Appendix 3B. Diseases of fishes and water-borne diseases: Representative diseases of potential concern related to biota transfers associated with water diversions

The list of biota of concern developed in collaboration with Reclamation and Technical Team included species of microorganisms that are considered in this appendix as representative disease or toxin-producting agents. As a subset of biota of concern (Table 1), these species complement those species previously considered in Appendix 3A.

Table 1. Diseases of fishes and waterborne disease agents as representative biota of concern[1]

Protozoa and Myxozoa	***Bacteria and viruses***
Myxobolus (*Myxosoma*) *cerebralis*	Enteric redmouth (ERM)
Polypodium hydriforme	Infectious hemtopoietic necrosis virus (IHNV)
*Cryptosporidium parvum**	*Escherichia coli* (various serotypes)*
*Giardia lamblia**	*Legionella* spp.*
	Salmonella spp. (including, but not limited to *S. typhi, S. typhmurium,* other *Salmonella* serotypes and other water-borne infectious diseases)*
Cyanobacteria	
*Anabaena flos-aquae**	
*Microcystis aeruginosa**	
*Aphanizomenon flos-aquae**	

The species considered in this appendix represent biota which are dependent on dispersal of host (primary or intermediate) to expand their current range (e.g., fish diseases such as *Myxosoma cerebralis* and *Polypodium hydriforme*) or agents of waterborne disease that currently have a presence in both Missouri River and Red River watershed (hence, shifts in metapopulations may

[1]Asterisk "*" indicates that these species occur in both source and receiving systems, but interbasin water transfers may infer risks beyond baseline levels, if shifts in metapopulations are realized consequent to water diversion.

be related to interbasin water transfers). Here, we briefly summarize life history information for each representative, particularly those attributes that influence their potential for being transferred between Missouri River and Red River watersheds consequent to potential interbasin water diversions.

While considering the role of epizootic disease or low occurrence infection, and the potential for cross-species transmission of disease agents is always a possibility, interactions between hosts and infectious agent may be subject to stochastic events that influence cross-species infectivity and agents "jumping" from one species to another (see, e.g., Krauss et al 2003). For the current effort, we consider disease events as "simple" outcomes associated with interbasin water transfers, given the biota may move collaterally from source waters to receiving systems. The brief life histories of biota of concern that follow are intended to provide background and context for the evaluation of risks considered in this work (Section 3 and Section 4), and as such are organized as two major breakout groups: those disease agents primarily associated with fishes and those disease agents primarily associated with terrestrial and wetland vertebrates, including humans. The inclusion of disease agents having well-defined roles in human health should not be interpreted as public health issues being primary drivers in this analysis. Rather, agents of human disease, often being zoonotic in character, serve as representative biological agents included in the range of disease processes potentially of interest when biota transfers are concerned, especially when transfers could potentially be associated with increased likelihoods for occurrence of emerging infectious disease.

Overview: Disease of fishes

Fishes are susceptible to a number of parasites and infectious diseases which potentially yield individuals with reduced health and lifetimes, if not premature death. If apparent within populations, reductions in individual health can be expressed as increased morbidity and mortality within a region. If widespread, the disease may be characterized as an epizootic. From a quantitative perspective, disease-related mortality is best documented for hatcheries and aquaculture facilities, although field observations of disease outbreaks are not uncommon for

some diseases related to causal agents frequently associated with human interventions (e.g., intentional or accidental poisonings, fish-kills related to environmental conditions such as temperature extremes, toxic chemical releases). Diseases of freshwater fishes commonly associated with ichthyofauna of the Upper Missouri River and the Red River are listed in Table 1. Selected disease agents included in this list were considered as biota of concern from interbasin water transfers between these river basins.

Table 1. Commonly encountered fish diseases of concern to resource managers during biota transfers that potentially result from interbasin water diversions of various magnitudes.

• Lamprey infestation	• Sessile, solitary, ectocommensal ciliate infestations
• Leech infestation	• Sessile, colonial, ectocommensal ciliate infestations
• Copepod infestation/infection	• Typical water mold infection
• Branchiuran infestation	• Atypical water mold infection
• Monogenean infestation	• Branchiomycosis
• Protozoan ectoparasites: general features	• Columnaris infection
• Ich infection	• Bacterial cold water disease
• Trichodinosis	• Bacterial gill disease
• *Chilodonella* infestation	• Lymphocystis
• Tetrahymenosis	• Epitheliocystis
• Freshwater velvet disease	• Miscellaneous skin and gill diseases
• Ichthyobodosis	• Incidental findings (e.g., stressor interactions manifested as disease states)
• Gill Cryptobia infestations	
• Gill amoebic infestations	

External parasites of fishes. External parasites of fishes that could be transfered between watersheds interconnected by natural (e.g., flooding) or anthropogenic “bridges” are numerous, but following the lead of Noga (1996), the simple listing in Table 1 illustrates potential disease-

causing organisms as species spearheading biological invasion (e.g., an emerging disease previously not recorded to the receiving system) or shifts in metapopulations between watershed (e.g., an increase occurrence of a disease previously characterized for the receiving system). A facet of biota transfers that is incompletely understood is that associated with the loss of parasites and disease agents upon invasion – the so-called “Enemy Release Hypothesis” (see, e.g., Colautti et al 2004, Poulin and Mouillot 2003,Wolfe 2002) which may be similar to the loss of parasites observed when anadromous fishes move from freshwater to marine habitats. Subsequent to movements from one basin to another, other parasites are likely going to be acquired from the “new” environment, and the variety of parasites may increase among the invasive species emigrating to receiving waters.

Bacterial, viral, and fungal diseases of fishes. Fishes are susceptible to numerous bacterial, viral, and fungal diseases, and some of the more common bacterial diseases to North American waters include furunculosis, bacterial kidney disease (BKD), coldwater disease (CWD), vibriosis, and enteric redmouth disease (ERM) (see, e.g., Noga 1996, Hoffman 1999, Wolf 1988). Whether considering disease agents and their impacts on wild stocks or stocks in hatcheries or controlled facilities, the manifestations of disease can be conveniently assigned to categories, e.g., acute infectious disease, chronic infectious disease, diseases characterized by skin lesions, and diseases characterized at post-mortem conditions. For the most part, but especially in wild stocks, frank disease is difficult to characterize unless altered states of morbidity or mortality are evident (e.g, acute episodes manifested at “fish kills” or skin lesions presented as signs of acute or chronic disease). For example, fishes encounter a range of fungi during their various life stages with *Saprolegnia* spp. being a common opportunistic infection in a wide range of aquatic vertebrates and invertebrates. Similarly, furunculosis caused by *Aeromonas salmonicida*) can be a problem in freshwater fishes, and the disease is widespread across numerous species (see, e.g., Cipriano and Bullock 2001). No natural waters with resident fish populations are considered free of disease, and furunculosis can be a source of significant mortality in wild populations, e.g., if water temperatures in a river become unusually high for extended periods. And, bacterial kidney disease (BKD) is a chronic infection of salmonids fishes, especially in culture environments (e.g., hatchery

and aquaculture), which spreads both vertically and horizontally. Once established, BKD may be difficult to control and virtually impossible to cure. BKD is present in Canada as well as the US (see, e.g., Bullock and Herman 1988). Prevention, and in the absence of prevention, control of any disease process – bacterial, fungal, viral – under field conditions is challenging, and under cultured conditions, while more manageable, still requires time and resources that ultimately reflect investments that may not be fully appreciated in long-term gains anticipated by resource managers.

For the current investigation, biota of concern included Enteric redmouth (ERM) and Infectious hemtopoietic necrosis virus (IHNV).

[2]**Enteric Redmouth Disease.** Enteric redmouth disease (ERM) is a systemic bacterial disease caused by *Yersinia ruckeri* (Family Enterbacteriacae). Salmonids such as rainbow trout, *Oncorhynchus mykiss*, are particularly responsive to infection, and ERM occurs in salmonids throughout Canada and US waters in both wild populations and in culture environments. ERM generally expresses itself by sustained low-level mortality, eventually resulting in high losses. Epizootics can occur if chronically infected fish are stressed during hauling (e.g., transport of hatchery-reared fish), or exposed to other poor environmental conditions (e.g., altered water quality for wild populations) in the wild. In hatcheries, treatment for ERM is accomplished with medicated diets or by intraperitoneal injections for adult fish or through commercially-available vaccines and surface disinfection of eggs in culture facilities.

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[2]Original material derived from Fish Disease Leaflet 82: Enteric Redmouth Disease of Salmonids, G. L. Bullock and R. C. Cipriano, U.S. Fish and Wildlife Service, National Fisheries Research Center-Leetown, National Fish Health Research Laboratory, Box 700, Kearneysville, West Virginia. 25430 United States Department of the Interior, Fish and Wildlife Service. 1990 (Revision of Fish Disease Leaflet 57 (1979), same title, by G. L. Bullock and S. F. Snieszko and Fish Disease Leaflet 67 (1984) by G. L. Bullock.)

