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The Transmission of Mycobacterium Avium Among Swine: An Immunologic and Bacteriologic sSudy

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THE TRANSMISSION OF *MYCOBACTERIUM AVIUM* AMONG SWINE: AN IMMUNOLOGIC AND BACTERIOLOGIC STUDY

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Advisor Date

Head, Microbiology Department Date

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Introduction

The incidence of tuberculosis in swine at federally inspected abattoirs has steadily declined since 1922 when 16.38% of all carcasses were retained because of this infection (54). This decline has been attributed to many factors, most notably, the reduced incidence of tuberculosis in cattle and poultry due to eradication programs and changes in animal husbandry and management practices (54). These factors have combined to reduce the retention of swine carcasses because of tuberculosis to less than 0.85% in 1976 (44).

Before 1972, infected tissue in carcasses with localized tuberculoid lesions was simply removed and the carcasses were processed as usual. Only carcasses with generalized infections were condemned (6). In 1972, tuberculosis in swine again became a major economic problem when USDA Meat and Poultry Inspection Division regulations regarding the handling of carcasses with localized infections were changed. The new regulations require carcasses with tuberculoid lesions in two primary sites (regional lymph nodes) to be cooked 30 minutes at 77 C before being used for human food. The entire carcass is condemned if lesions are found in more than two primary sites or if evidence of a generalized infection is found (6,45). Since a cooked carcass loses two-thirds of its original value, the new regulations greatly increase the economic loss due to porcine tuberculosis. The loss from the 81,777,000 swine slaughtered at federally inspected abattoirs in the United States in 1974 was estimated to be $5,485,070, of which $3,223,270 (59%) was due to the cooking of carcasses (17).

Mycobacterium avium is the major cause of tuberculosis in swine (53). The natural reservoir of this organism is assumed to be birds, particularly poultry. Federal meat inspection records show that efforts to reduce tuberculosis in poultry over the last 55 years have resulted in an accompanying decline of tuberculosis in swine (54). Studies have shown, however, that *M. avium* infections in swine cannot always be traced to birds, and the disease is common in those regions where tuberculosis in poultry is rare (53). Also serotypes not normally pathogenic for birds are commonly isolated from swine (40,51, 53). Indeed, many swine farms today appear to have recurring tuberculosis with no obvious source of infection. Sources of infection other than poultry need to be identified and their importance determined.

In the past, swine were not considered to be an important source of infection among themselves. Results of limited studies have shown that there is a possibility that swine can acquire mycobacterial infections from infected penmates (15,35,54). The study presented here was designed to: 1. determine if infected swine can transmit *M. avium* to uninfected penmates, 2. provide information as to possible mechanisms of transmission, and 3. determine how soon after exposure swine become infected and how long an animal remains infected and a source of infection.

Literature Review

Swine mycobacterial infections in the United States are caused primarily by organisms of the M. avium complex (53). M. avium complex is the name proposed for the slow-growing, nonchromogenic, acid-fast bacteria that were formerly divided into two species, M. avium and M. intracellulare (60). The two organisms can be differentiated only by serotyping or, to some extent, by animal pathogenicity (61). M. avium is pathogenic for chickens and rabbits, but rarely for man. M. intracellulare is relatively nonpathogenic for laboratory animals and chickens, but causes an "atypical" mycobacterial infection in man (40). M. bovis and M. tuberculosis may also infect swine, but can be readily differentiated from organisms of the M. avium complex by biochemical and colony characteristics. Some rapid-growing mycobacteria occasionally infect swine (51,53).

Because swine are not often tuberculin skin tested and rarely have clinical signs of the disease, tuberculosis in swine is usually discovered during inspection at slaughter (54). The disease may occur either as a localized or as a generalized infection. Localized infections are usually caused by organisms of the M. avium complex. This infection is nonprogressive and is characterized by thinly encapsulated, caseous to slightly calcified lesions in one or more lymph nodes, usually of the head and digestive tract. Lesions are microscopic to 4 or 5 mm in diameter and may coalesce to involve the entire lymph node. Swine with localized infections apparently suffer no adverse effects and are normal in appearance and growth (24,54). Localized infections accounted

for nearly all of the swine retained for tuberculosis at federally inspected abattoirs in 1976 as only 0.9% of the swine retained were condemned due to a generalized infection (44). Generalized tuberculosis, more commonly caused by M. bovis, is characterized by small, well-encapsulated lesions in the liver, spleen, kidney, and lungs. It may progress into extensive nodular lesions involving these organs, and signs such as dyspnea, diarrhea, and anorexia may occur in the latter stages. Enlarged lymph nodes may interfere with organ function (54).

Sources of Mycobacterial Infections for Swine

It is generally assumed that the sources of mycobacterial infections for swine are reservoirs of the organisms in other species. Evidence for this is found in numerous studies showing the ease with which swine may become infected with tubercle bacilli, either by direct contact with other animals or by ingesting animal byproducts and wastes. Results of studies have shown that swine can become infected with M. avium, the avian tubercle bacillus, after contact with tuberculous chickens or an environment previously occupied by tuberculous fowl (13,15,24,41,50, 51). In addition the incidence of M. avium infections in swine continues to be greatest in those regions of the United States where the incidence in poultry remains highest (51). Outbreaks of swine tuberculosis caused by the human tubercle bacillus, M. tuberculosis, have been traced to feeding swine garbage from a tuberculosis sanatorium and improper disposal of human sewage (24). Outbreaks of tuberculosis in swine due to the bovine tubercle bacillus, M. bovis, have been traced to feeding them offal from abattoirs or milk from tuberculous

cows (24,54). It has been suggested that the species of tubercle bacillus that infects swine in a region might reflect the species infecting other animals in the region (24,54). Results of surveys done since the 1920's have shown that M. avium caused the majority of mycobacterial infections in swine in the United States (52,54).

Efforts to control tuberculosis in other animals have resulted in a corresponding decline of tuberculosis in swine. For example, modern poultry management practices have resulted in reducing the condemnation of mature chicken carcasses because of tuberculosis from 274/100,000 in 1962 to less than 10/100,000 in 1976. During the same period, the incidence of tuberculosis in swine decreased from 2.25% to 0.78% (44,51). A similar decrease in the incidence of M. bovis infections in swine accompanied the reduction in number of infections in cattle. M. bovis infected almost 5% of the cattle in the United States in 1918 and caused as much as 6.6% of the swine tuberculosis detected during the 1920's and 30's (44,54). As a result of the Cooperative State-Federal Bovine Tuberculosis Eradication Program, M. bovis infections in cattle were reduced to less than 0.08% of the total population by 1975 (44). During the same period, the infection in swine has become very rare (53). Cooking garbage and offal intended for feed, the practice of raising one animal species on a farm, and segregating swine from other farm animals have also played important roles in reducing the incidence of swine tuberculosis (24,54).

Results of studies have shown, however, that all M. avium complex infections of swine cannot be traced to poultry. Using serologic and

chicken pathogenicity tests, Schaefer (40) studied 81 M. avium complex organisms isolated from swine. Thirty-nine (49%) of the isolates were found to be serotypes 1 or 2, those serotypes mainly associated with tuberculosis in poultry. Twenty-eight of these isolates were injected into chickens and 25 were infective. The other 42 isolates were distributed among nine other serotypes. Twenty-four of these were tested, and only six were pathogenic for chickens. Schaefer concluded that such a high incidence of infection with strains not pathogenic for chickens indicated sources other than tuberculous birds and suggested soil related sources. Mallman (25) tested 43 M. avium swine isolates and only 28 were pathogenic for chickens.

Several outbreaks of swine tuberculosis due to non-avian serotypes have been reported (9,26,32,36,43). From 40 to 70% of the swine in these reports had gross tuberculous lesions. In some cases, the source of infection was determined to be contaminated saw dust bedding which was a continuous source of infection until removed. In other cases, no source of infection could be found.

Between July 1971 and June 1974, personnel of the Animal and Plant Health Inspection Service of the USDA isolated mycobacteria from the lymph nodes of 1547 (78%) of 2036 swine carcasses retained as tuberculous at federally inspected abattoirs (53). M. avium complex organisms accounted for 97% of the mycobacteria isolated. Seventy-six percent of the M. avium complex organisms were serotypes 1 and 2. The incidence of infections with serotypes 1 and 2 varied with different regions of the country. In the west-north central region, for example,

87% of the M. avium complex isolations were serotypes 1 or 2, while in the south central region, these serotypes accounted for only 35% of the isolates. It is of interest that in the western and south central regions, where 71% and 35% respectively of the isolations were serotypes 1 or 2, tuberculosis in chickens is very rare (51). This indicates that even for those serotypes normally associated with poultry there are other sources of infection for swine.

