

EPIZOOLOGY OF ERYSIPELOTHRIX RHUSIOPATHIAE
INFECTION IN TURKEYS

by

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EPIZOOLOGY OF ERYSIPELOTHRIX RHUSIOPATHIAE
INFECTION IN TURKEYS

INTRODUCTION

Oregon turkey producers have experienced severe losses in recent years from Erysipelothrix rhusiopathiae infections in their birds. The disease usually manifests itself during the cold and rainy months. However, during the 1949-50 season of infection outbreaks occurred from August 1949 through April 1950. During that period 42 different outbreaks of the disease were diagnosed by the Department of Veterinary Medicine at Oregon State College. The majority of cases appeared within the Willamette Valley region.

A diagnosis of erysipelas was established in all cases by the following procedure: Organs of birds showing typical septicemic lesions were cultured on nutrient agar slopes fortified with peptone and gelatin and in Brewer thioglycollate medium. If a morphologically typical organism was recovered from these media, a mouse protection test using anti-swine erysipelas serum of equine origin was employed for identification. Mice receiving serum and the isolated culture survived the inoculation while mice receiving the culture only died in about three

to five days. The organism was again recovered from the tissues of the dead mice by culturing.

The prevalence of the infection is important to other livestock producers and to public health workers. Man will quite readily serve as a host to the bacterium, in which case the disease is called erysipeloid. The disease can be expected to appear more frequently in the swine of this region as the hog population increases. Erysipelothrix rhusiopathiae infections have been diagnosed in Oregon sheep, and the condition may be of considerable importance in that specie.

PART I

ERYSIPELOTHRIX RHUSIOPATHIAE AS A SOIL SAPROPHYTE

It has been reported by Hesse (3, pp.168-191) and Vallee (8, pp.857-858) that Erysipelothrix rhusiopathiae is both a soil saprophyte and a virulent pathogen. The idea of a soil saprophytic phase for the organism has been used as an explanation for the continuity of the infection from year to year in western Europe's concentrated swine raising regions. These investigators stated that soils in regions where the disease was an enzootic problem would serve as favorable growth media for the organism, whereas soils from nonaffected areas would not serve as favorable growth media for Erysipelothrix rhusiopathiae.

In view of the prevalence of the disease on Oregon's turkey ranges a program was initiated to determine the validity of the soil saprophyte concept for the bacterium. It was thought that if a relationship to the soils of the region could be found the information might be useful as the basis for control measures.

PROCEDURE

In preparation for soil studies with the organism it was considered imperative that a technique be developed

wherein the organism, if present, could be recovered even though associated with the multiple flora of soil. Dale (1, p.228) demonstrated that growth of Erysipelothrix rhusiopathiae could still be supported in media containing a concentration of 0.25 per cent phenol. Media phenolated in that concentration would suppress most bacteria. Because of the microaerophilic physiology of Erysipelothrix rhusiopathiae, Brewer thioglycollate medium was chosen to obtain the maximum number of cells in fluid culture. It was found that Brewer thioglycollate medium containing 0.25 per cent phenol supported luxuriant growth of pure cultures of Erysipelothrix rhusiopathiae when incubated at room temperature with only a slight lengthening of the initial stationary phase of the growth curve as compared with nonphenolized media. However, phenolized cultures incubated at 37 degrees centigrade were almost completely inhibited by the phenol. Many of the active cultures of Erysipelothrix rhusiopathiae presented characteristic colony growth in Brewer thioglycollate medium within 12 hours. The nonmotile organisms remained suspended in the slush agar, presenting a characteristic feathered appearance. Because of these characteristics, Brewer thioglycollate medium was considered ideal for research purposes with the soil and for primary isolation from diseased birds.

Dale's (1, p.228) procedure for separating Erysipelothrix rhusiopathiae from contaminating bacteria was to pour phenolized culture over the surface of plates and then to pick the Erysipelothrix colonies from the plate. Rosenwald (6, p.16) found it useful to inject the phenolized cultures into laboratory mice (Mus musculus). The pathogen was then recovered from the tissues of the mice that died. Since the biochemical properties of Erysipelothrix rhusiopathiae are unreliable as criteria for identification (4, pp.5-10), it was decided to use both plating and animal inoculation techniques in each step in the study that required recovery of the specific organism. Henceforth throughout the soils study when reference is made to animal inoculations it will refer to subcutaneous injections in the following dosages:

1 mouse	0.3 ml. mixed culture
1 mouse	0.3 ml. mixed culture and 0.5 ml. antiserum
1 mouse	0.1 ml. mixed culture
1 mouse	0.1 ml. mixed culture and 0.5 ml. antiserum

The antiserum used was commercially produced anti-swine erysipelas serum of equine origin. Antiserum was very effective in affording passive immunity to the mice. Therefore, it was very useful in the protection tests to demonstrate the serological identity of the cultures. Mice that did not receive antiserum were cultured from the heart blood, liver, spleen, and kidneys after death. In

most instances the organism was recovered from all of those tissues in mice dead of erysipelas. Of the large number of mice injected with serum and culture only five mice failed to be protected throughout the four to five week period that the animals were held after inoculation.

Three of the five animals had received pure culture and antiserum. They all lived longer than their cage mates who received culture only, and all of them yielded pure cultures of Erysipelothrix rhusiopathiae at autopsy.

Mice that received culture only were usually dead within two to five days. The two mice that died after receiving mixed culture and antiserum did not succumb until 20 and 24 days after inoculation, at which time the passive antibodies had apparently disappeared. No further reference will be made to this irregularity in the results since it in no way affected the proposed outcome of the trials.

