SPECIES IDENTITY AND EPIDEMIOLOGY OF *BRUCELLA* STRAINS ISOLATED FROM ALASKAN ESKIMOS

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Huntley et al (1963) recently reported on the occurrence of brucellosis among Eskimos inhabiting the Arctic slope of Alaska. An epidemiologic survey conducted by these investigators established the source of the disease for the Eskimos as direct contact with infected carcasses of the wild reindeer, *Rangifer tarandus*, which they use for milk, meat, and clothing. Questions that remain unanswered in the report of Huntley et al are: (1) What is the specific identity of these strains of *Brucella*, and (2) what was the source of infection for Alaskan reindeer? Huntley et al thought that the cultural and antigenic characteristics of the strains suggested a closer relationship to *Brucella melitensis* than to either *B. suis* or *B. abortus*, but they declined to offer a more precise identity. The only other reported isolation of *Brucella* organisms in Alaska was made by Edwards (1959), and at that time the organism was identified as *B. suis* type 2. The strain isolated in 1959 as well as 2 of the strains reported by Huntley et al were obtained for metabolic and bacteriophage examination with the hope that these additional methods would help to provide their specific identity. The purpose of this paper is to present evidence to support the conclusion that the organisms isolated from Alaskan Eskimos are members of the species *B. suis* and also to provide epidemiologic information concerning the source of infection for reindeer herds in Alaska.

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METHODS

Each of the 3 strains of *Brucella* used in this investigation was isolated from Eskimos in Alaska. Strain number A-1959 is the strain that was isolated by Edwards in 1959, and strains 2-0151 and AHRL-2 were isolated by Huntley et al (1963). Strains 1330 and 16 M are World Health Organization standard reference strains. The biotypes of *B. suis* have been reported on previously (Meyer and Cameron, 1963).

Conventional procedures for ascertaining carbon dioxide requirements, hydrogen sulfide production, and the bacteriostatic action of basic fuchsin and thionin on the growth of the organism were carried out as recommended by the Joint FAO/WHO Expert Committee on Brucellosis (1953, 1958). The method of Jones (1958) was used for the preparation of monospecific antisera.

Methods for preparation of *Brucella* bacteriophage and its use in the speciation of the genus *Brucella* have been detailed by Jones (1960), Meyer (1961a, 1962), and Meyer and Morgan (1962).

Resting cell suspensions for manometric determinations were prepared as described by Meyer and Cameron (1958). The substrates were dissolved in Sorensen's 0.06 M phosphate buffer and, when necessary, the pH of the solution was adjusted to 7 by the addition of sodium hydroxide. Conventional manometric techniques were employed to determine oxygen uptake (Umbreit et al, 1957). Each flask contained 1 ml

TABLE 1.—Differentiation of *Brucella melitensis* from the biotypes of *Brucella suis* by the conventional biochemical tests

Species and strain number	H_2S production on day:				Dye bacteriostasis, μg per ml medium								Agglutination with monospecific antisera against antigens		Lysis by concentrated phage*
					Thionin					Basic fuchsin					
	1	2	3	4	10	15	20	25	50	10	15	20	A	M	
B. melitensis type 1, 16 M	−	−	−	−	+	+	+	−	−	+	+	+	−	+	−
B. suis type 1, 1330	+	+	+	+	+	+	+	+	+	−	−	−	+	−	+
B. suis type 2, 570	−	−	+	+	+	−	−	−	−	+	−	−	+	−	+
B. suis type 3, 686	−	−	−	±	+	+	+	+	+	+	+	+	+	−	+
Alaskan, 1959	−	−	−	±	+	+	+	+	+	+	+	+	+	+	+
Alaskan, 2-0151	−	−	−	±	+	+	+	+	+	+	+	+	+	+	+
Alaskan, AHRL-2	−	−	−	±	+	+	+	+	+	+	+	+	+	+	+

None of these strains required CO_2 for initial isolation.
* None were lysed by the routine test dilution of phage.

cell suspension, 0.5 ml substrate, 1.4 ml buffer, and 0.1 ml KOH. All experiments were performed in duplicate and repeated on separate lots of media. The figures given in the results are typical $Q_{O_2}^{N_2}$ values with the endogenous respiration rates subtracted.

