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Project Summary

Cross Transmission of *Giardia*

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Giardia cysts isolated from fecal samples obtained from humans (*Homo sapiens*), beaver (*Castor canadensis*), dogs (*Canis familiaris*), cats (*Felis domesticus*), bighorn x mouflon sheep (*Ovis canadenis x O. musimon*), guinea pig (*Cavis porcellus*), muskrat (*Ondatra zethica*), and mule deer (*Odocoileus hemionus*) were given to a variety of experimental animals. Human source *Giardia* cysts established infections in dogs, cats, beaver, rats (*Rattus norvequicus*), gerbils (*Gerbillus gerbillus*), guinea pig, raccoon (*Procyon lotor*), bighorn x mouflon sheep, and pronghorn antelope (*Antilocapra americana*). *Giardia* cysts from naturally occurring beaver successfully infected dogs. A dog was infected with *Giardia* cysts from a bighorn x mouflon sheep which had been infected with human source *Giardia*. Human source *Giardia* cysts were used to infect cats and cysts from these cats were used successfully to infect dogs.

Evidence exists that once dogs are treated with metronidazole and then reexposed to *Giardia* cysts they become infected yet do not shed cysts. This most likely occurs in natural cases of giardiasis in dogs where the animal stops shedding cysts yet has a latent infection. Female dogs and cats may start shedding *Giardia* cysts 3-4 weeks after parturition.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Giardia (Protozoa: Hexamitidae) has been known as a parasite of humans since Leeuwenhoek found trophozoites of the protozoan in his own feces A recent report indicates that *Giardia lamblia* is the most common parasite in stool specimens submitted for examination in the United States, with prevalences ranging from 2 to 20% with the average at 3.8%. Within Colorado, giardiasis exists in two forms: endemic and epidemic. Previous parasitological state surveys showed that *Giardia* is the most common parasite identified, with prevalence rates of 5% and 5 6%. A third survey in Colorado reported 3% of the people examined were infected with *Giardia*. Infected persons had diarrhea which lasted an average of 3.8 weeks A correlation between seasonal distribution of cases and fecal contamination of mountain streams indicates drinking untreated water is an important cause of endemic giardiasis

Numerous epidemics of giardiasis have occurred in Colorado. The precise source of the *Giardia* in these outbreaks is not known, but *Giardia* cysts have been recovered from samples of public water supplies in various other localities. Cross connections between water and sewage lines were determined to be the cause of one Colorado outbreak, others were associated with incompletely treated surface water. Beaver infected with *Giardia* were found below the

water inlets for the water system in a Washington State outbreak.

Sylvatic giardiasis has been described in Colorado with beaver (18%), cattle (10%), domestic cat (25%), and dogs (13%), being positive for *Giardia*. Two of 34 coyotes *(Canis latoans)* from northern New Mexico were also positive for *Giardia*.

Early parasitologists, describing species of *Giardia* from various hosts, named species after the host in which they were found irrespective of morphologic similarities between *Giardia* in the different hosts In the absence of cross-transmission experiments to determine the validity of speciations, this probably was the safest approach. However, as early as 1952 investigators could not find any morphologic differences between species of *Giardia* described from the laboratory rat and a number of wild rodents. A review of the literature determined that most experimental cross-transmission studies were questionable. This prompted the proposal of two species, *G. muris* in the mouse, rat, and hamster, and *G. duodenalis* in the rabbit, man, dog, cat, cattle, and various rodents.

Although this proposed speciation was based on morphology, the implication that other animals could serve as reservoirs for man was extremely important. Although the author of this proposed speciation did not accept the success obtained by two other investigators in infecting laboratory rats with *Giardia* from man, these early investigators were aware that the various *Giardia* might not be host-specific.

Researchers gave human-source *Giardia* to dogs and reported establishing infections with the prepatent period ranging from 3 to 40 days. However, this experiment was not well controlled. Another experiment, not adequately controlled, infected six dogs with human-source *Giardia* cysts and found the prepatent period was 6-9 days. The dogs used in this experiment were examined for *Giardia* for two weeks, without positive findings, prior to inoculation. All of these results strongly suggest that the premise of only a few species of *Giardia* was probably correct. In another cross-transmission study it was reported that *G. muris* from laboratory mice, *G. simoni* from laboratory rats and *G. peromysci* from deer mice were very host-specific, while *G. microti* and *G. mesocricetus* were not host-specific

The present cross-transmission studies were stimulated by the increasing number of unexplained epidemics of giardiasis in humans, all apparently waterborne, but not readily traceable to human contamination of the water supplies. They pointed to another possible source of infection, a wild or domestic mammal This, of course, necessitated more extensive cross-transmission studies involving a multitude of wild animal hosts to determine if, indeed, a wild animal species was responsible for the epidemics.

Results

Animals exposed to *Giardia* cysts from clinically-ill humans produced data which varied both within and among experimental groups. Hamsters, domestic rabbits, laboratory mice, wapiti, mule deer, white-tailed deer, black bear, domestic sheep, and domestic cattle were not infected successfully with human source *Giardia* cysts. Animals which did become infected were laboratory rats, gerbils, guinea pigs, beaver, dog, raccoon, bighorn x mouflon sheep and pronghorn antelope. Cysts from all animals, with the exception of some of those from rats, ranged in size from 9.5 to 11.0 μm x 8.0 to 9.5 μm. After intubated animals became patent, the in-group control often started shedding cysts 8-20 days after exposed animals became patent. This indicated transmission of *Giardia* from the exposed animals to the in-group controls. None of the control groups, held in the same facilities as the exposed animals and the in-group control, became positive for *Giardia*.

