ISOLATION OF BRUCELLA SUIS FROM AIR OF SLAUGHTERHOUSE

Marvin M. Harris, Ph.D., Stanley L. Hendricks, D.V.M.
George W. Gorman, B.S., Joe R. Held, D.V.M.

BRUCELLOSIS is considered an occupational disease, since its chief mode of transmission to humans is by direct contact with infected animals or their tissues. Outbreaks among slaughterhouse workers have been common, and transmission has been assumed to be by direct contact. Airborne transmission of brucellosis has been considered of minor importance (*1*).

Rosebury and associates (*2*), who produced *Brucella* infection experimentally in guinea pigs by the aerial route, showed that *Brucella suis* is unique compared with several other nonsporulating organisms tested; that is, it is not destroyed by drying to the degree that others are. Its yield in aerosol form was found to be both relatively high and consistent.

During a recent epidemic of brucellosis among the employees of a swine-slaughtering plant in Iowa, 128 persons were affected over a period of approximately 9 months. Because epidemiologic evidence (which will be reported elsewhere) suggested that airborne transmission from hog to man may have been a prominent factor in this epidemic, attempts were made to recover *Brucella* from the air of the plant. Isolation of *Brucella* from the air under field conditions had not been previously reported.

Since the highest attack rates occurred among the employees of the "kill" and "casings" department, air sampling was confined to the room in which they worked. Very turbulent air conditions prevailed in this room, which was approximately 300 feet long by 100 feet wide by 20 feet high. Hot air and steam mixed at several ports in the room with cold air coming from the outside. Many floor fans were placed at different points within the room. Ceiling and wall exhaust fans carried off some of the hot air and steam. At various points in the room where different procedures were employed in dressing the carcass and processing the viscera, heavy aerosols were conceivably produced. With the probability of such heavy aerosols and marked air turbulence, it seemed likely that airborne particles were being carried to all parts of this room.

The devices used to sample the air of the "kill" room included the all-glass impinger, the Andersen sampler, the slit sampler (*3*), settling plates, and 300 cotton swab samples taken from machinery, floors, equipment, and carcasses.

Air samples and swab samples from surfaces were taken during 1 week in February 1960, while plant operations were in full force. Air sampling began in the morning and continued throughout the workday. The Andersen and slit samplers were operated at an airflow rate of 1 cubic foot per minute. Agar plates were exposed in the samplers for 10-minute in-

Dr. Harris, Mr. Gorman, and Dr. Held are with the Public Health Service. Dr. Harris, formerly with the Technical Development Laboratories, is presently with the Virology Research Resources Branch, National Cancer Institute. Mr. Gorman is a bacteriologist at the Technical Development Laboratories, Communicable Disease Center, Savannah, Ga. Dr. Held is with the Epidemiology Branch, Communicable Disease Center, Atlanta, Ga. Dr. Hendricks is a public health veterinarian at the Iowa State Department of Health, Des Moines.

tervals. The all-glass impingers were operated at the rate of 0.5 cubic foot per minute for 10-minute intervals throughout the day. The Andersen, slit, and all-glass impinger air samplers were operated on a stand 5 feet above the floor in the central area of kill operations. A total of 480 cubic feet of air was collected by these volumetric samplers.

Settling plates were set out each day at various sites in the "kill" room for periods varying from 1 to 5 hours. A total of 110 settling plate samples were collected. Under the conditions encountered in the environment sampled, heavy overgrowth occurred in a majority of the plates.

The solid medium used in the slit and Andersen samplers and settling plates was serum dextrose agar with bacitracin, polymyxin B, and actidione added (*4*). The collecting fluid in the all-glass impinger was 20 ml. of clear sterile tapwater, pH 7.5, with a total solid content of 174.4 ppm.

The 300 swabs of fomites were placed in serum dextrose broth containing the same antibiotics used in the agar and incubated at 37° C. for 12 days. The broth cultures were then streaked on serum dextrose agar plates containing antibiotics, incubated at 37° C. in cans containing CO_2, and examined periodically for 30 days for *Brucella*-like colonies. The air samples collected in the liquid impingers were recovered in 20 ml. of sterile tapwater, which was placed in an equal quantity of the serum dextrose broth at double strength.

All cultures were incubated at 37° C. in cans containing 10 percent CO_2 for at least 5 days before inspection and then reincubated for 7 to 12 days.

The incubation of the cultures in cans containing 10 percent CO_2 was performed under field conditions in an incubator improvised in a small storage room at the plant. After the week of sampling, all cultures were transported by automobile, 1,200 miles to the base laboratory in Savannah, Ga.