The ecology of M. avium complex organisms is not well understood. The organisms have been isolated from many sources, and they are able to persist in the environment for considerable time. Shchpilov and L'vova (42) were able to isolate virulent organisms from litter in a poultry house four years after it was contaminated by tuberculous ducks. Temperature extremes of -40 C to +30 C had occurred. Shalk et al (41) buried tuberculous poultry carcasses outside in soil at depths up to three feet. Almost two and one-half years later, material removed from the deeper levels was still infective to chickens. M. avium has survived as long as two years on straw and hay stored outdoors (1,20). There are reports of isolations of M. avium from saw dust and litter bedding (4,19,39), certain swine feed (fish meal and protein meal and concentrate) (19), soil (59), water (14), and ticks and bedbugs found in chicken coops (7,51). M. avium has also been isolated from many species of wild birds (8). Conceivably, all of these could be sources of infection to swine, although the role each might play in the overall problem has not been determined.

Swine to Swine Transmission of M. avium

It has been suggested that infected swine may transmit M. avium

to penmates. Circumstantial evidence for this was provided as far back as 1926 by Graham and Tunnicliff (15). They observed that pigs negative to the tuberculin skin test could become positive after continuous association with tuberculin-test positive pigs, although transmission from infected poultry to pigs occurred more readily. Results of recent investigations have shown that swine to swine transmission of M. avium can occur. In 1972, Ray et al (35) demonstrated that previously uninfected swine may develop positive tuberculin skin reactions and tuberculous lesions after being housed with swine orally inoculated with 2 mg of an M. avium isolate of porcine origin. Two of six artificially inoculated animals killed 57 days post inoculation (PI) had lesions limited to the mesenteric lymph nodes. The two contact animals also had mesenteric lymph node lesions when they were necropsied 177 days after first contact. Three of the six inoculated animals were tuberculin skin test positive 42 days PI and all were positive when tested 98 days PI. The contact pigs were negative when tested after 42 days contact but were positive after 98 days. Recently, Thoen et al (55) reported two pigs became infected when kept in contact with two pigs orally infected with 50 mg M. avium serotype 8. The same serotype organism was isolated from cervical and mesenteric lymph nodes of pigs of both groups 92 days after inoculation and exposure. The two inoculated swine were skin test positive and positive for lymphocyte immunostimulation to the purified protein derivative (PPD) of M. avium tuberculin when tested 10 and 12 weeks PI. One contact pig was skin test positive 10 weeks after exposure and positive for lymphocyte

immunostimulation after 12 weeks. Both pigs were skin test positive after 12 weeks contact.

A likely source of infectious material is feces from infected penmates. Thoen and Karlson (54) described lesions caused by M. avium in the submucosa of the intestinal tract of swine and postulated that such lesions could ulcerate and discharge bacilli into the feces. Graham and Tunnicliff (15) infected tuberculin-test negative chickens by injecting them with rectal scrapings from ten tuberculin-test positive pigs. Twenty-two (17%) of 77 such samples obtained over a three month period proved infective to chickens. During a study of the effectiveness of a BCG (M. bovis strain bacille Calmette-Guerin) vaccine in swine, Tammemagi and Simmons (46) cultured feces and urine from 12 swine every two weeks for four months after the animals had been orally inoculated with 0.2 mg M. intracellulare serotype VI (M. avium complex serotype 6). The organism was isolated from the feces of one animal two and four weeks PI and from another six weeks PI. The organism was not isolated from any of the urine samples. In 1971 (47) the same authors reported isolating the same serotype from feces and urine of a pig orally inoculated 89 days previously with 10 mg of the organism. Tuberculous lesions were present in the mesenteric lymph nodes at necropsy nine days later. The organism was isolated from mesenteric and gastrohepatic lymph nodes and from kidney. The authors were unable to isolate the organism from the urine or feces of another animal injected intramuscularly with the same inoculum. No uninoculated animals were kept in contact with these animals. However, in the study by Thoen et al (55) M. avium

was not isolated from feces and urine collected at necropsy from either the inoculated or the contact swine that became infected.

In Vitro Lymphocyte Immunostimulation (LI)

Antigens which cause delayed hypersensitivity also cause in vitro transformation of lymphocytes derived from animals sensitized to the antigen. This transformation is a cellular blastogenesis characterized by an increased rate of metabolism with synthesis of protein, RNA, and DNA, and results in cell enlargement and division (34). The lymphocyte immunostimulation test (LI), which quantitatively measures the transformation of lymphocytes, is an in vitro correlate of the skin test. LI has been shown to be specific and to correlate with the skin test in humans and animals naturally or experimentally infected or sensitized with various species of mycobacteria (5,10,29,30,31).

In the LI test, a known number of lymphocytes is cultured with the antigen (test culture) and the same number is cultured without the antigen (control culture). Lymphocytes in the test culture are transformed if the animal from which they were derived has been previously sensitized to the antigen. Tritiated thymidine added to the culture medium is incorporated into the synthesized DNA of the lymphocytes. The amount of thymidine incorporated during the incubation period is proportional to the rate of lymphocyte metabolism. The metabolic rate of both the test culture and the control culture are measured by collecting the lymphocyte DNA and measuring the radioactivity of each in a scintillation spectrometer. A stimulation index is then calculated by dividing the counts per minute of the test culture by the counts per minute of the

control culture. A stimulation index of 2.0 or higher has been considered a positive indication of immunostimulation by tuberculin (10,28).

Because of shared antigens, one species of mycobacteria which sensitizes an animal may cause hypersensitivity or positive LI indexes to mycobacterins[1] prepared from other species. In such cases, LI may be better than multiple skin tests for identifying the infecting species. For example, using LI, several test cultures each containing a mycobacterin of a different species can be tested at one time and compared to a single control culture. With experimentally infected animals, it has been shown that a homologus mycobacterin produces a higher LI index than do heterologus mycobacterins (2,10,28,29,30,31). The results of simultaneous skin test reactions may not be quantitated as easily. Two factors may alter simultaneous skin test reactions: 1. the total amount of antigen injected into all sites, which influences the size of each reaction (3,23), and 2. the relative concentration of the mycobacterins, wherein a reaction to the more dilute sensitin may be masked (3). In addition the size of a skin test reaction may vary with the injection site (22).

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[1]The conventional use of the term tuberculin applies only to those sensitins prepared from tubercle bacilli (38). Similar preparations from other mycobacterial species are named by adding "in" to the end of the species name, e.g., gordonin from M. gordonae. Mycobacterin is a collective term indicating such a testing agent prepared from any species of Mycobacterium.

In a skin test, most of the tuberculin leaves the injection site within a few hours (33). The LI test may give a more definite reaction than the skin test because all of the antigen remains available to the lymphocytes throughout the incubation period (11). It has been suggested that certain factors which would not alter the results of the *in vitro* test could influence the results of the skin test. Among these are: concurrent infections, the state of the disease, changes in internal milieu, and stress (2,29,30). For example, although an individual may be rendered anergic to the skin test by a severe, active tuberculous infection, the lymphocytes from that individual will give a positive *in vitro* LI reaction (16).

Monitoring the spread and development of disease by repeatedly skin testing individuals during a short period could produce erroneous results. It has been shown that uninfected individuals skin tested with tuberculin can become sensitized and produce false-positive responses to a test given two days later. Conversly, sensitized individuals who react to a skin test may fail to react or have reduced reactions on another test given two days later (49). It has also been shown that skin testing sensitized or non-sensitized individuals can either enhance or suppress the *in vitro* transformation of lymphocytes from the blood of these individuals for as long as three weeks following the skin test (18,48). Because the LI test would not have these effects on the individual's system, it is ideally suited for monitoring disease spread and development when it is necessary to test the individual several times.

Materials and Methods

Source of Animals

The swine used in this study were of mixed breed and were obtained from a local herd with no history of swine tuberculosis. Upon receipt, all animals were skin tested with avian old tuberculin and tested for lymphocyte immunostimulation with M. avium PPD to insure uninfected animals were obtained (procedures described later).

Housing and Care of Animals

The swine were housed for the duration of the study in a building which had several concrete-floored isolation rooms on both sides of a central hallway. Each room could be entered either from the hallway or from outside. The air in the rooms was constantly circulated by small wall fans and fresh air was brought into each room by a ceiling ventilation fan. The door leading outside was often left open to allow fresh air into the room in warm weather.

In two pens in a large room (Figure 1), uninfected swine were housed in direct and indirect (aerosol) contact with the infected swine. The partition separating the pens consisted of two 3-feet high, three-quarter inch plywood walls, one foot apart, which were sealed at the floor to prevent fluid or fecal material in one pen from seeping to the other. At one end of the room was a drainage gutter and conduit common to both pens. The pigs in Pen B were prevented access to the gutter by a metal grating. No measures were taken to exclude the swine in Pen A from the gutter. A group of swine in Part I of the study was housed in a room separate from the others while being artificially infected.

