Tests demonstrated that normal animals do not acquire nocardial mastitis by oral intake of infectious milk. The organism is also effectively destroyed in commercially processed milk which meets the minimum pasteurization standards.

Oral Infectivity and Thermal Resistance of Nocardia asteroides in Milk

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THE RECOGNITION of *Nocardia asteroides* as an etiological agent of bovine mastitis was reported from California in 1957 (*1,2*). Since that time the disease has been recognized in six California and three Hawaiian herds and has been reported from Texas (*3*), Massachusetts (*4*), and Alabama (letter from C. S. Roberts to Dr. L. Ajello, Communicable Disease Center). Previous reports of the thermal resistance of the organism (*5,6*) and of its infectivity for man (*7–11*) suggested public health complications might arise from this disease in cattle.

The most probable site of primary human infection appears to be the lungs (*8,9,11,12*), although injuries to the teeth and gums may also serve as portals of entry (*13,14*). In addition, it has been stated that *N. asteroides* may be transmitted to man in milk (*15*). Thus, the oral infectivity for susceptible species and the susceptibility of the organism to pasteurization were important factors to consider when the presence of the organism in a universally consumed food became known.

This paper reports the results of experiments to investigate the susceptibility of swine, calves, and guinea pigs to this organism when exposed by the oral route and to study the thermal resistance of *N. asteroides* in milk.

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Oral Transmission Test Methods

All oral transmission trials were conducted with milk from infected cows. Weekly cultural analyses of the milk were made, and the population of *N. asteroides* was estimated by plate counts. Guinea pigs, calves, and young swine were fed fresh milk from infected bovine mammary glands. All three species had previously been shown to be susceptible to infection by the intravenous and intraperitoneal routes.

Calves. Five dairy calves 1 week to 1 month of age were fed milk from infected cows for 1 to 5 months. Approximately 2 to 4 million organisms were consumed daily. These ani-

mals were skin tested prior to and subsequent to the feeding period. They were bled periodically and their serums tested for complement-fixing antibodies for *N. asteroides* (*16*).

At necropsy cultural examinations were made of swabs from the larynx, esophagus, trachea, rumen, omasum, small intestine, and tissues from mesenteric lymph nodes. Two control calves receiving normal milk were handled in an identical fashion. One calf which had received infectious milk for 3½ months without visible effect was switched to normal milk and injected both intravenously and intraperitoneally with 2 billion organisms. Subsequently, the clinical response, skin test reactions, and complement fixation titers of this animal were observed.

Guinea pigs. Three guinea pigs were fed milk containing 20,000 to 30,000 *Nocardia* organisms per milliliter. Milk consumption by these animals was estimated at 10 ml. per animal per day. These animals were killed at intervals over a 4-month period and examined for the presence of *Nocardia* in the alimentary canal or for lesions of nocardiosis.

Five guinea pigs were anesthetized, their pharyngeal mucous membranes were scarified, and infectious milk was introduced into the oral cavity. These animals were subsequently fed infectious milk ad lib as described for the above group.

Aspiration of infectious milk was induced in nine guinea pigs. The guinea pigs were anesthetized and 0.5 ml. of milk containing 100,000 *Nocardia* organisms per milliliter was placed in the oral cavity. The nostrils of each animal were manually blocked until audible aspiration occurred. The guinea pigs of this group were examined culturally and histologically for infection after a period of 3 weeks, unless death occurred earlier.

Swine. Five weanling swine weighing 35 to 40 pounds were placed on a ration of grain and milk from infected udders which contained approximately 1,000 viable *Nocardia* organisms per milliliter. During a 3-month period these animals consumed 2 to 4 liters of milk daily containing 1 million viable nocardial elements per liter. These animals were observed for clinical illness and were culturally examined at necropsy. Cultural examination included swabs taken from the esophagus, stomach, small intestine, large intestine, and cecum plus aseptically harvested specimens of mesenteric lymph node, spleen, liver, lung, heart blood, and bile.

