ROUTES OF ELIMINATION OF ERYSIPELOTHRIX INSIDIOSA FROM INFECTED SWINE

by

Richard Lee Wood

A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
MASTER OF SCIENCE

Major Subject: Veterinary Bacteriology

Approved:

Signatures have been redacted for privacy

Iowa State University
Of Science and Technology
Ames, Iowa

1966

TABLE OF CONTENTS

INTRODUCTION

The bacterium Erysipelothrix insidiosa is the causative organism of swine erysipelas, a disease of considerable economic importance to the swine industry in Europe, Asia, and North America. The organism was first isolated in Europe in 1878 from an experimentally infected mouse, and in 1882 was found to be associated with the disease of swine.

In spite of the long history of swine erysipelas, and the vast amount of literature on the disease, there is relatively little specific information on its epizootiology. Statements in veterinary literature on this aspect of the disease have been based largely on observations of the occurrence and characteristics of natural outbreaks. It is generally accepted that the erysipelas organism can be transmitted by way of feed, water, or soil, and that it can be found in feces and urine of pigs with the disease, which provides a means for contamination of the environment. However, a complete determination of the routes and times of elimination of the organism from clinically affected pigs has not been reported.

The purpose of this project was to determine the routes by which E. insidiosa is eliminated into the environment, and when elimination occurs during the course of clinical illness in experimentally infected swine.

REVIEW OF LITERATURE

The bacterium now known as Erysipelothrix insidiosa (5), formerly Erysipelothrix rhusiopathiae, was first discovered in 1878 in Germany by Robert Koch (25, pp. 83-87), who isolated from an experimental mouse an organism which he called the "bacillus of mouse septicemia." Further studies on this bacillus were reported by Löffler (30) in 1881. A similar organism isolated by Pasteur and Thuillier from swine affected with rouget was briefly described by Pasteur in 1882 (38). Löffler is credited with giving the first accurate description of Schweinerotlauf, or swine erysipelas, and its causative organism in 1882, although this work was not published until 1886 (29), by which time other reports had appeared in European literature (8, 45).

In the United States, the organism was found in a pig in 1885 by Theobald Smith of the United States Department of Agriculture (60, p. 196), and again in 1888 by Moore (32). Swine erysipelas was not recognized as a serious disease in this country, however, until 1931 (10, 57), although reports of its occurrence had appeared in the intervening years (9, 15, 61, 65).

Throughout the history of swine erysipelas, the epizoctiology of the disease has not been fully understood. Various factors relating to its occurrence have been sug-

gested, but are without proof. Pasteur (38) believed that "improved" white breeds of pigs were more likely to contract the disease than the more common breeds. Friedberger and Fröhner (13) also mentioned breed as a factor in susceptibility, although Harrington (19) saw no such relationship. Nutritional deficiencies (59) and weather conditions (24) have been related to occurrence of the disease. Aitken (1) reported outbreaks which appeared to be related to accidental access to a corn field, feeding of new corn, or introduction into new pasture. Kurek (28) reported that susceptibility was increased by a high protein diet. Variations of incidence related to cyclic years and to season of the year have been mentioned (1, 17, 26). Early workers observed that the disease appeared to be confined to certain geographical areas. Nocard and Leclainche (33) stated that although diffusion from "permanent" infection zones into "secondary" zones sometimes occurred, the disease did not tend to persist in the latter. Vallée (62) and Francke and Goerttler (12) also stated that the disease was confined to certain districts.

The repeated occurrence of swine erysipelas in certain areas, sometimes with several years elapsing between outbreaks, led to the belief that the causative organism persisted for long periods in the soil. This view still commonly is held, although it is supported only by circumstan-

tial evidence. Hesse (21) reported experiments in which E. insidiosa survived only a few days in acid soils, but could be recovered for at least 90 days from alkaline soils. He concluded that the organism could live a year or more in soil of alkaline reaction. Marmorstein (31) reported that the organism would grow at 37° C. in sterile earth mixed with alkaline liquid stable waste or hay infusion. Vallée (62) reported that culture media containing filtrates of soil taken from areas where erysipelas was enzootic would support growth of E. insidiosa; but samples from areas where the disease did not occur would not support growth. The behavior of E. insidiosa in soil under laboratory conditions has been investigated also by Rosenwald (41), Rowsell (43), Sukhoretskii (53), Gurova (18), and Szynkiewicz (56). Their results indicated that the organism does not persist in the soil. Aitken (2) pointed out an apparent correlation between alkalinity of soil and the occurrence of natural outbreaks. On the other hand, Connell and Langford (7) stated that they found no evidence of geographical distribution of the disease according to soil type. They reported the incidence was proportional to swine population.

There are no reports of isolation of E. insidiosa from soil under natural conditions. Vallée (62) and Obreshkov (34) failed in attempts to find the organism in soil of areas where swine erysipelas had occurred. Rowsell (43) could not

establish a permanent population after repeated inoculations into soil taken from enzootic areas. Szynkiewicz (55) reported that E. insidiosa added to soil samples was destroyed by protozoa, and postulated that this was the probable fate of many pathogenic bacteria in the soil. Sukhoretskii (53) stated that the failure of the erysipelas organism to survive in soil for long periods is comparable to results reported for other non-spore-forming bacteria, and that the soil can be regarded as a source of infection only when continuously and directly contaminated. Doyle (11) believed that it is doubtful that the soil is the main source of infection, although E. insidiosa may survive long enough after being shed by infected animals to be a hazard to susceptible pigs.

The existence of asymptomatic carriers of E. insidiosa among swine has been known for many years. Olt (36), Bauermeister (4), and Pitt (39) demonstrated the presence of the organism in the tonsils and intestines of slaughtered pigs that had no clinical evidence of erysipelas infection. These findings have been confirmed by a number of investigators (3, 6, 7, 14, 20, 42, 54, 58, 68). Furthermore, E. insidiosa has been isolated from tonsillar secretions taken from live healthy pigs (27). Spears (52) isolated the organism from femoral red marrow of slaughtered pigs.

The carrier rate among healthy pigs is estimated to vary from 30 to 50% (11), but the significance of such animals as

disseminators of the organism has not been conclusively determined. Outbreaks of swine erysipelas frequently coincide with the introduction of apparently healthy pigs to a herd, indicating that the carrier pig may have an important part in the spread of the disease. Hutyra et al. (23) considered asymptomatic carriers to be as infectious as clinical cases and suggested that they were the cause of apparently spontaneous outbreaks. Gledhill (16) stated that subclinical infections in pigs are common in enzootic areas, and suggested that such animals can spread the disease. On the other hand, Francke and Goerttler (12) believed that carriers do not create a serious hazard to susceptible pigs. Rowsell (43) reported experiments indicating that E. insidiosa could resist the acidity of the stomach long enough to pass into the more favorable environment of the intestinal tract. He believed this to be the most likely route of elimination by the tonsillar carrier. Kurek (28) isolated E. insidiosa from 3 out of 454 fecal samples from 67 healthy unvaccinated swine. He concluded that such animals excrete the organism in the feces only sporadically.

Excretion of the erysipelas organism by clinically affected swine has been reported. Cornevin (8) stated that he could transmit the disease with fecal material from dead or dying pigs, but could not do so with urine. Hutyra et al. (23) and Preisz (40) mentioned observations by European

workers that erysipelas could be transmitted through feces and urine of pigs during illness and for several days after recovery. Rowsell (44) demonstrated the presence of the organism in feces of pigs with acute erysipelas induced by oral infection. Kurek (28) found E. insidiosa in feces of 8 out of 10 pigs ill with spontaneous erysipelas. Such contaminated body excretions no doubt provide a common source of infection, since it is known that susceptible pigs can contract erysipelas by ingestion of contaminated feed, water, and soil, as well as through superficial skin wounds (48). Transmission by direct contact also is a possibility, according to Van Es and McGrath (63), although Goerttler (17) believed that this seldom occurs.

The ubiquity of E. insidiosa in nature is a factor which complicates attempts to delineate methods of transmission. The organism has been isolated from many species of mammals, birds, and insects, and from both marine and fresh-water fish (48). Hettche (22) found the organism in city sewage from abattoirs and suburban stables. Olsuf'ev et al. (35) isolated the organism from stream water and from rodents inhabiting the banks. Wellmann (67) found that rodents, birds, and insects could act as intermediate carriers, but stated that their significance remained to be proved.

METHOD OF PROCEDURE

The general plan of these experiments was to infect pigs with selected strains of Erysipelothrix insidiosa and to collect samples from the blood and various body excretions for cultural examination until death or 10 days post-exposure.

Materials

Animals

Eighteen specific-pathogen-free (SPF) pigs were used in these experiments. Twelve of the pigs were males, and 6 were females. Three of the males were of the Hampshire breed, and the remainder of the pigs were of mixed breeding, and predominantly white in color. All pigs had been obtained by hysterectomy, given no colostrum, vaccines, antiserums, or antibiotics, and reared in isolation on concrete floors. When used in the experiments, the pigs were 4 1/2 to 8 months of age, and weighed 89 to 187 pounds.

Equipment for animal confinement

A galvanized steel metabolism crate[1] designed for sheep was modified for use with swine. Modification consisted of the installation of removable rigid aluminum panels at the sides and rear of the crate, sloping inward in order to limit

---

[1]Wahmann Manufacturing Co., Baltimore, Md.

movement by the pig when in the standing position, while providing sufficient space for the animal to lie down (Figs. 1, 2). The front of the crate was equipped with a stanchion (Fig. 2) for restraint of the head when necessary for sample collection. The floor of the crate was made from expanded metal of sufficient strength to support the animal, while allowing urine of males to pass through into collecting equipment (Figs. 2, 3).

Equipment for feces collection

An opening was maintained in the floor of the crate at the rear for droppings to fall through. Droppings from males were collected in a disposable 12 by 13 by 24-inch bag of 0.0015-inch polyethylene (10-gallon garbage can liner). The opening of the bag was stretched over a 12 by 14-inch wood frame which was inserted into the opening in the floor at the rear of the crate (Figs. 1, 3). Droppings from females were deflected with a sloping, trough-shaped piece of 1/4-inch mesh galvanized wire cloth into a pail lined with a disposable polyethylene bag (Fig. 6).

Equipment for urine collection

Urine was collected from males by means of a 14 by 22-inch rectangular catching funnel under the floor of the crate (Figs. 1, 4). The funnel was made from 1/4-inch mesh galvanized wire cloth supported by a wood frame at the periphery

of the base, and with a 2 by 2-inch opening in the wire cloth at the apex. The inner surface of the funnel was lined with a double layer of disposable 0.0015-inch polyethylene sheet with a small opening at the apex. To prevent splashing of urine, cheesecloth was laid over the top of the funnel and fastened to the wood frame with thumbtacks. From the catching funnel, urine passed through a sterile glass funnel into a sterile 2000-ml. aspirator bottle.

Urine was collected from females by means of a 14 by 14-inch catching funnel of construction similar to that for males. The funnel was suspended at the rear of the crate under the sloping wire cloth used to deflect fecal droppings (Figs. 5, 6). From the funnel, urine passed into a sterile 1000-ml. wide-mouth jar.

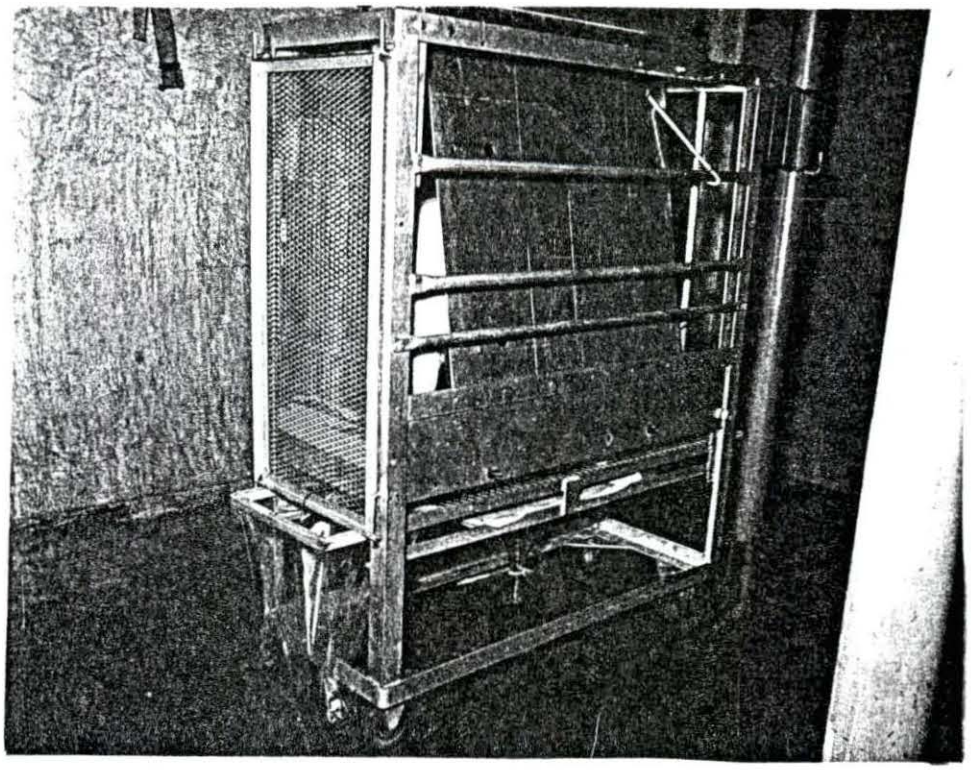
Media and solutions

Tryptose serum broth Tryptose broth base was prepared as described by Packer (37). Sterile horse serum was added to give a concentration of 5%. The medium was dispensed aseptically into flasks, incubated overnight at 37° C. to determine sterility, and stored at 4° C. The final pH range was 7.3 to 7.4.

Selective liquid medium A liquid enrichment medium utilizing high concentrations of antibiotics for the selective culture of E. insidiosa was developed, and has been

Fig. 1. A view of the metabolism crate used for confinement of pigs. The sloping panels restricted movement by the animal when in the standing position. The crate is fitted with equipment for separate collection of feces and urine from males.

Fig. 2. Front view of the metabolism crate, showing the sloping side panels, stanchion, and expanded metal floor



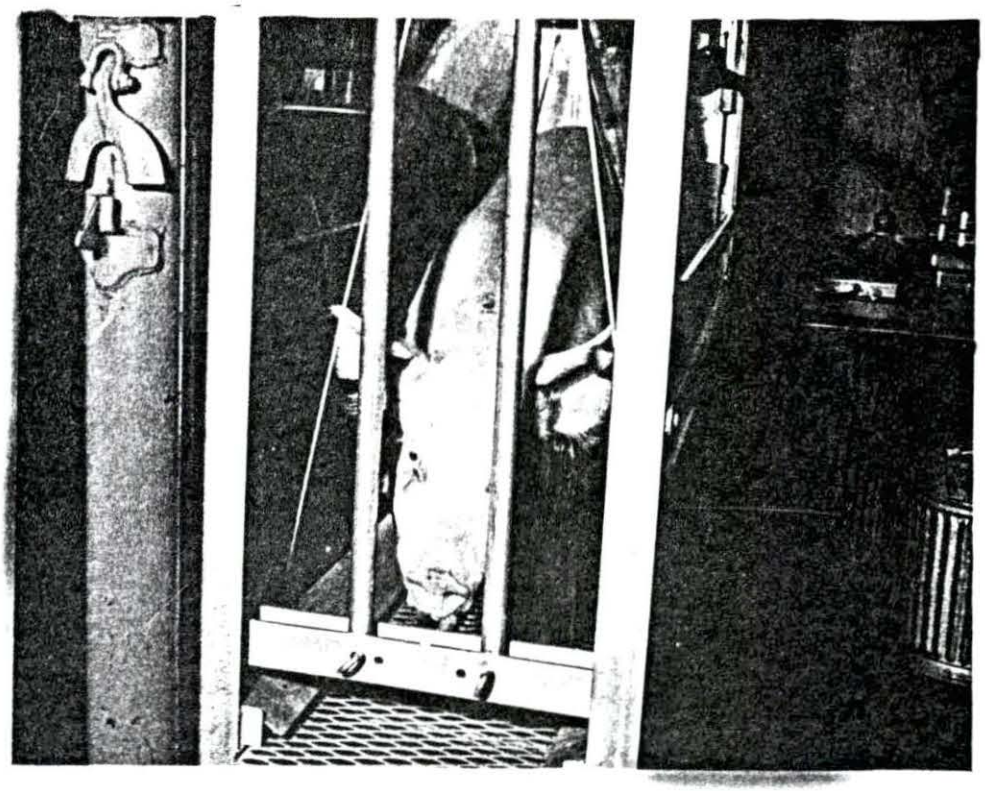
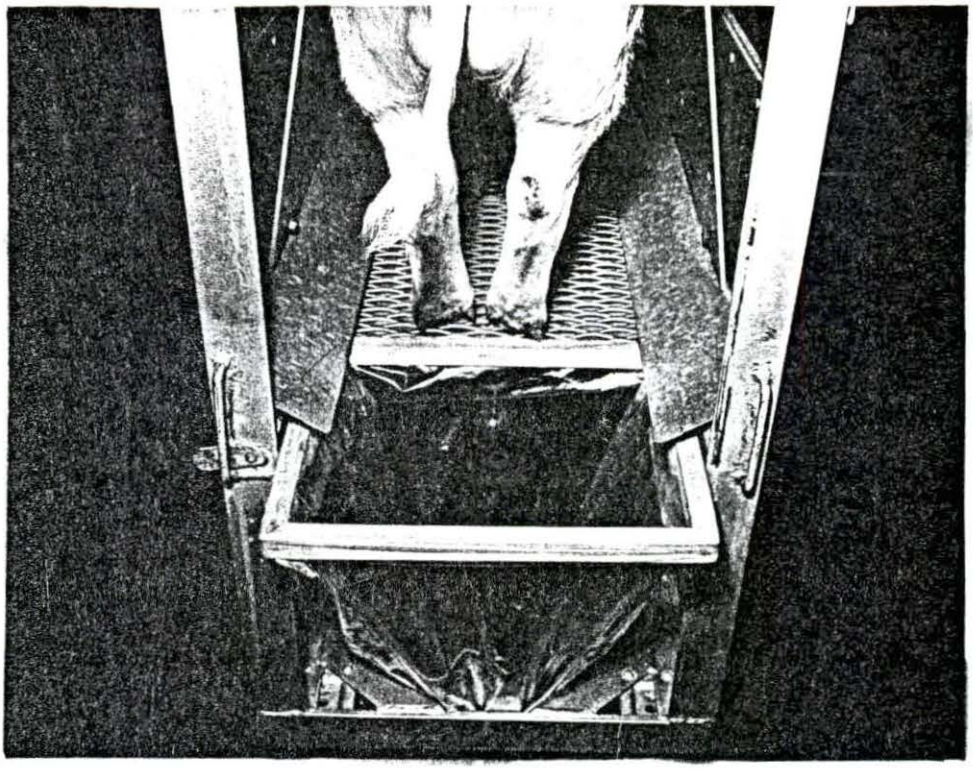
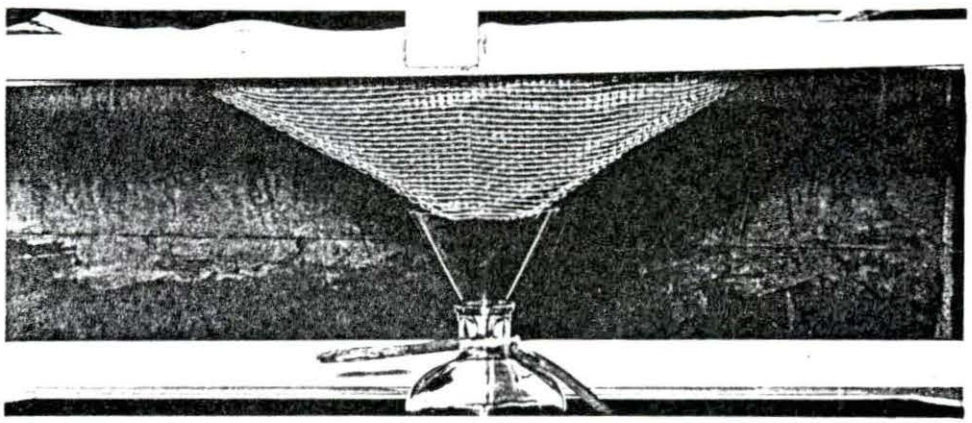


Fig. 3. Rear view of the metabolism crate with rear panel removed to show the expanded metal floor and the feces collecting bag used for male pigs

Fig. 4. Close-up view of equipment used for collection of urine from male pigs





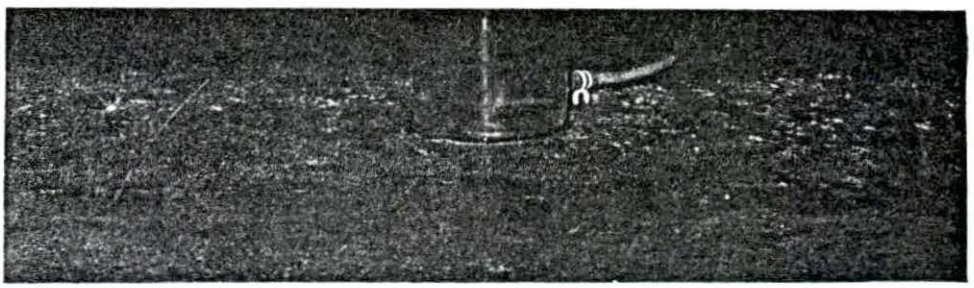
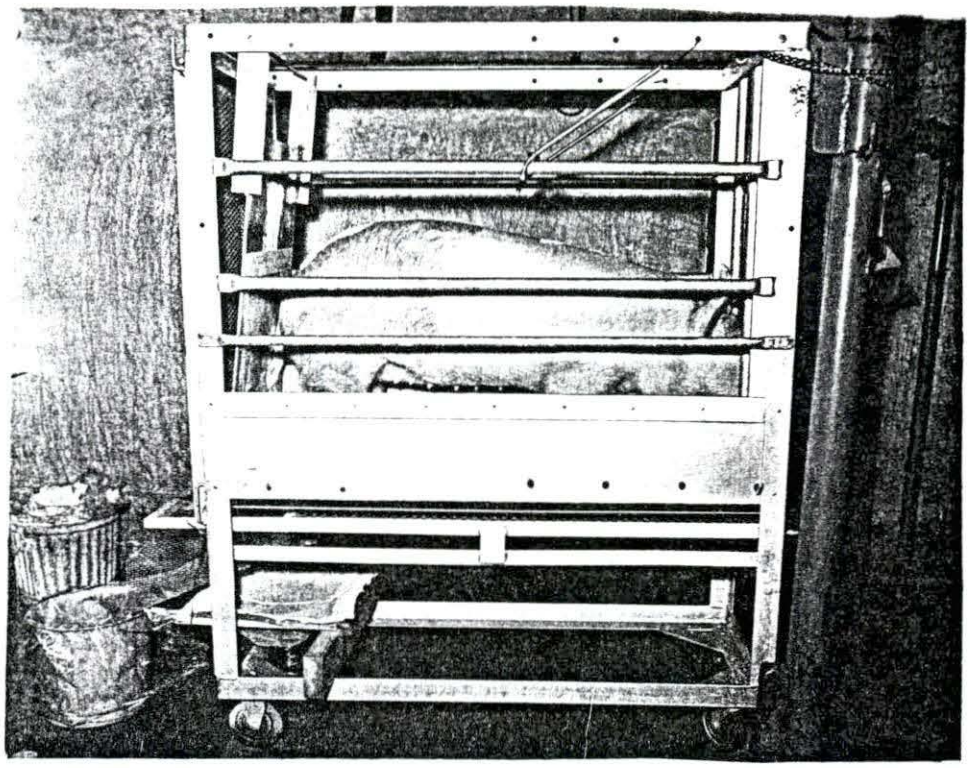
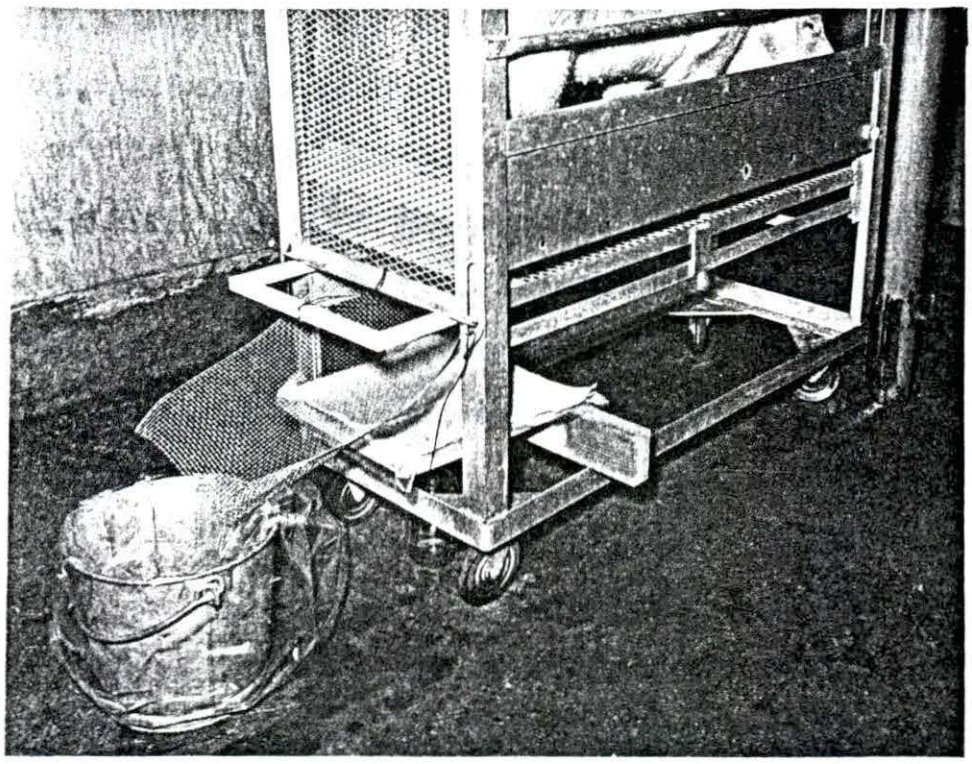


Fig. 5. Side view of the metabolism crate with side panels removed, showing the sloping rear panel. The crate is fitted with equipment for separate collection of feces and urine from female pigs.

