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# CONTAMINATION OF NATURAL WATERS AND MUD WITH *Pasteurella tularensis* and TULAREMIA IN BEAVERS AND MUSKRATS IN THE NORTHWESTERN UNITED STATES

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## CONTENTS


| | Page |
|---|---|
| Introduction | 1 |
| Methods | 4 |
| Field data | 6 |
| Montana | 6 |
| Bitter Root Valley area (Ravalli County) | 6 |
| Gird Creek | 6 |
| Cattail Creek | 7 |
| Other Gird Creek data | 13 |
| Bitter Root River and tributaries other than Gird Creek | 15 |
| Lewistown area (Fergus County) | 18 |
| Warm Springs Creek | 18 |
| Big Spring Creek | 20 |
| Other Montana areas | 25 |
| Idaho | 29 |
| Warm Spring Creek | 29 |
| Other Idaho areas | 30 |
| Wyoming | 32 |
| Oregon | 32 |
| Washington | 33 |
| Muskrats and beavers as sources of human infection in other areas | 33 |
| Experimental investigations | 37 |
| Infected animals as possible sources of contamination of water and mud | 38 |
| Contaminated water as a possible source of infection for animals | 44 |
| Miscellaneous experiments | 49 |
| Discussion | 54 |
| Summary | 58 |
| References | 59 |

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## INTRODUCTION

Jellison, Kohls, Butler, and Weaver (1942) reported their observations, made in 1939 and early 1940, of a widespread tularemia epizootic in beavers, *Castor canadensis*, in the western and central portions of the southern half of Montana [1] and in Sheridan County, Wyo.[2] Beavers in certain streams were found dead, and *Pasteurella tularensis* was repeatedly recovered from beaver carcasses. This organism was also recovered from the only muskrat (*Ondatra zibethica*) examined from the waters of four streams in which such animals had been found dead, and from the mud in one of these streams.

Subsequent information suggested that during the period covered by this report beavers may have been affected in other Montana streams also, and that muskrats were probably far more extensively involved than had been indicated (Parker, Steinhaus, and Kohls, 1943). This information also suggested that there had been a similar widespread occurrence of tularemia in beavers and muskrats in 1941.

Further studies of *P. tularensis* in beavers, muskrats, water, and mud and studies of the relationship of the occurrence of these organisms in such situations to the occurrence of tularemia in man were initiated by the Rocky Mountain Laboratory in March 1942 and were continued on a fairly extensive scale until the early summer of 1943. Limited observations were made subsequently. The project consisted chiefly of: (1) More or less intensive study of a small stream, Gird Creek, and one of its tributaries, Cattail Creek, near Hamilton, Mont., which were continuously contaminated for a period of 16 months; (2) repeated studies of certain streams near Lewistown, Mont., and of a large marshy area near May, Idaho; (3) single attempts to isolate *P. tularensis* from various streams and from beavers and muskrats in Montana and Idaho; (4) field and laboratory experiments.

The occurrence of *P. tularensis* in natural waters was first reported from Russia by Miller (1935), who mentioned the work of his colleague, Somoff, concerning the possible spread of tularemia through this medium. Some years prior to this, in May 1926, the first human

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[1] This report covered a cooperative study by the Rocky Mountain Laboratory, the Montana Livestock Sanitary Board, and the Montana State Fish and Game Commission.

[2] The first reports of the spontaneous occurrence of tularemia in beavers were by Hammersland and Joneschild (1940) and Scott (1940). Spontaneous infection of muskrats was first recognized by Green and Shillinger (1933) in Iowa. The first evidence of the disease in muskrats was supplied by Schwartz (1929) who reported that two Japanese became infected after skinning a muskrat in Montana in 1928.

epidemic known to be due to contact with water rats, *Arvicola amphibius*, occurred near Astrakhan in southern Russia. There were about 200 recorded cases, all of which recovered. In May 1928 an epidemic of 800 cases was observed farther north in the Province of Riazan. These epidemics coincided with the spring floods and occurred exclusively among rat hunters and those engaged in skinning the animals. Subsequent to these epidemics several other outbreaks were observed and investigations of the water rats themselves showed the disease to be prevalent in these animals even in districts where human cases had not been reported. In general, many of the epidemiological aspects noted by the Russian investigators are similar to those reported in this paper.

Apparently no association between the infected water rats and the possibility of contaminated water was noted during these first outbreaks. It was not until after Somoff's isolation of *P. tularensis* from natural water in 1934 that the first reported outbreak of tularemia due exclusively to water was observed in 1935 by Karpoff and Antonoff (1936). This outbreak occurred among people who were mowing hay and using water from a contaminated stream for drinking purposes. Of the 43 cases examined, the anginal form of disease predominated. Some cases were of the typhoidal and the oculo-glandular types. Tests of the stream water by inoculation into guinea pigs showed *P. tularensis* to be present. Cultures isolated from the water did not differ in virulence from those obtained from the lymph glands of the patients. Water rats and mice were known to frequent the vicinity of the stream concerned.

According to Schmidt (1947), a great extension of the disease took place in Russia in 1940–1942 and the number of cases rose to hundreds of thousands in some districts. In the Rostov district alone the number of cases increased from 8,500 in November 1941 to 14,000 in January 1942. Almost all of the inhabitants in some areas were affected and the disease was stated to have been a serious cause of disability in the Russian Army. These outbreaks were believed to have arisen from streams and wells that were contaminated by water rats. The ulcero-glandular form of the disease predominated.

An urban epidemic which resulted from diversion of river water into an artesian water distributing system following destruction of the well during the war has been reported by Tsareva (1945). Contamination of the water was confirmed bacteriologically, and the epidemic ceased when the supply of river water was cut off.

According to Sinai and Voskresensky (1943) tularemia outbreaks of water origin have occurred repeatedly in the Soviet Union: Rostev region, 1934–1937; Novosibirsk region, 1935, 1936, 1939, and 1940; Saratov region, 1939; and Ordzhenikidzevsky border, 1940. Except

for the epidemic described above little information relative to these outbreaks is available to us. Sinai and Voskresensky state, however, that they appeared as a consequence of epizootics among mouse-like rodents through contamination of wells and brooks by rodent excrement and corpses. Human infections were thought to result from drinking infected water and from other uses such as bathing. The outbreaks were limited to persons making use of the infected water supply, without reference to age, sex, or profession. The epidemics developed rapidly and declined abruptly when use of the infected water ceased. Maisky (1945), in discussing the types of epidemic outbreaks of tularemia, stated that the predominant clinical form associated with water was anginous-bubonic, with frequent development of buboes in the region of the cheeks.

Of the many animals involved in the epizootiology of tularemia in relation to man in the Soviet Union, the water rat is regarded as the one of basic importance (Voskresensky, 1943). This animal is widely distributed and is present on all the principal river systems and lakes. It is extremely susceptible to the disease, which ranges from acute fatal infections to the chronic unapparent forms considered by Russian workers to be responsible for continuance of the disease during interepizootic periods. The muskrat, *Ondatra zibethica*, is regarded as being of lesser importance. It is an introduced species, first seen in Russia in 1927, but as yet it is neither as abundant nor as widely distributed as the water rat. Spontaneous infections in muskrats in the Novosibirsk region were demonstrated in 1939.

Some experimental data have been obtained in the Soviet Union on the survival of *P. tularensis* in media other than living vertebrates and arthropods. These observations have been summarized by Khatenever (1943) but details of techniques employed are largely lacking. In damp soil the organisms were said to remain virulent for not less than 30 days; in ice for 32 days; in hydrant water for as long as 95 days. In a 3-liter vessel containing hydrant water in which five mice dead of tularemia were placed, the water remained infectious for test mice up to 92 days, but with progressive reduction in virulence. In another experiment, two portions of unsterile well water of 4 liters each were seeded with *P. tularensis* to a concentration of 5 million organisms per cc. One portion was held at 9° C. and the other at 21° C. to 24° C. In the former, virulence for guinea pigs was maintained for about 60 days; in the latter, for more than 12 and less than 17 days. The organism was capable of surviving in hides of water rats dead of tularemia, and kept at a temperature varying from 15° C. to 20° C. for at least 20 days.

The role of frogs and toads in the epizootiology of tularemia was investigated by Novikova and Lalazarov (1940). They found that

*Rana esculenta* and *Bufo viridis* are experimentally susceptible to infections which for the most part were chronic or latent. A culture of the organism was isolated from water in the jars in which the animals were kept. Only rarely could typical cultures be isolated from the animals. For the most part the organisms were obtained in the form of a feebly virulent brown or unpigmented culture, differing in several respects from typical *P. tularensis*.

In 1937 Öz (1938) and Hüseyin independently demonstrated the presence of *P. tularensis* in a stream in Turkey. This stream was located in the vicinity of Lüleburgaz in Thrace, where numerous cases had occurred among soldiers the previous summer. The exact mode of infection in these cases was never determined with certainty, although transmission by arthropods and contact with contaminated water was suspected. Wild hares were reported to be present in great numbers, but none of the patients had contact with these animals (Hüseyin, 1937). Field mice and house mice were also plentiful. Attempts to demonstrate natural infections in hares and mice were unsuccessful.

The susceptibility of the water buffalo to experimental infection was reported by Kamil and Bilal (1938) and by Bilal-Golem (1946). The urine of the water buffalo was virulent to mice by subcutaneous inoculation for more than a month after the infection. It was pointed out that buffalo could contaminate streams and pools, and thus play a part in the epidemiology of the disease.

Bilal (1939) studied the susceptibility of the frog, *Rana ridibunda*, to tularemia. Frogs inoculated with a virulent strain died without any characteristic macroscopic lesions. It was found that infected frogs contaminate the water in which they live and that normal frogs can become infected by water contaminated by frogs infected with *P. tularensis*.

According to Bilal-Golem (correspondence, R. R. Parker, May 3 and June 5, 1946), another outbreak occurred at Lüleburgaz during the summer of 1945. Water from a brook was stated to be the sole cause of the epidemic.

## METHODS

Water samples were collected in sterile 200 ml. bottles. The test for contamination with *P. tularensis* was made by injecting each of four guinea pigs intraperitoneally with 10 ml. of water.

Mud samples were collected in 500-ml. bottles and consisted of 3 tablespoonfuls of top mud. To each sample 300 ml. of tap water were added, the bottle was thoroughly shaken, and the suspended material allowed to settle. The supernatant water was tested in the same manner as the water samples. Soil samples were handled in

the same manner as mud samples. The interval between collecting and testing of samples in the Bitter Root Valley area was usually about 2 hours and seldom over 4, but was 1 to 3 days for those from distant points. These latter were transported by automobile and were kept iced insofar as possible. Some of the remaining portions of water samples were used for other tests noted in the section of this paper dealing with experimental investigations (p. 40).

Some of the isolated water samples from streams, a number of which were positive, were obtained on trips to areas visited only once, or while en route to areas visited repeatedly. Such samples were taken at a point where the streams concerned crossed a main highway. The status of most such streams with respect to the presence or absence of muskrats or beavers is unknown.

It is obvious that "top mud" was in direct contact with the overlying water and that it was also lifted through the water when being removed. Therefore, mud samples are reported as positive with this factor in mind. However, positive mud samples were sometimes obtained at the same time and at the exact point at which the overlying water was negative, while in one stream the water was repeatedly positive and mud samples were consistently negative.

The test guinea pigs were observed for a period of 21 days. Surviving animals were sacrificed on the twenty-second day. The tissues of all animals that died and those of all sacrificed animals exhibiting lesions that were at all suggestive of tularemia were cultured on cystine-heart-agar. Transfer of tissues from test animals to fresh animals was made frequently. No test was considered positive unless a culture of *P. tularensis* was isolated from at least one of the four guinea pigs inoculated with the test sample or from an animal inoculated with tissue from one of these guinea pigs. Five animals infected with tularemia died on the twenty-first day after inoculation; two of these exhibited typical lesions of tularemia. Only two sacrificed animals yielded cultures of *P. tularensis*. Deaths due to causes other than tularemia, particularly to peritonitis, were common among the guinea pigs used in testing mud samples.

Many of the water and mud samples containing *P. tularensis* infected all four test guinea pigs but in numerous instances only one, two, or three animals became infected. In the text the results of the test are frequently expressed as +1, +2, +3, or +4 to indicate the number of guinea pigs that became infected. For the purpose of this paper, samples that failed to infect any animal are considered to indicate that the water or mud represented was not contaminated at the time the sample was taken. However, it is probable that at least some of the negative tests might have proved positive had a greater number of test guinea pigs been used.

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The streams from which positive water and mud samples were taken ranged from small, short tributaries only a few feet wide and quite shallow to relatively large streams many miles long, up to at least 75 feet in width (at the point where the sample was taken) and from one to several feet deep. Some of the streams, even the larger ones, were rushing torrents at the time the test was made.

Repeated attempts were made to isolate *P. tularensis* directly from naturally contaminated water by inoculating cystine-heart-agar plates with 1 ml. of water, but no definitely positive results were obtained.

Techniques which apply only to the experimental studies are given in the introductory paragraphs of section on experimental investigations (p. 40) or under the experiment to which they applied specifically.

## FIELD DATA

### *Montana*

#### BITTER ROOT VALLEY AREA (RAVALLI COUNTY)

The Bitter Root Valley, which has been the site of the more intensive studies, is drained by the Bitter Root River and its tributaries. The investigations were particularly concerned with one of the lesser tributary streams, Gird Creek, and with Cattail Creek, a small stream emptying into Gird Creek.

#### GIRD CREEK

Gird Creek is a relatively small stream rising in the Sapphire Mountains on the east side of the Bitter Root Valley and, after following a somewhat winding northwestward course for a distance of about 12 miles through agricultural land, joins the Bitter Root River just northwest of Corvallis. It divides at a point about 2 miles west of the mountains and the two resulting channels reunite farther north to complete a loop about 3½ miles long and 1 mile wide. Parts of the two channels forming this loop are used to carry water from irrigation ditches. Three small tributaries join Gird Creek at the loop, Grimes Creek and Spring Creek at the north end, and Cattail Creek near the south end. The courses of the last two lie entirely within the loop. A fourth small tributary, Jones Creek, joins Gird Creek from the south about 1 mile south of the loop. (See fig. 1.)

During the period March 17, 1942, through mid-November 1943, materials were tested from 230 stations along Gird Creek and its tributaries. These stations consisted of 143 for water samples, 58 for mud, and 29 for soil samples. Tests were made regularly from some stations, and only occasionally from others. *P. tularensis* was isolated one or more times from 95 of the water stations, 19 of the mud stations, and from 2 of the soil stations.

FIGURE I. MAP OF GIRD CREEK AREA SHOWING PRINCIPAL COLLECTING STATIONS. STATIONS REFERRED TO IN THE TEXT BY NUMBER ARE NUMBERED.

STREAMS

MARSHES

CATTAILS

● STATIONS YIELDING CULTURES OF PASTEURELLA TULARENSIS ONE OR MORE TIMES.

□ STATIONS NOT YIELDING CULTURES OF PASTEURELLA TULARENSIS ONE OR MORE TIMES.

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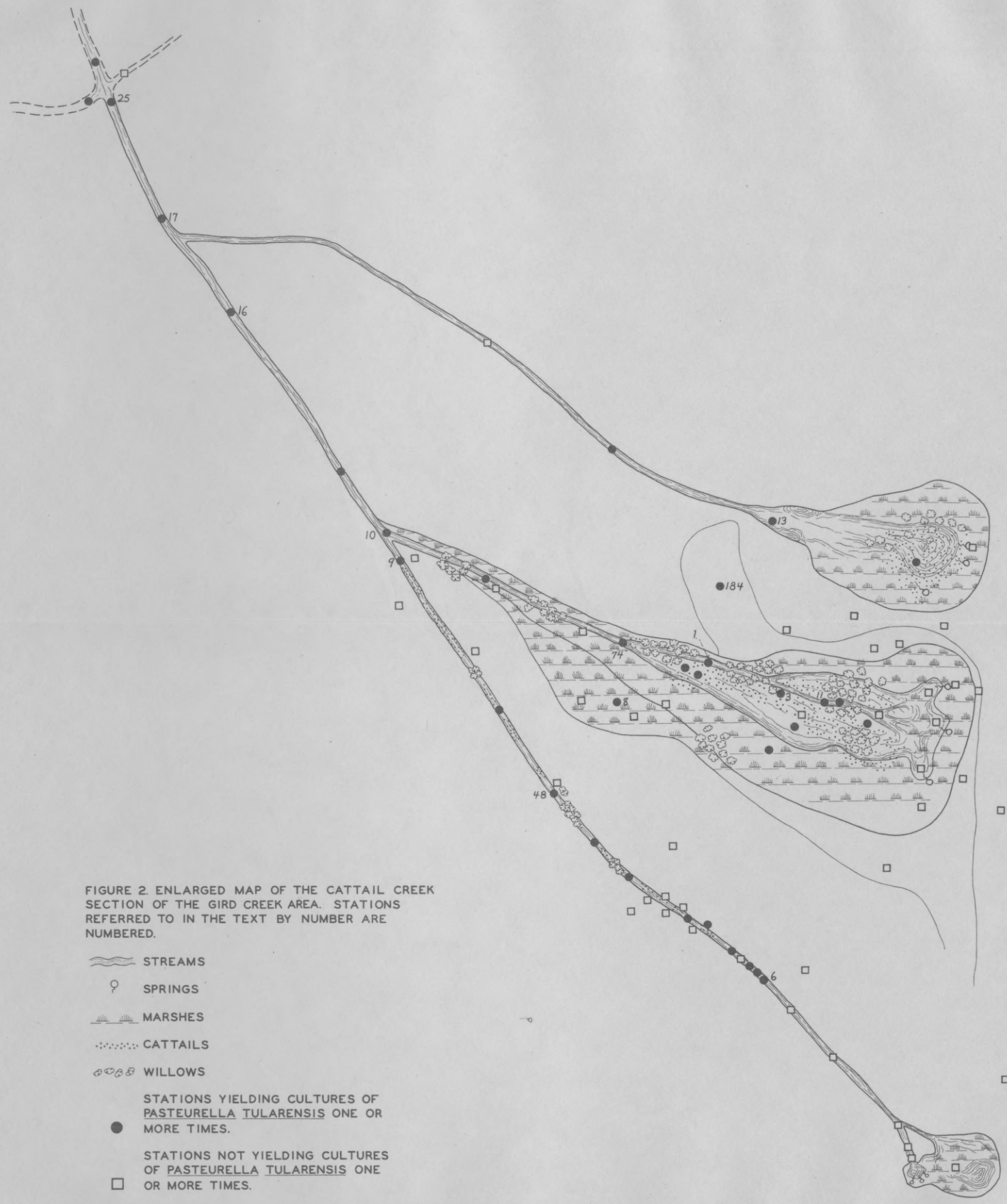


FIGURE 2. ENLARGED MAP OF THE CATTAIL CREEK SECTION OF THE GIRD CREEK AREA. STATIONS REFERRED TO IN THE TEXT BY NUMBER ARE NUMBERED.

