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A METHOD OF DETERMINING THE POLLUTION
OF SURFACE WATERS BY THE EGGS
OF ECHINOCOCCUS

TECHNICAL DOCUMENTARY REPORT AAL-TDR-63-37

July 1964

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ARCTIC AEROMEDICAL LABORATORY
 AEROSPACE MEDICAL DIVISION
 AIR FORCE SYSTEMS COMMAND
 FORT WAINWRIGHT, ALASKA

Project 8246. Task 8246-1

(Prepared under Cross Service Agreement CSA 61-1 by
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ABSTRACT

A continuous upward flow sedimentation unit was developed to test surface waters at remote U. S. Air Force radar stations in Alaska for the presence of eggs of Echinococcus. Chlorination employed at these water supplies is not an effective protection. Many animals in these areas are infected with the tapeworm, and man can serve as a host for the larval form. Diagnoses of echinococcosis in the native population caused concern by the Air Force for health of Air Force personnel. Lack of early disease symptoms and unreliability of clinical tests necessitate physical surveillance of water supplies. Laboratory tests validated methodology. One field test showed negative results. Further tests are recommended.

PUBLICATION REVIEW



HORACE F. DRURY
Director of Research

A METHOD OF DETERMINING THE POLLUTION
OF SURFACE WATERS BY THE EGGS
OF ECHINOCOCCUS

SECTION I. INTRODUCTION

Many U. S. Air Force remote radar sites in Alaska use surface ponds for sources of drinking water. Chlorination is the only treatment provided. The presence of canine animals infected with cestodes of the genus Echinococcus on the watersheds, and the discovery of cases of echinococcosis in humans on St. Lawrence Island and other places on the western coast of the Alaskan mainland were of interest to the Arctic Aeromedical Laboratory because of the Air Force personnel stationed in these areas.

The presence of infected canines near the Air Force installations was of public health significance because man can serve as a host for the larval stage of the cestode. In Alaska, dogs, red and arctic foxes, and wolves serve as hosts for the adult form of the worm while moose, caribou, and voles serve as the natural hosts for the larval form, depending upon the species involved. Man is not a host for the adult tape worm; however, the oncospheres will survive in man, penetrate the wall of the intestine and be carried in the blood stream to the liver or other organs (depending upon conditions and species) and develop into a larva that often is pathogenic.

Rausch (1952) reported that human cases were diagnosed frequently in the native population in Alaska. He stated that the most probable method of infection occurs from accidental portage of eggs to the mouth from hands contaminated in handling dogs, articles contaminated by fecal deposits of infected dogs, and improperly cured furs. He mentioned the possibility of water which is contaminated with eggs as a possible source of infection. In addition, Rausch (1958) quoted Yamashita (1956) as expressing the opinion that contaminated water supplies might be of importance in infections on Rebun Island, Japan. Rausch and Schiller (1954) reported on the incidence of Echinococcus on St. Lawrence Island and again indicated the possibility of the presence of Echinococcus eggs in water supplies, suggesting that they might be a source of human infection. They successfully infected voles with the eggs of E. sibiricensis (= E. multilocularis) which had been kept in pond water at room temperature for 22 days. In testing the viability of the eggs at low temperatures they found that some eggs were still infective after being exposed to temperatures as low as -56° C. Tulloch (1960) also cited water supplies as a possible source of infection and expressed the belief that of the

animal parasites. Echinococcus represented the most serious health hazard to military personnel operating in the East Yukon Flats area of Alaska.

Several serological and intradermal techniques have been investigated as a means of early human diagnosis, since five to ten years may elapse before symptoms are apparent and then symptomatic diagnosis is difficult, but these have not proven entirely satisfactory. Consequently, it is especially important that measures be employed to prevent infection of Air Force personnel who are not familiar with the problem. To this end the Alaskan Air Command has promulgated regulations prohibiting shipment of dogs from St. Lawrence Island and restricting activities of dogs on the radar sites. Also, newly arriving personnel are presumably indoctrinated to the hazard of handling native dogs, furs, etc., and are reminded to wash before meals. Although this represents the most serious hazard, the possibility of a contaminated water supply must be considered. Douglas (1959) felt that this was not a hazard at fixed installations, but was a threat during field installations. Actually, a hazard does exist at fixed installations since chlorination is the only positive method of treatment employed, and the resistance of Echinococcus eggs to chlorine has been demonstrated. Meyers at the Arctic Health Research Center found that both iodine and chlorine up to 100 mg/l for ten minutes contact would not affect the viability of the eggs (unpublished report). If storage is provided at fixed installations, some eggs might be removed by sedimentation and hence the hazard might be less than in the field; however, this is not a positive control. Consequently, an investigation was initiated to determine the presence or absence of Echinococcus eggs in the surface water supplies of remote Air Force stations in Alaska.