Fig. 6. Close-up view of equipment used for separate collection of feces and urine from female pigs





described and tested for its efficiency (71). The medium consistently permitted cultural detection of the organism in pig feces containing 6 to 11 viable cells/Gm.

For use in the present experiments, the medium, modified by the addition of buffering salts, was prepared in quantities of 25 to 30 liters as follows: A broth base was prepared, containing 0.3% beef extract, 1.5% tryptose, and 0.5% NaCl. To each liter of broth base was added 5.4 Gm. of Na_2HPO_4, 0.64 Gm. of KH_2PO_4, and 50 ml. of horse serum. The preparation was sterilized by filtration with a Hormann model ST-80 filter press.[1] After filtration, 400 μg./ml. of kanamycin,[2] 50 μg./ml. of neomycin,[3] and 25 μg./ml. of vancomycin[4] were added aseptically. The pH range of the final preparation was 7.3 to 7.4. The medium was dispensed aseptically into sterile flasks and tubes and stored at 4° C. Medium not used within 2 weeks was discarded.

Beef infusion broth Ground defatted beef was added to distilled water at the rate of one pound/liter. After standing overnight at 4° C., the mixture was cooked for one hour at 80° to 90° C., allowed to stand for 2 hours, and

---

[1]F. R. Hormann and Co., Newark, N. J.

[2]Kantrex, Bristol Laboratories, Syracuse, N. Y.

[3]Mycifradin, the Upjohn Co., Kalamazoo, Mich.

[4]Vancocin, Eli Lilly and Co., Indianapolis, Ind.

filtered through a layer of muslin. For each liter of infusion, 10 Gm. of Bacto-peptone[1] and 5 Gm. of NaCl were added. The pH was adjusted to 7.6 with 4% NaOH. The preparation was autoclaved at 121° C. for 30 minutes and filtered through No. 2 Whatman filter paper. The medium then was dispensed into flasks, autoclaved again, and stored at 4° C. The final pH range was 7.3 to 7.4.

Beef infusion agar An infusion of beef, supplemented by 10 Gm. of Bacto-peptone and 5 Gm. of NaCl/liter, was prepared as described for beef infusion broth. Agar was added at the rate of 20 Gm./liter, and the pH was adjusted to 7.6. The medium was autoclaved at 121° C. for 30 minutes and filtered through non-absorbent cotton. The medium then was autoclaved again, dispensed into sterile petri plates, and allowed to harden. The plates were incubated overnight at 37° C. and stored at 4° C. in covered cans. The final pH range was 7.3 to 7.4.

Packer's medium (37) Bacto-azide violet blood agar base[1] was prepared according to the manufacturer's directions, except that horse serum at the rate of 5% was added instead of whole blood. The medium was dispensed into sterile petri plates and allowed to harden. The plates were incubated overnight at 37° C. and stored at 4° C. in covered

---

[1]Difco Laboratories, Detroit, Mich.

cans. The final pH range was 6.8 to 7.0.

Commercially prepared media Bacto-nutrient gelatin[1] tubes and Bacto-triple sugar iron agar[1] slants were prepared according to the manufacturer's directions, and stored at 4° C.

Base medium for fermentable carbon compounds A base medium, containing Andrade's indicator, appropriate fermentable carbon compounds, and a 10% concentration of horse serum, was prepared as described by White and Shuman (70), dispensed into tubes, and stored at 4° C.

Buffered NaCl solution Buffered NaCl solution (pH 7.5) was prepared by dissolving 8.5 Gm. of NaCl, 5.4 Gm. of Na_2HPO_4, and 0.64 Gm. of KH_2PO_4 in each liter of distilled water and autoclaving at 121° C. for 20 minutes.

Buffered peptone solution One per cent buffered peptone solution (pH 7.5) was prepared by dissolving 10 Gm. of Bacto-peptone, 5.4 Gm. of Na_2HPO_4, and 0.64 Gm. of KH_2PO_4 in each liter of distilled water and autoclaving at 121° C. for 20 minutes.

Exposure cultures

Three freeze-dried smooth strains of E. insidiosa, serotype "A", were used for exposure of pigs. The strains, iden-

---

[1]Difco Laboratories, Detroit, Mich.

tified as E1-6P, HC-585, and de Castro, were from the stock culture collection at the National Animal Disease Laboratory, Ames, Iowa. They were selected for their ability to induce swine erysipelas in susceptible pigs with a reasonable degree of predictability as to the clinical course of the disease.

Strain E1-6P had been obtained as follows: A virulent strain identified as E1-6[1] was passed serially in 2 pigs, both of which died of the infection. A pure culture of the organism recovered from the spleen of the second pig was freeze-dried and designated stock strain E1-6P. This strain was used to produce severe illness with death or recovery.

Strain HC-585 was from a stock of freeze-dried culture that had been used for percutaneous exposure of pigs and for other experimental purposes at the National Animal Disease Laboratory (46, 47, 49, 50, 69). This strain was used to produce less severe illness with development of characteristic urticarious rhomboid skin lesions and subsequent recovery.

Strain de Castro was a culture received from Brazil,[2] where it had been isolated from the tonsils of an apparently

---

[1]Original culture kindly supplied by Dr. K. F. Lawson, Connaught Medical Research Laboratories, University of Toronto, Toronto, Canada.

[2]Original culture kindly supplied by Dr. A. F. Pestana de Castro, Instituto Biológico, São Paulo S. P., Brazil.

healthy pig (6). This strain had been found to be of low virulence in swine (51), and was used to produce a mild form of the disease.

A fresh ampule of freeze-dried stock culture was used for exposure of each pig. Each culture was prepared as follows: The contents of an ampule were reconstituted and inoculated into 100 ml. of tryptose serum broth. The broth culture was incubated 24 hours at 37° C. and used for direct inoculation of the animal. A count of the viable bacterial cells/ml. of each culture was made by plating tenfold dilutions in triplicate. The average count of each strain is given in Table 1.

Table 1. Number of viable cells of E. insidiosa in exposure cultures

Strain	No. of cultures	No. of viable cells/ml. Range	Average
E1-6P	9	5.10 to 9.90 x 10^8	6.73 x 10^8
HC-585	4	1.25 to 2.60 x 10^9	1.75 x 10^9
De Castro	5	1.27 to 1.30 x 10^9	1.28 x 10^9

Animal Handling and Sampling Schedule

The experiments were conducted with one pig at a time. Each pig was maintained in the crate continuously during observation. Samples from the mouth, tonsils, nasal passages, conjunctival sacs, skin, and blood were taken once daily, between 8:30 and 9:30 A.M. Feces and urine were collected continuously, and were removed from the collecting equipment at 8:30 A.M. and between 4:00 and 5:00 P.M. daily. In addition, clinical observations were recorded twice daily. These included rectal temperature, appetite, general attitude, and the appearance and characteristics of urticarious lesions.

Feed and water were given after the morning and afternoon sample collections. The ration at each feeding consisted of 1 1/4 to 2 pounds of commercial pig grower (14% crude protein); the amount depending on size of the pig.

For purposes of data recording, each experimental day was designated as the 24 hours between completion of sample collections and feeding at 9:30 A.M. At this time each morning the crate, the surrounding area, and all equipment used for animal handling and sample collection were cleaned with a 3% solution of a commercial detergent-phenolic disinfectant,[1] and replaceable components were changed. The feces collecting bag was changed after each afternoon col-

---

[1]Ves-phene, Vestal Laboratories, Inc., St. Louis, Mo.

lection also.

Samples collected from each pig during the first experimental day were used for pre-exposure control data and the pig then was exposed to infection at the beginning of the second experimental day (9:30 to 9:45 A.M.). Observation was terminated by death from acute erysipelas or by euthanasia 10 days after exposure. Post-mortem examinations were made, and from each pig the tonsils, gall bladder, and a segment of the terminal part of the ileum including the ileocecal valve, were removed and refrigerated in sterile containers until cultural examination. In addition, post-mortem samples were taken with sterile cotton-tipped applicators from the mouth, nasal passages, conjunctival sacs, and anus of each pig that died of the disease.

Exposure Procedures

Exposures to E. insidiosa were made by parenteral injection. The number of pigs given each strain, amounts of culture given, and routes of injection are shown in Table 2. Intradermal injections were given on the left side, about 4 inches lateral to the dorsal midline and just posterior to the shoulder; intramuscular injections were given in one or both hind legs; and intravenous injections were given in an ear vein. Before injections were given, the hair was clipped from the area and the skin was cleaned with three or four

Table 2. Routes of injection, amounts given, and number of pigs exposed to each strain of E. insidiosa

Exposure strain	Route of injection[a]	Ml. of culture	Number of pigs exposed		
			Male	Female	Total
El-6P	I.D.	0.1	3	1	4
El-6P	I.M.	4.0	2	1	3
El-6P	I.M.	6.0	1	0	1
El-6P	I.M.	8.0	1	0	1
HC-585	I.D.	0.1	2	2	4
De Castro	I.D.	0.1	1	0	1
De Castro	I.V.	5.0	2	2	4

[a]I.D. = intradermal; I.M. = intramuscular; I.V. = intravenous.

applications of ether, and allowed to dry. After completion of an intradermal injection, the site was covered with a patch of transparent polyethylene sheet fastened to the skin with adhesive tape (Fig. 7). This was done to prevent possible contamination of the environment from the site of injection, while allowing observation of the development of a local skin lesion. Sites of intramuscular and intravenous injections were covered with a small pad of absorbent cotton held in place by adhesive tape.

Sampling Procedures

Feces

In addition to the samples removed twice daily from the collecting equipment, feces eliminated by a pig while being attended at the close of each experimental day (between 8:30 and 9:30 A.M.) were caught in sterile hand-held containers. The feces eliminated during each experimental day thus were divided into 3 samples (afternoon, overnight, and morning) for separate examination. Feces eliminated by females while being attended at 4:00 to 5:00 P.M. were caught in sterile hand-held containers and included with the afternoon sample, but examined separately. Each sample was distributed into sterile 8-ounce covered plastic cups[1] at the time of collection, and refrigerated at 4° C. within one hour. The samples were kept under refrigeration until prepared for cultural examination.

Urine

The samples of urine removed twice daily from the collecting equipment were refrigerated within one hour. The two collections (afternoon and morning) during the first day after exposure of a pig were separately examined. All other collections were combined into single daily samples, except

---

[1]Falcon Plastics, Los Angeles, Calif.

that urine eliminated by females while being attended at 8:30 to 9:30 A.M. and at 4:00 to 5:00 P.M. was caught in sterile hand-held containers for separate examination.

Mouth

Secretions in the mouth were sampled by swabbing the areas lateral to the upper and lower molars and under the anterior portion of the tongue with sterile cotton-tipped applicators (Fig. 8).

Tonsils

The surfaces of the tonsils were scraped with the end of a sterile wood tongue blade (Fig. 9). The material collected was transferred to a sterile cotton-tipped applicator.

Conjunctival sacs

Lacrimal secretions were sampled by drawing the lower eyelid away from the eyeball with forceps and swabbing the conjunctival surface near the medial canthus with cotton-tipped applicators moistened with sterile buffered NaCl solution (Fig. 10).

Nasal passages

Samples were collected from the nasal passages with the head of the pig restrained in a lowered position. The surface of the snout was cleaned with 70% ethanol. Each nasal passage then was washed twice with approximately 20 ml. of

sterile buffered NaCl solution. The solution was forced into the nasal passages with a sterile rubber bulb and allowed to flow out into a sterile plastic cup (Fig. 11). The collected washings (approximately 75 ml.) were immediately transferred to a sterile 250-ml. screw-capped flask.

Skin

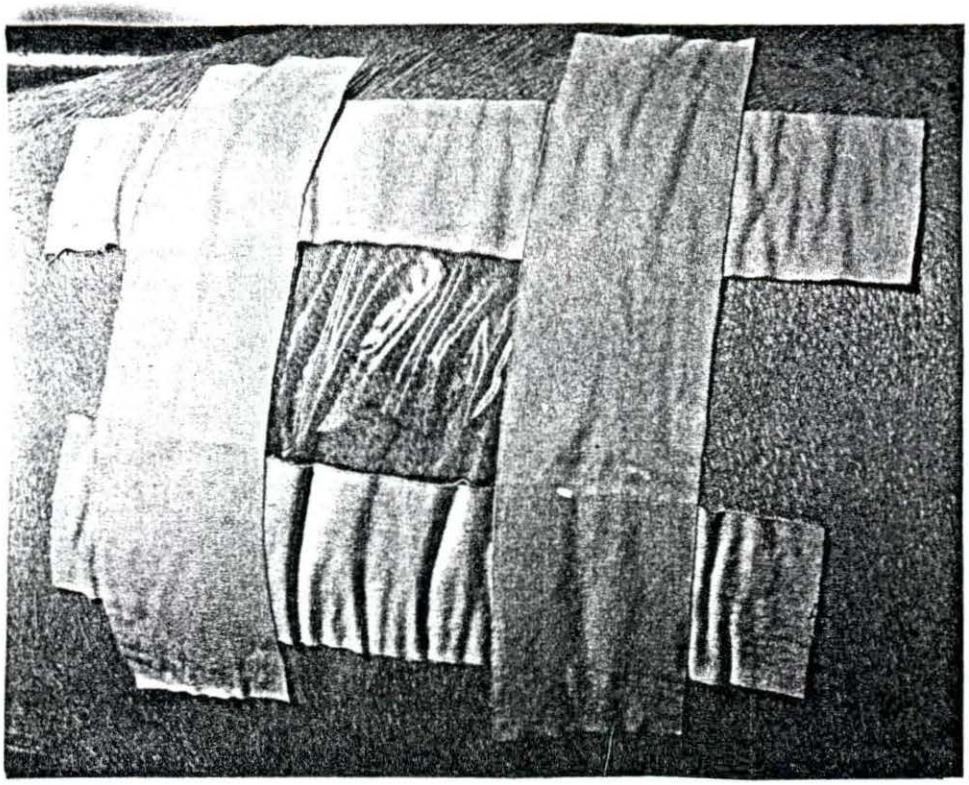
Samples from the surface of the skin were taken routinely from areas of the neck posterior to the ears and from the dorsal midline in the sacral region. The hair was clipped from the latter area before sampling was begun on the first experimental day. On the second day, immediately after exposure of each pig, the surface of this area was cleaned with ether. On 4 of the pigs, a 4 by 4-inch covering patch of polyethylene sheet was attached to the skin by adhesive tape with the anterior side left open (Fig. 12). This was done in an attempt to prevent external contamination of the area to be sampled.

Samples were taken by rubbing the skin vigorously with cotton-tipped applicators moistened with sterile buffered NaCl solution.

The surfaces of urticarious lesions were cultured when present.

Fig. 7. Transparent polyethylene patch covering the site of intradermal injection of E. insidiosa (strain HC-585), photographed at 48 hours post-exposure. Note the dark area of local inflammation at the site of injection.

Fig. 8. Procedure for obtaining samples from the mouth



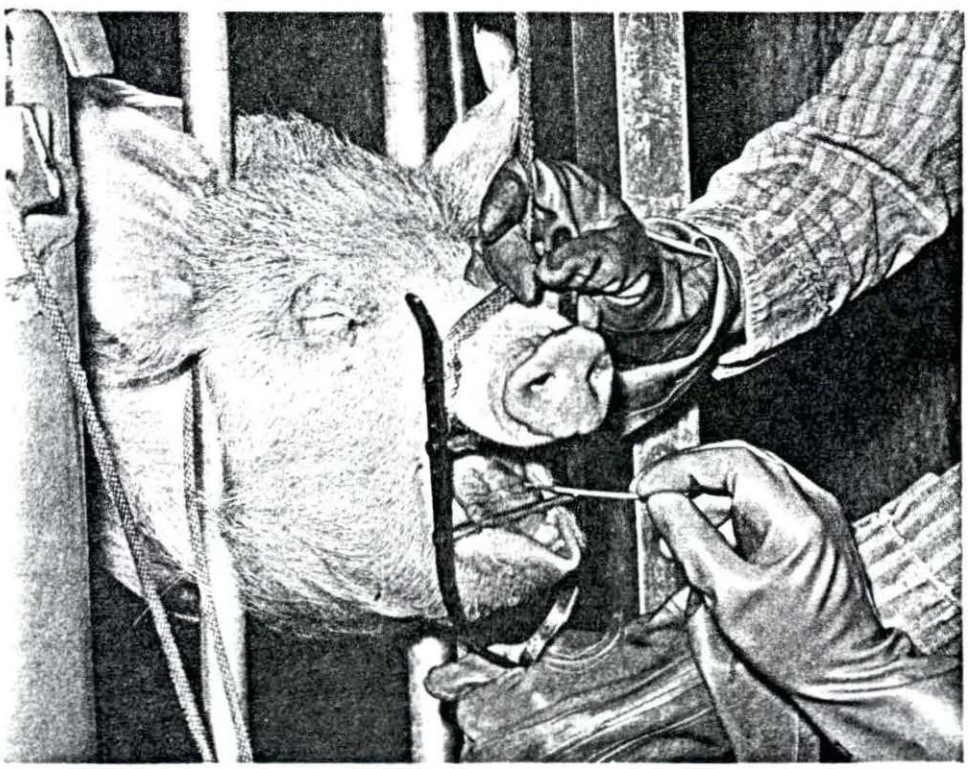
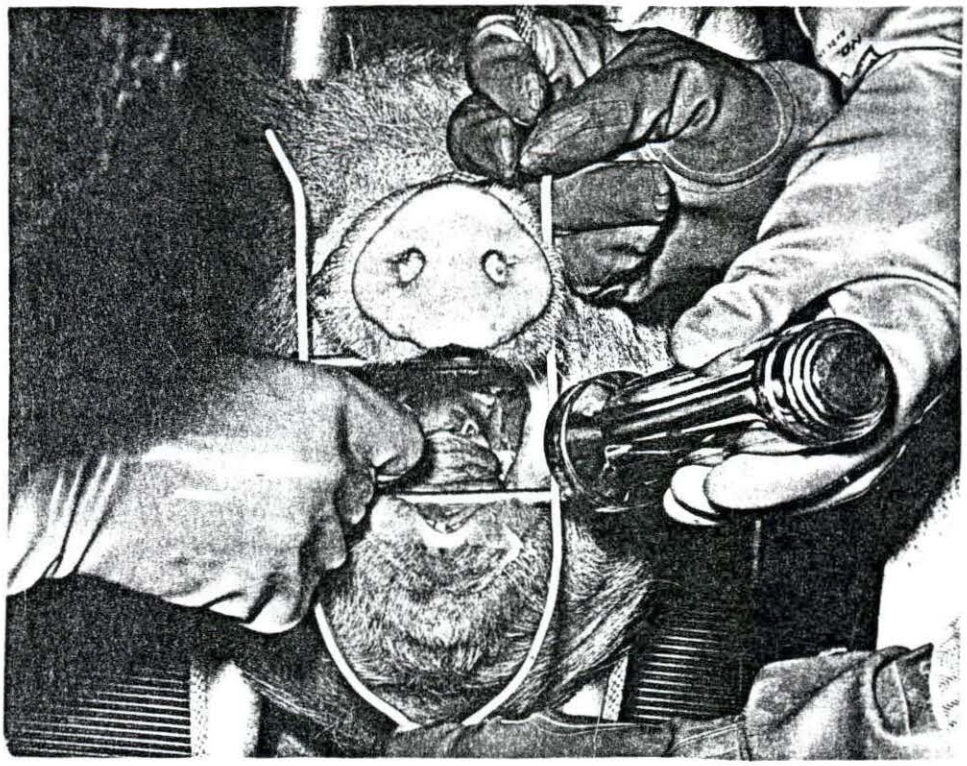


Fig. 9. Procedure for obtaining samples from the tonsils by scraping the surfaces with a wood tongue blade

Fig. 10. Procedure for obtaining samples from the conjunctival sacs



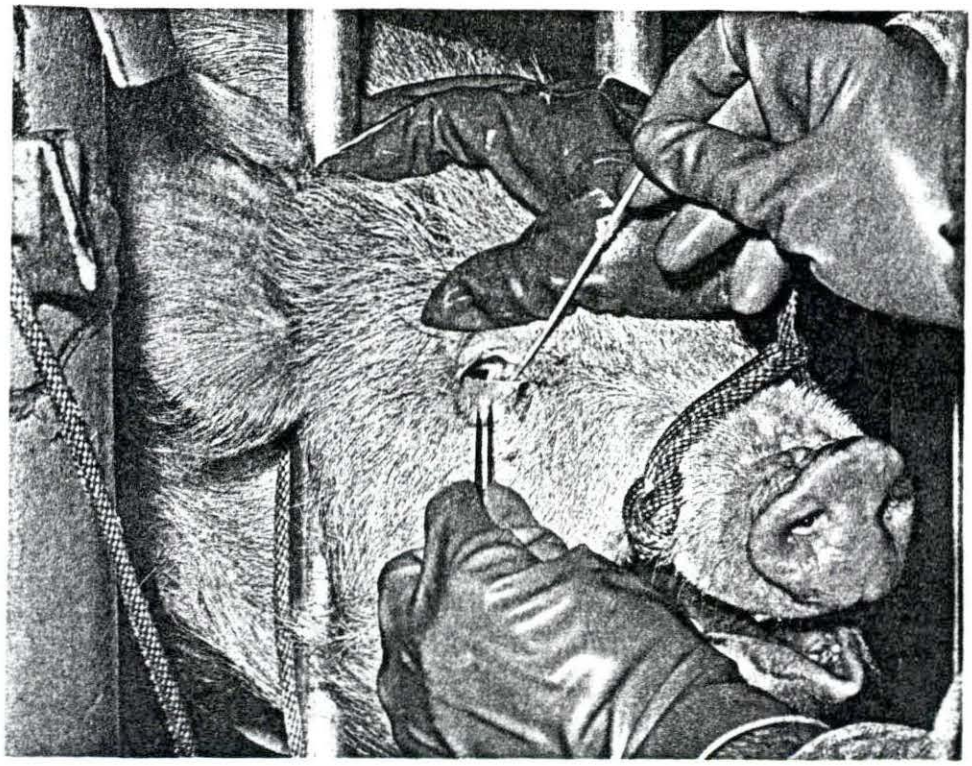
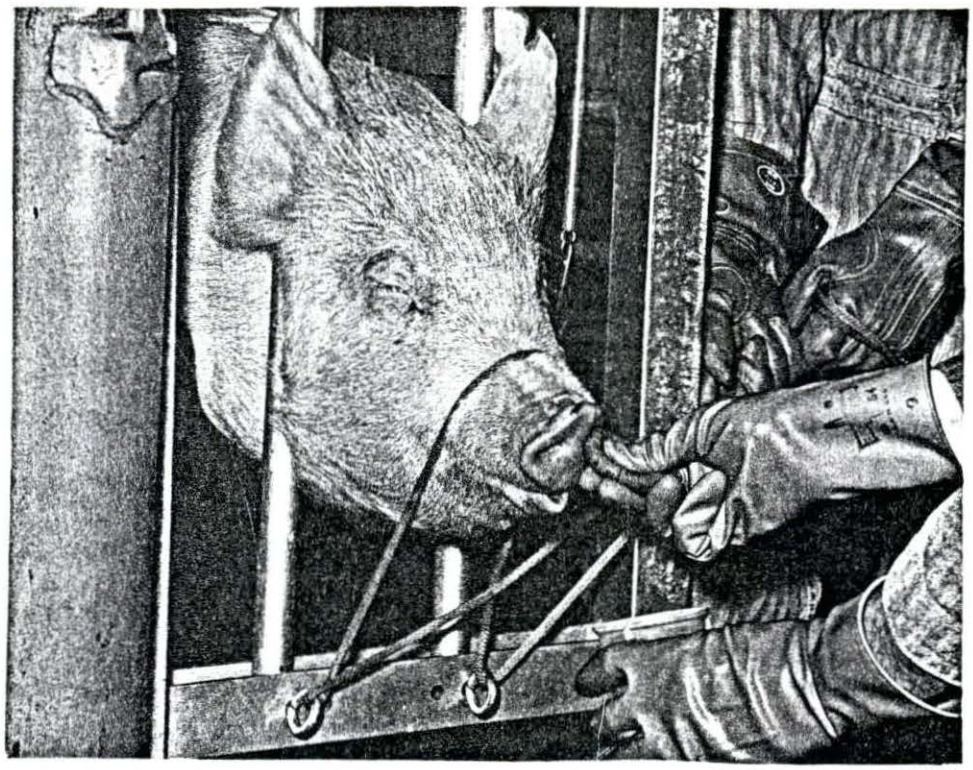
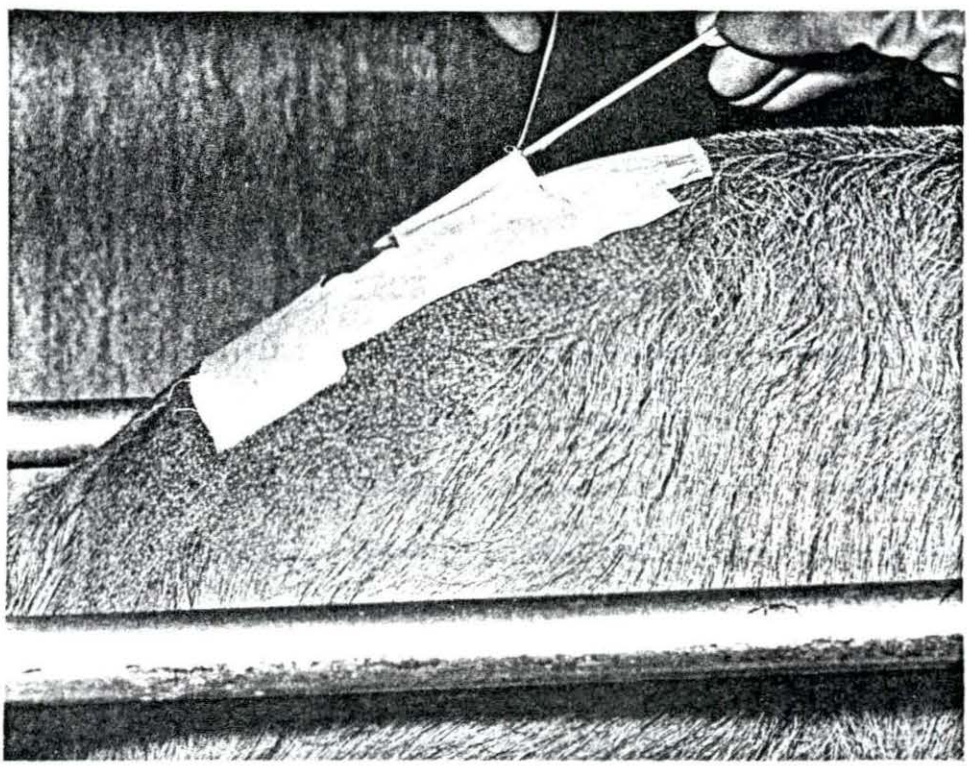


Fig. 11. Procedure for obtaining washings from the nasal passages

Fig. 12. Procedure for obtaining samples from an area of the skin covered by a polyethylene patch





Blood

Three to 4 ml. of blood were drawn aseptically from the anterior vena cava.

