

ERYSIPELOTHRIX RHUSIOPATHIAE
INFECTION IN TURKEYS

by

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A THESIS

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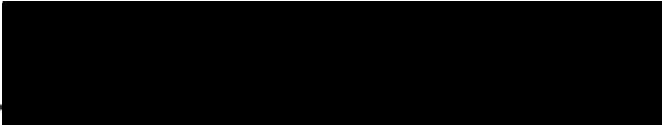
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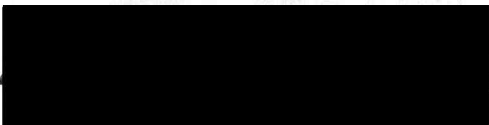
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ERYSIPELOTHRIX RHUSIOPATHIAE INFECTION IN TURKEYS

INTRODUCTION

Though infections with Erysipelothrix rhusiopathiae have been known in the United States since before 1900, the septicemic form of the disease has become prevalent only in the last fifteen years. It was first noted as a disease attacking swine. Marsh (15) showed that some polyarthrititis of sheep was due to localized infection by this organism.

For the sake of clarity, the term "erysipelas", as used in this paper, refers to infection with the organism, Erysipelothrix rhusiopathiae unless otherwise stated. In human medicine the term "erysipelas" refers to an infection characterized by reddening of the skin and caused by the organism, Streptococcus pyogenes. In veterinary literature, infections due to Erysipelothrix rhusiopathiae have been termed "swine erysipelas". There is no evidence to indicate that hogs are primary hosts or reservoirs for this pathogen.

In man, Erysipelothrix rhusiopathiae infections are known as "erysipeloid". Such infections may cause localized lesions, transitory arthritis, or fatal endocarditis. The term "erysipeloid" is used in this paper in the sense of human infections and also as an adjective to describe a

purplish, localized skin lesion sometimes found in turkeys.

The first recorded American outbreak of erysipelas in turkeys occurred in 1934 in New Jersey (3). Since then numerous turkey outbreaks have been reported. Infections with Erysipelothrix rhusiopathiae have also been reported in ducks, quail, pheasants, and various avian species.

From specimens submitted to the Poultry Disease Laboratory of the Department of Veterinary Medicine at the Oregon Agricultural Experiment Station, three outbreaks of the disease were diagnosed in 1937 (18). Since that time, diagnoses of the disease in various turkey flocks in the state have been made every fall and winter.

The work reported here covers a survey of Erysipelothrix rhusiopathiae infections in Oregon turkeys since 1937, bringing up to date the work previously reported (18) (19). Since 1940 the basic source of the organism in the turkey outbreaks has been studied. Investigations to determine the viability of the pathogen in soil under various conditions are reported.

The experimental use of bacterins as prophylactic agents is discussed.

PREVIOUS INVESTIGATIONS AT OREGON AGRICULTURAL EXPERIMENT STATION

Much basic information about erysipelas as it occurs in Oregon was reported by Rosenwald and Dickinson (19) in

1940. There is no need to repeat the statements pertaining to the history of the disease, its economic and public health aspects, the characteristics of the causative organism, as well as the symptomatology, lesions and epizootology of erysipelas in turkeys.

Data presented in 1940 indicated that, in Oregon, erysipelas occurred as a septicemic infection of turkeys during the months from October through February. The vast majority of the affected birds were males. The epizootology of the disease was erratic, with an average mortality of about five per cent and slightly higher morbidity. The disease was diagnosed only from flocks in that portion of Oregon known as the Willamette Valley. Injuries through the mucous membrane or epidermis seemed the most probable and usual portal of entry for the infection. Soil, harboring virulent organisms, was the most likely "suspect" as the source and reservoir of infection.

Laboratory and field trials indicated that commercial anti-Swine Erysipelas serum (from hyperimmune horses) has no practical value as a therapeutic or prophylactic agent. However, birds which survived injections of living organisms and such antiserum were resistant to further infection. Limited trials with sulfanilamide indicated that this drug has no curative value when administered to turkeys sick with erysipelas.

The use of vaccines containing live organisms and an irritant glucoside, saponin, conferred a permanent immunity on birds that survived the inoculation of such vaccine. As only two of nine injected birds lived, the mortality risk was too great to indicate further use of this vaccine.

The virulence of Erysipelothrix rhusiopathiae was apparently enhanced by serial passage through turkeys.

OTHER INVESTIGATIONS

Zochowski, Roginski, and Teklinska (25) reported the successful use of a special "sero-vaccine with Yatren" against erysipelas in swine, but no details of preparation are available.