SECTION 2. DEVELOPMENT OF THE METHOD

It was expected that surface water supplies contaminated with Echinococcus eggs would contain a small concentration of the eggs. They might be present only in the bottom layer of water in the reservoir because of the removal of the eggs from the water by settling. Therefore, the common flotation methods for separating parasite eggs from liquids were necessarily ruled out. Rowan and Gram (1959) reported on a method of recovering eggs of Schistosoma from large quantities of sewage using a continuous flow horizontal sedimentation basin. The eggs were removed from the sediment by flotation and the sample was then filtered. The eggs were stained with the use of ninhydrin. Meyers (1955) making use of Stoke's Law, was able to determine the specific gravity of Echinococcus eggs as averaging 1.12 with a range of 1.10 to 1.14. These determinations were made on eggs having a diameter varying from 31 to 36 microns. A vertical flow settling chamber was considered preferable to a horizontal chamber since it could be employed

in the field without as much concern for leveling the apparatus, and the effects of short circuiting were considered to be less. Since the specific gravity and the diameter of the *Echinococcus* eggs were known, it was possible to design a vertical system specifically for removal of these particles, whereas in horizontal flow sedimentation chambers, removal of specific particle sizes from a mixture of sizes cannot be accomplished.

Stoke's Law states that when a discrete spherical particle falls through a fluid due to the force of gravity in such a way that the flow of the fluid with respect to the particle is non-turbulent (laminar flow), the particle will accelerate until an ultimate velocity (settling velocity, v_s) is achieved due to the viscous resistance of the fluid (dynamic, or absolute, viscosity, μ). This relationship is established in the following equation:

$$\text{Equation I.} \quad v_s = \frac{g(\rho_s - \rho) d^2}{18\mu}$$

where g = gravity constant = 982 cm/sec^2
 ρ_s = mass density of particle, g/cm^3
 ρ = mass density of fluid, g/cm^3
 d = effective diameter of particle

Assuming the most critical basis for designing the chamber, the smallest egg expected to be removed was assigned a diameter of 25 microns ($2.5 \times 10^{-3} \text{ cm}$) and a mass density of 1.05 g/cm^3 . Since cold water has a greater viscosity, the viscosity of water at 5°C was used, i. e., $1.52 \times 10^{-2} \text{ dyne-sec/cm}$. Substituting in Equation I establishes the minimum v_s as:

$$\frac{982 (1.05 - 0.999) (2.5 \times 10^{-3})^2}{18 (1.52 \times 10^{-2})} =$$

$$1.14 \times 10^{-3} \text{ cm/sec}$$

Any particle larger or heavier would have a greater v_s and would also settle. And if the viscosity were decreased due to warmer water, then v_s of this particle would be greater. However, in order to conform with Stoke's Law, the flow must be laminar. Laminar flow is considered to exist when the Reynolds number N_r is less than 0.5. N_r is determined from the following relationship:

$$\text{Equation II.} \quad N_r = \frac{\rho v_s d}{\mu}$$

Substituting for the variables.

Equation II. (continued)

$$\frac{N_p = 0.997 \times 1.14 \times 10^{-3} \times 2.5 \times 10^{-3}}{1.52 \times 10^{-3}}$$
$$1.87 \times 10^{-4}$$

which is well within the laminar range, and Stoke's Law is applicable.

The chamber selected had a cross sectional area, A_c , of 555 cm^2 which dictated the flow rate, Q from the well known relationship:

Equation III. $Q = AV$

In order to maintain the upward velocity of the water at a value just below v_g so that the particles would settle, Q was determined as:

$$Q \leq A_c v_g$$
$$Q \leq 555 \times 1.14 \times 10^{-3}$$
$$Q \leq 0.633 \text{ cc/sec} = 38 \text{ cc/min}$$

Actually, any Q less than 38 cc/min would have been satisfactory, but in order to partially overcome the inevitable occurrence of currents and short circuits, the Q was set at 30 cc/min. Thus, in one hour the quantity pumped would be 1.8 liters, and in one day would be over 43 liters. The tank height was selected at approximately four times the width (Figure 1) and contained, when full, approximately 60 liters of water.