Preparation of Samples for Culture

Feces

The samples of feces collected during each experimental day were prepared for cultural examination within 6 hours after the close of the day. Each sample was weighed and divided into portions of 40 to 50 Gm., and each portion was placed in a pint mason jar. Sterile one per cent buffered peptone solution was added to make a total volume in each jar of approximately 220 ml. A stirring rod with a 2-inch propeller was placed in each jar, which then was covered with a flat mason lid that had a hole in the center for the shaft of the stirring rod. The lids were secured with standard mason rings. The contents were mixed for 10 minutes with stirring motors operated at 1600 R.P.M., transferred to 250-ml. glass centrifuge bottles, and centrifuged for 10 minutes at 1950 R.P.M. (1000 R.C.F.) in an International model UV centrifuge.[1] The cloudy supernatant fluid in each bottle was decanted into a 250-ml. screw-capped polypropylene centrifuge bottle and the residue was discarded. The fluid was centrifuged for 10

---

[1]International Equipment Co., Boston, Mass.

minutes at 9000 R.P.M. (13,200 R.C.F.) in a Sorvall SS-3 centrifuge[1] with a type GSA rotor. The supernatant fluid was discarded, and each pellet of residue was resuspended in 500 ml. of selective liquid medium.

Fecal samples weighing less than 20 Gm. were inoculated directly into 500 ml. of selective liquid medium after first being broken up and suspended in a small volume of the medium. Samples weighing 20 to 40 Gm. were suspended in five times their weight of buffered peptone solution and then were handled in the same manner as described for the 40 to 50-Gm. portions.

All mixing jars, lids, stirring rods, centrifuge bottles, and other equipment coming in contact with the fecal material were cleaned and sterilized by autoclaving after each use, and kept in covered pans.

Urine

The urine collected during each experimental day was prepared for cultural examination within 4 hours after the close of the day. The volume of each sample was measured, and the urine was centrifuged. Samples of more than 1000 ml. were centrifuged in a Sorvall SS-1A/KSB-1 four-tube "St. Gyorgyi and Blum" continuous flow system[1] operated at 12,000 R.P.M. (17,300 R.C.F.). Samples of 1000 ml. or less were

---

[1]Ivan Sorvall, Norwalk, Conn.

distributed into 250-ml. screw-capped polypropylene bottles and centrifuged for 10 minutes in the SS-3 centrifuge with the type GSA rotor operated at 9000 R.P.M. (13,200 R.C.F.). After centrifugation, the clarified urine was discarded and the pellets of residue were combined and resuspended in 100 to 500 ml. of selective liquid medium, depending on the amount of residue.

All centrifuge bottles, tubes, components of the continuous flow system, and other equipment coming in contact with the urine were cleaned and sterilized by autoclaving after each use, and kept in covered pans.

Nasal washings

Within one hour after collection, each sample of nasal washings was distributed into sterile 50-ml. nylon centrifuge tubes, which were capped and centrifuged for 6 minutes in the SS-3 centrifuge with a type SS-34 rotor[1] operated at 12,000 R.P.M. (17,300 R.C.F.). The supernatant fluid was discarded and the residue was resuspended in 100 ml. of selective liquid medium.

Blood

Each sample of blood was placed directly into 50 ml. of beef infusion broth immediately after being obtained.

---

[1]Ivan Sorvall, Norwalk, Conn.

Other samples

Cultures from the mouth, tonsils, conjunctival sacs, and skin were made immediately after sampling by snapping off the cotton tip of each applicator into a tube of selective liquid medium.

Post-mortem specimens

Specimens removed from pigs at post-mortem were prepared as follows:

Tonsils The surrounding tissues were trimmed away and the oral surfaces of the tonsils were scored with a flamed scalpel by making numerous cuts of 1/8 to 1/4 inch deep. The entire structure was placed in 100 ml. of selective liquid medium.

Gall bladder The attached tissues were trimmed away and the gall bladder was opened with flamed scissors. After the bile was drained out, the entire structure was placed in 100 ml. of selective liquid medium.

Intestine The lymphoid tissue near the ileocecal valve and in the wall of the adjacent ileum was scraped off with a flamed scalpel, and the material obtained was placed in 100 ml. of selective liquid medium.

Other post-mortem cultures Cultures from the mouth, nasal passages, conjunctival sacs, and anus of each pig that died from acute erysipelas were made immediately after sampling by snapping off the cotton tip of each applicator into

a tube of selective liquid medium.

Cultural Examinations

Culture procedure

After inoculation, all liquid cultures were incubated at 37° C. for 48 hours and subcultured onto plates of Packer's medium. One plate was inoculated from each flask or tube of liquid culture by spreading the contents of a 20-gauge wire loop (i.d. 3 mm.) evenly over the surface of the agar. The plates were incubated at 37° C. for 72 hours and examined under a dissecting microscope for colonies of E. insidiosa.

Identification of E. insidiosa

Tentative identification of the organism was based on colonial characteristics on Packer's medium. From each plate that had bacterial growth considered to be E. insidiosa, pure culture isolations were made on beef infusion agar and in beef infusion broth. Positive identification of the organism was based on: (1) characteristic growth in gelatin stab cultures (5); (2) reaction in triple sugar iron agar (64); and (3) the pattern of acid production from 20 fermentable carbon compounds (Table 12), using the method reported by White and Shuman (70). From each pig, the cultures subjected to these tests consisted of: (1) the first culture of E. insidiosa recovered from each source of samples; (2) a pooled culture

from each source of samples, comprising all isolations of the organism made during the observation period; and (3) each culture recovered from specimens obtained at post-mortem examination. In addition, each culture prepared from stock for exposure of a pig was subjected to the same tests to provide a basis for comparison with cultures recovered from the animal.

FINDINGS

Clinical Response after Exposure to *E. insidiosa*

The clinical response of each pig is given in Table 3, and includes the peak temperature and presence or absence of other signs of infection. In addition, individual charts for the 18 pigs can be found in the Appendix (Figs. 25-42), and include temperatures as recorded twice daily and the time of appearance of other signs of infection.

Pigs exposed to strain E1-6P

Each of the 9 pigs exposed to strain E1-6P developed local inflammation at the site of injection within 24 hours. The pigs became visibly ill 24 to 48 hours post-exposure, and had peak temperatures of 106.4° to 108.4° F. within 24 to 79 hours. Death occurred 48 to 96 hours post-exposure in 4 pigs exposed by intradermal injection, and approximately 78 hours post-exposure in one pig exposed by intramuscular injection. Four pigs survived after exposure by intramuscular injection. Inappetence and depression were observed in all 9 pigs exposed to strain E1-6P, and 3 pigs developed urticarious skin lesions 72 hours post-exposure. The skin lesions were slightly raised, irregularly shaped areas of erythema, which were spread diffusely over the body. The 4 surviving pigs regained normal appetite and attitude, with disappearance of skin lesions, by the fifth to ninth day post-exposure.

Table 3. Clinical response of pigs after exposure to E. insidiosa

Pig no.	Sex[b]	Exposure			Peak temp. (°F.)	Visible signs of infection[a]			
		Strain	Route[c]	Amount		Inappetence	Depression	Skin lesion	Death
1452	M	El-6P	I.D.	0.1 ml.	107.6	+	+	-	+
1454	M	El-6P	I.D.	0.1 ml.	107.6	+	+	+	+
1654	M	El-6P	I.D.	0.1 ml.	106.4	+	+	-	+
1455A	F	El-6P	I.D.	0.1 ml.	107.6	+	+	-	+
1459	F	El-6P	I.M.	4.0 ml.	107.8	+	+	-	+
981	M	El-6P	I.M.	6.0 ml.	108.4	+	+	+	-
990	M	El-6P	I.M.	8.0 ml.	106.9	+	+	-	-
994	M	El-6P	I.M.	4.0 ml.	107.4	+	+	-	-
1332	M	El-6P	I.M.	4.0 ml.	107.6	+	+	+	-
1334	M	HC-585	I.D.	0.1 ml.	107.5	+	+	+	-
1450	M	HC-585	I.D.	0.1 ml.	106.6	+	+	+	-
1333A	F	HC-585	I.D.	0.1 ml.	108.0	+	+	+	-
1452A	F	HC-585	I.D.	0.1 ml.	105.8	-	-	+	-
1446	M	DeCastro	I.V.	5.0 ml.	105.0	-	-	+	-
1447	M	DeCastro	I.V.	5.0 ml.	104.2	-	-	+	-
1449	F	DeCastro	I.V.	5.0 ml.	104.2	-	-	+	-
1451A	F	DeCastro	I.V.	5.0 ml.	104.4	-	-	+	-
1345	M	DeCastro	I.D.	0.1 ml.	105.2	-	-	-	-

[a]Presence or absence is indicated by + or -, respectively.

[b]M = male; F = female.

[c]I.D. = intradermal; I.M. = intramuscular; I.V. = intravenous.

Pigs exposed to strain HC-585

Each of the 4 pigs exposed to strain HC-585 by intradermal injection developed local inflammation at the site of injection within 24 hours. Signs of generalized infection were observed 31 to 72 hours post-exposure. Three pigs had inappetence and depression, and subsequently returned to normal appetite and attitude on the sixth to ninth day post-exposure. One pig (No. 1452A) had no loss of appetite or change in attitude. Peak temperatures of 105.8° to 108.0° F. were recorded 3 to 6 days post-exposure. One pig (No. 1333A) had pronounced diphasic temperature peaks of 106.4° F. at 31 hours and 108.0° F. at 6 days post-exposure (see Fig. 36). All 4 pigs exposed to strain HC-585 developed urticarious skin lesions over the shoulders, back, rear quarters, and sides 48 to 72 hours post-exposure. These were well defined, raised, rhomboidal areas of erythema. The lesions began to recede on the seventh to ninth day post-exposure, but were still visible at the time of euthanasia on the tenth day.

Pigs exposed to strain de Castro

In the 4 pigs exposed to strain de Castro by intravenous injection, no deviations from normal appetite and attitude were observed. Temperatures remained in the normal range (see Figs. 38-41), except for a brief rise to 105.0° F. recorded 7 hours post-exposure in one pig (No. 1446). Urti-

carious skin lesions were observed on all pigs 2 to 5 days post-exposure. These consisted of one to five slightly raised, pale, irregularly shaped areas of erythema in the region of the shoulders. The lesions began to recede on the eighth to ninth day post-exposure, and generally were not visible at the time of euthanasia on the tenth day.

Exposure of one pig (No. 1345) to strain de Castro by intradermal injection produced local inflammation at the site of injection in 24 hours. This consisted of a slightly raised, pale area of erythema approximately 3/4 inch in diameter. The lesion persisted for less than 24 hours. No signs of generalized infection were observed. A brief rise in temperature to 105.2° F. was recorded during the sixth day post-exposure (see Fig. 42).

Recovery of *E. insidiosa* from Infected Pigs

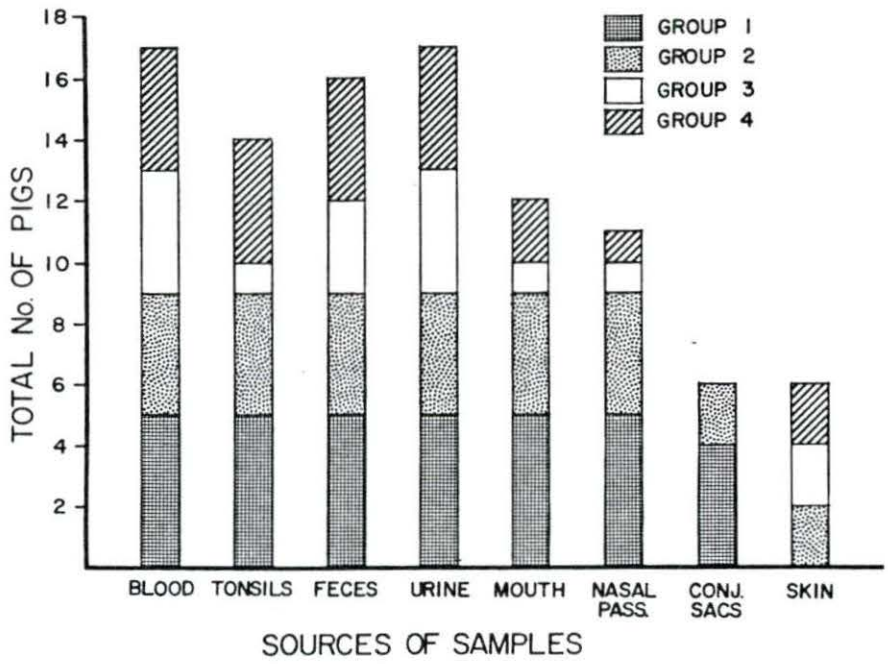
Sources of recovery

Erysipelothrix insidiosa was not found in any pre-exposure samples. After exposure of 18 pigs, the organism was recovered on one or more days from the blood of 17, urine of 17, feces of 16,[1] tonsils of 14, mouths of 12, nasal passages of 11, skin of 6, and conjunctival sacs of 6 (Fig. 13).

---

[1]A positive fecal sample from pig No. 1333A on day 4 post-exposure was not counted because accidental contamination from urine was suspected (see Fig. 36).

Fig. 13. Number of pigs from which E. insidiosa was recovered after exposure. In group 1, recoveries from tonsils and conjunctival sacs of 3 pigs were made at post-mortem only (see Table 10).


GROUP 1
GROUP 2
GROUP 3
GROUP 4
TOTAL No. OF PIGS
18
16
14
12
10
8
6
4
2
BLOOD
TONSILS
FECES
URINE
MOUTH
NASAL PASS.
CONJ. SACS
SKIN
SOURCES OF SAMPLES

Graphic records of the incidence of recovery of E. insidiosa from each pig during the observation period are shown in the Appendix (Figs. 25-42).

In order to provide convenient means of identification and to make comparative observations, data from the 18 pigs were divided into 5 groups, according to the strain of E. insidiosa used for exposure and the severity of clinical response. The group assignments, by pig numbers, are given in Table 4.

Erysipelothrix insidiosa was recovered on one or more days post-exposure from all sources of samples in group 1 except the skin, from all sources in group 2, and from all sources in groups 3 and 4 except the conjunctival sacs (Fig. 13). In group 5, consisting of one pig (No. 1345), E. insidiosa was recovered only from the skin at the site of injection. The patch covering the site of injection on this pig was removed on the third day post-exposure, and the area was swabbed daily for cultural examination during the remainder of the observation period. The organism was recovered on days 3, 4, and 5 post-exposure.

Time of first recovery from each source of samples

Erysipelothrix insidiosa was recovered from the blood stream 24 hours post-exposure in all pigs of groups 1, 2, and 4, and in 3 pigs of group 3. In one pig of group 3 (No.

Table 4. Assignment of data from pigs into groups, according to exposure strain of E. insidiosa and clinical response

Pig numbers	Exposure strain	Clinical response	Group
1452 1454 1654 1455A 1459	E1-6P	Severe generalized infection, fatal	1
981 990 994 1332	E1-6P	Severe generalized infection, non-fatal	2
1334 1450 1333A 1452A	HC-585	Less severe generalized infection, non-fatal	3
1446 1447 1449 1451A	DeCastro	Mild generalized infection, non-fatal	4
1345	DeCastro	No signs of generalized infection	5

1333A), the organism was detected in the blood 72 hours post-exposure (Fig. 36). In pig No. 1345 (group 5), all blood cultures were negative (Fig. 42).

Data from the 16 pigs that had bacteremia 24 hours post-exposure were used to determine the median day of first recovery of E. insidiosa from the feces, urine, tonsils, mouth, nasal passages, and skin. For data on the first appearance

of the organism in feces, each day was divided approximately into thirds to correspond with the times of sample collection (afternoon, overnight, and morning). Similarly, the time of collection of the afternoon urine sample during the first day post-exposure was designated as approximately 1/3 day post-exposure.

The median day post-exposure of first recovery of E. insidiosa from each source was as follows: feces and urine, 2; tonsils, mouth, and nasal passages, 3; and skin, 4 (Table 5). The organism was recovered from the conjunctival sacs on days 2 to 4 post-exposure in 4 pigs of group 1, and on days 9 and 10 in 2 pigs of group 2. A median day of first recovery from the conjunctival sacs could not be determined.

Time of first recovery of E. insidiosa in relation to first observation of signs of infection

In the 16 pigs that had bacteremia 24 hours post-exposure, one or more visible signs of infection (inappetence, depression, urticarious skin lesions) appeared 1 to 5 days post-exposure (median: 2 days). In these pigs, the incidence of first recovery of E. insidiosa before, simultaneously with, and after the appearance of visible signs of infection is given in Table 6.

Table 5. Times of first recovery of E. insidiosa from pigs that had bacteremia 24 hours after exposure

Source of samples	No. of pigs positive	Time of first recovery (No. of days post-exposure) Range	Median
Blood	16	1	1
Tonsils	14	1-6	3
Feces	16	1/3-5 2/3	2
Urine	16	1/3-4	2
Mouth	12	1-4	3
Nasal passages	11	1-5	3
Conjunctival sacs	6	2-4[a] 9-10[b]	None[c]
Skin	5	4-5	4

[a]Group 1 (4 pigs).

[b]Group 2 (2 pigs).

[c]No median, due to wide variation between groups 1 and 2.

Incidence of recovery on each day post-exposure

Combined data from groups 2, 3, and 4, consisting of 12 pigs that had non-fatal generalized infection, were used to determine the incidence of recovery of E. insidiosa from each source of samples on each of 10 days post-exposure. Such data were not available from pigs that died of acute erysipelas (Group 1) or that failed to develop generalized infec-

Table 6. Time of first recovery of *E. insidiosa* in relation to the first observation of signs of infection in pigs that had bacteremia 24 hours after exposure

Source of samples	No. of pigs positive	First recovery before signs of infection[a]		First recovery simultaneous with signs of infection		First recovery after signs of infection[b]	
		No. of pigs	Per cent	No. of pigs	Per cent	No. of pigs	Per cent
Blood	16	14	87.5	2	12.5	0	0.0
Tonsils	14	2	14.3	2	14.3	10	71.4
Feces	16	7	43.8	1	6.2	8	50.0
Urine	16	6	37.4	5	31.3	5	31.3
Mouth	12	1	8.3	3	25.0	8	66.7
Nasal passages	11	1	9.1	2	18.2	8	72.7
Conjunctival sacs	6	0	0.0	0	0.0	6	100.0
Skin	5	0	0.0	0	0.0	5	100.0

[a]Range of days: 1/3 to 4.

[b]Range of days: 1/3 to 4.

tion (Group 5).

Blood The organism was recovered from the blood of 3 or more pigs on each day. Recoveries were made from 8 to 11 pigs on days 1 through 5, and from 3 to 5 pigs on days 6 through 10 (Fig. 14).

Tonsils The organism was recovered from the tonsils of one or more pigs on each day. Recoveries were made from 1 to 4 pigs on days 1 through 3, and from 6 to 8 pigs on days 4 through 10 (Fig. 15).

Feces The organism was recovered from feces of 2 or more pigs on each day. Recoveries were made from 2 pigs on day 1, 4 pigs on day 2, and from 6 to 9 pigs on days 3 through 10 (Fig. 16).

Urine The organism was recovered from urine of 6 to 9 pigs on each day (Fig. 17).

Mouth The organism was recovered from the mouth of one or more pigs on each day. Recoveries were made from 1 pig on day 1, 2 pigs on day 2, and from 4 to 5 pigs on days 3 through 10 (Fig. 18).

Nasal passages The organism was recovered from the nasal passages of one or more pigs on each day. Recoveries were made from 1 pig on day 1, 2 pigs on day 2, and from 4 to 5 pigs on days 3 through 10 (Fig. 19).

Conjunctival sacs The organism was recovered from the conjunctival sacs of one pig on days 9 and 10 only (Fig.

20).

Skin The organism was recovered from the surface of the skin of one or more pigs on days 3 through 10. Recoveries were made from 1 pig on day 3, 5 pigs on days 4 and 5, 4 pigs on day 7, and 2 pigs on days 6, 8, 9, and 10 (Fig. 21). Recoveries from the skin include those made both from routinely sampled areas and from urticarious lesions. Of 23 positive cultures, 7 were from the surfaces of urticarious lesions.

Total recoveries from six routes of elimination on each day post-exposure From a combination of six sources considered to be routes of elimination (feces, urine, mouth, nasal passages, conjunctival sacs, skin), the total number of recoveries on each day is shown for groups 2, 3, and 4 in Fig. 22. The largest number of recoveries occurred on day 4 in group 2, on day 3 in group 3 and on day 7 in group 4.

Total recoveries from each source of samples

From the 12 pigs comprising groups 2, 3, and 4, the total number of daily recoveries of E. insidiosa from each source of samples during 10 days post-exposure is given in Table 7 and is shown graphically in Fig. 23. A total of 67 recoveries was made from the blood, and 56 from the tonsils. From the six sources considered to be routes of elimination, the total numbers of recoveries were as follows: urine, 70; feces,

Table 7. Total number of recoveries of E. insidiosa in groups 2, 3, and 4 during 10 days post-exposure

Source of samples	Group 2 (4 pigs)	Group 3 (4 pigs)	Group 4 (4 pigs)	Total (12 pigs)
Blood	31	16	20	67
Tonsils	30	1	25	56
Feces[a]	34	17	16	67
Urine	35	17	18	70
Mouth	27	6	5	38
Nasal passages	28	7	2	37
Conjunctival sacs	2	0	0	2
Skin	9	8	6	23

[a]One or more positive fecal samples from a pig during one day was counted as one recovery.

67;[1] mouth, 38; nasal passages, 37; skin, 23; and conjunctival sacs, 2.

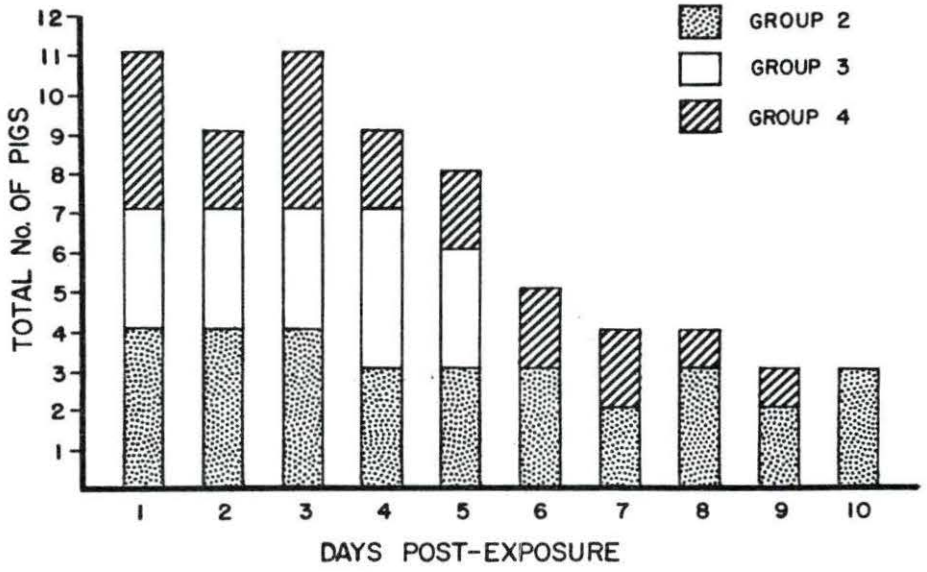
Total recoveries from six routes of elimination in each of groups 2, 3, and 4

The total number of daily recoveries from the six routes of elimination during 10 days post-exposure is shown for each of groups 2, 3, and 4 in Fig. 24. A total of 135 recoveries

[1]One or more positive fecal samples from a pig during one day was counted as one recovery.

