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The Effects of Penicillin on E. Rhusiopathiae Infected Pigeons

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UNIVERSITY OF NEBRASKA COLLEGE OF AGRICULTURE
AGRICULTURAL EXPERIMENT STATION

Research Bulletin 141

The Effects of Penicillin on *E. Rhusiopathiae* Infected Pigeons

L. Van Es, J. F. Olney and I. C. Blore
Department of Animal Pathology and Hygiene

LINCOLN, NEBRASKA
OCTOBER, 1945

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The Effects of Penicillin on *E. Rhusiopathiae* Infected Pigeons

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ADVANTAGE was taken of an opportunity to study the effects of penicillin on *Erysipelothrix Rhusiopathiae*, the causative organism of swine erysipelas. Owing to the fact that swine can not readily be infected with erysipelas by artificial methods, pigeons which are highly susceptible to the disease, were selected to serve as experimental animals.

It was the purpose of these studies to ascertain whether or not the swine erysipelas bacillus is sensitive to the drug and incidentally to determine the possibility of finding application of penicillin in the treatment of swine.

To this end the pigeons were injected with the culture of this organism and with the penicillin solution. Penicillin was given either simultaneously or at stated intervals. The latter varied in accordance with the plan of the separate experiments. Culture control pigeons were added to each experiment. All the injections were made intramuscularly.

Until an approximately correct penicillin dosage could be established, the amount used was based on the net body weight of the pigeons used, i.e. after the subtraction of the weight of the biologically inert feathers from the gross body weight. As determined by previous observations, the weight of feathers amounted to an average of 1/17 to 1/13 of the gross body weight apparently because of seasonal influences.

The cause of deaths occurring in the experiments was always ascertained by the microscopic examination of the blood or by culture methods.

Experiment C-59721 (A)

In this experiment each of ten pigeons of an average net weight of 303.43 G. was injected with a solution of penicillin-sodium at the rate of approximately 8000 Oxford units per K.G. at each injection.

These injections were made on six successive days. On the third day of the experiment, each of the ten pigeons and four culture control pigeons were inoculated with 0.25 cc. of a 48 hours' broth culture of *E. Rhusiopathiae*. The omission of culture inoculations on the first two days was to determine whether or not penicillin *per se* may have any effect on the experimental birds. These injections were not followed by any reaction.

All the pigeons which were tested as planned survived in good health, whereas three of the four culture control pigeons succumbed within five days.

In order to determine whether or not the ten pigeons which survived had acquired any resistance, they were inoculated with 0.50 cc. of a 48 hour broth culture 14 days after the primary inoculations. The original control pigeon survived whereas all the penicillin treated birds succumbed to the culture inoculations.

Results

The administration of penicillin protected all the pigeons used in this experiment against the virulent inoculations. A subsequent inoculation with culture showed that they had failed to develop any immunity.

Experiment C-59721 (B)

For the purpose of this experiment a number of pigeons were divided into four groups and all of them were inoculated with 0.25 cc. of a 48 hours broth culture of *E. Rhusiopathiae* and penicillin-sodium in ascending doses as indicated in Table I.

TABLE I.

Group No.	No. of pigeons	Doses of penicillin in Oxford Units.	Doses of Culture cc.
I	4	1200	0.25
II	4	2400	0.25
III	8	3600	0.25
IV	4	Culture Controls	0.25

Results

The injection of 1200 Oxford units of penicillin-sodium failed to protect three of the four pigeons simultaneously inoculated with the culture.

Three of the four pigeons which were injected with 2400 Oxford units withstood the infective inoculations.

All of the eight pigeons which received 3600 Oxford units each of the penicillin used survived the culture inoculations but three of the four culture control pigeons failed to survive the culture injections.

Two weeks after the primary treatment with culture and penicillin, the surviving pigeons had their immunity challenged by a subsequent inoculation with 0.50 cc. of a 48 hours culture of *E. Rhusiopathiae*. This resulted in the death of twelve of the pigeons thus treated. They had not developed any resistance.

Experiment C-59721 (C)

This experiment duplicates the one of C-59721 (B). The pigeons were divided into five groups of four birds each and they were all simultaneously injected with 0.25 cc. of a 48 hours broth culture of *E. Rhusiopathiae* and penicillin-sodium as scheduled in Table II.