RESULTS AND DISCUSSION

Table 1 compares the biochemical and serological characteristics of the Alaskan strains with standard strains of *B. melitensis* and with 3 biotyes of *B. suis*. Table 2 shows their comparative oxidative metabolic patterns.

Table 1 shows that the Alaskan strains are identical to *B. suis* type 3 in their biochemical characteristics and in their growth patterns on basic fuchsin and thionin. By these conventional determinative methods, one of the critical features that differentiates *B. melitensis* from *B. suis* type 3 is the ability of the latter type to grow on 4 to 5 times the concentration of thionin that is inhibiting for all the biotypes of *B. melitensis* (Meyer, 1961b; Meyer and Morgan, 1962; Meyer and Cameron, 1963). The Alaskan strains also are similar to all biotypes of *B. suis* in their response to *Brucella* bacteriophage. Only *B. abortus* is lysed at the routine test dilution; *B. melitensis* is resistant to lysis; and lawns of *B. suis* show areas of clearing when concentrated (10,000 times the routine test dilution) or stock suspensions of phage are used. The host-phage relationship in the species of *B. suis* is currently disputed by Stableforth and Jones (1963) who report that the clearing observed with concentrated phage is lysis from without rather than

TABLE 2.—Differentiation of *Brucella melitensis* from the biotypes of *Brucella suis* by oxidative metabolic patterns

Species and strain number	Substrates										
	Amino acids								Carbohydrates		
	D-alanine	L-alanine	L-asparagine	L-glutamate	DL-ornithine	DL-citrulline	L-arginine	L-lysine	L-arabinose	D-galactose	D-ribose
B. melitensis type 1, 16 M	208	198	222	412	19	4	8	10	0	0	30
B. suis type 1, 1330	122	30	8	17	165	139	76	113	210	113	342
B. suis type 2, 570	36	38	35	156	144	156	87	19	239	70	420
B. suis type 3, 686	82	38	0	87	175	138	69	73	30	16	287
Alaskan, 1959	151	28	0	100	158	165	85	56	20	12	282
Alaskan, 2-0151	150	13	6	90	210	131	51	52	31	8	266
Alaskan, AHRL-2	101	37	0	91	200	186	140	45	21	0	222

true plaque formation and by Calderone and Pickett (1964) who believe that it is true lysis. Irrespective of the mechanism involved, the important point here is that the Alaskan strains respond in a manner identical to *B. suis* and dissimilar to *B. melitensis.*

Table 2 shows that the organisms of Alaskan origin display a pattern of oxidative metabolism that is uniform among the 3 strains, is distinguishing for the species of *B. suis*, and is distinctly different from the oxidative pattern of *B. melitensis.* The rates of utilization on D- and L-alanine, L-asparagine, L-glutamic acid, DL-ornithine, DL-citrulline, L-arginine, L-lysine, and D-ribose are within the rate ranges which characterize *B. suis* and differentiate this species from *B. abortus* and *B. melitensis* (Meyer and Cameron, 1958, 1961a, b, 1963; Meyer, 1961b). The biotypes within the species of *B. suis* can be differentiated by differences in carbohydrate utilization. The Alaskan strains oxidize the same carbohydrates as *B. suis* type 3.

By use of the biochemical, phage, and metabolic tests recommended by the Subcommittee on Taxonomy of *Brucella* of the International Committee on Bacteriological Nomenclature (Stableforth and Jones, 1963) for the differentiation of the species and biotypes of *Brucella*, the Alaskan strains are indistinguishable from *B. suis* type 3. They differ from this biotype serologically in that they agglutinate both *B. abortus* and *B. melitensis* monospecific antisera, while *B. suis* type 3 agglutinates with only *B. abortus* monospecific antisera. The simultaneous presence of both antigens is not unique to only these strains in this genus. Five of the 9 biotypes of *B. abortus* agglutinate with *B. melitensis* monospecific antisera and 2 of the 3 biotypes of *B. melitensis* agglutinate with *B. abortus* monospecific antisera. The ability to agglutinate with either, or both, types of antisera is not a species-identifying characteristic but is one of the features used to define a biotype within a species. In view of the overall characteristics displayed by the *Brucella* organisms isolated by Huntley et al (1963) and in accord with the current classification system for this genus, it is suggested that these strains and others fitting the same description be classified as *B. suis* type 4.