ERM was first reported in rainbow trout from Idaho in the 1950's, then described by Rucker in 1966 (Rucker, 1966). In epizootics fish typically present with clinical signs of lethargy, anorexia, and subcutaneous hemorrhages in and around the mouth, oral cavity, and isthmus (hence, the disease's common name), and at the base of fins. Gill filaments may be hemorrhagic, and petechial hemorrhages may occur on the surface of the liver, pancreas, pyloric caeca, and swim bladder, and in the lateral musculature. Splenomegaly is generally observed, with the organ being highly friable. Inflammation occurs throughout the gastrointestinal tract, with the lower intestine becoming charged with a thick, yellowish exudate. Exophthalmus occurs, commonly accompanied by hemorrhages around the ocular cavity and iris, with the eyes frequently becoming ruptured as the disease progresses. Histologically, tissues from infected trout display acute bacteremia and attendant inflammatory responses in virtually all tissues. Upon microscopic examination, bacteria are conspicuous in vascular tissues and in zones of petechial hemorrhage (Rucker 1966). Bacterial colonization occurs in the capillaries of highly vascularized tissue (e.g., gill and kidney) which is followed by dilation of small blood vessels; petechial hemorrhages; erythrocyte congestion; and edema of the kidneys, liver, spleen, heart, and gills. Focal necrosis may occur in the liver, and marked accumulations of mononuclear cells in periportal areas. Hemorrhages develop in outer portions of the digestive tract, and the lining or mucosa becomes edematous and necrotic, frequently sloughing off into the lumen (Busch 1983). Atypical infections sometimes occur, in which case hemorrhages do not develop on the mouth and gill cover, and fish merely become dark and swim near the surface (Frerichs et al. 1985). If fish survive, their skin darkens and their behavior becomes altered, e.g., the typical survivor shuns other fish and seeks shelter (Busch 1983; Rucker 1966). Busch and Lingg (1975) showed that 25% of the rainbow trout surviving an experimental ERM challenge became asymptomatic carriers in which the bacterium was localized in the lower intestine. Such trout serve as reservoirs of infection.

There are two serotypes of *Y. ruckeri*, the first isolated from salmonids in the Hagerman Valley, Idaho (Type I; Ross et al. 1966; Busch 1983). At present, most *Y. ruckeri* linked to ERM are Type I, although O'Leary (1977) has described another serotype of *Y. ruckeri* isolated from Pacific salmon (*Oncorhynchus* spp.; Type II). Additional research has characterized as many as

five distinct serotypes (Pyle et al. 1985, 1987; Stevenson and Airdrie 1984), but only Types I and II are causally linked to ERM epizootics. The original source of *Y. ruckeri* is uncertain, since the isolate from Idaho was contemporaneous with isolates in West Virginia and Australia in the 1950's (Bullock et al. 1977).

Natural infections spread from fish to fish by direct contact with infected fish or carriers. Rucker (1966) transmitted the disease by exposing healthy rainbow trout to waterborne bacteria shed by infected trout, but vertical transmission has yet to be documented and probably does not occur. Stressors have been shown to play a significant role in triggering ERM outbreaks (Hunter et al. 1980). Experimentally, incubation takes 5 to 10 days at13 to 15°C, although the time course for outbreaks in the wild are undoubtedly affected by temperature, pH, and dissolved oxygen among other chemical and physical stressors.

Since its early characterization, geographic ranges of host and disease have increased. Since its initial isolation in Idaho which was associated with transportation of carrier fish, ERM has spread to virtually all trout-producing regions of the United States and Canada. The host range has also expanded to include other salmonids (e.g., Atlantic salmon and Pacific salmon) and non-salmonids such as emerald shiners, *Notropis atherinoides* (Mitchum 1981); fathead minnows, *Pimephales promelas* (Michel et al. 1986); goldfish, *Carassius auratus* (McArdle and DooleyMartyn 1985); and farmed whitefish, *Coregonus* spp. (Rintamaki et al. 1986).

Given the nearly 50 years since its description, management tools have been developed to control, and when possible, prevent ERM. A practical and commercially available bacterin was developed in the early 1960's and 1970's (Klontz 1963; Ross and Klontz 1965; Anderson and Ross 1972; Anderson and Nelson (1974). Then, Croy and Amend (1978) demonstrated that fish could be immunized by immersion in a hyperosmotic solution of sodium chloride, followed by immersion in the bacterin. Commercial ERM bacterin was first licensed in 1976. Other management tools have also been developed, including antibacterials (e.g., oxytetracycline, erythromycin, quinolones (e.g., initial reports of Ceschia et al. 1987; Bullock et al. 1983).

Summary of occurrence of ERM in Missouri River and Red River watershed in the US and Canada. In Figure 1the dot map summarizes the current status of known occurrences of ERM (red dots are sample locations with known occurrence; green dots are sample locations known to be negative) in Missouri River (HUC 10) and Red River (HUC 09). ERM in Canada is widespread, but much of the data reflect disease occurrence in hatchery-reared salmonids and discussions regarding the risks potentially associated with infected hatchery stock entering wild

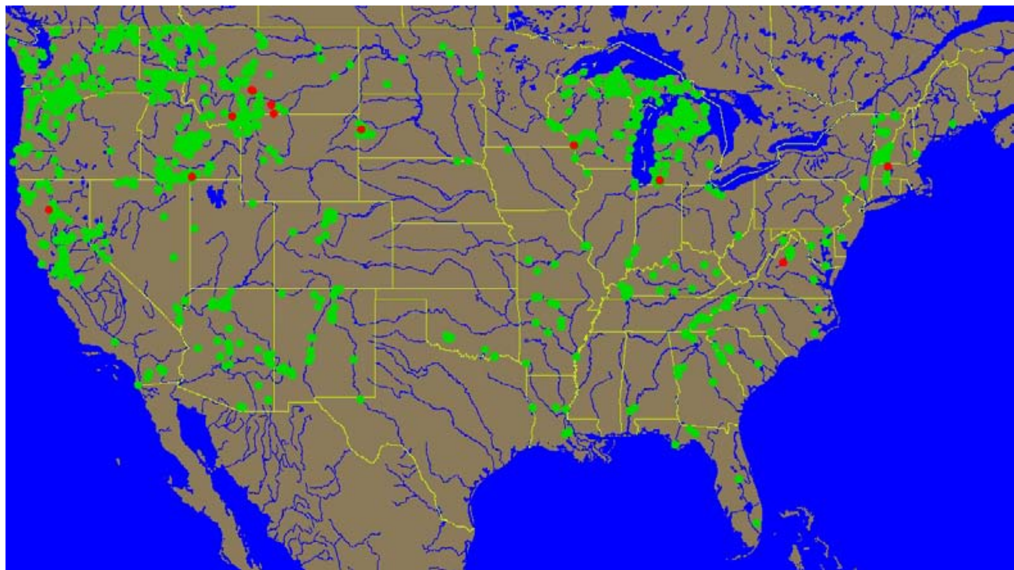

Figure 1. Dot map summarizing sampling locations (green dots) and known occurrences of ERM (red dots) in US surface waters (see US Fish and Wildlife Service, National Fish Health Survey, available online at http://wildfishsurvey.fws.gov/).

populations (see, e.g., http://www.intrafish.com/laws-and-regulations/report_bc/vol3-vi.htm; http://home.hisf.no/SVENJO/Undervis%20fra%20C/FISKESYK/fishhlth.htm#1).

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Viral diseases of fishes. Fishes exhibit a number of viral diseases manifested in the wild and under controlled conditions in hatcheries or other culture facilities (see, e.g., Wolf 1988, Hoole et al 2001 on cyprinds, Roberts and Shepherd 1997 on salmonids). For example, in hatchery culture salmonids frequently diagnosed viral diseases (see, e.g., Roberts and Shepherd 1997) include infectious pancreatic necrosis virus (IPN), salmon papilloma, and infectious hematopoietic necrosis virus (IHNV), the latter disease identified as one of the biota of concern in the current investigation. Each of these diseases may also be observed in wild stocks.

[3]**Infectious hematopoietic necrosis virus (IHNV)**. Infectious Hematopoietic Necrosis Virus (IHNV) is a bullet shape rhabdovirus that effects primarily in salmonid populations. IHNV is endemic to the Pacific Northwest where the virus was first isolated from a disease outbreak in 1953 at two fish hatcheries in the state of Washington. IHNV was reported through the remainder of 1950's and 1960's throughout the Pacific Northwest and caused unprecedented high mortality in salmon production (Wolf, 1988). IHNV received its name because the primary histological manifestation is the necrosis of hematopoietic tissue of the anterior kidney (Amend et al., 1969). However, this virus presents with an acute, systematic infection and causes viremia with hemorrhage and necrosis of many organs and tissues. Other names that have been given to this virus were: British Columbia Virus, the Oregon Sockeye Virus, and the Sacramento River Chinook Disease Virus. (Chiou, 1996).