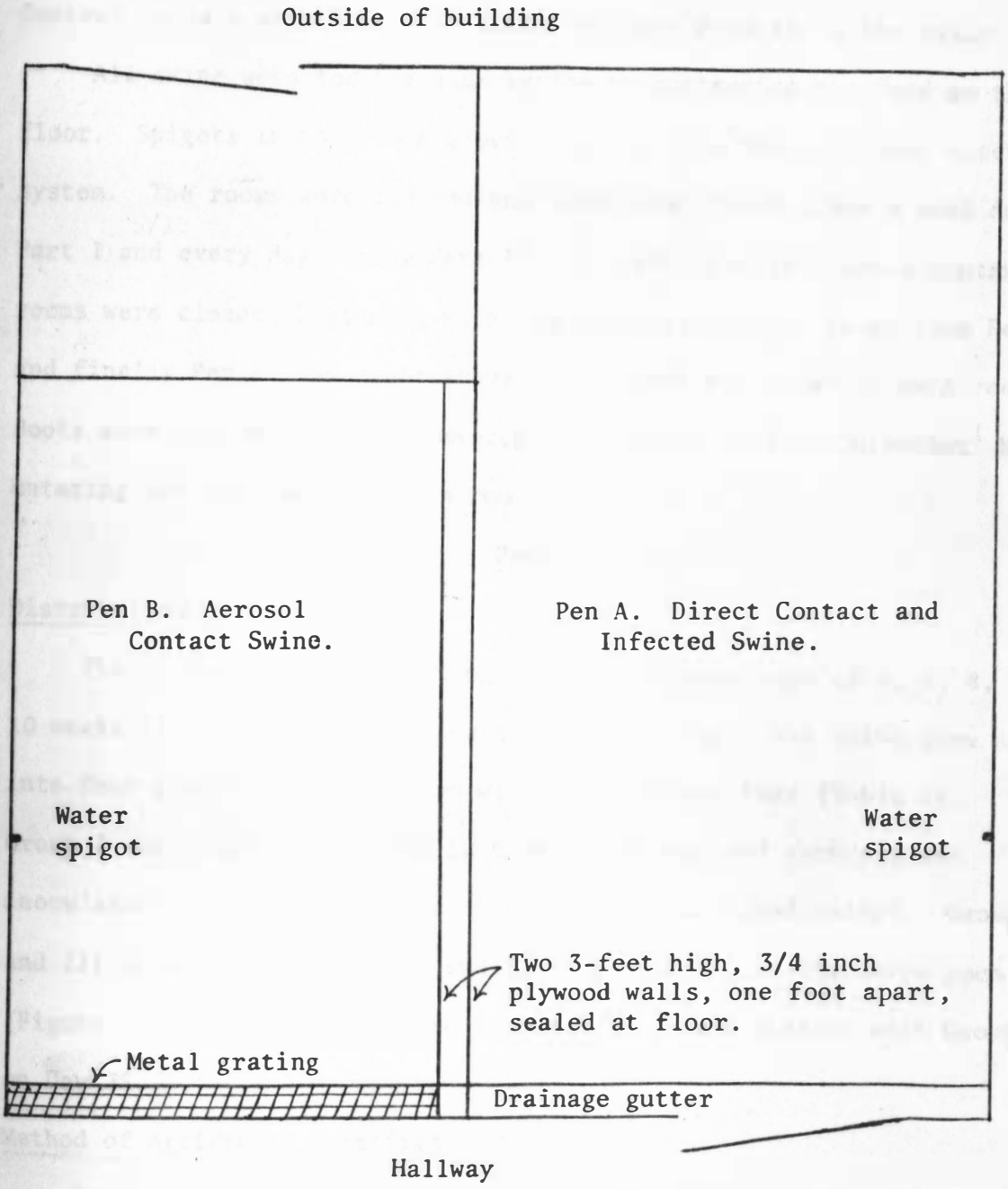

Figure 1. Large room divided into two pens for exposing infected animals to uninfected animals. Scale: 5/16 inch = 1 foot.

Control animals were housed in rooms separate from the other swine.

All swine were fed the same ration by scattering the feed on the floor. Spigots in each room provided water from the municipal water system. The rooms were cleaned and hosed down three times a week during Part I and every day during Part II. To avoid possible cross contamination, rooms were cleaned beginning with the control animals' room, then Pen B, and finally Pen A. Separate shovels were used for cleaning each room. Boots were scrubbed with a commercial tuberculocidal disinfectant[1] before entering and upon leaving each room.

Part I

Distribution of Animals

Thirty-two swine, consisting of eight animals each of 4, 6, 8, and 10 weeks of age, were used in Part I of the study. The swine were divided into four groups and identified with numbered ear tags (Table 1). Group I was originally housed in a separate room and each pig was inoculated with M. avium on Day 0 (procedure described later). Groups II and III were housed in Pens A and B, respectively, in the large room (Figure 1). Group I was placed in Pen A in direct contact with Group II on Day 35.

Method of Artificial Infection

On Day 0, the swine in Group I were inoculated with M. avium serotype 1[2]. The inoculum was prepared by pooling 5 seven-day-old cultures

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[1] Vestal Laboratories (Environ[r]), St. Louis, MO.

[2] Veterinary Services Laboratory, NADL, US Department of Agriculture, Ames, IA.

Table 1. Swine Used in Part I of the Transmission Study.

Group	Animal	Age on Day 0
Group I (Artificially infected). Each animal inoculated with 2.5 mg M. avium on Day 0. Housed in separate room until Day 35, then moved to Pen A for duration of Part I.	110	4 weeks
	118	
	114	6 weeks
	115	
	116	8 weeks
	117	
	111	10 weeks
	112	
Group II (Direct contacts). Housed in Pen A for duration of Part I. In direct contact with Group I after Day 35.	120	4 weeks
	127	
	121	6 weeks
	126	
	122	8 weeks
	125	
	123	10 weeks
	124	
Group III (Aerosol contacts). Housed in Pen B for duration of Part I. In indirect contact with Group I after Day 35.	130	4 weeks
	132	
	133	6 weeks
	136	
	135	8 weeks
	137	
	131	10 weeks
	134	
Group IV (Control animals). Housed in separate room for duration of Part I.	101	4 weeks
	102	
	103	6 weeks
	104	
	105	8 weeks
	106	
	108	10 weeks
	109	

of the organism grown in 7 ml Dubos broth[1] at 37 C. A 2 ml sample was placed in a Fitch-Hopkins tube and centrifuged 20 minutes at 2350 x g to determine the wet weight of the organisms by their packed cell volume (37). The remainder of the pooled culture was then diluted with phosphate buffered saline (0.01 M, pH 7.6) to 2.5 mg organism (approximately 2.5 x 10^8 organisms) per 10 ml. Ten ml of the adjusted inoculum was then deposited in the pharnyx of each animal with a syringe.

Method of Exposing Contact Swine to Infected Swine

On Day 35, Group I swine were removed from their room one at a time, placed in a farrowing pen, and scrubbed with the tuberculocidal disinfectant to reduce as much as possible the number of organisms on their bodies. After a thorough rinse, they were put in Pen A with Group II for the remainder of Part I.

Monitoring Disease Spread and Development

Each pig in Groups I, II, and III was tested for lymphocyte immunostimulation by M. avium PPD on Days 0, 10, 25, 38, 52, 108, 122, 138, and 180. The swine in Group IV were tested only on Days 0, 143, and 180. Fecal material collected from each animal on Days 32, 64, 96, and 120 was cultured for mycobacteria (procedure described later). All of the swine were skin tested on Days 0, 82, and 187. The pigs were slaughtered when it was determined that Group II had become infected with M. avium. On Day 143, the two or three largest animals in each group were necropsied. These were swine numbers 111, 112, 117,

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[1]Difco Laboratories (0385-17), Detroit, MI.

123, 124, 131, 134, 135, 106, 108, and 109. On Day 195, the remaining animals in Groups I, III, and IV were necropsied. The remaining six animals in Group II were retained for use in Part II of the study.

At necropsy, the animals were examined for grossly visible tuberculoid lesions. Portions of the following tissue were collected for mycobacteriological culturing (procedure described later): tonsil, cervical lymph nodes, mediastinal lymph nodes, mesenteric lymph node, and liver. Pig 114 (Group I) was necropsied on Day 84 after chronic diarrhea had led to emaciation.

Part II

Distribution of Animals

In Part II of the study, the six naturally infected swine from Part I (Group II) were used as a source of infection. These swine were now between 10 and 11 months of age. All of these animals were positive for lymphocyte immunostimulation and five of the six were skin test positive when tested with M. avium tuberculin one month before Part II of the study began. M. avium had been isolated from fecal samples from five of these swine and from tissue obtained from the two Group II swine necropsied in Part I.

Twenty-four 4-week-old swine were obtained for use as either uninfected contact swine or as controls. These were divided into three groups of eight and identified with ear notches (Table 2). The isolation rooms were thoroughly cleaned and disinfected with the commercial tuberculocidal disinfectant and then fumigated with formaldehyde before Part II began. On Day 0 of Part II, Group V was placed in Pen A of the large

Table 2. Swine Used in Part II of the Transmission Study.

