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Giardia in Denali National Park.

A preliminary study.

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Abstract: Cysts of Giardia lamblia were sought by immunoflourescent technique in water and fecal specimens in Denali National Park, Alaska. Water from a beaver pond contained Giardia cysts. Fecal samples from caribou and arctic ground squirrel contained Giardia cysts.

Giardia was first identified by Van Leewenhouk in his own diarrheal stool in 1681 (1). Only recently has it again been recognized as a significant cause of human illness (2). Currently is is the most commonly identified human parasite, (3). Ingestion of *Giardia lamblia* cysts leads to infection of the small intestine (4) in man and many other mammals. It has increasingly become recognized as a source of waterborne epidemics of diarhea associated with both municipal water sources (5,6) and backpackers, (7,8).

A wide variety of wild and domestic animals have been demonstrated to carry *Giardia lamblia* (8,9,10,11,12). It is a strong possibility that animals serve as a source of backpackers' giardial infections. It is not currently possible to trace the presence of waterborne cysts to a specific source (13).

The National Park Service warns hikers of the potential of waterborne infection by *Giardia* in Denali National Park in Alaska (DNP). The Park Service recommends that hikers in the park treat their water by

boiling or halogen disinfection. Several cases of diarrhea associated witl drinking untreated backcountry water have occurred. They were clinically consistant with giardiasis. Some cases have been confirmed by means of stool examination for ova and parasites (14). Yet no prior search for evidence of Giardia infestation of the water and animals of Denali National Park has been made.

This study has two parts, water sampling and examination of both human and animal fecal smears to determine if the presence of Giardia lamblia cysts could be confirmed.

Approach

Rather than trying to survey or randomly sample the many water systems of Denali Park, which spans over 6 million acres, a decision was made to systematically study one water system, Jenny Creek. Samples were taken along its course over a period of time. The goal was to observe if Giardia cysts were present and whether they were associated with the home range of a particular animal. Samples were taken by filtering stream water through a 5 micron filter and examining the residue for Giardia cysts.

Previous studies have implicated beaver, microtenes and other animals as reservoirs of Giardia (11,12). In Denali, due to an interest in animals that might transmit Giardia from one watershed to another and a decision not to trap animals in a national park on a preliminary study, samples were taken from large and middle sized animals. Moose, caribou, grizzly bear, dall sheep, wolf, marmot, arctic ground squirrels and pica were sampled. One Ptarmigan was sampled (impulse shopping). Five

additional animal samples were collected from sources not positively identified.

In addition 10 samples were taken from human volunteers on the staff at D.N.P.. All were people in frequent contact with the environment of the park. Anecdotally one had previously endured a long bout of diarrhea thought to be giardial in origin. He reported drinking untreated water in the park prior to his illness. He was subsequently treated with Flagyl and symptoms gradually disappeared.

Description of Study Area

The Jenny Creek water system originates on the northern face of foothills of the Alaska Range in DNP, east of the Savage River. Unlike the many glacial water sources in Denali, it arises from two springs. The water is thus clear and attractive for drinking. Nineteen water samples were taken, 17 from Jenny creek and 2 from beaver ponds that are fed by the Savage River, a glacial river too silty to be sampled by the field filtration procedure used in this study. The Savage River is joined by Jenny Creek several miles above the beaver ponds. The water of the beaver ponds themselves is clear and are circumstantially connected with episodes of human diarrhea.

Jenny Creek was chosen because the headwaters are in a area closed to human use, (map 2). The terrain is alpine tundra and is inhabited seasonally by dall sheep and caribou. The headwater region is permanently occupied by arctic ground squirrel, marmot and small rodents. The entire course of the stream is inhabited transiently by grizzly bear and wolf. The water flows down through the closed area into a dense

willow thicket heavily populated by moose. The creek emerges from the closed area to join with Caribou Creek, another clear creek, and travels along the southern border of Savage Campground. The area around the campground is an zone of heavy human use though it should be noted that both clean water and toilet facilities are provided and so human interaction with the water supply is perhaps limited.

Within two miles of joining Caribou Creek the combined flow joins the heavily silted glacial runoff of the Savage River. Approximately 6.5 miles downstream a series of active beaver ponds are fed by the Savage River. In them the water is clear due to settling. The water is still and has a dust on the surface. The beavers are quite active and were in sight during sampling.