Five serotypes of IHNV have been classified on the basis of the molecular weight differences of the viral components. The distribution of the virus first characterized from the Northwest has subsequently been observed throughout the United States and Canada and has been identified in

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[3]Original material prepared by Dr. Jo-Ann Leong from Oregon State Universiy, Dept. of Microbiology; Chiou, P.P. (1996), A Molecular Study of Viral Proteins in the Pathogenesis of IHNV, (PhD dissertation, Department of Microbiology, Oregon State University; Infectious Hematopoietic Necrosis Virus (IHNV): IHNV Factsheet (February 11, 2002); Linda Bootland, Assistant Professor at Oregon State University.

Minnesota, Montana, South Dakota, Alaska, West Virginia and British Columbia. It has also been observed in Europe and Asia with outbreaks reported in France, Italy, Belgium, Japan, Taiwan, and Korea. The spread of IHNV is believed to have originally been from the practice of feeding fry with meal composed of ground adult fish and viscera, but more recently it has been a consequence of shipping IHNV contaminated eggs and fry from the Pacific Northwest of the United States and from Canada (Wolf, 1988).

IHNV infections may cause severe mortalities in young fish, generally as fry or fingerlings. Survival and percent mortality from IHNV are directly correlated to the age and size of the fish. The younger the fish, the more susceptible they are to this disease. Young fish infected with the virus present with external signs of infection within a week of exposure. Moralities usually begin four or five days after exposure with peak counts about ten days after exposure. Generally, after 40 or 50 days there are usually no more moralities (Chiou, 1996).

The most accepted route of infection is through the gills, skin or GI track. When fish first contract IHNV, they may die without any clinical signs but usually they are moribund and lethargic with periods of sporadic whirling or hyperactivity (Wolf, 1988). Fry may also be dark in color, have a distended abdomen, pale gills, expothalmia, hemorrhaging of musculature and vase of fins, and cast-like excretion trails. Internally fish appear anemic, there is petechial hemorrhaging of mesenteries or visceral tissues and musculature, and the stomach or intestine is filled with milky or watery fluids (Reno, 1998). Additionally, there is extensive necrosis of the hematopoietic tissues of the anterior kidney and spleen. Necrosis is usually observed throughout most internal organs of infected fish.

The host range of IHNV is relatively broad and is known to naturally infect various salmonids, including rainbow trout (*Oncorhynchus mykiss*), chinook salmon (*O. tshawytscha*), sockeye salmon (*O. nerka*), pink salmon (*O. gorbuscha*), chum salmon (*O. keta*), coho salmon (*O. kisutch*), amago salmon (*O. rhodurus*), yamame salmon (*O. masou*), Atlantic salmon (*Salmo salar*), and cutthroat trout (*S. trutta*). Bull trout (*Salvelinus confluentus*), mountain whitefish

(*Prosopium williamsoni*) and the marine fish seabream (*Archosargus rhomboidalis*) and turbot (*Scopthalmus maximus*) have been experimentally infected with IHNV. Other hosts may include mayflies, copepods and leeches.

IHNV transmission. The epizootiology of IHNV is not completely understood and the source of the virus infecting salmonids is still unknown (Bootland and Leong, 1999). Transmission of fish diseases may occur through vertical or horizontal vectors. Horizontal transmission, from infected to uninfected fish, may occur through feces, urine, and ovarian or seminal fluid. Additionally, IHNV has demonstrated that it may survive for several months in water and infect fish (Mulcahy et al., 1983). IHNV is then either absorbed through the skin and gills or it is consumed orally. Therefore, infected water may also be a potential source of viral infections, but it is not likely that IHNV could survive a winter in the environment.

There are many factors that may influence transmission and virulence. Susceptibility to the virus decreases with an increase in fish age and weight (LaPatra et al., 1994). Other factors that influence virulence or transmission are geographic location, genetics, fish health or stress, fish density and temperature (Bootland and Leong, 1998). One of the most important factors that influence epizootics is temperature. IHNV outbreaks usually occur in water temperatures from 10º C to 12º C, but IHNV has been known to kill trout fry from 3 to 18 Cº (Bootland and Leong, 1998).

IHNV epizootics usually occur in very young salmonid fingerlings and fry, but may also infect older fish. It usually causes an acute disease in young fish with mortalities of fry and fingerlings as high as 90% (Bootland and Leong, 1998). Interestingly, the virus may only be isolated up to approximately fifty days after viral exposure and thereafter is usually not isolated again until the fish nears sexual maturity (Bootland and Leong, 1998). The survivors of epizootics are presumed to be carriers, apparently for life. However, the virus is difficult to isolate during this stage.

Horizontal transmission is a major component of viral spreading during an epizootic, both in

nature and in husbandry (Wolf, 1988). Horizontal transmission is most likely to occur from contact between adults during spawning, from fry to fry, or from eating fish excrement floating in the water. Vertical transmission, from an adult to its progeny, occurs through ovarian or seminal fluid. However, vertical transmission has not been well documented. Other sources of infection other than fish may exist and transmission may be occurring from an unknown reservoir or host. For example, several invertebrates have been shown to carry this virus. IHNV has been isolated from copepods, mayflies and salmon leaches (Mulcahy, 1990).

The most frequent evidence for vertical transmission is the association between the shipment of infected eggs into new geographic areas and resultant outbreaks of IHNV (Bootland and Leong, 1998). However, there are conflicting views as to how vertical transmission occurs. The controversy lies in whether the virus is on the egg surface or within the egg. Observations that disinfecting does not always prevent IHNV infection of progeny suggest that the virus is within the egg (Amend, 1975). However, examination of egg shells for IHNV has yet been done (Bootland and Leong, 1998).

There are two possible mechanisms for the observance of IHNV in spawning adults. The first possibility is that the virus may be entering a latent state in fish that survive an IHNV epizootic. Then the virus is reactivated when the fish becomes sexually mature. The second possibility is that fish surviving an epizootic are then completely clear of the virus but become reinfected prior or during their spawning migration. Currently, there is still much to be learned about the state of the virus in fry and smolts that survive IHNV epizootics. Some studies have shown that IHNV could be isolated from adult sockeye salmon during the ocean phase of their life cycle (Traxler et al., 1997). Other studies have also shown that IHNV survivors are latently infected with IHNV, but it has not been possible to show a reactiviation of the latent state or identify conditions that trigger reactivation (Bootland and Leong, 1998). Thus, there is a possibility that a combination of the two hypotheses accounts for the infection of sexually mature fish (Bootland and Leong, 1998). There is also the possibilty of alternate reseviors, both marine and fresh water.

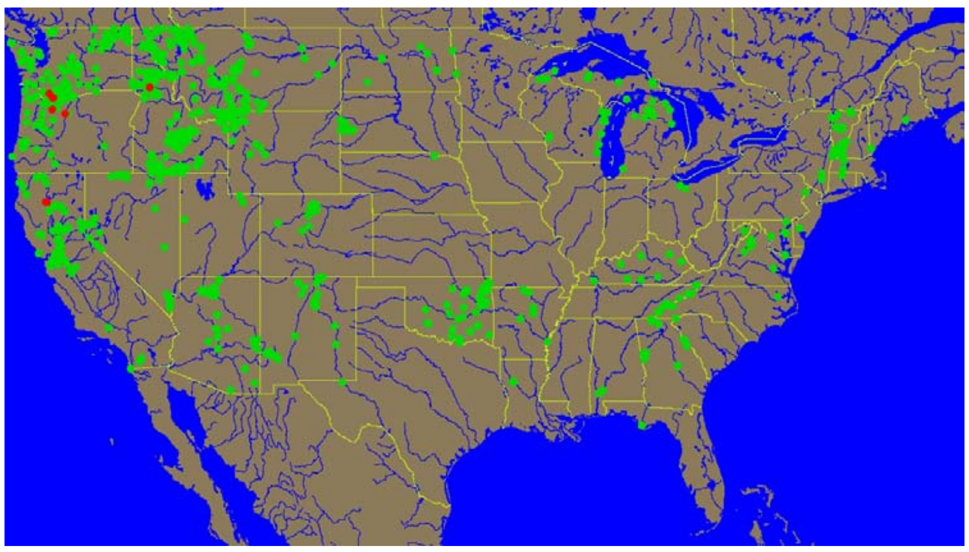
Sources of IHNV other than salmonid fish have not been identified, but potential sources include freshwater and marine invertebrates, sediment and other fish species (Bootland and Leong, 1998). IHNV has been isolated from several different types of invertebrates; such as parasitic copepods, mayflies and leeches. The virus has also been isolated from sturgeon and suckers. The transmission of the virus from these potential reseviors could occur through the salmonids actually eating the infected organisms or the virus could be transmitted through the water. Transmission from invertebrates to salmonid fish has not been demonstrated, but the role of alternate resevoirs can not be ruled out (Bootland and Leong, 1998).