STREAMS

SPRINGS

MARSHES

CATTAILS

WILLOWS

● STATIONS YIELDING CULTURES OF PASTEURELLA TULARENSIS ONE OR MORE TIMES.

□ STATIONS NOT YIELDING CULTURES OF PASTEURELLA TULARENSIS ONE OR MORE TIMES.

944770 O - 51 (Face p. 7)

Isolations of *P. tularensis* were made from 703 of the 1,198 samples tested during the 16-month period from March 17, 1942, to July 15, 1943. During this period, of 981 water samples examined, 610 (62 percent) were positive and 371 (38 percent) were negative, of 178 mud samples, 89 (50 percent) were positive and 89 (50 percent) negative, and of 39 soil samples 4 (10 percent) were positive and 35 (90 percent) negative. No isolations were made from 104 water and mud samples tested during the 4-month period from July 15 to November 15, 1943. Samples were taken at 26 stations each month during this latter period and negative results were uniformly obtained even from those stations which had yielded positive results throughout the entire preceding 16-month period.

Of 5,208 guinea pigs used in the entire series of tests, 2,078 died of tularemia. Of the 610 positive water samples, 99 infected only 1 test guinea pig, 83 infected 2, 109 infected 3, and 319 infected all 4 test animals. Of the 89 positive mud samples, 30 infected 1 guinea pig, 24 infected 2, 16 infected 3, and 19 infected all 4 test animals. Of the 4 positive soil samples, 1 infected 2 guinea pigs, 2 infected 3, and 1 infected all 4 test animals.

Guinea pigs that became infected died in from 3 to 21 days. The average days to death from positive water samples was 9.5, from positive mud samples, 9, and from positive soil samples, 11 days. On two occasions *P. tularensis* was recovered from water-injected guinea pigs which showed suggestive lesions when sacrificed on the twenty-second day.

## CATTAIL CREEK

Cattail Creek, which is approximately ½ mile long, has its source in three small adjacent marshes fed by underground springs draining the bench land on the east. Each marsh has an effluent stream. These unite just below the marshes to form the main stream, which in turn flows into one of the main channels of Gird Creek. (See fig. 2.) The course of Cattail Creek traverses two pastures. The three marshes lie entirely within one of these which was used for horses but had been used for sheep until shortly before the start of our observations. The other pasture was used for cattle.

The marshes lie in a north and south line, the middle one being the largest. It is about 300 feet long by 90 feet wide. Each marsh contains several inches of surface water underlaid by a few inches to 2 feet of black mud containing a high proportion of decaying organic matter. The water is semistagnant but there is a more or less perceptible central current. A heavy growth of cattails occurs in each marsh. Moss is abundant and algae are numerous. During spring and early summer the water level is raised due to melting snow and irrigation and the rate of flow is consequently increased. The bed of

the effluent stream draining the middle marsh was lowered in October 1942 by the owner of the farm. This resulted in a lowering of the water level and some decrease in the area under water (fig. 3).

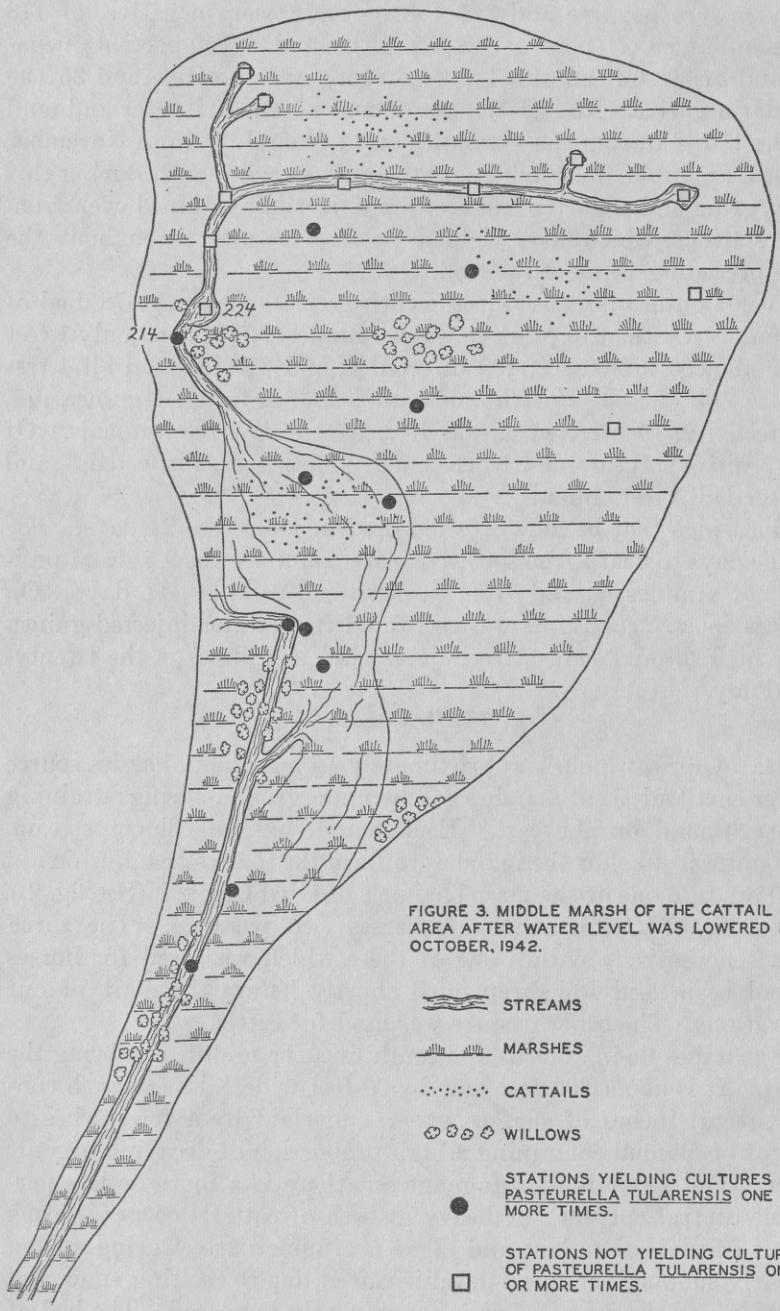


FIGURE 3. MIDDLE MARSH OF THE CATTAIL CREEK AREA AFTER WATER LEVEL WAS LOWERED IN OCTOBER, 1942.

The temperature of the water, mud, and air was taken periodically at various points in the marshes and along Gird Creek. The low and high temperatures observed were as follows:

| | *Water* | *Mud* | *Air* |
|---|---|---|---|
| Low (Feb. 16, 1943) | 0° to 4° C. | 0° to 9° C. | −0.5° to 4° C. |
| High (Aug. 3, 1943) | 10° to 21° C. | 10° to 18° C. | 28° to 30° C. |

As determined by Hellige colorimeter standards, the reaction of the water was definitely alkaline, varying from pH 7.4 to 8.2 with an average of about pH 7.8.

We are indebted to Mr. H. B. Foote, director of the Division of Water and Sewage of the Montana State Board of Health, for the following analysis of a water sample collected February 19, 1943 from Cattail Creek. This water had been treated with hypochlorite in connection with experiments to determine the effect of chlorination on *P. tularensis* and the analysis was made 3 days after the sample was taken.

| | *P. p. m.* | | *P. p. m.* |
|---|---|---|---|
| Silica ($SiO_2$) | 19.6 | Carbonate radicle ($CO_3$) | 0.0 |
| Iron (Fe) | 0.0 | Bicarbonate radicle ($HCO_3$) | 290.0 |
| Calcium (Ca) | 60.0 | Sulphate radicle ($SO_4$) | 11.0 |
| Magnesium (Mg) | 23.0 | Chloride (Cl) | 8.0 |
| Sodium and potassium calculated (Na—/—K) | 8.0 | Total solids | 270.0 |
| | | Total hardness | 244.0 |

*Local animal population.*—In March 1942, there was evidence that there had been a heavy field mouse (*Microtus pennsylvanicus modestus*) population during the preceding winter in the area of the marshes and their effluent streams. There were numerous mouse nests, mostly unused, in the grass and in the cattails. When studies were initiated a considerable number of mice were still present. They became scarce in early April, having migrated to nearby fields. A few muskrats had been present in the middle marsh up to about March 16, but not thereafter. Native animals, other than birds, were relatively scarce during the late spring and summer, but there was a limited influx of field mice after the nearby hay was cut in early July. A few shrews (*Sorex vagrans monticola*) were also present at that time. No turtles, only a few frogs, and no fish other than minnows were seen.

In the early fall there was a second influx of field mice. By early November the population was heavy. This influx was accompanied by the arrival of owls, hawks, and magpies. The subsequent abundance of field mice followed the same variation as described for the previous year.

The mud and water contained a heavy and varied population of snails, leeches, fairy shrimps, water beetles, and immature insects

during the warmer months of 1942. Turbellaria occasionally were observed in abundance in association with algae even in midwinter.

*Tularemia in local animals.*—*P. tularensis* was recovered from the tissues of one of three muskrats (the other two were not examined) found dead on March 16, 1942, in the middle marsh. No muskrats were present after this date although 60 had been trapped in the marshes during the preceding year.

*P. tularensis* was also recovered from the tissues of a field mouse found dead on April 7, 1942, close to the middle marsh. During August the tissues of 27 field mice and 3 shrews trapped near the marshes were tested with negative results. During the period November 5, 1942, to April 7, 1943, 162 field mice and 23 shrews were tested. Ninety-one of the mice and 20 of the shrews were trapped close to the marshes and from these animals *P. tularensis* was recovered from separate pools of 10 field mice and 3 shrews taken on November 5 (Kohls and Steinhaus, 1943), 5 field mice taken on November 11, and 5 field mice taken on December 24. Seventy-one of the field mice and 3 of the shrews were trapped in hay fields adjacent to the pasture land containing the marshes. The only positive test among this group of animals was from a pool of 3 field mice taken December 30.

Snails, fairy shrimp, leeches, dragon fly nymphs, small bivalve mollusks, water beetles, and frogs were tested for the presence of *P. tularensis*. One lot of 6 snails and one of 30 fairy shrimp, collected October 29, 1942 in the north marsh, each yielded cultures of *P. tularensis*.[3] None of the other animals tested were found to be infective.

*Water and mud contamination.*—Beginning March 17, 1942, water and mud samples were tested at intervals from established stations. The stations were located in the springs which supply the middle marsh, at numerous points in the marshes (particularly the middle one), and at points on the three effluents and the stream which these form. The location of the stations is shown on the accompanying map (fig. 2) and the stations which are referred to in the text are numbered. Mud samples were taken mainly from the middle marsh and the effluent stream of the south marsh, which was itself somewhat marshy.

Samples were tested at weekly intervals from March 17, 1942, to December 30, 1942, at about 2-week intervals until March 24, 1943, and at monthly intervals until March 17, 1944. Samples were taken quite regularly from certain stations, from others only occasionally.

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[3] Before testing, each lot was first washed in several changes of tap water and finally in two changes of sterile saline solution. The last wash material of each lot was tested in a guinea pig and in no instance caused tularemia. After trituration in sterile saline the tissue of each lot was injected into four guinea pigs—two subcutaneously, two intraperitoneally. In each of the positive tests *P. tularensis* was cultured from the heart blood of one of the test guinea pigs.

*P. tularensis* was obtained from the first water and mud samples collected from the marshes and their effluents and from water samples taken between the marshes and the junction of Cattail Creek with Gird Creek. Positive tests were obtained more or less consistently from many of the stations regularly sampled until April 1943. At that time 25 stations were selected for subsequent sampling. Twenty of these were positive in April, 3 in May, 7 in June, 5 in July, and none were positive in either August, September, or October of 1943. Therefore, Cattail Creek water was shown to be contaminated from mid-March of 1942 to mid-July of 1943, a period of 16 months. Contamination was much more marked during the first 13 months. How long it may have been present prior to March of 1942 there is no means of judging.

It is of interest to refer to the findings after the level of the middle marsh was lowered in October 1942 and the water receded. The springs which previously discharged under water were now exposed and the water from them flowed in channels to form a common stream that flowed into what was now left of the marsh (fig. 3). New stations were then established along the channels from the springs to the marsh and water samples from these stations were taken over a period of several months. In no instance was a positive test obtained above station 214, which was located in a group of willows. Stations below this point were quite consistently positive. It appeared probable therefore that contamination first occurred in the immediate vicinity of station 214 or between it and station 224 only 15 feet above. However, no clue was obtained as to the source of the contamination.

During the period that *P. tularensis* was so consistently present the infectivity of the water in the marsh as a whole did not falter even during periods of markedly increased water flow.

It is of possible interest to give the results from a few of the individual stations of the Cattail Creek area. Both water and mud samples were taken from stations 1 and 3 in the middle marsh from March 17, 1942, to October 6, 1943, except that the mud samples were omitted on March 17 and March 24, 1942. There were 54 water samples and 52 mud samples from each station. The results of these tests are summarized in table 1.

These figures show the water positive and the mud negative about 2½ times as often as the water was negative and the mud positive. However, it should be pointed out that this difference may be more apparent than real since a large proportion of the guinea pigs that received mud samples died of peritonitis soon after being injected. It is quite possible that some of these animals might otherwise have shown evidence of tularemia.

**Table 1.—*Results of Tests of Water and Mud Samples from Stations 1 and 3, Cattail Creek Area, Mar. 17, 1942, to Oct. 6, 1943***

| Station | Water | | Mud | | Water and mud | | | |
|---|---|---|---|---|---|---|---|---|
| | + | − | + | − | Both+ | Water+, mud− | Water−, mud+ | Both− |
| 1 | 43 | 11 | 37 | 15 | 32 | 9 | 5 | 6 |
| 3 | 44 | 10 | 33 | 19 | 29 | 13 | 4 | 6 |
| Total | 87 | 21 | 70 | 34 | 61 | 22 | 9 | 12 |

+=Positive for *P. tularensis.*

Water station 9 was at the mouth of the stream draining the south swamp, and water station 10 was just below the mouth of the stream arising from the middle swamp. The water at the latter point represented both swamps. Thirty-five samples from each station were tested concurrently between March 1942 and July 1943. Concurrent samples were positive 25 times and negative twice. Station 9 was negative 7 times when station 10 was positive. Only once was station 10 negative and station 9 positive. The results suggest that a greater concentration of *P. tularensis* was present in the water from the middle swamp.

Water station 17, on the main channel of Cattail Creek just below the mouth of the effluent stream from the north marsh, served to test the water from all three marshes. Samples were tested 27 times from March 1942 to November 1943. Nineteen samples were positive. The last 4 samples taken in August, September, October, and November 1943 were negative as were all other water samples taken in the Gird Creek area during that period. Excluding these last samples, 5 others were negative when samples in the marshes were positive. For example, water from station 10 was positive each of these 5 times and on one of those times water from station 13 in the north marsh was positive. On the other hand, on 5 occasions when station 13 was negative, station 17 was positive. Station 25, the one next below 17, was negative on the same days as station 17.

Station 6 was located on the tributary originating in the south marsh and at a point about one-fifth of the distance from the marsh to the mouth of the stream. As previously noted this effluent stream was marsh-like. Eighteen samples taken at station 6 between March 24 and September 1, 1942, were all negative, whereas, of 17 samples tested from September 9 to December 30, 16 were positive. The water at station 48 downstream from 6 was positive throughout the entire period (April to December). Obviously a new source of contamination appearing between September 1 and 9 was responsible for the

contamination present on and after the latter date, but this source was not determined.

Of 668 guinea pigs used to test water samples from the Cattail Creek area during the warmer months of June, July, and August 1942, 168 died of tularemia, the average days to death was 11±3. Of 848 guinea pigs used to test water samples taken from September 1942 through March 1943, 645 died of tularemia; average days to death was 8±2. It is not apparent whether the more marked evidence of contamination during the colder months was due to the presence of more tularemia bacteria or to an enhanced virulence of the bacterium during the colder months.

*Tests of soil samples.*—Soil samples from the vicinity of the marshes were tested in order to determine whether *P. tularensis* was resident in the soil. Thirty-nine samples from 29 stations were tested in the same manner as mud samples. Most samples were of soil immediately underlying field mouse runs or nests and contained mouse feces. The only positive samples, four in number, were from stations within the overflow area of the middle swamp, one from the north side (station 184) and three from the south (station 8). All were taken after the recession of overflow water and before the soil had become dry. A water sample taken March 24, 1942, from a hoofprint at station 8 was positive. Damp soil taken at the same point on April 7 was also positive but six samples of dry soil taken between April 17 and May 19 were negative. A moist soil sample taken April 2, 1943, following an overflow from the marsh was again positive.

## OTHER GIRD CREEK DATA

Following the finding of *P. tularensis* in Cattail Creek water in mid-March 1942, the water in the west channel of the Gird Creek Loop, into which Cattail Creek flows, was tested, and contamination was shown to a point 1½ miles below the junction of the east and west channel, i. e., to a distance of about 2 miles (station 34) below the marshes that head Cattail Creek. Contamination at this farthest point was demonstrated each week until April 14. Thereafter, until November 1942, *P. tularensis* was recovered to a distance of 1 mile (station 33) below the junction but not beyond. To what degree contamination at these downstream stations had its origin in Cattail Creek is uncertain, since *P. tularensis* was demonstrated on June 30, 1942, and on four subsequent occasions in water samples taken from the Gird Creek channel just above its junction with Cattail Creek. However, several tests made during that period at points farther up the loop were negative.