After holding the animals four to five weeks they were destroyed by crushing the skull. Mice that had not received antiserum and had survived for that period of time were then cultured to be certain that no Erysipelothrix cells were present in the tissues.

Each trial involved several cultures and methods which are described in the tables. The four mice injected with each culture within a given trial were held in high-walled metal cages. The cages and all their contents

were sterilized at the completion of each trial by autoclaving for one hour at 16 pounds pressure and 120 degrees centigrade.

For each culture within a trial a pure culture control group was placed through the identical procedure as were the various mixed cultures. This was always done to establish the fact that the cells were still pathogenic after growth through phenol and various incubation procedures. In all instances the pure cultures appeared on the streaked plates. They killed all the nonprotected mice except three mice that failed to die from culture #636 which had become avirulent. Two of those mice yielded spleen cultures on autopsy.

METHOD I. CULTURE AND SOIL IN PHENOLIZED MEDIUM

Each strain of Erysipelothrix rhusiopathiae used in method I was handled in the following manner:

A loopful of 48 hour culture of Erysipelothrix rhusiopathiae grown on Brewer thioglycollate medium was placed in a tube containing 9.5 milliliters of Brewer thioglycollate medium to which 0.5 of a milliliter of a 5 per cent phenol solution had just been added. This made a final concentration of 0.25 per cent phenol in the medium. To this mixture was added 0.25 of a gram of soil. The soil particles gravitated to the bottom of the

tube with ample opportunity to inoculate the medium with their multiplicity of organisms during settling.

All tubes were held at room temperature for 96 hours, at which time a loopful from each culture was streaked on a petri dish containing a nutrient agar medium fortified with gelatin and peptone¹. Mouse injections were made from the tubes. Table I presents a summary of results obtained by this method of recovery.

The mice receiving antiserum were protected in all cases. Erysipelothrix rhusiopathiae infection was considered the cause of death in mice when the organism was recovered in pure culture, while animals receiving passive immunization survived. Gram's staining and cultural characteristics were also used to determine the identity of the isolate from the dead mice. Erysipelothrix rhusiopathiae was recovered from all dead mice. Culture #636 failed to kill the mice and was, therefore, the only strain that was not recovered by the mouse injection technique. It was recovered on the streaked plate. Later experiments showed that this culture was losing its pathogenicity for mice.

1. Thirty-one grams of nutrient agar (Difco), 20 grams of gelatin, and 15 grams of peptone per 1000 milliliters of water.

Table I. Erysipelothrix rhusiopathiae
and Soil in Phenolized Medium.

Trial	Technique	Culture						
		#643	#353	#636	#618	#767	#774	#777
I Soil pH 6.1	Mouse 0.3 ml.	+						
	Mouse 0.1 ml.	+						
	Plate	+						
IV Soil pH 5.2	Mouse 0.3 ml.		+	-	+			
	Mouse 0.1 ml.		+	-	+			
	Plate		+	+	+			
VI Soil pH 7.2	Mouse 0.3 ml.				+	+	+	+
	Mouse 0.1 ml.				+	+	+	+
	Plate				+	+	+	+

+ = Erysipelothrix rhusiopathiae recovered either from dead mice or from plate.

- = Erysipelothrix rhusiopathiae not recovered.

Identification of the erysipelas organism from the streaked plates was based upon a typical Gram's stain made from minute dewdrop-like colonies along the line of streak. The plates were always incubated at room temperature to minimize overgrowth by soil contaminants. The typical discreet Erysipelothrix colonies were usually apparent within 72 hours of incubation. In some instances rough colony forms would appear which were somewhat larger and opaque. These colonies could easily be confused with a soil organism that was frequently encountered in the experiments. This soil organism seemed to have a phenol tolerance equal to that of Erysipelothrix. Differentiation of the two organisms was based on the observation that the contaminant appeared on the plates about 24 hours before Erysipelothrix colonies were expected, and staining demonstrated that the soil bacterium was a Gram positive, short-chained, streptococcus. Erysipelothrix rhusiopathiae was recovered from all of the plates used in this method of recovery.

The experiments in method I employed three soil samples. The soil used in trial 1 was taken from a plot that had been repeatedly used for vegetable gardening. It had a pH value of 6.1. The soil sample used in trial 4 had a pH value of 5.2 and was taken from a turkey range where erysipelas had never been known to occur. The soil

used in trial 6 had a pH value of 7.2 and was taken from a plot of land used for growing vegetable crops.

The soils in trials 1 and 6 were used the day after being taken into the laboratory. The soil sample in trial 4 had been in the laboratory in a covered beaker for 42 days. All pH determinations were made on a Beckman potentiometer at the time that the trials were begun. The buffer activity of the Brewer thioglycollate medium kept the hydrogen ion concentration of the medium at about pH 6.9 even after 0.25 of a gram of these soils had been added.

Seven different strains of Erysipelothrix rhusiopathiae were recovered eight times with no failures from a medium contaminated with unsterilized soil. This demonstrated that when the erysipelas organism was cultivated under the described conditions, it could compete favorably and grow in the presence of soil organisms. The value of both plating and animal inoculation in recovery methods was demonstrated in the case of culture #636 which was obtained only on the streaked plate. On the other hand, plating alone could not be relied upon because soil organisms sometimes overgrew the plate before Erysipelothrix colonies had appeared. The mice served well as live media for the separation of nonpathogenic microorganisms from the pathogenic Erysipelothrix rhusiopathiae.

