

MYCOBACTERIA ISOLATED FROM APPARENTLY NORMAL SWINE LYMPH NODES IN URUGUAY¹

A. Saenz² and F. Errico³

Nontuberculous mycobacteria such as Mycobacterium kansasii and M. avium intracellulare currently pose significant problems for human health. The epidemiologic mechanisms involved in their transmission are uncertain, but recent evidence suggests swine could be involved. The work presented here sought to isolate nontuberculous mycobacteria from apparently normal swine lymph nodes in Uruguay, to identify the isolates, and to assess the isolated organisms' pathogenicity in laboratory animals.

Introduction

At present, increasing importance is being ascribed to the study of nontuberculous mycobacteria in both humans and animals (1-3). The principal reason is that as more is learned about these organisms' culture requirements, morphology, pigment formation, and responses to biochemical tests, they are being encountered with increasing frequency. It is true that the tuberculous mycobacteria *Mycobacterium tuberculosis* and *Mycobacterium bovis* continue to pose relatively major human and animal health problems in Latin America (4), and that the less-known nontuberculous mycobacteria have thus far been of relatively slight epidemiologic importance. Nevertheless, the problems posed by the latter types may become more important in the future—as is suggested by increasingly frequent isolation of strains from human patients that pose difficult clinical diagnostic problems and that are hard to treat because of resistance to most currently employed antituberculosis drugs.

The epidemiologic mechanisms producing sources of infection are still not clear in many cases. It is evident, however, that the inci-

dence of human and animal infections caused by nontuberculous mycobacteria that proliferate in the environment (water, soil, and food) is growing (1, 2, 5). It is also evident that swine, because of their susceptibility to various mycobacterial species (particularly *M. bovis*, *M. avium*, and *M. tuberculosis*), are also capable of constituting an important reservoir and source of mycobacterial transmission (4, 6, 7).

As early as 1963, Scannon et al. reported the presence of nonchromogenic bacteria at a hog-breeding facility (6). Later, in 1969, Kleeberg highlighted the significance of finding nontuberculous mycobacteria in swine, considered as a reservoir, and the value of this finding for subsequent epidemiologic work, especially work on cattle (1). Then, a few years thereafter, Gotijo and colleagues isolated nontuberculous mycobacteria from 14.5 per cent of 200 lymph nodes taken from apparently healthy hogs in Brazil (5). However, a bacteriologic study performed by Kantor and Leslie in Argentina, using 715 hog lymph nodes with granulomatous lesions, found that nontuberculous mycobacteria accounted for only 3.7 per cent of the lesions, the remainder being infected with *M. bovis* (88.6 per cent) and *M. avium* (7.5 per cent) (4); while another survey, based on bacteriologic study of granulomatous lesions in Uruguayan swine, reported that 63.9 per cent of the 187 specimens examined yielded *M. bovis*, 31.7 per cent yielded *M. avium*, and only 4.4 per cent yielded other mycobacteria (8).

¹Also appearing in Spanish in the *Boletín de la Oficina Sanitaria Panamericana* 94(4), 1984.

²Doctor of medicine; Laboratory Chief and Bacteriologist, Zoonoses Section, Ministry of Public Health of Uruguay.

³Veterinary doctor; Technician, Department of Bacteriology, Miguel C. Rubino Veterinary Research Center, Casilla Correo 6577, Montevideo, Uruguay.

The purpose of the present work was to determine whether or not nontuberculous mycobacteria were present in the lymph nodes of a sample of apparently healthy swine, to identify each type of mycobacteria isolated, and to study the isolates' pathogenicity in laboratory animals.

Materials and Methods

Over a period of 18 months, 250 specimens of apparently normal hog lymph nodes were obtained at two slaughterhouses—one in the capital city of Montevideo and the other in Canelones, a department capital some 45 kilometers from Montevideo. All the specimens collected from each hog (including the submaxillary, retropharyngeal, bronchial, mediastinal, and mesenteric nodes) were placed in aseptic plastic bags. They were then transported under refrigeration (4-7°C) to the laboratory, where they were dissected and classified as being normal in appearance, edematous, hemorrhagic, or anthracic. Each specimen was then treated according to the method described by Thorel and Boisvert (9). After treatment and neutralization, 0.3 ml of the specimen was placed in each of six tubes containing Lowenstein-Jensen media and six containing Stonebrink media; and two tubes of each type were then incubated at 22°C, 37°C, and 45°C. The cultures were read after 4, 8, 15, 30, and 60 days of incubation. In addition, smears were prepared from each specimen and stained by the Ziehl-Neelsen method.