Animal fecal samples were obtained from the eastern half of the original 1.8 million acre wilderness region of Denali National Park. The sampling zone stretched from Jenny Creek westward to Moose Creek including the central valley of DNP and its rim, (Map 1). The central valley is formed by the edge of the main Alaska Range and the Outer Range. It contains primarily tundra with patches of spruce and thickets of brush. Glacial valleys from the main range and river valleys from the outer range pierce the mountains to join the central valley. The mountains themselves are covered with alpine tundra below snowline.

Materials and Methods

Water Sampling: Field Techniques.

Water was sampled on clear days without recent rain to avoid turbid conditions which would prevent effective filtration. Prior to sampling all materials were rinsed thoroughly in water from the sample site. Water was collected in a 5 gallon plastic bucket and pumped through a 5 micron 142 millimeter diameter polycarbonate membrane filter (Nucleopore, Pleasanton Ca.). An electric bilge pump coupled to a rechargable battery provided the flow. When the filtration reached the point of diminishing returns the pump was stopped, the filter removed from the cartridge and placed on a pane of plexiglass resting in a plastic collection pan. Loose water was shaken from the filter to the pan and then the filter's surface wetted with sample water from a wash bottle. The filter was squeegeed dry into the pan with a flexable rubber blade to remove and capture potential cysts. This process was repeated at least twice. The filter was then reinserted into the holder and further water was pumped. The cleaning process and collection was repeated until cleaning no longer improved the filtration rate. The filter was squeegeed a final three times and the contents of the collection pan were poured into a 250 ml conical tip sealable centrifuge bottle. The collection pan itself was rinsed and squeegeed 2-3 times and the contents added to the sample. The sample was preserved with 6 drops of formalin (37%) and stored at ambient temperature. A new filter and squeegee was used for processing each sample.

On return to Seattle water samples were processed and examined by microscopy within 15-42 days of sampling (mean 26).

Water Samples: Lab Methods, Summary.

Water samples were concentrated by centrifugation and decanted. Particles of Giardia density were collected by density separation. The concentrated particles were deposited onto a small filter. Three stains were applied for immunoflourescence and viability assay. A polyclonal rabbit anti Giardia cyst serum was followed by a flourescein conjugated goat anti rabbit serum. The final stain was ethidium bromide (EB) which selectively stains the interior of dead cysts. The stained filter was transferred to a slide and sealed in Elvinol for microscopic examination.

Details of Technique

Water samples were returned to Seattle in 250 ml centrifuge tubes. They were centrifuged at 650xg for 15 minutes, the volume was then reduced to 10 ml by aspiration. The pellet was resuspended and transferred to a 50 ml centrifuge tube. The 250 ml tube was rinsed three times with .22 micron filtered Phosphate Buffered Saline (PBS) and the contents added to the 50 ml tube. The 50 ml tube was refrigerated over night to assist in the removal of algae. The sample was then centrifuged at 650 G for three minutes, volume was reduced to 5 ml by aspiration and the pellet resuspended.

Each sample was then layered on 7 ml of .22 micron filtered potassium citrate, specific gravity 1.13, in 6-12 15 ml centrifuge tubes. No more than 3 mls of sample was placed in a tube and less if the sample was dirty. The 50 ml tube was rinsed three times with PBS and the rinse was layered as well. Layered samples were centrifuged at 650 G for 2 minutes. The denser material penetrated the citrate leaving the Giardia and lighter material on top. Two mls were harvested from the citrate water interface taking an equal amount of each to recover potential Giardia cysts.

A filter cartridge (swinnex, Millipore) containing a 5 micron 13 mm diameter polycarbonate membrane filter (Nucleopore) was selected. The outlet was sealed and the cartridge was filled with .3 ml of .22 micron filtered 1% Bovine Serum Albumin (BSA) in PBS. The BSA served to block nonspecific protein binding sites to reduce background staining. The filter cartridge was connected to an aspirator chamber set at 10 PSI via a hypodermic needle. The sample was placed into an open syringe connected to the filter cartridge. The syringe and cartridge were then rinsed three times with 10 ml of PBS, transferring the sample onto the filter. The cartridge was removed and the outlet plugged with Parafilm. The cartridge was then filled with .3 ml PBS, sealed and refrigerated until staining.