TABLE II.

Group No.	No. of pigeons	Doses of penicillin in Oxford Units.	Doses of culture cc.
I	4	1200	0.25
II	4	2400	0.25
III	4	3600	0.25
IV	4	4800	0.25
V	4	Culture Controls	0.25

The following results were recorded:

Three of the four pigeons which were each injected with 1200 Oxford units of penicillin-sodium failed to be protected against the culture inoculations.

Of the four pigeons injected with 2400 Oxford units, three proved to be adequately protected against the virulent inoculations.

Of the four pigeons each of which received 3600 Oxford units, three proved to be protected against the culture injected.

Of the four pigeons which were each injected with 4800 Oxford units of penicillin-sodium, one succumbed to the culture inoculation, another one died from intercurrent causes and two birds proved to be adequately protected.

Of the four culture control pigeons, three died of *E. Rhusiopathiae* infection and one proved to be resistant.

Eighteen days after the primary culture inoculations the immunity of the ten surviving pigeons was challenged by a subsequent injection of 0.50 cc. of a 48 hours broth culture of *E. Rhusiopathiae*. This resulted in the death of eight birds. One of the two pigeons which survived was the culture control, which had proved to be resistant in the primary experiment.

Experiment C-60196 (A)

For the purpose of comparison this experiment is a repetition of Experiment C-59721 (A). To this end each of ten pigeons was injected with 2400 Oxford units of penicillin-sodium on six successive days. On the third day each of the ten pigeons and four culture control birds were inoculated with 0.25 cc. of a 48 hours

broth culture of *E. Rhusiopathiae*. In order to ascertain whether or not the penicillin injections had any effect *per se* the culture inoculations were delayed until the third day of the series. No such influence became apparent.

Results

All of the pigeons treated with penicillin survived in the experiment, whereas the four culture control birds succumbed to the culture inoculations. Twenty-three days after the last penicillin injection all the surviving pigeons had their resistance challenged by a subsequent inoculation of 0.50 cc. of a 48 hours broth culture of *E. Rhusiopathiae*. As a result nine of the ten surviving pigeons died of the infection and one of the birds proved to be resistant.

Experiment C-60196 (B)

This experiment was undertaken in order to establish the approximate limit of time when pigeons could still be protected by the injection of penicillin after having been inoculated with a virulent culture of *E. Rhusiopathiae*.

For this purpose the pigeons utilized in this experiment were divided into thirteen groups of four birds each, one group to serve as culture controls. On 2-26-45 the entire lot of 52 pigeons was inoculated with 0.50 cc. each of a 48 hours broth culture of *E. Rhusiopathiae*.

TABLE III.

Schedule for penicillin injections and a record of their results.			
Dates and hours of proposed penicillin injections		Nos. of pigeons dead or alive on 3-9-45	
		Alive	Dead (Blood-Positive)
2-26-45	9 a.m.	3	1
	5 p.m.	2	2
2-27-45	9 a.m.	0	4
	5 p.m.	0	4
2-28-45	9 a.m.	0	4
	5 p.m.	} All these pigeons had succumbed to the virulent inoculations before they could be injected with penicillin.	
3- 1-45	9 a.m.		
	5 p.m.		
3- 2-45	9 a.m.		
	5 p.m.		
3- 3-45	9 a.m.		
	5 p.m.		
2-26-45	Controls	0	4

Beginning 2-26-45 and concluding on 3-3-45 each pigeon in the thirteen groups was injected with approximately 2400 Oxford units of penicillin-sodium (8000 Oxford units per K.G. net body weight.) The injections were made as nearly as possible at 9 a.m. and 5 p.m. of each day in accordance with the schedule shown in Table III.

Results

The results obtained in this experiment revealed that of the four pigeons injected with culture and penicillin-sodium at 9 a.m. on 2-26-45, three of the birds survived and of the four pigeons injected with penicillin at 5 p.m. of the same day only two of these pigeons withstood the culture inoculations.

With only one exception, all the pigeons which were subsequently injected with penicillin, died because of the culture inoculations.