Fig. 14. Number of pigs in groups 2, 3, and 4 having *E. insidiosa* in the blood on each day post-exposure

Fig. 15. Number of pigs in groups 2, 3, and 4 having *E. insidiosa* in the tonsils on each day post-exposure


GROUP 2
GROUP 3
GROUP 4
TOTAL No. OF PIGS
1
2
3
4
5
6
7
8
9
10
11
12
1
2
3
4
5
6
7
8
9
10
DAYS POST-EXPOSURE

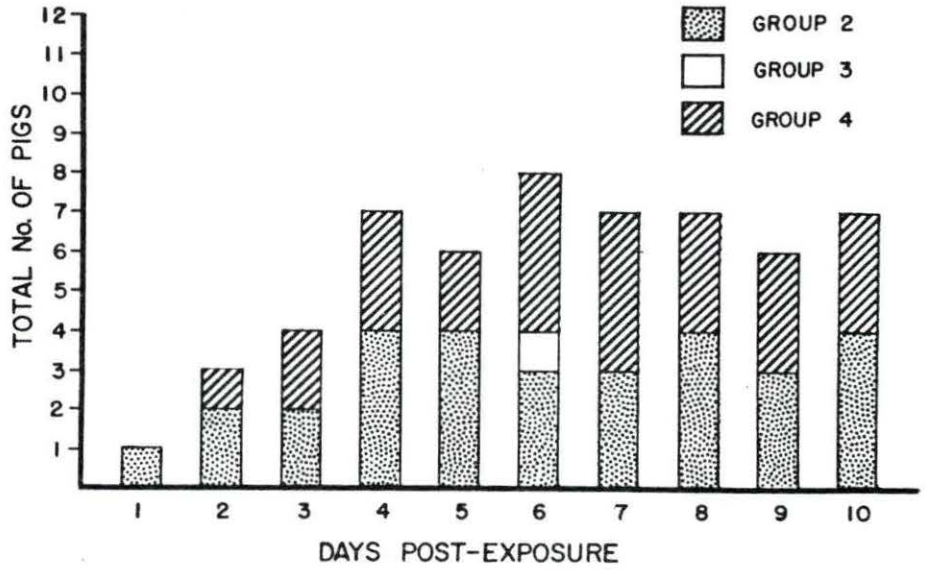
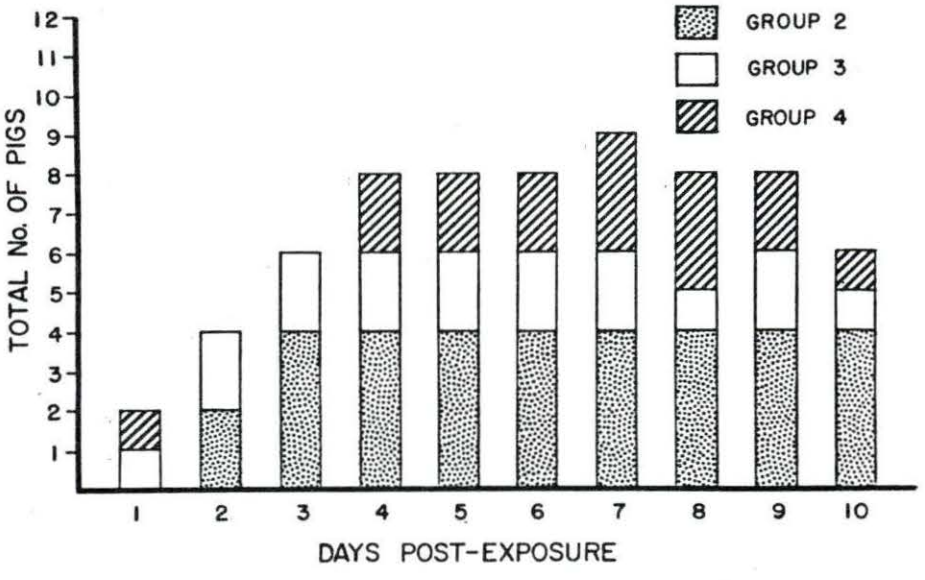

GROUP 2
GROUP 3
GROUP 4
TOTAL No. OF PIGS
1
2
3
4
5
6
7
8
9
10
11
12
1
2
3
4
5
6
7
8
9
10
DAYS POST-EXPOSURE

Fig. 16. Number of pigs in groups 2, 3, and 4 having E. insidiosa in the feces on each day post-exposure

Fig. 17. Number of pigs in groups 2, 3, and 4 having E. insidiosa in the urine on each day post-exposure


GROUP 2
GROUP 3
GROUP 4
TOTAL No. OF PIGS
1
2
3
4
5
6
7
8
9
10
11
12
1
2
3
4
5
6
7
8
9
10
DAYS POST-EXPOSURE

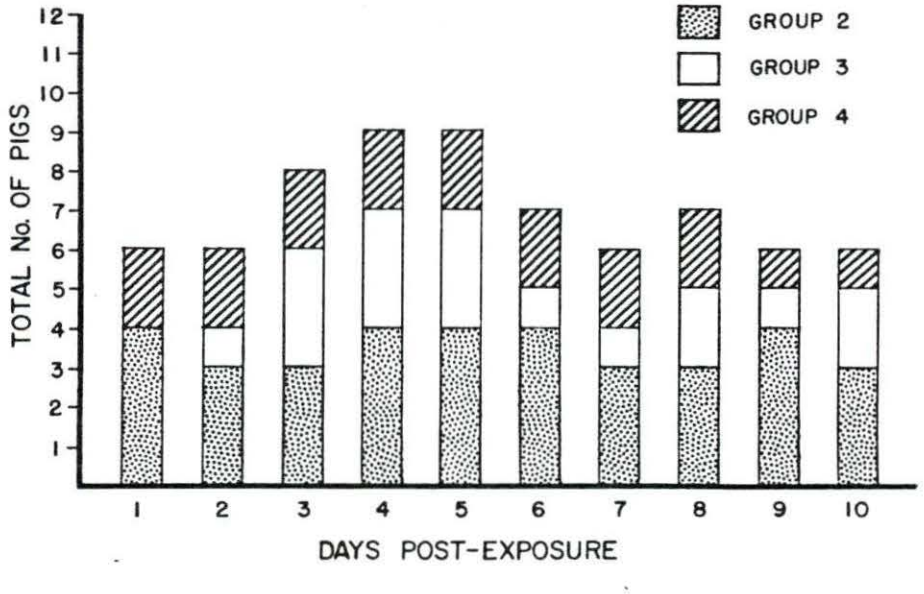
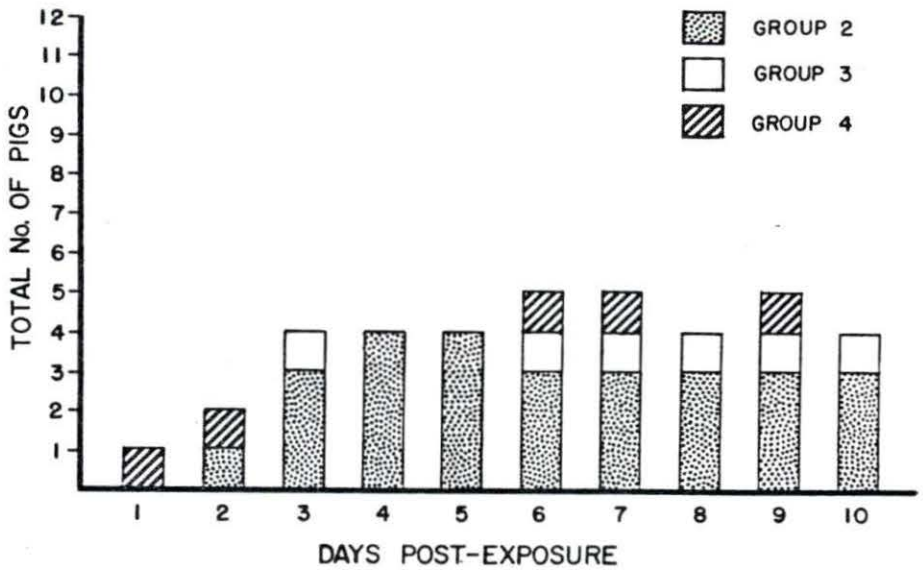

GROUP 2
GROUP 3
GROUP 4
TOTAL No. OF PIGS
1
2
3
4
5
6
7
8
9
10
11
12
1
2
3
4
5
6
7
8
9
10
DAYS POST-EXPOSURE

Fig. 18. Number of pigs in groups 2, 3, and 4 having E. insidiosa in the mouth on each day post-exposure

Fig. 19. Number of pigs in groups 2, 3, and 4 having E. insidiosa in the nasal passages on each day post-exposure


GROUP 2
GROUP 3
GROUP 4
TOTAL No. OF PIGS
1
2
3
4
5
6
7
8
9
10
11
12
1
2
3
4
5
6
7
8
9
10
DAYS POST-EXPOSURE

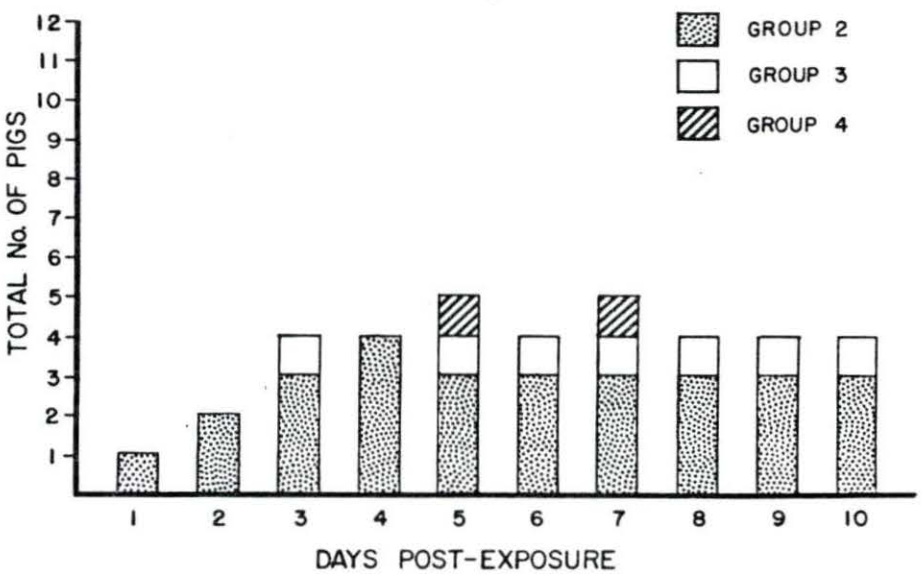
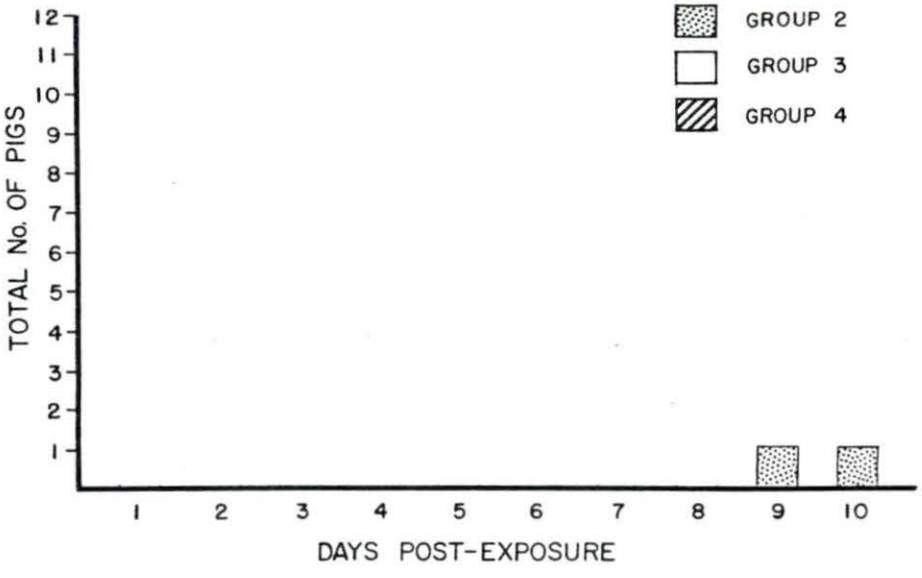

GROUP 2
GROUP 3
GROUP 4
TOTAL No. OF PIGS
1
2
3
4
5
6
7
8
9
10
11
12
1
2
3
4
5
6
7
8
9
10
DAYS POST-EXPOSURE

Fig. 20. Number of pigs in groups 2, 3, and 4 having *E. insidiosa* in the conjunctival sacs on each day post-exposure

Fig. 21. Number of pigs in groups 2, 3, and 4 having *E. insidiosa* on the skin (including urticarious lesions) on each day post-exposure


GROUP 2
GROUP 3
GROUP 4
TOTAL No. OF PIGS
12
11
10
9
8
7
6
5
4
3
2
1
1 2 3 4 5 6 7 8 9 10
DAYS POST-EXPOSURE

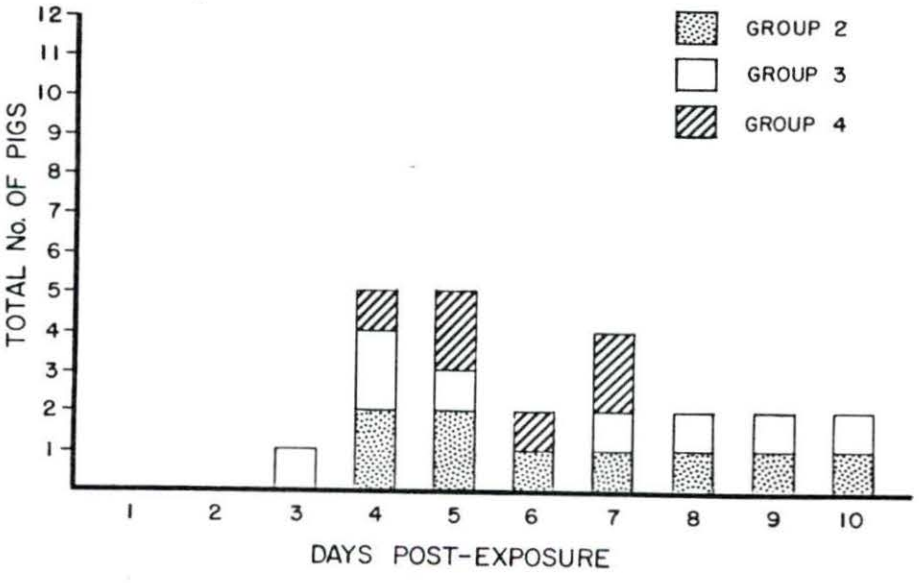

GROUP 2
GROUP 3
GROUP 4
TOTAL No. OF PIGS
12
11
10
9
8
7
6
5
4
3
2
1
1 2 3 4 5 6 7 8 9 10
DAYS POST-EXPOSURE

Fig. 22. Total number of recoveries of E. insidiosa from six routes of elimination (feces, urine, mouth, nasal passages, conjunctival sacs, skin) on each day post-exposure

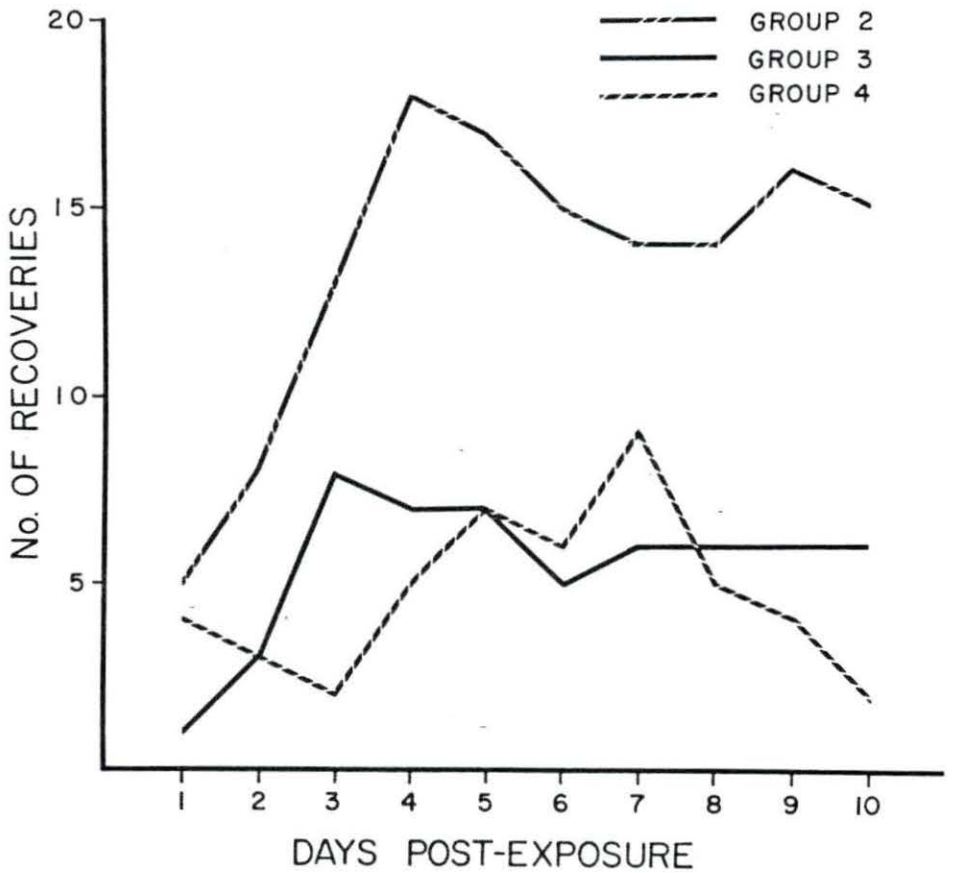
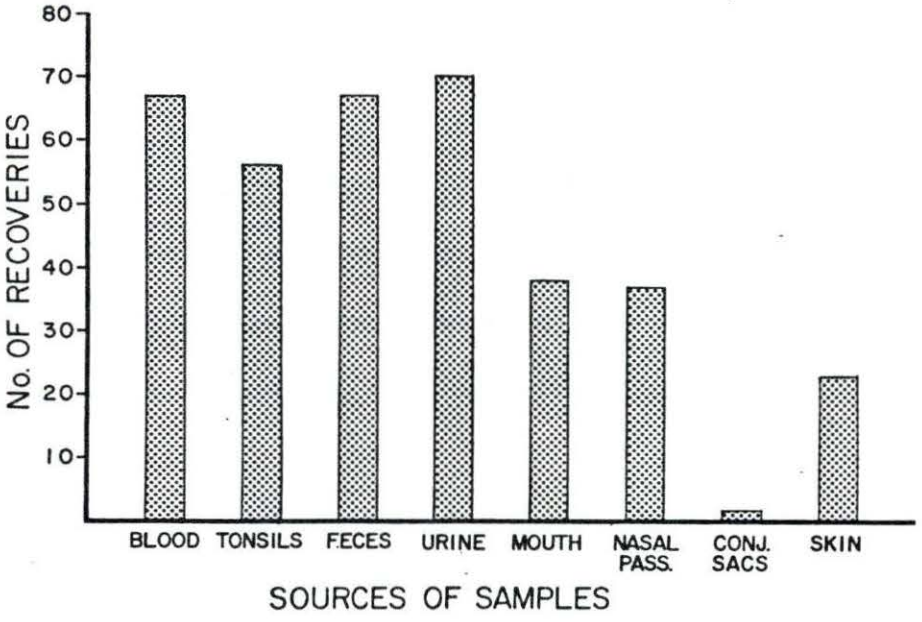
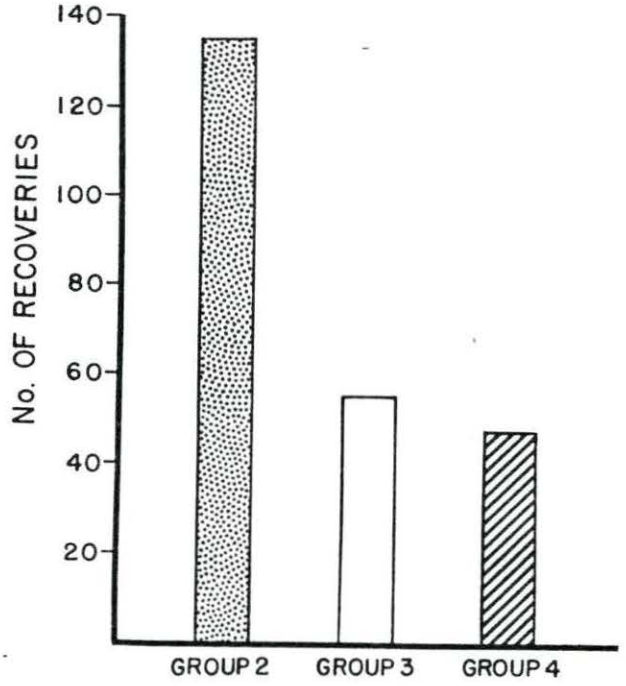

GROUP 2
GROUP 3
GROUP 4
No. OF RECOVERIES
20
15
10
5
1
2
3
4
5
6
7
8
9
10
DAYS POST-EXPOSURE

Fig. 23. Total number of recoveries of E. insidiosa from each source of samples in groups 2, 3, and 4 during 10 days post-exposure

Fig. 24. Total number of recoveries of E. insidiosa from six routes of elimination (feces, urine, mouth, nasal passages, conjunctival sacs, skin) during 10 days post-exposure


80
70
60
50
40
30
20
10
No. OF RECOVERIES
BLOOD
TONSILS
FECES
URINE
MOUTH
NASAL PASS.
CONJ. SACS
SKIN
SOURCES OF SAMPLES


140
120
100
80
60
40
20
No. OF RECOVERIES
GROUP 2
GROUP 3
GROUP 4

was made from group 2, 55 from group 3, and 47 from group 4.

Comparison of recoveries from fatal and non-fatal cases of swine erysipelas

To compare the frequency of recovery of E. insidiosa during fatal and non-fatal illness, data collected during the first 72 hours post-exposure were taken from 4 of the 5 pigs in group 1 and from the 4 pigs in group 2. Data from pig No. 1452 were excluded because the pig died 48 hours post-exposure (see Fig. 25).

In each of these groups, the total number of recoveries from each source, as well as the number from all sources combined, are given in Table 8. A few of the recoveries from fatal cases were made shortly after death, which occurred at 72 hours. A total of 40 recoveries was made from fatal cases, and 43 from non-fatal cases.

Quantities of feces and urine examined

The total amount of feces and urine examined pre-exposure and the average amount examined per day post-exposure from each pig are given in Table 9. The quantities given reflect the total amount eliminated by each animal during observation.

Post-mortem findings

Results of post-mortem cultural examinations for the presence of E. insidiosa in the 5 pigs that died of acute erysipelas (Group 1) are given in Table 10.

Table 8. Number of recoveries of E. insidiosa during 72 hours post-exposure from fatal and non-fatal cases of swine erysipelas induced by strain E1-6P

Source of samples	Recoveries from fatal cases (4 pigs)	Recoveries from non-fatal cases (4 pigs)
Blood	12[a]	12
Tonsils	4[b]	5
Feces[c]	7	6
Urine	5	10
Mouth	5[a]	4
Nasal passages	5[a]	6
Conjunctival sacs	2[a]	0
Skin	0	0
Total	40	43

[a]One recovery was made from a pig shortly after death at 72 hours.

[b]Two recoveries were made from pigs shortly after death at 72 hours.

[c]One or more positive fecal samples from a pig during one day was counted as one recovery.

Results of post-mortem cultural examinations of the 13 pigs that survived after exposure (groups 2, 3, 4, and 5) are given in Table 11.

Table 9. Quantities of feces and urine examined

Pig no.	Weight (pounds)	Grams of feces examined		Milliliters of urine examined	
		Total amount pre-exposure	Average per day post-exposure	Total amount pre-exposure	Average per day post-exposure
1452	132	582	323	1045	1100
1454	161	866	188	1070	1275
1654	89	466	324	810	952
1455A	137	567	419	1160	850
1459	139	781	257	1355	1393
981	187	268	238	2010	1776
990	185	918	403	1850	1474
994	135	238	286	1270	1205
1332	127	392	189	1060	842
1334	160	334	211	1120	948
1450	150	599	361	1200	1651
1333A	145	521	256	1120	1067
1452A	136	974	321	2550	2108
1446	120	578	431	1940	2637
1447	143	535	344	2080	1755
1449	143	840	355	2620	2011
1451A	170	706	373	1500	1247
1345	123	904	442	2200	961

Table 10. Results of post-mortem cultural examinations for E. insidiosa[a] in pigs that died from infection by strain E1-6P

Pig no.	Approximate time of death[b]	Tonsils	Gall bladder	Intestine	Mouth	Nasal passages	Conjunctival sacs	Anus
1452	48 hours	+	+	+	+	+	+	+
1454	96 hours	+	+	+	+	+	+	+
1654	72 hours	+	+	+	+	+	+	+
1455A	72 hours	+	+	+	+	+	-	+
1459	78 hours	+	+	+	+	+	-	+

[a]Presence or absence of the organism is indicated by + or -, respectively.

[b]Number of hours post-exposure.

Table 11. Results of post-mortem cultural examinations for *E. insidiosa*[a] at 10 days post-exposure in pigs that survived infection by strains E1-6P, HC-585, and de Castro

Pig no.	Exposure strain	Tonsils	Gall bladder	Intestine
981	E1-6P	+	-	+
990	E1-6P	+	+	+
994	E1-6P	+	-	+
1332	E1-6P	+	+	+
1334	HC-585	-	-	-
1450	HC-585	+	-	+
1333A	HC-585	-	-	-
1452A	HC-585	-	-	-
1446	De Castro	+	-	-
1447	De Castro	+	-	+
1449	De Castro	+	-	+
1451A	De Castro	-	-	-
1345	De Castro	-	-	-

[a]Presence or absence of the organism is indicated by + or -, respectively.

Identification of E. insidiosa

The stock cultures of exposure strains E1-6P, HC-585, and de Castro and the cultures of these strains recovered from the animals produced identical results in the tests used for identification. All tested cultures produced characteristic "test tube brush" growth in gelatin stab cultures (5). Hydrogen sulfide was produced in triple sugar iron agar slants, with a sharp line of demarcation as described by Vickers and

Bierer (64). The pattern of acid production (Table 12) from fermentable carbon compounds by each culture was identical to that described by White and Shuman (70).

Table 12. Acid production from fermentable carbon compounds by *E. insidiosa*, strains E1-6P, HC-585, and de Castro

Compound	Reaction[a]	Compound	Reaction[a]
Arabinose	-	Maltose	+
Dextrin	+	Mannitol	-
Dulcitol	-	Mannose	+[b]
Fructose	+	Raffinose	-
Galactose	+	Rhamnose	-
Glucose	+	Salicin	-
Glycerol	-	Sorbitol	-
Inositol	-	Sucrose	±[c]
Inulin	-	Trehalose	-
Lactose	+	Xylose	-

[a]Presence or absence of acid in 48 hours is designated by + or -, respectively.

[b]Acid production is slow (5 to 10 days).

[c]Slight acid production.

DISCUSSION

Erysipelothrix insidiosa was recovered from all sources investigated as possible routes of elimination from infected swine. The most frequent and consistent sources of recovery were the urine and feces, as indicated by the number of pigs with one or more positive samples (Fig. 13), and by the total number of recoveries during 10 days post-exposure (Table 7, Fig. 23). A moderate number of recoveries were made from the mouth and nasal passages, whereas recoveries from the skin were comparatively infrequent. The organism was found only occasionally in the conjunctival sacs.