Three stains were used. Polyclonal rabbit anti Giardia cyst (RAG) prepared by Dr. Ongerth, flourescein conjugated goat anti rabbit (GAR) (Gibco) and ethidium bromide (EB) (SIGMA). EB is a nucleic acid stain that is excluded efficiently by viable cysts. Thus it only stains the interior of dead cysts. The RAG was stored frozen in 25 microliter aliquots. It was mixed prior to use with 3.75 ml PBS to achieve a

dilution of 1/150. The GAR was diluted from 25 microliter aliquots into 2 ml of .22 micron filtered 5% goat serum in PBS to achieve a dilution of 1/80. The goat serum served to bind to nonspecific protein binding sites on particles in the sample and reduce background staining. The prepared GAR was stored in the dark in the refrigerator at 5 C and used within 4 days. EB was diluted 50 micrograms/ml in PBS.

The filter cartridge was rinsed 3 times with 10 ml PBS over the aspirator. The outlet was then plugged and the cartridge filled with .3 ml RAG. The inlet was sealed and the cartridge was incubated for 30 min at 37 C. The cartridge was then rinsed 3 times with 10 ml PBS. The outlet was again stoppered and the cartridge was filled with .3 ml of GAR and incubated for 30 minutes at 37 C. The cartridge was rinsed 3 times with 10 ml PBS, filled with .3 ml EB and incubated 10 min in the refrigerator at 5 C.

The cartridge was rinsed a final time, then opened and the filter carefully transferred to a clean, flamed microscope slide. One drop of Elvanol mounting media was placed onto the filter and covered with a coverslip. The Elvanol was allowed to set for 30 minutes before viewing. Slides were stored in the refrigerator to prevent the flourescein from fading.

Fecal Samples

Field Technique.

Fecal samples were collected in either a sealed plastic bag or sealed plastic test tube. Twenty-four of 127 samples (19%) were collected from the Jenny Creek watershed from which the water samples were taken. The remainder were taken from other areas in the sample region. Samples were taken from a wide range of habitat, from the top of low mountains on the edge of the Alaska range to the low area surrounding beaver ponds. An effort was made to take fresh samples as judged by color, texture and moisture. Many were taken with the animal in sight.

Samples were emulsified in commercially prepared distilled water and rolled onto a clean microscope slide with a new wooden applicator. The slides were allowed to air dry, then heat fixed by passing them through the flame of a pocket lighter (Bic) three times. Slides were subsequently flooded with 100% ethanol and covered with upturned paper plates to allow evaporative drying to take place slowly. They were stored in sealed slide boxes with silica dessicant at ambient temperature.

Upon return to Seatle slides were frozen (4-42 days after collection). They were subsequently thawed and prepared individually.

Lab Methods.

To stain fecal smears an 18mm x 18mm area on each slide was surrounded by nail polish to form a well and dried in the incubator at 37 C. The well was flooded with .15ml of RAG and the slide placed in a incubator at 37 C which was humidified to prevent drying. The slide was gently rinsed with PBS three times and soaked in PBS for 3 minutes. The slide was then allowed to air dry. The slide was subsequently flooded with .15ml GAR and agin incubated in a humid chamber at 37 C for 30 minutes, rinsed and soaked in PBS and air dried. Two drops of elvinol were placed in the well and a coverslip applied.

Microscopy

Water and fecal slides were scanned at 200x under UV illumination. Detailed identification was performed at 400x. Fecal smears were further evaluated under phase microscopy. Water slides were additionally examined under bright light. Possible cysts were compared to positive controls and judged by the following criteria:

Shape: elliptical. This could approach round when a cyst was wedged vertically into a pore of the filter on a water slide.
Size: 5-6 micron x 10-12 microns.
Stain quality: specifically stained cysts produce an even, flourescent green color.
Cyst Wall: smooth, of uniform thickness without indentation.
Abscence of other flourescein stained structures within the cyst.
Water slides were examined for evidence of EB staining. Full staining of internal structures indicated a dead cyst.
Animal slides were evaluated under phase microscopy for internal structures inconsistant with the presence of enclosed trophozooites.
Water slides were evaluated under bright light. Cysts would fade to a very faint image without disappearing completely.

Results

Water Samples:

Two of the 19 water samples had detectable Giardia cysts, #7 and #8. They were taken from the Savage beaver ponds on subsequent days. Several cysts were found in each sample. Cysts did not stain with EB.

Table 1

Sample #	Date	Vol. L.	result.
1	8/3	40	No cysts detected
2	8/4	101	"
3	8/5	6	"
4	8/8	59	"
5	8/9	71	"
6	8/9	55	"
7	8/10	26	Cysts detected
8	8/11	36	Cysts detected
9	8/24	108.5	No cysts detected
10	8/24	58	"
11	8/25	102	"
12	8/25	101	"
13	8/25	107	"
14	8/25	64	"
15	8/27	100	"
16	8/27	100	"
17	8/28	105	"
18	8/28	100	"
19	8/28	102	"

Fecal Samples:

Five of the fecal smears were positive. Three caribou samples and two ground squirrel samples contained cysts. No other samples, animal or human, had detectable cysts.