Group	Animal
Group II (Direct contact swine from Part I)*. Housed in Pen A from Day 1, Part II. It had been 74 days since these were last exposed to the artificially infected swine (Group I) in Part I.	120 127 121 126 122 125
Group V (Direct contact swine)**. Housed in Pen A with Group II from Day 1 until end of study.	4-1 4-2 4-3 4-4 4-5 4-6 4-7 4-8
Group VI (Aerosol contact swine)**. Housed in Pen B from Day 1 until end of study.	3-1 3-2 3-3 3-4 3-5 3-6 3-7 3-8
Group VII (Control animals)**. Housed in separate room for duration of Part II.	1-1 1-2 1-3 1-4 1-5 1-6 1-7 1-8

*Between 10 and 11 months of age.
**Four weeks old.

room (Figure 1) in direct contact with Group II, and Group VI was placed in indirect contact in Pen B. This was 234 days after Group II was exposed to Group I in Part I. Group VII swine were controls and were housed in another room for the duration of the experiment.

Monitoring Disease Spread and Development

On Day 0 Part II, swine in each group were tested for lymphocyte immunostimulation and skin tested with M. avium tuberculin. After Day 30, swine in Group V were tested for lymphocyte immunostimulation approximately once every two weeks and those in Groups II and VI once a month. Fecal material from swine in Groups II and V was cultured for mycobacteria on Days 0, 31, and 93. Before necropsy, all swine were tested for lymphocyte immunostimulation and those in Group V were skin tested with M. avium tuberculin.

Pig 127 (Group II) was necropsied on Day 240 after lameness and a leg infection, attributed to slipping on the concrete, had led to deteriorated health. Group VI swine were necropsied on Day 253, Group VII swine on Day 254, Group V swine on Day 266, and Group II swine on Day 280. At necropsy, the animals were examined for tuberculous lesions and portions of the following tissue for mycobacteriologic culturing were collected from the animals in groups II, V, and VI: tonsil, cervical lymph nodes, mediastinal lymph nodes, mesenteric lymph node, lung, liver, spleen, and kidney. In addition to the tissues listed, urine and feces from Group II animals were also cultured. No tissue was cultured from Group VII swine.

Tuberculin Skin Test

The swine were skin tested by injecting 0.1 ml of M. avium tuberculin[1] intradermally on the top side at the base of the left ear. Forty-eight hours later, the injection site was examined for erythema and induration. The zone of induration (if present) was measured at its greatest extent and then measured at a right angle to the first measurement. An area of induration 5 x 5 mm or greater was considered a positive reaction.

Lymphocyte Immunostimulation Test

By puncture of the anterior vena cava, approximately 20 ml of peripheral blood were collected into a vacutainer tube containing approximately 500 units of preservative-free heparin. Lymphocytes were separated from the blood by ficoll-hypaque density centrifugation (30). Ten ml of blood were carefully layered upon 8 ml of ficoll-hypaque solution (specific gravity 1.090) in a 15 x 150 mm tube. The tube was centrifuged 40 minutes at 400 x g and the lymphocyte layer, which formed at the interface of the ficoll-hypaque solution and serum , was removed with a pipet. This 1 to 2 ml cell suspension was washed twice by mixing with 3 ml of Hank's balanced salt solution in a 12 x 75 mm tube. The mixture was centrifuged 10 minutes at 175 x g and the supernatant fluid discarded. Following the second washing, the cell pellet was resuspended in 3 ml of RPMI 1640 tissue culture medium[2] containing 15% fetal calf serum,

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[1]Avian Old Tuberculin (Lot 8), Veterinary Service Laboratory, NADL, US Department of Agriculture, Ames IA.

[2]Grand Island Biological Company, Grand Island, NY.

penicillin (100 units/ml), and streptomycin (100 ug/ml). The lymphocyte concentration in this suspension was determined with a cell counter[1], and RPMI medium was added to adjust the concentration to 2.5 x 10^6 cells/ml.

The lymphocytes were cultured in wells of microtitration plates[2]. Each plate contained eight rows of 12 wells, each well having a capacity of 0.4 ml. Each row was used for testing one lymphocyte suspension. To each well in a row was added 0.2 ml of lymphocyte suspension (5.0 x 10^5 cells). To wells one through four was added 0.05 ml of RPMI medium. These were the control cultures. In wells five through eight the cells were tested for immunostimulation (blastogenesis). Each of these wells received 0.05 ml (40 ug/ml) of the purified protein derivative (PPD) of M. avium tuberculin[3] in RPMI medium. To wells nine through twelve was added 0.05 ml (1 mg/ml) phytohemagglutinin-M (PHA-M)[4] in RPMI medium. These cultures provided positive controls for lymphocyte immunostimulation as PHA-M elicits maximum blastogenesis from lymphocytes independent of sensitization.

Plastic covers were placed on the culture plates which were incubated 50 hours at 37 C in a 5% CO_2, 100% humidified atmosphere. After 50 hours incubation, 0.05 ml of tritiated thymidine[5] (specific

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[1]Coulter Electronics Inc. (Model F), Hialeah, FL.

[2]Falcon (3040), Oxnard, CA.

[3]Veterinary Services Laboratory, US Department of Agriculture, Ames, IA.

[4]Difco Laboratories (0528-56), Detroit, MI.

[5]ICN Pharmaceuticals, Inc., Irvine, CA.

activity 66.9 Ci/mmole, 0.5 uCi/well) in RPMI was added to each culture. The cultures were incubated an additional 18 hours, then immediately processed or refrigerated at 4 C. Using a lymphocyte harvester[1] (Figure 2), the DNA of the lymphocytes in each well was collected on glass fiber filters[2] and washed ten times with distilled water. The filters were dried two hours at 80 C. Spots containing lymphocyte DNA on the filters were punched out, placed in miniature scintillation vials, and 3 ml of scintillation fluor (0.08 g bis-MSB (p-bis(o-methylstyryl)-benzene) and 3.92 g PPO (2,5-diphenyloxazole) per liter of toluene) were added to each vial. Radioactive decay of the ^{3}H in each sample was counted for one minute in a liquid scintillation spectrometer[3]. A stimulation index was calculated for each lymphocyte sample by dividing the average count per minute of the four cultures grown in the presence of the antigen (wells five through eight) by the average count per minute of the four control cultures (wells one through four). An index of 2.0 or higher was considered positive for lymphocyte stimulation if the index remained above 2.0 on the subsequent testing. An index was calculated for the positive control cultures (wells nine through twelve) by dividing their average count per minute by the average count per minute of wells one through four. An index of 2.0 or higher indicated that conditions were proper and that the lymphocytes were capable of blastogenesis.

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[1]Microbiological Associates (MASH II), Bethesda, MD.

[2]Whatman, Inc. (Grade 934 AH), Clifton, NJ.

[3]Packard Instrument Company, Inc. (Model 3375), Downers Grove, IL.

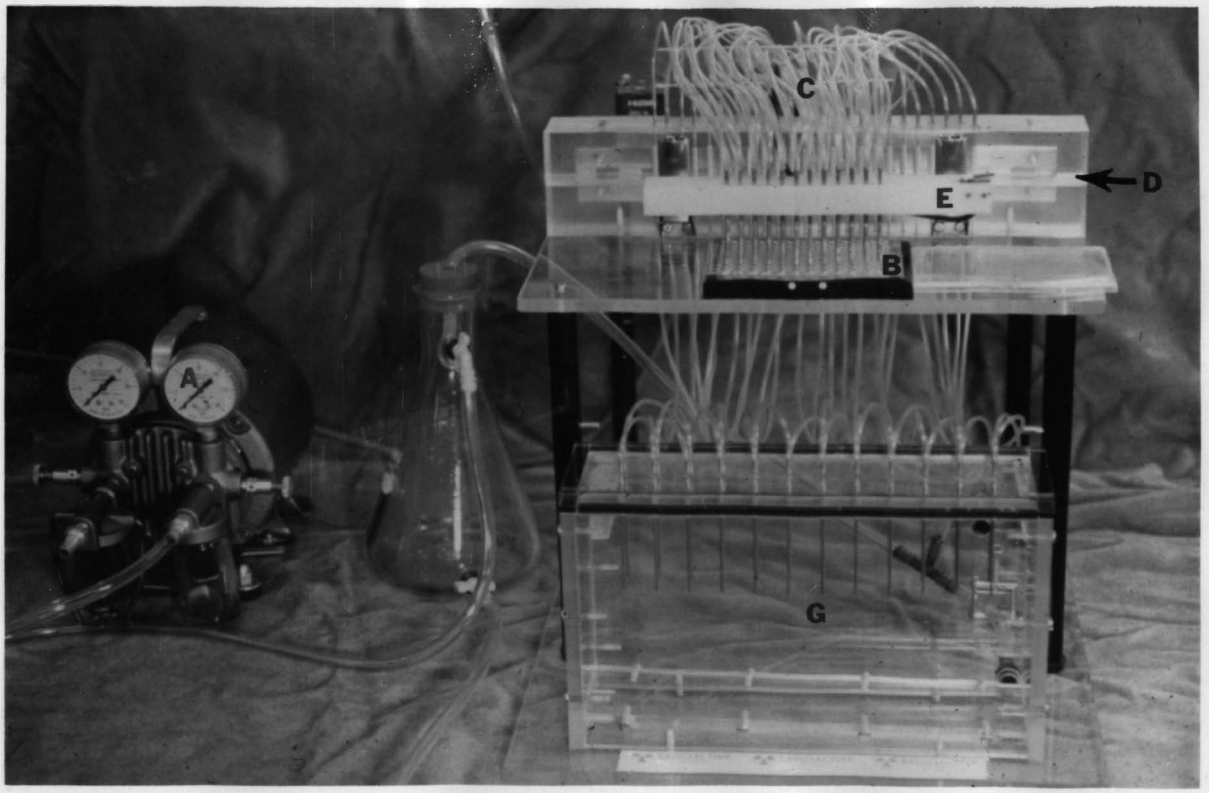

Figure 2. Lymphocyte Harvester. The pump (A) provides a continuous suction which draws the contents out of the wells of the culture plate (B), through tubes (C), and through the fiber glass filter (D). The wells of the culture plate are filled with distilled water ten times by means of a solenoid switch (E) which controls the water flow from an external reservoir (F), and emptied each time by the suction. The water from the rinses passes through the filter before it empties into a reservoir (G). The water rinses the culture wells, lyses the lymphocytes, and washes the filter of extraneous tritiated thymidine. The lymphocyte DNA is retained on the filter.