SECTION 3. PROCEDURE

Initially, the sediment obtained from several hundred liters of water was to be drawn off from the bottom of the settling chamber and recovered on filter paper. The eggs would then be stained with ninhydrin to facilitate identification. However, it was found that the eggs did not take the ninhydrin stain, and with some water samples the membrane filter with a pore size of five microns clogged fairly rapidly. Consequently, the sample was centrifuged so that most of the sediment was eventually contained in a volume of approximately 1 ml. Several loops of sediment were then deposited on each of several slides which were viewed at 100X to determine the presence of the eggs. Suspected particles were examined at higher powers. Because of their characteristic shape and fairly dark color, the eggs were readily identified without staining.

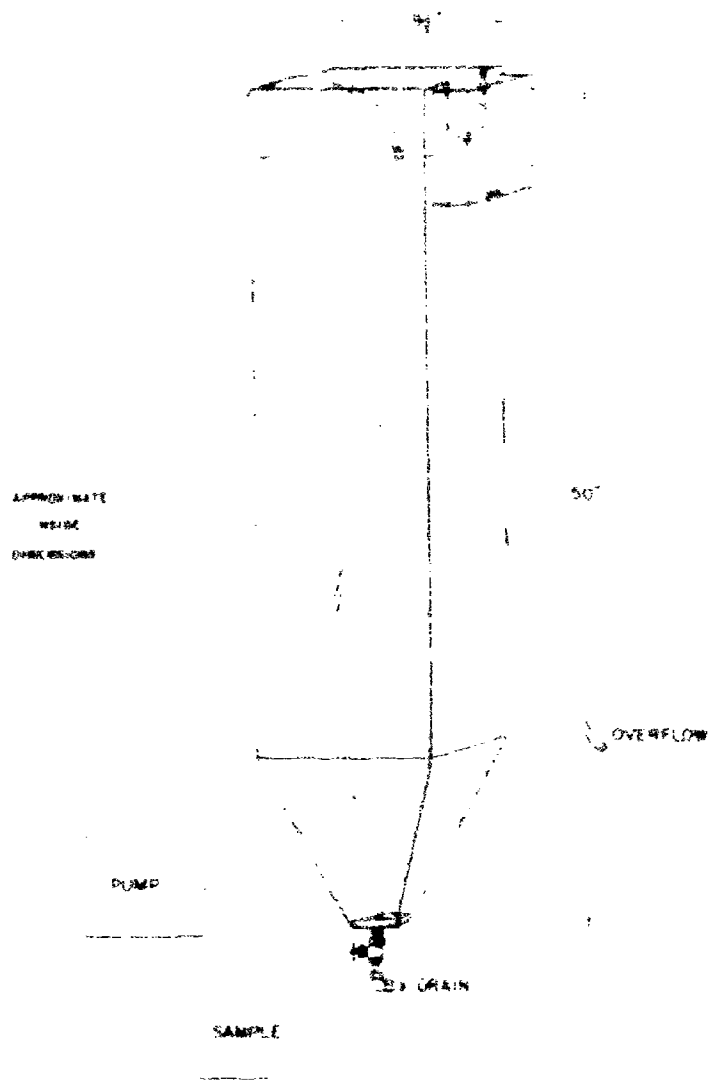


FIGURE 1

Continuous flow sedimentation chamber for removal of Echinococcus eggs from surface water supplies

Before recovery of Echinococcus eggs from the water supply was attempted, several trial runs were conducted using plastic spheres (obtained from the Dow Physical Research Laboratory and shown in Figure 2) which had a specific gravity of 1.05 and varied in diameter from 28 to 56 microns.