Table 2

Animmal:	Caribou	Squirrel	Sheep	Moose	G Bear
# pos.	3/27	2/14	0/20	0/29	0/16
%	11	14	0	0	0

Animal:	Wolf	Marmot	Pica	Ptarm	unidentified
# pos.	0/3	0/6	0/6	0/1	0/5
%	0	0	0	0	0

Human Samples	
# pos	0/10
%	0

Discussion

The two water samples in which cysts were detected were both from the large Savage beaver pond, the sample site farthest downstream. The association between *Giardia* and Beaver has been made before(6,15,16). It is likely that the local beavers are shedding cysts. It is unfortunate that the beaver habit of defecating in the water prevented collection of their scat during this study. It would be impossible to determine, however, whether the beaver were the source of infection of the pond or were themselves infected by cysts in the pond.

The sampling method employed has a limit of detection of approximately 1 cyst in 10 L (23). Cyst concentrations in this range have been demonstrated in the Cascade mountains of Washington (23). Both caribou and ground squirrels, species identified by this study to carry

Giardia elsewhere in the park, inhabit the watershed supplying these ponds. It is possible that the stream above is infected at less than the level of detection. The ponds might serve as a point of accumulation allowing cyst detection.

Cysts failed to stain with EB which suggests that they were viable at the time of staining in Seattle. Cold water prolongs cyst viability, (13). Individual cysts are probably infectious for a prolonged period of time in Alaska's cold water.

Three caribou samples were positive. The samples were from the adjacent regions of Stony Hill and Highway Pass. Two were collected on the same day 8/7/87 and one a week later, 8/14/87. All three samples were noted to be older than most, having a hard exterior and a soft interior. Two positive arctic ground squirrel specimens were found. They were collected the same day, 8/7/87 from the entrance to different burrows approximately 100 yards away from Eilson Visitor Center. Twenty-one/127 samples were collected on 8/7/87 all in the region between Toklat and Eilson Ranger Stations. Four/5 of positive specimens were found on 8/7/87. All 5 positive samples were found west of Toklat. Positive samples were collected in watersheds that fed the Thoroughfare and Toklat Rivers as well as Little Stony Creek. While all positive fecal samples were found west of Toklat Ranger Station the finding of Giardia cysts in water of the eastern end of the park suggests that it may be found in animals in the east as well.

While other members of the deer family have been found to carry Giardia, elk (18,19), mule deer (20), this is the first finding in caribou known to the author. The finding of caribou carrying Giardia

cysts is significant. The caribou, genus Rangifer, is well named, migrating over hundreds of miles of arctic and sub arctic terrain. Their travels take them from low river valleys to high mountain passes. They come into contact with a great number of northern water sources. If they carry Giardia cysts they have the potential to transfer the organism from one water system to another and at any point along its course. Caribou range above the headwaters of many water systems, leading to the potential for infecting even the sources of some rivers and streams.

Arctic ground squirrels may act as a reservoir within a watershed. Ground squirrels live above the source of some water systems providing the potential to infect a stream at its beginning. Their coprophagic behavior may be responsable for their initial infection. It may also serve to perpetuate their infection, as it does in some other small mammals, by ingestion of their own cysts, (17,18).

The prevalence of Giardia findings in the scat of caribou and ground squirrel was 11% and 14% respectively. While no other animal species was demonstrated to carry Giardia the small number of samples taken does not in any way rule out the possibility.

Identification of Giardia by microscopy of fixed slides is problematic, There is not general agreement on speciation or morphological characteristics of subspecies of Giardia lamblia,(1,16). While some strains have been shown to be human infective, they are impossible to distinguish by microscopy from other strains, (2). Strains of different virulence for humans have been found, (21). It is also not currently possible to determine the source of a cyst found in water or

stool, (2). What can be said with certainty in Denali is that in a region where people drinking untreated water have manifested symptoms of giardiasis and some have had confirmed cases, Giardia cysts have been found in water and animal fecal samples. While highly suggestive that the cysts found were human infective this is not conclusive.

Future directions for study in Denali might include sampling inflow and outflow streams to the beaver ponds to determine if the beaver ponds are a source or merely a collecting point for Giardia.

