, 1965), even though Dorsey and Harshfield (1959) had reported that fowl cholera was one of three most important poultry disease problems in South Dakota.

The National Turkey Federation in 1970 held a National Symposium on fowl cholera in turkeys. Their findings indicated that fowl cholera was increasing in incidence in turkey flocks. An estimated 14% of all turkeys in the United States, ranging from 2% in California to

50% in Texas, were affected by fowl cholera. It was estimated that $14 million was lost due to fowl cholera in 1969 through weight loss, mortality, and vaccination programs.

Vaccination programs in this country vary from the use of bacterins to a live attenuated vaccine. The bacterin provides some immunity to turkeys if the vaccinated birds are exposed to a homologous challenge, but with more than sixteen strains involved the bacterin is only partially effective. The live vaccine offers greater protection than the bacterin but the use of this vaccine gives a 2 to 4% mortality rate and therefore is only recommended for use when a farm has a history of fowl cholera outbreaks (Bierer and Derieux, 1972). Furthermore, the use of this live vaccine is not yet approved by all states.

The use of a live vaccine without the problem of mortality due to the vaccination would be a vital step towards reducing the problem of fowl cholera in commercial poultry flocks. Research was undertaken to develope such a vaccine using a streptomycin dependent mutant of *Pasteurella multocida*.

# LITERATURE REVIEW

## Organism

Fowl cholera is a disease caused by the bacterium *Pasteurella multocida*. The genus *Pasteurella* is named in honor of Louis Pasteur for his classical study using this organism in his work on vaccines. The species multocida means "many killing", due to the many species of animals this organism can affect.

The organism had many names before Rosenbusch and Merchant proposed the name *Pasteurella multocida* in 1939. When Pasteur worked with the organism in 1880 it was known as the virus which caused fowl cholera. In 1893 the organism was being called *Bacterium bipolare multocida* due to its unusual staining properties. In 1929 the name *Pasteurella septica* was suggested in honor of Pasteur. This name was used until 1939 when the name was finally standardized as *Pasteurella multocida* (Smith, 1974).

The *Pasteurella multocida* organism is a Gram-negative, non-motile rod. It occurs singly, in pairs or as chains. In freshly isolated cultures, it has a large capsule and stains bipolar with Wayson's or Wright's stain. Upon prolonged growth on artificial media the organism tends to lose these properties (Heddleston and Rhoades, 1978).

Fowl cholera may occur in two forms. One form is localized causing edema in the wattles, sinuses, or feet. This form usually is not fatal. The birds that recover may become a reservoir or carrier of the organism.

The other form of the disease is a systemic or acute form. Initially, infected birds will have ruffled appearances and marked decreases in feed and water consumption. Greenish-yellow diarrhea is usually seen. The birds eventually die of dehydration. A 90-100% mortality rate in a turkey flock is possible.

Upon post-mortem examination the liver of an infected bird will have small, white, necrotic foci on its surface. A blood or liver smear stained with Wayson stain will show small (0.25 to 0.4 μm in length), bipolar organisms (Heddleston and Rhoades, 1978).

The spread of the disease may be attributed to wild animals and birds or to mechanical methods such as machinery, boots, clothing, or feed. Mixing old and young birds together may also spread the disease.

The organism has not been found to be transmissible through the egg (Heddleston and Rhoades, 1978).

## Classification

Since Pasteur's time, different strains of *Pasteurella multocida* have been identified, but the strains were separated by the species of animal from which they were isolated. Cornelius (1929) used an agglutinin absorption test to differentiate strains of *Pasteurella*. He did not try to separate the strains by which animals they infected since it had been shown that a strain of *Pasteurella* isolated from a buffalo did infect pigs. He identified four types of *Pasteurella multocida* and concluded that there was no relationship between the serological grouping and the animal origin of the strains.