Recently cooperative field studies have been conducted by the state and Federal Bureaus of Animal Industry in certain areas to determine the feasibility of immunizing swine against erysipelas by the use of specific anti-serum and living culture (20) (4). The results of this work are not yet available, but apparently there have been some instances of vaccination breaks following such treatment. That use of virulent material is dangerous and contrary to the best practices of sanitary science should be recognized.

Breed (4) in a review of the disease in swine mentions

that, "provocative stimulus has been mentioned relative to the appearance of an acute outbreak of the disease". He suggests that concentration of infection present in the soil, hot humid weather, age, and possibly the lack of certain nutritive factors may be provocative stimuli. Premises contaminated with Erysipelothrix rhusiopathiae are said to retain the contamination for ten or twenty years. He further mentions that there are two types of the organism, with differences in pathogenicity and antigenicity.

Breed's observation on the two antigenic types of Erysipelothrix rhusiopathiae is at variance with the findings of Barber (2) and Julianelle (12) who report only one antigenic type, unrelated serologically to Listeria monocytogenes (Pirie) (16). Listeria monocytogenes does resemble Erysipelothrix rhusiopathiae in some respects. Karlson and Merchant (13) in reviewing the biological and biochemical characteristics of the organism, found Erysipelothrix rhusiopathiae very resistant to age and drying.

Creech (5) reports that Stockman states that "the microbe passes a saprophytic existence in the soil". He mentions that the causes for stepped-up virulence are not known. No evidence is mentioned supporting Stockman's statement.

Porter and Hale (17) testing the efficacy of sulfanilamide and sulfapyridine on Listeria monocytogenes and Erysipelothrix rhusiopathiae infections in mice, found that these drugs were of use in treating mice infected with fatal doses of Listeria monocytogenes but were of no help in the case of Erysipelothrix rhusiopathiae.

Lindenmayer and Hamilton (14) report that five outbreaks of erysipelas in turkeys have been diagnosed in Washington in the last three years. Epizootology corresponded to the Oregon outbreaks reported. Three of the outbreaks occurred on the same farm in three successive years. They reported apparent success in treating sick turkeys with turkey serum containing many organisms. This serum was obtained by bleeding a turkey, sick with erysipelas, and sterilizing the serum by the addition of formalin.

However, only a small number of birds were used in these trials. Only two birds were tested in the laboratory and the test dose of virulent Erysipelothrix rhusiopathiae was injected eight days after the use of the turkey serum.

These workers found sulfanilamide of no value.

DIAGNOSTIC PROCEDURE

Birds submitted to the Department of Veterinary Medicine from flocks in which erysipelas was diagnosed were autopsied and the lesions noted. If losses occurred

particularly in the males of flocks approaching marketable age and condition, special note was made of lesions suggestive of erysipelas. Such lesions are swollen, turgid, cyanotic caruncles, subcutaneous ecchymotic and suffusion hemorrhages, congested viscera and injected mesenteric blood vessels.

Smears made from the liver, heart's blood, and spleen were stained by Hucker's Gram-staining technique. The presence of small, curved, beaded Gram-positive rods, occurring singly or in clumps, justified a tentative diagnosis of erysipelas.

Bacteriological cultures were made to nutrient agar slants and generally to Difco Tryptose Phosphate broth or half-percent agar semi-solid medium from the spleen, liver, heart's blood, bone marrow of the long bones, testes, and in certain cases, from the subcutis underlying erysipeloid lesions or from the turgid caruncle. Isolation of Erysipelothrix rhusiopathiae from sick birds that were killed was neither as easy nor as positive as isolation of the organisms from birds dead of the disease.

If the cultures yielded typical dewy colonies, growing readily but discreetly, transfers were made to differential media and from the solid or semi-solid medium to Difco Tryptose Phosphate broth. The differential media were incubated at least a week before final determinations

were made. Erysipelothrix rhusiopathiae regularly ferments dextrose and galactose and usually acidifies lactose and levulose, all without the formation of gas. Mannite, dulcitate, maltose, and sucrose are unchanged. Though lead acetate medium is occasionally browned by the formation of hydrogen sulfide, this medium usually suppresses growth.

Meantime, as soon as 24-hour broth culture was available, animal protection tests were set up. One or more mice (Mus musculus) were injected with 0.1 to 0.5 cubic centimeter of broth culture of the suspected organisms, while control mice were protected with an inoculation of 0.5 to 1.0 cubic centimeter of commercial anti-Swine Erysipelas serum and then injected with the broth culture of the bacteria.

If the protected mice survived while the others died in from two to ten days, yielding typical erysipelas organisms on culture, and if the biochemical reactions were characteristic of Erysipelothrix rhusiopathiae, a diagnosis of erysipelas was made.