Prevention and control. While IHNV primarily is a disease of salmonid fishes, commonly steelhead trout (*O. mykiss*), sockeye salmon (*O. nerka*), chinook (*O. tshawytscha*), chum (*O.keta*), coho (*O. kisutch*), and Atlantic salmon (*Salmo salar*), the disease has also been observed in pike fry (*Esox lucius*) and other species under experimental conditions. Historically, the geographic range of IHNV was limited to the Pacific Rim of North America but, more recently, the disease has spread to continental Europe and Asia. Disease control in the field is singly focused on prevention from stocked fish, but in hatchery settings, control methods currently rely on disinfection of fertilized eggs. Eggs, alevins and fry should be reared on virus-free water supplies in premises completely separated from possible IHNV-positive carriers. Broodstock from sources with a history of IHNV outbreaks should also be avoided wherever possible. At present, vaccination is only at an experimental stage. As with viral haemorrhagic septicaemia virus (VHSV), good over-all fish health condition seems to decrease the susceptibility to overt IHNV. Handling and other types of stress frequently cause sub-clinical infection to become overt.

In Canada, Fisheries and Oceans Canada maintains a complete listing of the occurrence of this virus in wild stocks in British Columbia. IHNV has been recently identified as the cause of significant mortalities in farmed and enhanced salmon in the Pacific Northwest. Outbreaks of the disease in wild salmon have been reported primarily in juvenile sockeye and occasionally chum salmon in freshwater. IHNV has also affected immature kokanee (freshwater sockeye) adults.

Outbreaks of this disease in Atlantic salmon farms in British Columbia occurred in 1992, 1995, 1996,1997 and 2001. All reported cases occurred within the Campbell River area. IHNV is present in wild fish stocks, in particular, sockeye salmon. It is likely that the disease is transferred from wild fish to farmed salmon, but given the disease is present in wild salmon, additional risk of impact on wild stocks from IHNV farm outbreaks is considered relatively low, provided health status of released fish is documented.

Current status of IHNV in Missouri River and Red River watersheds in the US and Canada. Figure 2 summarizes the current status of IHNV throughout the US (red dots are sample locations with known occurrence; green dots are sample locations without known occurrence).



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Iridovirus.[4] At the October 28, 2003 stakeholder meeting in Fargo, North Dakota, concerns of biota transfers of iridovirus of fishes were expressed. Request was made of USGS/BRD/CERC to briefly review existing information on these disease agents as potential biota of concern for interbasin biota transfers. In part, these concerns stemmed from observations of transfers potentially linked to hatchery-reared sturgeon from the fish hatchery located near Garrison Dam in North Dakota.

Numerous sturgeon species have been integral components of significant fisheries within North America and have played an important historical role in recreational and commercial fisheries of various riverine and Great Lakes communities throughout the United States. Present commercial fisheries for sturgeon species are virtually non-existent, in part due to overexploitation coupled with an inherently long period of time for sturgeon species to become sexually mature.

Two species within the Missouri River basin – pallid sturgeon (*Scaphirhynchus albus*), which was considered as biota of concern in the current investigation, and shovelnose sturgeon (*Scaphirhynchus platorynhus*) – have been recently found to harbor a suspect virus (currently being referred to as the Missouri River Sturgeon Iridovirus, or MRSIV), very similar to but different from the White Sturgeon Iridovirus (WSIV; see Figure 3). Currently, MRSIV has been detected only in captive propagated sturgeon in US Fish and Wildlife Service facilities and in wild shovelnose sturgeon collected in the Missouri River below Ft Peck. Both shovelnose and pallid

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[4] See also:

Williamson, D.F., 2003, Caviar and Conservation, Status, Management, and Trade of North American Sturgeon and Paddlefish, TRAFFIC North America, World Wildlife Fund, Washington, D.C., 252pp.

LaPatra, S.E., J.M Groff, G.R. Jones, B. Munn, T.L. Patterson, R.A. Holt, A.K. Hauck, and R.P. Hedrick. 1994. Occurrence of white sturgeon iridovirus infections among cultured white sturgeon in the Pacific Northwest. Aquaculture 126:201-210.

http://fwp.state.mt.us/fwppaperapps/wildthings/2002annualreport.pdf

sturgeon have been diagnosed with the iridovirus agent. In USFWS Region 6, three Service facilities have cultured sturgeon in which the iridovirus was detected: Gavins Point National Fish Hatchery, Valley City National Fish Hatchery, and Garrison Dam National Fish Hatchery.

Current information regarding the significance of the iridovirus in Missouri River sturgeon species is lacking in the following areas: a) its host and geographic range in wild populations, b) its transmissibility to other species of sturgeon and the question of vertical transmission from parents to progeny, c) the utility of existing sturgeon cell fines and primary cell cultures, and d) applicable diagnostic and monitoring procedures for both latent and patent infections.

Figure 3. White sturgeon iridovirus sample locations (green dots, sampled but no record of occurrence for WSIV)

As noted in Appendix 3A and main body of this report, Pallid sturgeon are Federally listed as an endangered species, not legally catch-able and subject to a multi-agency recovery effort. The current recovery plan calls for supplemental propagation programs to provide absolutely essential recruitment in the Upper Missouri River basin where natural recruitment is non-existent and has been so for over 20 years. Without releasing hatchery propagated sturgeon into the wild, to pass on the gene pool from the aging pallid sturgeon population, the species will become extinct in the upper basin. Service facilities in Region 6 have implemented culture programs and management

activities to assist in the recovery effort. The intensive culture of the pallid and shovelnose sturgeon presents fish health concerns. As with most fish pathogens, the iridoviral agent can be associated with mortalities in cultured sturgeon but has not been identified as a mortality factor in the wild.

The significance of the iridoviral agent in shovelnose and pallid sturgeon is not entirely known, primarily as a function of our lack of knowledge regarding the epizootiology and life cycle of the viral agent. Management decisions relative to both species, must be based on good science with regard to pathogen detection and significance. Improved management decisions can be made if we have a good understanding of the naturally occurring presence of this virus in wild populations of both species. Lack of thorough information is currently resulting in management decisions that err on the side of caution regarding stocking of positive or suspect sturgeon. In the not too distant future, decisions will need to be based on the need to prevent extinction of the species as the wild population continues to age toward senility and death.

Fungal diseases of fishes[5]

Fungal diseases of fishes were not included as biota of concern, yet are briefly consider for completeness. While fungal disease agents potentially transferred as a consequent of interbasin water diversions are already present in both source and receiving basins, the potential for shifts in metapopulations does exists, although at a relatively low level. Fungi can become a problem, if fish are stressed by disease, by poor environmental conditions, receive poor nutrition, or are injured. If these factors weaken the fish or damage its tissue, fungus can infest the fish. Fungi can also prevent successful hatching when it invades fish eggs (see, e.g., Bruno and Wood 1999,

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[5]Material as excerpt of: Technical Fact Sheet VM 97 (College of Veterinary Medicine, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida (February 1996); see http://edis.ifas.ufl.edu/BODY_VM033, last accessed December, 12, 2004.

McVicar 1999).

Fungi are grouped by the morphology of various life stages. All fungi produce spores which readily spread disease, since fungal spores are relatively resistant to heat, drying, disinfectants and the natural defense systems of fish. The three most common fungal diseases are Saprolegniasis (see Bruno and Wood 1999), Branchiomycosis, and Ichthyophonus disease (McVicar 1999).

Saprolegniasis. Saprolegniasis is a fungal disease of fish and fish eggs most commonly caused by the Saprolegnia species, the "water molds." The disease is common in fresh fishes, especially in species occupying warmer waters. *Saprolegnia* spp. can grow at temperatures ranging from 32˚ to 95˚F but seem to prefer temperatures of 59˚ to 86˚F. The disease will attack an existing injury on the fish and can spread to healthy tissue. Poor water quality (e.g., water with low circulation, low dissolved oxygen, or high ammonia) and high organic loads, including the presence of dead eggs, are often associated with *Saprolegnia* infections. The presence of *Columnaris* spp. bacteria or external parasites are also common with Saprolegniasis.

Disease Signs. Saprolegniasis is often observed as fluffy tufts of cotton-like material – colored white to shades of gray and brown – on skin, fins, gills, or eyes of fish or on fish eggs. These areas are scraped and mounted on a microscope slide for proper diagnosis. Under a microscope, Saprolegnia presents as hyphae.