Rosenbusch and Merchant (1939) found two distinct types of *Pasteurella multocida* and a less distinct third type based on biochemical and serological differences. Immunological tests performed by Little and Lyon (1943) indicated that there were three types of nonhemolytic *Pasteurella*. Roberts (1947) found four distinct types based on immunologic studies. Carter (1952) was able to classify three types of *Pasteurella multocida* (A,B,C) by serological tests on the capsular antigen. Later biochemical studies showed that 3 types of *Pasteurella multocida* could be identified (Dorsey, 1963). Heddleston (1966) reported three distinct immunological types of *Pasteurella multocida* based on cross challenges of immunized birds. As many as sixteen different serotypes have been reported. The differences are based on biochemical reactions and gel diffusion differences (Heddleston *et al.*, 1972a; Heddleston *et al.*, 1972b).

Capsule

The relationship of the bacterial capsule of *Pasteurella multocida* and its ability to cause disease in avian species has been reported. The encapsulated organisms are more virulent than the unencapsulated organisms (Priestly, 1936; Heddleston *et al.*, 1964). Whether the capsule contains virulent factors or is just a protective shield against the bird's immune systems has not been determined (Mahreswaran *et al.*, 1973).

The involvement of the capsule in an immunogenic response in avian species is not clear. Early experiments involving the capsule indicated that there was a direct relationship between the size of the capsule and immune response (Priestly, 1936; Carter, 1950).

The results of later studies have indicated that the capsule may be involved in the immune response but is not necessarily the only factor in immunity (Yaw and Kakavas, 1957; Heddleston *et al.*, 1964; Rebers and Heddleston, 1974).

## Nature of the Immunogenic Response

Carter (1951), while experimenting with a chicken embryo bacterin, found that bacterins made of *Pasteurella* organisms with large capsules stimulated better protection against a homologous challenge than did an unencapsulated organism preparation. He noted, however, that the large encapsulated organisms produced less cross protection than did the organisms with smaller capsules.

In a later study, Carter (1952) stated that the capsule is type specific, but there may be a somatic antigen common to all species of *Pasteurella multocida*.

The results from the studies of the somatic antigens have shown that they do cause an immune response in chickens, but not in mice (Yaw and Kakavas, 1957; Heddleston *et al.*, 1964; Heddleston *et al.*, 1966). This immune response does not necessarily protect chickens from heterologous challenges, therefore, indicating that there may be different somatic antigens in different species (Doubley, 1956; Yaw

and Kakavas, 1957). However, Rebers and Heddleston (1974) found that free endotoxin was cross reactive *in vitro* to 15 other serotypes.

Studies on the nature of cross protection factors (protection against different immunological types of *Pasteurella multocida*) indicate that growing *Pasteurella multocida* on artificial media causes a loss of cross protection factors (C.P.F.'s). Loss of these C.P.F.'s may be due to lack of essential nutrients or growth at 37°C rather than 41°- 44°C (temperature of a bird with acute fowl cholera) (Heddleston and Rebers, 1972; Rebers *et al.*, 1975; Rebers and Heddleston, 1977; Rimler *et al.*, 1979b).

C.P.F.'s were enhanced if the organisms used for a vaccine were grown in the host species to be vaccinated. This indicates that the antigen is host specific, or that the antigen inducing C.P.F.'s is easily lost (Heddleston and Rebers, 1974; Rebers and Heddleston, 1977; Rimler *et al.*, 1979b).

## Early Development of Vaccines

Pasteur (1880) reported that on prolonged culturing of *Pasteurella multocida* in the laboratory the organism becomes less virulent over time on artifical media. His work indicated that although virulence could be decreased by this method, the decrease could not be expected to occur with a reproducible regularity. This was the major problem with his method (Hadley, 1910; Heddleston and Rhoades, 1978).

To avoid the problem of irregularities in virulence, Kitt in 1892 used injections of immune chicken blood for passive immunization. The problem of virulence was eliminated, but the effectiveness of passive

immunization was not satisfactory (Hadley, 1910).