Thermal Resistance Test Methods

To determine the resistance of *N. asteroides* to pasteurization, two types of milk samples were prepared for use in the time-temperature trials. The first consisted of normal milk to which culture material of a strain of *N. asteroides* was added. This strain had been isolated from a dairy cow infected with a severe form of nocardial mastitis. The strain was grown on tryptose agar containing 5 percent washed bovine erythrocytes and had been transferred on this medium innumerable times over a 2-year period. Microscopic examination of the organisms added to the milk showed that many of the mycelial elements were fragmented into rod and coccoid forms.

The second sample consisted of fresh milk drawn from infected bovine mammary glands. These glands were naturally infected or had been experimentally infected as described previously (*17*). All of these glands showed marked clinical signs of infection. Microscopic examination of this milk showed predominantly branched mycelial forms of *Nocardia.*

Heating procedure. To simulate commercial pasteurization, we adopted a finely controlled method of heat exposure similar to the technique used by Enright and co-workers (*18*) in studying thermal inactivation of *Coxiella burnetii.* This method employed a large-capacity water bath equipped with a mechanical stirring device and mercury thermostat capable of maintaining the temperature of the water within ±0.2° F. Milk samples containing the organisms were sealed in containers, immersed in the water bath, and vigorously agitated during the heating period. When the critical milk temperature was reached, the holding period was determined by stopwatch timing and rechecked against the time-temperature record of each test. At the end of the holding period the milk samples were immersed in a circulating cold water bath (40° F.). A thermistor-actuated, continuously recording potentiometer recorded the exact

temperature changes during heat-up, holding, and cool-down periods. The time sequences were measured both manually and by means of a mechanical timing device arranged to record at 1-second intervals. After cooling, the samples were plated in duplicate on tryptose agar containing 5 percent washed bovine erythrocytes. These preparations were incubated for 96 hours at 37° C., after which time colony counts were made.

Survival and destruction end points. As used in this study, the end point of survival was the maximum time a sample could be heated at a given temperature and still contain demonstrably viable elements of *N. asteroides.* The time of destruction, or negative end point, was the minimum time a sample had to be heated at a given temperature to destroy detectable evidence of viable elements of the organism.

Time correction. Burton's formula (*19*), based on the present pasteurization curve, was used to estimate the duration of heat exposure of the organisms occurring during heating and cooling. This time was then added to the time at the holding temperature in order to estimate the total, or corrected, thermal inactivation time at the holding temperature. While this method is not theoretically correct, it provides an estimate in which the error is relatively small.

The cultural techniques (*2*) and the skin test and complement fixation techniques (*16*) have been described in previous papers.

Oral Transmission Results

Calves which consumed infectious milk for 1 to 5 months showed no evidence of clinical illness, nor were lesions detected at necropsy. Neither skin test reaction nor positive complement fixation titer was demonstrable in these animals at the conclusion of the trial. At necropsy, *N. asteroides* was isolated from the larynx, esophagus, rumen, and omasum of some of the exposed animals but not from the small intestine. The organism was isolated from the trachea of one calf, but no attendant lesions of the trachea, bronchi, or lungs were seen.

Calf 431, which had been fed nocardial milk for 3½ months, was switched to normal milk and was then injected intravenously and intraperitoneally with 2 billion *Nocardia.* A positive clinical response followed, marked by a temperature rise which peaked at 105.3° F. 2 days after inoculation. Four weeks after inoculation the calf had a positive skin test and a complement fixation titer of 1:32. Both of these tests had given negative results when used prior to feeding milk containing the organism, and they repeatedly gave negative results during the 3½ months that the infectious milk was fed. The calf was killed and examined 5 weeks after inoculation. *N. asteroides* was cultured from an encapsulated lesion occurring at the peritoneal inoculation site.