Since the mice receiving anti-swine erysipelas serum were protected in all instances while mice receiving only mixed culture died within two to five days (except the avirulent #636), this method was considered satisfactory for combining a diagnostic and isolation technique in one operation.

ISOLATION EXPERIMENTS FROM SOIL
SEEDED WITH ERYSIPELOTHRIX RHUSIOPATHIAE

Since method I demonstrated a technique for separating viable Erysipelothrix rhusiopathiae from soil microorganisms, the method was used to attempt recovery from soil samples that were seeded with Erysipelothrix rhusiopathiae and allowed to stand for varying periods.

There were slight variations in procedure for the various trials in the seeded soil recovery experiments. In trial 4 the cultures of Erysipelothrix rhusiopathiae were first grown in nonphenolized Brewer thioglycollate for 48 hours. One-half milliliter of each culture and 10 grams of soil were mixed with a sterile mortar and pestle. The soil had a pH value of 5.2. After thorough trituration the mixture was placed in a sterile petri dish and allowed to stand at room temperature for 24 hours. The 10 gram masses of soil in the petri dishes were dark with moisture at the beginning of the holding period, but after 24 hours they had dried to firm clods. Particles

of the dried soils were taken from all surfaces and from the centers of the masses of soil until 0.25 of a gram from each soil mass had been accumulated. The 0.25 gram lots of seeded soil were then added to tubes of Brewer thioglycollate medium containing 0.25 per cent phenol. All weighing and handling was done with sterile equipment. The tubes were incubated at room temperature for 96 hours after which plates were streaked and mice were injected. The organism was not recovered from this phase of trial 4.

Erysipelothrix rhusiopathiae was not recovered from the highly acid soil used in trial 4. Therefore, it was decided to use a neutral soil in trial 5. A soil of pH 7 was obtained from a turkey range where clinical cases of the disease had been occurring¹. In trial 5 the phenolized tubes were inoculated with 0.5 gram portions of seeded soils which were obtained in the same manner as described for trial 4. It had been observed that the phenol would satisfactorily inhibit the soil flora in the greater amount of soil. This permitted the use of a larger inoculum of Erysipelothrix cells. The procedure was otherwise identical to that of trial 4. Live Erysipelothrix cells were not recovered.

1. Attempts to recover the bacterium from this soil had been unsuccessful and will be discussed later.

In trial 6 a soil of pH 7.2 was used. It was theorized that the Erysipelothrix cells might undergo some phase of adjustment when added to the soil which would require a period of days before the cells could become acclimatized to the new environment and begin dividing rapidly. Therefore, in trial 6 the seeded soils were allowed to stand at room temperature for ten days before being sampled. Another deviation from trials 4 and 5 was the addition of a few drops of sterile distilled water to the 10 gram soil masses whenever they showed evidence of drying around the edges. It was felt that moisture was necessary for the greatest amount of microbiological activity in the soil. After allowing the moist seeded soils to stand at room temperature for ten days particles were removed from each 10 gram mass from all surfaces and the center of the mass until 0.5 of a gram had been accumulated. The accumulated 0.5 gram lots were then added to tubes of phenolized Brewer thioglycollate which were incubated at room temperature for 96 hours. Plates were streaked and mice were injected. Table II presents the results of the experiments.

The experiments demonstrated that by the techniques used culture #618 could not be recovered after 24 hours from an acid soil, it could not be recovered after 24 hours from a neutral soil, and it could not be recovered

Table II. Soil Seeded with Erysipelothrix rhusiopathiae
and Added to Phenolized Medium.

Trial	Technique	Culture						
		#618	#353	#636	#454	#412	#767	#774 #777
IV Soil pH 5.2	Mouse 0.3 ml.	—	—	—				
	Mouse 0.1 ml.	—	—	—				
	Plate	—	—	—				
V Soil pH 7	Mouse 0.3 ml.	—			—	—		
	Mouse 0.1 ml.	—			—	—		
	Plate	—			—	—		
VI Soil pH 7.2	Mouse 0.3 ml.	—					—	—
	Mouse 0.1 ml.	—					—	—
	Plate	—					—	—

— = Erysipelothrix rhusiopathiae not recovered.

after ten days from a neutral soil in which it was kept moist. Seven other culture strains responded in a similar manner.

Since 0.5 of a milliliter of Brewer thioglycollate medium filled the inoculating loop 90 times, it was determined that 0.25 of a gram of seeded soil contained 2.25 times more Erysipelothrix cells than were used to inoculate the tubes in method I. When 0.5 of a gram of seeded soil was used there were 4.5 times more Erysipelothrix cells in the inoculum than were used in method I. Nevertheless, in all ten attempts to recover the organism from seeded soil by both plating and animal injection methods the results were uniformly negative.

STERILIZED SOIL EXPERIMENT

The results suggested that the soils were destroying the Erysipelothrix cells by some antagonistic effect.