Excretion of E. insidiosa from the body appeared to be a result of the presence of bacteremia. From 12 pigs that had non-fatal generalized infection, the highest incidence of elimination of the organism occurred during days 3 through 10 post-exposure, following a high incidence of bacteremia during days 1 through 4 (Figs. 14-21). This suggests a causal relationship of bacteremia to excretion of the organism from the body. Further evidence lies in the fact that no recoveries from external sources were made before bacteremia was detected, except from the feces of pigs 1452A and 1449 (Figs. 37, 40) and the urine of pig 981 (Fig. 30) during the first 24 hours post-exposure. These early recoveries were believed to be due to the existence of bacteremia before the first

post-exposure blood sample was taken.

The times of first appearance of E. insidiosa in the external sources varied within ranges of several days, but a determination of the median days of first recovery revealed that the organism generally appeared first in the urine and feces. This was followed by recovery from the tonsils, mouth, and nasal passages, and later from the skin.

The presence of E. insidiosa in the feces of 7 pigs and in the urine of 6 pigs before clinical signs were evident indicates that the practice of isolating visibly ill pigs in herd outbreaks of erysipelas would not remove all of the animals shedding the organism. If pigs are isolated on the basis of clinical signs only, including elevated temperatures, a number of shedders may go unnoticed, since they can be detected only by examination for presence of the organism in the blood or body excretions.

Considerable difference in the incidence of elimination was observed between pigs exposed to different strains of E. insidiosa, and although there was some relationship to virulence, the differences were not entirely commensurate with the severity of clinical illness. The number of recoveries in group 3, exposed to strain HC-585, was less than half the number in group 2, exposed to strain E1-6P, and only slightly more than the number in group 4, exposed to strain de Castro (Fig. 24). In view of the severity of illness induced by

strain HC-585, the comparatively infrequent and inconsistent shedding by the pigs exposed to this strain was unexpected. These characteristics may have been related to the comparatively short duration of bacteremia, which did not persist beyond the fifth day post-exposure in any pigs of group 3 (Fig. 14).

In addition to the differences in total recoveries observed between pigs exposed to different strains, there were differences in the time of highest number of recoveries. The total number of daily recoveries from all routes of elimination increased in groups 2 and 3 to peaks at 4 and 3 days post-exposure, respectively (Fig. 22). Recoveries from group 4, however, increased to a peak at 7 days post-exposure, after having decreased during the first 3 days.

Dissemination of E. insidiosa from pigs can be brought about by the establishment of bacteremia without a state of clinical illness, as indicated by the results from pigs exposed to strain de Castro. The few urticarious lesions observed on these pigs were the only visible evidence of infection, and could have been overlooked without close examination. Excretion of a strain of such low virulence by a pig, however, may depend upon the ability of the organism to reach the blood stream after natural exposure of the animal.

No significant difference in the extent of shedding was observed between fatal and non-fatal cases of swine erysip-

elas produced by strain E1-6P. Although the factors which cause death of a pig after exposure to a virulent strain of E. insidiosa remain unknown, there is apparently no relationship between death or survival of the animal and the incidence of shedding.

Results of post-mortem cultural examinations of the tonsils, gall bladder, and intestine varied with the exposure strains. Erysipelothrix insidiosa was recovered from the tonsils and intestines of all pigs exposed to strain E1-6P, but from the gall bladders of only those which died of infection or still had bacteremia at the time of euthanasia. Cultures from the gall bladders of all other pigs were negative. Differences between strains in the establishment of the organism in tonsils and intestinal lymphoid tissue were not related to virulence, as indicated by the results in pigs exposed to strains HC-585 and de Castro. Although strain de Castro was capable of producing only a mild clinical infection, it had a greater tendency to become established in these areas than did the more virulent strain HC-585. Strain HC-585 seems to be an exception to the suggestion of Rowsell that E. insidiosa may have a predeliction for tonsillar tissue (44).

These experiments have shown that a strain of E. insidiosa of low virulence can become localized in the tonsils and other lymphoid tissue of a pig after intravenous exposure,

and that when bacteremia is present, such a pig can be a persistent shedder of the organism by way of the urine and feces. This suggests a possibility that, if an asymptomatic carrier pig underwent intermittent phases of bacteremia originating from a localized depository of the organism such as the tonsils, the animal could be also an intermittent shedder.

Rowsell's belief that the most likely route of excretion of E. insidiosa by the tonsillar carrier is through the digestive tract (43) was not supported by the results of these experiments, nor did it appear that the tonsils were a source of excretion by way of the mouth or nasal passages. An examination of Figs. 25 through 41 reveals that E. insidiosa was recovered from the mouths of 7 pigs, the nasal passages of 6 pigs, and the feces of 12 pigs either before being recovered from the tonsils or without being recovered from that source at all. However, it may not be valid to extrapolate results obtained from pigs with clinical evidence of infection in order to form a hypothesis concerning asymptomatic carriers. Further research is necessary to determine the significance of the carrier pig as a disseminator of the erysipelas organism.

In the pigs exposed to virulent strains in these experiments, clinical responses, including the time of appearance of E. insidiosa in the blood and the appearance of clinical signs, were similar to descriptions of acute erysipelas given

by Wellmann (66). In order to induce the desired clinical response with each strain, it was necessary to expose pigs by different parenteral routes. Exposure to strain El-6P by intramuscular injection generally resulted in non-fatal illness, although death of one pig occurred. The amount of culture given 2 pigs (Nos. 981, 990) was increased in unsuccessful attempts to produce death. The route of exposure was changed to intradermal, and fatal illness was induced consistently in 4 pigs. Exposure to strain HC-585 by intradermal injection induced the clinical response desired. However, exposure to strain de Castro by this route failed to induce generalized infection, and it was necessary to use the intravenous route in order to obtain a satisfactory clinical response.

Regardless of the route of exposure or the amount of culture given, bacteremia was detected 24 hours post-exposure in 16 of the 17 pigs that had generalized infection. This consistent factor indicated that neither the routes of parenteral injection nor the amounts of culture given were significant variables in the experimental procedure, except as related to the presence or absence of subsequent generalized infection.

The pronounced diphasic clinical response of one pig in group 3 (No. 1333A), although not typical of the clinical signs seen in these experiments, was similar to observations

reported by Wellmann (66). The delayed appearance of bacteremia in this pig was similar to observations of percutaneously infected pigs reported by Rowsell (44).

Since no evidence of generalized infection was found in pig 1345, exposed to strain de Castro by intradermal injection, the brief rise in temperature recorded on day 6 post-exposure was not believed to be associated with infection. This pig was unusually restless during confinement in the crate throughout the experiment, and made persistent efforts to escape. The increased temperature may have been due to such a period of agitation. Generally, the pigs used in the experiments became quite tractable after one or 2 days of confinement.

The source of E. insidiosa found on the surfaces of normal skin and urticarious lesions could not be determined conclusively, although there was some evidence that the organism can be exuded through the skin during generalized infection. Of the 4 pigs on which plastic patches were fastened in attempts to exclude external contamination (Nos. 1450, 1333A, 1447, 1451A), recoveries were made from one (No. 1451A). Regardless of the source of the organism, however, the demonstration of its presence on the skin in these experiments indicates a possible source of dissemination by direct contact, as suggested by Van Es and McGrath (63).

The use of more males than females in the experiments

was due to the availability of animals at given times during the several months' duration of the project, and also because of anticipated difficulty in obtaining continuous fecal and urine samples from females without cross-contamination. However, due to the habits of elimination developed by these pigs in confinement, it was possible to collect more than half of the fecal and urine samples separately in hand-held containers at the time of elimination. In addition, it developed that the risk of cross-contamination of feces and urine from the 6 females was of little consequence, for the following reasons: Two females (Nos. 1452A and 1449) had no fecal and urine samples in which E. insidiosa was detected simultaneously. In 3 females (Nos. 1451A, 1459, and 1455A), all simultaneously positive fecal and urine samples had been collected separately by hand. Only one pig (No. 1333A) had simultaneously positive fecal and urine samples that were suspected of being cross-contaminated (Fig. 36). Since the organism was recovered from one subsequent urine sample, but from no other fecal samples, the positive culture in question was considered to be from the urine only.

From the results of these experiments, it was concluded that pigs are generally frequent shedders of E. insidiosa during generalized swine erysipelas infection. Dissemination of the organism into the environment is associated with the presence of bacteremia, and often occurs before infection can

be detected visually. The urine and feces are the earliest and most persistent routes of elimination during clinical illness, and therefore are the most significant methods of dissemination by the infected pig. Excretion of the organism by way of the mouth and nasal passages and its presence on the skin may constitute significant methods of dissemination, but these sources are secondary in importance. Lacrimal secretions are not a significant route of elimination.

SUMMARY

Eighteen specific-pathogen-free (SPF) pigs were confined individually in a restraining crate and exposed to Erysipelothrix insidiosa by parenteral injection. Three strains of the organism were used to induce clinical illness of different degrees of severity. Samples from the blood, tonsils, mouth, nasal passages, conjunctival sacs, and surface of the skin were taken daily for cultural examination. Feces and urine were collected continuously and examined culturally. Collection of samples extended from one day before exposure until death from acute erysipelas or until euthanasia 10 days after exposure.

Nine pigs exposed to strain E1-6P developed severe clinical illness, from which 5 pigs died. Four pigs exposed to strain HC-585 developed less severe clinical illness with recovery. Five pigs were exposed to strain de Castro, which induced a mild form of the disease in 4 pigs, and no evidence of generalized infection in one pig.

Erysipelothrix insidiosa was not found in any pre-exposure samples. After exposure of the 18 pigs, the organism was recovered from the blood of 17, urine of 17, feces of 16, tonsils of 14, mouths of 12, nasal passages of 11, skin of 6, and conjunctival sacs of 6. From 12 pigs that had non-fatal generalized infection, the following numbers of daily recoveries were made during 10 days post-exposure: blood, 67;

urine, 70; feces, 67; tonsils, 56; mouth, 38; nasal passages, 37; skin, 23; and conjunctival sacs, 2. The median day post-exposure of first recovery from each source was as follows: blood, day 1; urine and feces, day 2; tonsils, mouth, and nasal passages, day 3; and skin, day 4. A median day could not be determined for the conjunctival sacs. Excretion of the organism from the body was preceded or accompanied by bacteremia.

It was concluded that pigs are generally frequent shedders of E. insidiosa during generalized erysipelas infection. The urine and feces are the earliest and most persistent routes of elimination, and often contain the organism before infection can be detected visually. Excretion of E. insidiosa by way of the mouth and nasal passages and its presence on the skin may be significant methods of dissemination, but these sources are secondary in importance. Lacrimal secretions are not a significant route of elimination.

LITERATURE CITED

1. Aitken, W. A. Acute swine erysipelas in northwestern Iowa. American Veterinary Medical Association Journal 116: 41-45. 1950.

2. Aitken, W. A. How ubiquitous is the erysipelas bacillus? American Veterinary Medical Association Journal 132: 402-403. 1958.

3. Arsov, R. Healthy swine as carriers of swine erysipelas bacteria (translated title). Nauchni Trudove Vissh Veterinarno-Meditsinski Institut "Prof. Dr. G. Pavlov" 4: 185-190. 1956.

4. Bauermeister, C. Ueber das ständige Vorkommen pathogener Mikroorganismen, insbesondere der Rothlaufbacillen in den Tonsillen des Schweines. Archiv für Wissenschaftliche und Praktische Tierheilkunde 28: 66-101. 1902.

5. Breed, R. S., Murray, E. G. D., and Smith, N. R. Bergey's manual of determinative bacteriology. 7th ed. Baltimore, Maryland, The Williams and Wilkins Co. 1957.

6. Castro, A. F. P. de, Santa Rosa, C. A., Troise, C., e Gissoni, R. H. Isolamento de Erysipelothrix rhusiopathiae de suínos aparentemente normais abatidos em matadouro. Arquivos do Instituto Biológico, São Paulo 30: 115-117. 1963.

7. Connell, R. and Langford, E. V. Studies of swine erysipelas. V. Presence of Erysipelothrix rhusiopathiae in apparently healthy pigs. Canadian Journal of Comparative Medicine 17: 448-453. 1953.

8. Cornevin, C. Première étude sur le rouget du porc. Paris, France, Asselin et Houzeau. 1885.

9. Creech, G. T. The bacillus of swine erysipelas isolated from urticarial lesions of swine in the United States. American Veterinary Medical Association Journal 59: 139-150. 1921.

10. DeFosset, A. J. Swine erysipelas. Veterinary Medicine 27: 224-229. 1932.

11. Doyle, T. M. Can swine erysipelas be eradicated? Epidemiological and immunological aspects. Veterinary Reviews and Annotations 6: 95-103. 1960.

12. Francke, G. und Goerttler, V. Allgemeine Epidemiologie der Tierseuchen. Stuttgart, Germany, Ferdinand Enke. 1930.

13. Friedberger, E. and Frȍhner, E. Veterinary pathology. 4th ed. Vol. 1. London, England, Hurst and Blackett, Ltd. 1904.

14. Geissler, S. Untersuchungen über das Vorkommen von Rotlaufbakterien in den Tonsillen gesunder Schlachtschweine. Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. Originale 159: 335-338. 1953. Original available but not translated; abstracted in Veterinary Bulletin 24: 2218. 1954.

15. Giltner, L. T. A fatal disease of young pigs apparently caused by the bacillus of swine erysipelas. American Veterinary Medical Association Journal 61: 540-543. 1922.

16. Gledhill, A. W. Swine erysipelas. In Stableforth, A. W. and Galloway, I. A., eds. Infectious diseases of animals. Vol. 2. Diseases due to bacteria. pp. 651-670. New York, New York, Academic Press, Inc. 1959.

17. Goerttler, V. Probleme der Rotlaufforschung. Deutsche Tierärztliche Wochenschrift 67: 285-288. 1960.

18. Gurova, E. I. Factors influencing the survival of pathogenic bacteria in soil (translated title). Nauchnye Trudy Ukrainskiĭ Institut Éksperimental'noĭ Veterinarii 25: 269-280. 1959. Original available but not translated; abstracted in Veterinary Bulletin 30: 1740. 1960.

19. Harrington, C. F. Field observations on erysipelas in swine herds. American Veterinary Medical Association Journal 82: 492-503. 1933.

20. Hartwigk, H. und Barnick, K. F. Ein Beitrag zur Frage der Schaffung von Rotlaufbakterienträgern durch die Simultanimpfung. Deutsche Tierärztliche Wochenschrift 61: 241-243. 1954. Original available but not translated; abstracted in Veterinary Bulletin 25: 24. 1955.

21. Hesse, E. Einfluss der Reaktion, insbesondere des Bodens, auf die Fortentwicklung des Rotlauf-Murisepticus Bacillus. Archiv für Wissenschaftliche und Praktische Tierheilkunde 50: 168-191. 1924.

22. Hettche, H. O. Zur Ätiologie der Rotlaufinfektion. II. Mitteilung. Archiv für Hygiene und Bakteriologie 119: 178-183. 1937.

23. Hutyra, F., Marek, J., and Manninger, R. Special pathology and therapeutics of the diseases of domestic animals. 5th English ed. Vol. 1. Chicago, Illinois, Alexander Eger. 1946.

24. Kalich, J. Rotlauf als Umweltproblem. Tierärztliche Umschau 14: 232-234. 1959.

25. Koch, R. Gesammelte Werke von Robert Koch. Vol. 1. Leipzig, Germany, Georg Thieme. 1912.

26. Kucsera, G. Adatok a börskarifikációval végzett mesterséges sertésorbáncfertőzés időszakos sikertelenségének kérdéséhez. Magyar Állatorvosok Lapja 12: 222-223. 1957. Original available but not translated; abstracted in Veterinary Bulletin 28: 2019. 1958.

27. Kucsera, G. Untersuchungen über Rotlaufbakterienträger sowie über die biologischen Eigenschaften der aus Bakterienträgern gezüchteten Rotlaufstämme in einem mit Adsorbat-Vakzine immunisierten Schweinebestand. Acta Veterinaria Academiae Scientiarum Hungaricae 8: 129-135. 1958. Original available but not translated; abstracted in Veterinary Bulletin 29: 1297. 1959.

28. Kurek, C. Nowsze poglądy na patogenezę różycy w świetle badań własnych. Medycyna Weterynaryjna 14: 523-529. 1958.

29. Löffler, F. Experimentelle Untersuchungen über Schweine-Rothlauf. Arbeiten aus dem Kaiserlichen Gesundheitsamte 1: 47-55. 1886.

30. Löffler, F. Zur Immunitätsfrage. Mitteilungen aus dem Kaiserlichen Gesundheitsamte 1: 134-187. 1881.

31. Marmorstein, J. Ueber die Vermehrung tierpathogener Bakterien in steriler Erde. Tierärztliche Rundschau 30: 143-146. 1924.

32. Moore, V. A. Mouse septicemia bacilli in a pig's spleen, with some observations on their pathogenic properties. Journal of Comparative Medicine and Veterinary Archives 13: 333-341. 1892.

33. Nocard, E. et Leclainche, E. Les maladies microbiennes des animaux. 3rd ed. Vol. 1. Paris, France, Masson et cie. 1903.

34. Obreshkov, K. The preservation and propagation of swine erysipelas bacteria in the soil (English summary). Sbornik Nauchni Trudove Ministerstvo na Zemedelieto, Veterinarno Institut, Sofia 7: 21-27. 1956.

35. Olsuf'ev, N. G., Petrov, V. G., and Shlygina, K. N. The detection of the causal organisms of erysipeloid and listeriosis in stream-water. Journal of Microbiology, Epidemiology, and Immunobiology 30: 112-119. 1959.

36. Olt, A. Ueber das regelmässige Vorkommen der Rothlaufbacillen im Darme des Schweines. Deutsche Tierärztliche Wochenschrift 9: 41-43. 1901.

37. Packer, R. A. The use of sodium azide (NaN_3) and crystal violet in a selective medium for streptococci and Erysipelothrix rhusiopathiae. Journal of Bacteriology 46: 343-349. 1943.

38. Pasteur, L. Sur le rouget, ou mal rouge des porcs. Comptes Rendus de l'Académie des Sciences, Paris 95: 1120-1121. 1882.

39. Pitt, W. Beiträge zum regelmässigen Vorkommen der Rotlaufbacillen auf der Darmschleimhaut und in den Tonsillen gesunder Schweine. Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten. Originale 45: 33-37; 111-121. 1908.

40. Preisz, H. von. Rotlauf der Schweine. In Kolle, W. und Wassermann, A. von. Handbuch der pathogenen Mikroorganismen. Vol. 6. Part 1. pp. 449-482. Jena, Germany, Gustav Fischer. 1929.

41. Rosenwald, A. S. Erysipelothrix rhusiopathiae infection in turkeys. Unpublished M.S. thesis. Corvallis, Oregon, Library, Oregon State College. 1942.

42. Rowsell, H. C. A cultural and biochemical study of strains of Erysipelothrix rhusiopathiae with special reference to the carrier pig. Canadian Journal of Comparative Medicine 22: 82-86. 1958.

43. Rowsell, H. C. The effect of stomach contents and the soil on the viability of Erysipelothrix rhusiopathiae. American Veterinary Medical Association Journal 132: 357-361. 1958.

44. Rowsell, H. C. Studies on the experimental production of swine erysipelas. American Veterinary Medical Association Proceedings 1955: 143-148. 1955.

45. Schütz, J. Ueber den Rothlauf der Schweine und die Impfung desselben. Arbeiten aus dem Kaiserlichen Gesundheitsamte 1: 56-76. 1886.

46. Shuman, R. D. Experimental evaluation of culture and serum vaccination for the control of swine erysipelas. II. Baby pig vaccination. American Veterinary Medical Association Journal 123: 304-307. 1953.

47. Shuman, R. D. Experimental evaluation of culture and serum vaccination for the control of swine erysipelas. III. Weaning age vaccination. American Veterinary Medical Association Journal 123: 307-310. 1953.

48. Shuman, R. D. Swine erysipelas. In Dunne, H. W., ed. Diseases of swine. 2nd ed. pp. 409-452. Ames, Iowa, The Iowa State University Press. 1964.

49. Shuman, R. D. Swine erysipelas induced by skin scarification. American Veterinary Medical Association Proceedings 1951: 153-160. 1951.

50. Shuman, R. D., Wood, R. L., and Cheville, N. F. Sensitization by Erysipelothrix rhusiopathiae (insidiosa) with relation to arthritis in pigs. II. Pretreatment with dead cells of serotypes A and B and challenge with live homologous or heterologous cells. Cornell Veterinarian 55: 387-396. 1965.

51. Shuman, R. D., Wood, R. L., and Monlux, W. S. Sensitization by Erysipelothrix rhusiopathiae (insidiosa) with relation to arthritis in pigs. III. Pretreatment with dead cells of serotype B and challenge with live or dead homologous or live heterologous cells. Cornell Veterinarian 55: 397-411. 1965.

52. Spears, H. N. Carriers of swine erysipelas. Journal of Comparative Pathology and Therapeutics 64: 152-156. 1954.

53. Sukhoretskii, B. S. Survival of the swine erysipelas bacterium in soil (translated title). Vychonyía Zapiski Vitebsk Veternyarna-Zoatekhnichnyĭ Instytut 16: 18-22. 1958.

54. Szynkiewicz, Z. M. Nosicielstwo włoskowców różycy na migdałkach świń zdrowych poddanych ubojowi. Roczniki Nauk Rolniczych, Ser. E, 66: 535-542. 1955.

55. Szynkiewicz, Z. M. Protozoa as fundamental factors for the destruction of Erysipelothrix insidiosa in soil. Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. Originale 192: 347-352. 1964.

56. Szynkiewicz, Z. M. Viability of Erysipelothrix insidiosa in different types of nonsterilized soil. Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. Originale 192: 336-346. 1964.

57. Taylor, J. B. Swine erysipelas. American Veterinary Medical Association Journal 79: 813-814. 1931.

58. Tenbroeck, C. Studies on Bacillus muriseptícus, or the Rotlauf bacillus, isolated from swine in the United States. Journal of Experimental Medicine 32: 331-343. 1920.

59. Trautwein, K. Épidemiologie et prophylaxie du rouget du porc. Bulletin, Office International des Épizooties, Paris, France 32: 222-231. 1949.

60. United States Department of Agriculture. Bureau of Animal Industry. Second annual report, for the year 1885. 1886.

61. United States Department of Agriculture. Bureau of Animal Industry. Twelfth and thirteenth annual reports, for the fiscal years 1895 and 1896. 1897.

62. Vallée, M. Sur l'étiologie du rouget. Toulouse, France, Imprimerie Ouvriere. 1930.

63. Van Es, L. and McGrath, C. B. Swine erysipelas. Nebraska Agricultural Experiment Station Research Bulletin 128. 1942.

64. Vickers, C. L. and Bierer, B. W. Triple sugar iron agar as an aid in the diagnosis of erysipelas. American Veterinary Medical Association Journal 133: 543-544. 1958.

65. Ward, A. R. The etiology of polyarthritis in swine. American Veterinary Medical Association Journal 61: 155-161. 1922.

66. Wellmann, G. Auftreten von Rotlaufbakterien im Blut von experimentell mit Rotlauf infizierten Schweinen. Deutsche Tierärztliche Wochenschrift 60: 366-369. 1953.

67. Wellmann, G. Summaries of experiments in swine erysipelas in Germany. American Veterinary Medical Association Journal 127: 331-333. 1955.

68. Wellmann, G. Vorkommen und Virulenz von Rotlaufbakterien in nicht an akutem Rotlauf erkrankten Schweinen. Deutsche Tierärztliche Wochenschrift 61: 357-363. 1954.

69. White, T. G. Type specificity in the vaccination of pigs with killed Erysipelothrix rhusiopathiae. American Journal of Veterinary Research 23: 752-755. 1962.

70. White, T. G. and Shuman, R. D. Fermentation reactions of Erysipelothrix rhusiopathiae. Journal of Bacteriology 82: 595-599. 1961.

71. Wood, R. L. A selective liquid medium utilizing antibiotics for isolation of Erysipelothrix insidiosa. American Journal of Veterinary Research 26: 1303-1308. 1965.

ACKNOWLEDGMENTS

This project was conducted under the United States Government Employees' Training Act, Public Law 85-507.

The author wishes to thank Dr. R. A. Packer and Dr. R. D. Shuman for their counsel and helpful editorial suggestions.

Thanks are extended to Mr. H. K. Hand and Mr. H. J. Fleming for their assistance in handling animals, and to Mr. N. A. Nord for assistance in the cultural examination of samples.

Appreciation is expressed also to Mr. R. M. Glazier and Mr. W. A. Romp for the preparation of graphs and for the photographs which appear in the thesis.

APPENDIX

Figures 25 through 42 consist of individual charts summarizing the data obtained from each of the 18 pigs used in the experiments. Temperatures are given as recorded twice daily (8:30 A.M. and 4:00-5:00 P.M.). In each chart, the presence of one or more visible signs of infection (see Table 3) is represented by a gray bar showing the period during which consecutive observations were positive. The presence of E. insidiosa in each source is represented by a stippled bar showing the periods during which recoveries were made from consecutive samples. Open spaces in bars represent negative results. Each chart is accompanied by descriptive information on the individual pig, including breed, sex, age, weight, exposure strain used, and group assignment.

Fig. 25. Summary of data from pig No. 1452

Breed: Mixed; white in color

Sex: Male

Age: 8 months

Weight: 132 pounds

Exposure:

Strain: E1-6P

Route: Intradermal

Amount of culture: 0.1 ml.

