A STUDY OF THE TYPES OF TUBERCLE BACILLI FOUND IN HUMAN TUBERCULOSIS IN ALASKA

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TUBERCULOSIS is the major health problem in Alaska. Table 1 (*Alaska's Health*, 1955) compares active tuberculosis newly reported per 100,000 population in the United States and its Territories for 1954. The records of the Division of Tuberculosis Control, Alaska Department of Health, prior to 1954 do not contain a breakdown of bone and joint, pulmonary, and other forms of tuberculosis. During 1954 a total of 760 new cases were reported, and 40 of these were extrapulmonary. There were 20 meningitis, 10 bone and joint, 2 genito-urinary, 2 pericarditis, 1 peritonitis, and 5 unclassified (throat). A case with extrapulmonary and pulmonary disease was recorded as one, but as pulmonary tuberculosis. Weiss (1953) shows that the majority of the cases are among Eskimo, Aleuts, and Indians living in the outlying areas. Aboriginal health problems in Alaska are those of a people who maintain their primitive culture and who have never known high standards of living, or high quality health services. The high rates of skeletal tuberculosis due to human type tubercle bacillus are the results of contact with highly infectious cases of pulmonary disease in isolated communities.

Table 1. Active tuberculosis cases newly reported per 100,000 population United States and Territories, 1954.

Political division	*Cases per 100,000*
Alaska	370.0
Puerto Rico	157.6
Arizona	154.3
District of Columbia	139.0
New Mexico	89.5
Kentucky	83.4
Hawaii	77.2
All other States	Below 70.0[1]
Av. for the United States and Territories	51.0

[1] Idaho reports the lowest number–14.0.

This study was made to try to determine the types of *Mycobacterium tuberculosis* giving rise to the high prevalence of bone and joint tuberculosis in Alaska. The annual reports of the Alaska Department of Health list the physician and clinic treatment of children under 21 years of age for tuberculosis of the bone and joints as shown in Table 2. The highest morbidity exists among the aboriginal races of Alaska. The study was initiated in 1947 as an adjunct to an extensive skeletal tuberculosis program and streptomycin

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chemotherapy study under the direction of Dr. Philip H. Moore, Orthopedic Surgeon, Alaska Department of Health, Mt. Edgecumbe, Alaska. An additional phase was to observe if types, strains, variations, and mutations different from typical *M. tuberculosis* isolated in other regions of the world were producing extrapulmonary disease and the relative frequence of these in human tuberculosis among Alaskans.

Table 2. Tuberculosis of the bone and joints active and unspecified.

	1948	*1949*	*1950*	*1951*	*1952*	*1953*	*1954*
Cases, skeletal (under 21 years)	102	105	151	196	155	182	180

Attention was first directed to the types found in the extrapulmonary cases in small children and young adults brought to medical centres for surgery from all parts of Alaska. Later, series of sputum specimens were included in the study. Although bovine tubercle bacilli are rarely the etiological agents in pulmonary tuberculosis, we thought it was advisable to include samples as part of this investigation.

Materials and methods

The majority of specimens from extrapulmonary tuberculosis, especially bone and joint cases, were received at the Juneau Central Laboratory of the Alaska Department of Health from Dr. Philip H. Moore for diagnostic purposes. Specimens from a limited number of extrapulmonary and all the pulmonary cases were received at random from private physicians as part of their routine practice. Specimens were received, for the most part, in sterile, screw-cap bottles through the mail as soon as possible after collection; however, some were brought directly to the laboratory from the operating room, hospital or physicians' clinics. Some of the material from bone and joint cases was placed in sterile physiological saline to prevent excessive drying while enroute to the laboratory.