In some instances pigeons were used for the protection test instead of mice. The diagnoses reported here are based on the isolation from turkeys of organisms morphologically, biochemically, and antigenically like Erysipelothrix rhusiopathiae, with virulence typical of this organism.

RECENT OUTBREAKS IN OREGON

Table I summarizes the outbreaks in Oregon since the report in 1940 (19). The first outbreak in the 1940-1941 season occurred in mid-september, at the same time that fowl cholera (Pasteurella avicida infection) was diagnosed in the flock. Though many birds from this same flock were autopsied during the next five months, only at this period was there evidence of Erysipelothrix rhusiopathiae infection. Fowl cholera continued to cause losses. The last diagnosis of erysipelas during that season was made in December, 1940.

During the 1941-42 turkey season, only four outbreaks of turkey erysipelas have come to the attention of the Poultry Disease Laboratory. The first occurred in a flock of 1000 birds of both sexes ranging in an apricot orchard. A loss in excess of ten per cent was reported. Three other flocks of 1000 each, owned by the same grower, apparently were not affected. This outbreak is the first diagnosed in that portion of Oregon east of the Cascade mountains.

The outbreak in Flock 24 is of interest in that the necropsy was made because several of the birds had been rejected when marketed because they showed numerous blue-black discolorations on the skin. One bird, sick when

Table I. Epizootiological Data on 9 Flocks of Turkeys
Affected with Erysipelas since July 1, 1940.

Flock No.	Total No. Birds	Age (Mo.)	Sex	♂	♀	Approx. mortality Total	Other stock previously	Approx. duration (days)	Management and control measures	Effect of control
17	1,200	3	♂-♀			40	Turkeys - goats	4-5	Change range	No further loss
18	5,000	5.5	♀	30	0	30 ¹	No report	5	Cull & market	Salvage
19	1,400	5.5	♂-♀	35	5	40 ¹	No report	7	Move, cull & market	No report
20	1,500	6	♂-♀			20	Sheep - turkeys	7 wk.	Cull & market	Salvage
21	1,750	6.5	♂	100	0	100	Turkeys - Sheep, cattle	30	All ♂ but breeders sold 1 wk. before autopsy	2
22	1,000	5	♂-♀	96	4	100 ¹	No report	7	Cull & market	No report
23	2,200	6	♂-♀	100 ¹	0	100 ¹	Sheep	7	Cull, segregate breeders, sell	Breeder ♀ normal Salvage ³
24	80	6	♂-♀	4	1	5	No report	7	Cull & market	Salvage ⁴
	350	6	♂-♀						Segregate	Breeders stayed normal
25	1,350	6	♂-♀	20	5	25	Turkeys - Sheep	7	Cull, segregate breeders. Market well birds.	Little further loss
TOTAL: 14,830				385	15	460				

1 - Mortality up to the time birds were brought to laboratory. No further report.

2 - Losses continued in toms saved for breeders.

3 - Many sick birds

4 - Loss of grade. Rejects due to poor dressing.

killed, was sent to the laboratory. Careful bacteriological examination of bone marrow, femoral blood vessels, spleen and liver failed to reveal any infection. Culture of the edematous subcutis beneath the discolored areas yielded a few colonies of a filamented, readily decolorized Gram-positive rod which fermented dextrose, but not lactose, sucrose, maltose, dulcitol, nor mannitol. Injection of broth cultures of this organism into mice resulted in fifty per cent mortality among those not protected by commercial anti-Swine Erysipelas serum. Reinjection of the culture recovered from the dead mice into other mice killed all unprotected mice. The serum-protected animals died within twelve hours after injection due to trauma. Pigeons were also killed, while those protected by anti-serum survived. A diagnosis of Erysipelothrix rhusiopathiae infection was made. The breeder birds in this flock remained healthy with no loss.

In general, the recent outbreaks present the same puzzling epizootology previously reported. Culling sick birds and marketing the remainder still seems the most practical way of handling flocks in which erysipelas occurs.

EXPERIMENTAL USE OF BACTERINS

Since the use of commercial anti-Swine Erysipelas serum did not seem the practical answer to the need for some prophylactic against the infection in turkeys, and since the production of serum from hyperimmunized turkeys is not commercially feasible, it appeared desirable to attempt immunization by other means.

Difco Tryptose Phosphate broth to which 0.05 per cent agar had been added was prepared in rubber-stopped prescription bottles, with 100 cubic centimeters in each bottle. These were sterilized, checked for sterility, then inoculated with one cubic centimeter of a twenty-four hour culture of Erysipelothrix rhusiopathiae, recently isolated from a turkey. (Culture 121). After forty-eight hours' incubation, the cultures were checked for purity. The five bottles of culture, which were proved pure, were used for the preparation of bacterins.