Management and Control. Saprolegniasis is best prevented by good management practices – such as good water quality and circulation, avoidance of crowding to minimize injury (especially during spawning), and good nutrition. Once Saprolegnia is identified in an aquatic system, sanitation should be evaluated and corrected. Disease outbreaks are common under cultured conditions (e.g., hatcheries), and if mortality is observed under these conditions, medication is appropriate.

Branchiomycosis. Branchiomyces demigrans or "Gill Rot" is caused by the fungi *Branchiomyces*

sanguinis and *B. demigrans*. Branchiomycosis is a pervasive problem in Europe, but has been only occasionally reported by US fish farms. As with diseases in general, both species of fungi are found in fish suffering from an environmental stress, e.g., low pH (5.8 to 6.5), low dissolved oxygen, or a high algal bloom. *Branchiomyces* spp. grow at temperatures between 57˚ and 95˚F with optimal growth occurring between 77˚ and 90˚F. The main sources of infection are the fungal spores carried in the water and detritus on pond bottoms. Again, disease is infrequent observed in field settings, and is more commonly observed in culture facilities.

Disease Signs. *B. sanguinis* and *B. demigrans* infect the gill tissue of fish. Symptomatically fish may appear lethargic and may be seen gulping air at the water surface (or piping). Gills appear striated or marbled with the pale areas representing infected and dying tissue. High mortalities are often associated with this infection.

Management and Control. Avoidance is the best control for Branchiomycosis. Good management practices will create environmental conditions unacceptable for fungi growth. If the disease is present in hatchery stock, infected fish should not be transported, and care must be taken to prevent movement of the disease to uninfected areas. In hatcheries, formalin and copper sulfate have been used to control the disease, but all tanks, raceways, and aquaria must be disinfected and dried as prophylactic measures. Ponds should be dried and treated with quicklime (calcium oxide). Prevention in wild populations is the only practice management tool to avoid disease outbreaks.

Icthyophonus Disease. Icthyophonus disease is caused by the fungus, ***Icthyophonus hoferi*** . The fungus grows in fresh, and wild and cultured fish are equally likely to harbor the disease. The disease, however, tends to be restricted to cool temperatures (36˚ to 68˚F). Spread of the disease occurs via fungal cysts which are released in the feces and by cannibalism of infected fish.

Disease Signs. Because the primary route of transmission is through the ingestion of infective spores, fish with a mild to moderate infection will show no external signs of the disease. In severe

cases, the skin may have a "sandpaper texture" caused by infection under the skin and in muscle tissue. Some fish may show curvature of the spine. Internally, the organs may be swollen with white to gray-white sores.

Management and Control. There is no cure for fish with *I. hoferi*, and infected fish carry the disease for life. Prevention is the only control, and to avoid introduction of infective spores, never feed raw fish or raw fish products to cultured fish. In culture systems, complete disinfection of tanks, raceways, or aquaria must be completed, if eradication of disease agent is a management goal. For ponds with dirt or gravel bottoms, months of drying are required to totally eliminate the fungus.

Regardless of the water source, fish fungi disease agents are cosmopolitan in surface waters. Fungal diseases are often indicative of a more serious water quality problems. Saprolegniasis is the most common fungal disease, but can be eliminated easily after the primary cause of illness has been identified and corrected. On the other hand, Branchiomycosis, a relatively new problem in the US, has caused high mortalities in cultured fish, and is difficult to control. Ichthyophonus disease is a systemic fungal disease and once it enters the fish, there is no cure. The best control for all fungal infections is good management: good water quality, good nutrition and proper handling.

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[6]Protozoan and myxozoan diseases of fishes

The taxonomy of the entire myxozoan group (Lom and Noble 1984) and the *Myxobolus*/*Myxosoma* group in particular (Landsberg and Lom 1991) is very dynamic and the object of much work. While a detailed review of the species current taxonomic status lies beyond the scope of the current investigation, a very brief summary provides the phylogenetic context beneficial to the analysis.

Myxozoa attained phyllum status from some workers, while others regard them as a highly specialized, reduced forms of parasites having complex life cycles as member of the Cnidaria (see, e.g., Roberts and Janovy 2005). Regardless of these higher level taxonomic disputes, the myxozoans are comprised of approximately 1200 species, with nearly 50 genera having been characterized. Regardless of the fundamental issues underlying the taxonomy of the group, the Myxozoa are all parasitic life forms, generally presenting parasitic life-cycles dependent on teleost fish, although representatives are also known from invertebrates, amphibia, and turtles. When fully characterized, their life cycles are relatively complex. A highly abridged overview of life cycle focuses on spores being multicellular in origin, with each spore comprised of 1 to 3 valves (rarely more), each containing one to many sporoplasms. Each sporoplasm contains one to many polar capsules, with each bearing a polar filament that is expelled during invasion of the host.

A generalized myxozoan life-cycle generally finds the earliest stages developmental stages of the parasite in fish (intermediate host) as trophozoites within cells. Depending upon species, the cell

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[6]Original material prepared by Maria E Markiw, U.S. Fish and Wildlife Service, National Fisheries Research Center-Leetown National Fish Health Research Laboratory, Box 700, Kearneysville, West Virginia 25430.

type targeted will tend to be specific, e.g., cartilage for the causative agent of whirling disease. Growth of the trophozoite yields amoeboid cytoplasm eventually increasing in size, while the nucleus undergoes repeated karyokinesis to form a plasmodium (large cytoplasmic mass with many nuclei). Plasmodia grow attached to the epithelium in the coelomic area (i.e. urinary bladder, swim bladder) in coelozoic forms or occur within tissues in species histozoic types. During winter, many coelomic forms (e.g., *Myxobilatus microspora* in urinary bladder of largemouth bass) plasmodia form sheet-like aggregates, then in late spring and summer form long, finger-like extensions that bud off as free-floating plasmodia. Spores then form within the fingers and buds which pass out with the urine (see Booker and Current 1981).

Some cells are destined to form spores within the plasmodia, and a range of sporulation processes have been characterized (e.g., disporoblastic spore formation in *Henneguya exilis* in channel catfish; Current and Janovy 1977). In all types of sporulation, an envelope cell encapsulates a sporogonic cell where multiple cell divisions of sporogonic cell to form new cells that eventually form 2 separate spores within the envelope cell. The number of spores developing from the same cells varies, but ultimately spores liberated into environment where they are ingested by tubificid oligochaetes (definitive host). Once in the definitive host, polar filaments are expelled, valves of the spore separate, and sporoplasm invades gut (usually, intracellular spaces between intestinal epithelial cells). Sporoplasm subsequently undergoes asexual division, often times by multiple fission, which is then followed by gamogony where cells derived from the fusion of different plasmodia. Cell fusion yields 3-valved triactinomyxon spores which are subsequently released into the lumen of the gut and are eventually released upon defecation. Once the triactinomyon spores are reach water, they attach to fish via polar filaments, and sporoplasms invade cells of primary host. Fish can also become infected by ingesting the intermediate hosts, the infected oligochaetes.

Given the number of species in the Myxozoa, only a handful of species are completely characterized with respect to life cycle and identification of primary and intermediate hosts. In aquatic vertebrates (fishes and amphibians), representative genera and species of myxozoans include: *Chloromyxum trijugum* in the gall bladder of centrarchids in North America, *Henneguya*

exilis in the gill filaments of channel catfish in North America, *Myxidium serotinum* in the gall bladder of anurans in the Western hemisphere, *Myxobilatus mictospora* in the urinary bladder of large mouth bass in North America, *Myxobolus cyprini* in muscles of carp in Europe, *Sphaerospora renicola* in the renal tubuli of European carp, *Thelohanellus nikolskii* in cysts on the fins of carp in Europe, and *Myxosoma cerebralis* which infects cartilage tissues in young trout, causing deformities, neurological dysfunction, and death. The current investigation focuses on the causative agent of whirling disease which may have been introduced to North America with brown trout transplants from Europe (see Bartholomew and Wilson 2002).

Myxobolus cerebralis and whirling disease. Whirling disease is a parasitic infection of trout and salmon by the myxosporean protozoan *Myxobolus cerebralis* (Syn. *Myxosoma cerebralis*). This parasite targets cartilagenous tissue, and infection can cause deformities of the axial skeleton and neural damage that results in "blacktail." The disease is named for the erratic, tail-chasing, "whirling" in young fish that are startled or fed. Heavy infection of young fish can result in high mortalities or unmarketable, deformed individuals.

Although the parasite was first reported in 1903 in central Europe (Hofer 1903), its complete life cycle was not described until the early 1980's. Whirling disease occurs throughout Europe (Halliday 1976) where it probably originated. It occurs in the former Soviet Union (Uspenskaya 1955) and was seemingly introduced into British surface waters where it is now common (Elson 1969; O'Brien 1976; Hudson and Holliman 1985). It was accidentally introduced into New Zealand (Hewitt and Little 1972) and into the United States. The detailed history of the disease and its introduction into the United States (into Pennsylvania and Nevada in about 1955) were discussed in a recent review by Hoffman (1990). Although Hoffman provided an extensive list of the present worldwide distribution of the infection, the cited occurrence in several countries may be suspect, because of the methods applied in the detection and identification of spores in earlier studies. *Myxobolus cerebralis* was probably established in North America much earlier than reported because the parasite requires several years to become established at sufficiently high intensity for clinical signs to appear in fish.