To classify the types of mycobacteria isolated, the macroscopic appearance of the prepared smears was assessed; the appearance of the cultures was noted; and determinations were made regarding the cultures' chromogenicity, photochromogenicity, development time, optimal growth temperature, and growth in the Lowenstein-Jensen tubes as compared to growth in the Stonebrink tubes. In addition, a number of cytochemical tests were performed to determine the specimens' levels of beta glucosidase, catalase (at room

temperature and at 68°C), niacin, urease, iron uptake, nitrate reduction, potassium tellurite reduction, tween 80 hydrolysis, and growth in 5 per cent sodium chloride.

Experiments to assess certain isolates' pathogenicity were performed using the triple inoculation method developed by Saenz and colleagues (10) with merions,⁴ hamsters, and mice. The merions and hamsters were inoculated intraperitoneally with bacillary masses ranging from 0.5 to 1.0 mg. The mice were given 0.03 mg intravenously and 0.5 mg intraperitoneally. All the inoculated animals were then kept under observation for a period of one year following their inoculation.

Results

In all, 30 sets of specimens (12 per cent of the 250 tested) yielded isolates of mycobacteria. These were classified as indicated in Table 1. A more detailed listing of the test criteria used to arrive at these classifications is provided in Table 2.

Pathogenicity experiments were performed on the 14 isolates listed below.

Strains of isolated mycobacteria tested for pathogenicity	No. of isolates tested
<i>M. avium intracellulare</i>	4
<i>M. terrae-triviale</i>	4
<i>M. scrofulaceum</i>	1
<i>M. kansasii</i>	1
<i>M. chelonae</i>	1
<i>M. vaccae</i>	1
<i>M. aurum</i>	1
<i>M. gastri</i>	1

The detailed findings of these pathogenicity tests on the 14 isolates are to be described in a subsequent communication. The basic results, however, can be summarized as follows:

1) The five isolates in the MAIS group (*M. avium intracellulare*, *M. scrofulaceum*) were found

⁴Members of the rodent genus *Meriones* belonging to the subfamily of gerbils.

Table 1. Strains of mycobacteria isolated from 250 sets of apparently normal swine lymph nodes.

Strains of mycobacteria isolated	No. of isolates	% of isolates
<i>M. avium intracellulare</i>	14	46
<i>M. scrofulaceum</i>	2	7
<i>M. terrae-triviale</i>	5	17
<i>M. kansasii</i>	3	10
<i>M. gastri</i>	2	7
<i>M. vaccae</i>	2	7
<i>M. chelonae</i>	1	3
<i>M. aurum</i>	1	3
Total	30	100

to produce similar lesions in gerbils, hamsters, and mice, the gerbil being the most sensitive. During the first three months a pathologic picture of a Yersin type developed; but continued observation showed that the animals could present pulmonary pictures of a bronchopneumonia type at the time of death.

2) The tested *M. kansasii* isolated produced a picture similar to the classical one described by Buhler and Pollak (11). The animal of choice for pathology testing of *M. kansasii* is the hamster.

3) Certain of the strains classically regarded as saprophytic (*M. terrae-triviale*, *M. chelonae*, *M. vaccae*, and *M. aurum*) were found to possess important pathogenic abilities, producing specific and significant lesions.

4) The *M. gastri* strain tested showed no evidence of pathogenicity.

Discussion

The proportion of swine (30 of 250) yielding mycobacterial isolates should be considered significant, especially since the samples were obtained from animals destined for human consumption.