Group assignment: 1

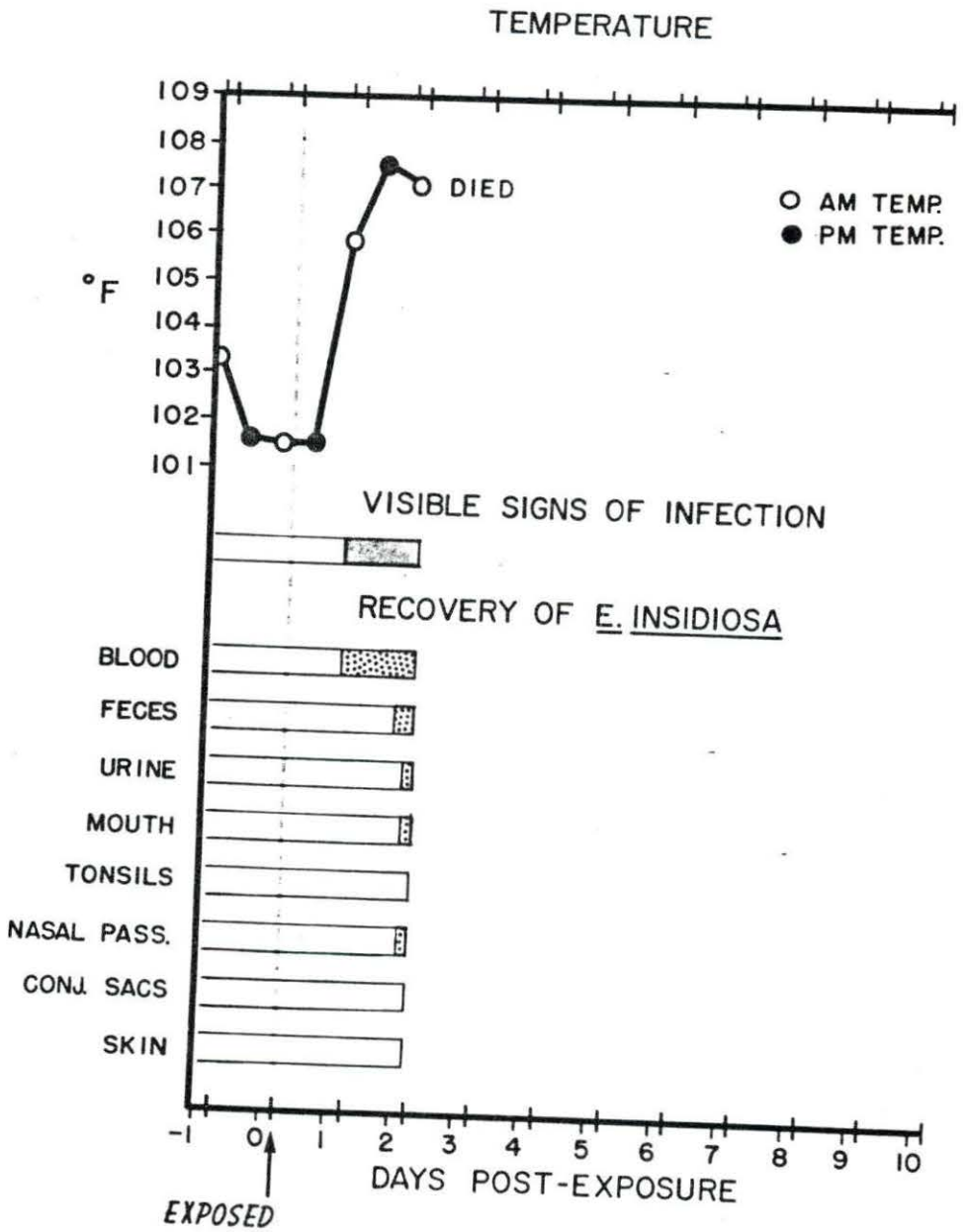

TEMPERATURE
109
108
107
106
105
104
103
102
101
°F
DIED
AM TEMP.
PM TEMP.
VISIBLE SIGNS OF INFECTION
RECOVERY OF E. INSIDIOSA
BLOOD
FECES
URINE
MOUTH
TONSILS
NASAL PASS.
CONJ. SACS
SKIN
-1
0
1
2
3
4
5
6
7
8
9
10
DAYS POST-EXPOSURE
EXPOSED

Fig. 26. Summary of data from pig No. 1454

Breed: Mixed; white in color

Sex: Male

Age: 8 months

Weight: 161 pounds

Exposure:

Strain: E1-6P

Route: Intradermal

Amount of culture: 0.1 ml.

Group assignment: 1

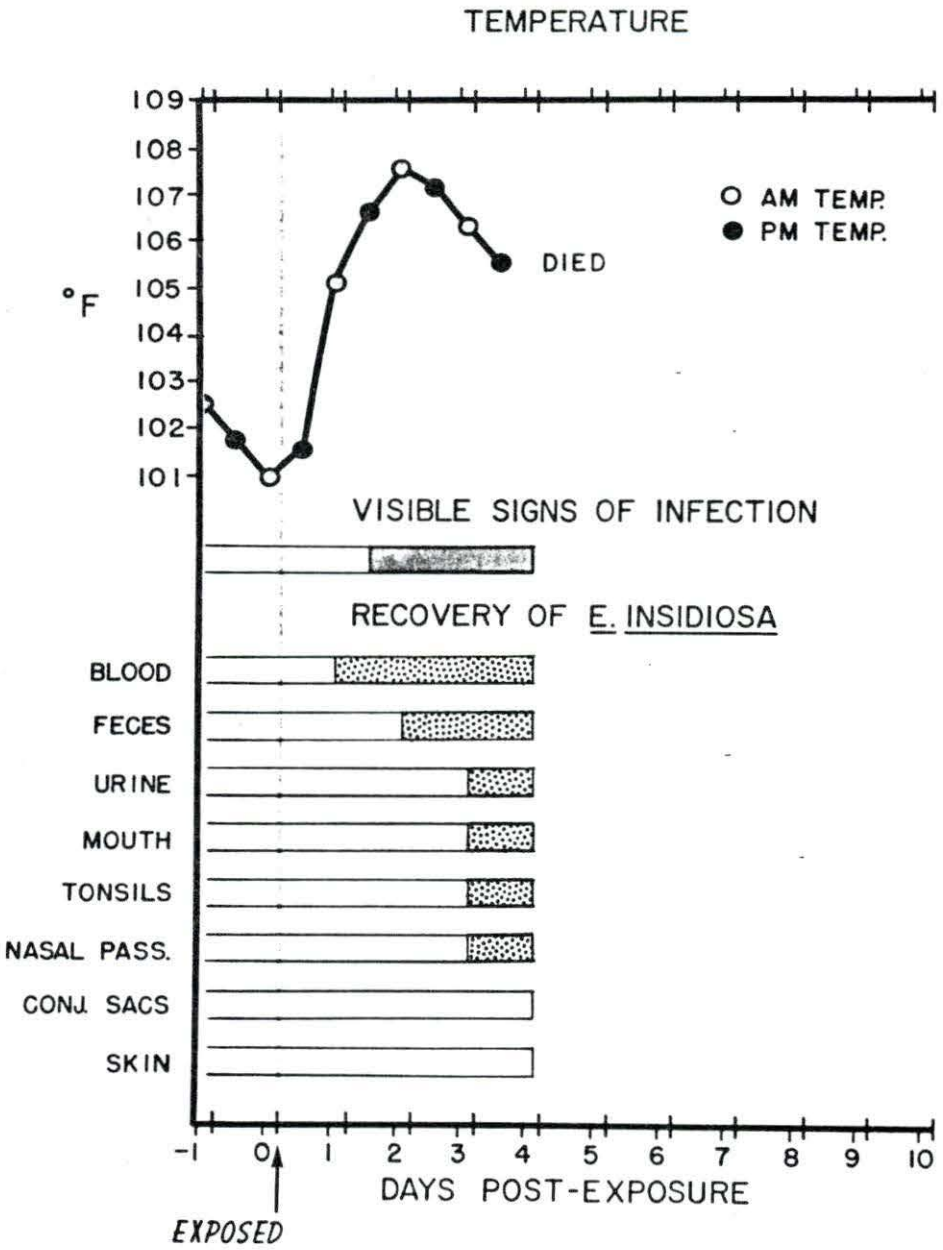

TEMPERATURE
109
108
107
106
105
104
103
102
101
°F
AM TEMP.
PM TEMP.
DIED
VISIBLE SIGNS OF INFECTION
RECOVERY OF E. INSIDIOSA
BLOOD
FECES
URINE
MOUTH
TONSILS
NASAL PASS.
CONJ. SACS
SKIN
-1
0
1
2
3
4
5
6
7
8
9
10
DAYS POST-EXPOSURE
EXPOSED

Fig. 27. Summary of data from pig No. 1654

Breed: Mixed; predominantly white in color

Sex: Male

Age: 5 months

Weight: 89 pounds

Exposure:

Strain: E1-6P

Route: Intradermal

Amount of culture: 0.1 ml.

Group assignment: 1

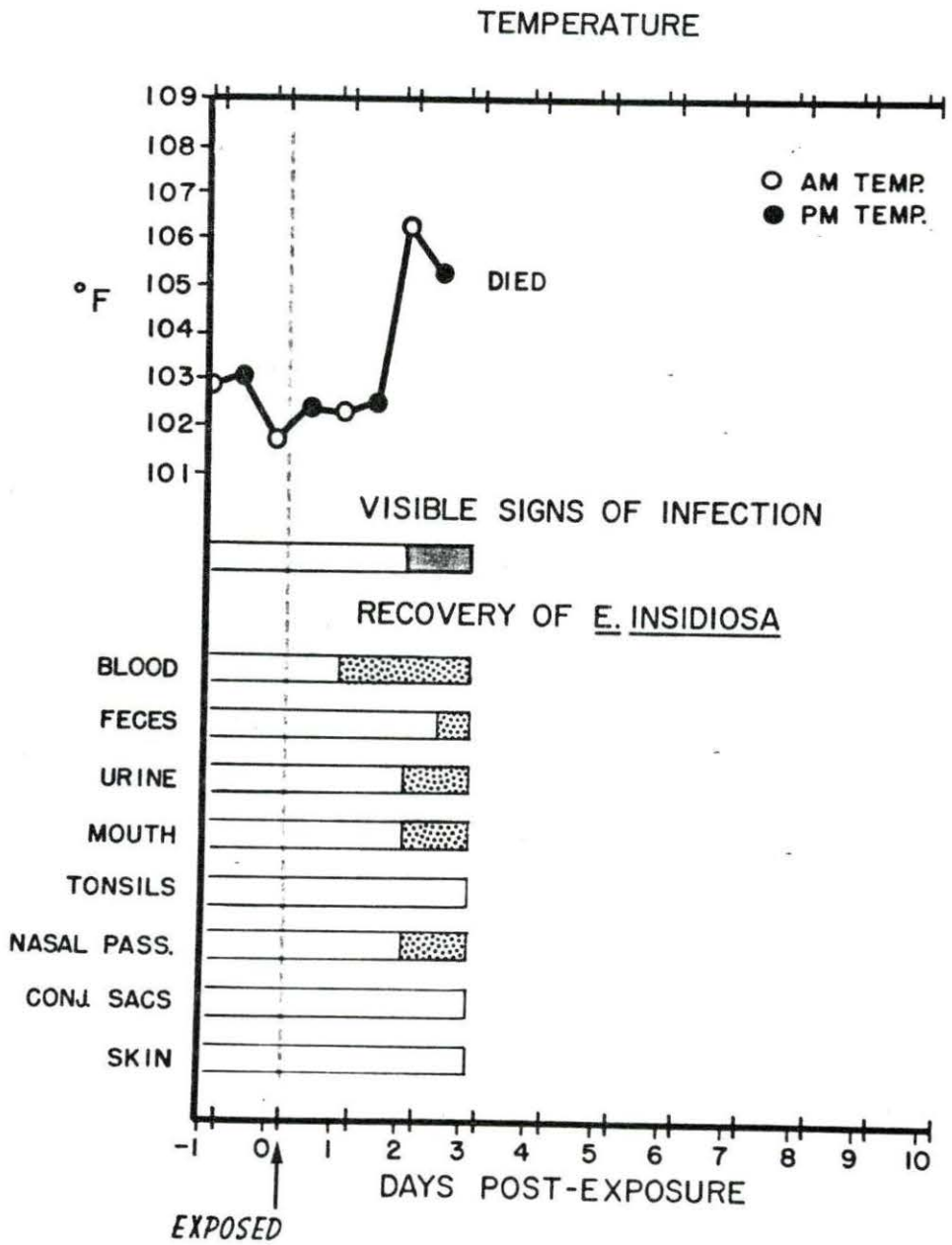

TEMPERATURE
109
108
107
106
105
104
103
102
101
°F
O AM TEMP.
● PM TEMP.
DIED
VISIBLE SIGNS OF INFECTION
RECOVERY OF E. INSIDIOSA
BLOOD
FECES
URINE
MOUTH
TONSILS
NASAL PASS.
CONJ. SACS
SKIN
-1
0
1
2
3
4
5
6
7
8
9
10
DAYS POST-EXPOSURE
EXPOSED

Fig. 28. Summary of data from pig No. 1455A

Breed: Mixed; white in color

Sex: Female

Age: 8 months

Weight: 137 pounds

Exposure:

Strain: E1-6P

Route: Intradermal

Amount of culture: 0.1 ml.

Group assignment: 1

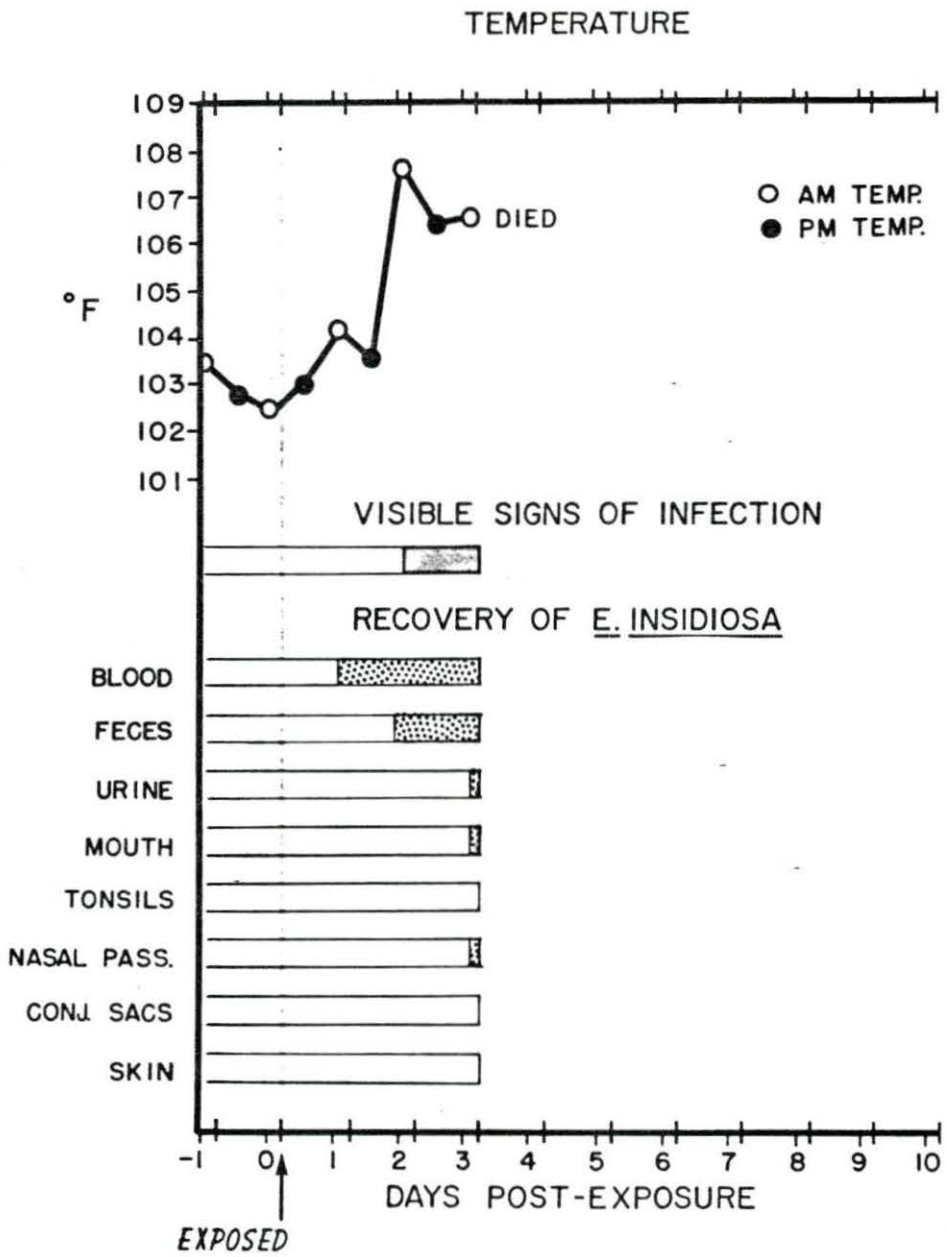

TEMPERATURE
109
108
107
106
105
104
103
102
101
°F
DIED
AM TEMP.
PM TEMP.
VISIBLE SIGNS OF INFECTION
RECOVERY OF E. INSIDIOSA
BLOOD
FECES
URINE
MOUTH
TONSILS
NASAL PASS.
CONJ. SACS
SKIN
-1
0
1
2
3
4
5
6
7
8
9
10
DAYS POST-EXPOSURE
EXPOSED

Fig. 29. Summary of data from pig No. 1459

Breed: Mixed; predominantly white in color

Sex: Female

Age: 8 months

Weight: 139 pounds

Exposure:

Strain: E1-6P

Route: Intramuscular

Amount of culture: 4.0 ml.

Group assignment: 1

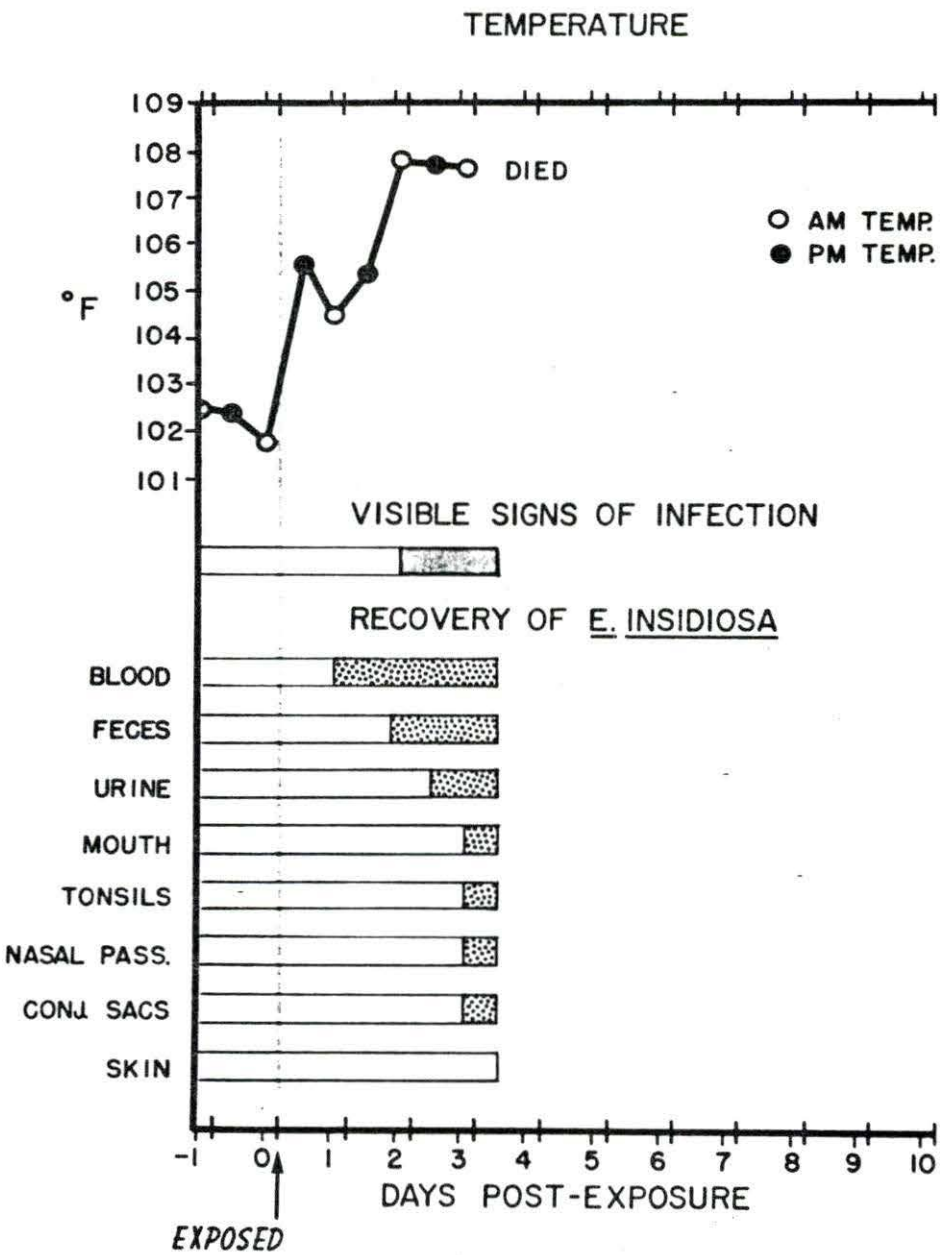

TEMPERATURE
109
108
107
106
105
104
103
102
101
°F
DIED
AM TEMP.
PM TEMP.
VISIBLE SIGNS OF INFECTION
RECOVERY OF E. INSIDIOSA
BLOOD
FECES
URINE
MOUTH
TONSILS
NASAL PASS.
CONJ. SACS
SKIN
-1
0
1
2
3
4
5
6
7
8
9
10
DAYS POST-EXPOSURE
EXPOSED

Fig. 30. Summary of data from pig No. 981

Breed: Hampshire

Sex: Male

Age: 6 1/2 months

Weight: 187 pounds

Exposure:

Strain: E1-6P

Route: Intramuscular

Amount: 6.0 ml.

Group assignment: 2

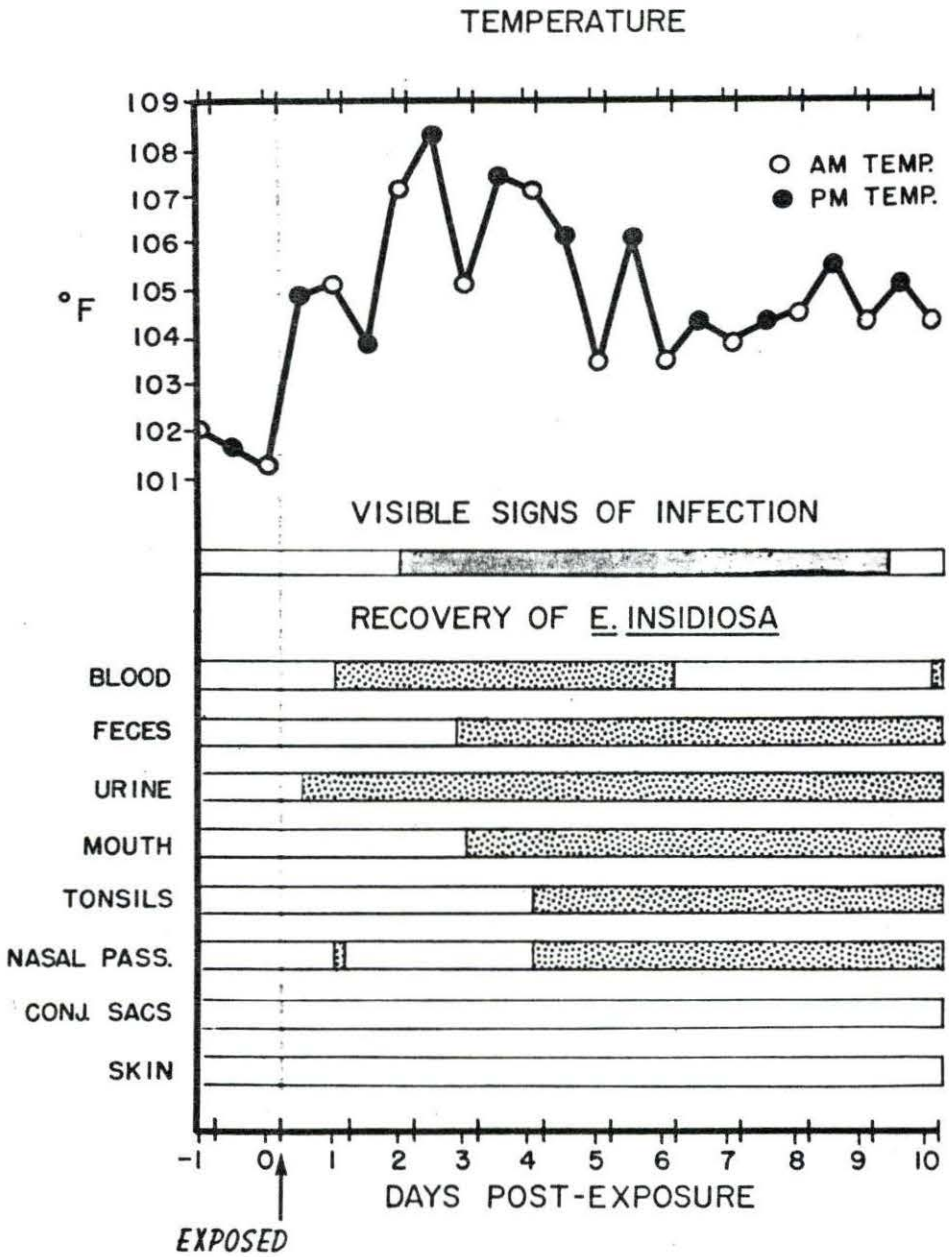

TEMPERATURE
109
108
107
106
105
104
103
102
101
°F
O AM TEMP.
● PM TEMP.
VISIBLE SIGNS OF INFECTION
RECOVERY OF E. INSIDIOSA
BLOOD
FECES
URINE
MOUTH
TONSILS
NASAL PASS.
CONJ. SACS
SKIN
-1
0
1
2
3
4
5
6
7
8
9
10
DAYS POST-EXPOSURE
EXPOSED

Fig. 31. Summary of data from pig No. 990

Breed: Hampshire

Sex: Male

Age: 8 months

Weight: 185 pounds

Exposure:

Strain: E1-6P

Route: Intramuscular

Amount of culture: 8.0 ml.