Sampling other water systems on a greater scale would be valuable. Of particular interest would be the streams associated with the positive fecal samples such as those at Highway Pass and Stony Creek. It would be useful to follow some water sources over a greater period of time to note whether water contamination is intermittent or continous.

In regards to animal sampling it would be important to live trap microtenes and other small mammals which have been implicated as a reservoir of Giardia in other studies, in order to identify and sample their scat. Similarly it would be valuable to acquire beaver scat by either live trapping beaver or scooping feces from the bottom of the pond to test for Giardia. Sampling of a greater number of scat from many species to further determine what species were infectied and to what degreee would be informatiive.

An ELISA technique has been developed for Giardia since these samples were mounted, (22). Future animal samples might be collected in tandem. One fixed in formalin, which makes the cysts easier to detect (22), and one fresh. The fixed samples could be screened by ELISA in a fraction of the time of microscopy and then confirmed by microscopy. The

fresh sample could be used as a source of parasites to infect lab animals. Cysts from infected lab animals could be used to determine human infectivity.

Conclusion

Giardia cysts have been identified in both water and fecal samples in Denali National Park. Caribou and arctic ground squirrel have been shown to carry Giardia spp.. Caribou may be capable of spreading the parasite widely. While this study does not determine if the Giardia found is human infective, circumstantial evidence in the form of confirmed cases of giardiasis associated with park waters suggest that it is. The National Parks Service's warning to treat the water is appropriate.