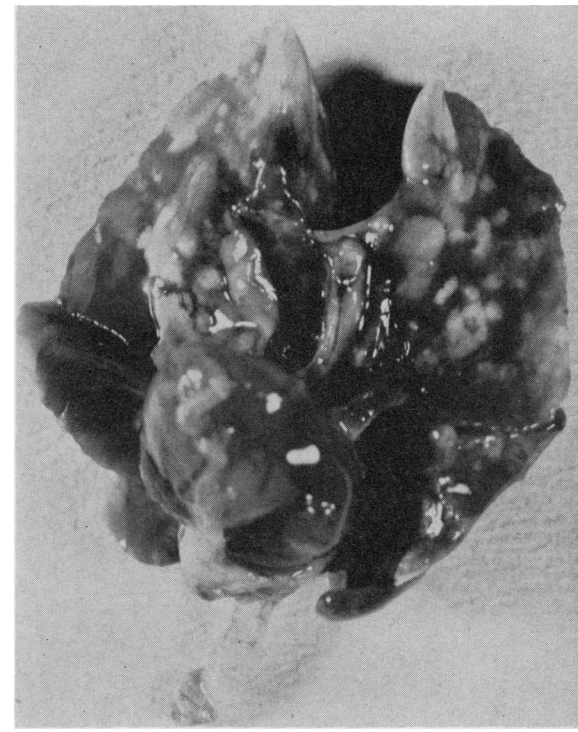

Figure 1. Lung lesions observed in guinea pig after experimentally induced aspiration of milk containing Nocardia asteroides

Guinea pigs fed infectious milk ad lib for 1 to 4 months showed neither illness nor lesions at necropsy. *N. asteroides* was isolated from the esophagus but not from the stomach, small intestine, or cecum. Two of the five guinea pigs whose pharyngeal mucous membranes were scarified prior to receiving infectious milk died within 48 hours. The inframandibular region of each was greatly swollen from a streptococcal cellulitis. The remaining three guinea pigs showed neither illness nor lesions at

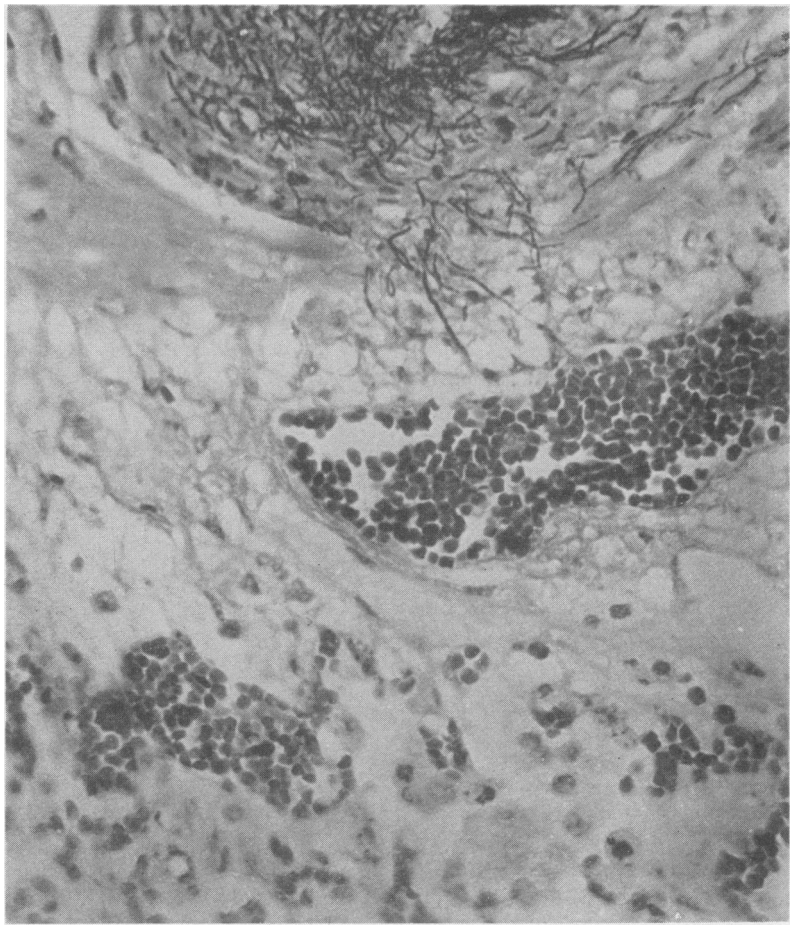

Figure 2. Lung lesion in guinea pig: extension of the mycelial elements from the bronchiolar lesion at the top into the vessel at the center (Gram-Weigart stain X 400)

necropsy, nor were *Nocardia* organisms recovered by cultural methods. Of the nine guinea pigs inoculated by aspiration, four died of nocardial pneumonia and another had well-developed lesions of pulmonary nocardiosis when examined at necropsy, 20 days after aspirating the infectious milk.