Early in the study, pathological materials received were macerated in sterile pyrex tubes containing sterile quartz sand or were ground in a one-inch steel mortar with pestle. The material was directly transferred and rubbed into the surface of slants of Löwensten-Jensen Medium (LJM) (Holm and Lester, 1941) to make good contact between bacilli and medium. The LJM[1] is capable of supporting the growth of human, bovine, avian strains, and other *Mycobacteria*; at the same time growth on this medium permits differentiation of tubercle bacilli from acid-fast and alcohol-fast saprophytes. Sterile diluent was used at first to recover macerated tissues. Later, however, because of contamination and suspected contamination of the pathological specimens it was necessary to use chemical homogenization followed by con-

[1]The Löwenstein-Jensen medium is a solid egg-potato medium, and in the Alaska Department of Health Laboratories we follow Holm and Lester's directions almost verbatim. The failure of most bovine strains to grow in the original isolation, in the presence of glycerine, while the human type prospers on mediums containing this substance, is of the utmost importance in distinguishing the two types by cultural methods. For this reason, all materials suspected of containing bovine tubercle bacilli should be cultured on both glycerinated and non-glycerinated mediums.

centration and cultivation on LJM. It is appreciated that where possible, homogenization with sodium hydroxide or other chemical should be avoided in the isolation of *Mycobacteria* from solid tissues and lesions, because of the marked reduction in the numbers of viable organisms that can be recovered by culture methods. We used a concentration of 3 per cent sodium hydroxide with equal quantities of the macerated and ground tissues with an incubation of 20 minutes at 37°C, before the addition of acid neutralizer (2 ml. H_2SO_4–2 ml. H_3PO_4 to 100 ml. distilled water) and brom thymol blue indicator. The prepared homogenates were centrifuged and the sediment transferred to and well rubbed into the surface of 5 slants of LJM (3 containing 0.75 per cent glycerine and 2 without glycerine). All culture tubes were examined at the end of the second week and every week thereafter for a period of eight weeks. All cultures which were negative at the end of twelve weeks but which were inoculated with material containing moderate to many numbers of bacilli by smear, were washed with sterile diluent and recultured on fresh LJM and additional smears examined for acid-fast bacilli. All slants were incubated in a position horizontal to the surface of the slant for 24 to 48 hours, to bring the seeding sediment into contact with all parts of the exposed surface. The tubes were then held in an upright position for the remaining period of incubation.

The techniques used for the pulmonary specimens are as follows: sputum was collected in sterile 1 oz. glass bottles with tightly fitted screw caps. The specimens as received were decanted into 250 ml. erlenmeyer flasks with rubber or ST glass stoppers, and an equal volume of 4 per cent sodium hydroxide was added to each. The mixture was held at 37°C for 20 minutes with manual shaking at intervals to assure thorough homogenization. Brom thymol blue was added and the contents were brought to a definite yellow colour by slow addition of the acid neutralizer. The contents were then back titrated with 4 per cent sodium hydroxide until the first persistent greenish-blue tinge appeared. The contents were balanced in 50 ml. centrifuge tubes and centrifuged at 2,000 to 3,000 r.p.m. for 15 minutes, after which the supernatant fluid was discarded. Concentrates thus prepared were used for microscopy and seeding of LJM slants. As with the pathological specimens 5 slants (3 with 0.75 glycerine and 2 without glycerine) were seeded and cultivation carried out in an identical manner.

Since tuberculosis in man is almost always caused by mammalian type tubercle bacilli no attempts were made to incubate cultures at temperatures below or above 37°C. Human disease caused by the avian type bacilli are so infrequently encountered (Feldman, 1938, pp. 357–410; Bradbury and Young, 1946) we did not consider that the relative frequency warranted incubation of slants at the higher temperatures.

Smears prepared from the concentrates were made by spreading approximately 0.1 to 0.2 ml. of the material on new glass slides. The smears were stained according to the Ziehl-Nielson technique and on examination not less than 100 microscopic (oil immersion) fields (Corper and Nelson, 1949) were covered. Corper (1928) and Cummings (1949) have estimated that where

fewer than 100,000 acid-fast bacilli per ml. of sputum are present the detection is very difficult by microscopy.

Results

A comparison of the results of the cultural and animal pathogenicity studies as applied to 180 extrapulmonary and pulmonary specimens containing tubercle bacilli is shown in Table 3. The series subjected to animal inoculations at the Tuberculosis Evaluation Laboratory, Communicable Disease Center, U.S. Public Health Service, Chamblee, Georgia, did not contain any bovine type bacilli. However, one of these cultures from sputum on LJM had the typical dysgonic colonial morphology of the bovine type.[1] This culture resembles the transitional forms described by Jensen (1950, p. 68), who believes that bovine can change to human type tubercle bacilli after a period of residence in the human body. Our strain was found to conform with the pathogenicity of the human type and classified as such.