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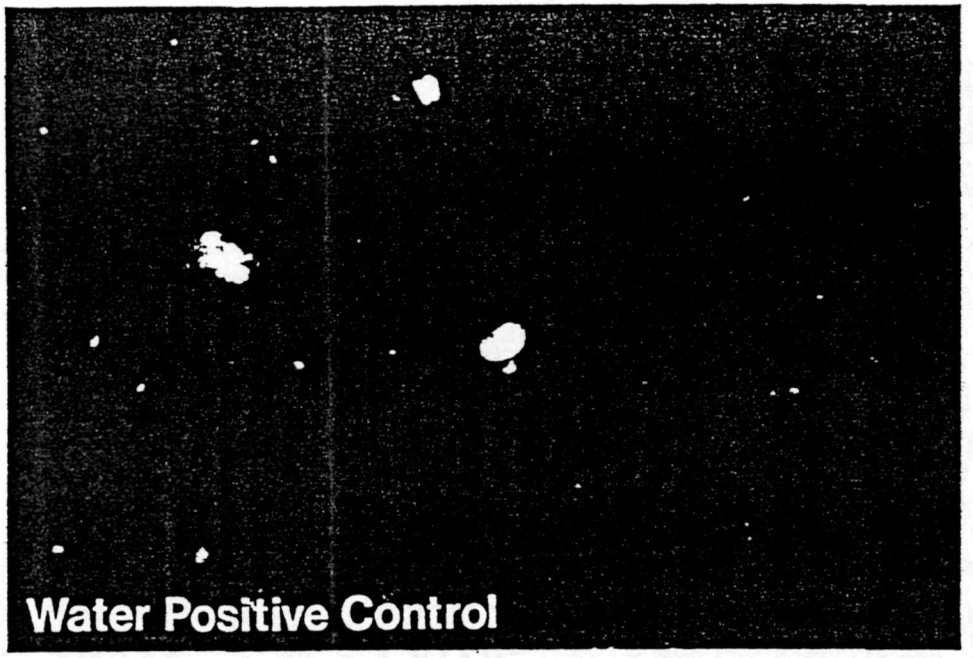
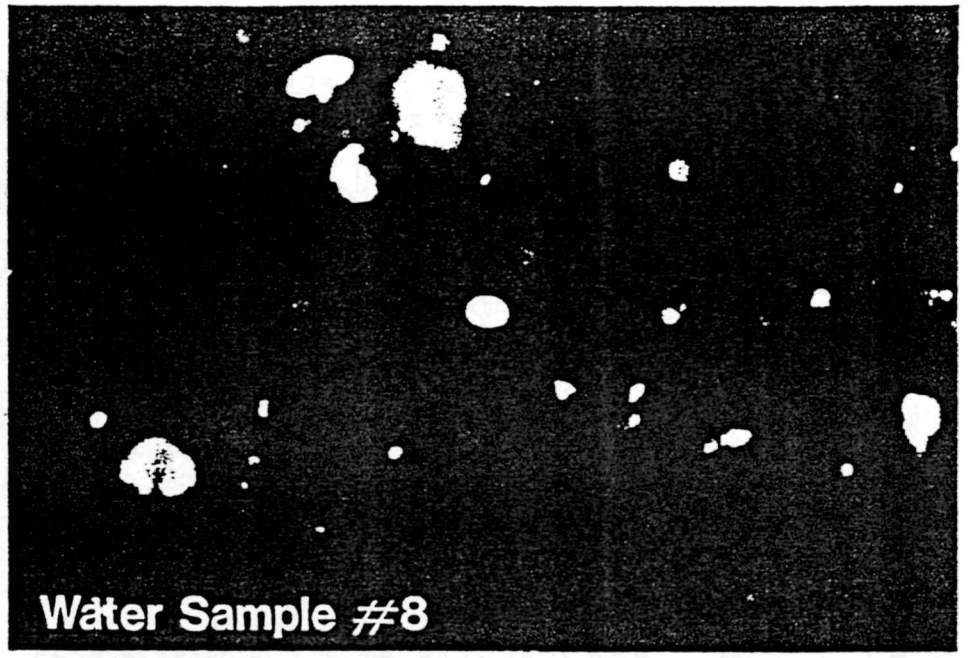