Group assignment: 2

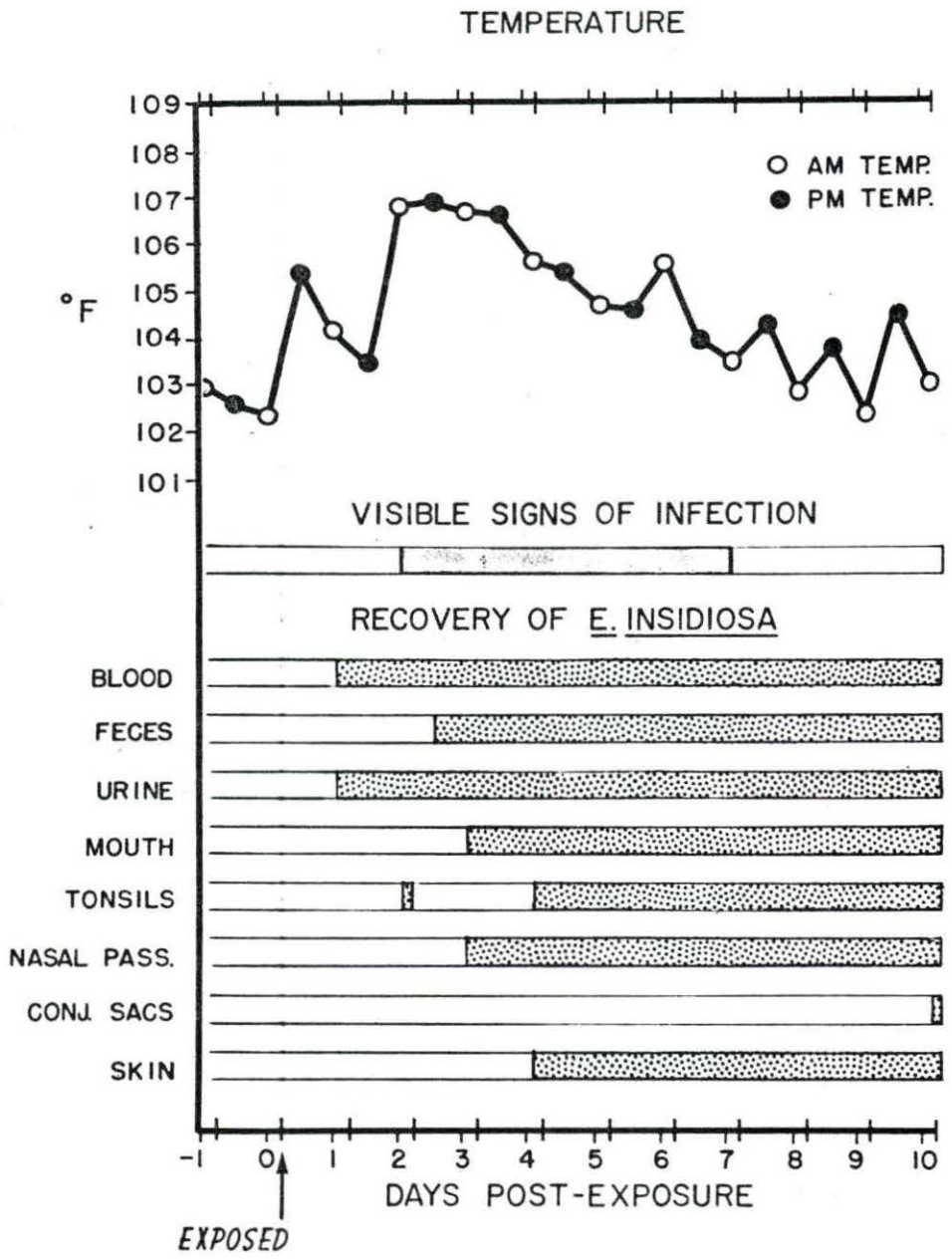

TEMPERATURE
109
108
107
106
105
104
103
102
101
°F
AM TEMP.
PM TEMP.
VISIBLE SIGNS OF INFECTION
RECOVERY OF E. INSIDIOSA
BLOOD
FECES
URINE
MOUTH
TONSILS
NASAL PASS.
CONJ. SACS
SKIN
-1
0
1
2
3
4
5
6
7
8
9
10
DAYS POST-EXPOSURE
EXPOSED

Fig. 32. Summary of data from pig No. 994

Breed: Hampshire

Sex: Male

Age: 6 months

Weight: 135 pounds

Exposure:

Strain: E1-6P

Route: Intramuscular

Amount of culture: 4.0 ml.

Group assignment: 2

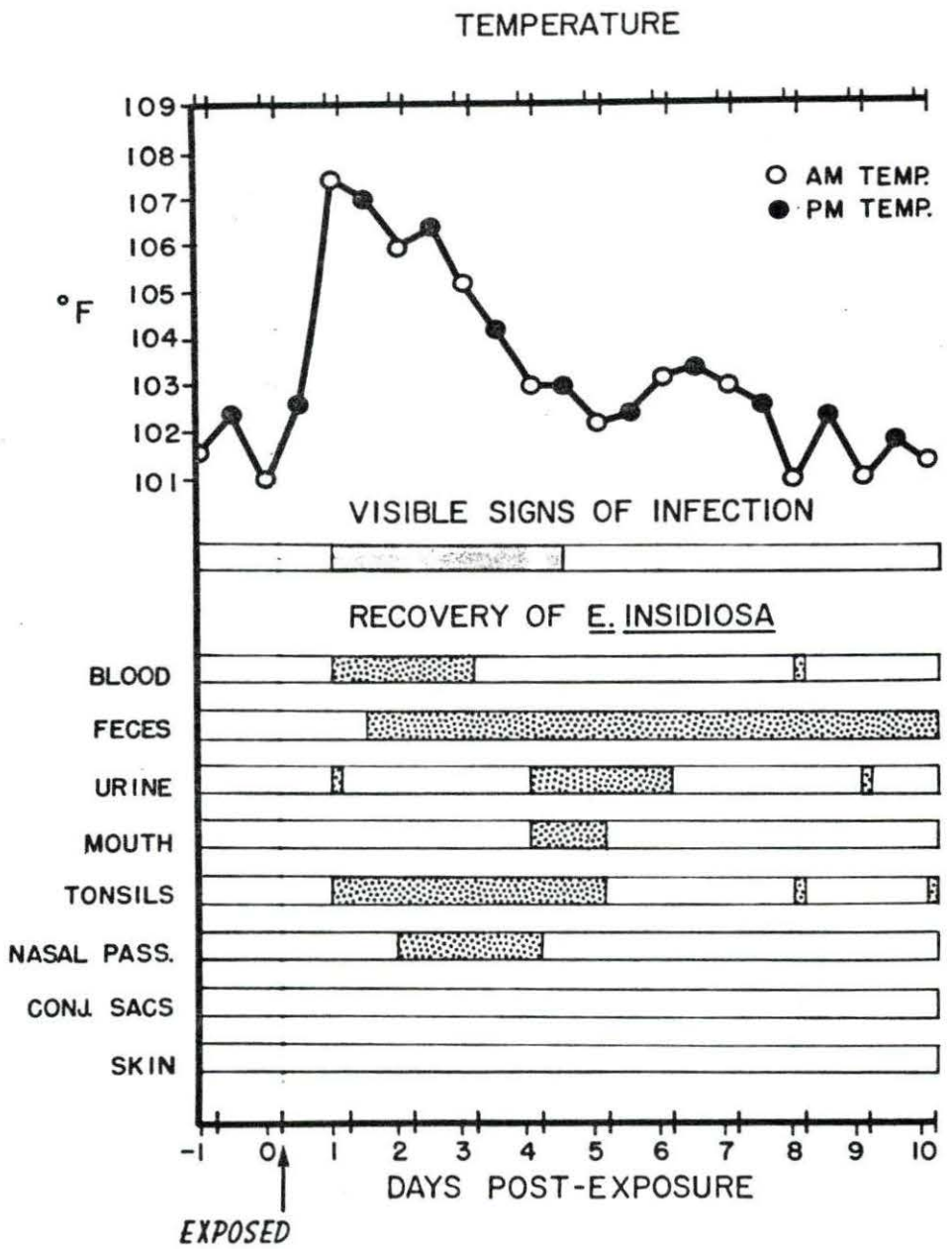

TEMPERATURE
109
108
107
106
105
104
103
102
101
°F
AM TEMP.
PM TEMP.
VISIBLE SIGNS OF INFECTION
RECOVERY OF E. INSIDIOSA
BLOOD
FECES
URINE
MOUTH
TONSILS
NASAL PASS.
CONJ. SACS
SKIN
-1
0
1
2
3
4
5
6
7
8
9
10
DAYS POST-EXPOSURE
EXPOSED

Fig. 33. Summary of data from pig No. 1332

Breed: Mixed; predominantly white in color

Sex: Male

Age: 4 1/2 months

Weight: 127 pounds

Exposure:

Strain: E1-6P

Route: Intramuscular

Amount of culture: 4.0 ml.

Group assignment: 2

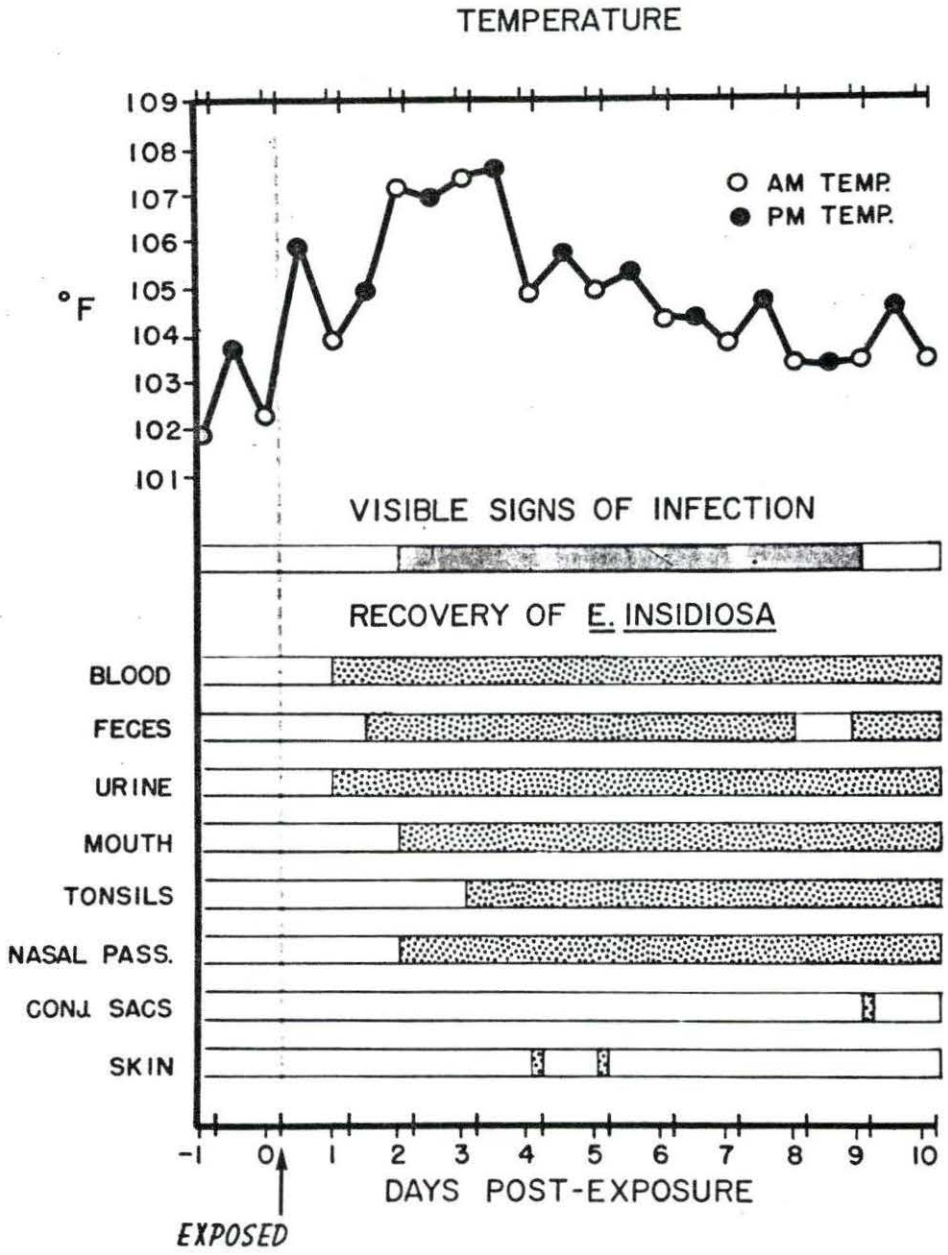

TEMPERATURE
109
108
107
106
105
104
103
102
101
°F
AM TEMP.
PM TEMP.
VISIBLE SIGNS OF INFECTION
RECOVERY OF E. INSIDIOSA
BLOOD
FECES
URINE
MOUTH
TONSILS
NASAL PASS.
CONJ. SACS
SKIN
-1
0
1
2
3
4
5
6
7
8
9
10
DAYS POST-EXPOSURE
EXPOSED

Fig. 34. Summary of data from pig No. 1334

Breed: Mixed; predominantly white in color

Sex: Male

Age: 5 months

Weight: 160 pounds

Exposure:

Strain: HC-585

Route: Intradermal

Amount of culture: 0.1 ml.

Group assignment: 3

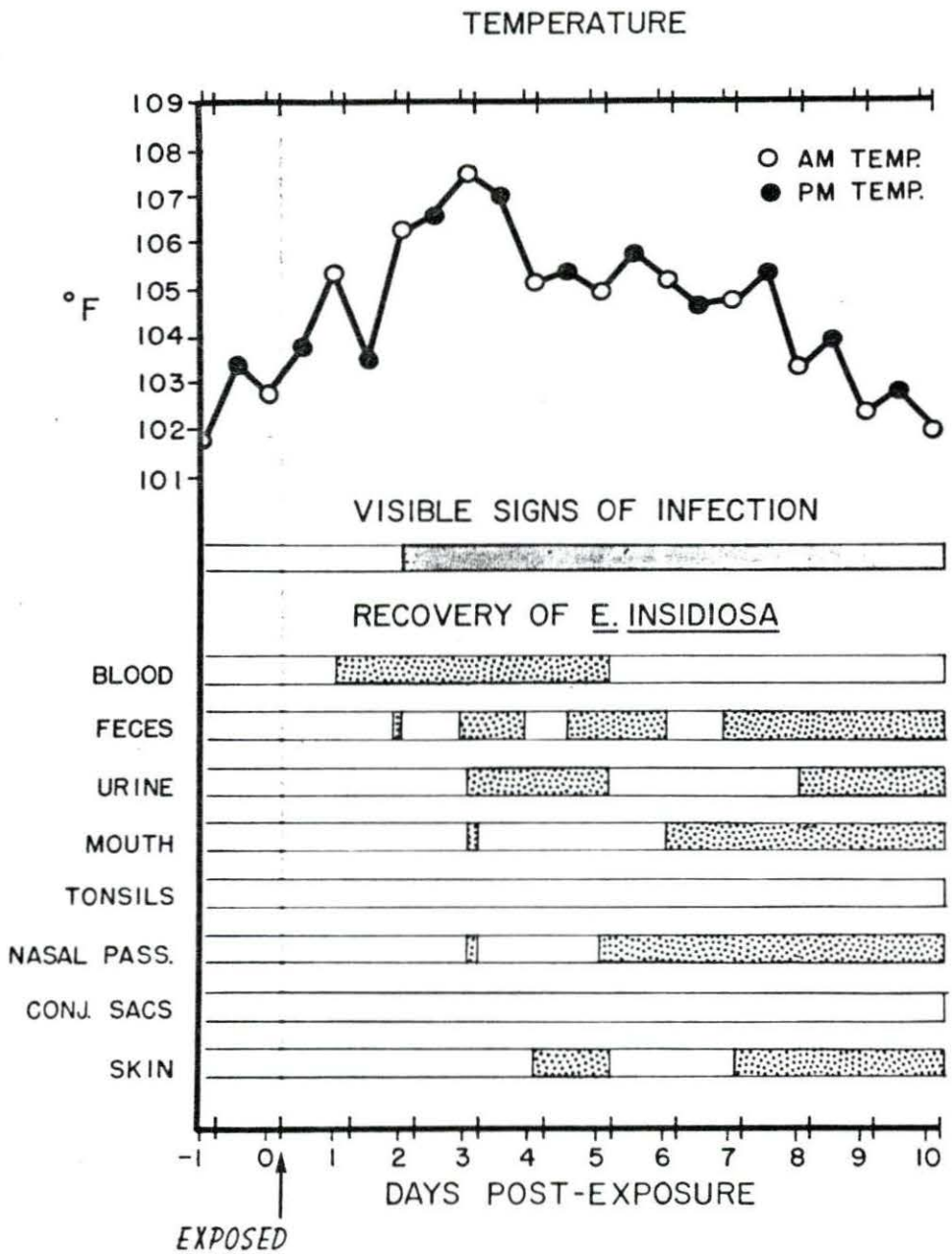

TEMPERATURE
109
108
107
106
105
104
103
102
101
°F
AM TEMP.
PM TEMP.
VISIBLE SIGNS OF INFECTION
RECOVERY OF E. INSIDIOSA
BLOOD
FECES
URINE
MOUTH
TONSILS
NASAL PASS.
CONJ. SACS
SKIN
-1
0
1
2
3
4
5
6
7
8
9
10
DAYS POST-EXPOSURE
EXPOSED

Fig. 35. Summary of data from pig No. 1450

Breed: Mixed; predominantly white in color

Sex: Male

Age: 6 1/2 months

Weight: 150 pounds

Exposure:

Strain: HC-585

Route: Intradermal

Amount of culture: 0.1 ml.

Group assignment: 3

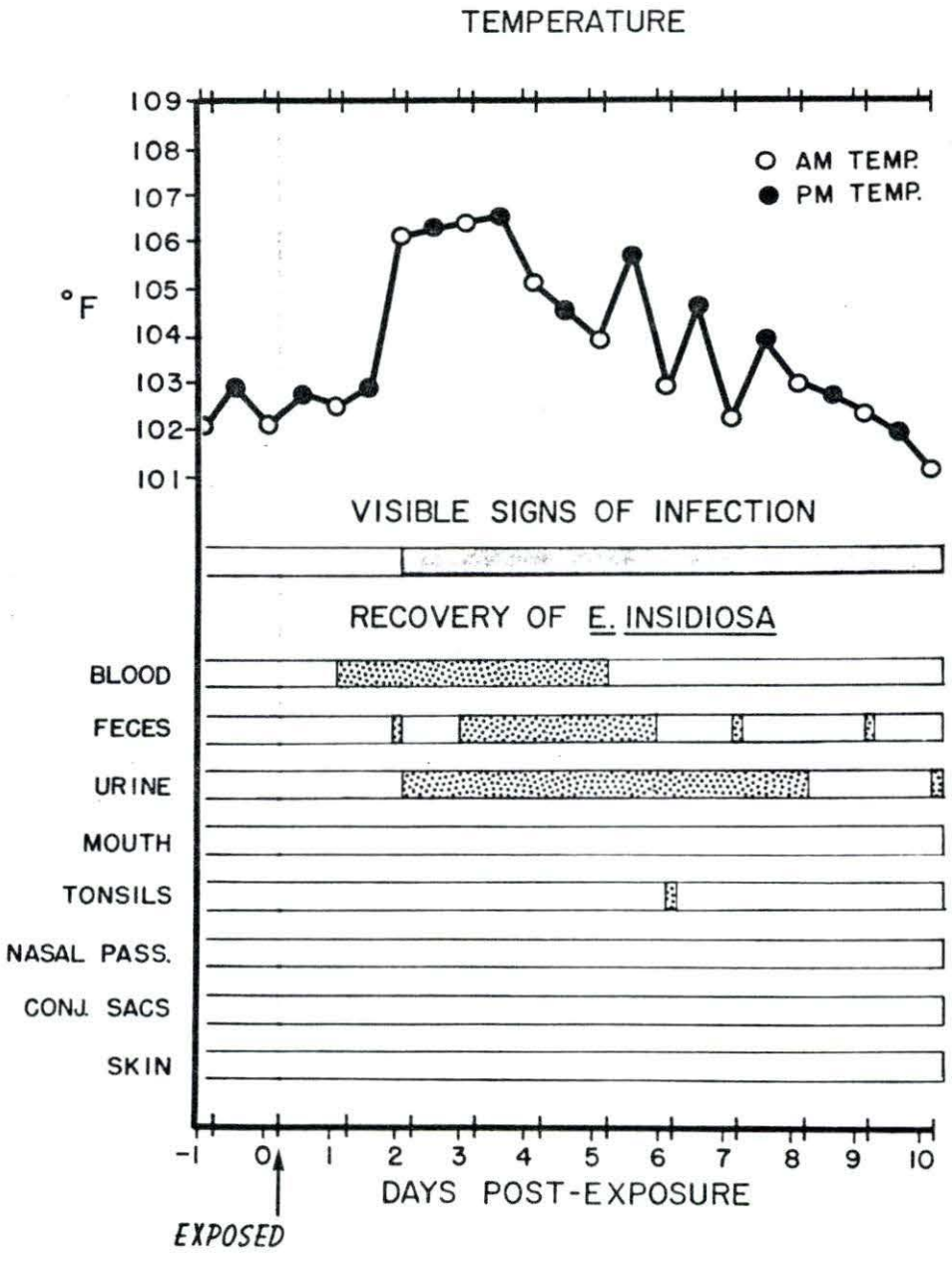

TEMPERATURE
109
108
107
106
105
104
103
102
101
°F
AM TEMP.
PM TEMP.
VISIBLE SIGNS OF INFECTION
RECOVERY OF E. INSIDIOSA
BLOOD
FECES
URINE
MOUTH
TONSILS
NASAL PASS.
CONJ. SACS
SKIN
-1
0
1
2
3
4
5
6
7
8
9
10
DAYS POST-EXPOSURE
EXPOSED

Fig. 36. Summary of data from pig No. 1333A

Breed: Mixed; predominantly white in color

Sex: Female

Age: 5 1/2 months

Weight: 145 pounds

Exposure:

Strain: HC-585

Route: Intradermal

Amount of culture: 0.1 ml.

Group assignment: 3

Note: The positive fecal sample shown on day 4 post-exposure was considered to be the result of contamination from urine.

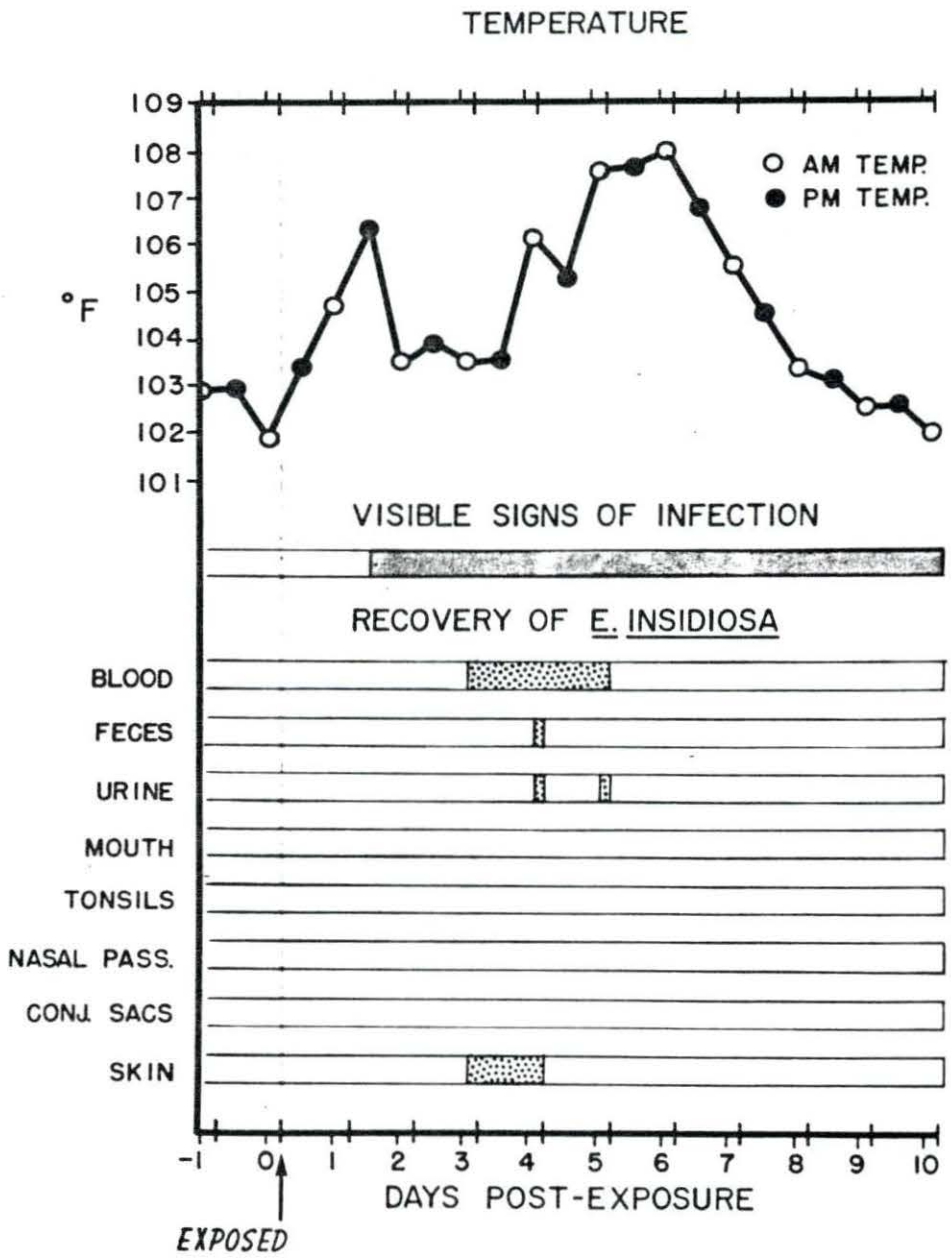

TEMPERATURE
109
108
107
106
105
104
103
102
101
°F
AM TEMP.
PM TEMP.
VISIBLE SIGNS OF INFECTION
RECOVERY OF E. INSIDIOSA
BLOOD
FECES
URINE
MOUTH
TONSILS
NASAL PASS.
CONJ. SACS
SKIN
-1
0
1
2
3
4
5
6
7
8
9
10
DAYS POST-EXPOSURE
EXPOSED

Fig. 37. Summary of data from pig No. 1452A

Breed: Mixed; predominantly white in color

Sex: Female

Age: 7 1/2 months

Weight: 136 pounds

Exposure:

Strain: HC-585

Route: Intradermal

Amount of culture: 0.1 ml.