Necropsy of the guinea pigs developing nocardial pneumonia after aspiration of the infectious milk showed firm, red, atelectatic areas throughout the lungs, and numerous 1 to 2 mm. foci containing thick pus were observed (fig. 1). Adhesions between the lungs and the thoracic cage were noted. Tissue sections from the lungs showed numerous necrotic foci with early fibrotic changes occurring at the periphery of the lesion. Extensive edematous infiltration of the alveoli was seen with some alveoli being packed with exudate as were the neighboring bronchioles. Extension of the lesions to adjacent vessels was noted (fig. 2). *N. asteroides* was isolated from and demonstrated histologically in the lung lesions of the animals. Metastatic infection of the spleen of one animal was observed.

It was thought that the results of oral transmissibility studies on the normal ingestion of infectious milk by calves might have been altered by the complexity of the bovine stomach. Guinea pigs, while susceptible to *N. asteroides* and possessing a simple stomach, did not consume milk readily. Swine were investigated as a third species for oral transmissibility studies because they possessed a simple stomach, would consume milk readily, and were susceptible to infection by parental inoculation.

The five swine fed infectious milk averaged a 150-pound gain in body weight during the 90 days' observation. No signs of illness were noted during the feeding period, nor were lesions detected in any of the swine at necropsy. Cultural examinations resulted in recovery of the organism from the esophagus of one animal, but none was isolated from lower areas of the digestive canal.

The results of the oral transmissibility trials are summarized in table 1.

Pasteurization Results

Early trials using culture-inoculated milk that was heated in an ordinary water bath at 147.2° F. and 150.8° F. for 30 minutes indicated that *N. asteroides* survived these exposures which exceed the minimum recommendations for commercial pasteurization. These results were in agreement with those of others (*5*, *6*) who used culture-inoculated milk in their trials.

When fresh milk from infected udders was heated in a carefully controlled system, in no instance did the organisms drawn from infected glands survive the recommended pasteurization conditions of 145° F. for 30 minutes or 161° F. for 15 seconds. These results are presented in figure 3. When milk from infected udders was held at 40° F. for 3 days or frozen for 3 months, the organisms did not appear to have a heat resistance differing from that of organisms in fresh milk from infected udders.

In table 2 are presented the pertinent results of heating milk from infected cows and of heating culture-inoculated milk. These results are arranged according to certain selected temperatures and the maximum time of survival and minimum time of destruction at these temperatures. These times have been corrected to include the lethal effect of the heat-up period as previously described. The times are estimations of the length of the holding period at a certain temperature if the holding temperature was attained instantaneously. The negative end points, using milk from infected cows, are below those of the presently recommended minimum standards for the pasteurization of milk.

It is unlikely that milk from infected cows being pasteurized commercially would contain organism populations which exceeded those in this study. Infected glands observed during

Table 1. Transmissibility of Nocardia asteroides through milk to susceptible animals

Species	Method of exposure	Clinical illness	Necropsy	*Nocardia* recovered	Immune response	
					Skin test	Average complement fixation titer
3 guinea pigs.	Fed ad lib 1–4 mo.	None	Neg	Esophagus (2)[1]		
5 guinea pigs.	Scarified pharyngeal mucosa, fed ad lib 4 mo.	2 died within 48 hrs.	Streptococcal cellulitis.	None.		
		3 none	Neg	None.		
9 guinea pigs.	Aspiration	4 died; 1 ill	Lung lesions	Lungs (5); spleen (1).		
5 calves	Fed 1–5 mo.	None	Neg	Esophagus (2); rumen (2); larynx (1); trachea (1); omasum (1).	Neg.	Neg.
Calf 431	Fed 3½ mo., then challenged intravenously and intraperitoneally.	Noted after 2 days' temperature 105.3° F.	Lesion at inoculation site.	Lesion	Positive.	1 : 32
2 calves	Fed normal milk 3–5 mo.				Neg.	Neg.
5 swine	Fed 1–3 mo.	None	Neg	Esophagus (1)		

[1] Numbers in parentheses indicate the number of animals in which the finding was made.

this study did not shed in excess of 500,000 organisms per milliliter of milk. Glands shedding 10,000 or more *Nocardia* organisms per milliliter are usually quite indurated and are not likely to be milked for commercial purposes. The dilution factor from bulk handling of milk would be sufficient to hold the count well below the range encountered in these tests.