To eliminate the possibility of a physical or chemical effect from the soil itself, a sterilized soil experiment was conducted. To tubes of nonphenolized Brewer thioglycollate medium 0.25 gram portions of soil were added. A soil sample was used that had demonstrated its ability to eliminate Erysipelothrix cells in less than 24 hours. The tubes were autoclaved at 16 pounds pressure and 120 degrees centigrade for 20 minutes. Sterility of

the contents of the tubes was determined by incubation at 37 degrees centigrade for 48 hours. A loopful of 48 hour culture of Erysipelothrix rhusiopathiae was then added to each tube. The tubes were incubated at room temperature for 72 hours. In order that the cultures would receive the same handling as those used in previous methods, a loopful of culture from each tube was next placed into phenolized medium and incubated at room temperature for 96 hours. The inoculating and plating procedure was followed as described in previous experiments. Results are recorded in Table III:

Table III. Erysipelothrix rhusiopathiae
added to sterile soil in medium.

Technique	Culture		
	#618	#353	#636
Mouse .3 ml.	+	+	-
Mouse .1 ml.	+	+	-
Plate	+	+	+

+ = Erysipelothrix rhusiopathiae recovered
either from dead mice or from plate.

- = Erysipelothrix rhusiopathiae not
recovered.

The results seemed to indicate that toxic products from the soil itself were not concerned with the maintenance of Erysipelothrix rhusiopathiae in the presence of soil. Culture #636 did not kill mice in this

experiment because it had lost its virulence, but it was recovered on the plate. This sterilized soil experiment was very similar to those performed by many of the early workers. Such experiments were presented as evidence that the organism was a soil saprophyte (3, pp.168-191).

ANTIOBIOTIC STUDY

Attention was next focused upon an effort to demonstrate a soil antibiotic or bacteriophage. In the first antibiotic experiment 0.25 gram amounts of soil were added to tubes of nonphenolized Brewer thioglycollate medium and allowed to incubate at room temperature for 72 hours. A tremendous amount of biological activity took place in the tubes as was evidenced by turbidity and gas production. The contents of the tubes were then subjected to filtration through both Berkefeld N and Seitz filters. The filtrates were tested for bacterial sterility by inoculating from them to nutrient broth and nutrient agar slopes. Streaked plates of three strains of Erysipelothrix rhusiopathiae were then prepared. Cylindrical plugs of medium were aseptically removed from the plates as described in the agar cup-plate technique for antibiotic studies. The fresh filtrates were pipetted into the holes in the medium. The plates were then incubated upright at room temperature. Twenty-four hours later

more filtrate was added to the holes. After 48 hours a zone of opacity 6 millimeters wide was observed around the agar holes containing the Seitz filtrate. This, therefore, demonstrated the extent to which the filtrate had diffused into the medium, but colonies of Erysipelothrix rhusiopathiae had grown right to the edge of the agar holes. Colonies also grew in the presence of the filtrate on the plates containing the Berkefeld N filtrate.

Since an antibiotic may be produced and then rapidly destroyed in a relatively short period of time, a second antibiotic experiment was instituted in which filtrates were made from the soil at 24, 48, 72, and 96 hour intervals. All filtrations were made in Seitz equipment in the second experiment. Poured plates were used instead of streaked plates in order that a more even colony distribution could be obtained. Three separate strains of the organism were again used and again inhibition failed to occur in the zone into which the filtrates had diffused.

These experiments were both conducted with filters of negative electrical charge. Some of the known antibiotics such as penicillin should have passed through the filters since penicillin will form salts with cations. A bacteriophage should also have appeared in the filtrate. However, an antibiotic of anionic character would probably be retained in the filter.

It is felt that these negative results do not in any way eliminate the possibility of some form of biological antagonism to Erysipelothrix rhusiopathiae, which may be a factor common to many or all soils.

NONPHENOLIZED MEDIUM CULTIVATIONS

Two experiments were conducted to see how well Erysipelothrix rhusiopathiae could compete with the varied organisms of soil under conditions of artificial cultivation.

In the first experiment 0.25 gram portions of a soil with a pH of 5.9 were added to tubes of nonphenolized Brewer thioglycollate. To each tube of the mixture a loopful of 48 hour Erysipelothrix rhusiopathiae culture was added. The tubes were incubated at room temperature for 72 hours. Since phenol was not present as an inhibitor, a great deal of turbidity and gas was produced. If Erysipelothrix cells had been able to compete with the soil organisms for nutrients and had not been killed by the activities of the soil flora, it seemed reasonable that the soil bacteria could next be screened out by adding the mixed culture to phenolized medium while the Erysipelothrix cells would flourish. This step was performed in order that sufficient colony separation would occur on the plates and to avoid the death of mice from

toxins or soil pathogens which might have been present. Plating and mouse inoculations were performed as in other experiments from the phenolized tubes after 96 hours of incubation at room temperature. The results are recorded on Table IV. The passage through phenolized medium was considered necessary. In preliminary work, mice had been injected with soil and Erysipelothrix culture grown in nonphenolized media. The mice in most cases would die in less than 24 hours and their tissues would yield a variety of organisms. Streaked plates of such material were rapidly overgrown with soil bacteria and molds. If the plates were incubated at 37 degrees centigrade, the overgrowth by contaminants was even more rapid.