By 1910, Hadley was working on a fowl cholera vaccine in the United States. Cultures of *Pasteurella multocida* which were slightly virulent were held at either 44°C or 63°C. It was found that the culture held at 44°C produced protection when injected into rabbits while the organisms held at 63°C produced little protection unless inoculations were repeated. It was also found that a subcutaneous inoculation was better than an intermuscular inoculation (Hadley, 1912).

In another experiment, Hadley (1914) inoculated rabbits with an avirulent strain of *Pasteurella multocida* and then challenged the rabbits with eight different strains of *Pasteurella multocida*. It was found that the rabbits were resistant to five of the eight challenge strains. It was not reported how effective this avirulent strain was in chickens.

During the same period Mack and Records (1916) were experimenting with killed cultures of *Pasteurella multocida*. The method was to isolate the organism from a flock that had fowl cholera, kill it with phenol and then inoculate the same flock with this bacterin. This method supposedly had stopped the disease in fifteen of sixteen flocks, but was used as a treatment and not used for preventative purposes.

## Bacterins

The media selected for the growth of the bacteria may be the most important aspect of making an effective bacterin. Bacterins prepared from cultures grown in tryptose broth were not as effective

in inducing immunity in chickens as were bacterins prepared from cultures grown in chicken embryos (Carter, 1950).

Preparations grown on turkey blood or liver induced immunity in turkeys but preparations grown on artifical media or when washed did not (Heddleston and Rebers, 1972; Rimler *et al.*, 1979a).

Repeated transfers of bacteria on artifical media reduced the bacteria's effectiveness as a bacterin to induce immunity (Rebers and Heddleston, 1977).

Bacteria grown in chicken embryos were not as effective as bacteria grown in turkey embryos for inducing immunity in turkeys (Heddleston and Rebers, 1974; Rimler *et al.*, 1979).

Heddleston and Rebers (1972) have shown that heating, drying, or adding formalin, betapropiolactine or phenol to kill *Pasteurella* does not alter their antigenic characteristics, although they found that 0.5% gluteraldehyde did alter these characteristics.

The major emphasis in preparing fowl cholera bacterins has not been on the method of killing the *Pasteurella* organisms but on the suspension of the dead organisms to produce better immunity.

Organisms emulsified in oil gave better immunity in chickens than did organisms suspended in water (Heddleston and Hall, 1958).

Aluminum hydroxide absorbed bacterins have not been found to be superior to oil emulsified bacterins, but are easier to prepare (Heddleston and Reisinger, 1960; Bhasin and Biberstein, 1968).

Heddleston (1962) did note that a bivalent absorbed bacterin was not as effective as a bivalent emulsified bacterin; possibly more organisms were needed in the absorbed bacterin.

## Vaccination

The number of organisms and the route of administration for a fowl cholera bacterin has not been clearly defined. It has been found that a subcutaneous injection of $1.3 \times 10^{10}$ organisms is as effective as a subcutaneous injection of $1.3 \times 10^{7}$ organisms (Heddleston and Reisinger, 1959).

## Live Vaccines

Bierer *et al.* (1968) reported that a type 3 avirulent strain of *Pasteurella multocida* administered via the drinking water provided protection to 90% of the turkeys when challenged with a homologous strain of *Pasteurella multocida.* The live vaccine provided better immunity than an injected oil based bacterin or other commercial bacterins (Bierer and Scott, 1969; Bierer, 1969; Bierer and Derieux, 1971). Brown *et al.* (1970) confirmed these results and also found that the live vaccine administered via the drinking water was more effective than when a bacterin was administered through the drinking water.

The live vaccine called the Clemson University (C.U.) vaccine has been found to provide immunity against a homologous challenge (type 3) and, also, against heterologous challenges (type 1 and type 2) in turkeys much more so than oil base bacterins (Bierer and Derieux, 1972).