Culturing Feces for Mycobacteria

A cotton-tipped applicator was used to collect feces from pigs up to approximately 150 pounds weight. The applicator was inserted into the pig's rectum as far as possible (2 to 3 inches) and moved back and forth to collect as much fecal material as possible. The collected material was decontaminated (57) by swirling the swab in 5 ml of 0.3% aqueous benzalkonium chloride[1] in a 10 ml, screw-capped, polyethylene centrifuge tube. The mixture was shaken vigorously by hand every few minutes for one-half hour and then allowed to stand 24 hours. The mixture was then centrifuged 20 minutes at 3000 x g and the supernatant fluid discarded. To inhibit fungal growth, the sediment was resuspended in 1 ml of an aqueous solution of amphotericin B (250 ug/ml)[2]. A few drops of the suspension were inoculated onto slants of Lowenstein-Jensen, Stonebrink's, and Herrold's egg yolk (with malachite green, without mycobactin) media (57). The tubes were incubated at 37 C and examined for growth weekly for eight weeks.

Rectal swabs from pigs larger than approximately 150 pounds yielded little, if any, fecal material. Fecal samples were collected from these swine by placing the animal in a farrowing crate (2 by 6 feet) and catching naturally voided feces in a cardboard container placed on the floor behind the animal.

Approximately 5 g of feces were placed in a 200 ml bottle containing

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[1]Winthrop Laboratories (Zephiran^{r}), New York, NY.

[2]Grand Island Biological Company (Fungizone^{r}), Grand Island, NY.

100 ml sterile distilled water. This mixture was shaken vigorously by hand a few minutes and then filtered through filter paper. Twenty ml of the filtrate were collected in a 50 ml screw-cap centrifuge tube, then centrifuged 20 minutes at 3000 x g. The supernatant fluid was discarded, and the sediment was resuspended in 40 ml of 0.3% aqueous benzalkonium chloride and allowed to stand 24 hours. The sample was centrifuged, the supernatant fluid discarded, and the sediment resuspended in 1 ml aqueous solution of amphotericin B (250 ug/ml). The suspension was inoculated onto media as above.

Culturing Tissue for Mycobacteria

Tissue samples collected during necropsy were placed in sterile plastic bags, labeled, and either kept frozen (-15 C) until cultured or immediately cultured. At the time of culture, tissue specimens were trimmed of fat and placed in 1:1000 sodium hypochlorite overnight at 4 C (57). The following day, the tissue was removed with sterile forceps, dissected with sterile scissors, and examined for gross lesions. Approximately 10 g of tissue and 50 ml nutrient broth[1] containg 0.04% phenol red indicator were homogenized two minutes in a sterile blender jar in a biological safety cabinet. The suspension was then inoculated onto one slant of Herrold's egg yolk medium with a sterile cotton swab. For decontamination, 5 ml of the homogenate were added to 5 ml 0.5 N NaOH in a centrifuge tube. After 10 minutes, 6 N HCl was added a drop at a time until the indicator turned yellow. The sample was brought

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[1]Difco Laboratories (0003-01), Detroit, MI.

back to a neutral pink by adding, dropwise, 1N NaOH. Another 5 ml of the homogenate were placed in a sterile tube and frozen to be used with further decontamination if the original treated sample remained contaminated. The tubes were removed from the cabinet and the NaOH treated sample was centrifuged 20 minutes at 2700 x g. All but 1 ml of the supernatant fluid was decanted, the sediment was resuspended in the remaining fluid, and inoculated with a swab onto a slant of each of the media listed under fecal culturing. Tubes were incubated at 37 C and examined for growth weekly for eight weeks.

Culturing Urine for Mycobacteria

Urine was collected from the Group II swine during necropsy by drawing the urine from the bladder into a sterile evacuated 500 ml bottle. In order to concentrate the organisms which it might contain, the urine was placed in a separatory funnel and 1 ml of 30% acetic acid and 1 ml of 5% tannic acid per 500 ml urine were added (21). After the mixture stood overnight at 4 C, the floculum, which had formed and settled to the bottom of the funnel, was drawn into a 50 ml centrifuge tube and centrifuged 20 minutes at 2700 x g. All but 5 ml of the supernatant fluid was discarded and 5 ml of 4% H_2SO_4 was added to decontaminate the sediment (21). The contents were mixed and the tube was allowed to stand 15 minutes. The tube was then filled with sterile water and centrifuged as before. All but 1 ml of the supernatant fluid was discarded, 1 drop of 0.01% phenol red was added as an indicator, and the sample was neutralized by adding, dropwise, 1N NaOH. Slants of media were inoculated and incubated as above.

Identification of Isolates

On primary isolation at 37 C, M. avium usually developed visible colonies in two to three weeks and mature colonies in four to five weeks. At least one M. avium-like colony was picked from each set of slants and subcultured in 5 ml Dubos broth. This was incubated seven days at 37 C and observed for typical mycobacterial growth. A smear from this culture was stained by the Ziehl-Neelson method to confirm that the isolate was pure and acid-fast. A diluted sample from this culture was inoculated onto two slants of Herrold's egg yolk medium. These cultures were used for pigmentation study, to determine precisely the appearance time and colonial characteristics, and to provide cultures for biochemical tests. One tube was covered with foil to exclude light, then both tubes were incubated at 37 C until colonies formed on the uncovered slant. The top portion of the covered slant was exposed and examined for colonial pigmentation. The presence of pigment at this time indicated that the isolate was a scotochromogen. If no pigment was present, the uncovered part of the slant was exposed one hour to the light of a 60 watt bulb 10 inches away (58). The tube was incubated overnight and the colonies examined for pigment the following day. The presence of pigment in the colonies at this time indicated that the isolate was a photochromogen. If no pigmentation developed, one slant was left exposed to the light of the laboratory for two weeks to check for delayed photochromogenicity. A slow growing, nonchromogenic isolate was considered to belong to either Runyon Group III or the M. avium complex.

The isolate was identified as belonging to the M. avium complex if it reduced tellurite within three days but did not reduce nitrate or hydrolyze Tween 80, if it produced pyrazinimidase but not urease, and if it had little or no catalase activity (57,58). Tissue isolates were tested for pathogenicity for chickens by injecting 0.1 mg wet weight of the isolate (grown in Dubos broth) intraperitoneally into two young chickens (five to eight weeks old) (37). The chickens were necropsied and examined for tuberculous lesions when they died or eight weeks PI.

Results

Part I

Results of Skin Tests and Lymphocyte Immunostimulation Tests

The results of the skin test reactions of the swine in Group I are shown in Table 3. All of the swine were negative to the initial test. On Day 82, seven of the swine had positive skin test reactions, and the remaining animals were still positive when tested again on Day 187.

The LI test results of the Group I swine are shown in Table 4. Six of the seven remaining swine were positive when tested on Day 108, and all were positive when tested on Day 122 and thereafter.

The skin test reactions of the swine in Group II are shown in Table 5. Five of the eight swine in this group were skin test positive when tested 47 days after exposure to Group I, and all six of the remaining swine were positive when again tested 152 days after exposure. Five of these swine were still positive when Part II of the study began 234 days after exposure to Group I.

As shown in Table 6, positive LI indexes developed after the skin tests became positive. Three of the swine had positive indexes when tested 73 days after exposure to Group I, and the indexes of all were positive when again tested 87 days after exposure and thereafter during Part I.

None of the swine in Groups III and IV reacted to the skin tests or developed positive LI indexes during the study.

Isolation of M. avium from Feces

The isolations of M. avium from fecal samples from the Group I

Table 3. Skin Test Reactions of Group I Swine at Various Times Following Oral Inoculation with *M. avium*.