Because of some delay in processing the cultures as a result of transportation difficulties, most of the cultures appeared overgrown with various types of airborne saprophytic organisms, and suspicious-looking *Brucella*-like colonies could not be readily picked from the original plates. Therefore, the surface growth of each plate was washed off with 1 ml. of pH 7.0 buffered saline, and 0.1 ml. of this suspension was placed in a tube containing 10 ml. of buffered saline (or tryptose broth). One-tenth of a milliliter of this suspension was then streaked on the surface of a serum dextrose agar plate containing antibiotics. The plates were incubated in cans containing 10 percent CO_2 at 37° C. for 5 days and examined for colonies resembling *Brucella*.

More than 200 colonies were picked from the plates, transferred to tryptose agar slants, and incubated at 37° C. for 5 days in cans containing 10 percent CO_2. A suspension of each culture was made in 0.1 percent merthiolated saline and adjusted to a density of 60 percent transmission at 600 Å in a spectrophotometer. This antigen was used for tube agglutination tests. Tube agglutination tests in 1 ml. final volumes were set up in dilutions of 1:100 through 1:3,200 using a *B. suis* antiserum (titer 1:3,200). The tubes were incubated in a water bath at 50° C. for 5 hours and refrigerated overnight and then read.

Two isolates gave positive agglutination tests to a titer of 1:1,600 with the *Brucella* antiserum. One isolate was recovered in 10 cubic feet of air from a slit sampler, and the other from a settling plate that had been placed on top of a fluorescent light fixture 8 feet above the floor for 4 hours.

After tentative identification of *Brucella* by the agglutination test, additional confirmatory tests were performed on each isolate with the following results. Gram stains showed a small gram-negative rod. They were nonmotile. Growth was equally good both aerobically and in 10 percent CO_2 at 37° C. Growth was typical on blood agar; that is, good growth in the heavy part of the streak with no isolated colonies until after 24 hours' incubation. Smooth growth occurred on acriflavine. Immune serums prepared from isolates agglutinated antigens of *B. suis* to a high titer; antigens prepared from isolates agglutinated in *Brucella abortus* absorbed antiserum but produced no agglutination in *Brucella melitensis* antiserum. Immediate urease activity was observed. Growth occurred on 1:50 and 1:100,000 thionin but did not occur on 1:50 and 1:100,000 basic fuchsin. H_2S was produced

throughout a 4-day period. Isolates were found virulent in rabbits and guinea pigs. Final identification was *B. suis*.

Summary

During an epidemic of brucellosis affecting 128 employees of a swine-slaughtering plant in Iowa, 300 swab samples were taken from surfaces, and a total of 480 cubic feet of air was sampled by three sampling devices. All samples were taken during plant operations in 1 week of February 1960.

Despite the heavy overgrowth of saprophytic organisms on the mediums containing antibiotics, *Brucella* was recovered in 10 cubic feet of air from a slit sampler, and another *Brucella* isolation was made from a settling plate. The suspect *Brucella* isolates were biochemically and serologically identified as *B. suis*. The virulence of both strains was demonstrated in rabbits and guinea pigs.

REFERENCES

(*1*) Spink, W. W.: Nature of brucellosis. University of Minnesota Press, Minneapolis, 1956, p. 89.

(*2*) Rosebury, T., et al.: Experimental air-borne infection. Williams & Wilkins Co., Baltimore, Md., 1947, p. 147.

(*3*) Wolf, H. W., et. al.: Sampling microbiological aerosols. PHS Publication No. 686 (Public Health Monograph No. 60). U.S. Government Printing Office, Washington, D.C., 1959.

(*4*) Jones, L. M., and Brinley Morgan, W. J.: A preliminary report on a selective medium for the culture of *Brucella*, including fastidious types. Bull. World Health Organ. 19: 200–203 (1958).

Institutes in the Care of Premature Infants

In the fall of 1962 the institutes for physicians and nurses in the care of premature infants at the New York Hospital-Cornell Medical Center will begin their 14th year of operation. The institutes, under the sponsorship of the New York State Department of Health and the Children's Bureau, are designed to meet the needs of physicians and nurses in charge of hospital premature nurseries and special premature centers and of medical and nursing directors and consultants in State and local premature programs.

Attendance at each institute is limited to six physician-nurse teams. The program for physicians lasts 2 weeks; the one for nurses, 4 weeks. Participants pay no tuition fee, and stipends are provided to help cover expenses during attendance.

Institutes for the 1962–63 year are scheduled to begin September 17, November 5, January 7, March 18, and May 13. Early application is essential since plans are contingent on the number of applications received.

Additional information may be obtained by writing to Box 143, Institute in the Care of Premature Infants, New York Hospital, 525 East 68th Street, New York 21, N.Y.