Bierer and Scott (1969) and Bierer and Derieux (1972) found that $7 \times 10^{5}$ organisms given to turkeys via drinking water was sufficient

for immunity for 5 weeks, but caused a 4.2% mortality in the flock when the vaccine was administered. A 6.7% mortality was noted when the C.U. vaccine was administered at $1.2 \times 10^7$ organisms/ml orally while no mortality was noted when $1.5 \times 10^5$ organisms were administered. Both doses gave satisfactory immunity at three weeks after vaccination (Coates *et al.*, 1977).

The major problem with the C.U. vaccine is the 4.2% mortality rate (Bierer and Derieux, 1972) and also, it may establish a reservoir in the turkey flock (Matsumoto and Helfer, 1977).

To overcome the problems with a live avirulent vaccine, researchers started mutating strains of *Pasteurella* for use as a vaccine. The first mutant was a high temperature mutant (M-2283) selscted from an encapsulated type 4 organism (V-2283) isolated from a turkey which had died from fowl cholera. It was found that the mutant gave a high degree of protection to homologous and heterologous challenges if the challenge was inoculated via the same route as the vaccine was administered, this indicated that the mutant only produced a local immunity (Mahreswaran *et al.*, 1973).

Chengappa *et al.* (1979) mutated a strain of *Pasteurella multocida* (type 3) with nitrosoguanidine (N.T.G.) and selected a streptomycin dependent (str-d) mutant. The results indicated that the str-d mutant was effective when given either orally or parenterally at doses of $10^9$ organisms per dose in turkeys with no deaths reported from the vaccination.

Another N.T.G. mutant was developed by Herman *et al.* (1979). This mutant was temperature sensitive and was found to be an effective vaccine against homologous and heterologous challenges (Michael *et al.*, 1979). This vaccine is still in the early stages of testing and is not commercially available.

## MATERIALS AND METHODS

### Turkeys

The turkeys used in all experiments were obtained from Janssen's Turkey Hatchery, Zeeland, Michigan, at one day of age. The birds were straight run and were housed on the floor in 12 x 14 foot pens at the MSU Poultry Science Teaching and Research Center.

Commercial turkey starter (28% protein) was fed to the turkeys from one day to four weeks of age. Commercial turkey grower (22% protein) was fed from four weeks of age until termination of the experiment. All feed was given *ad libitum*. Drinking water was supplied *ad libitum* except when indicated.

### Reference Vaccine

A commercial avirulent live vaccine (type 3) was obtained from American Scientific Laboratories, Madison, Wisconsin. This vaccine was used in experiments II, III, and IV. This vaccine was administered through drinking water, when the turkeys were 6 and 8 weeks of age, as recommended by the manufacturer. Each turkey received approximately $10^9$ organisms per vaccination.

### Experimental Vaccine

The experimental vaccine was a streptomycin dependent (str-d) mutant strain of P-1059 (type 3), obtained from Dr. G.R. Carter and M.M. Chengappa, Department of Microbiology and Public Health, Michigan State University.

Maintenance:

The culture was maintained on Tryptose Agar (TA) (Difco) plus 400 μg/ml streptomycin (str) plates. For experiments I and IV an eighteen hour growth of str-d P 1059 was aseptically removed from a TA plus str (400 μg/ml) plate and passaged through mice injected with 25 μg of str. After 24 hours the mice were sactificed.

A culture of the str-d P1059 was reisolated and streaked onto three TA plus 400 μg str plates. These plates were incubated for 18 hours. At that time the growth was aseptically removed from the agar and suspended in normal saline plus 400 μg/ml str. The suspension was diluted until $10^9$ organisms per ml was attained. The number of organisms was calculated using a spectrophotometer at 600 nm.

The handling of the str-d P1059 in experiments II and III were the same as above with the exception that the culture was not passed through mice before inoculation.

The str-d P1059 vaccine was administered in the drinking water in which 0.5% skim milk plus 400 μg str was added. This vaccine was given to the turkeys when they were 6 and 8 weeks of age.