A second experiment with nonphenolized medium was performed with soil seeded with Erysipelothrix rhusiopathiae. To each 10 gram mass of soil of pH 5.4 was added 0.5 of a milliliter of 48 hour culture of Erysipelothrix rhusiopathiae. These mixtures were triturated with a sterile mortar and pestle and then placed in sterile petri dishes for 24 hours at room temperature. To tubes of nonphenolized Brewer thioglycollate medium were added 0.25 grams of these soil and culture mixtures. They were held at room temperature for 72 hours. A loop of the cultures was then placed in phenolized medium and incubated at room temperature for 96 hours after which plates were streaked

Table IV. Nonphenolized Medium Cultivations

Method		Technique	Culture		
			#618	#353	#636
<u>Erysipelothrix rhusiopathiae</u> and soil added to nonphenolized medium.	Soil pH 5.9	Mouse 0.3 ml.	—	—	—
		Mouse 0.1 ml.	—	—	—
		Plate	—	—	—
Soil seeded with <u>Erysipelothrix rhusiopathiae</u> and added to nonphenolized medium.	Soil pH 5.4	Mouse 0.3 ml.	—	—	—
		Mouse 0.1 ml.	—	—	—
		Plate	—	—	—

— = Erysipelothrix rhusiopathiae not recovered.

and mice were injected. Results of the experiment are recorded on Table IV.

TURKEY RANGE RECOVERY EXPERIMENTS

Four attempts were made to recover Erysipelothrix rhusiopathiae from turkey ranges upon which the disease was occurring. The exact area on which losses began was sought in each case. The character of the ranges was found to vary. One range consisted of a creek bottom that flooded each year. Gravel was the chief constituent of the soil with some clay and top soil that had been added with each successive flood. The soil had a pH value of 5.6. The second range was a gully that collected surface water and had been packed firm by the turkeys following a previous rainstorm. The land was seeded to alta fescue and clover. The top two inches of soil had a pH value of 5.5. Turkeys had also died of erysipelas on that range three years previously. The third range was a logged-off coniferous wood-lot whose soil had a pH value of 7. The fourth range was a muddy and much trampled field upon which a few areas of a grass cover crop could still be seen. It had a pH value of 6.7. Soil samples were taken in three places from each of the ranges. One gram of each sample was placed in Brewer thioglycollate medium containing 0.25 per cent phenol and incubated at room temperature

for 96 hours. Plates were then streaked and mice were injected. Erysipelothrix rhusiopathiae was not recovered from any of the soils.

DISCUSSION

These findings place serious question on the generally accepted idea that Erysipelothrix rhusiopathiae plays the dual role of saprophyte and pathogen.

Analysis of the literature reveals that this very basic idea concerning the epizootology of Erysipelothrix infections stems from the work of a few European research laboratories. Hesse (3, pp.168-191) made a very lengthy study of the problem in 1923, and his work has been widely quoted. However, the bulk of his experiments were conducted in sterilized soil with mature boullion culture of Erysipelothrix rhusiopathiae added to "weak super-saturation". He found that growth failed in both naturally and artificially acidified soils. Instead of demonstrating that the organism was a soil saprophyte he had, in effect, established the pH range of growth for the organism by using a sterilized soil and an unstated quantity of boullion as media. He recovered the organism from these media for 93 days at which time he discontinued the experiment.

In his experiments with nonsterilized soil he used no

inhibiting agent to suppress soil organisms and reported that "in comparison to the analogous sterile experiments, no deviations worth mentioning were determined".

In Hesse's procedure enough medium containing pure culture of Erysipelothrix was added to the soil to furnish a great deal of readily available nutrients. That addition should have resulted in a great increase in the population of the normal soil flora. Poured plates were made of his mixture, and the Erysipelothrix colonies were then to have been picked and identified. No specific details were given in his work as to the means of identification in the nonsterile soil experiments. In other experiments he seemed to rely very strongly upon Gram's staining. That cannot be considered a sound criterion for identification because of the pleomorphism of the organism and its variability in Gram reaction.

Nowhere in his work did he employ the mouse protection test along with staining and cultural characteristics to determine the exact identity of the cells that he presumed were Erysipelothrix rhusiopathiae. Hesse also reported the spontaneous appearance of Erysipelothrix rhusiopathiae in decaying organic matter which had been previously sterilized. Air-borne contaminates were not excluded in the "spontaneous appearance" experiments.

Preisz (5, p.455) quoted the work of Marble in which

sterile earth was mixed with slightly alkaline liquid manure or hay infusion. Erysipelas organisms in the mixture were said to increase strongly at 37 degrees centigrade and to grow through a 10 centimeter layer in 10 to 13 days. No other details were offered. However, it was noted that here again sterile soil was used.

An abstract of the work of Maurice Vallee (8, pp.857-858) stated that he found alluvial quaternary soils highly favorable for the growth of the organism. It is not known whether he worked with sterile or nonsterile soil.

Rosenwald (6, pp.14-26) reported recovery of Erysipelothrix rhusiopathiae on the 48th day from an unsterilized soil seeded with that organism. Apparently recovery was not made before or after the 48th day. In another experiment he recovered the organism seven days after seeding an unsterilized soil. His mixture consisted of one part of mature culture of Erysipelothrix rhusiopathiae grown in tryptose phosphate broth to 1.6 parts of soil. The soil had been air-dried in the laboratory for 15 days prior to its use. The seeded soil was first grown in a medium that did not contain an inhibitor and was next transferred to a phenolized medium. He could not recover the organism from sterilized soil in the same experiment.

The findings of the present study should be considered in analyzing the data that is available for

explaining the perpetuation of Erysipelothrix rhusio- from year to year by a sojourn through soil. On the turkey ranges of Oregon the disease does not commonly appear on the same range on successive years. There has been no conclusive evidence that infection has followed previous use of the land by swine or sheep. However, the very fact that the organism can infect such a multiplicity of hosts suggests the possibility of living reservoirs of infection. The organism has been recovered from man, sheep, swine, fish, mink, quail, ducks, geese, pheasants, rats, turtle doves, black birds, green finches, gold finches, and charfinches. It would seem that the flight of migratory birds or the habits of certain animals that might be carriers could bear investigation as mechanisms for the perpetuation of Erysipelothrix infections.