One lot was retained as the virulent control. To the second lot one per cent (by volume) of formalin (40 per cent formaldehyde) was added; to the third lot one per cent (by volume) of chloroform was added; to the fourth lot one cubic centimeter of a sterile one per cent aqueous solution of Merthiolate (Lilly) was added. The final dilution of Merthiolate in the culture was one to ten thousand.

The fifth lot was heated in a water bath for thirty minutes at 65 to 70 degrees, Centigrade. After three days' incubation at 37 degrees, Centigrade, and two weeks at room temperature, all lots were tested for sterility and all proved sterile, except the virulent control.

One month after preparation, two-tenths of a cubic centimeter of each bacterin was injected into each of four mice (Mus musculus). Uninjected controls were left with each group of mice. Two mice injected at the same time with the same amount of the virulent culture (0.2 cc.) died in 88 hours. Ten days later two mice from each of the groups were injected with a test dose (0.2 cc. each) of the virulent culture. Two "unprotected" control mice were also injected at this time. All died between 65 and 140 hours and Erysipelothrix rhusiopathiae was recovered from each mouse.

The balance of the mice, two from each group, were placed in the contaminated cages and left there until December 15, 1940, thirty-two days after they were injected with the bacterins. They were then inoculated with 0.1 cc. each of the virulent culture. All died in from five to seven days. The bacterins apparently did not confer any protection.

It is interesting to note that seventeen months later the untreated virulent control culture was still pathogenic

for mice.

THE VIABILITY OF ERYSIPELOTHRIX RHUSIOPATHIAE IN SOIL:
PRELIMINARY STUDIES

GENERAL CONSIDERATIONS. Formation of bacteriostatic and bactericidal agents by certain soil organisms has recently been demonstrated by Dubos (7, 8), Dubos and Hotchkiss (9, 10), Hoogerheide (11), and Waksman and Woodruff (22, 23, 24). This has been substantiated by other workers. In a recent review, Allcroft (1) summarizes the work since the time of Pasteur on antibacterial agents derived from microorganisms.

Though no evidence by American workers substantiates the belief, there is widespread supposition that Erysipelothrix rhusiopathiae exists as a saprophyte in the soil. A French worker, Vallee (21), is frequently cited as having shown that this organism survives for a shorter period in acid than in alkaline or neutral soils.

Since the erratic behavior of erysipelas in turkeys indicates that the pathogen may be resident in the soil, it was deemed desirable to check the behavior of Erysipelothrix rhusiopathiae in soil under various conditions.

Search of the literature fails to reveal any American work proving that the organism maintains itself in soil. In studying the epizootology of erysipelas, it would be helpful

to be able to isolate the organism from soil suspected of harboring Erysipelothrix rhusiopathiae.

Dale (6) showed that Erysipelothrix rhusiopathiae would withstand a concentration of 0.25 per cent phenol for long periods. He was able to purify this organism from mixed cultures by seeding the cultures, which were first enriched in broth, into a medium containing one quarter per cent phenol. After incubation at room temperature for several days, this seeded, phenolized broth was plated and characteristic colonies picked and identified.

PROCEDURE AND RESULTS. Preliminary investigations were set up to determine the feasibility of using this technique for the recovery and isolation of Erysipelothrix rhusiopathiae from soil.

Two lots of unsterilized, mixed soil were prepared and placed in sterile one-inch test tubes. The hydrogen ion concentration of this soil (expressed as pH) was 7.35. One lot was seeded with a broth culture of a virulent strain of Erysipelothrix rhusiopathiae. The other lot was seeded with the same amount of sterile broth. At intervals of from one to 297 days, samples of each of these soils were inoculated into Difco Tryptose Phosphate broth containing 0.25 per cent phenol and held at room temperature for five to sixty days. Nutrient agar plates were then

streaked with the cultures. Early in the work it was noted that the phenolized broth, when incubated at 37 degrees, Centigrade, suppressed the growth of Erysipelothrix rhusiopathiae as well as contaminants.

Colonies were picked from the streak plates and checked by staining. Though many colonies were picked, no isolations of Erysipelothrix rhusiopathiae were made. Some soil bacteria which were not suppressed grew in colonies very much like those of Erysipelothrix rhusiopathiae.

During these preliminary investigations, white mice (Mus musculus) were injected with the phenolized cultures at various intervals. One was killed by the injection of broth seeded with soil forty-eight days after the soil was contaminated with Erysipelothrix rhusiopathiae. A pure culture of the organism was recovered from the heart's blood of this mouse. A cage mate died nine days later from "contact" erysipelas.