Challenge Strains

Three challenge strains were used in these experiments; P1059 (type3), P1662 (type 4), and X-73 (type 1). The cultures were maintained on stock culture agar. Before using each strain for challenge, it was passed through young turkeys and reisolated.

In experiments I and II, the challenge was given by swabbing the

nasal cleft of each bird. In experiments III and IV the challenge was given through the drinking water to which 0.5% skim milk was added for three successive days. The challenge was given at 12 weeks of age.

When vaccines or challenges were given through the drinking water, water was withheld from the turkeys for eight hours prior to inoculation. Four hours after the birds were inoculated, fresh drinking water was provided.

Mortality was recorded daily for 14 days after the birds were challenged. Birds which died were necropsied and a liver and a blood smear were stained with Wright's stain. A pure culture of *Pasteurella multocida* and a smear showing bipolar stained organisms indicated the bird died from fowl cholera.

Experiment I

Sixty six male and female turkeys were used in this experiment as shown in Table 1. Each bird in the three treatment pens ingested approximately $1.9 \times 10^9$ str-d P1059 organisms over one four-hour period at 6 and 8 weeks of age.

Challenge doses were given at 12 weeks of age by the nasal swab method. Each bird received an average dose of $5.0 \times 10^7$ organisms.

Experiment II

Ninty nine turkeys either male or female were used for this experiment (see Table 2). Pens 1, 2 and 3 received the experimental vaccine as in experiment I. Pens 4, 5 and 6 were given the commercial

Table 1. Experimental design of experiment I.

| Pen | Number of Turkeys | Immunizing Agent | Challenge strain of *P. multocida* |
|---|---|---|---|
| 1 | 11 | str-d vaccine | P-1059 |
| 2 | 11 | str-d vaccine | X-73 |
| 3 | 11 | str-d vaccine | P-1662 |
| 4 | 11 | non vaccinated control | P-1059 |
| 5 | 11 | non vaccinated control | X-73 |
| 6 | 11 | non vaccinated control | P-1662 |

Table 2. Experimental design of experiment II.

| Pen | Number of Turkeys | Immunizing Agent | Challenge strain of *P. multocida* |
|---|---|---|---|
| 1 | 11 | str-d vaccine | P-1059 |
| 2 | 11 | str-d vaccine | X-73 |
| 3 | 11 | str-d vaccine | P-1662 |
| 4 | 11 | C.U. vaccine | P-1059 |
| 5 | 11 | C.U. vaccine | X-73 |
| 6 | 11 | C.U. vaccine | P-1662 |
| 7 | 11 | non vaccinated control | P-1059 |
| 8 | 11 | non vaccinated control | X-73 |
| 9 | 11 | non vaccinated control | P-1662 |

C.U. vaccine in a manner as recommended by the manufacturer. Pens 7, 8 and 9 were control pens.

Wild type doses of P1059 and P1662 were given at 12 weeks of age with each bird being given $5 \times 10^7$ bacteria. The birds given the X-73 challenge received an average dose of $5 \times 10^6$ organisms.

Experiment III

Nine pens with 8 turkeys in each pen were used in this experiment (see Table 3). The experimental vaccine was given to turkeys in pens 1, 2 and 3 on two successive days at 6 and 8 weeks of age. Each bird ingested approximately $3.75 \times 10^8$ organisms per day. Turkeys in pens 4, 5 and 6 received the commercial vaccine as in experiment II. Turkeys in pens 7, 8 and 9 were used as control pens.

At 12 weeks of age the birds were challenged through the drinking water. The challenge was given for three successive days. Each bird ingested an average of $10^9$ organisms per day.

Experiment IV

This experiment was very similar to experiment III except that 10 turkeys were placed in each of nine pens (see Table 4). The experimental vaccine was given to pens 1, 2 and 3 for 2 successive days at 6 and 8 weeks of age, but prior to vaccination the experimental vaccine was passaged through mice to increase the antigenic capability of the vaccine. The commercial vaccine was administered the same as in experiment III, and the challenge was given as in experiment III.