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In January 1943, because of the greater evidence of contamination in Cattail Creek during the preceding few months, the water tests were extended both up and down Gird Creek and to the tributaries previously mentioned. The water throughout the loop was found contaminated. Tests upstream from the loop were made at 10 stations on Gird Creek (over a distance of 2 miles) and at 5 on a short tributary, Jones Creek (1 mile). Six Gird Creek stations above the confluence with Jones Creek were all negative; the one farthest upstream was a beaver pond within the mountains. Four of five stations on Jones Creek were positive. These stations were the farthest upstream stations at which *P. tularensis* was demonstrated. Two of four Gird Creek stations between this tributary and the loop were positive, the one nearest the mouth of Jones Creek and the one nearest to the loop. On the three-fourths mile section of the loop north of Cattail Creek five of six stations were positive.

At the north end of the loop a station on Grimes Creek just before it joins Gird Creek was positive. No tests were made further up this stream.

Twenty-nine stations were established on Spring Creek in early 1943. This small stream, about one-half mile long, is of interest because conditions were so different from those on Cattail Creek. (See fig. 1.) Its source is a spring. The course is over sand and gravel between steep banks about 2 to 4 feet high. There are mud pockets along the banks that are deposits from the waters of numerous small springs and from runoff water during periods of melting snow or heavy rain. Water from 6 stations was positive over the full period of 8 weeks that tests were made, but tests of 17 mud pockets were consistently negative. Water from the spring at the very head of the stream was negative but one positive sample was obtained on February 10, 1943, about 10 yards downstream.

Water was tested from 14 stations along the 6 miles of Gird Creek between the north end of the loop and the Bitter Root River over a period of 12 weeks beginning in January 1943. All these tests were consistently positive. In addition, water taken during this period at points in the river one-quarter and one-half mile below the mouth of the creek were positive but not those taken downstream 1 mile. One sample taken 100 feet above the mouth was also positive.

The last positive test of Gird Creek water below the loop was obtained from a sample taken at station 46 on July 2, 1943. Above the loop the last such sample was taken at station 124 on April 2, 1943. Monthly samples taken at both stations after these dates until March 16, 1944, were consistently negative.

*Animal population.*—It was not possible to make a detailed study of the animal population along the course of Gird Creek other than

in the Cattail Creek area. In general it was typical of the local fauna. There were beavers in the upstream section beyond the point at which the water was found contaminated, and a very few below the loop. Muskrats were usually present along the stream where conditions were suitable, especially in associated marshy areas. Ducks frequented certain portions.

*Tularemia in animals.*—*P. tularensis* was recovered from the tissues of a beaver found dead in Gird Creek on April 3, 1943, at a point about half way between the loop and the Bitter Root River. Hundreds of muskrats along the course of Gird Creek died during the early months of 1943.

## BITTER ROOT RIVER AND TRIBUTARIES OTHER THAN GIRD CREEK

After finding Gird Creek so extensively contaminated, tests were made in February 1943 of all larger streams flowing into the Bitter Root River between the towns of Florence on the north and Darby on the south, a distance of about 50 miles. Two simultaneous samples were taken from each stream, from some streams on February 18, and from the remainder on February 26. Sixteen streams joining the river from the west and nine joining it from the east were sampled. Of the former, five were found contaminated—Mill, Bear, Sweathouse, Blodgett, and Gold Creeks (all +1 except Gold Creek which was +3); of all the latter, six in addition to Gird Creek were found contaminated—north and south branches of Burnt Fork Creek and Willow, Three Mile, Eight Mile, Willoughby, and Sardine Creeks. In addition, the water in three large irrigation ditches on the east side was contaminated. Two later samples were taken from Gold Creek (March 30, +3, and April 23, +3) but no further tests were made after August 13, when the waters of all these streams except Sardine Creek (+1) were negative.

The town of Stevensville is located in the northern part of that portion of the east side of the Bitter Root Valley in which many streams were contaminated. The town obtained its water from Swamp Creek, a small tributary of Burnt Fort Creek, which rises in a marshy area close to the mountains several miles to the east. A water sample taken on April 7, 1943, from Swamp Creek just above the point at which water is diverted for city use was positive (+3). Other samples taken after the water had passed through the chlorination plant and from taps in the city were negative.

In addition to the waters of the creeks, the river water was tested at six road crossings. These tests were all negative. Positive tests of river water have been noted as obtained below the mouth of Gird Creek in January 1943. A sample taken just above the mouth of the stream at the same time was also positive.

There are a number of low, flat, brushy, more or less marshy areas varying in size along the course of the river. One of these areas is just west of the city of Hamilton. It is marshy in part and is traversed by several small streams. Some beavers and muskrats were present locally in the fall of 1942. Part of this area was used as a city park and an enlargement of one of the streams was used for swimming. Two water samples taken on March 9, 1943, one just above the swimming area, the other in another channel, were positive (+1 and +1, respectively). Eight other samples taken in the same general area were negative. Samples taken 14 days later from 9 of these 10 stations were negative, the positive test (+1) being from a station previously negative. About ⅓ of an acre of the west portion of the land occupied by the Rocky Mountain Laboratory adjoins this area and is marshy. A water sample taken here on March 23 was positive (+1). Positive water samples were collected February 26, 1943 from river sloughs in two similar areas a few miles north of Darby (+4 and +4, respectively).

In late February and early March 1943, 31 water samples were taken from 22 creeks joining the Bitter Root River between a point near the headwaters of the West Fork on the south and Darby on the main river 50 miles to the north. The only positive sample (+1) was one of four from the most southerly tributary (Chicken Creek), deep in the Bitter Root Mountains.

Other tests of water samples taken during the same period from the Bitter Root River and tributaries, over a distance of 15 miles north of points heretofore mentioned, were all negative. There were a few reports of dead beavers and muskrats along this portion of the river.

Our tests thus covered a portion of the Bitter Root River and most of its tributaries from the mouth of the Bitter Root Valley upstream for a distance of about 115 miles. While only isolated tests were made in many instances, the evidence is sufficiently conclusive to show that water contamination during the late fall of 1942 and until the spring of 1943 was most prevalent in that portion of the valley between Florence and Hamilton where it is the widest (4 to 10 miles), that is, where the flora, fauna, soil, and topography are typical of valley country and not of mountain country.

In the spring of 1943 tests were made of the waters of 26 pools in meadow and pasture land in that portion of the east side of the Bitter Root Valley in which at that time most of the local tributary streams of the Bitter Root River and the irrigation ditches that traversed it were positive. None of the pools were in close proximity to the river nor did they contain any overflow water from any irrigation ditch. The largest pool was about 50 feet in diameter and 10 inches deep; the smallest, about 15 feet in diameter and 6 inches deep. The water in

some of the pools had drained from areas of at least one-eighth mile square. All of the drainage areas presented habitat conditions suitable for field mice. Tests of all of these pools were negative.

These findings, together with the known local conditions at the time and the results of observations in other areas as reported in this paper, appear to indicate that contamination with *P. tularensis* is a phenomenon more likely to be associated with flowing waters than with temporary accumulations of runoff water. Even marsh waters that have been found positive were from marshes that were integral parts of permanent water courses.

*Tularemia in animals.*—A muskrat found dead in the Hamilton city park on March 9, 1943, and one near Woodside on March 8, 1944, yielded pure cultures of *P. tularensis*. *P. tularensis* was also recovered from a beaver carcass found April 5, 1943, in a river slough 3 miles south of Hamilton; but tests of another found July 2, on the river 4 miles north of Victor were negative. Again, on February 14, 1948, a dead beaver was found on the river immediately west of Hamilton and the organism was recovered from the tissues.

In February 1949 an epizootic occurred in muskrats in a small slough tributary to the Bitter Root River near Florence. Fourteen animals were found dead, 5 of them in 1 muskrat house. The tisses of 12 were tested and *P. tularensis* was isolated from 10. Two trapped muskrats were obviously diseased and a culture of the organism was established directly from 1 of them. Tissues of 2 of 3 field mice (*Microtus* sp.) found dead in the area were also positive. Two dead beavers were found in the general area in March 1949. One was positive for *P. tularensis;* the other was so badly decomposed that no tests were made.

The recoveries of *P. tularensis* reported above and in earlier sections of this paper convey no adequate idea of how extensively the beaver and muskrat populations of the north half of the Bitter Root Valley were affected by epizootic tularemia during the period covered by this report, especially during the winter of 1942–43 and the following spring when the populations of these animals were virtually annihilated in numerous localities. Reports of dead beavers were numerous and some trappers reported having seen hundreds of dead muskrats. The assumption that the deaths of these animals were at least in part due to tularemia is supported by the occurrence of at least eight cases, as reported below, among local trappers who had skinned dead muskrats. River sloughs, marshy areas, ponds along the courses of creeks, and other habitat areas known to have been populated by numerous muskrats in the fall of 1942 and the early winter of 1943, and marked for trapping operations in the spring, were found completely depopulated when the trapping season opened in March.

It is probably safe to assume that deaths of muskrats from tularemia numbered in the thousands and those of beavers at least in the hundreds.

*Human infections.*—In late March and early April 1943, eight cases (J. M., F. M., E. H., E. S., R. F., H. M., R. M., and C. M.) of tularemia occurred among local trappers operating along the Bitter Root River and tributary streams and in marshy areas between the town of Florence on the north and Gold Creek, a few miles south of Hamilton. This was during the period that contamination of natural waters was general in the area, and widespread mortality was reported among local beaver and muskrat populations. Six of these cases came to attention the week beginning April 10, the others a few weeks later. Other illnesses among trappers, quite possibly due to tularemia, occurred at this same time, but the persons concerned did not consult physicians and blood samples could not be obtained.

The eight verified cases were very interesting because of their relative mildness. One patient was confined to bed 4 days, two others for 2 days each, and the others were not sufficiently ill to require bed rest. Three of the individuals did not consult a physician. The mild character of the illness during the early portion of its course led some of the patients to forego consulting a physician until, after a period of weeks, persistent local lesions and continued malaise prompted them to do so. Local lesions were present on one or both hands of each patient except one who had a tender edematous finger for a considerable period. In only two of the other patients were typical primary ulcers present. The lesions in the remaining five persons consisted of multiple pustular areas about 0.5 cm. in diameter which failed to ulcerate. Enlargement of either or both the axillary and epitrochlear lymph nodes was present in all cases and in one there was lymphangitis. One patient developed a rash on his neck and arms. A prolonged period of malaise accompanied each case.

## LEWISTOWN AREA (FERGUS COUNTY)

### WARM SPRINGS CREEK

This creek, a tributary of the Judith River, has its source in Maiden canyon about 18 miles northeast of Lewistown. Our observations were made along about 200 yards of its course where it flows through a narrow mountain meadow immediately below the source of the stream. Here it was 2 to 3 feet wide except where its course was broken by a series of beaver dams. The water in the lower ponds formed by the dams was semistagnant and the stream bed was dry for about a mile below the lowermost pond. Here the flow resumed and the stream continued in a northwesterly course to the Judith River.

Visits were made to the area on October 13 and 14 and November 18, 1942, January 7 and March 24, 1943, and June 25, 1947.

*Animal population.*—According to local reports there had been no beavers in this portion of the stream before 1938, but in September 1942, 8 or 10 were supposed to have been present. At the time of the first visit there were two beaver houses, both occupied. In March 1943, one, possibly two, beavers were left. A few muskrats frequented the ponds and there was abundant evidence of a heavy field mouse population in the adjacent meadow. At the time of the last visit (June 1947) there were no beavers present. The dams had washed out during the intervening years and the ponds formed by them had disappeared.

*Tularemia in animals.*—Six dead beavers were found along this portion of the stream. The first was found on September 22, 1942, by the local game warden. The five others were found by us, one each on October 13 (badly decomposed), October 14 and November 18, and two on January 7, 1943 (both partially eaten by dogs). The tests of the badly decomposed carcass and those of one of the partially eaten carcasses were negative, the others were positive.

A dead muskrat found on January 7 was too badly decomposed to test. The tissues of a field mouse found dead the same day were negative.

The dogs that fed on two of the carcasses (at least one was infected) exhibited no ill effects according to the owners.

*Water and mud contamination.*—Beginning October 13, 1942, and on subsequent visits thereafter, water and mud samples were collected from stations established along the 200-yard portion of the stream where the dead beavers were found. The results of these tests are summarized in table 2.

**Table 2.—*Results of Tests of Water and Mud Samples from Stations on Warm Springs Creek, Fergus County, Montana***

| Date | Number of samples | Water | | Mud | |
|---|---|---|---|---|---|
| | | Positive | Negative | Positive | Negative |
| Oct. 13, 1942 | 13 | 4 | 3 | 3 | 3 |
| Nov. 18, 1942 | 12 | 4 | 2 | 6 | 0 |
| Jan. 7, 1943 | 4 | 1 | 2 | 0 | 1 |
| Mar. 24, 1943 | 5 | 1 | 4 | 0 | 0 |
| June 25, 1947 | 6 | 0 | 5 | 0 | 1 |
| Total | 40 | 10 | 16 | 9 | 5 |

During the period of October 1942 to March 1943, 21 tests of water samples and 13 of mud samples were made. Ten of the former and

9 of the latter were positive. In the 10 positive water tests, 15 of 40 guinea pigs became infected and in the 9 positive mud sample tests, 25 of 36 became infected. The samples taken in June 1947, long after the dams had washed out and all of the beavers had either died or had migrated elsewhere, were negative.

*Other data.*—The section of the creek just discussed is joined from the north by a small, short, spring-fed tributary. On March 24, 1943, a positive water sample was taken from the latter stream at the point where it flowed past a dwelling which had been occupied by two men, one of whom had died the preceding December; the other still used it. These men did their own housekeeping, and water for all purposes was taken from the stream. At this point the flow about half filled a 3-inch pipe. While no previous tests of the water of this stream had been made, experience elsewhere suggests that it had probably been contaminated for a considerable period. Beavers had been present in a pond near the head of this stream but not for the past several months at least. It therefore appeared probable that the surviving occupant of the dwelling had used contaminated water for some time and that the one who died might have done so. Inquiry was made as to the cause of the latter's death but no data suggestive of tularemia were obtained. The surviving occupant had had a brief illness in February. A blood sample taken April 17 did not agglutinate *P. tularensis.*

A rancher on Warm Springs Creek, living 2 miles downstream, found three dead beavers on his ranch in late November 1942. Spleen and liver tissue obtained from one of the carcasses by Dr. H. F. Wilkins of the Montana State Livestock Sanitary Board was forwarded to us. *P. tularensis* was recovered. Two dead beavers and several dead muskrats were reported found in early 1943 about 13 miles still farther down stream.

## BIG SPRING CREEK

This stream, which empties into the Judith River, has its chief source about 5 miles southeast of Lewistown in large springs discharging about 60,000 gallons a minute. The city water supply is piped from these springs. The main creek meanders for about 20 airline miles, passing through Lewistown and finally flowing into the Judith River. At station 4 (see tabulation below), approximately half way downstream, the creek is normally about 30 feet wide and 2 to 3 feet deep, but during the spring runoff the flow of the stream is greatly increased. There are some 19 tributary streams and marshes draining into the main creek.

*Tularemia in animals.*—On September 18, 1942, a beaver carcass was found in a slough tributary to the creek by the local game warden who placed it in cold storage. The tissues were tested at the Rocky Mountain Laboratory October 16, and *P. tularensis* was recovered. There

were no beaver colonies in this slough or nearby. Muskrats were present and on November 18 one was found dead about 75 yards farther up the slough. Its tissues were also positive. Another dead muskrat, too decomposed to test, was found January 8, 1943, near this same point.

It is apparent that late in 1942 and early 1943 considerable numbers of beavers and muskrats died in this stream and its tributaries. One report stated that "1,500 dead muskrats were found" along a few miles of the main creek above Lewistown between March 1 and 24, 1943. Early in the same month a trapper reported that he found more dead beavers than he caught live ones. A ranch hand reported having seen beaver carcasses floating downstream in the late summer of 1942.

The local game warden has informed us that most of the tributaries south of Lewistown were populated to capacity with beavers and muskrats prior to the epizootic under discussion and that the loss approximated 80 percent.

*Water and mud contamination.*—Data for water and mud samples from the main creek are presented in table 3.