Discussion

In view of these findings it appears unlikely that infection will result from the consumption of commercially processed milk from udders shedding *N. asteroides*. Oral consumption of infectious milk was not an effective means of transmission. Three susceptible species, subjected to diets of infectious milk for several months, consistently failed to develop detectable infection from simple oral exposures. Even deliberate injury of the pharyngeal epithelium of guinea pigs coupled with immediate superimposition of infectious milk on the traumatized area failed to produce nocardial infection. However, the possibility of infection resulting from the aspiration of small quantities of milk

Figure 3. Thermal resistance of Nocardia asteroides in milk from infected mammary glands

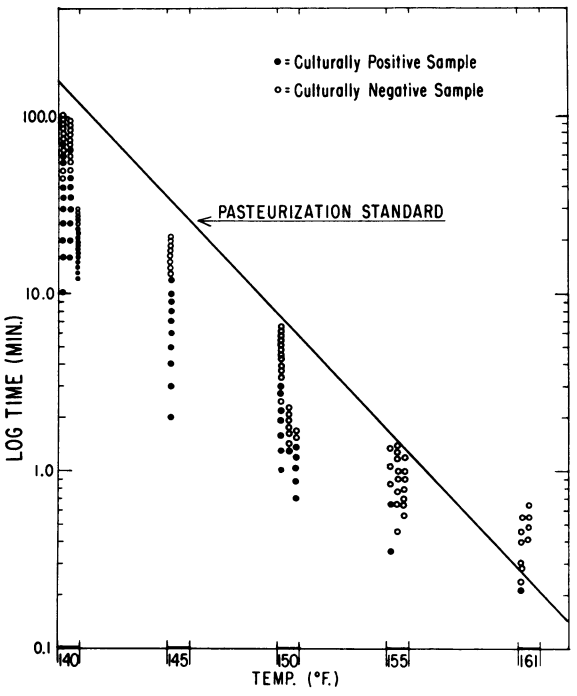

Table 2. Pertinent times and temperatures resulting in survival or destruction of Nocardia asteroides in milk

Organism source	Organism count before heating (per milliliter)	Holding temperature (° F.)	Maximum survival time [1] (minutes)	Minimum destruction time [1] (minutes)
Infected cow (fresh milk)	154, 000	140	70. 0	75. 0
	220, 000	145	12. 0	13. 0
	114, 000	150	3. 1	3. 4
	323, 000	155	. 6	. 8
	40, 000	161	. 21	. 23
Infected cow (milk held 3 days at 40° F.)	5, 800	145	2. 0	3. 0
Culture-inoculated milk	165, 000	145	40. 0	--------
	430, 000	161	. 33	. 49

[1] Times were corrected for the heat-up interval.

laden with *Nocardia* organisms has been established. These results tend to support the observation of Weed (*11*), who considers the lung as the most probable organ for primary infection in man.

Previous reports of the survival of *N. asteroides* beyond the limits of pasteurization were derived from experiments in which the milk was inoculated with undefined numbers of organisms from culture media. While there appear to be differences in heat susceptibility between organisms from infected glands and those from culture media, our results indicate that comparatively high numbers of *N. asteroides* in fresh or refrigerated milk from infected udders are susceptible to current pasteurization techniques.

A possible threat to public health remains because many persons, especially dairymen and their families, consume raw milk. This practice has been observed in dairies where the disease in animals is known to exist. Furthermore, since there are indications that some of the bovine isolates are of unusually high virulence (*17*), personnel handling infected animals should use precautions against self-contamination. For this reason, and the danger of possible aspiration of infectious milk, the practice of consuming raw milk from infected herds should be condemned, and the slaughter of animals known to be infected should be recommended.