In the United States, whirling disease has been detected in 22 states: Alabama, California, Colorado, Connecticut, Idaho, Maryland, Massachusetts, Michigan, Montana, Nevada, New Hampshire, New Jersey, New York, Ohio, Oregon, Pennsylvania, Utah, Virginia, Washington, West Virginia, and Wyoming (Figure 4). Figure 4 illustrates a state-based summary of whirling disease occurrence from its initial characterization in fish from Pennsylvania through its dispersal throughout surface waters of the US.

Figure 4. Spread of whirling disease as indicated by state records (from Bartholomew and Reno 2002)

Figure 5 and Figure 6 present dot maps capturing occurrence data, which illustrate uncertainties associated with "fish disease registration," given the spatial requirements of GIS-based predictive tools such as GARP. For example, the resolution of map projections such as those of Figure 4 are spatially biased and do not readily translate into decision-making processes focused on watershed level management models. Although more data intensive, the strengths of point data and its role in analysis, e.g., of invasive species and their potential for interbasin transfer and their subsequent establishing sustainable populations would be fully supported, if a formal registry of disease occurrence would be maintained for fish pathogens such as *M. cerebralis*.

Figure 5. Dot map ca 2002 supporting state-records map of Figure 4 (from Bartholomew and Reno 2002).

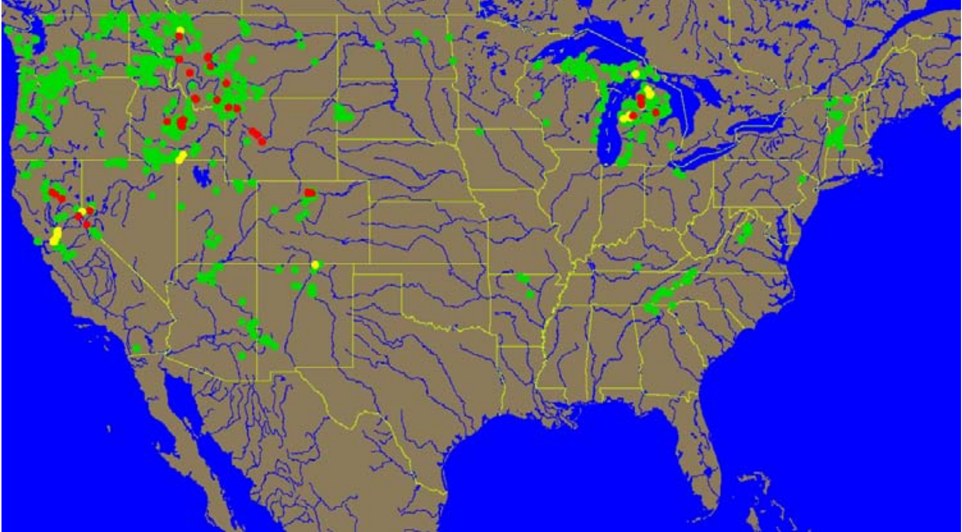

Figure 6. Dot map ca 2004 derived from US Fish and Wildlife Service, National Wild Fish Health Survey at http://wildfishsurvey.fws.gov/.

Diagnosis and clinical signs. Moderate or heavy clinical infection of fish with whirling disease can be presumptively diagnosed on the basis of changes in behavior and appearance. When

alarmed or feeding, some infected individuals show an abnormal whirling behavior. The caudal peduncle and tail may become dark or even black, but these characteristics fade in preserved specimens. The whirling behavior is believed to be the result of impaired coordination caused by neural damage from lesions and disintegration of cartilaginous tissue around the organs of equilibrium. These clinical signs appear, depending on temperature and intensity of the infection, about 35 to 80 days after initial infection and can persist for about a year. Deformities of the axial skeleton or head, shortening of the snout, and cranial depressions persist through the life of the infected fish. These signs are not conclusive, however, since injury, exposure to toxic chemicals, or deficiency in dietary tryptophan and ascorbic acid can evoke similar signs (Wolf et at. 1981). The collective appearance of all signs throughout a population are suggestive of a clinical infection with whirling disease.

To date, no reliable nondestructive serological procedures have been developed for detecting the causal organism of whirling disease in fish. Nonspecific, false positive, and false negative reactions have been found in tested fish (Griffin and Davis 1978; Markiw, unpublished data). The long life cycle of the parasite, about 3 months in fish and 3.5 months in tubificid worms, may result in continual changes of antigenic components.

In gross pathological examination, internal organs appear normal. Histological sections of cartilage, particularly skull, gill, and vertebrae, show areas of lysis and inflammation. Although hematoxylin and eosin stains are routinely used in histology, these stains do not enhance the appearance of spores of *M. cerebralis*, so methylene blue, Giemsa or May-Grünwald Giemsa, or Ziehl-Neelsen stains are recommended because the polar capsules react strongly and make the spores prominent. If the infection has existed for 3 to 4 months (depending on temperature), spores of the myxozoan *M. cerebralis* have had sufficient time to form in or around the cartilage lesions, and the presence of *M. cerebralis* spores in cartilage areas is pathognomonic for whirling disease.

Identification. *Myxobolus cerebralis* is the only myxosporean found in the cartilage of salmonids.

The mature spore is lenticular in side view and nearly circular in front view. The spores are 8 to 10 mm in greatest diameter and have two prominent ovate polar capsules with coiled filaments that may be extruded. Aberrant spores, either in shape or with unequal polar capsules, may be encountered (Lom and Hoffman 1971; Markiw and Wolf 1974a), and the iodinophilous vacuole may not be present. However, the absence of the iodinophilous vacuole is not always reliable taxonomically and can be observed only in fresh spores. Preserved spores are usually about 10% smaller than fresh spores, and may contribute to misidentification of species. Sole identification by morphology may be difficult because *M. cerebralis*-infected fish may have mixed infections with other myxosporeans from the central nervous system, muscle, or skin. For example, spores of other species of *Myxobolus* are similar to *M. cerebralis*, and can occasionally be isolated from the head, but not in the cartilage or bone, of salmonids. These species include:

Myxobolus kisutchi – in the central nervous system of coho salmon (*Oncorhynchus kisutch*) and chinook salmon (*Oncorhynchus tshawytscha*). The preserved spores (formalin) are 7 to 8 mm in diameter, appear uniform in shape, and contain an iodinophilous vacuole.

Myxobolus squamalis – in the scales of rainbow trout (*Oncorhynchus mykiss*) and salmon from the western United States. The preserved spores (formalin) are 8 to 9 mm in diameter and appear uniform, with equal polar capsules and with a narrow ridge that parallels either side of the sutural ridge.

Myxobolus arcticus – in the central nervous system of coho salmon, sockeye salmon (*Oncorhynchus nerka*), Dolly Varden char (*Salvelinus malma*), lake char ("Neyva," *Salvelinus neiva*), Arctic grayling (*Thymallus arcticus*), Arctic char (*Salvelinus alpinus*), and whitefish (*Coregonus clupeaformis*), the fresh spores are large, 14.3 to 16.5 x 7.6 to 7.7 mm, with large, elongated polar capsules (recent description by Pugachev and Khokhlov 1979).

Myxobolus neurobius – in the central nervous system of brown trout (*Salmo trutta*); Arctic grayling; European grayling (*Thymallus*) from central Europe, Eurasia, and North America; and arctic char and wild young Atlantic salmon (*Salmo salar*) in Newfoundland (Maloney et al. 1991). The preserved spores (glycerin) are oval and appear in a wide range of sizes, 10 to 12 x 8 mm (Schuberg and Schroeder 1905); but fresh spores are larger, 13.4 to 14 x 8.5 to 9.2 mm, according to a recent description by Pugachev and Khokhlov (1979).

Myxobolus insidiosus – in the muscle of cutthroat trout (*Oncorhynchus clarki*), chinook salmon, and coho salmon from the western United States. The fresh spores are about the same size and shape as *M. arcticus*, 12.8 to 17.3 x 9 to 11.5 mm.

Histological location and identification of *M. cerebralis* spores in lesions of skeletal tissue, particularly of the head, have been recommended for confirmation of diagnosis, but this may not be reliable for fish infected with only a few spores. A presumptive diagnosis may be based on location, size, and morphology of the spores and epizootiological data such as geographical location and history (e.g., of the reach or hatchery in wild or cultured fish, respectively), the species of fish, and the clinical signs. Diagnosis is usually confirmed by the identification of spores, generally from a direct fluorescent antibody test with rabbit antiserum against *M. cerebralis* or *Triactinomyxon* spores conjugated with fluorescein isothiocyanate (Markiw and Wolf 1978; Markiw 1989a; antiserum prepared at the National Fish Health Research Laboratory has shown cross reactivity with *Myxosoma cartilaginis* of bluegills, *Lepomis macrochirus*).