**Table 3.—*Results of Tests of Water and Mud Samples From Big Spring Creek, Fergus County, Mont.***

(—=negative; +=positive. The figure after + sign shows the number of test guinea pigs that became infected)

| Station | Sample | Oct. 13, 1942 | Nov. 18, 1942 | Jan. 8, 1943 | Mar. 24, 1943 | May 16, 1943 | June 25, 1947 | Feb. 26, 1950 | Location |
|---|---|---|---|---|---|---|---|---|---|
| | Downstream from Lewistown | | | | | | | | |
| 1 | Water | +4 | (—) | (—) | | | (—) | (—) | Slough 4½ miles north west of Lewistown, point where dead beaver found Sept. 18. |
| | Mud | (—) | (—) | | | | | | |
| 2 | Water | | +4 | +1 | +4 | | (—) | | 75 yards farther up same slough. Dead muskrat found Nov. 18. |
| 3 | Water | | +2 | (—) | +4 | | (—) | (—) | On creek 50 yards below slough. |
| 4 | Water | | +3 | (—) | +4 | | (—) | (—) | On creek, 50 yards above slough. |
| | Mud | | | (—) | | | | | |
| 5 | Water | | | +2 | | | | | At ranch 1½ miles farther downstream. |
| | Mud | | | (—) | | | | | |
| 6 | Water | | | +2 | +3 | (—) | | | At Hanover, 1½ miles farther downstream. |
| | Mud | | | +2 | +3 | | | | |
| 7 | Water | | | +1 | +4 | (—) | (—) | (—) | ½ mile downstream from Hanover. |
| | Mud | | | (—) | (—) | | | | |
| 8 | Water | | | (—) | | | | | 1 mile upstream from 4. |
| | Mud | | | (—) | | | | | |
| 9 | Water | | | (—) | +4 | | (—) | (—) | Bridge at Arrow refinery 1 mile upstream from 8. |
| | Mud | | | (—) | | | | | |
| 10 | Water | | | (—) | | | | | At bridge 400 yards upstream from 9. |

Table 3.—*Results of Tests of Water and Mud Samples From Big Spring Creek, Fergus County, Mont.*—Continued

| Station | Sample | Oct. 13, 1942 | Nov. 18, 1942 | Jan. 8, 1943 | Mar. 24, 1943 | May 16, 1943 | June 25, 1947 | Feb. 26, 1950 | Location |
|---|---|---|---|---|---|---|---|---|---|
| | Downstream from Lewistown | | | | | | | | |
| 11 | Water | ---------- | ---------- | (−) | ---------- | ---------- | ---------- | ---------- | ½ mile upstream from 10, Cattail swamp adjacent. |
| | Mud | ---------- | ---------- | (−) | | | | | |
| 12 | Water | ---------- | ---------- | (−) | +4 | (−) | (−) | (−) | Bridge near city limits of Lewistown. |
| | Within city limits of Lewistown | | | | | | | | |
| 19 | Water | ---------- | ---------- | ---------- | ---------- | (−) | ---------- | ---------- | |
| | Upstream from Lewistown | | | | | | | | |
| 13 | Water | ---------- | ---------- | (−) | +3 | (−) | ---------- | ---------- | Beaver colony near city limits. |
| | Mud | ---------- | ---------- | (−) | ---------- | ---------- | | | |
| 14 | Water | ---------- | ---------- | +1 | +3 | (−) | (−) | (−) | Bridge 1 mile upstream from 13. |
| 15 | Water | ---------- | ---------- | +1 | +4 | ---------- | (−) | (−) | 2 miles upstream from 14. |
| 16 | Water | ---------- | ---------- | (−) | ---------- | ---------- | ---------- | ---------- | Bridge 1 mile upstream from 15. |
| 17 | Water | ---------- | ---------- | (−) | +3 | ---------- | (−) | (+) [1] | Bridge 1 mile upstream from 16. |
| 18 | Water | ---------- | ---------- | (−) | ---------- | ---------- | ---------- | ---------- | Spring at source of creek. |

[1] Each of 10 white mice received 2 ml. of water intraperitoneally. Four died of tularemia.

In view of the recovery of *P. tularensis* from the one water sample taken on October 13 and from three of the four water samples taken on November 18 (all of these samples taken about 4½ miles downstream from Lewistown) it was decided to sample other portions of the stream. This appeared desirable since the creek flows through Lewistown, a city of about 6,000 population, and since the city water supply is from the springs that are the main source of the stream.

On January 8, 1943, 18 water samples were taken at stations spaced one-half to 2 miles apart, from the springs above Lewistown to a point one-half mile below Hanover, an airline distance of about 12 miles. Eight mud samples were also taken. On March 24, water samples were taken from 11 of these stations and mud samples from 2 of them. Water samples from 5 of these same stations were taken May 16, from 10 on June 25, 1947, and from 9 on February 26, 1950.

The results of the tests of the water samples afford an interesting comparison. Of the 18 samples of January 8, only 6 were positive, of which 2 infected 2 guinea pigs each and 4 infected 1 animal each; while of the 11 samples of March 24, every one was positive and 7

infected all 4 test guinea pigs and 4 infected 3 animals each. All of the 6 samples of May 16 were negative, as were the 10 taken June 25, 1947, 4 years later. However, 1 of 9 samples taken on February 26, 1950, was positive. This sample was from station 17, about 1 mile below the mouth of Castle Creek which was also positive at this time (see below).

The positive samples of January 8 and March 24, 1943, showed that an 8 to 10 mile portion of the creek upstream and downstream from Lewistown was contaminated with *P. tularensis*. No samples were taken within the city limits on the latter date or earlier but the presence of contamination was suggested by the positive tests of samples from stations 12 and 13. Both of these stations were within a few yards of the city limits, on the north and south of the city, respectively, and about a mile and a half apart.

The results are particularly interesting because on both January 8 and May 16, 1943, the water flow was normal, whereas on March 24, when all of the 11 water samples tested were positive, the flow was at least twice normal due to melting snow and the water was very turbid with suspended soil particles.

*Tributaries.*—A number of tributary creeks join Big Spring Creek along its course. Water samples from five larger tributaries of the seven that are upstream from Lewistown were tested, but none from those downstream. Casino Creek joins the main creek from the west at the south edge of town; Castle and Hansen Creeks join it from the west near the springs. Pike Creek and the East Fork merge with it from the east about 2 and 2½ miles, respectively, from Lewistown. Repeat samples were taken at approximately the same point as the original ones. The results of these tests are presented in table 4.

**Table 4.—*Results of Test of Water and Mud Samples from Five Tributaries of Big Spring Creek, Fergus County, Mont.***

(—=negative; +=positive. The figure after + sign shows the number of test guinea pigs that became infected)

| | Sample | Jan. 8, 1943 | Mar. 24, 1943 | May 16, 1943 | June 25, 1947 | Feb. 26, 1950 | Location |
|---|---|---|---|---|---|---|---|
| Casino Creek | Water | +4 | +3 | (—) | (—) | | Just above mouth. |
| Castle Creek | Water | +4 | +3 | +2 | (—) | +[1] | Pond with beaver and muskrats just above mouth. |
| | Mud | | +1 | | | +[1] | |
| Do | Water | +3 | | | | | At ranch 5 miles upstream. |
| Hansen Creek | do | | +4 | | | | Just above fish hatchery. |
| Pike Creek | do | | +4 | (—) | | | Just above mouth. |
| East Fork Creek | do | | +4 | | | | Do. |
| Do | do | | | +1 | | | 3 miles upstream. |

[1] Each of 10 white mice received 2 ml. of water intraperitoneally. Five died of tularemia.

Beavers and muskrats are reported to have been found dead on all of these tributaries during the 1942–43 epizootic and the estimated loss according to the local game warden was about 80 percent. On Castle Creek beavers and muskrats were present in early January 1943 in the vicinity of the sampling station just above the mouth of the stream but the game warden reported that most of the muskrats died prior to the next visit on March 24, 1943. Whether any of the beavers died during this period is not known. This site was not visited again until June 25, 1947, when the presence of beavers was again noted. No evidence of either beavers or muskrats was observed here at the time of the last visit on February 26, 1950.

*Discussion.*—The evidence of increased and more general contamination of the water of the main creek on March 24, 1943, was surprising in view of the fact that the volume of water being carried was over twice that noted on the other visits. This phenomenon suggests the existence of an extensive reservoir of *P. tularensis* from which the bacteria were released by some factor associated with high water. The only two possibilities that have occurred to us are (a) contaminated mud from the stream bed, particularly beaver ponds and sloughs, and adjacent marshes and/or (b) contaminated soil adjacent to the banks of the stream over or through which runoff water drained into the creek.

With respect to the first possibility, the results of the tests of eight samples of mud taken from Big Spring Creek prior to March 24 (only one was positive) do not suggest that mud contamination was consequential. While the actual volume of mud tested was infinitesimal yet there are few mud deposits. The stream bed is mostly gravel. However, this does not obviate the possibility that contamination may have been heavy in mud of the tributary streams and marshes. These tributaries have mud bottoms for much of their courses exclusive of numerous mud-bottomed beaver ponds and marshes tributary to this stream. The tabulated results of the tests of water from the five largest of the seven tributaries upstream from Lewistown suggest that water contamination on January 8 was heavier than in the main stream (only three tributaries were tested on that date), and that on March 24 (all five tributaries tested) it was at least as heavy as in the main stream.

With respect to the second possibility, there is little to say. Soil contamination, so far as there is any reason to believe, would certainly have its source in animals and would be derived from their carcasses, their urine, and perhaps their feces. While tularemia may be present in extensive winter and spring populations of field mice living in close proximity to streams (as shown by our observations and those of Jellison et al. (1942)) and may cause the death of many mice, and

while the urine of infected mice is known to be infectious, it does not appear likely that contamination from this source would produce a reservoir of the bacterium sufficiently extensive to maintain the degree of contamination which the tests showed to be present in the water of Big Spring Creek on March 24 and which may have continued over a considerable period. Other animals might contribute to such a reservoir but such contributions would in all probability, be relatively minor.

So far as can be judged from information now available, and having in mind the observations and experimental studies made on Cattail Creek near Hamilton (see section under Gird Creek and under Experimental Investigations) it is thought that soil contamination derived from animals is unlikely to have played more than a very minor part at the most in the increase in the contamination of the water of of Big Spring Creek.

It is pointed out elsewhere in this paper that it is improbable that carcasses of beavers or muskrats are a factor of any real consequence in the maintenance of contamination of stream water with *P. tularensis.*

## OTHER MONTANA AREAS

Information from various sources, including a questionnaire sent to over 2,000 trappers, indicates that beavers and muskrats have been found dead in at least 150 Montana streams in 40 of the 56 counties during the period 1939 to March 1943. While smaller streams predominate, all the main rivers are included. For some streams there have been reports of dead animals having been found only in restricted areas, while for others there are reports that animals have been found dead over long stretches of the water courses and that in some instances mortalities occur nearly every year. On the other hand, men who have trapped along certain streams for many years report never having found a dead beaver or muskrat.

The data received are of such a nature that it is impossible to make any accurate estimate of numbers of animals that died each year. That the aggregate for the state may be large is definitely shown by the fact that hundreds of beavers are known to have died on the Little Big Horn River and its tributaries in 1939. And in this particular area the data of Scott (1940) and Jellison et al. (1942) suggest rather definitely that tularemia was the main factor concerned.

In March 1943, the local deputy game warden expressed the opinion that beaver and muskrat populations in the streams in a considerable area tributary to Lewistown, Mont., were being decimated.

Table 5 presents data concerning contamination of various Montana streams not specifically mentioned in earlier sections of this report, animals infected, and all known human cases of tularemia in Mon-

Table 5.—*Data Concerning Contamination of Various Montana Str eams; Animals Infected, and Human Cases of Tularemia Resulting From Beaver and Muskrat Contact (Except 8 Bitter Root Valley Cases)*

| Stream | County | Animals infected | Laboratory Tests | | Human cases from beaver or muskrat contact in county concerned |
|---|---|---|---|---|---|
| | | | Animal tissue | Water | |
| Not known | Powder River | Not known | None | None | 1, April 1940. Skinned muskrats. |
| Do | Yellowstone | do | None | None | 1, May 1943. Skinned beavers. |
| Deep Creek, tributary of Missouri River. | Broadwater | Dead beavers reported found May 1942 | (+) | −May 1942 | 1, April 1949. Skinned muskrats. |
| Smith River, tributary of Missouri River. | Meagher | Several beavers, muskrats, and mice reported found dead early in 1942. | None | −June 1942 | 1, April 1942. Skinned beavers and muskrats. |
| Judith River, tributary of Missouri River. | Fergus | Beavers reported found dead, December, 1942. | (+) | −January 1943 | 3, April 1943. Sorted beaver hides, handled beavers, and skinned muskrats found dead, respectively. |
| Cottonwood Creek and Beaver Creek tributaries of Judith River. | do | Muskrat | None | +March 1943 | |
| Box Elder Creek, tributary of Flat Willow Creek. | do | Three beavers reported found dead September–November 1942. All beavers on upper portion of creek said to have died in 1939 and 1940. | None | +January 1943 | |
| McDonald Creek, tributary of Box Elder Creek. | do | One beaver reported found dead, June 1942, another October 1942. Population said to have been decimated. | None | +November 1942 and May 1943. | |
| Judith River, tributary of Missouri River. | Judith Basin | Not known | None | +May 1943 | None known. |
| Running Wolf Creek, tributary of Judith River. | do | Beaver, August 1942 | (+) | −October 1942 | Do. |
| Surprise Creek, tributary of Arrow Creek. | do | Dead muskrats reported found December 1942. | None | +March and May 1943. | Do. |
| Otter Creek, tributary of Belt Creek | do | Not known | do | +March and May 1943. | Do. |
| Belt Creek, tributary of Missouri River. | Cascade | do | do | +March 1943 | Do. |
| Flatwillow Creek, tributary of Musselshell River. | Petroleum | Several beavers reported found dead from early 1941 to November 1943. | do | +March 1943 | Do. |
| Jefferson River | Madison | Two muskrats reported found dead in spring of 1942. | do | −June 1942 | 1, December 1937. Muskrat bite. |
| Moore Creek, flows into Meadow Lake | do | Muskrat found dead, March 1944 | (+) | None | 1, April 1941. Skinned muskrats. |
| Pipestone Creek, tributary of Jefferson River. | Jefferson | Muskrats reported found dead in April 1942. | None | −June 1942 | 1, April 1942. Skinned muskrats found dead. |

| | | | | | |
|---|---|---|---|---|---|
| Dry Creek, Bear Creek and other tributaries of East and West Gallatin Rivers. | Gallatin | Muskrats reported found dead in spring of 1943. Number of beavers reported to have died in spring of 1940. | do | None | 1, June 1940; 2, April 1943; 1, August 1946; 2, March, April 1949. Skinned trapped muskrats, and muskrats and beavers found dead. |
| | | Three beavers found dead November 1949. | 1+. 2 not tested (Montana State Veterinary Laboratory). | + from at least 6 separate streams (Jellison et al. 1950). | |
| Beaverhead River, tributary of Madison River. | Beaverhead | Beaver found dead February 1943. Two muskrats found dead March 1943. Numerous dead beavers and muskrats found during this period. See Jellison et al. (1942) for earlier. reports. | 1+, 1− | Some +, others −, March 1943. | 2, March 1940; 1, March 1943; 1, February 1946. Skinned trapped muskrats and muskrats found dead. |
| Clark Fork River, tributary of Columbia River. | Granite, Powell, and Deer Lodge. | Eight muskrats reported found dead in March 1943. | None | None | See Powell County. |
| Willow Creek, tributary of Clark Fork River. | Granite | Dead beavers and muskrats reported at various times since 1939. | do | + June 1943 | 1, spring 1939; skinned muskrats. 2, April 1943; skinned muskrats. |
| Lost Creek, tributary of Clark Fork River. | Deer Lodge | Not known | do | + March 1943, − May 1943. | None known. |
| Not known | Powell | do | do | None | 2, October 1928; skinned muskrats; 1, April 1943; skinned muskrats. |
| O'Keefe Creek, tributary of Clark Fork River. | Missoula | do | do | + April, May, June 1943. | None known. |
| Ninepipe Reservoir | Lake | Many muskrats reported to have died in spring 1943. | do | − May 1943 | 1, April 1943; skinned muskrats found dead in potholes in immediate vicinity of reservoir. 2, April and October 1949, locality where infected not known. |
| Irrigation ditch, 4 miles east of Ravalli | do | Not known | do | + April 1943, − May 1943 | |
| Flathead River, tributary of Clark Fork River. | Sanders | do | do | + April 1943 | 1, April 1948; "muskrats, beavers, or mink." |
| Swamp draining into Jocko River, tributary of Flathead River. | do | do | do | + April 1943 | 1, March 1949; skinned beaver. 1, March 1949; skinned muskrats and beavers. |

tana resulting from beaver and muskrat contact except eight Bitter Root Valley cases, previously discussed (p. 20). In April and May 1943, single water samples all of which were negative, were tested from 71 sources, including river, river sloughs, creeks, marshes, irrigation ditches, and potholes in Missoula, Mineral, Sanders, Flathead, Lake, and Lincoln counties.

*An outbreak of tularemia of undetermined source in eastern Montana.* Dr. J. H. Garberson, of Miles City, Mont., reported concurrent cases of tularemia in the late summer of 1942 in four employees of a large sheep ranch in Rosebud County. The onsets all occurred during the 3-day period July 19 to 21. Since all four patients exhibited tonsillar lesions, it appeared possible that the source of infection had been some material contacted by all at about the same time. Contaminated food or water seemed the most likely possibilities.

Conditions on this ranch were investigated on August 16 and 17. It contained 18,000 acres on which 20,000 sheep were ranged. Coyotes, rabbits, mice, snakes, and ducks were abundant. Large numbers of rabbits had died in the spring. Scattered over the ranch were a number of reservoirs and natural depressions that held run-off water. There were also several small, eroded stream beds in which water flowed following precipitation. The drinking water for the ranch house was stored in a cistern supplied from two sources, rain and natural ice. The ice was obtained from a nearby reservoir during the winter and lasted through the following summer.

The following data were obtained concerning the tularemia cases: From June 29 to July 3, K., R., and R. O. repaired ranch fences about 6 miles from the ranch house. On the second day their water jug, which contained cistern water, was broken and they drank water from West Jack Creek and from two reservoirs. On the third day K. and R. again drank from these two sources. From July 6 to 11, K. and R. worked in another part of the ranch and again drank water from one of the reservoirs. On July 11, the three men began working in the hay fields. Mrs. K., the fourth patient, began working with them on July 13 and during this period cistern water was used. On July 19, R. became definitely ill. On July 20, K. developed a severe sore throat accompanied by a high fever, as did Mrs. K. on July 21. Mr. and Mrs. K. were then hospitalized. R. O. became ill on July 21.

Water and mud samples taken from the various storage reservoirs all proved negative for *P. tularensis*. West Jack Creek and other creeks could not be tested since water was not flowing at the time of our visit. The reservoir waters were badly polluted and were accessible to all types of wild life. A dead rabbit was noted in one of the reservoirs.

A sample of cistern water taken August 17 was negative.

Though the negative test of the cistern water had little significance

because it was made about 3 weeks after the first onset, nevertheless the fact that this water was used by four other persons, who did not become ill, discounted the likelihood that it was responsible. The only occasion on which the three men drank water from a common source other than the cistern was June 30. The incubation periods of 19 to 21 days that would be entailed if water ingested on that date was the source of infection are unreasonably long, even granting the chance that the fourth patient, who was the wife of one of the men, might have acquired her infection by mouth to mouth contact with her husband. Perhaps the most likely period for infection to have been acquired was the one when all four patients were working in the hay fields. During this period, however, only cistern water was used for drinking purposes.