The occurrence of brucellosis in reindeer has been reported previously by several Russian investigators. Davydov (1961) isolated 72 strains of *Brucella* from *R. tarandus*, presented the same description of these organisms as did Huntley et al, and suggested that they be classified as a new species, *Brucella rangiferi tarandi.*

Cherchenko and Bakaeva (1962) who isolated 16 strains from reindeer in Siberia described them as having the same characteristics as the Davydov strains, but they suggested that such strains be classified as a stable variant of *B. melitensis.*

Pinigin and Petukhova (1962) who studied 41 strains isolated from reindeer in the Taimur and Magadan Regions of Russia described the organism indentically as had the other investigators and urged that such strains be known as *Brucella rangiferi.*

All available literature reports indicate that the *Brucella* organisms isolated from the reindeer species *R. tarandus* in Siberia and other parts of Russia are of similar description to those isolated from the same species of reindeer and from human beings exposed to infected reindeer on the North American continent. While it is not possible to offer irrefutable proof that the strains isolated in Russia are indeed *B. suis* type 4, epidemiologic information drawn from the movement of rein-

deer from Siberia to Alaska can be offered as substantiative evidence.

Wead (1937) reported that, due to the combination of a severe winter in 1890–91 and a failure of the walrus to return to coastal waters, the Eskimos of King Island and the northern coastal area of Alaska were facing starvation. In fact, 200 Eskimos on King Island perished from starvation that winter, and those that survived subsisted on dog meat and seaweed. To alleviate the immediate starvation and to provide the Eskimos with foundation stock for a continuing source of meat and skins, Congress authorized the captain of the U. S. Revenue Marine cutter *Bear* to purchase reindeer from Russia when the ship was next in a Siberian port. In 1891, 10 reindeer were imported from Siberia and in the following year an additional 171 animals were transported on the *Bear* from Siberia to Alaska. All of the animals that were obtained in Siberia were delivered to and cared for by the newly established Alaskan Reindeer Station at Port Clarence, Alaska. The herd population steadily increased and by 1896 the animals were distributed among 3 reindeer stations. During 1896, 133 deer were herded south to the Yukon to alleviate starvation among the gold prospectors. In 1930–1935, 2100 head of the reindeer were driven from Alaska to the MacKenzie Delta in Canada to provide a food supply for Canadian Eskimos. Significantly, Toshach (1955) isolated *Brucella* from 2 Eskimos who normally resided in northern Canada and suggested reindeer as the possible source of these infections. The serological and biochemical characteristics described by Toshach indicate that her strains were similar to those isolated in Alaska and Siberia.

Because of the recurrent threat of starvation among peoples of the Arctic, the importation of *R. tarandus* from Siberia and their subsequent geographical distribution have been remarkably well documented. Relative to this knowledge of the movement of the reindeer is the unanimity among Russian, American, and Canadian investigators in their description of the characteristics of the *Brucella* strains isolated from the reindeer. In overall characteristics these strains fit the species description of *B. suis*, but they are serologically distinguishable from all other biotypes of *B. suis*. The concomitancy of these circumstances warrants the conclusion that the organism inducing brucellosis in reindeer in the Arctic areas of North America and in Russia are the same type and that the infection was imported initially from Siberia.

SUMMARY

Three strains of *Brucella* isolated from Alaskan Eskimos were examined by all the methods recommended by the Subcommittee on Taxonomy of *Brucella* of the International Committee on Bacteriological Nomenclature and were found to have characteristics that identify them as members of the species *Brucella suis*. By their biochemical features, oxidative metabolic pattern, and response to concentrated *Brucella* bacteriophage, they were indistinguishable from *B. suis* type 3. Because they differed in the serological characteristic of agglutinating with *B. melitensis* monospecific antisera as well as that of *B. abortus*, the recommendation was made that these strains and all others fitting the same description be classified as *B. suis* type 4.

Previous investigators had established that the source of infection for the Eskimos was direct contact with infected reindeer, *Rangifer tarandus*. Epidemiologic evidence was presented showing that the initial source of infec-

tion for reindeer in Alaska was importation of reindeer from Siberia.

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