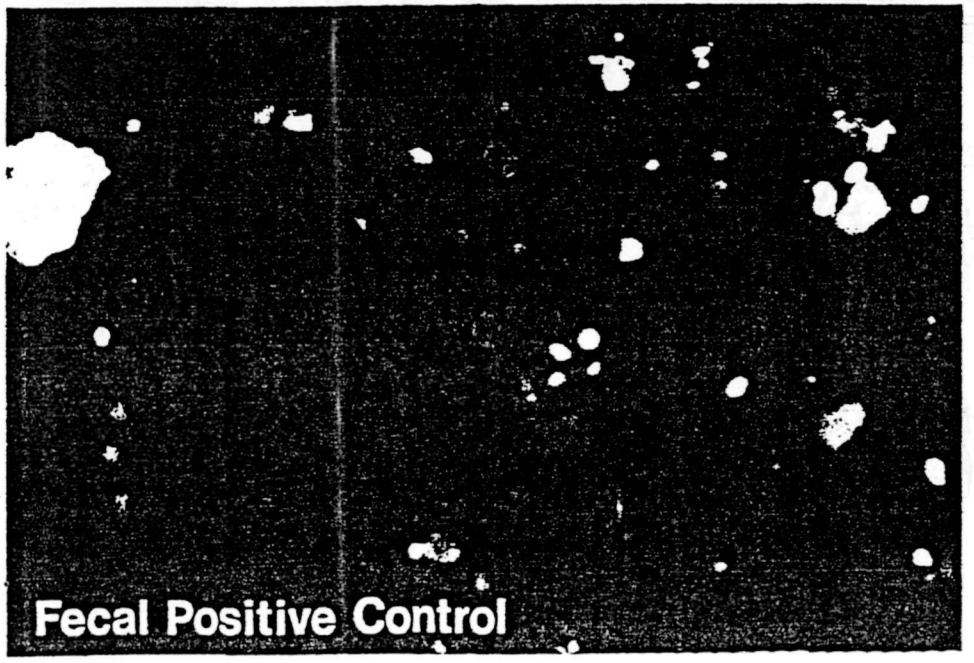
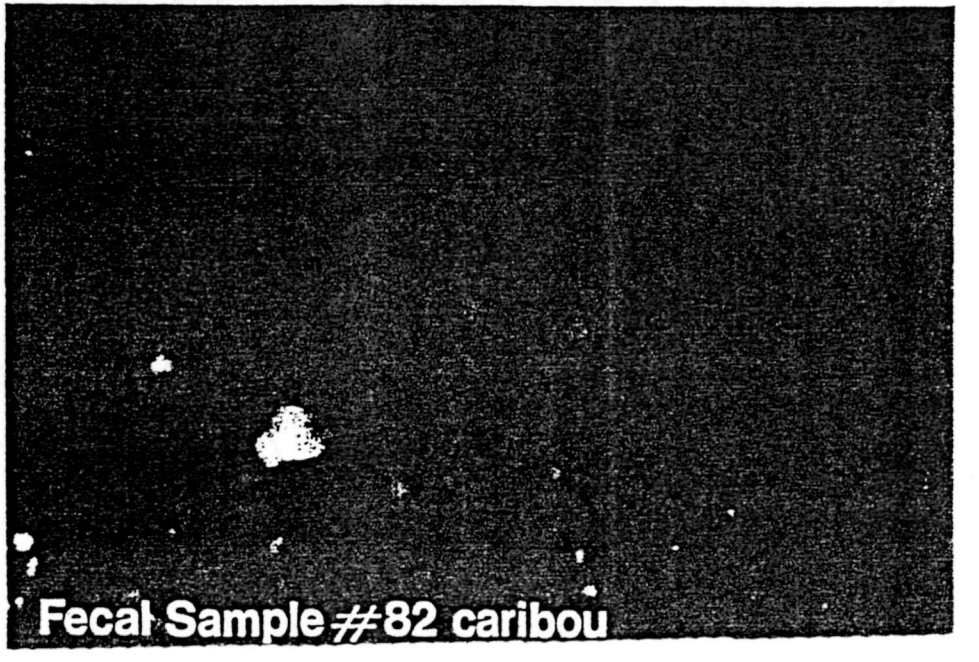
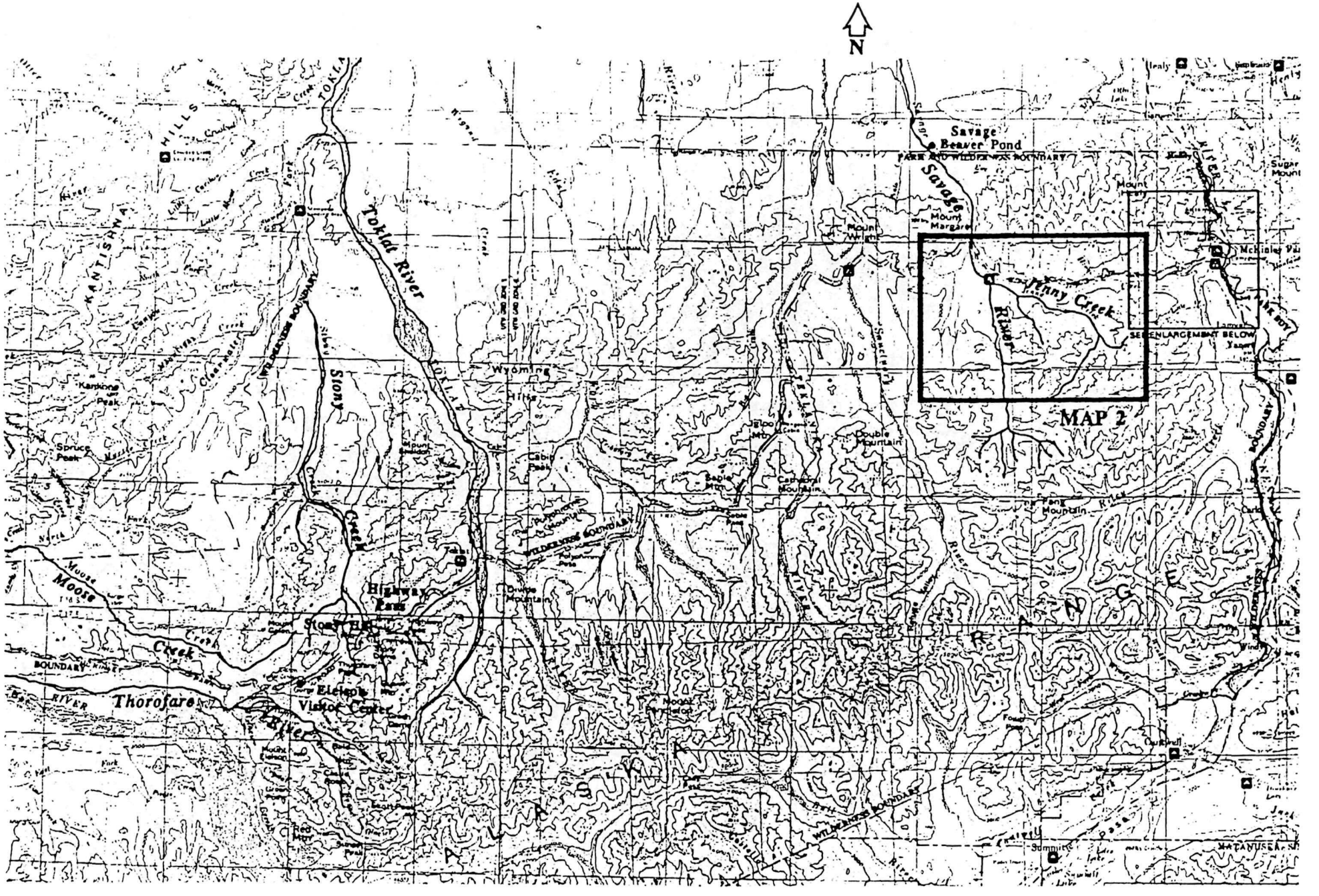
Appendix A Photographs Magnification 400X