Injections of phenolized broth cultures seeded with soil 127 days, 132 days, 188 days, and 297 days after the soil was contaminated with Erysipelothrix rhusiopathiae did not kill mice.

The use of Dale's phenolized selective medium in conjunction with streak plating is not as satisfactory a technique for isolation of Erysipelothrix rhusiopathiae from the soil as the use of this medium followed by

injection into susceptible mice. Under some conditions Erysipelothrix rhusiopathiae will survive in soil for at least 48 days.

FURTHER STUDIES ON THE ISOLATION AND VIABILITY OF
ERYSIPELOTHRIX RHUSIOPATHIAE

GENERAL CONSIDERATIONS. Experience at the Oregon State College Department of Veterinary Medicine has indicated that uninoculated mice in contact with those sick with erysipelas often contracted the infection. This suggested the possibility of checking various turkey ranges for the presence of Erysipelothrix rhusiopathiae by placing mice on soil from the areas, provided mice exposed to soil containing the organism would contract a lethal infection with any degree of regularity. The pathogen could then be isolated from the hearts' blood of the dead rodents and identified.

Experiments were set up to check the effect of soil bacteria and dessication on erysipelas organisms seeded into soil. Other trials were designed to determine how regularly mice in contact with soil containing Erysipelothrix rhusiopathiae would become infected and yield the organism on culture.

The two series of tests were run concurrently. All equipment used was first sterilized by autoclaving at 15

pounds pressure for 30 minutes, or by boiling for the same length of time, or by exposure to live steam at 100 degrees Centigrade for one hour on each of three successive days.

Soil samples were obtained and thoroughly mixed. The hydrogen ion concentration of this soil was determined before it was sterilized. The hydrogen ion concentration of the soil before sterilization, expressed as pH, was 6.55. The pH of that portion of the soil which was sterilized by autoclaving at 15 pounds pressure for 140 minutes and 200 minutes on successive days was 6.29. These determinations were made with a Beckman Potentiometer. Both sterilized and unsterilized soils were allowed to dry at room temperature for 15 days.

A moderately virulent strain of Erysipelothrix rhusiopathiae, tested for pathogenicity, was grown in 1500 cubic centimeters of Difco Tryptose Phosphate broth for 96 hours. This same culture was used for all soil contamination and seeding.

DIRECT ISOLATION FROM SOIL. After air-drying for 15 days at room temperature, two hundred and fifty grams of the soil, sterilized by autoclaving as noted, and an equal amount of the unsterilized soil were placed in separate containers. One hundred and fifty cubic centimeters of the 96-hour broth culture were thoroughly mixed with the sterile sample while 160 cubic centimeters of the

culture of Erysipelothrix rhusiopathiae were mixed with the unsterile soil. More culture was mixed with the unsterile soil because it contained less residual soil moisture than the sterilized soil. The amounts of the broth culture were completely taken up by the soil samples.

Each seeded soil sample was divided into approximately equal amounts and placed in two previously-sterilized porous, clay flowerpots. Each of the flowerpots was placed in a number ten, lacquered fruit can (from which the top had been cut), covered with a paper towel, and appropriately identified. One of the two cans containing sterilized, seeded soil in flowerpots was allowed to remain dry, while water was placed in the other can to keep the soil moist by capillary action. The pots containing unsterilized, seeded soil were similarly treated -- one left dry, one kept moist. Air-born flora were not excluded.

Bacteriological cultures made at the time of seeding yielded a pure culture of Erysipelothrix rhusiopathiae from the sterilized soil and a mixed culture containing the pathogen from the unsterilized soil.

Soil samples from each of the four flowerpots were taken at regular intervals indicated in Table II. All samples were handled in a uniform manner. The soil was taken with sterile forceps or soil drill, inoculated into Difco Tryptose Phosphate broth and incubated at 37 degrees

Table II. Results of Direct Soil Isolation

Amount and treatment of soil	Amount of culture cc.	Results***		
		Interval between seeding of soil and testing#	7 days	14 days
125 grams unsterile soil allowed to dry	75	Positive	Negative	Negative
125 grams sterile soil allowed to dry	80	Negative	Negative	Negative
125 grams unsterile soil kept moist	75	Positive	Negative	Negative
125 grams sterile soil kept moist	80	Negative	Negative	Negative

* Tested by routine procedure described

*** Results are reported as negative if Erysipelothrix rhusiopathiae was not isolated from mice dying within 4 weeks of injection. If this organism was thus isolated, results are reported as positive.

Centigrade for 24 hours. Two cubic centimeters of this culture were then added to 4 cubic centimeters of phenolized Difco Tryptose Phosphate broth containing 0.375 per cent phenol. These cultures, containing a final dilution of 0.25 per cent phenol, were held at room temperature for 3 to 5 days.