Table 3. Type classification by culture and pathogenicity studies.

Source of materials	*Cases*	*Type*		*Number of cases in different age groups (bovine type bacilli examined for).*		
		Human	*Bovine*	*Less than 5 yrs.*	*5–15 yrs.*	*16 yrs. and over*
Cervical gland	2	2	0	0	0	2
Bone and joint	62	62	0	13	38	11
Genito-urinary	3	3	0	0	0	3
Pulmonary	106	106	0	0	41	65
Miscellaneous	7	7	0	1	1	5
Total	180	180	0	14	80	86

The central and three regional laboratories of the Alaska Department of Health made a total of 43,990 cultural examinations on specimens of all kinds from July 1944 to June 1955 (Table 4). Of this number 5,113 were typical eugonic colonies and 1 was a dysgonic colony of *M. tuberculosis.* These were tentatively classified as human type on the basis of colonial morphology except the one strain which was later classified with the human type on the basis of pathogenicity. Cummings (1949) has pointed out that through routine use of a specific culture medium such as LJM, a qualified bacteriologist can tentatively identify, by colonial morphology, typical human, bovine, and avian species and distinguish these from chromogenic acid-fast saprophytes. Bergey's

Table 4. Cultures of all sources from laboratories of Alaska Department of Health, 1 July 1944 to 30 June 1955.

Mycobacterium isolated	*Negative culture*	*Unsatisfactory culture*	*Total*
5,114	34,469	2,407	43,990

[1]Most of the typical human type strains grow readily and luxuriantly on LJM appearing as large, rough, "cauliflower" colonies. Such growth is called "eugonic". It is dry and friable when picked up with a needle. Bovine colonies grow poorly and appear later than the human type as small, smooth, and pale to cream coloured colonies. This is known as "dysgonic" growth. These growth differences are important in distinguishing the two types of mammalian tubercle bacilli.

Manual (Breed and others, 1948, pp. 876–91) in the key to the genus *Mycobacterium* lists a considerably greater number of acid and alcohol-fast bacilli, other than those responsible for human tuberculosis, the majority of which are saprophytes or non-pathogenic for mammals or birds. It has been recently and adequately demonstrated (Cummings, 1949; Patnode and others, 1954), that sole reliance on colonial morphology is not advisable and the final determinations of the types of *M. tuberculosis* should always be made through the use of healthy laboratory animals.[1]

Table 5. Number or frequency of human and bovine tubercle bacilli in 2,227 cases of human tuberculosis, prepared from figures collected by Möllers.

		0—15 years		*16 years and over*	
Source of materials	*Cases*	*Human*	*Bovine*	*Human*	*Bovine*
Bone and joint	203	108	44	50	1
All forms	2,227	682	195	1,301	29

A total of 30 strains of chromogenic saprophytic *Mycobacteria* were isolated in our laboratories during the period 1 July 1944 to 30 June 1955 from the 43,990 field speciments obtained. The colonies of the acid-fast saprophytes appeared quickly on LJM, producing well-marked differences and thus presented few difficulties in differentiation from genuine mammalian tubercle bacilli. These cultures were classified by the Tuberculosis Evaluation Laboratory to be avirulent by pathogenicity studies.

No attempts were made in this study to pick, for pure-culture specific identification, colonies of acid and alcohol-sensitive commensal microorganisms which had escaped alkaline homogenization.

The incidence of human infections, including pulmonary tuberculosis, due to bovine tubercle bacilli is not known in the United States, according to Feldman and Karlson (1947, p. 239). However, an early study by Park and Krumwiede from 1910 to 1912 (1910; 1911; 1912) indicated the incidence of bovine type tuberculosis in children to be about 6 to 10 per cent. Price (1932) studying tuberculosis in Canada found the incidence of bovine type bacilli to be 14.1 per cent of 268 tuberculous children, while the percentage frequency in 168 tuberculous adults from the same area was only 3.5 per cent. His investigation showed that the individuals with bovine type tuberculosis were using raw milk at the time their illness was diagnosed. Möllers (1928) reviewed the literature on the incidence of human and bovine types of tubercle bacilli in 2,244 cases of tuberculosis in human beings of known groups. Table 5 lists the cases of bone and joint disease and the totals for all forms of tuberculosis. Seventeen cases had infections from which both types were isolated. These were not included in the final tabulations. Studies of recent years show a definite decline in the relative frequency of bovine type tubercle bacilli in human tuberculosis where pasteurization or boiling of milk is practised.