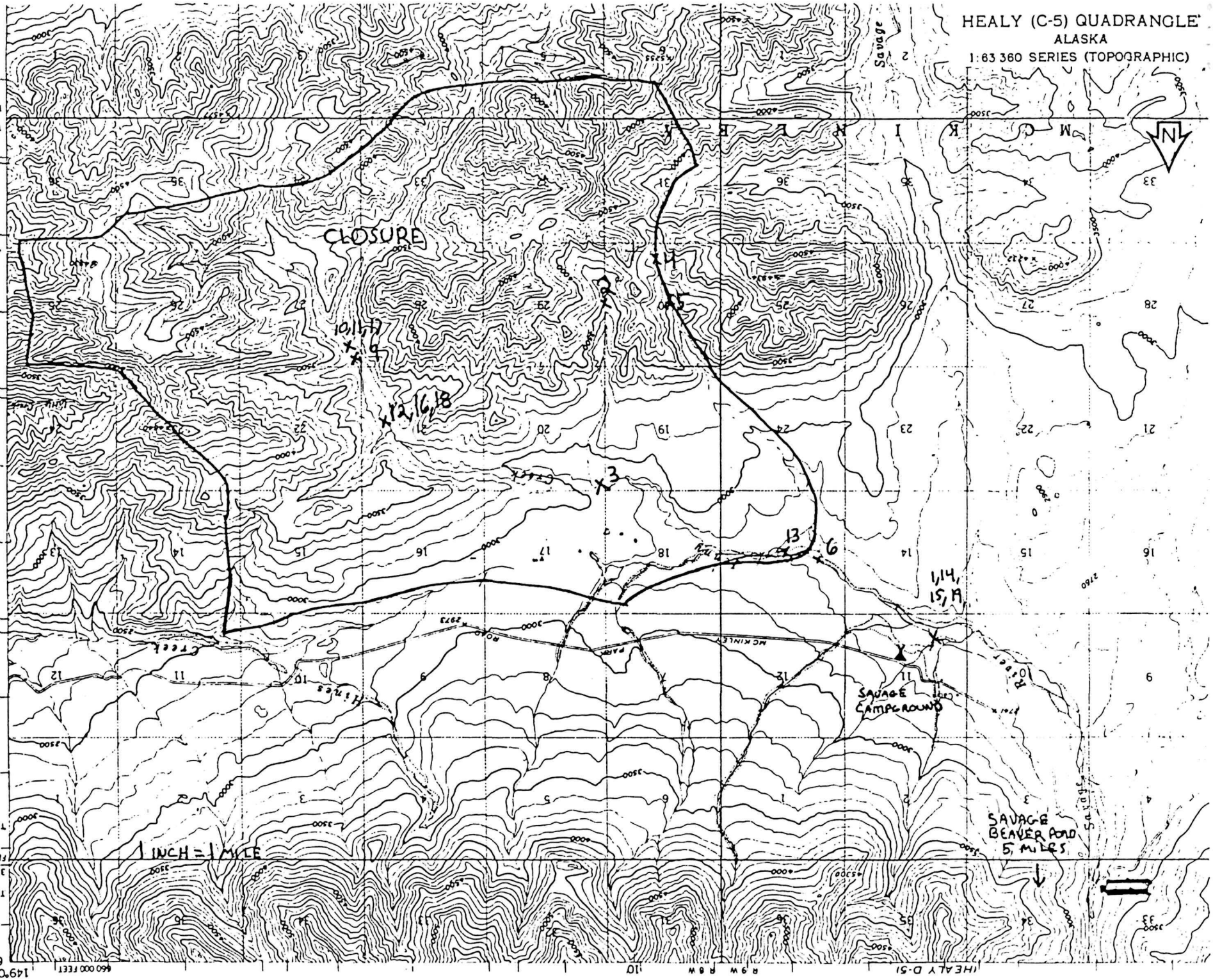








DENALI NATIONAL PARK AND PRESERVE
ALASKA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ALASKA
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CLOSURE
SAVAGE CAMPGROUND
SAVAGE BEAVER POND 5 MILES
1 INCH = 1 MILE
Savage
McKINLEY
PARK
ROAD