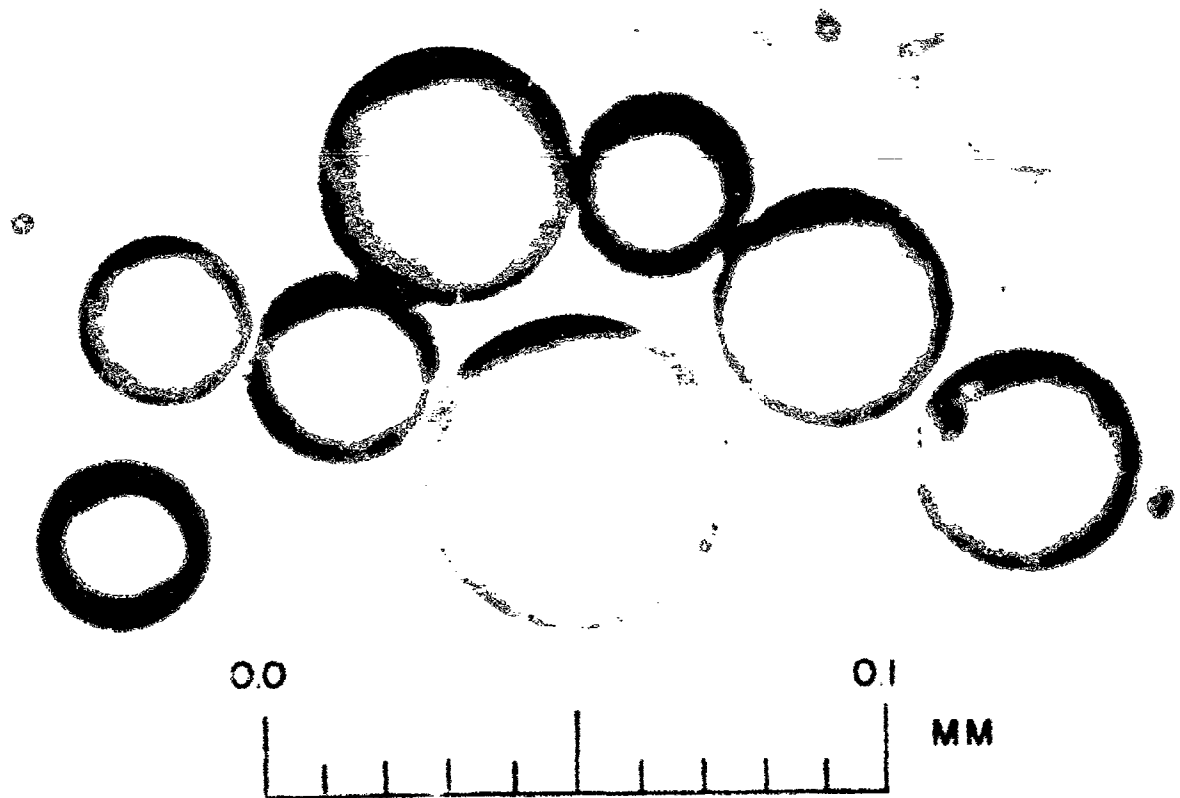


FIGURE 2

Plastic spheres recovered from unit

Since the settling velocity is a function of specific gravity and diameter, the larger but lighter plastic spheres behaved similarly to the Echinococcus egg. In the first laboratory test of recovery one cc of the suspension of plastic spheres was introduced into a large tank of tap water, and after pumping for 24 hours two liters were withdrawn from the bottom of the chamber and centrifuged. The sediment in the centrifuge tube was obvious, and a loop spread on the slide confirmed the presence of the plastic spheres. Although only about 10 per cent of the solids added was recovered, the method was considered acceptable even though it was not quantitative. The procedure was repeated using a suspension of nonviable (preserved) eggs and proglottides which had been removed from the intestines of a canine animal at time of autopsy (obtained from Dr. Robert Rausch, Arctic Health Research Center, Anchorage, Alaska). The first loopful of sediment removed from the

centrifuge tube and deposited on the slide contained a large number of Echinococcus eggs (Figure 3). This demonstration was considered sufficiently accurate to warrant use of the method in the field.



FIGURE 3

Nonviable Echinococcus eggs recovered from unit

Since none of the Air Force installations on St. Lawrence Island use surface water supplies, a station on the western coast of the mainland was chosen for the test. The Air Force station near the native village of Unalakleet obtains water from a small reservoir which is located at some distance from the main station, and hence does not receive as much surveillance as a closer facility would. Also, reports of foxes on the watershed as well as dogs from the nearby village are not uncommon. In fact, on the day the test was conducted, a native dog was encountered right at the water's edge. The schematic drawing of the water supply (Figure 4) shows that there are several built-in, although unintentional, safeguards in the system. It is quite probable that if any Echinococcus eggs are carried into the reservoir from the watershed they settle out in storage. Again, the wet well in the pump house provides another place for sedimentation, although admittedly it would be less pronounced due to the greater flow rates and turbulence. And, thirdly, there is an excellent opportunity for removal by sedimentation in the

75,000 gallon storage tank at the camp site, as shown by an accumulation of very fine particles in the bottom of the tank. The experimental Echinococcus sedimentation unit was installed at the pump house on the reservoir and the water was sampled as it entered the bottom of the wet well in the pump house. The pump operated for 24 hours before the first sample was taken, and samples were taken thereafter every 12 hours for the next four days.