[1]In our study, the 180 cultures of the tubercle bacillus and the chromogenic *Mycobacterium* spp. were referred to the Tuberculosis Evaluation Laboratory, Atlanta, Georgia, for animal confirmation of our cultural findings. The one dysgonic culture and some of the 5,113 isolated prior to the study were likewise subject to animal study, but the remaining cultures were tentatively classified as human type on the basis of colonial morphology.

Table 6. Total tuberculin tests of cattle in Alaska with reactors. Fiscal years 1917–1942.

	Herds tested	*Cattle tested*	*Reactors found*	*Per cent reactors*
1917–42	467	4,756	75	1.6

Total tuberculin tests of cattle in Alaska with reactors

Year	*Cattle tested*	*Reactors found*	*Per cent reactors*
1941	958	4	0.41
1942	1,064	2	0.18
1943	1,302	2	0.15
1944	1,243	3	0.24
1945	1,382	7	0.50
1946	688	0	0.00
1947	1,254	2	0.15
1948	1,017	1	0.09
1949	1,558	2	0.13
1950	1,436	0	0.00
1951	1,192	0	0.00
1952	1,059	0	0.00
1953	1,811	0	0.00
1954	1,436	0	0.00
1941–54	17,400	23	0.13

Gernez (1939) demonstrated that there is a high incidence of tuberculosis in the cattle of France, but a low incidence of human tuberculosis caused by the bovine type tubercle bacilli. He attributes this to the general practice of boiling milk to preserve it, as the percentage rises in areas where such preservation of milk is not a common practice.

In the parts of Alaska where all forms of tuberculosis are highly prevalent, cattle for milk production are exceedingly rare or non-existent. Even though raw milk may not be used by the aboriginal peoples living in these regions, the people are closely associated with animals and handle large volumes of meat which is eaten partly cooked or raw. No cases of tuberculosis have been reported in herds of Alaskan reindeer slaughtered under veterinary supervision, but there are no data on the tuberculosis rates among the other animals which these people use as food.

The low incidence of infections in Alaska, predominately among caucasians and non-natives, living in or near the population centres where dairy cattle are most common, has been determined by tuberculin testing and evaluated by Weiss (1953). Pasteurization is the common practice for most of the milk and milk products commercially distributed in these more populated centres and the sections immediately adjacent. Tuberculin-testing of cattle in Alaska was instigated in 1917 by the Bureau of Animal Industry, U.S. Department of Agriculture, and veterinarians of the Alaska Department of Agriculture have carried on this program since 1941 as shown in Table 6. The upper part of the table lists the numbers of tuberculin-tested cattle with reactors during fiscal years 1917 to 1942, as tested by the B.A.I. and the lower part shows the total numbers of cattle tested by the territorial veterinarians annually from 1941 through to 1954. J. W. Wilson, Commissioner of Agriculture, Alaska Department of Agriculture, Palmer, says that no laboratory studies were conducted on specimens collected at post mortem examination of the slaughtered or destroyed reactors (personal communication).

McPhedran and Opie (1935) state that the spread of tuberculosis occurs in large part by long drawn out family or household epidemics in which the

disease is slowly transmitted from one generation to the next. The source of infection in Alaska appears to be mainly person to person by direct contact in overcrowded living establishments. To a lesser degree, transmission occurs by indirect contact with infected discharges of human beings and animals, especially in the areas of high incidence where substandard environmental conditions are major problems in communicable disease control. Sled dogs may further spread human type bacilli through discharges from their bodies, exposing human beings to infection (Williams, 1946).

Table 7 is compiled from similar studies already published (Blacklock, 1929; Griffith, 1907, 1930; Mitchell, 1914; Price, 1939) in various countries of the world. These figures (not frequencies) indicate that although bovine type bacilli are not a frequent cause of human tuberculosis they play a definite role, especially in countries where pasteurization or boiling of milk is not common and where tuberculin-testing of cattle is not a routine procedure to eliminate positive reactors from herds. The limited numbers of cattle and the annual tuberculin-testing programs in Alaska result in low bovine type tuberculosis case rates, and it is evident that bovine type *M. tuberculosis* is of minor significance in the problems of over-all tuberculosis control in Alaska.