Group assignment: 3

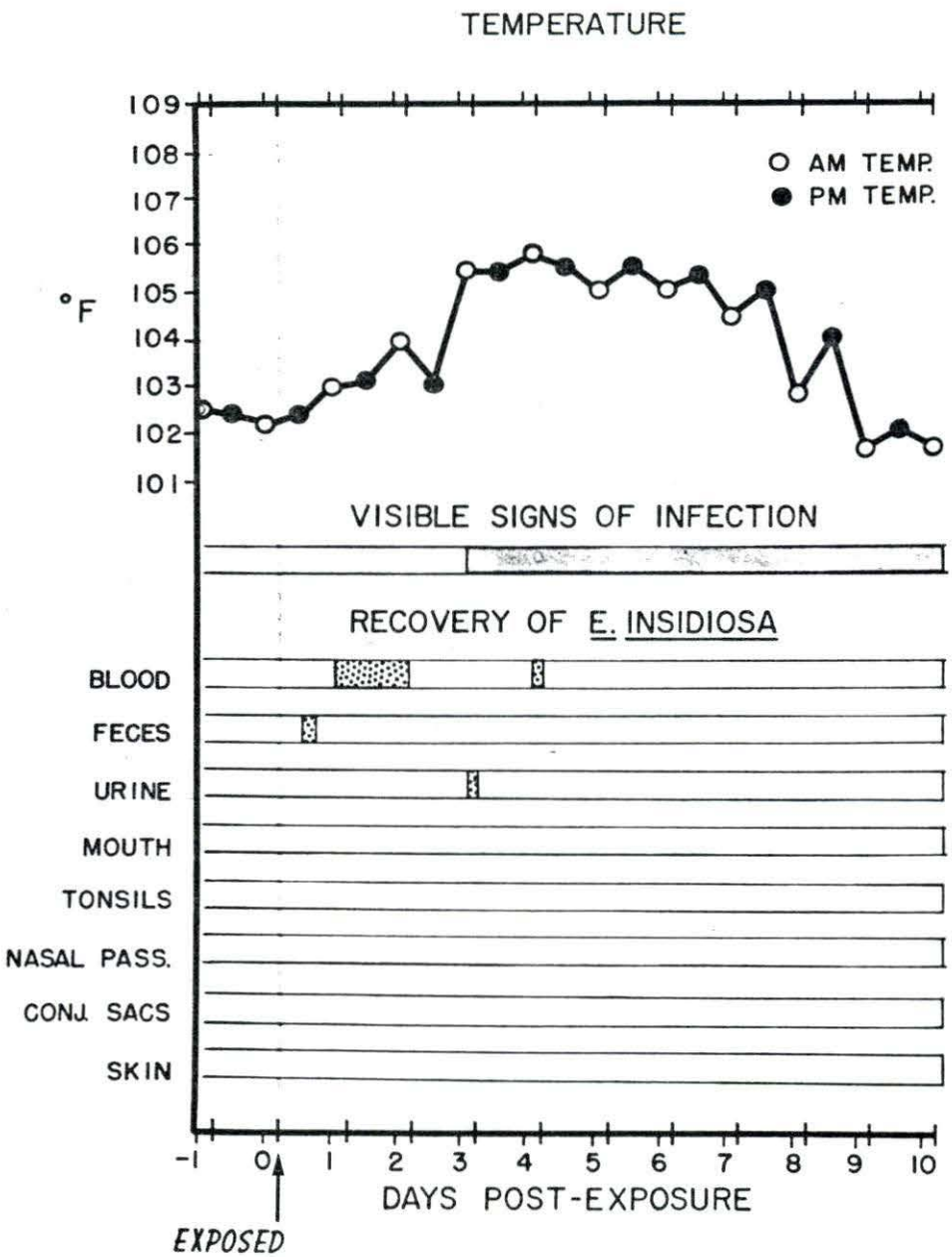

TEMPERATURE
109
108
107
106
105
104
103
102
101
°F
AM TEMP.
PM TEMP.
VISIBLE SIGNS OF INFECTION
RECOVERY OF E. INSIDIOSA
BLOOD
FECES
URINE
MOUTH
TONSILS
NASAL PASS.
CONJ. SACS
SKIN
-1
0
1
2
3
4
5
6
7
8
9
10
DAYS POST-EXPOSURE
EXPOSED

Fig. 38. Summary of data from pig No. 1446

Breed: Mixed; white in color

Sex: Male

Age: 5 months

Weight: 120 pounds

Exposure:

Strain: de Castro

Route: Intravenous

Amount of culture: 5.0 ml.

Group assignment: 4

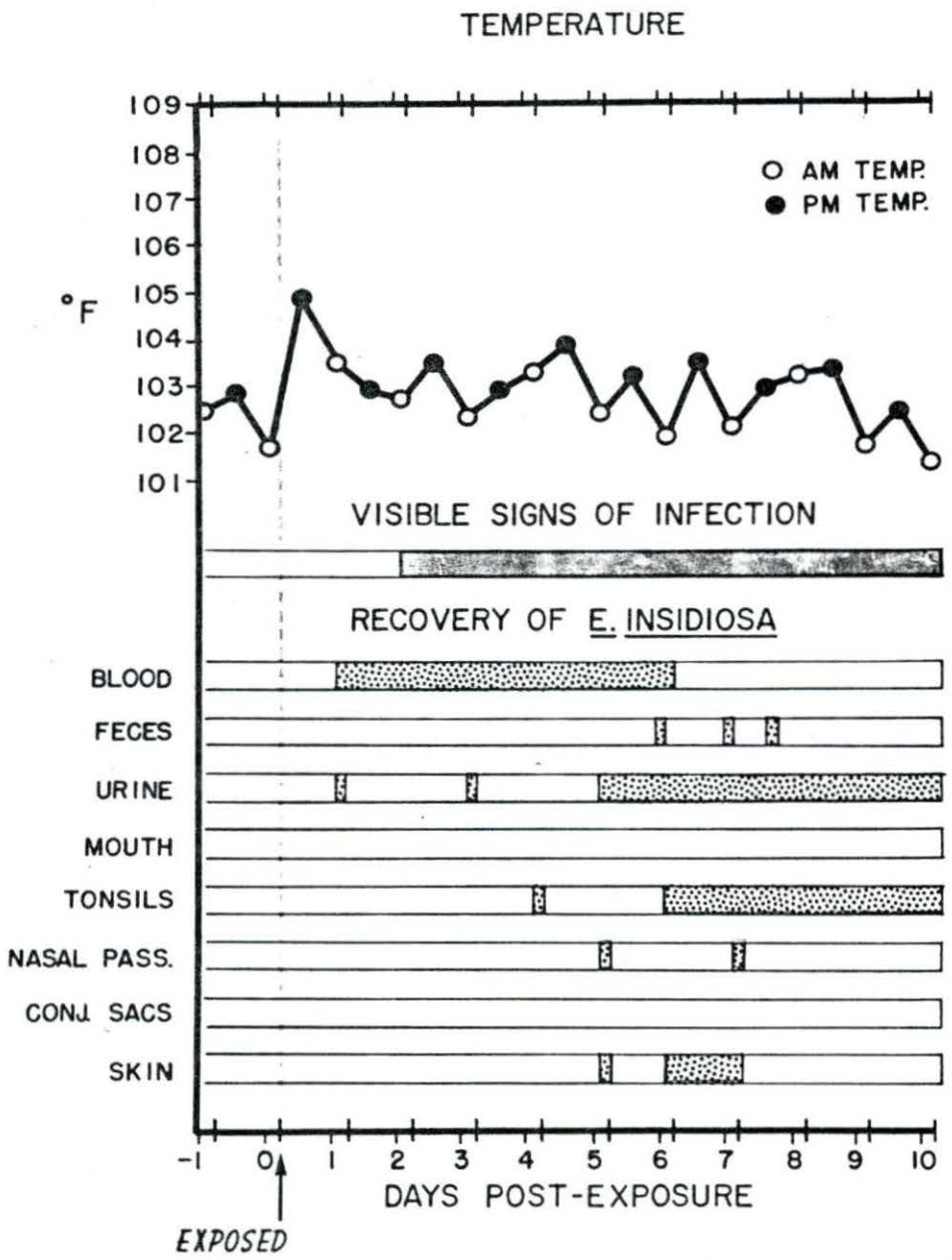

TEMPERATURE
109
108
107
106
105
104
103
102
101
°F
O AM TEMP.
● PM TEMP.
VISIBLE SIGNS OF INFECTION
RECOVERY OF E. INSIDIOSA
BLOOD
FECES
URINE
MOUTH
TONSILS
NASAL PASS.
CONJ. SACS
SKIN
-1
0
1
2
3
4
5
6
7
8
9
10
DAYS POST-EXPOSURE
EXPOSED

Fig. 39. Summary of data from pig No. 1447

Breed: Mixed; white in color

Sex: Male

Age: 6 months

Weight: 143 pounds

Exposure:

Strain: de Castro

Route: Intravenous

Amount of culture: 5.0 ml.

Group assignment: 4

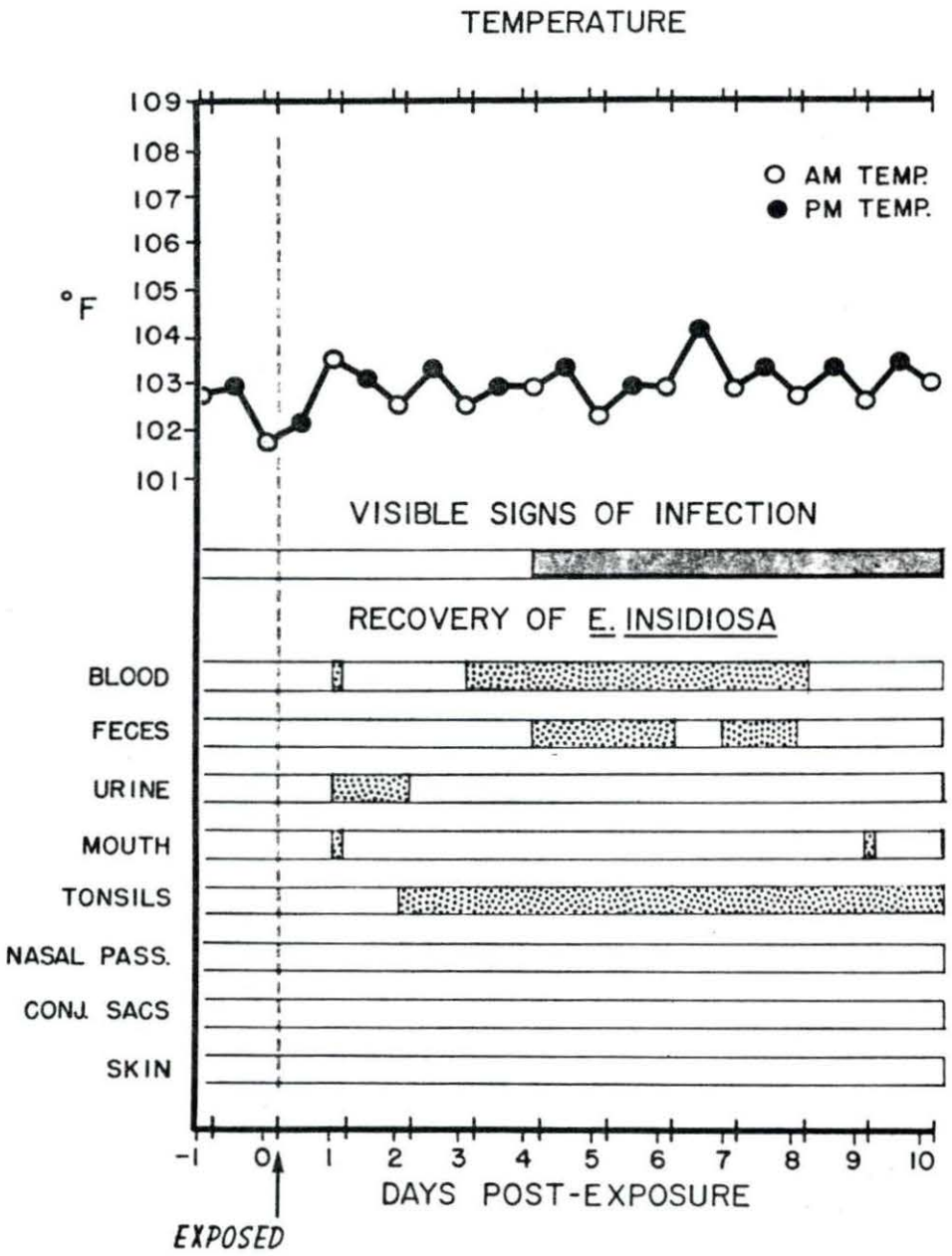

TEMPERATURE
109
108
107
106
105
104
103
102
101
°F
O AM TEMP.
● PM TEMP.
VISIBLE SIGNS OF INFECTION
RECOVERY OF E. INSIDIOSA
BLOOD
FECES
URINE
MOUTH
TONSILS
NASAL PASS.
CONJ. SACS
SKIN
-1
0
1
2
3
4
5
6
7
8
9
10
DAYS POST-EXPOSURE
EXPOSED

Fig. 40. Summary of data from pig No. 1449

Breed: Mixed; white in color

Sex: Female

Age: 5 1/2 months

Weight: 143 pounds

Exposure:

Strain: de Castro

Route: Intravenous

Amount of culture: 5.0 ml.

Group assignment: 4

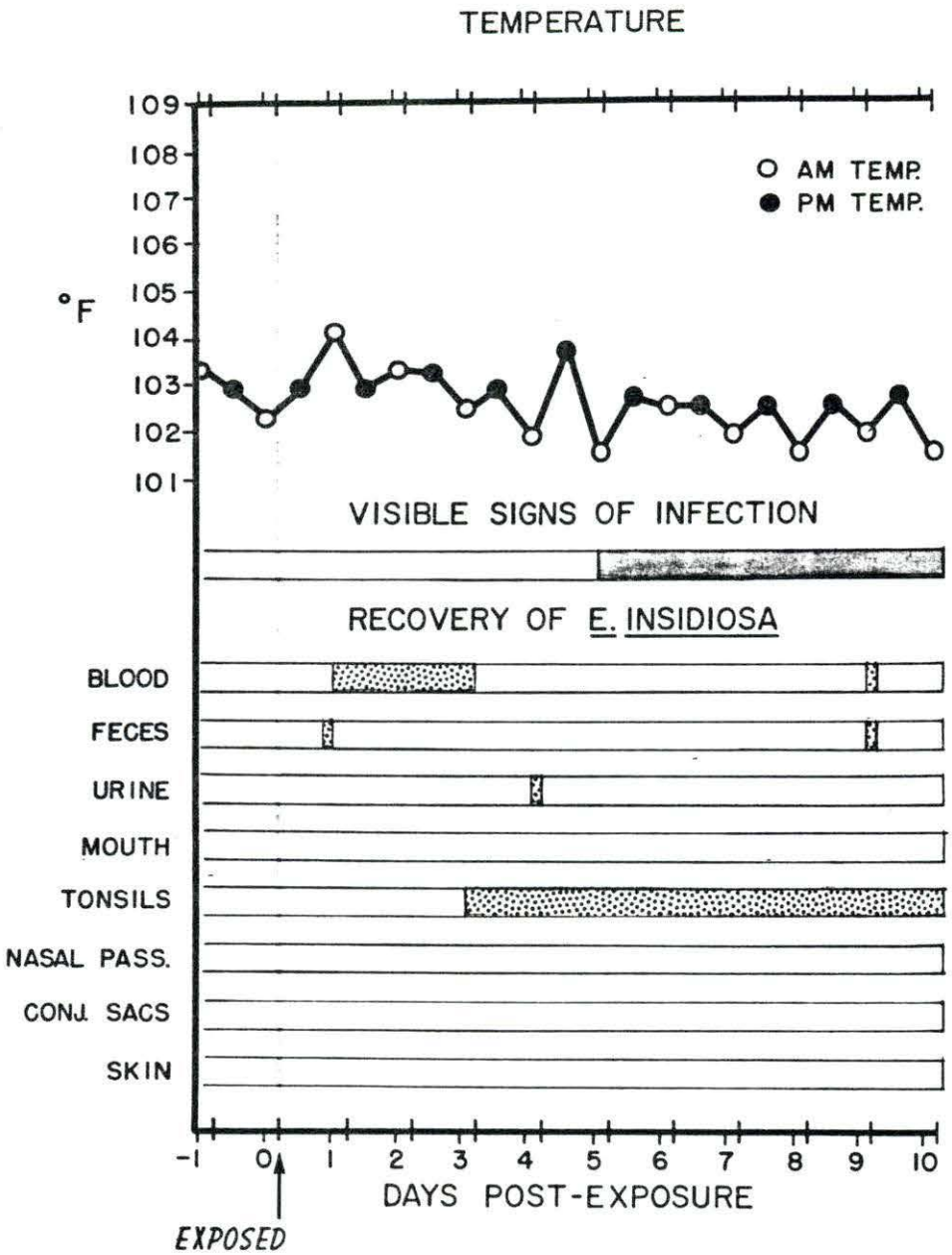

TEMPERATURE
109
108
107
106
105
104
103
102
101
°F
O AM TEMP.
● PM TEMP.
VISIBLE SIGNS OF INFECTION
RECOVERY OF E. INSIDIOSA
BLOOD
FECES
URINE
MOUTH
TONSILS
NASAL PASS.
CONJ. SACS
SKIN
-1
0
1
2
3
4
5
6
7
8
9
10
DAYS POST-EXPOSURE
EXPOSED

Fig. 41. Summary of data from pig No. 1451A

Breed: Mixed; white in color

Sex: Female

Age: 6 months

Weight: 170 pounds

Exposure:

Strain: de Castro

Route: Intravenous

Amount of culture: 5.0 ml.

Group assignment: 4

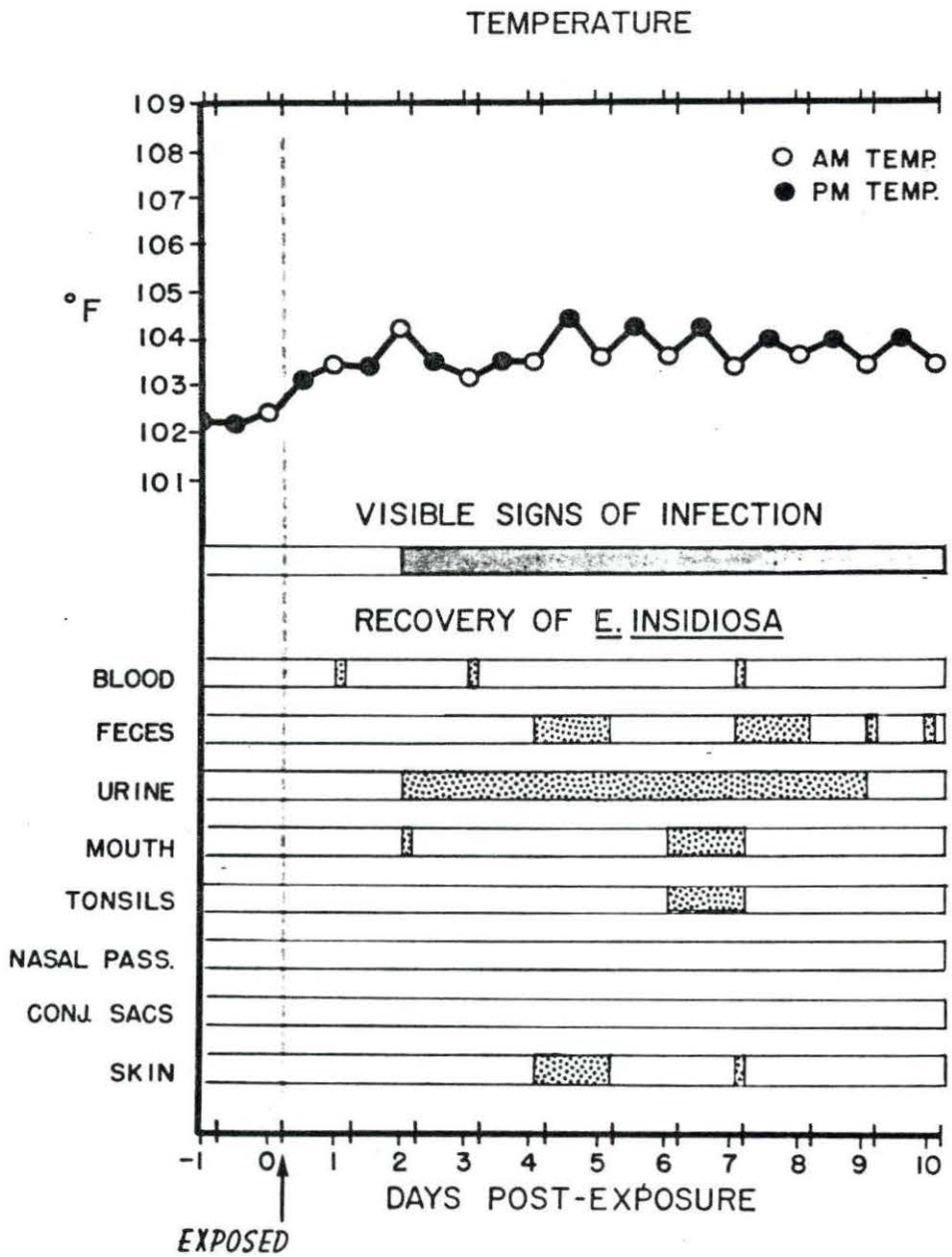

TEMPERATURE
109
108
107
106
105
104
103
102
101
°F
AM TEMP.
PM TEMP.
VISIBLE SIGNS OF INFECTION
RECOVERY OF E. INSIDIOSA
BLOOD
FECES
URINE
MOUTH
TONSILS
NASAL PASS.
CONJ. SACS
SKIN
-1
0
1
2
3
4
5
6
7
8
9
10
DAYS POST-EXPOSURE
EXPOSED

Fig. 42. Summary of data from pig No. 1345

Breed: Mixed; white in color

Sex: Male

Age: 6 months

Weight: 123 pounds

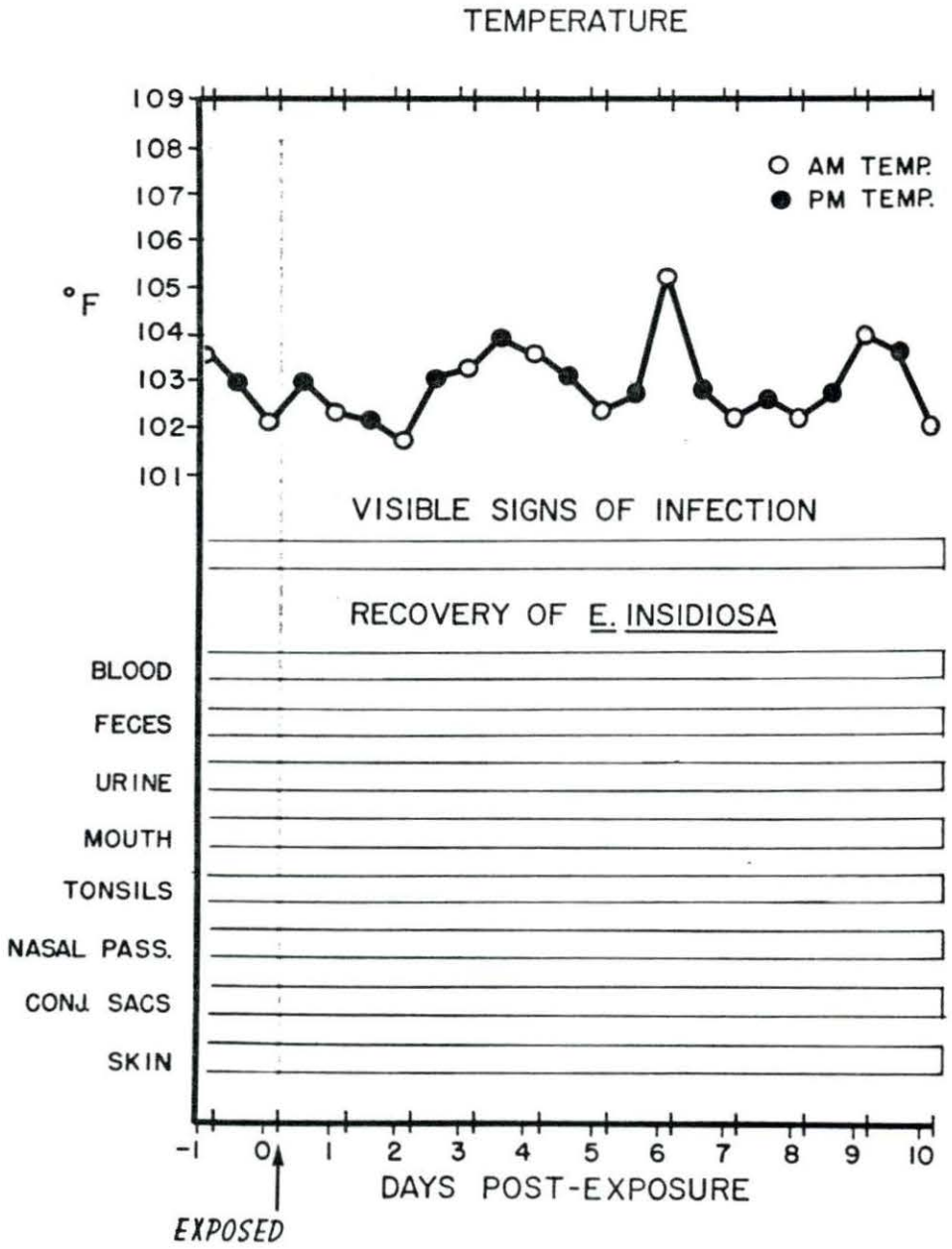
Exposure:

Strain: de Castro

Route: Intradermal

Amount of culture: 0.1 ml.

Group assignment: 5


TEMPERATURE
109
108
107
106
105
104
103
102
101
°F
AM TEMP.
PM TEMP.
VISIBLE SIGNS OF INFECTION
RECOVERY OF E. INSIDIOSA
BLOOD
FECES
URINE
MOUTH
TONSILS
NASAL PASS.
CONJ. SACS
SKIN
-1
0
1
2
3
4
5
6
7
8
9
10
DAYS POST-EXPOSURE
EXPOSED