Group	Animal	Days since inoculated 0	82	187
I	110	-	45 x 55*	70 x 120
	118	-	11 x 12	50 x 50
	114	-	4 x 4	NT**
	115	-	45 x 45	60 x 80
	116	-	10 x 17	10 x 10
	117	-	25 x 30	NT
	111	-	50 x 50	NT
	112	-	10 x 15	NT

*Cross-sectional measurement in millimeters of area of induration 48 hours after injection of 0.1 ml avian old tuberculin. A reaction of 5 x 5 mm or greater considered positive; - = no reaction.

**NT = not tested.

Table 4. Results of Lymphocyte Immunostimulation Tests of the Group I Swine at Various Times Following Oral Inoculation with M. avium.

Group	Animal	Days since inoculation								
		0	10	25	38	52	108	122	138	180
I	110	1.3*	0.9	1.6	1.6	1.2	7.8	NT**	28.5	32.7
	118	1.2	0.9	1.1	0.6	1.0	2.5	2.5	1.6	2.4
	114	1.4	1.3	1.7	1.0	1.1	NT	NT	NT	NT
	115	1.1	0.6	1.1	1.7	1.1	11.4	60.1	6.4	10.0
	116	1.0	0.7	2.6	1.1	0.9	1.0	5.2	2.9	4.0
	117	1.2	1.6	2.1	2.8	1.6	16.7	14.4	12.7	NT
	111	1.2	0.9	1.6	1.0	1.3	9.7	19.4	13.3	NT
	112	1.5	0.9	2.1	1.0	2.0	15.9	2.1	2.7	NT

*An index of 2.0 or higher considered positive if the index remained 2.0 or higher on subsequent tests.
**NT = not tested.

Table 5. Skin Test Reactions of the Group II Swine at Various Times After Direct Contact with Group I.

Group	Animal	Days after exposure 0	47	152	234*
II	120	-	5 x 7**	25 x 40	20 x 20
	127	-	-	20 x 20	20 x 30
	121	-	9 x 11	60 x 110	20 x 20
	126	-	-	100 x 110	20 x 40
	122	-	-	20 x 20	-
	125	-	10 x 10	30 x 30	15 x 15
	123	-	9 x 10	NT+	NT
	124	-	7 x 10	NT	NT

*Day 0, Part II (74 days after last exposure to Group I).
**Cross-sectional measurement in millimeters of area of induration 48 hours after injection of 0.1 ml avian old tuberculin. A reaction of 5 x 5 mm or greater considered positive;
- = no reaction.
+NT = not tested.

Table 6. Results of Lymphocyte Immunostimulation Tests of the Group II Swine at Various Times Following Exposure to Group I.

Group	Animal	Days since exposure						
		0	3	17	73	87	103	145
II	120	1.3*	0.7	0.9	2.0	4.1	4.5	6.2
	127	1.3	1.4	1.0	1.1	6.8	6.2	10.0
	121	1.0	1.3	1.2	1.2	5.5	10.8	8.1
	126	1.3	1.2	1.3	4.0	46.4	15.2	17.7
	122	0.9	1.4	1.0	1.2	16.0	3.6	9.0
	125	1.1	0.9	0.9	1.9	5.7	11.6	14.4
	123	0.8	1.1	NT**	0.8	2.5	5.2	NT
	124	1.2	1.5	NT	5.9	4.8	8.9	NT

*An index of 2.0 or higher considered positive if the index remained 2.0 or higher on subsequent tests.
**NT = not tested.

swine are shown in Table 7. M. avium was isolated from feces of seven of the eight swine 32 days after oral inoculation. The number of animals from which M. avium was isolated in this group decreased on each successive test until the organism was isolated from only one of the animals 120 days PI.

M. avium was isolated from fecal samples from five of the eight Group II swine 29 days after their exposure to Group I (Table 8). When fecal samples from this group were cultured 61 and 85 days PI the organism was isolated from two.

M. avium was not isolated from any fecal samples from the swine in Groups III and IV.

Grossly Visible Lesions and M. avium in Tissues Collected at Necropsy

The presence of grossly visible tuberculoid lesions and the isolations of M. avium from tissues collected at necropsy are summarized in Table 9. Lesions were found in and M. avium isolated from tissues from all of the swine in Group I. The organism was isolated from the mesenteric lymph nodes of all eight animals and from the cervical lymph nodes of seven. M. avium was isolated from the liver as well as the lymph nodes and tonsil of animal 116.

Although grossly visible lesions were discovered in only one, M. avium was isolated from the cervical lymph nodes of both and the mesenteric lymph nodes and tonsil of one of the Group II swine necropsied 108 days after exposure.

Lesions were not seen in the tissues from any of the swine in Groups III and IV, although M. avium was isolated from the tonsil of animal 131 of Group III.

Table 7. Isolations of M. avium from Feces of the Group I Swine at Various Times Following Oral Inoculation.

Group	Animal	Days after inoculation 32	64	96	120
I	110	+*	-	-	-
	118	+	+	-	-
	114	+	+	NT	NT**
	115	+	-	-	-
	116	+	+	+	-
	117	-	+	+	+
	111	+	-	-	-
	112	+	+	+	-

*+ = one or more colonies of M. avium isolated;
- = M. avium not isolated.
**NT = not tested.

Table 8. Isolations of M. avium from Feces of the Group II Swine at Various Times After Direct Contact with Group I.

Group	Animal	Days after exposure 0	29	61	85
II	120	-	+*	-	-
	127	-	+	-	+
	121	-	+	-	-
	126	-	+	-	-
	122	-	-	-	-
	125	-	-	+	+
	123	-	+	+	-
	124	-	-	-	-

*+ = one or more colonies of M. avium isolated;
- = M. avium not isolated.

Table 9. Isolation of M. avium and Grossly Visible Lesions in Tissue Collected at Necropsy from Swine Inoculated with M. avium (Group I) and Swine Exposed to Inoculated Swine (Group II).

Group	Animal	Mesenteric Lymph Node		Cervical Lymph Node		Mediastinal Lymph Node		Tonsil		Liver		Day in Experiment Necropsied	Days since Group II Exposed to Group I
		G*	I	G	I	G	I	G	I	G	I		
I	114	+	+	+	+	-	-	-	+	-	-	84	
	112	+	+	+	+	+	+	-	+	-	-	143	
	117	+	+	+	+	-	-	+	+	-	-	143	
	111	+	+	-	+	-	-	+	+	-	-	143	
	118	-	+	-	+	-	-	+	+	-	-	195	
	115	+	+	+	+	-	-	+	-	-	-	195	
	110	+	+	-	-	-	-	+	-	-	-	195	
	116	+	+	+	+	-	+	+	+	-	+	195	
II	123	-	+	-	+	-	-	-	+	-	-	143	108
	124	-	-	+	+	-	-	-	-	-	-	143	108

*G = Grossly visible tuberculoid lesions seen in tissue.
I = M. avium isolated from tissue.

Part II

Results of Skin Tests and Lymphocyte Immunostimulation Tests

As shown in Table 5, five of the six swine in Group II had positive skin test reactions when tested on Day 0, Part II (74 days after last exposure to Group I in Part I). All of these swine remained positive for lymphocyte immunostimulation by M. avium PPD during Part II (Table 10). None of the swine in Groups V, VI, and VII had a positive reaction to the initial skin test and none of the swine in Group V had a positive reaction when tested after 262 days exposure to Group II.

The results of the LI tests of the Group V swine are shown in Table 11. Animals 4-7 and 4-8 had very weak positive indexes when tested on Days 210 and 262. Indexes of 3.1 for animal 4-1 on Day 189 and 2.1 for animal 4-6 on Day 210 were not considered significant as the indexes did not remain above 2.0 on subsequent tests. None of the swine in Groups VI and VII developed positive LI indexes during the study.

Isolation of M. avium from Feces

M. avium was not isolated from fecal samples collected in Part II.

Grossly Visible Lesions and M. avium in Tissues Collected at Necropsy

The results of the necropsy of the swine in Groups II and V are shown in Table 12. Grossly visible lesions were found in tissues of five of the six Group II swine necropsied on Days 240 and 280, Part II (314 and 354 days after last exposure to Group I in Part I). The lesions were limited to mesenteric and cervical lymph nodes. M. avium was isolated from the lymph nodes of only two of these animals.

Grossly visible lesions were not seen in tissues of the Group V swine,

Table 10. Results of Lymphocyte Immunostimulation Tests of the Group II Swine During Part II of the Study.

Group	Animal	0/234*	49/283	77/311	105/339	147/381	168/402	240/474	280/514
II	120	8.6**	12.6	11.0	11.7	3.7	27.6	26.5	51.5
	127	4.8	14.6	11.4	7.5	6.3	21.9	19.4	NT+
	121	14.2	18.1	9.9	8.5	15.6	42.6	68.2	16.2
	126	18.4	12.7	22.5	27.4	14.0	50.9	91.0	8.5
	122	3.6	7.9	7.3	10.0	4.3	9.7	4.1	6.2
	125	37.7	13.7	19.2	18.5	9.2	26.4	36.9	36.5

*Day of experiment, Part II/days since [illegible] exposure to Group I in Part I (Group II exposed to Group I for 160 days in Part I).