Table 3. Experimental design of experiment III.

| Pen | Number of Turkeys | Immunizing Agent | Challenge strain of *P. multocida* |
|---|---|---|---|
| 1 | 8 | str-d vaccine | P-1059 |
| 2 | 8 | str-d vaccine | X-73 |
| 3 | 8 | str-d vaccine | P-1662 |
| 4 | 8 | C.U. vaccine | P-1059 |
| 5 | 8 | C.U. vaccine | X-73 |
| 6 | 8 | C.U. vaccine | P-1662 |
| 7 | 8 | non vaccinated control | P-1059 |
| 8 | 8 | non vaccinated control | X-73 |
| 9 | 8 | non vaccinated control | P-1662 |

Table 4. Experimental design of experiment IV.

| Pen | Number of Turkeys | Immunizing Agent | Challenge strain of *P. multocida* |
|---|---|---|---|
| 1 | 10 | str-d vaccine | P-1059 |
| 2 | 10 | str-d vaccine | X-73 |
| 3 | 10 | str-d vaccine | P-1662 |
| 4 | 10 | C.U. vaccine | P-1059 |
| 5 | 10 | C.U. vaccine | X-73 |
| 6 | 10 | C.U. vaccine | P-1662 |
| 7 | 10 | non vaccinated control | P-1059 |
| 8 | 10 | non vaccinated control | X-73 |
| 9 | 10 | non vaccinated control | P-1662 |

# RESULTS

## Experiment I

This experiment was a preliminary test to see if the str-d P1059 could be used as a vaccine against fowl cholera. The results (Table 5) were analyzed using the Chi square method.

When challenged with the wild type P1059 only 27% of the non vaccinated birds lived while 64% of the vaccinated birds lived. A haterologous challenge of X-73 killed all of the str-d P1059 vaccinated and non-vaccinated birds within 48 hours.

The heterologous challenge of P1662 killed 91% of the non vaccinated birds but only 45% of the vaccinated birds died.

The experimental vaccine significantly reduced mortality ($P<0.1$) in the P1059 and P1662 challenge groups. No reduction in mortality was seen in the X-73 challenge group.

## Experiment II

The results from experiment I suggested that the str-d P1059 may have some value as a vaccine. This experiment was conducted to test not only the value of the str-d P1059 as a vaccine but also to compare its efficacy to that of a live commercial vaccine.

The results (Table 6) differed greatly from those of experiment I. The P-1059 challenge killed 91% of the non vaccinated birds and 82% of the experimentally vaccinated birds. Only 18% of the birds that were vaccinated with the C.U. vaccine died.

Table 5. Mortality of Turkeys from Experiment 1 Challenged with Three Strains of *Pasteurella multocda*.

| Treatment | Challenge | Number of birds | Number of Deaths | % Mortality |
|---|---|---|---|---|
| Non vaccinated | P-1059 | 11 | 8 | 73 |
| str-d vaccine | P-1059 | 11 | 4 | 36* |
| Non vaccinated | X-73 | 11 | 11 | 100 |
| str-d vaccine | X-73 | 11 | 11 | 100 |
| Non vaccinated | P-1662 | 11 | 10 | 91 |
| str-d vaccine | P-1662 | 11 | 5 | 45** |

* significant at $P<0.1$
** significant at $P<0.05$

Table 6. Mortality of Turkeys from Experiment II Challenged with P-1059, X-73, and P1662 Strains of *Pasteurella multocida*.

| Treatment | Challenge | Number of birds | Number of Deaths | % Mortality |
|---|---|---|---|---|
| Non vaccinated | P-1059 | 11 | 10 | 91 |
| str-d vaccine | P-1059 | 11 | 9 | 82 |
| C.U. vaccine | P-1059 | 11 | 2 | 18* |
| Non vaccinated | X-73 | 11 | 11 | 100 |
| str-d vaccine | X-73 | 11 | 11 | 100 |
| C.U. vaccine | X-73 | 11 | 4 | 36* |
| Non vaccinated | P-1662 | 11 | 10 | 91 |
| str-d vaccine | P-1662 | 11 | 10 | 91 |
| C.U. vaccine | P1662 | 11 | 5 | 45* |

* significant at $P<0.05$

The X-73 challenge again killed all the non vaccinated birds and str-d P1059 vaccinated birds within 96 hours but only killed 36% of the birds vaccinated with the C.U. vaccine.