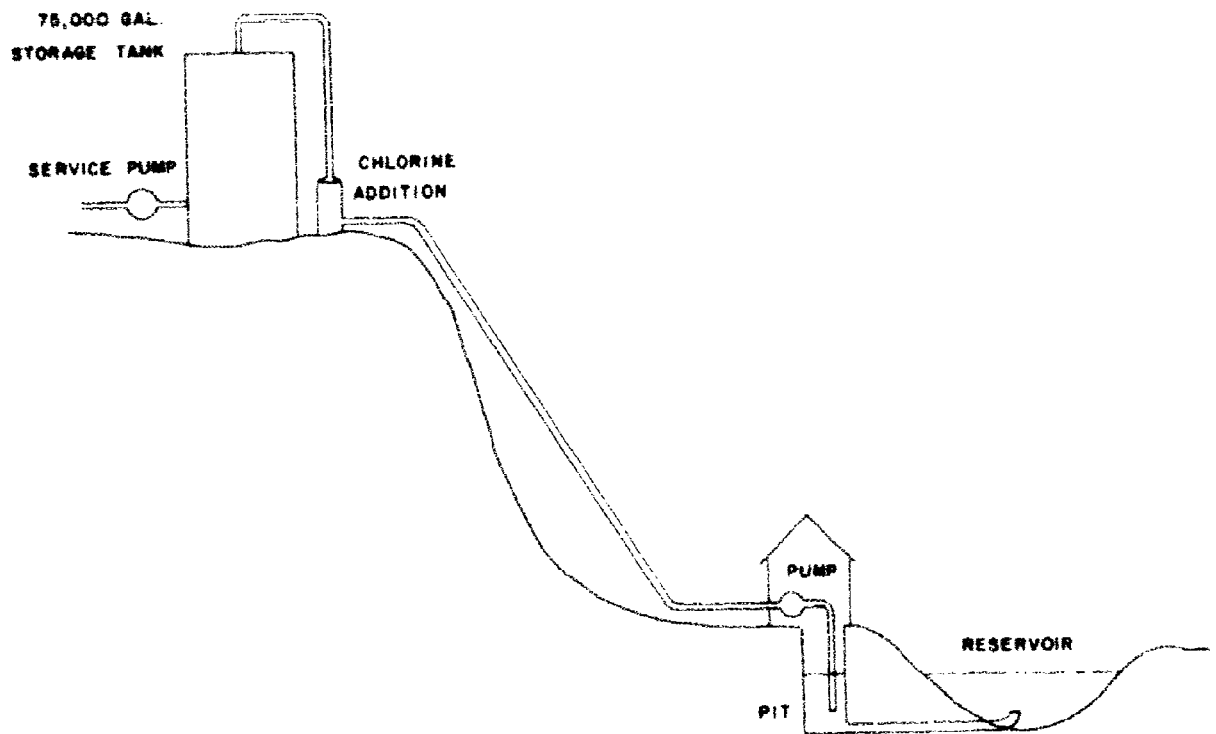


FIGURE 4

Schematic drawing of a remote Air Force site surface water supply.

SECTION 4. RESULTS

When the eight samples were analyzed in the same manner as the laboratory samples, none contained any evidence of Echinococcus eggs. Between tests, several samples of bottom sediment were removed from the reservoir near the inlet to the wet well. Common table salt was added to the bottom sediment slurry until the specific gravity was greater than 1.3. After thorough mixing the sample was allowed to sit undisturbed for an hour. The floating material was then removed with a microscope slide. Several examinations:

of the floating material showed no evidence of Echinococcus eggs. Since both the water samples and the mud samples contained many particles of the same size as the Echinococcus eggs and probably close to the same specific gravity, presumably any Echinococcus eggs present would have showed up in the sediment. It was, therefore, concluded that this particular body of surface water was not grossly contaminated with the eggs.

SECTION 5. FUTURE WORK

Although Air Force installations on St. Lawrence Island utilize ground water sources, some of the native residents employ surface waters as a source of drinking water. The experimental method should be useful in determining the presence of Echinococcus eggs in such water sources. Subsequent to the tests reported above the sedimentation unit was modified to include a centrally located inlet column and an elevated rather than a submerged inlet nozzle. In this way the pump surges did not cause nearly as much turbulence in the chamber, the water left the inlet column in a uniform flow pattern, and a more uniform upward flow resulted. The improved performance was vividly demonstrated with dye studies and documented by increasing the recovery of plastic spheres to over 30 per cent.

Until such time as all surface drinking waters are adequately filtered, or until the hazard of infection has been eliminated, the actual physical surveillance of the surface waters should be continued.

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