Giardia cysts were recovered from the only composite fecal sample from rats on days 22, 25, and 40 postexposure (PE) Cysts were of two sizes, 5 μm long and 10 μm long, but were identical in all other respects. All other experiments were performed with animals in individual cages. In another experiment using rats, cysts were shed for one day at 34 PE. Infected feces from this group were fed to an SPF dog which began shedding *Giardia* cysts eight days PE Control dogs remained negative

Gerbils exposed to human source cysts began shedding cysts 8, 13, and 18 days PE. In one test, the in-group control was positive 33 days PE The cysts were shed in varying numbers and not consistently in all samples Some exposed animals remained negative until the experiment was terminated in 42 days PE. One exposed guinea pig became positive for *Giardia* two days PE and continued shedding for 31 days. All other animals remained negative

Beaver exposed to human *Giardia* cysts from one human source were negative for 40 days preexposure and remained negative for 40 days PE. The control and one exposed beaver were inoculated with human *Giardia* from another source. These beaver started shedding *Giardia* cysts 25 days PE and continued shedding for 22 days, after which they shed cysts intermittently. The beaver used as a control for the second exposure remained negative. The *Giardia* from the first human source apparently were not infective, whereas those from the second source were infective.

SPF beagle puppies exposed to human source *Giardia* cysts began shedding cysts six to eight days PE and the in-group controls began shedding cysts 13 to 15 days after the exposed dogs were inoculated.

A young raccoon in a group of ten shed cysts for one day at eight days PE. A five-month old black bear cub remained negative for cyst shedding.

Bighorn x mouflon sheep exposed to human *Giardia* began shedding cysts nine days PE and shed cysts for four days. Sheep isolated cysts were inoculated to SPF beagles. A pup inoculated with cysts from one sheep started shedding cysts ten days PE, whereas a pup inoculated with cysts from another sheep remained negative for cyst shedding.

A young pronghorn antelope exposed to human *Giardia* cysts started shedding cysts 16-18 days PE and shed cysts for three days.

Wapiti, mule deer, white-tailed deer, and domestic sheep and cattle did not become infected with human *Giardia* cysts However, naturally-infected mule deer and cattle have been reported in the literature. Infection of muskrats was confounded since all animals including the control were found to be positive 18 hours after being exposed to human source *Giardia* cysts

Puppies and kittens exposed to human source *Giardia* cysts exhibited prepatent periods of 6-10 days and 6-27 days, respectively. Cysts isolated from one of the exposed kittens were inoculated into puppies and kittens. Most of these inoculated puppies and kittens began shedding cysts. All control animals remained negative throughout these experiments

Giardia cysts recovered from the feces of naturally-infected, free-ranging

beaver were given to mice, rats, hamsters, guinea pigs, and puppies Only the exposed puppies began shedding cysts eight days PE and the in-group control puppy began shedding cysts 17 days PE of the inoculated animals.

All dogs, including the in-group controls in one group, began shedding cysts at four days PE when exposed to *Giardia* isolated from muskrats. It is assumed that all of these dogs were exposed to *Giardia* before the experiment was begun A second group of dogs exposed to *Giardia* cysts from muskrats remained negative for 63 days.

Giardia cysts from a naturally-infected mule deer were given to SPF beagle puppies which remained negative for 28 days PE.

Reinfection with human *Giardia* cysts was attempted on positive dogs treated with metronidazole. Following six days of treatment, seven of the puppies were negative for *Giardia* shedding Seven days after treatment five of the six treated puppies were exposed to human *Giardia* cysts None of the puppies shed cysts for 40 days PE. The untreated puppy continued to shed; however, cysts were not observed in feces from the puppy which was negative when all of the dogs originally were obtained After 40 days PE, intestinal scrapings indicated *Giardia* trophozoites in three of the five exposed dogs The dog that was treated but not exposed to *Giardia* did not exhibit trophozoites in intestinal scrapings.

Giardia-free dogs and cats were difficult to obtain. Therefore, pregnant dogs and cats were obtained and held in clean rooms until they gave birth and the offspring were weaned The female dogs began shedding *Giardia* cysts two to four weeks after parturition and all of the offspring were positive seven days after the female started shedding cysts These female dogs were examined daily and criteria for selection included being *Giardia*-free for seven days prior to acceptance. None of these dogs shed cysts until two or four weeks after parturition. To solve this problem the adult animals were treated with metronidazole for five days and in this manner *Giardia*-free offspring. were produced and the adults did not shed cysts after parturition

Conclusions

1. *Giardia* cysts obtained from human, dog, cat, and beaver sources are not host specific
2 *Giardia* from human sources will readily infect dogs, cats, and beaver
3 *Giardia* from beaver sources will readily infect dogs
4 *Giardia* from dog or cat sources cross-transmit between these species.
5 Therefore, it must be assumed that *Giardia* from dog, cat, and beaver sources will infect humans

Recommendations

The results of this cross-transmission study showed that the *Giardia* found in humans, dogs, cats, and beaver sources are not host-specific and will readily establish in other animal species All three species are important as potential sources of *Giardia* for epidemics of waterborne giardiasis, but the beaver probably plays the most important role because of its closer association with water used by communities as their source of domestic supply Therefore, a study of the host-parasite relationship between beaver and *Giardia*, together with a study of the factors predisposing toward an epidemic of waterborne giardiasis (water pH, hardness, temperature, etc.) is the next logical step necessary to understand waterbrone giardiasis

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***T. H. Ericksen** is the EPA Project Officer (see below).*
The complete report, entitled "Cross Transmission of Giardia," *(Order No. PB 83-117 747; Cost: $8.00, subject to change) will be available only from:*
National Technical Information Service
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Springfield, VA 22161
Telephone: 703-487-4650
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