While none of the data obtained pointed to a probable source of infection it was felt that they did not completely eliminate the possibility the illnesses of the three men, at least, might have been caused by contaminated water.

## *Idaho*

### WARM SPRING CREEK

This area is about 3 miles west of May and is approximately 10 square miles in extent. It includes a number of spring-fed marshes with effluent streams that drain into Warm Spring Creek, which in turn flows into the Pahsimeroi River at the west end of the area. In some places in the marshes the mud is 10 feet deep, or more. There is a heavy growth of cattails in places.

Eight brief visits were made to this area—April 9, May 19, October 5, and December 15, 1942, February 16 and October 18, 1943, May 28, 1947, and April 21, 1949. Water and mud samples were taken and such general observations were made as a stay of a few hours permitted.

*Animal population.*—A considerable muskrat population is present in the marshes and effluent streams. On most of the trips, field mice were noted to be abundant. Shrews are likely present but were not seen. Other animals are undoubtedly present, although probably in small numbers.

*Tularemia in animals.*—Trappers who worked this area during the winter of 1941–42 stated that at least 150 dead muskrats were found. In some instances whole colonies had been destroyed. *P. tularensis* was recovered from scrapings from the hide and from the adherent flesh of a muskrat found dead in early February. This was the fourteenth animal of a single colony to be found dead. During the visits to the area, casual search was made for field mouse carcasses. None was found.

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*Water and mud contamination.*—From April 9, 1942, to April 21, 1949, water and mud samples were tested from a total of 29 stations established in the area. The location of the stations is shown on the accompanying map (fig. 4). During this period samples were taken at some stations as many as 7 times and at others only once. The results of the tests are presented in table 6.

**Table 6.—*Results of Tests of Water and Mud Samples From Warm Spring Creek, Lemhi County, Idaho***

| Date | Number of samples | Water | | Mud | |
|---|---|---|---|---|---|
| | | Positive | Negative | Positive | Negative |
| Apr. 9, 1942 | 18 | 8 | 10 | 0 | 0 |
| May 19, 1942 | 7 | 3 | 3 | 1 | 0 |
| Oct. 5, 1942 | 21 | 13 | 1 | 4 | 3 |
| Dec. 15, 1942 | 22 | 14 | 2 | 3 | 3 |
| Feb. 16, 1943 | 16 | 6 | 6 | 2 | 2 |
| Oct. 18, 1943 | 14 | 0 | 13 | 0 | 1 |
| May 28, 1947 | 12 | 2 | 10 | 0 | 0 |
| Apr. 21, 1949 | 13 | 0 | 13 | 0 | 0 |
| Total | 123 | 46 | 58 | 10 | 9 |

Contamination appeared to be at a maximum late in 1942. Of 14 water samples tested in October, 13 were positive, as were 14 of the 16 December samples.

*Human infections.*—Four muskrat trappers worked this area during early 1942. Three became infected, all apparently from skinning muskrats found dead. One had skinned the proved infected muskrat, above mentioned, a few days before onset of illness on February 6. In the case of the other two trappers, the onset for one was in late February or early March, and for the other, March 18.

## OTHER IDAHO AREAS

Data concerning the contamination of other Idaho streams, animals infected, and all known human cases of tularemia resulting from beaver or muskrat contact, except the three Warm Spring Creek cases discussed above, are presented in table 7.

Numerous dead muskrats were reported on Little Lost River, Butte County, in the spring and summer of 1942. During this period a patient ill with the typhoid type of tularemia claimed to have been infected by drinking water from this stream. However, other persons drinking water day after day from the same source did not become ill. Water from this stream was not tested.

Two trips, by different routes, were made across northern Idaho in April and May 1943, respectively. Beavers and muskrats were abun-

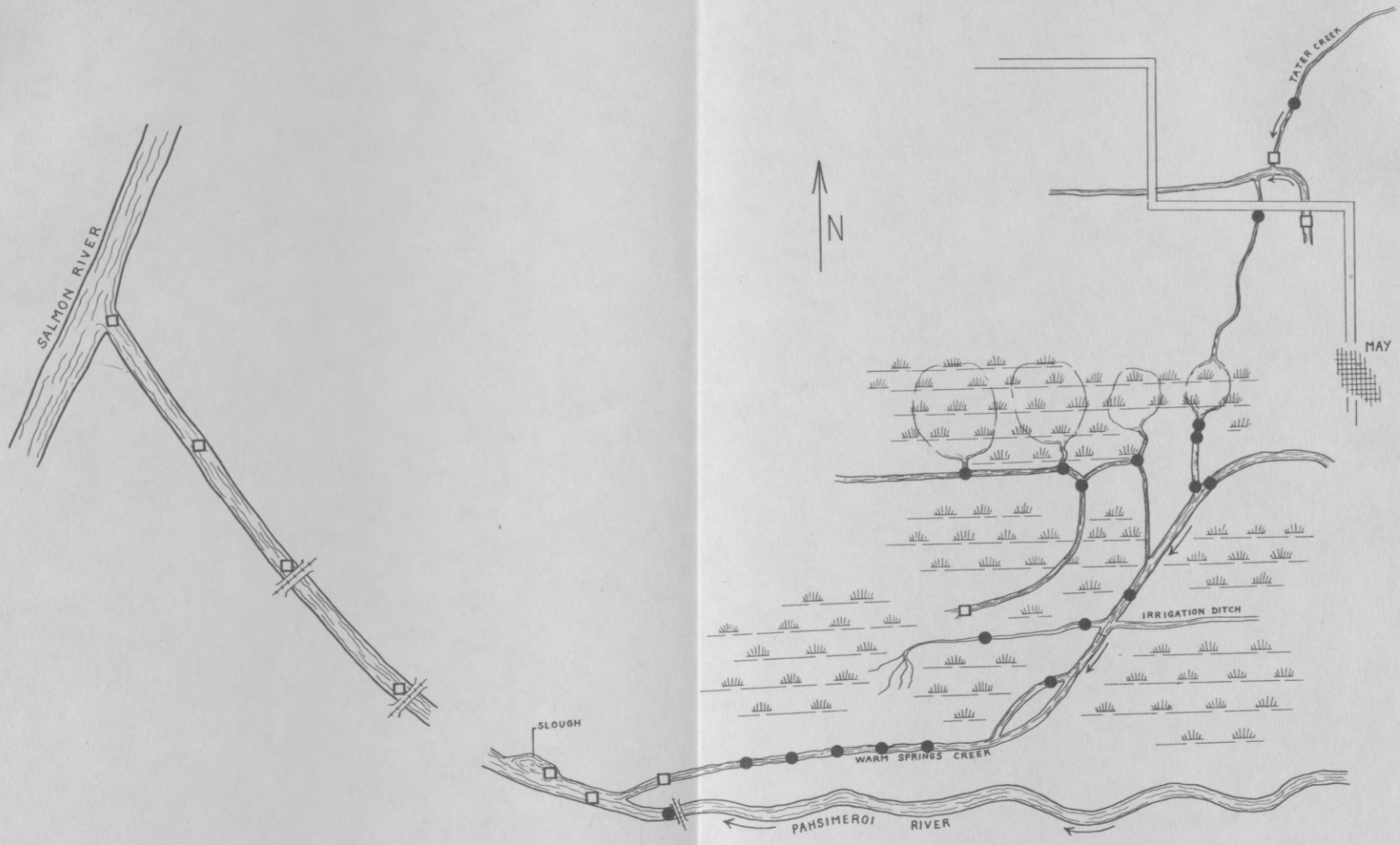


FIGURE 4. WARM SPRING CREEK AREA NEAR MAY, IDAHO. AREA SHOWN IS ABOUT 2 MILES WIDE AND 10 MILES LONG.

STREAMS

MARSHES

● STATIONS YIELDING CULTURES OF PASTEURELLA TULARENSIS ONE OR MORE TIMES.

□ STATIONS NOT YIELDING CULTURES OF PASTEURELLA TULARENSIS ONE OR MORE TIMES.

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Table 7.—*Data Concerning Contamination of Various Idaho Streams; Animals Infected, and Human Cases of Tularemia Resulting From Beaver or Muskrat Contact (Except Three Warm Spring Creek Cases)*

| Stream | County | Animals infected | Laboratory tests | | Human cases from beaver or muskrat contact in county concerned. |
|---|---|---|---|---|---|
| | | | Animal tissues | Water | |
| Not known | Fremont | Not known | None | None | 1, October 1934: Skinned muskrat. |
| Squaw Creek, tributary of Payette River. | Gem | Muskrat reported found dead in spring of 1942. | do | do | 1, spring 1942: Handled muskrat found dead. |
| Payette River (Seven Mile Slough area) tributary of Snake River. | do | "Muskrats died by the hundreds," in the spring of 1942. Four dead beavers reported found February 1942. | do | − May 1942 | 1, February 1942: Skinned beavers found dead. 2, March 1942: Handled beavers and muskrats. |
| Malad River (or Camas Creek), tributary of Big Wood River. | Camas | Several dead beavers reported found in April 1942. Three found in June 1942. | + June 1942 | + June 1942 (also mud). | None known. |
| Pearl Creek, tributary of Bear River | Bannock | Several reports that numbers of beavers and muskrats died in spring of 1943. | None | None | 1, April 1943: Skinned muskrat found dead. |
| Marsh Creek, tributary of Port Neuf River. | do | Not known | do | do | 1, March 1944: Skinned muskrats. |
| Not known | Bear Lake | do | do | do | 1, March 1950: Skinned muskrats. |
| Grays Lake | Bonneville | do | do | do | 1, April 1947: Skinned muskrat found dead. |
| Not known | Lemhi | do | do | do | 1, November 1949: "Muskrat." |

dant in the local streams, and numerous inquiries elicited no reports of animals having been found dead. Single water samples were collected from numerous creeks, rivers, river sloughs, lakes, marshes, and irrigation ditches, many of them in close proximity to beaver and muskrat houses. All were negative.

### *Wyoming*

We have been advised that in 1942 deaths of beavers were reported in Sheridan and Teton Counties. In a letter dated June 28, 1943, O. J. Murie, biologist, Fish and Wildlife Service, in Teton County stated that numerous beavers had been found dead that spring. He also sent preserved tissues showing suggestive lesions from a beaver found dead in the Snake River. Dead beavers were reported to have been found on Horse Creek, a tributary of the Green River in Sublette County and on Beaver Creek, in the spring of 1943.

*Human infections.*—Four persons are known to have contracted tularemia from beavers. Two occurred in April 1939 in connection with an epizootic in Sheridan County (Scott, 1940). In May 1943 a case occurred in a trapper who had skinned a beaver found dead on Horse Creek, about 2 miles north of Daniel in Sublette County. Another trapper contracted the disease in May 1946 from skinning beavers on Fall Creek, Teton County.

Two persons are known to have acquired the disease from muskrats. One skinned a muskrat that was trapped in October 1945 in Fremont County on the Little Wind River, halfway between Fort Washakie and Ethete. The other, a 6-year-old Indian boy, handled a muskrat found dead "on a tributary of the Wind River at Fort Washakie," Fremont County in April 1946. The grandmother is reported to have skinned the animal with no untoward effects.

### *Oregon*

We have been informed by Biologist A. V. Myers, of the Oregon State Game Commission, that in 1942, prior to June, muskrat deaths were reported from 12 counties (Lake, Harney, Malheur, Grant, Baker, Wallowa, Crook, Multnomah, Columbia, Clatsop, Jackson, and Klamath).

The extent to which tularemia was responsible for these fatalities is not known. However, according to "Tularemia * * *" (1942) and Wayson in correspondence (1942) many muskrats died in the spring that year at Klamath Lake, Klamath County, and *P. tularensis* was recovered from two animals examined. A local trapper, who became infected with tularemia in 1942 (see under *Human infections*) has reported that coincident with the epizootic in muskrats, "field mice" and "tule mice" died "by the millions" and were extremely scarce

during the following 2 years. Another trapper, who also contracted the disease that same year, has written that he "saw many dead coyotes, porcupines, field mice and muskrats." This same correspondent reported "many dead rats" in the early spring of 1945.

No reports of dead muskrats in the State were received by the State game commission in 1943 (letter from F. B. Wise, State game supervisor, November 10, 1943).

*Human infections.*—Eight definite cases of tularemia occurred during the season of 1942 among muskrat trappers operating in the vicinity of Klamath Lake. Unconfirmed reports suggest there may have been more.

A trapper (L. M.) claims to have contracted tularemia in February 1936 from muskrats caught on the North Fork of the Malheur River between Juntura and Beulah in Malheur County. His description of his illness suggests tularemia but he did not consult a physician.

### *Washington*

*Human infections.*—The only evidence of the occurrence of tularemia in muskrats or beavers or of the possible contamination of natural waters with *P. tularensis* in the State of Washington was a case (J. D.) due to skinning muskrats reported from Stevens County in December 1942. The patient was trapping within 1 mile of the town of Valley on Bulldog Creek and on the Colville River.

## Muskrats and Beavers as Sources of Human Infections in Other Areas

*Nevada.*—During the past few years reports have been received from various sources (1) that an unrecognized infection manifested by ulcers, fever, and glandular enlargement is prevalent among residents of the Ruby Valley and adjacent area in northern Nevada, (2) that those affected are principally persons who trap muskrats and beavers, and (3) that seasonal occurrence corresponds to the open season for trapping these animals. There is an apparent possibility that at least some of these cases may be due to tularemia.

*Utah.*—Twelve cases resulting from contact with muskrats have been reported.[4] One occurred in March 1948 and the others in February and March 1950. Eight of the patients gave a history of having skinned muskrats from Utah Lake, where muskrats were reported to have been found dead in the spring of 1948 and 1950; three others, a farmer and his son residing at Millville, and a farmer at Huntsville became infected from skinning muskrats from unspecified localities

[4] We are indebted to the Utah State Department of Health for information concerning some of these cases. See discussion by Jellison, Kohles, & Philip (1951).

in northern Utah. One was employed by a hide and fur company at Provo and had handled muskrat pelts from Utah Lake and probably elsewhere.

*Minnesota.*—Seven definite cases resulting from skinning muskrats have been reported from Minnesota.[5] The first two cases were infected in March 1930. One patient had trapped muskrats at Rice Lake (Morrison County), the other at Swan Lake (Nicollet County). The physician who attended the latter patient reported that several other trappers "had tender glands in the axilla, but were not as sick as this patient and did not report for treatment or diagnosis."

The third case was in February 1931 and was contracted from muskrats trapped on a farm near Motley. Infection may have been acquired in any one of three adjacent counties.

The fourth case occurred in April 1940. The patient had been trapping in Forest Prairie Township (Meeker County).

The fifth patient became ill in December 1943 after skinning muskrats presumably trapped near Dassel (Meeker County).

The sixth and seventh cases occurred in December 1945. The former was infected from muskrats from the Big Fork River (Itasca County), the latter from muskrats trapped near Verndale (Wadena County).

Six of these cases were of the ulcero-glandular type, one was of the glandular type. Case IV was described as having "numerous superficial ulcers" on both hands.

*Iowa.*—Only one case of tularemia in which muskrats were the source of infection has been reported (Jordan, 1940). Further information regarding this case is lacking.

*Wisconsin.*—Of tularemia cases occurring in Wisconsin, six have been reported as contracted from muskrats, two from beavers, and one from muskrats or beavers.[6]

The first muskrat-caused case was in March 1930 and was presumably contracted near Fond du Lac (Fond du Lac County). The second was in September 1941 and apparently became infected near Odanah (Ashland County).

Six other infections were all contracted in 1946—three muskrat-caused cases in the Horicon Marsh area (Dodge County), one muskrat-caused case in the Watertown Marsh near Richfield (Washington

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[5] The authors are indebted to Dr. O. McDaniel, of the Minnesota Department of Health, for the data concerning these cases.

[6] The authors are indebted to Drs. H. M. Guilford and A. R. Zintek of the Wisconsin Board of Health for information concerning cases in this State. The information furnished us differs in some respects from that published by Morgan (1949), who stated that "ten infected persons had killed and skinned muskrats * * * 2 cases each followed contact with skunks, horses, sick dogs which killed rabbits, foxes, muskrat or beaver, or possible contact with skunk, mink, muskrat, or raccoon. One case was recorded from exposure to a contaminated stream."

County), one beaver-caused case in Wood or an adjacent county, and the beaver- or muskrat-caused case was contracted in Wood, Portage, or Marathon Counties. In 1947 a case resulted from skinning beavers in Oneida County, and in 1949 a case occurred in a resident of Ladysmith (Rusk County) in which it was thought that the disease might have been contracted from handling muskrats in the local area but was questionable.

McDermid (1946) has reported both tularemia and Errington's disease as epizootic in muskrats, and tularemia as epizootic in beavers in the Horicon Marsh area where three of the 1946 infections were contracted.

*Michigan.*—Three cases resulting from contact with muskrats have been reported from Michigan.[7] All were contracted in December 1946 from muskrats at Pte. Mouileé marsh on Lake Erie in north Monroe County. The cases were of the ulcero-glandular type.

Trappers were reported to have found several dead beavers along streams in the vicinity of Escanaba, Delta County, in the spring of 1946, but the cause of death was not determined.

*Indiana.*—One definite and three possible cases due to contact with muskrats have been reported.[8] The former was infected in Putnam County in November 1946. The three possible muskrat-caused cases were infected in Vigo, Jaspar, and Wabash Counties in February, March, and November 1946, respectively. Each had a history of contact with other animals in addition to muskrats.