One-half cubic centimeter of each phenolized culture was then injected subcutaneously into each of two mice (Mus musculus), which were observed for at least four weeks or until they died. Dead mice were necropsied and bacteriological cultures were made to re-isolate Erysipelothrix rhusiopathiae. Organisms isolated from these mice were identified in the manner described under "Diagnostic Procedure".

Two mice were injected with culture made by seeding unsterilized soil, not contaminated with Erysipelothrix rhusiopathiae, into the same series of media as were used for the contaminated soils. Both mice survived.

The results of these attempts to isolate Erysipelothrix rhusiopathiae directly from contaminated soil are summarized in Table II.

MOUSE EXPOSURE TRIALS. Soil from the same mixed soil sample and the same culture of Erysipelothrix rhusiopathiae were used in this series of studies as were used for the direct isolation trials.

Three hundred and seventy-five grams of moist, mixed soil were placed in each of seven number 10 lacquered cans, from which the top had been cut. The cans were covered with paper towels, and three of them were sterilized by autoclaving for 140 and 200 minutes on successive days. All seven of the cans containing soil were allowed to air-dry at room temperature for 15 days. Each then contained about 250 grams of air-dried soil.

One hundred and seventy-five cubic centimeters of the 96-hour broth culture of Erysipelothrix rhusiopathiae were then added to each of the three sterilized soil samples and to three of the unsterilized samples. One can, containing unsterilized soil, was not contaminated with the broth culture of the pathogen, but was seeded with 175 cubic centimeters of sterile broth. The broth and the soil were thoroughly mixed and the cans were covered with paper towels. Air-born flora were not excluded.

Susceptible white mice were introduced into the cans at regular intervals as indicated in Table III. The mice were observed for at least thirty days or until they died. Those that died were autopsied and bacteriological examinations were made from the hearts' blood or livers to re-isolate the erysipelas organism. Organisms so recovered were identified as previously described.

Susceptible mice were introduced into the can con-

Table III. Results of isolation of Erysipelothrix rhusiopathiae
by mouse-soil contact

Amount and treatment of soil	Amount of culture cc.	Data regarding mice and results of exposure to seeded soil			
		Interval*	Number of mice	Treatment of mice	Results
250 grams unsterile soil	175	7 days	1	Injured	Survived
			1	Uninjured	Survived
250 grams sterile soil	175	7 days	1	Injured	Survived
			1	Uninjured	Dead 11 days; <u>E. rhusiopathiae</u> isolated
250 grams unsterile soil**	175	14 days	1	Injured	Dead 7 days; <u>E. rhusiopathiae</u> isolated
			1	Uninjured	Dead 13 days; <u>E. rhusiopathiae</u> isolated
250 grams sterile soil	175	14 days	1	Injured	Survived
			1	Uninjured	Survived
250 grams unsterile soil	175	28 days	1	Injured	Survived
			1	Uninjured	Survived
250 grams sterile soil	175	28 days	1	Injured#	Dead 26 days; <u>E. rhusiopathiae</u> isolated
			1	Uninjured	Survived
250 grams	175 of sterile broth	7 days	1	Injured	Survived
			1	Uninjured	Survived

* Interval between seeding of soil and exposure of mice by contact

** Soil became very wet 2 days after mice were introduced

Parturition 17 days before death

taining only the unsterilized soil, seeded with sterile broth. These survived.

Half of the mice exposed to soil contaminated with Erysipelothrix rhusiopathiae were first injured by abrading the skin of the feet until blood flowed. The remainder of the mice were not injured. Injuries due to fighting were unavoidable.

Provision was made for a drip water system in each can, but occasionally these did not work properly and allowed the soil to become overly wet. Such instances are noted in Table III. Feed was placed directly on the soil.

The results of this series of trials are given in Table III.

DISCUSSION. The results obtained in these trials fall into no consistent pattern. They indicate that although under some conditions Erysipelothrix rhusiopathiae will maintain itself in soil for a period of at least forty-eight days, under other conditions it may not be possible to recover the organism seven days after the soil was seeded. Possibly improvements in the technique used for isolation would lead to more consistent findings.

It should be pointed out that the hydrogen ion concentration of the soil in which the organism survived

for the longer period was considerably lower than in that from which the organism was not recovered one week after the soil was contaminated. This is an indication that the claims of Vallee (21) are correct, but additional data are needed to substantiate this work.