Swine, which are usually infected through the digestive tract (via food or water) or sometimes via fomites in the air, can serve as important mycobacterial reservoirs and transmission vehicles (2, 4, 6). There is thus a need to pursue further studies aimed at determining the importance of these animals in mycobacterial transmission and the type of lesions that transmitted mycobacteria can cause in both humans and animals. Until now, most studies on the isolation and classification of mycobacteria in swine have been done on specimens exhibiting granulomatous lesions (1, 3, 4, 6, 8, 12), with the result that these studies obtained a high frequency of *M. bovis* isolates. However, previous studies of swine lymph nodes without macroscopic lesions have not yielded isolates of *M. bovis*, and the overall percentages of mycobacterial species isolated were similar to those obtained by ourselves (5, 7, 13).

Fourteen of the isolates obtained in the present study (46 per cent of the total) consisted of *M. avium intracellulare*. This finding is consistent with the fact that this mycobacterium has accounted for a high percentage of the isolates obtained by other studies of lymph

Table 2. Test criteria used to identify the 30 strains of mycobacteria isolated from the lymph nodes of 250 apparently normal swine.

Strain isolated and number of isolates	Runyon group	Development time (slow or fast)	Development at			Pigment production in			Nitrate reduction	Catalase at		Tween 80 hydrolysis at		Tellurite reduction	β-glucosidase	Growth in 5% Iron NaCl uptake	
			22°C	37°C	45°C	Dark	Light	Niacin		22°C	68°C	Urease	5 days			10 days	(3 days)
<i>M. kansasii</i> (3)	I	S	+	+	-	-	+	-	+	+	-	+	-	-	-	-	-
<i>M. scrofulaceum</i> (2)	II	S	+	+	-	+	+	-	+	±	+	-	-	±	-	-	
<i>M. avium intracellulare</i> (14)	III	S	+	+	+	-	-	-	-	+	+	-	-	+	-	-	
<i>M. gastri</i> (2)	III	S	+	+	+	-	-	-	+	+	-	+	-	-	-	-	
<i>M. terrae-triviale</i> (5)	III	S	+	+	±	-	-	-	+	+	+	-	+	-	+	-	
<i>M. chelonae</i> (1)	IV	F	+	+	-	-	-	±	-	+	+	+	-	-	-	+	
<i>M. vaccae</i> (2)	IV	F	+	+	-	+	+	-	+	+	+	+	+	+	+	+	
<i>M. aurum</i> (1)	IV	F	+	+	-	+	+	-	-	+	+	+	+	+	+	+	

nodes, both with and without lesions (4, 5, 8, 12, 13).

It appears that humans contract *M. avium intracellulare* infections from some natural reservoir, since no transmission between humans has been found (4). The results of the present study suggest swine as a likely source of *M. avium intracellulare* infection in man.

There is also evidence that exposure to this mycobacterium may be fairly common. Specifically, partial sample surveys of the Uruguayan population's reaction to avian tuberculin have found positive response rates ranging from 5.3 to 36 per cent (2). Despite these high percentages, however, only four clinical cases produced by *M. avium intracellulare* have been reported (14).

In regard to the *M. scrofulaceum* and *M. kansasii* isolated in our study, these have been isolated previously from swine lymph nodes with and without lesions (4, 5, 7, 13). Bacilli of the *M. scrofulaceum* type have been implicated by authors in several countries as a cause of adenitis in children (10, 15, 16), and *M. kansasii* is known to cause severe illness in man. The natural habitat of *M. kansasii* is not known, but it has been isolated from cattle, swine, water, and milk (16).

The rest of the mycobacteria isolated in our study (*M. terrae-triviale*, *M. gastri*, *M. chelonae*, *M. vaccae*, and *M. aurum*) are considered saprophytic in both humans and animals; nevertheless, as noted above, we found some of these to demonstrate pathogenicity in laboratory animals. It is also worth noting that in two instances *M. vaccae* has been isolated from retropharyngeal nodes of dairy cattle with macroscopic lesions, and isolates from two

human hospital patients have also been identified by one of us (Errico) as consisting of *M. vaccae*.

Also, the finding of a relatively high percentage of atypical mycobacteria, regarded as nonpathogenic but capable of sensitizing humans and animals, indicates that a share of the positive human reactions to tuberculin may be due to paraspecific sensitization by these mycobacteria (2). This suggests that their possible role as sensitizing agents could prove a worthwhile subject of future study.