*New York.*—Four cases of tularemia that resulted from contact with muskrats have been reported from New York. One was in Oswego County in 1939. The patient, a trapper, became ill 3 days after having been bitten by a muskrat (Wadsworth, 1939). The other three cases were in Wayne County in 1942 (Wadsworth, 1942). The patients were members of a family group—a man, his wife, and the wife's father. Many of the muskrats handled—

"were said to have had subcutaneous abscesses, especially in the inguinal region which were often broken during skinning. Pus came in contact with a cut on the left hand of the younger man, which failed to heal and became swollen and painful within 2 days. He developed very mild symptoms consisting of malaise, anorexia, and slight headache. A gland in the left axilla became so swollen and painful that he consulted his physician on about the tenth day of illness. His temperature, pulse, and respiration were reported to be normal then, and at no time was it necessary for him to stop work as a laborer. Blood specimens collected on approximately the eleventh and twenty-fifth days of illness gave agglutination with *Bacterium tularense* in 1:10 and 1:1200 dilutions of serum, respectively. The wife, who helped the men and did most of the stretching of the hides, was reported to have symptoms similar to those of her husband, with

[7] The authors are indebted to Dr. S. C. Whitlock, of the Michigan Department of Conservation, for the data concerning these cases.

[8] The authors are indebted to Dr. James W. Jackson of the Indiana State Board of Health, for the data concerning these cases.

the exception of the local skin lesion. A blood specimen collected about 6 weeks later agglutinated *Bact. tularense* in a 1:640 dilution. The wife's father reported no symptoms of illness except two small skin lesions on his left hand, which were free of exudate but did not heal. His blood serum, however, gave agglutination in a 1:640 dilution."

*Maine.*—A fatal case of tularemia was contracted near Kokadjo Lake, Piscataquis County, Maine, in December 1933. This illness has been referred to by Badger (1939), Eckstein (1941), and Moore, Sawyer, and Blout (1944) as contracted while skinning a red fox. However, it is apparently not certain that this was true since Dr. E. A. Francis in correspondence has stated that the skinned carcasses of muskrats and a raccoon, in addition to those of two red foxes, were found in the hen houses of the patient. Furthermore, he was unable to recover *P. tularensis* from the fox carcasses. It appears obvious that all these animals had been but recently skinned and such data as are available suggest that this infection could as well have been acquired from one of the muskrats, or even perhaps from the raccoon, as from one of the red foxes.

*Canada.*—Johns (1933) reported three proved cases of tularemia in Ontario in 1932. One was quite definitely associated with muskrats, the other two apparently were. The first was the case of a laborer who lacerated the end of a middle finger and soon afterwards skinned four muskrats. Two days later, on April 20, he consulted a physician because of infection at the site of the lesion. The other two cases were a carpenter and his helper. They were trapping muskrats together. A muskrat was removed from a trap on April 4 and both men became ill on April 7. The carpenter also skinned raccoons and mink and the helper stated that he had occasionally handled rabbits. Although the muskrat contact was less clean cut than in the first case, the common day of onset of these two cases suggested a common source of infection and that the muskrat trapped 3 days before was the greatest probability.

The following report of human illness associated with an epizootic among beavers and muskrats appeared in the Fur Trade Journal of Canada, August 1950, page 6:

Thousands of dead muskrats and hundreds of dead beaver have been found in northern Manitoba's marshlands, victims of an epidemic which has greatly cut the expected spring fur harvest.

The disease is believed to be tularemia, and at least two trappers have contracted the disease from handling bodies of diseased animals. Several other cases among humans are suspected. However, the result of the illness in humans compares to influenza, and is not considered the serious aspect of the picture.

Department of game and fisheries spokesman, F. B. Chalmers, confirmed reports the disease has taken heavy toll of fur life. He said numerous diseased bodies have been sent to Winnipeg for laboratory examination and field parties of biologists are expected in the Pas during the summer to conduct detailed examinations.

*Alaska.*—Only one case of tularemia has been reported from Alaska (Williams, 1946). The patient, a meteorologist at Northway on the upper Tanana River drainage, gave a history of having skinned numerous muskrats for about 6 weeks prior to onset of symptoms June 5, 1945. Symptoms were "headache, orbital pain, generalized aching, fever of 104° F., and a dry bronchial cough. * * * The patient developed swollen, tender axillary and epitrochlear lymph nodes in his left arm. There was a small encrusted lesion on the back of the left middle finger which he stated had been there about a week." A blood sample taken June 20, 1945, agglutinated *P. tularensis* in a 1 : 1280 dilution. The patient reported that he had found no dead muskrats, beavers, mice, or rabbits in the area of trapping operations.

## EXPERIMENTAL INVESTIGATIONS

Reported in this section are a number of studies which relate directly to contamination of streams, and to the occurrence of tularemia in animals associated with an aquatic or semiaquatic environment. These studies were directed mainly toward determining the role of animals as sources of contamination and the degree to which they might be responsible for the persistence of the organism in contaminated streams. Consideration was given also to the possible role of contaminated water as a source of infection for animals living in or adjacent to such waters. In some of the experiments it was necessary to use guinea pigs instead of the native animals actually concerned.

Collection of urine samples and mouth washings from animals used in tests was required in some instances. Urine samples were obtained from live beavers and muskrats by holding them up by their tails over a collecting pan. When so held, these animals usually expelled urine. Urine from beavers, muskrats, and field mice that had died or were sacrificed was obtained by puncturing the bladder wall and drawing the urine into a syringe. If the bladder was empty, saline solution was injected into the bladder and then withdrawn in the same operation. In either case the same needle was used to inoculate the test guinea pigs. Mouth washings were obtained from living or dead beavers, muskrats, or guinea pigs by placing the animal on its back and expelling saline solution into the month with a pipette and then drawing it back, this being repeated several times with the same solution. Five ml. were used for a muskrat or guinea pig, and 10 ml. for a beaver.

The numbers of the stations from which water and mud samples were taken refer in all instances to stations on Cattail Creek in the Bitter Root Valley.

## INFECTED ANIMALS AS POSSIBLE SOURCES OF CONTAMINATION OF WATER AND MUD

*Occurrence of* P. tularensis *in the tissues, excretions, and mouth washings of muskrats and beavers dead of tularemia.*—This experiment was in the nature of a survey to determine if *P. tularensis* were present in various tissues, and in the feces, urine, and mouth washings taken immediately after death from a muskrat and a beaver, both of which died 4 days after receiving culture material, the former via the scarified skin, the latter by mouth. The results of the tests are presented in table 8 which also gives the nature and amount of the inoculum, route of inoculation, the number of test guinea pigs used, and the post-inoculation day on which each test animal died.

As seen from the table, all materials tested were positive. The average survival period of 8.7 days for guinea pigs receiving excretory

**Table 8.—*Occurrence of* P. tularensis *in Various Tissues, Excretions, and Mouth Washings Taken Immediately After Death From an Experimentally Infected Muskrat and Beaver***

| Source of inocula → | Muskrat | | | | Beaver | | | |
|---|---|---|---|---|---|---|---|---|
| Method of inoculation → | Subcutaneously | | Intraperitoneally | | All inoculations intraperitoneally | | | |
| Guinea pig number → | 1 | 2 | 1 | 2 | 1 | 2 | 3 | 4 |
| Days to death of test guinea pigs inoculated with: | | | | | | | | |
| Heart blood, 1 ml | 8 | 7 | 4 | 4 | 4 | 3 | -------- | -------- |
| Bone marrow, 1 ml. ss [1] | 6 | 5 | 4 | 4 | 3 | 3 | -------- | -------- |
| Liver, 1 ml. ss [1] | 8 | 5 | 3 | 3 | 5 | 5 | -------- | -------- |
| Spleen, 1 ml. ss [1] | 4 | 4 | 2 | 1 | 5 | 3 | -------- | -------- |
| Thoracic muscle, 1 ml. ss [1] | 9 | 8 | 8 | 7 | 5 | 5 | -------- | -------- |
| Subcutaneous tissue, 1 ml. ss [1] | 9 | 9 | 8 | 6 | 5 | 5 | -------- | -------- |
| Inguinal node, 1 ml. ss [1] | 8 | 7 | 5 | 3 | 7 | 7 | -------- | -------- |
| Brain, 1 ml. ss [1] | 8 | 8 | 7 | 4 | 6 | 6 | -------- | -------- |
| Hide scrapings, 1 ml. ss [1] | 9 | 8 | 7 | 6 | 10 | 5 | -------- | -------- |
| Mouth washing, 1 ml. ss [1] | 7 | 6 | 4 | 3 | 5 | 5 | -------- | -------- |
| Urine, muskrat: 0.1 ml; beaver: 1.0 ml | 12 | 8 | 12 | 9 | 8 | 7 | 8 | 7 |
| Feces from large intestine, 1 ml. ss [2] | 9 | 8 | 6 | 5 | 10 | 8 | 10 | 7 |
| | All intraperitoneally | | | | | | | |
| Feces last deposited before death 1, 2, 3, and 4 ml.[2] | 10 | 12 | 9 | 9 | (3) | 8 | 10 | 10 |

[1] ss=saline suspension.
[2] 5 grams feces were suspended in 5 ml. of saline.
[3] Died of intercurrent infection.

All the tissues used in the beaver experiment were tested culturally. *P. tularensis* was isolated only from the heart blood and bone marrow.

materials compared with 5.6 days for tissue-inoculated test animals is of interest but there was no basis for comparing the relative number or virulence of the organisms in the tissue and excretory materials tested. The mouth washings caused the death of guinea pigs in about the same time as the tissue materials.

*Infectiousness of the urine of mice, muskrats, and beavers.* Six field mice were inoculated by way of the scarified skin with a culture of *P. tularensis* isolated from water. One died on the sixth day post inoculation, two died on the seventh, two on the eighth, and one was sacrificed on the eighth day. The bladders of two of the mice that died yielded 0.2 ml. of urine; those of the other four contained no urine and each was washed with 0.5 ml. of salt solution. The material from each mouse was inoculated intraperitoneally into two guinea pigs. All 12 test animals died of tularemia.

One muskrat was inoculated by way of the scarified skin with a strain of *P. tularensis* isolated from water, and another was inoculated subcutaneously with 0.5 ml. of a suspension of a culture of a strain isolated from a snowshoe rabbit.

The muskrat inoculated by way of the scarified skin died on the eighth day after inoculation and *P. tularensis* was recovered from the spleen. Two guinea pigs similarly inoculated died on the eighth and ninth days, respectively. The muskrat urine was tested each day from the third to the seventh days by intraperitoneal injection into four guinea pigs. The amount of urine injected was 2 to 5 ml. None of these animals became infected. After the muskrat died, four guinea pigs were each injected intraperitoneally with 2 ml. of bladder washings. One died on the eighth day and three on the ninth day.

In the second test the muskrat died on the second day and *P. tularensis* was recovered from the spleen. Urine was collected the first day after inoculation and of the two guinea pigs that were injected with 1 ml. each, one died of peritonitis on the second day and the other remained well. After death of the muskrat, each of two guinea pigs received 1 ml. of bladder washings inoculated subcutaneously and intraperitoneally, respectively. One animal died of tularemia, the other remained well.

A beaver was inoculated by way of the scarified skin with a culture from a patient ill of tularemia. A urine sample was obtained 3 days later and two guinea pigs each received 1.5 ml. subcutaneously and two others each received the same amount intraperitoneally. These animals remained well. The beaver died of tularemia on the fourth day after inoculation. Four guinea pigs were each inoculated intraperitoneally with 1 ml. of urine obtained from the bladder and all four animals died of tularemia.

In summary, urine samples collected daily from the third to the seventh day from a muskrat ill with tularemia (died the eighth day after inoculation), a urine sample collected on the first day after inoculation from a muskrat that died on the second day, and urine obtained from a beaver that died of tularemia on the fourth day after inoculation, were all noninfectious for guinea pigs. However, bladder washings taken from the muskrat carcasses, urine from the beaver carcass, and bladder washings from six field mice dead of tularemia were all infectious. The infectiousness of urine taken after death from another beaver and another muskrat has been reported above.

That the urine in the bladder of a beaver dead of tularemia may remain infectious at least 4 days after death of the animal has been reported by Jellison et al. (1942). This urine, when stored at 38° to 40° F., remained infectious for 31 days, the supply then being exhausted.

In view of the negative results with urine excreted naturally during life, there appeared to be at least a chance that the positive results obtained with urine or bladder washings that had been drawn from the bladder after death were the result of having contaminated the needle by passing it through the bladder tissue. This possibility suggested the following experiment. Four guinea pigs were used that had just died of tularemia and which had empty bladders. Each bladder was punctured with the needle of a syringe that contained 1 ml. of sterile salt solution. The needle was then used to inject four successive guinea pigs intraperitoneally with ¼ ml. each. In three instances the first guinea pig injected died of tularemia and in one instance the first and second animal died. The other guinea pigs remained well. These results showed definitely that a needle contaminated by passing it through the bladder tissue of an animal dead of tularemia would infect one and sometimes two of a series of guinea pigs but not more. This finding appears to justify the conclusion that the urine and/or bladder washings taken after death from the field mice, muskrats, and the beavers were infectious.

*Determination of the period before death during which the feces of guinea pigs are infectious.*—Fecal pellets were collected daily from four separately caged guinea pigs inoculated subcutaneously with *P. tularensis*. One of these animals died of tularemia on the fourth day after inoculation and three on the fifth. The feces were tested by triturating two pellets in 4 ml. of salt solution and injecting each of two guinea pigs subcutaneously with half of the suspension. The pellets obtained during the first three days proved to be negative, while those passed on the fourth day were positive.

The intestinal contents of eight sacrificed guinea pigs which had been inoculated with *P. tularensis* subcutaneously were tested as above

except that a suspension of 0.5 ml. in 2 ml. of saline was used as the test inoculum. Two of the eight guinea pigs were sacrificed daily for 4 days. The intestinal contents on the first 2 days were negative while those on the third and fourth days were positive.

The results of the experiment indicate that in guinea pigs ill with tularemia, the contents of the large intestine may be infectious for at least 2 days before death and that infectious feces may be passed for at least 1 day before death. That the organism may also be present in the contents of the large intestine of beavers and muskrats immediately after death from tularemia, and in the last feces of these animals deposited before death, is demonstrated in an experiment above.

*Persistence of* P. tularensis *in the tissues and excretions in carcasses of guinea pigs exposed on the ground.*—The possibility that the decomposing carcasses of animals dead of tularemia might result in contamination of the soil and runoff waters was investigated. An experiment designed to explore this possibility was carried out in the winter and early spring because it is during this period that early run-off resulting from spring rains and melting snow takes place, and because of evidence suggesting that contamination of natural waters is more marked during the cooler months.

The carcasses of 18 guinea pigs that had been sacrificed while moribund from tularemia were placed on the ground February 22, 1944. Except for a covering of 12 x 12 mesh wire cloth, they were fully exposed to the varying meteorological conditions. Various tissues, feces from the large intestine, urine or bladder washings and mouth washings from two guinea pigs were tested for viable *P. tularensis* each week for 7 weeks. Each material was tested in two guinea pigs injected subcutaneously.

Decomposition was not sufficiently advanced that it appeared worth while to test soil samples from beneath the carcasses until the end of the seventh week. Tests made the seventh, eighth, and ninth weeks were negative. This result was to be expected, since the tests of carcass materials were negative by the seventh week. For test use, four samples of soil, totaling about 2 tablespoonfuls were taken from beneath each carcass. This soil was suspended in 50 ml. of tap water, thoroughly shaken, allowed to settle, and four guinea pigs each received 10 ml. of the supernatant water intraperitoneally.

The test data for the materials from the carcasses are given in table 9.

The data show that there was general survival of *P. tularensis* in all the carcass materials tested for at least 3 weeks but for less than 4 weeks. By the end of the fourth week the bacterium was absent from the mouth washings and liver, and by the end of the sixth week

Table 9.—*Tests Data on Persistence* of P. tularensis *in Tissues and Excretions From 18 Guinea Pig Carcasses Exposed on the Ground. Materials From 2 Carcasses Were Tested Each Week*

(+1=positive test from one carcass, +2=positive tests from both carcasses, —=negative tests from both carcasses)

| Number of weeks carcasses were exposed | Materials tested and results | | | | | | | | Meteorological data for the week immediately preceding each test | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Temperature | | | Precipitation | |
| | Spleen | Liver | Bone marrow | Feces | Muscle | Hide scrapings | Urine [1] | Mouth washings | Mean | High | Low | Inches, rain | Inches, snow |
| | | | | | | | | | °F. | °F. | °F. | | |
| 1 | +2 | +2 | +2 | +2 | +2 | +2 | +2 | +2 | 31.9 | 48.0 | 15.0 | 0.12 | 1.5 |
| 2 | +2 | +2 | +2 | +2 | +2 | +2 | +2 | +1 | 31.6 | 45.0 | 13.0 | .21 | 1.5 |
| 3 | +2 | +2 | +2 | +2 | +2 | +2 | +2 | +1 | 33.0 | 58.0 | 1.0 | Trace | Trace |
| 4 | +1 | (—) | +2 | +2 | +2 | +2 | +1 | (—) | 36.6 | 53.0 | 1.0 | .03 | 0 |
| 5 | (—) | (—) | (—) | +2 | +1 | +2 | (—) | (—) | 31.6 | 55.0 | 2.0 | .39 | .5 |
| 6 | (—) | (—) | (—) | (—) | +1 | (—) | (—) | (—) | 48.3 | 75.0 | 24.0 | 0 | 0 |
| 7 | (—) | (—) | (—) | (—) | (—) | (—) | (—) | (—) | 45.1 | 72.0 | 28.0 | .29 | 0 |
| 8 | ------ | ------ | ------ | ------ | ------ | ------ | ------ | ------ | 42.3 | 60.0 | 28.0 | .08 | 0 |
| 9 | ------ | ------ | ------ | ------ | ------ | ------ | ------ | ------ | 44.5 | 69.0 | 27.0 | .65 | 0 |

[1] Urine was used for the 1- and 3-week tests, and from 1 of the carcasses used for the 5-week test. Bladder washings were used for all other tests.

it was recovered only from hide scrapings of one guinea pig. The survival period of guinea pigs injected with 5-week-old material was only about 1 day longer than that of guinea pigs that received the 1-week-old material.

The results suggest that it is unlikely that under conditions similiar to those of this experiment, animal carcasses near streams play any consequential role in the contamination of natural waters.