Life Cycle. Whirling disease presents a two-host life cycle (Figure 7) involving a fish and the aquatic oligochaete *Tubifex* (Markiw and Wolf 1983; Wolf and Markiw 1984; Wolf et al. 1986), and two separate stages of sporogony occur, one in each host. Antigenic homology of the two morphologically distinct spore forms has been demonstrated serologically (Markiw 1989a).

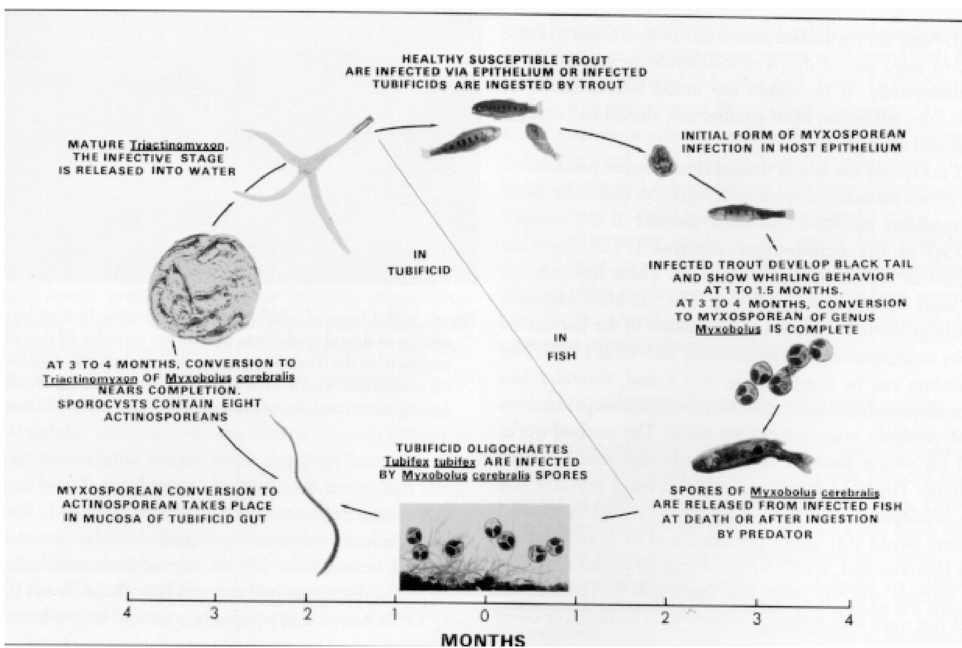

Figure 7. Diagram of 2-host life cycle of *Myxobolus cerebralis*, the causative agent of whirling disease (from Markiw 1992).

In brief, the life cycle of the causative agent of whirling disease begins with spores of *M. cerebralis* released to the aquatic environment when infected fish die and decompose or are consumed by predators or scavengers. The myxosporean-type spores are ingested by tubifex worms in whose gut epithelium the next phase of the life continues. In the oligochete gut epithelium, transformation into the actinosporean, or *Triactinomyxon*, occurs. This developmental stage is infective to fish, and takes about 3.5 months at 12.5ºC to complete development. Once fully developed, Triactinomyxon are released from infected worms into the water for several weeks. The Triactinomyxon spores are much larger and have three polar capsules and three grapple-like appendages, 170 to 180 mm long. Triactinomyxon enter susceptible fish such as rainbow trout through the epithelial cells of the skin, fins, buccal cavity (particularly at the base of the gills), upper esophagus, and lining of the digestive tract. Once infection occurs, transformation into *M. cerebralis* spores takes about 2.6 months at a water temperature of 12.5ºC. The life cycle for *M. cerebralis* was original documented by Markiew (see, e.g., Markiew and Wolf 1974a) and subsequently confirmed by El-Matbouli and Hoffmann (1989); a similar life cycle has been

characterized for *Myxobolus cotti* (El Matbouli and Hoffmann 1987). Although a two-host life cycle of the whirling disease organism is now widely accepted, the parasite has been recycled in fish or tubificids in the laboratory without loss of infectivity. Also, Hamilton and Canning (1987), Prihoda (1983), and Uspenskaya (1978) suggest direct transmission of the parasite from fish-to-fish by way of aged spores, although further study is indicated to detail fish-to-fish transmission.

Transmission. Salmonids contract whirling disease in two ways: by ingesting tubificids that harbor the specific actinosporean Triactinomyxon and by brief contact with waterborne Triactinomyxons released from infected tubificids. The experimentally produced actinosporean stage of *M. cerebralis* is short-lived, persisting 3 to 4 days at 12.5°C and fewer days at warmer temperatures (Markiw 1992b). Studies of the dynamics of the infective stage for fish (Markiw 1986) demonstrated that after a single exposure to *M. cerebralis* spores, a population of infected tubificids can release viable Triactinomyxon spores for as long as a year at a level detectable by only sentinel fish.

O'Grodnick (1975b) demonstrated that whirling disease cannot be transmitted vertically from infected brood stock to the egg. Shipments of salmonid eggs from waters contaminated with whirling disease are also unlikely to disseminate the parasite because rainbow trout are refractory to the infection during hatching and for a day afterward (Markiw 1991). Contrary to reports from eastern Europe and Russia (Prihoda 1983; Uspenskaya 1978), attempts to effect fish-to-fish transmission of whirling disease or through aged-spores of *M. cerebralis* in absence of tubificids have been unsuccessful in other studies.

Development. Development time for both stages of the whirling disease organism, myxosporean in fish and actinosporean in tubificids, is directly related to temperature. Trout fry fed infected worms or exposed to waterborne Triactinomyxon show blacktail after 35 to 45 days at a water temperature of 12.5°C. Whirling behavior first appears at about the same time or slightly later, and fully mature spores are detected after 2.6 to 3.5 months at 12.5°C. Under experimental conditions, following a 3-hour, single exposure to *M. cerebralis* to triactinomyxons, 2-month-old rainbow

trout became infected with spore counts in head cartilage ranging from less than 100 to nearly 2 million at 5 or 6 months and showed limitation of parasitism at the highest levels of infection (Markiw 1992a, 1992b). Development time is shortened or lengthened at temperatures above or below 12.5°C; about 50 days at 17°C and 120 days at 7°C (Halliday 1973).

Development time in the worm is defined as the interval between first contact with *M. cerebralis* spores and the release of the first Triactinomyxon. Experimentally, after single exposure of one population of tubificid worms to *M. cerebralis* spores at 12.5°C, Triactinomyxons were released in a consistent pattern that began at 104 to 113 days post exposure, subsequently peaking during the next 15 to 60 days. Spore production continued at trace levels for about 6 months, and during the next 3 months the infectivity was detectable by only sentinel fish (Markiw 1986). Whether the same infected worms are releasing Triactinomyxons for 11 months or a new generation of worms must become infected with *M. cerebralis* spores to produce infectivity is not known. One tubificid worm, at peak of productivity (about 130 days after exposure) can harbor 900 to 1,000 mature Triactinomyxons.

Reservoir of Infectivity. Trout and salmon can be infected with whirling disease and may harbor *M. cerebralis* spores. Predators and scavengers such as birds (Taylor and Lott 1978) that consume infected fish, can release viable *M. cerebralis* spores into the environment and may disseminate the parasite. The source of the infective agent for fish is usually the water supply or earthen ponds inhabited by aquatic tubificid worms. An outbreak of the disease can occur after stocking with infected fish or transferring fish from facilities where the infection had not yet been detected.

Susceptibility and Host Range. Young and adult trout and salmon are susceptible to *M. cerebralis* infection, but the severity of the infection decreases with age (Markiw 1992a). When fish are infected at an older age, they are usually asymptomatic, healthy-looking, and of normal size, but may carry the spores of *M. cerebralis*. Severe mortalities of 90% or more may occur among newly hatched fish exposed to the infective agent as sac fry; l-day-old rainbow trout are

refractory to the infection (Markiw 1991).