Conclusions

In our opinion, several findings emerging from the present study have implications of epidemiologic importance. These findings are (1) that swine appear to serve as a natural reservoir for nontuberculous mycobacteria; and (2) the relatively high rate of mycobacterial isolations from our specimens (12 per cent) supports comparable values reported by Gotijo et al. in Brazil (14.5 per cent) and García Rodríguez et al. in Spain (14 per cent) (5, 13).

It also seems noteworthy that 16 of the 30 isolates obtained from our swine specimens in Uruguay belonged to the MAIS complex, and that three others were identified as *M. kansasii*. This means that a total of 63.3 per cent of the mycobacteria isolated were identified as pathogens capable of infecting people. It also appears that these pathogenic species, like those that are saprophytic, may interfere with the diagnosis of tuberculosis by producing paraspecific sensitization in both humans and animals.

ACKNOWLEDGMENTS

We wish to express our appreciation to Mrs. Maria Alba Boga de Frost of the Ministry of Public Health Zoonoses Laboratory and to Mr. Miguel Castro, Laboratory Assistant at the Miguel C. Rubino Veterinary Research

Center, for their assistance in preparing material for isolation and classification. We are also very grateful to Dr. Isabel M. de Kantor of the Pan American Zoonosis Center's Tuberculosis Section for confirming our typing

of these isolates. In addition, we would like to thank Ms. F. Thorel of the Alfort School who provided confirmatory typing of two isolates

belonging to the old Runyon Group IV, one of these being the isolate of *M. aurum* and the other an isolate of *M. vaccae*.

SUMMARY

While the importance of nontuberculous mycobacteria such as *Mycobacterium avium intracellulare* and *M. kansasii* as a source of human and animal disease is far less than that of *M. tuberculosis* and *M. bovis* in the Americas, the former are being isolated with increasing frequency from human patients and are posing significant clinical diagnosis and drug treatment problems. Furthermore, though it appears that swine can serve as reservoirs and transmission vehicles for such bacteria, the epidemiologic mechanisms involved are still unclear. The purpose of the work reported here was to determine whether the lymph nodes of apparently healthy swine were harboring nontuberculous mycobacteria, to identify the types of mycobacteria isolated, and to assess the isolates' pathogenicity in laboratory animals.

Accordingly, sets of lymph nodes from 250 swine were obtained at Uruguayan slaughterhouses. Smears were prepared from these specimens, samples were cultured on Lowenstein-Jensen and Stonebrink media, and a number of cytochemical and other tests were performed.

Thirty of these swine (12 per cent) yielded isolates of mycobacteria, including 14 of *M. avium in-*

tracellulare; five of *M. terrae-triviale*; three of *M. kansasii*; two each of *M. scrofulaceum*, *M. gastri*, and *M. vaccae*; and one each of *M. chelonae* and *M. aurum*. Subsequent testing demonstrated that selected isolates of *M. avium intracellulare*, *M. scrofulaceum*, and *M. kansasii* exhibited pathogenicity in gerbils, hamsters, and mice, and also that isolates of certain types generally regarded as saprophytic (*M. terrae-triviale*, *M. chelonae*, *M. vaccae*, and *M. aurum*) produced significant lesions in laboratory animals.

The presence of these atypical mycobacteria in a relatively high percentage of the test animals suggests that such mycobacteria—both saprophytic and pathogenic varieties—might be sensitizing humans and animals to tuberculin, thereby interfering with the diagnosis of tuberculosis by means of tuberculin testing. In addition, the findings support the hypothesis that swine serve as a natural reservoir for nontuberculous mycobacteria. In this regard it appears significant that the swine tested were destined for human consumption, and that 19 of the 30 isolates obtained (63.3 per cent) consisted of pathogenic mycobacterial types capable of infecting people.