*Persistence of* P. tularensis *in the tissues of guinea pig carcasses (a) submerged in water, (b) submerged in water and mud, and (c) the occurrence of the bacterium in the water-mud medium.*—These experiments were designed to obtain information concerning the extent to which the carcasses of submerged animals that had died of tularemia may contaminate the surrounding water or mud with *P. tularensis.*

Thirteen carcasses of guinea pigs that had just died of tularemia were used for each of two experiments. Each carcass was placed in a separate galvanized metal container 14 inches in diameter and 24 inches deep. Tissues of one carcass and samples of the water or water and mud in which the carcass was submerged were tested on the first, fifth, tenth, fifteenth, twentieth, twenty-fifth, thirtieth, thirty-fifth, fortieth, forty-fifth, fiftieth, fifty-fifth, and seventy-fifth days. The tissue materials tested were spleen and liver, thoracic muscle, subcutaneous tissue scrapings from the hide, and bone marrow. Two

guinea pigs were used to test each material. The water used was from a beaver pond and the mud from a marshy area. Both were proved to be free of contamination with *P. tularensis*. The containers were kept out of doors under a roofed-over shed with side walls of wire cloth.

In the experiment using water only, the water was 12 inches deep. The water in each container was thoroughly mixed before a sample was taken for testing. It was tested by injecting each of two guinea pigs intraperitoneally with 10 ml. This experiment was initiated on March 16, 1942.

In the experiment in which the carcasses were under both mud and water, the mud was 12 inches deep and covered by 4 inches of water. The carcass was placed 4 inches deep in the mud. Four guinea pigs were used to test the water and four to test the mud. The water was not agitated before the sample was taken. Tests of the mud were made by injecting each quinea pig with 10 ml. of the washings (sterile saline solution was used) of 25 grams of pooled mud taken from 4 points of contact with the carcass. This experiment was started December 6, 1942.

The results of the two experiments are given in table 10.

**Table 10.—*Results of Tests of Tissues From Tularemia-infected Guinea Pig Carcasses That Had Been Submerged in Water From 1 to 75 Days, and Results of Tests of Water and Mud in Which the Carcasses Were Submerged***

(+=positive result; —=negative result. The sign to the left of the virgule is for the water test, that to the right is for the mud and water tests)

| Materials tested | Days materials were tested and results | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 50 | 55 | 75 |
| Water | + | + | + | — | — | — | — | — | — | — | — | — | — |
| Mud and water | —/+ | —/— | —/— | —/— | —/— | —/— | —/— | —/— | —/— | —/— | —/— | —/— | —/— |
| Spleen and liver | +/+ | +/+ | +/+ | +/+ | —/+ | +/— | +/+ | —/— | —/— | +/— | —/— | —/— | —/— |
| Hide scrapings | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | —/+ | —/+ | +/— | +/+ | —/— | —/— |
| Subcutaneous tissue | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/— | +/+ | —/+ | —/— | +/+ | —/— | —/— |
| Thoracic muscle | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | —/+ | +/+ | —/— | +/+ | —/— | —/— |
| Bone marrow | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/— | —/+ | —/— | +/+ | —/— | —/— |

The results indicate (*a*) that under the conditions of the experiments the carcass of an animal that has died of tularemia may contaminate with *P. tularensis* the water in which it is lying for a period of at least 10 days but not for 15 days, and (*b*) that if the carcass is submerged in mud overlain with water there is no consequential contamination of the mud and none of the overlying water.

## CONTAMINATED WATER AS A POSSIBLE SOURCE OF INFECTION FOR ANIMALS

*Amount of naturally contaminated water infectious for guinea pigs by intraperitoneal inoculation.*—An indication of the minimum number of tularemia organisms in naturally contaminated water that will cause infection in animals was obtained by the intraperitoneal inoculation of guinea pigs with decreasing amounts of water from Cattail Creek station 74. This single positive sample proved infectious for the animals in the amounts of 10, 5, 1.0, 0.1, and 0.01 ml.; but 0.001 ml. was not infectious.

Although no general conclusion can be drawn from the titration of this single water sample, the results do indicate that natural waters may contain sufficient tularemia bacteria to produce fatal infections in animals inoculated intraperitoneally with 0.01 ml. amounts. If a single bacterium were infectious this would mean that the particular water sample tested contained a minimum of 100 bacteria per ml.

*Number of organisms required to infect guinea pigs by intraperitoneal inoculation.*—Centesimal dilutions to 1:10,000,000,000 were made of an aqueous suspension of a strain of *P. tularensis* isolated from water. The stock suspensions and the five dilutions were each tested in four guinea pigs, each of which received 1 ml. subcutaneously. The animals receiving the stock suspension and the dilutions up to and including 1:1,000,000 all died of tularemia; those receiving the higher dilutions remained well. One-half and one-quarter ml. amounts of each suspension were also used for plate cultures. The colonies from the stock suspension and from the 1:100 dilution were too numerous to count. The average counts of the plates that received the 1:10,000 dilution indicated that this suspension contained a minimum of 345 bacteria per ml. There were no colonies on the plates that received the 1:1,000,000 dilution, although the guinea pigs that received this suspension died of tularemia.

The colony count for the 1:10,000 suspension indicates that each of the guinea pigs that were inoculated with the 1:1,000,000 suspension received a minimum of 3.45 bacteria. This suggests that a total of perhaps five to seven bacteria of the particular strain of *P. tularensis* used was sufficient to cause infection. However, the number that would have caused infection might actually have been smaller since the highest infectious dilution of the original suspension presumably was somewhere between 1:1,000,000 and 1:100,000,000.

*Infectiousness of naturally contaminated water when ingested by field mice, muskrats, beavers, and guinea pigs.*—In the following experiments, naturally contaminated water was provided for field mice in bottles fitted with glass tubes, and for beavers (except as noted below) and muskrats in bowls. This water was from various sta-

tions in the Cattail Creek area. In some of the tests with muskrats and beavers fresh contaminated water was supplied every 2 or 3 days. In tests in which the water was not renewed it is possible that it remained contaminated for at least several days.

Fifteen field mice were segregated in groups of five and each group was supplied with naturally contaminated water collected on November 24, 1942, from stations 1, 3, and 10. These samples were found to be positive by the usual test method, +3, +4, and +4, respectively. None of the mice died. They were sacrificed at intervals of from 8 to 19 days and tissue transfers made to two guinea pigs from each mouse. There were no gross lesions suggestive of tularemia in any of the mice, and none of the guinea pigs became ill. Two similar subsequent tests were valueless because one water sample used proved to be completely negative, while the other sample infected only one of the four test guinea pigs.

Two similar tests were made using tap water that was purposely heavily contaminated artificially. In each test 1 pint of water was contaminated with an entire plate culture of *P. tularensis*. Two strains isolated from water were used. In one test three mice died on the second day from some unknown cause. *P. tularensis* was not recovered from their tissues. The fourth mouse died on the fourth day and *P. tularensis* was recovered. The fifth mouse survived. In the other test all five mice died of tularemia, four on the fifth day and one on the seventh.

On March 24, 1943, a muskrat was given water for drinking that had been collected that same date from station 3. By the usual test this water was +3. The muskrat remained well. On May 3 it was given 8 ml. of artificially contaminated water by mouth by means of a pipette. It died 5 days later and *P. tularensis* was recovered from the spleen. Mouth washings taken after death were fatal for four test guinea pigs (each received 1 ml. intraperitoneally). One died on the sixth day, three on the ninth.

In another test 5 ml. of artificially contaminated water (culture was isolated from water) were given orally and the same water provided for drinking. This muskrat died on the sixth day and *P. tularensis* was recovered from the spleen. Of four guinea pigs that received mouth washings, two died of tularemia on the fourth day and two on the fifth day. Two others that received 1 ml. each of bladder washings (2 ml. of salt solution were injected into the bladder by syringe and immediately withdrawn) died of tularemia on the sixth day.

A supply of naturally contaminated drinking water from station 19 was provided a caged beaver. A month later the beaver was etherized and given orally, by pipette, 10 ml. of artificially contami-

nated water. It died of tularemia 4 days later. Four control guinea pigs that each received 0.05 ml. of this water subcutaneously died of tularemia on the fourth, fifth, sixth, and sixth days, respectively.

At various times in 1942, nine tests were made to determine the infectiousness of naturally contaminated water when given orally to guinea pigs. In six of these tests the contaminated water was given by pipette and in three it was made accessible in bottles.

In the six tests in which the water was given by pipette, five of the water samples were +4 and one was +3. Three samples were used the day of collection; one was used on the seventh day, and two on the fourteenth day after collection. The stored samples had been held at 46° F. Of each sample, four guinea pigs received 1, 2, 4, and 6 ml. respectively. Three of 24 test animals died of tularemia, 18 remained well, 2 died of intercurrent infections and the record of 1 animal was lost. Two of the tularemia infections occurred among the guinea pigs that received water (+3) from station 3 that had been stored for 14 days; the one that received 2 ml. died on the tenth day, and the one that received 4 ml. died on the eleventh day. The one that received 6 ml. of the same sample was the one for which the record was lost. The third tularemia infection was in a guinea pig that received 6 ml. of water (+4) just collected from station 10. This animal died on the twelfth day. The water samples from stations 3 and 10, as tested by injection intraperitoneally, caused the deaths of the test guinea pigs in 7, 8, and 8 and in 7, 7, 8, and 8 days, respectively.

In the three tests in which the contaminated water was made accessible in bottles, two of the water samples used were +4, the third was +3. The water was used as soon as collected. No infections resulted.

From the foregoing data it may be seen that of 15 field mice, 1 muskrat and 1 beaver given drinking water that was naturally contaminated with *P. tularensis* none became infected with tularemia, but animals of all three species did die of tularemia when water heavily contaminated artificially was used.

In 9 tests with guinea pigs, using naturally contaminated water, 3 of 24 animals that received the water by pipette died of tularemia but none of 12 that received the contaminated water provided in bottles. The 3 guinea pigs that died had received 2, 4, and 6 ml. of water, respectively, while animals that received 1 ml. were not affected. However, since numerous other guinea pigs that received 2, 4, and 6 ml. amounts of water samples that were equally as heavily contaminated (as far as the method used for testing for contamination permits a conclusion) did not become ill, it is not apparent whether it was the number or the relative virulence of the ingested bacteria that determined whether or not infection would result.

The results of the several tests with the various animals do not

justify a conclusion concerning the possibility that individuals of the three native species can become infected by ingesting naturally contaminated water, but they do suggest that it is by no means unlikely the infection could be contracted in this manner given the proper conditions.

*Infectiousness of naturally contaminated water for frogs, rainbow trout, and turtles.*—In 1942, several frogs, fish, and turtles were confined in wire cloth cages (2 by 2 mesh) 12 by 18 by 12 inches set several inches deep in water at the various Cattail Creek stations noted below. The cages were not in contact with the underlying mud. The animals were sacrificed at intervals and the tissues of each individual were segregated into two pools, one containing pieces of spleen, liver, heart, and kidney, the other pieces of lung and intestines (intestine only of trout). Pieces of intestines 1 to 1½ inches long were used from frogs and turtles; the whole intestine from the trout. Naturally some portion of the intestinal contents was used.

On June 29, 14 frogs (*Rana pretiosa pretiosa*) were placed in the water at station 48 on the effluent stream from the south marsh. A frog was sacrificed on the fourth day (July 3) and one every 2 or 3 days thereafter through the thirty-sixth day (August 4). All tests of the tissues were negative.

Water from the station was tested nine times during the course of the experiment. It was negative on July 3, 7, 10, 17, and 31. It was +2, +1, +3, and +4 on July 14, 21, 24, and 28, respectively.

On June 24, 24 rainbow trout (*Salmo gairdnerii*) 5 to 6 inches long, were placed at station 9 on the effluent stream from the south marsh and about 300 feet downstream from station 48. Beginning June 27, two trout were sacrificed each 2 or 3 days through July 28. All tests of materials of the trout sacrificed on the fourteenth day were negative except that of the air sac and intestine and its contents. One of the four guinea pigs that received this material died of tularemia on the twenty-second day after injection.

Tests of water samples taken from this station on June 23 and 30 and on July 7, 21, and 28 were negative; one taken on July 14, the same day the trout mentioned in the last paragraph was sacrificed, was +1, the one infected guinea pig dying of tularemia on the twenty-first day after injection.

The possibility that naturally contaminated water might be infectious for turtles was investigated by keeping six caged turtles (*Chrysemys picta bellii*) in water at three Gird Creek area stations. Individual animals were sacrificed at various times from the fourteenth to the fiftieth day thereafter and their tissues tested by guinea pig inoculation for evidence of infection. All of the tests were negative. Data relative to this experiment are presented in table 11.

Table 11.—*Tests of Tissues From Turtles, Caged for Varying Periods in Water at Gird Creek Area Stations, and Results of Concurrent Water Tests*

(—=negative tests; 0=no tests)

| Station | Turtle No. Inclusive dates of exposure | Duration of exposure (days) | Results of tissue tests | Results of concurrent water tests | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 6-30 | 7-3 | 7-7 | 7-14 | 7-17 | 7-21 | 7-24 | 7-28 | 7-31 | 8-4 | 8-11 | 8-18 | 8-25 | 9-2 |
| 3 | 1 (6-29 to 7-13) | 14 | (—) | (—) | +1 | (—) | +4 | | | | | | | | | | |
| 3 | 5 (7-13 to 8-10) | 28 | (—) | | | | +4 | +4 | +4 | 0 | +2 | 0 | (—) | +3 | | | |
| 3 | 6 (7-13 to 9-1) | 50 | (—) | | | | +4 | +4 | +4 | 0 | +2 | 0 | (—) | +3 | +1 | +3 | +1 |
| 10 | 2 (7-3 to 7-17) | 14 | (—) | +4 | 0 | +1 | +4 | | | | | | | | | | |
| 21 | 3 (7-3 to 7-24) | 21 | (—) | +1 | 0 | (—) | (—) | 0 | (—) | (—) | | | | | | | |
| 21 | 4 (7-3 to 7-31) | 28 | (—) | +1 | 0 | (—) | (—) | 0 | (—) | (—) | +2 | +1 | | | | | |

While it is unfortunate that these experiments with frogs, trout and turtles were initiated during a period in which, as later became apparent, the contamination of the water at the stations concerned was at a low ebb, the data at least suggest that these animals are not likely to become infected with tularemia as a result of frequenting water in which contamination with *P. tularensis* is no more marked than it was at the respective stations concerned.

The recovery of *P. tularensis* from the intestine and its contents and not from other tissues of a trout, suggests that the bacterium was likely present in ingested water rather than in the intestinal tissue. This possibility has some support in the fact that the test guinea pig concerned died on the twenty-second day after injection, while the guinea pig that died of tularemia after injection with water taken at station 10 on the same day that the trout was sacrificed succumbed on the twenty-first day. Furthermore, Morgan (1947) was unable to infect rainbow trout and five other species of fish by intraperitoneal injection was 1 ml. of a 48-hour culture of the organism.

## MISCELLANEOUS EXPERIMENTS

*Persistence of* P. tularensis *in water and mud stored at 7° C. in the laboratory.—Naturally contaminated water.*—Three tests using naturally contaminated water were made with water samples stored in a cold room. In no instance was the elapsed time between collection and refrigeration of the sample more than 5 hours.

The first test was made June 24, 1942, using 14 consecutive samples collected at weekly intervals (March 17 to June 23) from station 1. The last sample was refrigerated for 1 day, the first for 99 days. This test was made at an unfortunate time. While the samples through June 3 had been positive (+2, +3, or +4) at the time of collection the last three (June 10, 17 and 23) were negative. Samples taken after June 23 were again consistently positive. So, although all the stored samples were found negative, the findings for the 1-, 8-, and 15-day samples were without significance.

The second test was made August 27 using 22 consecutive weekly samples (March 17 to August 25) from station 3. When originally tested these samples were positive (+1, +2, +3, or +4) from March 17 through June 10, negative June 16 to July 7, positive (+2 or +4) July 14 to July 28, negative August 1, and positive (+3, +1, and +3) August 11 to August 25. The retests were completely negative, even for the 2-day sample.

The third test was made with samples collected December 23, 1942, from stations 1, 3, and 10. The samples were positive when collected; +3, +4, and +4, respectively. They were retested on the

twenty-third (January 15, 1943) and thirty-sixth days (January 28). The first retest was positive for each; +3, +4, and +2; the second retest was negative. This test showed survival of *P. tularensis* in naturally contaminated water for at least 23 days but not more than 35 days.

Unfortunately, the fact that the three most recently collected samples used in the first test were negative on the original test, made the results with the three samples collected before the twenty-ninth day without significance.

However, in the second test failure to survive was shown in four samples that had been stored for only 2, 9, 16, and 23 days; i. e., for the same period during which the samples used in the third test remained positive. No definite reason can be given for the difference of results in these two tests. It is possibly significant that the samples used in the second test were collected in the summer while those for the third test were collected in the winter.

*Naturally contaminated mud.*—Mud was collected April 7, 1942, from stations 1, 3, and 11 in the Cattail Creek area. That from 1 and 11 was stored at 7° C. in cylindrical jars 10 inches in diameter and 18 inches deep with the mud 12 inches deep. That from 3 was placed in a 6-inch by 9-inch battery jar with the mud 6 inches deep. After the initial test, top and bottom samples from each jar were tested separately in most instances. The results of the tests are presented in table 12. The numerator and denominator of each fraction show

**Table 12.—*Persistence of* P. tularensis *in Naturally Contaminated Mud Stored at 7° C.***

| Station | Original test Apr. 7 | Weeks | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 |
| 1 | 4 | 2 | 0/3 | 0/0 | 0/0 | 0/0 | 0 | 0 | 0 | -------- |
| 3 | 4 | 2/4 | 1/4 | 0/2 | 1/3 | 0/0 | 0 | 0/1 | 0 | 0/0 |
| 11 | 3 | 2 | 0/1 | 0/0 | 0/0 | 0/2 | 0 | 0 | -------- | -------- |

the number of the four test guinea pigs that were positive for top and bottom sample, respectively. If only one thoroughly stirred sample was tested, a whole number denotes the number of positive animals. If no test was made, the space is blank.