The work on the direct isolation from soil indicates that there is something in the unsterilized soil which permits Erysipelothrix rhusiopathiae to survive longer than in the autoclaved, sterilized soil. The difference in the acidities of the two soil samples is hardly great enough to account for the difference in the survival rate. It is possible that sterilization alters some soil components with the formation of substances toxic to Erysipelothrix rhusiopathiae or with the destruction of substances essential for its survival.

The logical expectation, in view of the work on bacterial antagonism, is that with soil as heavily seeded as were the soils in these trials, the sterilized soil would allow the longer period of survival or would even favor an increase in the numbers of Erysipelothrix rhusiopathiae present. The reverse of this expectation is what has occurred in the direct isolations from soil. The results of the mouse exposure trials are not consistent within themselves, nor with the results of the direct

isolation work.

There may, of course, be different survival rates in different strains of Erysipelothrix rhusiopathiae. Alteration of virulence may also occur. It is interesting to note that in the mouse-exposure trials the soil in the only lot from which both exposed mice died became extremely wet several days before the mice succumbed. This may have affected the resistance of the mice or the virulence of the organism. With as short a survival time as noted in the direct soil isolation work, the effect of the moisture content of the soil cannot be evaluated.

With as heavily contaminated soils as were used in these trials, the few mice which became infected with Erysipelothrix rhusiopathiae indicates that, as a test for the presence of relatively few organisms in the soil, the mouse-exposure method has little practical significance. Obviously the numbers of mice used and the number of trials run were too few to permit the interpretation of the results in terms of statistical or real significance.

Additional trials and more data, as well as improved methods for isolation of Erysipelothrix rhusiopathiae from soil, are needed to clarify the results reported in this thesis.

SUMMARY

The epizootology of Erysipelothrix rhusiopathiae infection in turkeys, as indicated by a survey of outbreaks previously reported from Oregon and other areas, and verified by further observations reported at this time, was highly erratic. Whether or not outbreaks follow provocative stimuli has not been determined.

Outbreaks of this infection in turkeys occurred during the late fall and early winter months. The vast majority of the birds affected were males.

Trials conducted with mice (Mus musculus) indicate that bacterins made by growing Erysipelothrix rhusiopathiae in broth and killing the organisms by various treatments, conferred no practical immunity against subsequent infection with the pathogen.

Methods are described for the isolation of Erysipelothrix rhusiopathiae from soil. The injection of phenolized broth, seeded with contaminated soil, into white mice and the isolation of the organism from the rodents appears feasible. That this method is not entirely satisfactory is indicated by the contrasting results of the direct soil isolation attempts and the mouse-exposure trials.

Mice (Mus musculus) exposed to soil contaminated with Erysipelothrix rhusiopathiae did not become infected

with sufficient regularity to justify the use of this method as a means of checking soil samples from suspected ranges for the presence of this pathogen. However, mouse exposure yielded cultures of Erysipelothrix rhusiopathiae on some occasions where the direct soil isolation attempts on similar soils did not.

The viability of Erysipelothrix rhusiopathiae in different soils varies, but is apparently not adversely affected by the presence of soil organisms.

If recovery of the pathogen by the direct soil-isolation method described be taken as an accurate criterion, Erysipelothrix rhusiopathiae maintained itself in unsterilized soil longer than in slightly more acidic, sterilized soil. The mouse-exposure results did not verify this conclusion.

REFERENCES

1. Allcroft, Ruth. Antibacterial Agents Derived from Micro-organisms with Special Reference to Gramicidin. Vet. Bull., Vol. XII, No. 1, pp. R1-R5, Jan. 1942.
2. Barber, Mary. A Comparative Study of Listerella and Erysipelothrix. The Jour. of Path. and Bact., Vol. XLVIII, No. 1, pp. 11-23, Jan. 1939.
3. Beaudette, F. R. and C. B. Hudson. An Outbreak of Acute Swine Erysipelas Infection in Turkeys. Jour. of the A.V.M.A., Vol. LXXXVIII, N.S. No. 41, No. 4, pp. 475-487, April, 1936.
4. Breed, Frank. Some Swine Problems of the Future. Jour. of the A.V.M.A., Vol. C, No. 778, pp. 27-32, Jan. 1942.
5. Creech, G. T., The Bacillus of Swine Erysipelas Isolated from Urticarial Lesions of Swine in the United States. Jour of the A.V.M.A., Vol. LIX, N.S. Vol. 12, pp. 139-150, 1921.
6. Dale, C. N. Studies on Phenol Tolerance of Erysipelothrix rhusiopathiae, Proceedings of Local Branches of the Society of American Bacteriologists. Jour. of Bact., Vol. 39, No. 2, pp. 228, Feb. 1940.
7. Dubos, R. J. Studies on a Bactericidal Agent Extracted from a Soil Bacillus. I. Preparation of the Agent. Its Activity in Vitro. II. Protective Effect of the Bactericidal Agent Against Experimental Pneumococcus Infection in Mice. J. Exp. Med. 70 1-10 and 11-17, (Abst. Vet. Bull., Vol. 10, page 806), Oct. 1940.
8. Dubos, Rene J. The Effect of Specific Agents Extracted from Soil Micro-organisms upon Experimental Bacterial Infections, Annals of Internal Medicine, Vol. 13, No. 11, pp. 2025-2037, May, 1940.