Only six pigeons survived in this experiment and these had their immunity challenged by a later inoculation of 0.50 cc. of a 48 hours broth culture of *E. Rhusiopathiae*. Five of these birds failed to show any resistance and the one which survived had shown resistance also in the primary experiment.

It appears therefore that a significant part of the pigeons inoculated with culture could no longer be saved by one injection of 2400 Oxford units of penicillin when they were even less than twelve hours advanced in their incubation period at the time of the penicillin treatment.

Experiments C-60196 (C, D, and E)

These experiments were an attempt to determine the effect on pigeons by the injection of a mixture composed of equal parts of a 48 hours broth culture of *E. Rhusiopathiae* and a solution of penicillin-sodium containing 5000 Oxford units per cc. dissolved in physiological salt solution.

MODE OF PROCEDURE

Experiment C—A group of ten pigeons was inoculated with 1 cc. each of the culture-penicillin mixture immediately after its preparation.

Experiment D—A group of ten pigeons was inoculated with 1 cc. each of the culture-penicillin mixture four hours after its preparation.

Experiment E—A group of ten pigeons was inoculated with 1 cc. each of the culture-penicillin mixture eight hours after its preparation.

Four culture control pigeons were each injected with 0.50 cc. of a 48 hours broth culture of *E. Rhusiopathiae*.

The mixtures to be used in Experiments D and E were kept at room temperature pending their injections.

Results

All of the ten pigeons survived which had been inoculated with the culture-penicillin mixture immediately after its preparation.

In the series of pigeons inoculated with the mixture four and eight hours after preparation, two pigeons in each group failed to be protected.

The four culture control pigeons died of *E. Rhusiopathiae* infection after normal incubation periods.

In order to determine whether or not the 26 pigeons which survived in the three groups had acquired any resistance engendered by the primary treatments, each of these birds were inoculated with 0.50 cc. of a 48 hours broth culture of *E. Rhusiopathiae*. All of these birds and four culture control pigeons succumbed to these inoculations.

Experiments C-60196 (F, G, and H)

These experiments were an attempt to determine the effects on pigeons of the injection of penicillin-sodium at the rate of 5000 Oxford units per cc. dissolved in 20 cc. of a 48 hours broth culture of *E. Rhusiopathiae*.

MODE OF PROCEDURE

Experiment F—A group of ten pigeons each of which was injected with 0.50 cc. of the culture-penicillin mixture immediately after its preparation.

Experiment G—A group of ten pigeons each of which was injected with 0.50 cc. of the culture-penicillin mixture four hours after its preparation.

Experiment H—A group of ten pigeons each of which was injected with 0.50 cc. of the culture-penicillin mixture eight hours after its preparation.

Four culture control pigeons each injected with 0.50 cc. of 48 hours broth culture of *E. Rhusiopathiae*, were included in the experiment.

The mixture to be used in experiments G and H was kept at room temperature pending its injection.

Results

Of the ten pigeons which were inoculated with the culture-penicillin mixture immediately after its preparation, three birds died of *E. Rhusiopathiae* infection and seven survived in good health.

All of the ten pigeons which were inoculated with the culture-penicillin mixture four hours after its preparation were adequately protected against the primary virulent injections.

Of the ten pigeons which were inoculated with the culture-penicillin mixture eight hours after its preparation, one bird succumbed to the culture injection and the remaining nine survived in good health.

With a view to establish whether or not any of the 26 pigeons which survived in the three experimental groups had developed immunity from the initial culture-penicillin injections, each of these birds was subjected to inoculation with 0.50 cc. of a 48 hours broth culture of *E. Rhusiopathiae* two weeks after the primary inoculations. Only one of these pigeons survived the test and the remaining 25 birds as well as four new control pigeons, died of the infection.

Experiments C-60197 (A, B, C, and D)

This is an attempt to determine the effects of the injection of 2400 Oxford units of penicillin-sodium into pigeons 48 hours after they had been inoculated with 0.50 cc. of a 48 hour broth culture of *E. Rhusiopathiae*. The penicillin was administered as single or repeated injections.