Persistence of *P. tularensis* was demonstrated for minimum periods of 4, 14, and 10 weeks, respectively. However, the fact that the bacterium was not recovered from the station 3 sample in the tenth- and twelfth-week tests but was recovered in that for the fourteenth week, and that there was a similar lapse in the recovery of the organism

from station 11 from the fourth to the tenth week, indicates that the technique did not give completely reliable negative results. This suggests that survival may have been for longer than the demonstrated periods.

It is of interest to note in the 16 positive tests in which both top and bottom mud were used that 20 of the 60 guinea pigs that received bottom mud became infected but only 4 of the 60 that received top mud; also that if only top mud had been tested, only 3 (all of station 3 mud) of these tests would have been positive instead of 8. It is not apparent whether this result was due entirely to chance or in part, at least, to some factor of possible significance in relation to these studies.

It is also of interest to note that *P. tularensis* inoculated into "normal" mud persisted in this medium for 7 but not for 14 days.

*Persistence of* P. tularensis *in naturally contaminated water and mud contained within a casing set in one of the Cattail Creek marshes.*—On December 15, 1942, a steel casing 23 inches in diameter and 35 inches high (made from a barrel from which both top and bottom had been removed) was forced down through the mud near station 3 till the bottom edge was in solid ground. When in place it extended 23 inches above the water level. The water overlying the mud inside was 4 inches deep. Water and mud samples were taken before the casing was put in place and thereafter from within the casing at varying intervals up to 108 days. The test data are given in table 13 together with the results of tests of mud and water samples taken at station 3 on the same dates. Blank spaces indicate negative results.

**Table 13.—*Persistence of* P. tularensis *in Naturally Contaminated Water and Mud Contained Within a Casing Set in One of the Cattail Creek Marshes***

| Date sample taken | Number of days | Water | | Mud | |
|---|---|---|---|---|---|
| | | Casing | Station 3 | Casing | Station 3 |
| Dec. 15 | 0 | +3 | +4 | +4 | +2 |
| Dec. 23 | 8 | | +4 | +4 | +2 |
| Dec. 30 | 15 | | +4 | +4 | +3 |
| Jan. 13 | 29 | | +4 | [1] +1 | +2 |
| Jan. 28 | 44 | | +3 | +1 | +4 |
| Feb. 10 | 57 | | | | +2 |
| Feb. 25 | 72 | | | | +1 |
| Mar. 11 | 86 | | +1 | | +1 |
| Mar. 24 | 99 | | +3 | | +1 |
| Apr. 2 | 108 | | +4 | | +1 |

[1] 2 test guinea pigs died from peritonitis.

Under the conditions of the experiment, the water and mud within the casing were isolated from contact with the slowly moving water and the mud of the marsh.

It is not apparent why, under these conditions, the water within the casing should have remained contaminated for less than 8 days, whereas the mud remained contaminated for at least 44 days but for less than 57 days (contamination was less marked after 15 days). Furthermore, these results were in marked contrast to those for station 3 where the slow-moving water and the mud were apparently continuously contaminated (except the water during February) throughout the entire 108-day period of the experiment.

A repetition of this experiment on a more comprehensive basis was planned because of the possibility that a knowledge of the factors responsible for the disappearance of *P. tularensis* from the water and mud within the casing would help in solving some of the problems with which this paper is concerned. However, the disappearance of contamination from the water and mud of the marsh prevented further studies of this nature.

*Persistence of* P. tularensis *in ice.*—Ice formed from naturally contaminated water. During early 1943 ice samples from five stations (1, 6, 9, 10, 44) in the Cattail Creek area were tested to determine whether or not they contained viable *P. tularensis.* These samples were melted and were tested in the same manner as water samples.

Ice samples from two (6 and 10) of the six stations were positive and in each instance resulted in the death of two of the test guinea pigs. One of the samples contained mud, and one was clear. For two stations (10 and 44) from which the water and ice samples were taken on the same dates, both water and ice were positive for the one and only the water for the other.

These data clearly indicate that ice formed from water contaminated naturally with *P. tularensis* may contain viable bacteria.

Ice formed from artificially contaminated water. In early March 1943, water from an uncontaminated stream was contaminated with a plate culture of a strain of *P. tularensis* isolated from mud. A shallow container holding several inches of this water was placed out-of-doors. At the end of 24 hours an inch of ice had formed. This was removed and stored in an electric refrigerator. The freshly contaminated water, the newly formed ice, and the water beneath it were each tested in four guinea pigs each of which received 10 ml. of water or of melted ice intraperitoneally. Samples of the stored ice were similarly tested on the third, eighth, twelfth, fifteenth, twenty-first, twenty-eighth, and thirty-fifth days.

The data obtained showed that under the conditions of the experiment *P. tularensis* survived in ice formed from artificially contam-

inated water for a period of not less than 12 days nor more than 14 days with a progressive loss of infectiousness.

*Tests of plants growing in naturally contaminated water.*—Moss and algae, mixed. Five samples of mixed moss and algae were tested from stations 3 and 94 during the winter and early spring of 1943. Concurrent tests of the station waters were positive. The test material was washed five times in sterile saline, then triturated in the same medium. Each resultant suspension was injected into four guinea pigs which received 1, 2, 3, and 4 ml., respectively. Most of the test animals died of peritonitis, Two animals, each of which received 3 ml. injections representing two samples from station 94, died of tularemia, one on the seventh day, the other on the eleventh day.

It is impossible to be certain whether infection was caused by *P. tularensis* on the surface of the plants or within the plant tissues.

*Cattails.*—The tissues of three cattail plants, *Typha* sp., taken December 10, 1942, in the Cattail Creek area (two from station 1 and one from station 3) were tested for the possible presence of *P. tularensis.* The surface of the plant was cleansed by washing it thoroughly in tap water, then in sterile saline. Tissue samples from the main portion of the root, from sprouts, and from the base of the stem were tested from each plant. The tissues for test use were cut from each plant and each piece, averaging ½ inch wide and 1 inch long, was triturated in saline and four guinea pigs each received intraperitoneally 5 ml. of the resultant suspension. All tests were negative.

Mud samples collected at the same time as the plant were +3 for station 1 and negative for station 3.

*Test of mud for −SH compounds.*—It has been fairly well established that *P. tularensis* needs substances containing potential or free −SH groups, notably cystine, for its growth on culture media. Since it seemed possible that the bacterium might be living in the marsh mud, samples of this mud were tested for some of these compounds. The Unna-Golodetz (1911) test for loosely bound sulphur was used to determine the presence of these substances. The reaction of this test is such that protein solutions in lye (NaOH), after the addition of a lead or bismuth salt (acetate), yield a brown to black color and the precipitation of lead sulfide in the presence of sulphur.

Several samples of mud and water from Cattail Creek stations 1, 3, and 10 were tested. Cystine and hair were used as controls. The results were uniform and were as follows: (1) All water samples gave negative tests for the presence of substances containing loosely bound sulphur; (2) all mud samples gave strongly positive tests; (3) all supernatant water completely separated from the mud samples by centrifuging gave negative tests; (4) tests of supernatant water from

which the mud had been only partially separated gave corresponding degrees of positiveness.

The results of this experiment indicate that if the Unna-Golodetz test is an acceptable one for the presence of cystine and other −SH compounds, the mud, but not the water taken from the marshes in the Cattail Creek area, contains appreciable amounts of these substances. Since the mud contained large amounts of decaying organic matter these results were to be expected.

## DISCUSSION

The localities and specific streams in the Northwestern United States in which either beavers or muskrats or both are known to have died during the period covered by this report lie within an area which includes central and western Montana, northern Wyoming, southern Idaho, northern Utah, and most of Oregon. That tularemia was at least partially responsible for the fatalities in Montana, Idaho, Wyoming, Utah, and the Klamath Lake region of Oregon is indicated by the recovery of *P. tularensis* from dead beavers or muskrats, and/or the occurrence of human cases resulting from skinning or handling one or the other of these animals trapped or found dead in the stream concerned. While beaver and muskrat fatalities have occurred over a large part of Oregon, infection has been recovered only from muskrats at Klamath Lake and human cases known to have resulted from contact with muskrats in Oregon have been reported from only that one portion of the State. It appears likely, however, that tularemia may have played some part in the animal fatalities reported from the 11 other Oregon counties from which no materials were tested.

That tularemia was largely responsible for deaths in beavers and muskrats in the region concerned is suggested by the fact that of materials from 28 beaver and 30 muskrat carcasses examined, *P. tularensis* has been recovered from the tissues of 15 of the former and 20 of the latter. No other infective agent was demonstrated in any of these animals.

The questions of chief interest brought up by the earlier studies of Jellison, Kohls, Butler, and Weaver (1942) and the present studies are (1) the source or sources from which semiaquatic animals acquire the infection, (2) the source of the contamination of stream water with *P. tularensis* and the factor or factors responsible for the persistence of the contamination for long periods, and (3) the degree to which contaminated water is a danger to man. None of these questions can be answered satisfactorily at the present time.

Jellison et al. (1942) expressed the opinion that infection in beavers is a "reflection of a concurrent epizootic among land-frequenting

animals living in proximity to affected streams" and that "some non-parasitic agency, water for example, may serve as a medium through which, or by which, infection can be conveyed from tularemia-infected land animals to water animals." This hypothesis appears to be the only tenable one on the basis of our present knowledge, but we still have no findings that will permit a conclusion as to the means of transfer of infection.

Results of tests to determine if infection in beavers and muskrats can result from ingestion of naturally contaminated water were inconclusive but they do suggest the possibility of infection being contracted in this manner. However, there are as yet no data to exclude means other than water as the possible bearer of the tularemia organism. Arthropod transmission is regarded as improbable because, in the region under consideration, beavers at least are not known to harbor blood-sucking ectoparasites and most of the fatalities occurred in the colder months when mosquitoes, deerflies and other biting insects are inactive.

Although our data indicate that land-frequenting animals can contaminate water with *P. tularensis* by means of their urine and carcasses, it is equally true that beavers and muskrats can do the same. Also, it is a possibility that land animals may become infected by drinking contaminated water. Although present information suggests that the primary guilt for contaminating natural waters lies with land-frequenting animals that live in close proximity to bodies of water and water courses, it nevertheless would be a case of the pot calling the kettle black to assume that this is always the case.

With respect to land-frequenting animals occupying habitats adjacent to affected bodies or streams of water as a possible initial source of water contamination and infection in beavers and muskrats, Jellison et al. (1942) reported that the only definite positive evidence of epizootic tularemia was in field mice. Six mouse carcasses were found at points along three streams where beavers and muskrats were known to have died of tularemia and *P. tularensis* was recovered from each. In the Gird Creek area we recovered *P. tularensis* from a single field mouse carcass found in April 1942 and from several field mice and shrews trapped during the ensuing months (Kohls and Steinhaus, 1943). Our observations revealed that field mice were abundant in the fall, winter, and early spring but were scarce in late spring and in summer.

The persistence of *P. tularensis* contamination of water and mud is an interesting phenomenon. Jellison et al. (1942) reported the persistence of contamination in a stagnant pond in the channel of the Musselshell River for a period of 31 days (samples taken November 30 to December 30, 1939). The next sample taken February 16,

1940, 48 days later, was negative. A mud sample collected from this pond on December 30 was positive and the sample was still positive when retested 31 days later. During the studies now reported *P. tularensis* persisted for months in both water and mud in the Gird Creek area in Montana and the Warm Spring Creek area in Idaho; in the former from March 1942 to July 1943 and in the latter from April 1942 to at least as late as February 1943. In the Gird Creek area the contamination seemed to become less marked during the late summer; samples from some stations proved noninfectious, those from others infected only one of the four test guinea pigs whereas earlier samples had infected all four, and infected animals lived longer. However, tests of autumn samples of both water and mud indicated that the contamination was at least as marked as during the spring and early summer. The Warm Spring Creek (Idaho) data also suggest that contamination was less marked during the late summer, though the test data do not afford as much evidence on which to base such an opinion. Whether the subsequent positive tests obtained in this and some other areas indicate persistence of contamination or reintroduction of the organism is impossible to state in view of infrequent testing during the intervening periods.

The factors responsible for the persistence of the contamination in water and mud of these areas are still obscure. As previously noted, there was no observed mammalian life in the Gird Creek area to suggest a continuing source of contamination from infected mammals. In fact, the contamination was so marked that, whatever its original source, it seems improbable that its persistence can be attributed to any factor or factors resident in land-frequenting animals. There seems to be no escaping the conviction that the factors governing persistence are resident in the water or mud or both. One hesitates to suggest the possibility of the multiplication of *P. tularensis* in a water-mud medium yet present information suggests such an hypothesis. This hypothesis is supported by the work of Gibby, Nicholes, Tamura, and Foshay (1948) who prepared extracts from mud samples furnished by Parker from the Gird Creek area. These extracts were reported to contain moderate amounts of substances having cultural properties similar to "blood cell extract" which supported excellent growth of the organism in simplified liquid media. The authors state:

> Hence, from a nutritional viewpoint there is little reason to doubt that *Bacterium tularense* could maintain itself in muds, and possibly even in waters, in the absence of any living host.

The question of the danger of human infection from the beaver-muskrat-water complex is an important one.

We have records of over 100 cases of tularemia in North America in which infection was contracted from skinning or handling infected beavers or muskrats. About 75 of these occurred in the Northwestern States and the remainder occurred elsewhere in the United States, and in Canada and Alaska. Infections contracted from muskrats have predominated in numbers by a ratio of about 5 to 1, which is not surprising in view of the relative abundance of the two animals and the opportunity for contact. The relative mildness of some cases resulting from beaver or muskrat contact, plus other factors, leads us to believe that the number of known cases attributable to these sources is only a fraction of the total number that have occurred.

Until recently there has been little evidence in this country of human infection resulting from stream water contaminated with *P. tularensis* either by ingestion or by way of the abraded or unabraded skin. At least 10 Montana cases in 1942 had initial throat lesions, but none could be definitely associated with water. Five of these were treated in one clinic, four of them being ill at the same time. There was reason to believe that three of the four cases concerned, all of which originated on a sheep ranch where tularemia is known to be quite prevalent, might have become infected by using water from a reservoir (dammed-up runoff water), but a detailed investigation failed to yield convincing evidence. In the Warm Spring Creek marsh area in Idaho, where three of four muskrat trappers became infected, the fourth escaped even though he had had his hands in contaminated water day after day. In the course of our studies of the Gird Creek area, one of us who has never had tularemia had contaminated mud and water on his hands hundreds of times without acquiring infection. Children seen playing in the creek when the water was known to be contaminated did not become ill. Yet, 2 ml. of the water from this stream given orally was capable of causing death in guinea pigs.

In 1950, however, Jellison, Epler, Kuhns, and Kohls reported the occurrence of four clinical cases of tularemia in Gallatin County, Mont., associated with one domestic rural water supply under circumstances that appeared to preclude other likely sources of infection. Three other individuals were probably infected from the same source but the evidence was less conclusive. All but one were previous residents of the ranch on which the water supply was located. The water supply concerned came from a marshy, spring area some 350 yards from the residence. It was fenced to keep out livestock and was grown over with water cress, weeds, and grass. There was evidence of field mouse and pocket gopher activity in proximity to the water source and there was abundant opportunity for contamination of the water by these rodents. A pipe fitted to a dam at the lower edge

of the marsh brought the water to a tank in the basement of the house where it was pumped into a pressure tank for distribution to the upper floors. Water from the kitchen tap was shown to be contaminated with *P. tularensis* on two consecutive tests. These tests were performed when a diagnosis of tularemia was made in two of the individuals currently using this water supply. Illness in these cases, as well as the two others definitely associated with this water supply, was characterized by severe and persistent sore throats. No history of illness could be elicited from the three other individuals but their agglutinin titers suggested infection with *P. tularensis* some years previously.

Concentrations of chlorine ordinarily used to treat water supplies apparently are adequate to kill or inactivate *P. tularensis* in naturally contaminated water. This was shown by Foote, Jellison, Steinhaus, and Kohls (1943), who treated the water of Cattail Creek at a point just below station 10. Two experiments were run, one on May 9, 1942, and one on February 19, 1943. In the first test the contaminated water was rendered harmless when treated to as low a dosage as to give a residual of 0.1 p. p. m. chlorine after 15 minutes contact. In the second experiment the various factors concerned (dosage, time, etc.) were better controlled and as much as 1.0 p. p. m. residual chlorine was found necessary to kill the organism in 15 minutes. However, 0.1 p. p. m. for 30 minutes did render the water noninfective for the test guinea pigs by injection. In general, it may be said that concentrations of chlorine which will kill the pathogenic bacteria ordinarily found in water will also render *P. tularensis* noninfective for guinea pigs.

## SUMMARY

In continuation of earlier reported investigations by the Rocky Mountain Laboratory, further studies of *P. tularensis* in natural waters and mud, and in muskrats and beavers, and of the relationship of the occurrence of the organism in such situations to the occurrence of tularemia in man were begun in the spring of 1942 and continued through 1950. The project consisted chiefly of (1) an intensive study of Gird Creek and one of its tributaries, Cattail Creek, near Hamilton, Mont.; (2) repeated studies of certain streams near Lewistown, Mont., and of a marshy area near May, Idaho; (3) attempts to isolate *P. tularensis* from various streams and from muskrats and beavers in Montana and Idaho; and (4) field and laboratory experiments directed primarily toward determining the role of animals in initiating and maintaining stream contamination, and to the possible role of contaminated water as a source of infection for animals living in and adjacent to such waters.

In general, the results and data gained from the field observations and laboratory experiments indicate that water and mud contamination, and the occurrence of tularemia in muskrats and beavers are widespread phenomena in the Northwestern States. Water and mud contamination may be present at any season of the year and may persist for at least 16 months. It is improbable that persistence of contamination can be attributed to factors resident in land-frequenting animals. Present information suggests that the factors governing persistence are resident in the water or mud or both, and suggests the hypothesis that the organism multiplies in the water-mud medium.

Over 100 North American human cases in which infection was contracted from skinning or handling infected muskrats or beavers are known. About 75 of these occurred in the Northwestern States and the remainder elsewhere in the United States and in Canada and Alaska. To date only seven cases in which the probable source of infection was contaminated water are known to have occurred in North America. All were attributed to a contaminated domestic rural water supply.

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