SUMMARY OF PART I

1. Erysipelothrix rhusiopathiae was grown in the presence of soil bacteria when phenol was used as an inhibiting agent and could be separated again from the soil bacteria.
2. Erysipelothrix rhusiopathiae was not recovered from soils seeded with the organism after standing for 24 hours and for 10 days.

3. Sterilized soil in the medium exerted no deleterious influence on the growth of the Erysipelothrix rhusiopathiae.

4. Erysipelothrix rhusiopathiae was not recovered from a medium containing soil in which phenol had not been added.

5. An attempt to demonstrate an antibiotic against Erysipelothrix rhusiopathiae from soil was unsuccessful.

6. Erysipelothrix rhusiopathiae was not recovered from turkey ranges where the disease was occurring.

PART II

IMMUNOLOGY OF ERYSIPELOTHRIX RHUSIOPATHIAE IN TURKEYS

ANTISERUM STUDY

A small experiment was devised to observe the protective effect of antiserum produced in a mammal for an avian specie.

Commercially produced anti-swine erysipelas serum of equine origin was first used in a mouse protection test to determine serum potency. Mice were inoculated with pure culture of Erysipelothrix rhusiopathiae and antiserum in the following dosages:

1 mouse	0.5 ml. culture
1 mouse	0.5 ml. culture and 0.5 ml. antiserum
1 mouse	0.1 ml. culture
1 mouse	0.1 ml. culture and 0.3 ml. antiserum

The two mice that received culture only were dead in less than 72 hours. Pure cultures of Erysipelothrix rhusiopathiae were recovered from the dead mice. The two mice that had received culture and antiserum remained healthy and were destroyed after 42 days.

Following the demonstration of adequate serum protection in the mice, a similar protection test was performed on four eight-month old turkey hens. They were inoculated subcutaneously in the following manner:

1 turkey	1.5 ml. culture
1 turkey	1.5 ml. culture and 10 ml. antiserum
1 turkey	0.5 ml. culture
1 turkey	0.5 ml. culture and 10 ml. antiserum

The two birds that received culture only were dead in less than 96 hours. Their tissues presented typical lesions and yielded pure cultures of Erysipelothrix rhusiopathiae. The two birds that received culture and antiserum suffered some depression and transitory edema about the face and neck from the first through the sixth days following inoculation. Daily temperatures were taken for 15 days following the inoculations and at no time was there a reading about 107.3 degrees Fahrenheit. The temperatures were considered within the normal range.

The two birds had demonstrated passive immunity to Erysipelothrix rhusiopathiae when a rather large dose of antiserum was given simultaneously with a virulent culture.

Fourteen weeks later the two birds were each inoculated with 1 milliliter of virulent culture to determine whether active immunity had resulted from their previous injections. They experienced no ill effects from the challenge culture and, therefore, demonstrated the production of active immunity to Erysipelothrix rhusiopathiae by the same procedure that is used for vaccination of swine in this country.

Erysipelothrix rhusiopathiae infection appeared in March of 1950 on the Oregon State College turkey unit. The organism was recovered from two dead toms and it was decided to protect other exposed toms with antiserum. Antiserum of the same lot used in the controlled experiment was injected into 29 mature toms in 10 milliliter doses. Twelve birds were held as noninoculated controls and were allowed to remain in contact with the injected birds. Fourteen days after inoculation one of the birds that had received antiserum died of erysipelas. None of the controls became infected and no other birds died. It was theorized that the passive antibodies had been lost by the serum-inoculated bird in less than two weeks.

VIRUS ETIOLOGY STUDY

Several diseases that were originally considered to have a bacterium as the sole etiological agent were later found to be caused by a virus or a virus and bacterium in synergism. Notable examples of such diseases are human influenza, hog cholera, canine distemper, and psittacosis.

Because of the difficulty in producing experimental infection in swine with Erysipelothrix rhusiopathiae, Doyle (2, pp.151-153) suggested a re-examination of the etiology of swine erysipelas. He particularly questioned the so-called chronic stage of erysipelas which results

in a rheumatoid condition in swine.

There are several reports in the literature of turkey flocks affected with erysipelas in which there was almost a 100 per cent morbidity but a much lower mortality. Diarrhea, an excessive amount of mucus in the oral cavity, and depression were observed in the turkeys that were sick but did not die. It was considered possible that a relatively mild virus disease might involve the whole flock and that some individuals could then easily experience invasion by Erysipelothrix rhusiopathiae which would develop to septicemic proportions and kill the host.

A bacterial filtration experiment was devised to search the possibilities of the hypothesis.

Material was obtained from two birds that had died in a flock of 900 eight-month old turkeys. Thirty birds had died in the flock. Liver and spleen tissues from the two birds were ground with a sterile mortar and pestle. The crushed tissue was then subjected to filtration through a Berkefeld N filter with just enough sterile distilled water added to permit passage of fluid through the filter. Sterility of the filtrate was determined by inoculation to nutrient broth and agar slopes. The filtrate was placed in the freezing unit of the refrigerator and the residue was also refrigerated. After 24 hours mice and turkeys were inoculated.