The forty pigeons utilized in this experiment were divided into four groups of ten pigeons each. All these pigeons, as well as the added four culture control pigeons, were each inoculated with 0.50 cc. of a 48 hours broth culture of *E. Rhusiopathiae*. The subsequent penicillin injections were made in accordance with the following schedule:

Experiment A—Ten pigeons at 10 a.m. on 3-14-45.

Experiment B—Ten pigeons at 10 a.m. and 4 p.m. on 3-14-45.

Experiment C—Ten pigeons at 10 a.m. and 4 p.m. on 3-14-45 and at 10 a.m. on 3-15-45.

Experiment D—Ten pigeons at 10 a.m. and 4 p.m. on 3-14-45 and 3-15-45.

Results

Experiment A—Of the ten pigeons in this experiment only two birds survived, seven died of acute *E. Rhusiopathiae* infection and one succumbed to an injury.

Experiment B—Of the ten pigeons comprising this group, eight birds died as a result of the virulent inoculations and only two birds survived.

Experiment C—Of the ten pigeons in this group, five succumbed to the culture inoculations and five survived.

Experiment D—Of the ten pigeons belonging to this group, six birds remained in good health and four died of *E. Rhusiopathiae* infection.

Controls. The four culture control pigeons died after incubation periods of three to four days.

On 3-30-45, the fifteen pigeons which had survived in the four groups were each inoculated with 0.50 cc. of a 48 hours broth culture of *E. Rhusiopathiae* in order to determine whether or not they had acquired a resistance as the result of the previous virulent inoculations. All these pigeons survived in good health, whereas the two culture control birds succumbed after normal incubation periods.

On 4-13-45, the fifteen pigeons were challenged as to immunity as before and again they all survived. Apparently these birds had acquired a manifest resistance. The two culture control pigeons added to the group died after an incubation period of three days.

COMMENT

Whereas in previous experiments the pigeons treated with penicillin by one or two injections, while surviving in the primary experiment, failed to show any resistance when challenged by a virulent inoculation subsequently made, it seemed possible that the delay of 48 hours in the administration of penicillin may have been a factor in the acquisition of resistance by the four pigeons which survived in Experiments A and B.

Results

The relatively high mortality caused by the virulent inoculations in the primary experiments can reasonably be attributed to the delay of 48 hours between the culture inoculations and the administration of the penicillin.

Even under the adverse influence of a more or less delayed penicillin treatment there is a conspicuous difference in the number of surviving pigeons among the two groups which received only one or two penicillin doses and the groups which were injected with penicillin three or four times.

When the fifteen pigeons which survived in the four groups were tested for immunity at fifteen and again twenty-nine days after the primary inoculations with *E. Rhusiopathiae* culture, they displayed a solid immunity on both occasions.

Experiments C-61391 C-62232 (A, B, C, D, E, and F)

This is a study to determine the relative influence of penicillin-sodium and penicillin-calcium on *E. Rhusiopathiae* infected pigeons.

SCHEDULE OF PROCEDURE

Experiment A—Each of ten pigeons were inoculated with 0.50 cc. of a 48 hours broth culture of *E. Rhusiopathiae* on the morning of 5-30-45.

On the morning and evening of 5-31-45 each of these birds was injected with 2500 Oxford units of penicillin-sodium dissolved in physiological salt solution. (2 doses.)

Experiment B—Each of ten pigeons were inoculated with 0.50 cc. of a 48 hours broth culture of *E. Rhusiopathiae* on the morning of 5-30-45.

Each of these pigeons was injected with 2500 Oxford units of penicillin-sodium dissolved in physiological salt solution, on the morning and evening of 5-31-45, 6-1-45, and 6-2-45. (6 doses.)

Experiment C—Each of ten pigeons was inoculated with 0.50 cc. of a 48 hours broth culture of *E. Rhusiopathiae* on the morning of 5-30-45.

On the morning and evening of 5-31-45 each of these pigeons was injected with 2500 Oxford units of penicillin-calcium dissolved in physiological salt solution. (2 doses.)

Experiment D—Each of ten pigeons was inoculated with 0.50 cc. of a 48 hours broth culture of *E. Rhusiopathiae* on the morning of 5-30-45.