The finding of saprophytic *Mycobacteria* in pulmonary and extrapulmonary specimens is not unusual. Pinner (1935), processing some 5,000 diagnostic sputum cultures at the Montefiore Sanatorium, New York, reported that 0.9 per cent contained colonies of chromogenic, saprophytic *Mycobacteria*; Lester (1939), isolated 130 strains of saprophytic *Mycobacteria* out of 26,343 specimens from human beings. Of the total 130 cultures, 75 (or 57.7 per cent) were gastric washings, 24 (or 18.4 per cent) were urinary specimens, and the remaining 13 (or 10 per cent) were from pulmonary sources. About one-fifth of the cultures resembled the typical eugonic and chromogenic *Mycobacteria* reported in literature. More recently Scott (1948) has isolated 15 strains of saprophytic *Mycobacteria* out of 1,000 consecutive cultures made for tubercle bacilli at Manitoba Sanatorium, Ninette, Manitoba. Of the 15 cultures, 11 (or 73.3 per cent) were from gastric washings, 2 (or 13.3 per cent) from pleural fluid and 1 (or 6.7 per cent) each from sputum and urine. All but 2 resembled the

Table 7. Human and bovine tuberculosis in man.

	Human		*Bovine*		
Political division	*Number*	*Per cent*	*Number*	*Per cent*	*Total*
Alaska	180	100.0	0	0.0	180
Australia	246	87.9	34	12.1	280
Canada	847	94.0	54	6.0	901
Denmark	4,832	88.2	644	11.8	5,476
England	3,592	97.8	79	2.2	3,671
France	1,055	97.4	28	2.6	1,083
Germany	1,007	86.4	158	13.6	1,165
Greece	327	100.0	0	0.0	327
Hungary	328	98.2	6	1.8	334
Italy	846	97.1	25	2.9	871
Japan	264	97.1	8	2.9	272
Netherlands	701	91.4	66	8.6	767
Norway	101	94.4	6	5.6	107
Poland	149	93.1	11	6.9	160
Spain	90	94.7	5	5.3	95
Sweden	14	100.0	0	0.0	14
Switzerland	201	92.2	17	7.8	218
Wales	201	99.0	2	0.98	203
Total	14,981	92.91	1,143	7.09	16,124

smooth, moist, creamy to orange eugonic strains described in the literature. Guinea pig inoculations were necessary to prove the lack of pathogenicity or virulence of the 2 cultures which did resemble the tubercle bacillus in staining reactions and growth stimulation. Of the 30 cultures isolated in our laboratories in Alaska only 4 (or 13.3 per cent) were from gastric washings, 1 (or 3.3 per cent) from spinal fluid, and the remaining 25 (or 83.3 per cent) were from pulmonary sources which constitute over 95 per cent of the specimen load. The volume and nature of our specimen load may account for the lower percentage of saprophytes isolated from gastric washings when compared with reports in the literature.

A total of 180 cultures isolated from human cases of tuberculosis were classified by colonial morphology and pathogenicity as to type. No bovine, only human type tubercle bacilli were found. The specimens were from the following sources: cervical gland 2, bone and joint 62, genito-urinary 3, pulmonary 106 and unclassified extrapulmonary cases 7.

The laboratories of the Alaska Department of Health isolated 5,114 cultures of *M. tuberculosis* from July 1944 through to June 1955. All these cultures except one have been characterized by the typical eugonic growth of the human type tubercle bacilli. The bovine type bacillus does not therefore have a major role as an etiological agent of public health significance in human tuberculosis in Alaska.

The author wishes to express appreciation to Dr. Philip H. Moore for the surgical specimens and to Dr. Martin M. Cummings and his associates for the valuable assistance through the additional biological and pathogenicity studies made on the cultures isolated. The technical assistance of Frank P. Pauls, James H. Savage, Mrs. Dixie M. Baade, Joseph Chiuminatto, and Miss Beatrice Shepard is greatly appreciated. Appreciation is expressed to Dr. Charles R. Hayman, Cecil Gronvall, Miss Helen Johnson, and James W. Wilson for statistical data used in this paper.

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