9. Dubos, Rene J. and Rollin D. Hotchkiss. The Production of Bactericidal Substances by Aerobic Sporulating Bacilli. Jour of Expt. Med., Vol. 72, No. 5, pp. 629-640, May 1, 1941.
10. Dubos, Rene J. and Rollin D. Hotchkiss. Properties of Bactericidal Fractions Extracted from a Sporulating Soil Bacillus. Jour. of Bact., Vol. 39, No. 1, pp. 49-50, Jan. 1940
11. Hoogerheide, J. C. An Agent, Isolated from a Soil Bacillus, which Inhibits Encapsulation of Friedlander's Bacterium and is highly Bactericidal for Gram-Positive Micro-organisms. Jour. of the Franklin Institute, Vol. 229, No. 5, p. 677, 1940.
12. Julianelle, L. A. The Identification of Erysipelothrix and its Relation to Listerella. Jour. of Bact., Vol. 42, No. 3, pp. 385-394, Sept. 1941.
13. Karlson, A. G. and I. A. Merchant. The Cultural and Biochemic Properties of Erysipelothrix rhusiopathiae. Am. Jour. of Vet. Research, Vol. II, No. 2, pp. 5-10, Jan. 1941.
14. Lindenmayer, J. E. and C. M. Hamilton. Treatment of Swine Erysipelas in Turkeys with Serum from a Turkey infected with Erysipelothrix rhusiopathiae. Jour. of the A.V.M.A., Vol. C, No. 780, pp. 212-213, March, 1942.
15. Marsh, Hadleigh. The Bacillus of Swine Erysipelas Associated with Arthritis in Lambs. Jour. of the A.V.M.A., Vol. LXXVIII, pp. 57-63, 1931.
16. Pirie, J. H. H. Listeria: Change of Name for a Genus of Bacteria. Nature, Lond., Vol. 145, p. 264, 1940. (Abst. Vet. Bull., Vol. X, No. 5, p. 325, May, 1940).
17. Porter, J. R. and W. M. Hale. Effect of Sulfanilamide and Sulfapyridine on Experimental Infections with Listerella and Erysipelothrix in Mice. Proc. Soc. Exp. Biol., N. Y. 42, 2 tables pp. 47-50. (Abst. Vet. Bull., Vol. 10, pp. 957-958, Dec. 1940).

18. Rosenwald, A. S. and E. M. Dickinson. A Report of Swine Erysipelas in Turkeys. Cornell Vet., Vol. 29, pp. 61-67, 1939. 10 refs.
19. Rosenwald, A. S. and E. M. Dickinson. Swine Erysipelas in Turkeys. Vol. II, No. 3, Am. Jour. Vet. Research, pp. 202-213, April, 1941.
20. Shore, J. H. Swine Erysipelas. Jour. of the A.V.M.A., Vol. XCIX, No. 775, pp. 295-296, Oct., 1941.
21. Vallee, Maurice. The Experiments Carried out in France on the Acidity of Soils and its Relation to Swine Erysipelas. Revue de Pathologie Comparee, Vol. XXX, No. 402-403, p. 857, 1930.
22. Waksman, S. A. and H. B. Woodruff. The Soil as a Source of Micro-organisms Antagonistic to Disease-Producing Bacteria. J. Bact., Vol. 40, p. 581, 1940.
23. Waksman, S. A. and H. B. Woodruff. Actinomyces antibioticus nov. sp., A New Antagonist Against Micro-organisms. J. Bact., Vol. 41, p. 32, 1941.
24. Waksman, S. A. and H. B. Woodruff, Actinomyces antibioticus, A New Soil Organism Antagonistic to Pathogenic and Non-pathogenic Bacteria, J. Bact. Vol. 42, p. 231, 1941b.
25. Zochowski, P., Z. Roginski and M. Teklinska. Immunization Against Swine Erysipelas by Sero-Vaccination and Yatren Vaccine, (Trans. Title) Pam. pans, Inst. Mauk. Cosp Wiej. Pulawy, Vol. 1, pp. 76-84, 1937. (Abst. Vet. Bull., Vol. 10, No. 7, pp. 494, 1940.)