On the morning and evening of 5-31-45, 6-1-45 and 6-2-45, each of these pigeons was injected with 2500 Oxford units of penicillin-calcium dissolved in physiological salt solution. (6 doses.)

Experiment E—Each of ten pigeons was inoculated with 0.50 cc. of a 48 hours broth culture of *E. Rhusiopathiae* on the morning of 5-30-45.

On the morning and evening of 5-31-45, each of these pigeons was injected with 2500 Oxford units of penicillin-calcium suspended in oil. (2 doses.)

Experiment F—Each of ten pigeons was inoculated with 0.50 cc. of a 48 hours broth culture of *E. Rhusiopathiae* on the morning of 5-30-45.

On the morning and evening of 5-31-45, 6-1-45 and 6-2-45, each of these pigeons was injected with 2500 Oxford units of penicillin-calcium suspended in oil. (6 doses.)

Controls—Four pigeons were each inoculated with 0.50 cc. of a 48 hours broth culture of *E. Rhusiopathiae* on the morning of 5-30-45 to serve as culture controls.

The results obtained in the primary experiments are summarized in Table IV.

TABLE IV.

Experimental Groups	Type of penicillin injected	Total of penicillin doses injected	No. of pigeons	No. of pigeons dead or alive (Final Results)	
				Survived	Died
A	Penicillin Sodium in phys. s. sol.	2	10	6	4
B	Penicillin Sodium in phys. s. sol.	6	10	10	0
C	Penicillin Calcium in phys. s. sol.	2	10	5	5
D	Penicillin Calcium in phys. s. sol.	6	10	10	0
E	Penicillin Calcium in oil	2	10	4	6
F	Penicillin Calcium in oil	6	10	10	0

Three of the four culture control pigeons died after an incubation period of three days and one died after seven days as a result of the *E. Rhusiopathiae* inoculations.

In order to ascertain whether or not any of the forty-five pigeons which survived in these experiments had acquired any resistance, each of these birds was, on 6-16-45, again inoculated with 0.50 cc. of a 48 hours broth culture of *E. Rhusiopathiae*. The results of the immunity test of 6-16-45 are exhibited in Table V.

On 6-30-45 the remaining pigeons were again subjected to inoculation with 0.50 cc. each of a 48 hours broth culture of *E. Rhusiopathiae* in an attempt to determine the stability of the resistance shown in the previous test. The results are recorded in Table VI.

TABLE V.

Experimental Groups	Type of penicillin originally injected	Total of penicillin doses originally injected	No. of pigeons	No. of pigeons alive or dead in 1st immunity test	
				Survived	Died
A	Penicillin Sodium in phys. s. sol.	2	6	6	0
B	Penicillin Sodium in phys. s. sol.	6	10	10	0
C	Penicillin Calcium in phys. s. sol.	2	3*	2	1
D	Penicillin Calcium in phys. s. sol.	6	10	10	0
E	Penicillin Calcium in oil	2	4	4	0
F	Penicillin Calcium in oil	6	10	9	1

* Two pigeons in Group "C" died from intercurrent causes.

TABLE VI.

Experimental Groups	Type of penicillin originally injected	Total of penicillin doses originally injected	No. of pigeons	No. of pigeons alive or dead in 2nd immunity test	
				Survived	Died
A	Penicillin Sodium in phys. s. sol.	2	6	6	0
B	Penicillin Sodium in phys. s. sol.	6	10	6	4
C	Penicillin Calcium in phys. s. sol.	2	2	2	0
D	Penicillin Calcium in phys. s. sol.	6	10	10	0
E	Penicillin Calcium in oil	2	4	4	0
F	Penicillin Calcium in oil	6	9	8	1

Results

Primary experiments. It appears that among the pigeons injected with only two doses of penicillin-sodium dissolved in physi-

ological salt solution, 60% of the birds survived. In the group which was treated with two doses of penicillin-calcium dissolved in physiological salt solution, 50% of the pigeons were protected. In the group injected with two doses of penicillin-calcium suspended in oil, 40% of the pigeons survived (Table IV).

On the other hand the pigeons in the three groups which received a total of six doses of penicillin each were all found to be adequately protected (Table IV).