**An index of 2.0 or higher considered positive if index remained 2.0 or higher on subsequent tests.

+NT = not tested.

Table 11. Results of the Lymphocyte Immunostimulation Tests of the Group V Swine During Exposure to Group II.

Group	Animal	Days since exposure to Group II													
		0	30	49	63	77	93	112	127	140	154	175	189	210	262
V	4-1	0.9*	0.9	1.0	1.4	1.3	1.0	1.1	1.1	1.2	1.0	1.9	3.1	1.3	1.3
	4-2	1.4	1.1	0.7	1.1	0.9	1.1	1.0	1.1	1.4	1.5	1.6	0.7	1.7	1.3
	4-3	1.1	1.4	1.9	1.4	0.8	1.3	1.2	1.1	1.4	2.2	1.3	NT**	1.5	1.7
	4-4	0.5	1.2	1.6	1.0	0.9	1.5	1.0	1.3	1.2	1.2	1.7	1.8	0.7	1.1
	4-5	0.7	0.7	1.4	1.3	1.1	1.2	1.2	1.3	1.0	1.6	1.8	1.4	1.3	1.1
	4-6	1.2	1.5	1.7	1.3	1.1	1.1	1.0	1.5	1.2	1.3	1.8	1.5	2.1	1.2
	4-7	0.5	1.1	2.0	1.7	1.2	1.0	1.0	1.3	1.0	NT	1.6	1.0	2.2	2.2
	4-8	0.8	1.5	1.2	1.2	1.3	0.9	1.9	1.5	1.3	1.4	1.8	1.5	2.2	2.0

*An index of 2.0 or higher considered positive if index remained 2.0 or higher on subsequent tests.
**NT = Not tested.

Table 12. Isolations of M. avium and Grossly Visible Lesions in Tissue Collected at Necropsy from the Swine in Groups II and V.

Group	Animal	Mesenteric Lymph Node G**	Mesenteric Lymph Node I	Cervical Lymph Nodes G	Cervical Lymph Nodes I	Kidney G	Kidney I	Other Tissues* G	Other Tissues* I	Description of Gross Lesions
II	120	+	-	-	-	-	-	-	-	One lesion in mesenteric lymph nodes, 4 mm.
	127	+	+	-	-	-	-	-	-	Three lesions in mesenteric lymph nodes, 2 to 4 mm.
	121	+	-	+	-	-	-	-	-	Two lesions in mesenteric lymph nodes, 4 mm; numerous lesions in cervical lymph nodes, pinpoint to 5 mm.
	126	+	-	+	-	-	-	-	-	Few lesions in mesenteric lymph nodes, 2 to 3 mm; numerous lesions in cervical nodes, pinpoint to 4 mm.
	122	+	+	-	-	-	-	-	-	Many lesions in mesenteric lymph nodes, 1 to 3 mm.
	125	-	-	-	-	-	-	-	-	No lesions found.
V	4-1	-	+	-	-	-	-	-	-	
	4-2	-	+	-	-	-	+	-	-	
	4-3	-	-	-	+	-	-	-	-	
	4-4	-	-	-	-	-	-	-	-	
	4-5	-	-	-	-	-	-	-	-	
	4-6	-	-	-	-	-	-	-	-	
	4-7	-	-	-	-	-	-	-	-	
	4-8	-	-	-	-	-	-	-	-	

*Mediastinal lymph node, tonsil, lung, liver, and spleen.
**G = Grossly visible tuberculoid lesions seen in tissue;
I = M. avium isolated from tissue.

although M. avium was isolated from mesenteric or cervical lymph nodes or kidney of three of these swine.

Grossly visible lesions were not seen and M. avium was not isolated from tissue of the aerosol contact swine (Group VI). Grossly visible lesions were not discovered in tissues of the Group VII swine (Control swine).

Discussion

The transmission of M. avium from artificially infected swine to uninfected contact swine has been reported by Ray et al (35) and Thoen et al (55). The present study differed from these trials in some very important respects:

1. In the other studies, the contact swine were exposed to the inoculated animals immediately after inoculation. With that technique, it is possible that the contact swine ingested organisms which were part of the original inoculum excreted shortly after oral inoculation and before the inoculated animals actually became infected. In the present study, there was a 35 day delay after inoculating the animals before exposing uninfected swine to them. This was an attempt to allow the inoculated swine to become infected before other swine were exposed to them and to prevent the contact swine from ingesting organisms from the inoculum. This possibility was further reduced by washing the inoculated animals and placing them in the pen housing the contact swine. This eliminated the chance of the contact swine ingesting M. avium from the pen contaminated while the inoculated swine were being infected. As discussed later, the possibility of the contact swine ingesting organisms from the inoculum may not have been eliminated in the present study.

2. Older swine (Group II) which had aquired an M. avium infection through contact, were used as a source of infection for other swine (Part II).

3. Disease transmission and development was monitored by lymphocyte immunostimulation and culture of feces at regular intervals following

inoculation or exposure.

4. Uninfected swine were placed in indirect (aerosol) contact as well as direct contact with infected swine.

5. In order to study the disease in older animals, swine which had aquired an infection (Group II) were kept beyond market age and weight. Information was gained concerning how long swine remain positive for in vitro lymphocyte immunostimulation and whether tuberculoid lesions in lymph nodes regress with age or harbor viable M. avium.

The results of the skin tests (Table 3), LI tests (Table 4), and necropsies (Table 9) of the inoculated swine in Part I (Group I) are similar to those reported by others who have inoculated swine orally with M. avium (5,26,30,35,46,47,55). The isolation of M. avium from the liver collected 195 days PI from animal 116 may indicate there was a generalized infection at some stage of disease development. The prevalence of lesions and M. avium in the alimentary lymph nodes as compared to the mediastinal lymph nodes is indicative of the oral route of inoculation.

It is apparent from Part I of the study that uninfected swine can aquire an M. avium infection from infected penmates under the conditions of this study. This was shown by the development in the Group II swine of positive skin test reactions (Table 5) and LI indexes (Table 6), and the development of grossly visible tuberculoid lesions from which M. avium was isolated (Table 9). This is similar to the results of Thoen et al (55) and Ray et al (35) who used direct contact swine in their studies.

The infection of swine of ages 4, 6, 8, and 10 weeks in Group I and swine of ages 9, 11, 13, and 15 weeks (age at time of mixing) in Group II

indicates that feeder pigs of all ages are susceptible to infection. Most of the animals in these groups had lesions for which their carcasses would have been either cooked or condemned at slaughter. Presumably this could happen to feeder swine of these ages in a commercial operation.

The confinement of lesions almost exclusively to lymph nodes of the alimentary tract in the contact swine indicates that the infection resulted from ingestion of the organisms. In the present study, M. avium was probably transmitted from infected swine to penmates by a fecal-oral mode. M. avium was isolated from fecal samples collected from all of the Group I swine on at least one occasion and from most of them more than once (Table 7). However, the organism was not recovered in abundance from any fecal sample. In most cases, there was one to five colonies per medium slant, and although the procedures used were not quantitative, this indicates that the number of M. avium organisms per gram of feces was very low. Merkal (27) states that, in cattle, at least 50 to 100 M. paratuberculosis organisms per gram of feces must be shed before they can be isolated using a procedure similar to that used in the present study. Considering the small number of organisms recovered from each sample and the decrease in the number of animals from which the organism was recovered on successive tests, it appears that the population of M. avium in the feces diminished until it was no longer possible to isolate the organisms. In addition to the small number of organisms in a sample, factors such as the variability in the amount of feces collected, the small amount of material actually cultured, and the loss of M. avium to dilution and decontamination, affected the recovery rate. A method of concentrating mycobac-

teria in a fecal sample, such as a pentane extraction of the organisms (52), might have resulted in recovering organisms for a longer time.

M. avium was present in fecal samples of the Group II swine as early as 29 days after exposure to Group I (Table 8). As in the case of the Group I swine, animals in Group II from which the organism was isolated also decreased on successive tests.

Under the conditions of this trial, there are two possibilities for the fecal shedding of M. avium by the infected swine: 1. the organisms were shed directly into the alimentary tract from lesions and excreted, or 2. the swine were continually ingesting and excreting organisms which came from the original inoculum. Considering the first possibility, it is unlikely that organisms in a tuberculous cervical or mesenteric lymph node would move retrogressively, i.e., from the lymph node to the lumen of the alimentary tract. However, surface lesions in the tonsils or mucosa of the intestines could provide the opportunity for M. avium to multiply and subsequently escape into the feces. In the present study, grossly visible lesions were found in the tonsils of six of the eight Group I swine, and M. avium was isolated from six, although not always from those with lesions. No lesions were seen in the tonsils of the Group II swine, although M. avium was isolated from one (Tables 9 and 12). The absence of tonsilar lesions in the Group II swine may explain their inability to infect the Group V swine to an appreciable extent. Thoen and Karlson (54) describe a tuberculous lesion in the intestinal submucosa of a pig and suggest that it could ulcerate and discharge M. avium into the feces. How prevalent such lesions are in naturally or experimentally

infected swine is not known.