The P-1662 challenge was very similar to the P-1059 challenge. The challenge killed 91% of both the nonvaccinated birds and the experimentally vaccinated birds. Only 45% of the birds vaccinated with the C.U. vaccine died.

The experimental vaccine did not significantly reduce mortality in any of the challenge groups while the C.U. vaccine significantly ($P<0.05$) reduced mortality in all three of the challenge groups.

Experiment III

This experiment was a repeat of experiment II except the challenge route was changed from a nasal swab method to an oral inoculation to beter simulate a natural infection. The results (Table 7) were similar to these in experiment II.

All of the control and str-d P1059 vaccinated birds died from the P1059 challenge while only 38% of the C.U. vaccinated birds died from the challenge.

The X-73 challenge killed 100% of the control birds and 88% of the str-d P1059 vaccinated birds, but, it didn't kill any of the C.U. vaccinated birds.

Challenge with the P1662 strain killed 88% of the control birds and 100% of the experimentally vaccinated birds. All of the C.U. vaccinated birds survived the challenge.

Table 7. Mortality of Turkeys from Experiment III Challenged with P-1059, X-73 and P-1662 Strains of *Pasteurella multocida*.

| Treatment | Challenge | Number of birds | Number of Deaths | % Mortality |
|---|---|---|---|---|
| Non vaccinated | P-1059 | 8 | 8 | 100 |
| str-d vaccine | P-1059 | 8 | 8 | 100 |
| C.U. vaccine | P-1059 | 8 | 3 | 38* |
| Non vaccinated | X-73 | 8 | 8 | 100 |
| str-d vaccine | X-73 | 8 | 7 | 88 |
| C.U. vaccine | X-73 | 8 | 0 | 0* |
| Non vaccinated | P-1662 | 8 | 7 | 88 |
| str-d vaccine | P-1662 | 8 | 8 | 100 |
| C.U. vaccine | P-1662 | 8 | 0 | 0* |

* significant at $P<0.05$

The experimental vaccine showed no significance ($P>0.05$) in reducing mortality but the C.U. vaccine showed significance ($P<0.05$) against all the challenge groups.

Experiment IV

The purpose of this experiment was to test whether the experimental vaccine had lost its ability to induce an immune response because of prolonged subculturing on artificial media. The str-d culture was passaged through mice before vaccinating the turkeys.

The results are shown in Table 8. All of the control birds died from the P1059 challenge and 90% of the str-d 1059 vaccinated birds died. Only 20% of the C.U. vaccinated birds died.

The X-73 challenge was less severe, killing only 60% of the controls and 50% of the str-d P1059 vaccinated birds. None of the C.U. vaccinated birds died.

The P1662 challenge was very mild, killing only 30% of the control birds and 40% of the experimentally vaccinated birds. Ten percent of the C.U. vaccinated turkeys died.

The str-d P1059 vaccine showed no significance ($P>0.05$) in reducing mortality in any challenge group. The C.U. vaccine significantly ($P<0.05$) reduced mortality against the P1059 and X-73 challenges but not against the P1662 challenge. This was due to the low mortality of the control birds challenged with the P1662 strain.