One group of mice received residue in the following dosages:

1 mouse	0.5 ml. residue
1 mouse	0.5 ml. residue and 0.5 ml. antiserum
1 mouse	0.1 ml. residue
1 mouse	0.1 ml. residue and 0.5 ml. antiserum

In less than 72 hours the two mice that had received residue only had died, and their tissues yielded pure cultures of Erysipelothrix rhusiopathiae.

Another group of mice received filtrate in dosages identical to the residue group. The mouse that received 0.5 of a milliliter of filtrate died in five days. Autopsy of the mouse revealed anasarca, hydrothorax, and ascites. The tissues yielded no bacteria. The heart, liver, spleen, lungs, and kidneys of the mouse were ground with a sterile mortar and pestle. The organs were filtered in a Seitz filter, and the filtrate was again checked for sterility. Mice were inoculated with filtrate and residue of the mouse organs but no adverse effect on the animals was observed. All mice were destroyed after being held for 24 days.

Two eight-month old turkey hens that had been raised under isolation conditions were inoculated with filtrate from the turkey organs. One bird received 1.5 milliliters of filtrate subcutaneously. The other bird received 1 milliliter of filtrate intravenously and 1 milliliter of filtrate intramuscularly.

It was thought that any deviations from the normal would be rather slight. Therefore, two noninoculated birds were housed and fed under identical conditions in order that a basis for comparison would exist. All four birds were watched carefully for signs of diarrhea, inappetence, depression, and excessive oral mucus. Temperatures were taken daily on all four birds throughout the following three weeks. No temperature elevations or symptoms developed in the birds.

Since the tissues of the mouse that died with extreme edema produced no ill effect in other mice, it was considered doubtful that a transmissible disease agent was involved. However, it will be impossible to exclude a virus etiology for turkey erysipelas until more work has been done.

AVIRULENT LIVE CULTURE VACCINE

Avirulent organisms have been used for prophylaxis against brucellosis in cattle and tuberculosis in man. Sandstedt and Swahn (7, pp.85-94) employed an avirulent strain of Erysipelothrix rhusiopathiae as a vaccine against erysipelas in swine. It had been observed in the Poultry Disease Laboratory of the Department of Veterinary Medicine at Oregon State College that some cultures of Erysipelothrix rhusiopathiae would lose their virulence

for turkeys after being grown on artificial media in the laboratory for some time.

A live culture vaccine which had lost its virulence for turkeys was used in two vaccination trials.

THE CULTURE. Culture #636 was recovered in routine autopsy from the heart blood of a turkey which had died of erysipelas on February 22, 1949. It was carried at monthly transfers on nutrient agar medium fortified with gelatin and peptone. Henceforth in this paper the avirulent live culture vaccine will be referred to as #636. The fact that this particular isolate was losing its virulence became known after use of the culture in the study of Erysipelothrix rhusiopathiae as a possible soil saprophyte. In experiments in which the culture was grown in a fluid medium containing 0.25 per cent phenol it killed only one out of the six mice used in the trials. The mouse that died did not succumb until the eighth day. The surviving mice were destroyed after 23 days and cultured. Live Erysipelothrix organisms were recovered from the spleens of two of the animals. Other cultures used in the soil experiments and handled under identical conditions killed all mice readily in from two to six days. In another soil experiment #636 failed to kill mice when grown in fluid medium that did not contain phenol.

Because of this apparent decrease in virulence of

#636 it was chosen for use in the turkey trials. However, inoculations of #636 into mice at the time of one of the vaccination trials in turkeys demonstrated that the strain was again capable of killing mice.

Cultures of Erysipelothrix rhusiopathiae used in these studies and those used as challenge cultures in the vaccine trials were identified not only on morphology and cultural characteristics but also by a mouse protection test employing anti-swine erysipelas serum of equine origin.

VACCINE TRIAL I. To determine whether #636 had lost its virulence for turkeys but had retained its antigenic properties, a preliminary experiment was devised:

The turkeys used had been raised from day-old poults by the experiment station under isolation conditions.

A 48 hour culture of #636 grown in Brewer thio-glycollate medium was injected subcutaneously in the sides of the necks of two eight-month old turkey hens. One bird received 0.5 of a milliliter of live culture and the other bird received 0.1 of a milliliter of live culture. Both birds were placed in an isolation cage with metal rod flooring.

Temperatures were taken at the time of injection and daily for the next 19 days. The temperature range ran from 104.7 degrees to 106.6 degrees Fahrenheit throughout

the experiment. This was well within the normal range. From the sixth through the twelfth days after vaccination with the avirulent culture there was noticeable edema of the head and face on both birds. No other adverse reaction was noted. Blood cultures taken ten days after inoculation were negative.

On the 20th day a virulent culture was inoculated subcutaneously into the birds and into two control birds of the same age which had not been inoculated previously but which were housed under identical conditions. The challenge culture was one that had been recovered from a field case the week before and had demonstrated its ability to kill mice in 48 hours. A Brewer thioglycollate culture of the challenge strain was used.

The two control birds died within 72 and 96 hours after challenge. They demonstrated typical lesions and yielded pure cultures of Erysipelothrix rhusiopathiae on autopsy. The two vaccinated birds remained normal.

VACCINE TRIAL II. Following the encouraging results of the first small experiment a larger trial was devised. The turkeys were nine months of age and came from the same source as those used in vaccine trial I. Six toms and ten hens were held on wood shavings litter in separate houses.