Summary

Oral infectivity of *Nocardia asteroides* was not demonstrable in normal guinea pigs, swine, and calves fed milk containing the organism. Injury of the pharyngeal epithelium of guinea pigs immediately preceding their exposure to infectious milk did not result in infection. Immunological reactions of calves did not imply that close association between host and parasite had developed during the period of feeding of the infectious milk. *Nocardia* organisms were cultured only from the upper alimentary tract of these animals.

Aspiration of small quantities of infectious milk resulted in the development of nocardial pneumonia and the death of several guinea pigs.

Milk from infected udders subjected to carefully controlled pasteurization trials of 145° F. for 30 minutes and 161° F. for 15 seconds was repeatedly shown to be free of viable elements of *N. asteroides.*

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Minnesota's Alcohol Education Program

To provide a sound program in alcohol education and to clear up misinformation regarding alcohol, the Departments of Health and Education of the State of Minnesota are publishing in *School Health News* a series of articles on alcohol education by Paul Riddle, Minnesota public health education consultant in school health. Several booklets have also been collected and are available for use in educating adults and teenagers alike that alcoholism is an illness that threatens individuals from all walks of life. These materials consist of statements of points of view about what alcohol is, why people drink and the personality of the alcoholic, effects on the individual and the community, and attitudes toward alcohol. A careful study of these booklets is intended to enable individuals to deal with the issue intelligently and to face the facts objectively, as a basis for the development of sound personal convictions and attitudes.

The series of articles in *School Health News* advocates formal instruction on alcohol which deals with its complexities and indicates how widespread is the responsibility for control of alcoholism. Parents and teachers are encouraged to provide objective and unprejudiced information about alcohol in relation to teenage beliefs, attitudes, and practices. As teenagers observe drinking among adults, it is important that they do not conclude that drinking is a way of emulating adult behavior.

"A teenager experimenting with drinking should not be condemned," one article states. "Adolescent drinking is a symptom of the growing pains of approaching adulthood."

Studies to determine student drinking attitudes and behavior indicate that the pattern and social context of drinking by adolescents reflects the pattern and social context of drinking among adults, states Riddle.

In one issue, *School Health News* encloses an article by Jean Libman Block, "Alcohol and the Adolescent," originally published in *Parents' Magazine.*

A child cannot be counseled with wisdom, states Block, until the parent examines his own attitude toward alcohol with complete honesty and decides what attitude he wishes his child to take. As children look to their parents for guidance in the matter of drinking, it is the duty of the parent to assist the child in developing a good attitude toward alcohol and to live by it.

The Minnesota program also employs educational materials obtained from other responsible sources, such as the Yale Center or the Public Health Service.

Raymond G. McCarthy has prepared the booklets, "Facts About Alcohol," Instructor's Guide to Facts About Alcohol," "Discussion Guides for Questions About Alcohol," and a kit, titled "Exploring Alcohol Questions," containing six leaflets, published by the Yale Center of Alcohol Studies, to be used not only by science and health teachers but also in other classes, such as biology, physiology, English, and social studies, in which the alcohol question can be attacked effectively.

The booklets contain detailed suggestions for presenting information on alcohol and provide a common background for students while focusing attention on the individual's decision about drinking and the factors that may influence him in making this decision.

The "Discussion Guides for Questions About Alcohol," a series of three booklets on the physiological effects of alcohol, community opinions on alcohol problems, and individual attitudes toward alcohol, indicate that the use of alcohol does contribute to social distress—but that it is drinking plus certain types of personalities plus certain kinds of situations that create alcoholism.

Booklets prepared by the Department of Health, Education, and Welfare and the Metropolitan Life Insurance Company, both titled "Alcoholism," discuss what alcohol is, the causes of alcoholism, and means of treatment and rehabilitation for the alcoholic. Medical treatment, psychotherapy, and Alcoholics Anonymous are discussed. The booklets offer data to suggest that 6 out of every 10 adults in the United States use alcoholic beverages, and that 1 out of every 15 persons who drink is prone to alcoholism.

The State of Minnesota believes that alcoholism can be prevented. The key to the task is to know what alcoholism is, how it develops, and where aid may come from. The primary goal of the alcohol education program is prevention.