The second possibility for the fecal shedding of M. avium (swine reingesting and excreting organisms from the inoculum) could explain the transmission of the organism as well as the extended period of shedding. It can be assumed that the Group I swine excreted in their feces the majority of the organisms given them in the inoculum (approximately 2.5×10^8 organisms per animal). Cleaning the pen three times a week would have resulted in a continual decrease in the number of organisms available for reingestion. However the swine would have had organisms in their intestines and excreted them after the pen was cleaned. Although attempts were made to remove all M. avium organisms from the external body surfaces, organisms being carried passively in the intestines would have contaminated the new pen when the groups were mixed 35 days PI.

The fact that M. avium was isolated from the feces of the swine in Groups I and II before they became sensitized to tuberculin tends to support the theory of passive transmission. For example, M. avium was isolated from the fecal samples of five of the eight Group II swine 29 days after exposure (Table 8). However these animals were probably not infected at this time since sensitization in the LI test did not develop until Days 73 to 87 (Table 6). In both groups, the number of swine from whose feces M. avium was isolated decreased on successive tests. This is in keeping with the idea that cleaning the pens decreased the number of organisms available for ingestion and subsequent excretion. On the other hand, it is possible that the swine stopped shedding M. avium because tonsilar or intestinal lesions healed.

Further study is needed to establish whether M. avium shed in the feces of exposed animals originates from lesions, or if the animals are simply recycling the organism. This might be accomplished by inoculating animals and restraining them in such a way that they cannot ingest their feces. Feces could be cultured periodically for M. avium to determine whether the animals continually shed the organism after inoculation. Animals so infected could also be placed with uninfected contact swine to see if transmission occurs. If it can be shown that M. avium persists in a swine herd because of a contaminated environment rather than one or more infected individuals, cleaning and disinfection of pig pens may be all that is necessary to eliminate the problem in many cases.

The swine in indirect contact (Group III) with the inoculated swine in Part I failed to develop either positive skin test reactions or positive LI indexes after 143 or 195 days exposure. Grossly visible lesions were not seen in any tissue, although M. avium was isolated from the tonsil of one animal. This animal may have been in a very early stage of infection, which could explain why it did not have positive skin or lymphocyte tests. It would appear that, under the conditions that existed, M. avium is not easily spread through an aerosol from a contaminated pen to an uncontaminated pen. This does not rule out the possibility that M. avium may be spread between pens where other conditions prevail.

None of the Group V swine (direct contact) had a positive skin test reaction when tested after 262 days exposure to Group II in Part II. Two of these swine, however, had very weak positive LI indexes when tested

after 210 and 260 days exposure (Table 11). When tissues were collected from the Group V swine after 266 days exposure, M. avium was isolated from three (Table 12). The two swine that had the weak positive LI indexes were not among the three from which M. avium was isolated. It is not known why the two swine developed these indexes. It is possible that the indexes were due to a non-specific response to an infection with another organism, or the swine were sensitized by ingestion of very low numbers of M. avium shed by the Group II swine. With regard to the former possibility, it should be noted that some of the swine in Group V had a mild skin infection caused by Corynebacterium pyogenes, a member of a genus which shares antigens with the mycobacteria (12).

The isolations of M. avium from the three Group V swine were from mesenteric and cervical lymph nodes, and kidney (Table 12). In all cases the number of organisms recovered was small, usually one colony per slant of medium, indicating that there were few organisms in the tissues. In no instance was a grossly visible lesion seen in these tissues. Perhaps these animals were in a very early stage of infection. Considering the low incidence of infection in the contact swine in Part II, it appears that the Group II swine were not a major source of infection. This is consistent with the fact that M. avium was not isolated from any of the fecal samples collected from the Group II swine during Part II. However, it is important to note that throughout the time they were with Group V, the Group II swine were indeed infected, as shown by positive skin test reactions at the beginning of Part II and positive LI indexes until the end of the study.

The presence of grossly visible lesions and viable M. avium in tissues

of the swine in Groups I and II long after inoculation or exposure is noteworthy. It has been suggested that lesions in swine lymph nodes regress or heal with age (24,26,35). Ray et al (35) reported that in swine intradermally inoculated with M. avium 113 and 175 days previously, lesions were fewer in number or absent, and the overall infection was less extensive than in swine examined 43 and 57 days PI. Luke (24) reported that he was unable to isolate mycobacteria from swine he had experimentally infected 112 days previously. He indicated that tissue in older infections probably would not contain viable organisms even though lesions may be present. On the other hand, Thoen et al (56) studied, by histologic examination and mycobacteriologic culture, the tuberculous lymph nodes of 23 brood sows retained at slaughter. M. avium was isolated from tissues of 21 and microscopic lesions were seen in tissues of 19 sows. A more detailed histologic study of tissues from some of the sows indicated that an active infectious process persisted, and the authors concluded that lesions in aged sows do not heal.

In the present study, the seven remaining Group I swine of Part I, necropsied 143 and 195 days PI, did not appear to be overcoming the infection. All had many grossly visible lesions, from pinpoint to five mm in diameter, in mesenteric or cervical lymph nodes or tonsil. Almost all the nodes contained viable M. avium and yielded a high population of colonies on slants. It is not known whether, with more time, these swine may have recovered from the infection caused by the very large inoculum given them. One animal appeared to be developing a generalized infection.

Perhaps the more naturally infected Group II swine were more

comparable to swine infected in the field. Grossly visible lesions were found in one of the two Group II swine necropsied after 108 days exposure to Group I (Table 9), and M. avium was recovered from the lymph nodes of both (40 or more colonies per slant). In contrast, while the location and number of lesions varied in the six Group II swine necropsied 354 days after their last contact with Group I (Table 12), M. avium was recovered from the lymph nodes of only two, and only one to 20 colonies grew per slant. This supports Luke's conclusion (24) that although lesions may be present in older animals, they may eventually become sterile.

Certain factors may influence both the infection of swine and the regression of M. avium lesions. These might include: 1. the number of organisms ingested, 2. the virulence or serotype of the infecting organism, 3. the age of the animal at the time of infection, and 4. the effects stresses have on the resistance to infection. For example, there may be differences in the virulence for swine of the various serotypes as there is for poultry. One or more of these factors may be responsible for the contradictory reports concerning regression of localized lesions in swine.

Luke (24) observed that, in swine experimentally infected and in older swine which presumably had long standing infections, the skin test reactions to avian tuberculin waned. He concluded that this phenomenon was directly related to the decreased number of viable organisms in the lymph nodes as the infection was overcome. The results of the skin tests of the Group II swine bear this out. As shown in Table 5, the reactions of five of the six swine were much smaller on Day 234 as compared to their reactions to the test on Day 152. One of the animals did not react at all to the later

test, even though M. avium was isolated from the mesenteric lymph node collected from the pig 280 days after the test. In contrast to the skin test reactions, the LI indexes remained fairly constant from the time the animals in Group II became sensitized until they were necropsied (Tables 6 and 10). It is not known if the LI indexes would have waned with more time, as the skin tests did.

Summary and Conclusions

1. After a 35 day delay between inoculating and mixing animals, M. avium was transmitted from orally inoculated swine to contact swine.
2. Five of the eight contact swine had positive skin test reactions after 47 days exposure and all developed positive LI indexes between 73 and 87 days.
3. The fact that grossly visible lesions and M. avium isolations were confined to lymph nodes along the alimentary tract of the contact swine indicated that the infection occured by ingestion.
4. M. avium was isolated from fecal samples of the inoculated swine, and it was concluded that M. avium in the feces of these swine was the source of infection for the contact swine.
5. It was not clearly determined if the organisms in the feces of infected swine were shed from surface lesions in the digestive tract or if the swine were reingesting and excreting M. avium from the original inoculum.
6. The fact that M. avium was isolated from the feces before the swine became sensitized to tuberculin favors the possibilty that the swine were reingesting organisms from the inoculum.
7. As early as 32 days after exposure, M. avium was isolated from the feces of the swine infected by contact in Part I; however, the number of animals from which it was isolated decreased on successive tests.
8. None of the swine in aerosol contact with the inoculated animals developed positive skin test reactions or LI indexes and M. avium was isolated from the tonsil of only one of the eight animals.

9. Although the swine infected by contact in Part I had positive LI indexes throughout Part II, they did not extensively infect swine in contact with them in Part II.
10. Although grossly visible lesions were discovered in only one, M. avium was isolated from both of the contact swine examined after 108 days exposure in Part I.
11. In contrast, while lesions of varying intensity were present in five of the remaining six swine of this group 354 days after last exposure, M. avium was isolated from only two.
12. Although lesions may not completely regress, the decrease in the number of isolations indicates that swine may be able to overcome a naturally aquired M. avium infection.
13. The tuberculin skin test reactions of these swine were less intense when tested 234 days after exposure than at 152 days; however, the LI indexes remained fairly constant after sensitization.

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