REFERENCES

- (1) Kleeberg, H. H., and E. Nel Ellen. Porcine mycobacterial lymphadenitis. *J S Afr Vet Assoc* 40 (3):233-250, 1969.
- (2) Saenz, A., W. Pedreira, et al. *Sensibilidad cutánea a los antígenos de micobacterias atípicas en el Uruguay*. Montevideo, 1979.
- (3) Thoen, C. O., J. L. Jarnagin, and M. L. Champion. Isolation and identification of mycobacteria from porcine tissues: A three year summary. *Am J Vet Res* 36(9):1383-1386, 1975.
- (4) de Kantor, I. N., and I. W. Lesslie. Aislamiento y clasificación de micobacterias de ganglios de cerdos en la Argentina. *Bol Of Sanit Panam* 77(6):495-499, 1971.
- (5) Gotijo, P. P. (filho), D. Do Nascimento, and L. Souza Fonseca. Isolamento de micobacterias atípicas a partir de ganglios linfáticos de suínos. *Revista Brasileira de Microbiologia* 5(3):59-62, 1974.
- (6) Scammon, L. A., M. J. Pickett, S. Froman, and D. W. Will. Nonchromogenic acid-fast bacilli isolated from tuberculous swine: their relation to *M. avium* and the "Battey" type of unclassified mycobacteria. *Am Rev Resp Dis* 87:97-102, 1963.
- (7) Viallier, J., J. Dabrigeon, and G. Viallier. Isolement de mycobactéries atypiques á partir de ganglions de porcs présumés sains. *Bulletin de la Société de Médecine Veterinaire Comparée* (Lyon) 78:137-140, 1976.
- (8) Errico, F., and J. Bermúdez. Identificación de micobacterias en suínos. *Revista de la Sociedad de Medicina Veterinaria del Uruguay* 16(74):117-119, 1980.
- (9) Thorel, M. F., and H. Boisvert. Action de l'acide sulfúrique a 4 per cent sur les diverses especes de mycobacteries. *Am Biol Clin* 34:431-435, 1976.
- (10) Saenz, A. C., R. M. Canto de Vallone, and E. F. Vallone. Poder patógeno experimental del *Mycobacterium scrofulaceum*. *Revista Uruguaya de Patología Clínica y Microbiología* 13(2):17-25, 1975.

(11) Buhler, V. B., and A. Pollak. Human infection with atypical acid-fast organisms: Report of two cases with pathologic findings. *Am J Clin Pathol* 23:363, 1953.

(12) Songer, J. G., E. J. Bicknell, and C. O. Thoen. Epidemiological investigation of swine tuberculosis in Arizona. *Can J Comp Med* 44:115-120, 1980.

(13) García-Rodríguez, J. A., F. Martín-Luego, M. C. Saenz-González, and M. Marreno Suárez. Isolement de mycobactéries atypiques a partir de ganglions bovins et porcins présumés sains. *Revue*

d'Epidémiologie, Médecine Sociale et Santé Publique 23:269-276, 1975.

(14) Tellerin, M., et al. Micobacteriosis. Unpublished document, 1982.

(15) Galiana, J., and W. Pedreira. Adenitis cervical por micobacterias atípicas. *Archivos de Pediatría del Uruguay* 42(2):65-86, 1971.

(16) Meyer, L., and H. David. *Mycobacteriologie en Santé Publique: Centre National de Référence pour la Tuberculose et des Mycobacteries*. Institut Pasteur, Paris, 1980.

RUBELLA SURVEILLANCE IN THE UNITED STATES

Although the incidence of rubella reported in the United States has fluctuated slightly over the past several years, a downward trend has been observed for most of the country. A review of data for the period 1 January 1980 through 24 September 1983 indicates that if no sudden change in reporting patterns occurs, the annual incidence of rubella in 1983 should be the lowest ever.

In 1980, a total of 3,904 cases of rubella were reported to the U.S. Centers for Disease Control; 2,077 cases were reported in 1981; and 2,325 cases were reported in 1982. During the first 38 weeks of 1983 (ending 24 September 1983), 791 cases were reported, a 61% decrease from the number reported during the same period in 1982.

Regarding congenital rubella syndrome (CRS), detailed reports of CRS cases are collected at the U.S. National Congenital Rubella Syndrome Registry. The cases reported are classified as confirmed or as compatible with CRS according to specific criteria and are reported by year of birth.

According to the registry, the incidence of confirmed and compatible cases has declined substantially since 1979. Fifty-five cases were reported in 1979, 14 in 1980, nine in 1981, and nine in 1982. California reported seven of the nine cases in 1982 and is the only state that has reported cases in 1983 (three cases, all with estimated dates of conception in 1982). Almost all CRS cases continue to be reported within the first year of birth.