Table 8. Mortality of Turkeys from Experiment IV Challenged with Three Strains of *Pasteurella multocida*.

| Treatment | Challenge | Number of birds | Number of Deaths | % Mortality |
|---|---|---|---|---|
| Non vaccinated | P-1059 | 10 | 10 | 100 |
| str-d vaccine | P-1059 | 10 | 9 | 90 |
| C.U. vaccine | P-1059 | 10 | 2 | 20* |
| Non vaccinated | X-73 | 10 | 6 | 60 |
| str-d vaccine | X-73 | 10 | 5 | 50 |
| C.U. vaccine | X-73 | 10 | 0 | 0* |
| Non vaccinated | P-1662 | 10 | 3 | 30 |
| str-d vaccine | P-1662 | 10 | 4 | 40 |
| C.U. vaccine | P-1662 | 10 | 1 | 10 |

* significant at $P<0.05$

## DISCUSSION

The purpose of the experiments reported in this thesis was to evaluate the effectiveness of a str-d P1059 mutant strain of *Pasteurella multocida* as a vaccine for fowl cholera. Field conditions were duplicated as much as possible to give results similar to what would occur in a commercial turkey operation. The results of these experiments are discussed here.

The first experiment gave evidence that the str-d P1059 strain does stimulate an immune response in turkeys and its protection was significantly effective in reducing mortality against a homologous challenge (P-1059) and also a heterologous challenge (P-1662). The birds challenged with X-73 died so quickly that an excessive dose of challenge was suspected.

The next experiment was conducted to test the effectiveness of the str-d P1059 vaccine as compared to the commercial C.U. vaccine. The C.U. vaccine significantly reduced mortality in all three challenge groups. The stp-d P1059 mutant, in contrast to experiment I, did not provide protection against any of the challenge strains.

At the time of this experiment it was thought that the turkeys receiving the str-d P1059 vaccine had access to other sources of water and therefore did not drink the medicated water quickly enough to be effective.

Experiment III was a repeat of experiment II except that the experimental vaccine was administered for 2 successive days via

the drinking water. This procedure was used to insure that all the turkeys in the group would receive a fair dose of the str-d P1059 vaccine.

The challenge strains were given to the turkeys through the drinking water for three successive days to imitate more closely the natural route of infection.

The results of this experiment were similar to those of experiment II. This suggests that either experiment I was an exception or that some change, such as, a capsular variation caused by the mutagenic drug which was not expressed in the first few generations after mutagenesis, (Chengappa, 1981), occurred to the streptomycin dependent P1059 strain in the time between experiment I and experiment II.

In the last experiment the str-d P1059 strain was passaged through a mouse to enhance its ability to stimulate an immunologic response. Also, the three challenge strains were passaged through mice instead of turkeys because of a shortage of turkeys on hand.

The P1059 challenge killed 100% of the control birds, 90% of the str-d P1059 vaccinated birds and only 20% of the C.U. vaccinated birds. This supports the evidence from experiments II and III.

In the other two challenge groups more than 20% of the control birds lived, indicating that a change in virulence occurred in the challenge strains P-1662 and X-73.

Possibly the decrease in virulence was caused by the passage of the challenge strains through mice instead of through turkeys. This could also explain why the str-d P1059 was only effective in experiment I.

# CONCLUSIONS

The use of vaccines for controlling fowl cholera is effective. There does remain room for improvement of the different types of vaccine. The bacterins are good in preventing outbreaks of fowl cholera if the infectious strain of *Pasteurella multocida* is homologous to the bacterin. Since there are so many strains of *Pasteurella multocida* it would be almost impossible and cost prohibitive to vaccinate flocks with bacterins of all known antigenic variants of *Pasteurella multocida.*

The live vaccines such as the C.U. vaccine are more effective against heterologous strains of *Pasteurella multocida* than the bacterin but the possibility of a disease outbreak of fowl cholera does exist when the live vaccine is used.

The only alternative to the two methods of vaccination listed above is the use of a mutant live strain of *Pasteurella multocida.* The first experiment discussed in this thesis indicates that a mutant live vaccine may be useful in controlling fowl cholera without the hazard of a disease outbreak. Further testing of this vaccine needs to be performed to enhance and stabilize its antigenic characteristics before it would be of use to the turkey producers.

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