The birds were vaccinated with a 48 hour culture of #636 grown in Brewer thioglycollate medium. Two hens and

two toms were held as nonvaccinated controls. These controls were left in the houses with the vaccinated birds to determine whether they would develop contact infection from those inoculated with the vaccine. Two toms and four hens received 0.5 of a milliliter of the vaccine while a like number of toms and hens received 0.1 of a milliliter of vaccine.

Temperatures were taken every other day for the next three weeks. They were all in the range of 104 degrees to 107 degrees Fahrenheit which was within the normal range. Two hens developed a transitory edema around the face which lasted three to five days and disappeared. The birds retained their vigor and appetite.

Tom #2779 died unexpectedly 51 days after vaccination. Autopsy revealed an acute hemorrhagic duodenitis. Cultures were made from heart blood, liver, spleen, and bone marrow, but no pathogens were found. The exact cause of death for the bird was not determined. However, the lesions and cultures were negative for Erysipelothrix rhusiopathiae.

At the end of 12 weeks all the birds were given a challenge inoculation. The challenge culture was a recent isolate and killed mice in less than 72 hours at the time it was used.

A 1 milliliter dose of 48 hour challenge culture

grown on Brewer thioglycollate medium was inoculated subcutaneously into all the birds in the trial. A mature tom which had been obtained from the field a few months previously was also inoculated as a control to further establish the virulence of the challenge culture. Results are recorded on Table V.

Erysipelas was considered the cause of death when typical lesions were observed on autopsy, and the organism was recovered in pure culture from the tissues. Active immunity was demonstrated in all the birds that received 0.5 of a milliliter of #636 vaccine culture. Death from erysipelas occurred in two of the birds that received only 0.1 of a milliliter of #636 culture. The virulence of the challenge dose was established since it killed three of the five controls and made the other two acutely ill. All vaccinated birds except the two that died from inadequate amounts of vaccine showed no inappetence or loss of vigor following the challenge inoculation.

SUMMARY OF PART II

1. Antiserum and virulent culture given simultaneously produced active immunity in two birds.

2. A turkey died of erysipelas 14 days after receiving 10 milliliters of antiserum.

Table V. Results of Turkey Immunization
with #636 Vaccine.

Bird Number	Sex	Vaccine Dosage	Result of Challenge Inoculation*
2768	Female	0.5 ml.	Normal
2771	Female	0.5 ml.	Normal
2785	Female	0.5 ml.	Normal
2772	Female	0.5 ml.	Normal
2773	Male	0.5 ml.	Normal
2760	Male	0.5 ml.	Normal
2780	Female	0.1 ml.	Normal
2769	Female	0.1 ml.	Normal
2766	Female	0.1 ml.	Normal
2776	Female	0.1 ml.	Died in 5 days of erysipelas.
2770	Male	0.1 ml.	Died in 5 days of erysipelas.
2779	Male	0.1 ml.	**
2759	Female	Non- vacci- nated	Died in 3 days of erysipelas.
2795	Female	Non- vacci- nated	Died in 5 days of erysipelas.
2778	Male	Non- vacci- nated	Acutely ill from fourth through tenth day after challenge - depressed, swollen snood, in- appetence, and deathly palor- recovered.
2782	Male	Non- vacci- nated	Acutely ill from second through tenth day after challenge - depressed, swollen face, in- appetence, and deathly palor- recovered.
95 Field Bird	Male	Non- vacci- nated	Died in 3 days of erysipelas.

*All birds challenged with a 1 milliliter dose of virulent culture 12 weeks after vaccination.

**Died 51 days after vaccination from undetermined cause - not challenged.

3. An attempt to demonstrate a virus etiology for turkey erysipelas was unsuccessful.

4. Active immunity was demonstrated against Erysipelothrix rhusiopathiae in two mature turkeys 20 days after vaccination with an avirulent live culture vaccine.

5. Active immunity was demonstrated in nine out of eleven mature turkeys 12 weeks after vaccination with the avirulent live culture vaccine.

6. Two vaccinated turkeys out of eleven died of erysipelas.

LITERATURE CITED

1. Dale, C. N. Studies on phenol tolerance of Erysipelothrix rhusiopathiae. Journal of bacteriology 39:228. 1940.
2. Doyle, L. P. Rheumatoid disease in swine. Proceedings fifty-third annual meeting of the United States livestock sanitary association: 151-153. 1949.
3. Hesse, Erich. Einfluß der Reaktion, insbesondere des Bodens, auf die Fortentwicklung des Rotlauf- bzw. Murisepticus-Bacillus. Archiv für Wissenschaftliche und Praktische Tierheilkunde 50:168-191. 1923-24.
4. Karlson, A. G. and I. A. Merchant. The cultural and biochemic properties of Erysipelothrix rhusiopathiae. American journal of veterinary research 2:5-10. 1941.
5. Preisz, H. V. Rotlauf der Schweine. Handbuch der Pathogenen Mikroorganismen. (Begründet von W. Kolle und A. V. Wasserman). Gustav Fischer und Urban and Schwarzenberg, 1929. 449-481.
6. Rosenwald, A. S. Erysipelothrix rhusiopathiae infection in turkeys. Thesis, Oregon State College Library: 1-31. 1942.
7. Sandstedt, H. and O. Swahn. Inoculation of small pigs with avirulent swine erysipelas vaccine. Skandinavisk veterinär-tidskrift för bakteriologi, patologi samt kött-och mjölkhygien 37:85-94. 1947.
8. Vallee, Maurice. Sur l'etiologie du rouget. Revue de pathologie comparee et d'hygiene generale 30:857-858. 1930. (abstract by A. Urbain)