Immunity test of 6-16-45. In this test the pigeons in the three groups which received only two doses of penicillin in the primary experiments made a good showing as only one bird of the thirteen tested failed to have developed resistance.

Of the thirty pigeons in the three groups which were originally treated with six doses of penicillin only two birds succumbed to the culture inoculations. (Table V.)

Immunity test of 6-30-45. All of the twelve pigeons in the three groups which were originally injected with two doses of penicillin proved to be still resistant to the virulent inoculations.

Of the twenty-five pigeons of the three groups which received six injections of penicillin each, five succumbed to the culture injections. Four of these were originally treated with penicillin-sodium dissolved in physiological salt solution and one treated with penicillin-calcium suspended in oil (Table VI).

The relative mortality rates in all groups is exhibited in Table VII.

TABLE VII.

Types of penicillin used in each group in primary experiment	Mortality in primary experiment		Mortality in test of 6-16-45		Mortality in test of 6-30-45	
	No. pigeons	Mortality rate	No. pigeons	Mortality rate	No. pigeons	Mortality rate
Penicillin Sodium in phys. s. sol.	20	20% (4 pigeons)	16	0% 0	16	25% (4 pigeons)
Penicillin Calcium in phys. s. sol.	20	25% (5 pigeons)	13	7.69% (1 pigeon)	12	0% 0
Penicillin Calcium in oil	20	30% (6 pigeons)	14	7.14% (1 pigeon)	13	7.69% (1 pigeon)
The combined penicillin calcium group	40	27.5% (11 pigeons)	27	7.41% (2 pigeons)	25	4% (1 pigeon)

CONCLUSIONS

1. *Erysipelothrix Rhusiopathiae* was found to be sensitive to penicillin. When the dosage of the latter was adequately adjusted the drug caused the pigeons to survive the inoculations of the virulent cultures. These observations confirm the results previously reported by Heilman and Herrell.*

2. The injection of 1200 Oxford units of penicillin was not sufficient to protect a majority of the pigeons against the culture inoculations. It became apparent that in penicillin experiments with pigeons the protective dosages range between 2400 and 3600 Oxford Units.

3. A significant number of the pigeons injected with 2400 Oxford units of penicillin succumbed to the culture inoculations when they were even less than twelve hours advanced in their incubation period at the time of the penicillin treatment.

4. When the penicillin treatment was delayed for 48 hours after the culture inoculations a high mortality was observed among the pigeons subjected to such treatment. In the type of experiments here considered, it is preferable to inject culture and penicillin simultaneously. The interval between the two injections should not exceed 24 hours.

5. Even under the adverse influence of a more or less delayed penicillin treatment there is a conspicuous difference in the number of surviving pigeons between the groups which received one or two penicillin doses and the ones which were injected three or more times with a decided advantage in favor of the latter.

6. The results of the injections with culture-penicillin mixtures did not greatly differ in the two groups. In each group only four pigeons succumbed to the inoculations. Twenty-six birds were adequately protected in each group in the primary experiments. In the immunity tests of a total of fifty-two pigeons, only one showed to be resistant, the remainder failing to acquire any immunity.

7. When the resistance of pigeons which survived after the primary inoculations with culture was challenged by one or two subsequent culture inoculations, it was found that a significant number of such birds had acquired a manifest immunity.

8. As there is no reason to believe that in the immunity test a penicillin influence persisted there probably is no ground for an attempt to determine the relative value of the two penicillin salts. The experimental data submitted in the text tend to show that any difference between them could not have been a conspicuous one.

9. Treatment by the administration of penicillin may be indicated for swine in the earliest stages of swine erysipelas. The fact that, in order to obtain the best results, repeated penicillin

* *Heilman and Herrell*—Proceedings Staff Meetings of the Mayo Clinic 19 June 1944.

injections seem to be necessary constitutes a considerable limitation on its employment in veterinary practice. Its use may, however, receive consideration in the treatment of erysipeloid in persons who are sensitized to horse serum.

The penicillin used in these experiments was generously supplied by the Norden Laboratories of Lincoln, Nebraska, and their kindness is hereby gratefully acknowledged by the authors.