Not all salmonid species are equally susceptible to infection. For example, rainbow trout are most susceptible to the disease and brook trout much less so. Lake trout apparently cannot be infected and do not acquire the disease (O'Grodnick 1979). Other salmonids can be infected, but clinical signs of the disease may or may not develop. In the following list, species are ranked in descending order of apparent susceptibility (O'Grodnick 1979; Hoffman 1990): rainbow trout, sockeye salmon, golden trout (*Oncorhynchus aguabonita*), cutthroat trout, brook trout, steelhead (*Oncorhynchus mykiss*), chinook salmon, Atlantic salmon, brown trout, coho salmon, lake trout (*Salvelinus namaycush*) and splake (hybrids between brook trout and lake trout). Lake trout and splake are refractory to infection with whirling disease. Susceptibility not only varies among species but also among strains and may vary tremendously among individual fish within a population (Markiw 1992a). Grayling (*Thymallus* sp.) and whitefish (*Coregonus* sp. and *Prosopium* sp.), which are generally regarded as salmonids, have yet to be tested and their susceptibility or resistance to whirling disease remains undetermined. Early accounts of whirling disease (Halliday 1976) suggested that non-salmonids were susceptible, but these early reports may be erroneous, and reexamination and identification of spores by current serological methods are necessary for confirmation (Markiew, personal communication).

Tubifex is the only tubificid that has been identified as susceptible to *M. cerebralis* (Wolf et al. 1986), and species of *Limnodrilus*, *Quistadrilus*, and *Ilyodrilus* in mixed populations with *Tubifex* did not produce Triactinomyxon when exposed to *M. cerebralis* spores. Other genera of oligochaetes have also been tested (*Dero*, *Stylaria*, and *Aeolosoma*) but do not produce infectivity for whirling disease (Markiw and Wolf 1983).

Prevention and Control. At the present time, control of *M. cerebralis* infections is difficult. However, application of preventive measures can decrease the intensity of the disease in fish culture facilities and perhaps eliminate the spread to non-enzootic areas. Because tubificids are essential intermediate hosts for development of the infective stage in fish, the avoidance of earthen

ponds for rearing fish should be considered. Tubificids are normal inhabitants of aquatic environments, and are particularly abundant in rich organic soils, occurring as dense red patches in settling basins and streams that carry effluent from trout hatcheries. The life span of *T. tubifex* is about 2.5 to 3 years depending on environmental conditions (USSR Academy of Sciences 1972), and the seasonal variation of oligochaete biomass is commonly observed with the greatest biomass in fall and the least in spring. The phenomenon might correlate with the intensity of reproduction, given breeding and development of oligochaetes are directly associated with temperature (USSR Academy of Sciences 1972).

Earthen ponds and raceways stocked with fish where cleaning is difficult or neglected are ideal habitats for worms and, once introduced, the whirling disease parasite becomes established. Techniques for prevention are periodical disinfection of the facility and the rearing of small trout indoors in pathogen-free water. Smooth-faced concrete or plastic-lined raceways that are kept clean and free of contaminated water keep the facility free of the disease. Disinfection of waterborne infectivity has also been effective and can be achieved by combining filtration to remove or reduce suspended contaminants with ultraviolet-irradiation (Hoffman 1974, 1975). Some chemotherapeutants reduced losses and infection of young trout, but none prevented or totally eliminated whirling disease. Development of spores decreased when young trout were fed furazolidone (Taylor et al. 1973); furoxone, benomyl, and fumagillin (O'Grodnick and Gustafson 1974, 1975); or proguanil and clamoxyquin (AIderman 1986). El-Matbouli and Hoffmann (1991) reported recently that fumagillin, fed to experimentally infected rainbow trout, defected morphology of *M. cerebralis* spores and could prevent a clinical outbreak of whirling disease. Chlorine (sodium hypochlorite), administered weekly for 4 months at concentrations of 0.5 ppm for 2 h to control waterborne infectivity (triactinomyxons) and infected tubificids, suppressed the prevalence of infection by 73% in one group of young trout and by 63% in another group of concurrently exposed young trout (Markiw, unpublished). This chlorine treatment regime was not toxic to trout.

Immune response of fish to the whirling disease pathogen is critical for vaccination against the

disease under culture conditions. Immune response in rainbow trout to *M. cerebralis* was studied early by Halliday (1974), Pauley (1974), and Griffin and Davis (1978), and these studies revealed some evidence that rainbow trout produce antibodies against *M. cerebralis*. Protection against infection, however, has not been demonstrated and the immune response to Triactinomyxon has not been examined. Subsequent studies, however, indicate that host tissue reaction against the pathogen decreased or even eliminated myxosporean infection in lightly infected rainbow trout (Markiw 1992a), which suggests that immunization against whirling disease may work with common specific antigenic components of both stages for producing an immunogen by genetic engineering.

In the past, radical methods of controlling the disease in affected trout hatcheries were used. Infected fish stocks were destroyed and buried and the entire facility disinfected. Present methods of control are less drastic, and approaches to managing hatcheries with infected fish are comprehensively discussed by Hoffman (1990). When fish of a hatchery are infected, the intensity of infection determines what can be done with infected individuals. Infected fish may be slaughtered and smoked for table use (smoking kills the spores; Wolf and Markiw 1982) or placed in enzootic areas. Such arrangements may reduce economic loss to fish culturists. Survey of watersheds for the source of infectivity with susceptible sentinel trout in floating cages (Hnath 1970; Horsch 1987) and the use of more sensitive methods of spore detection help pinpoint contaminated areas.

An evaluation of whirling disease should emphasize the intensity of infection, not simply the presence or absence of *M. cerebralis* in the environment. While a initial characterization of presence-absence is critical in the “discovery process” in identifying dispersal, in part, this more quantitative, intensive characterization focused on “how much” rather than occurrence data reflects the observation that control measures do not need to eradicate the parasite completely to be effective. Measures such as culturing resistant species, filtering the water supply, chemotherapy, and periodical disinfection of culture facilities reduce the potential for establishment of myxosporean infection in fish and actinosporean infection in tubificids, which

greatly reduce the number of infected individuals and the intensity of the infection. Whirling disease can also be reduced if hatchery-reared fish are inspected and certified as disease-free before transfer between facilities.

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Metazoan diseases of fishes: Phylum Cnidaria (Coelenterata) and Myxozoans

The parasitic members of the Cnidaria (Coelenterata) are systematically interesting, since their interrelationships with other members of the phyla are clouded, poorly characterized and poorly understood (see preceding section on *Myxobolus cerebralis*, the causative agent of whirling disease). For the representative disease-causing organism of interest to the current investigation, the genus *Polypodium* is a member of the Phylum Cnidaria. Relative to whirling disease and the literature available on *M. cerebralis*, few publications occur for *P. hydriforme*, yet the work reported for the parasite indicates the species currently occupies areas in both Missouri River and Hudson drainages (see, e.g., Holloway et al 1991, Dick et al 1991).

Cnidaria are aquatic, and most of their number are marine, although the freshwater representatives are well published in the literature. The phyllum is characterized by its radial symmetry, with most of its members have a gastrovascular cavity and tentacles at some stage of development. Tentacles are derivatives of extensions of body wall, with most species being armed with nematocysts (stinging cells). Most species are characterized by alternation of generations, with an asexual phase being polyploid in character and sexual generations taking the form of medusae. As a general feature, most species have one phase dominant their life cycle with the other being reduced or even absent (see Roberts and Janovy 2005).

The parasitic members of the phyllum are found in two of the three classes[7] that comprise the

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[7] Current convention lists Class Hydrozoa (four orders, including the hydras; Class Schyphozoa (four orders, including the jellyfish); and Class Anthozoa (two subclasses with 10-12 orders (depending upon authority), including sea anemones and corals). The current taxonomic status of the Myxozoa and their relationships to Cnidaria is uncertain. Herein, Myxozoa were considered separately in characterizing *Myxobolus cerebralis* as causative agent of whirling disease.

group, with *Polypodium hydriforme* being considered a hydrozoan. The taxonomic status of these parasitic forms remains disputed, but for our current investigation the "Myxozoa" includes the causative agent of whirling disease which was considered without particular attention afforded the basic research issues that suggests the group represents a fourth distinct class in the phyllum Cnidaria (Siddall et al 1995). As noted, *P. hydriforme* is a hydrozoan and infects the eggs of sturgeon and other primitive fish – *Acipenser* spp. (Acipenseridae), including sturgeon in Europe and North America, *Huso* spp. (Acipenseridae), the kaluga in Russia, and *Polyodon spathula* (Polydontidae), the paddlefish in North America (see, e.g., Hoffman et al 1974, Suppes and Meyer 1975, Holloway et al 1991, Dick et al 1991, Choudhury and Dick 1991, 2001; see Figures 8-10 for illustration of known occurrences).

In general, parasitic stages of the hydrozoans are found in oocytes throughout oogenesis, beginning with previtellogenesis up until spawning over one year later. Single cells in previtellogenic oocytes each have 2 nuclei (one large nucleus and one closely associated, but smaller haploid nucleus). As the life cycle progresses, the large nucleus engulfs the smaller nucleus which becomes enveloped within thin layer of cytoplasm within the larger nucleus. In essence, a small "cell" forms within a larger cell, where the engulfed nuclear content remaining haploid and the larger "cell" becomes polyploid. Eventually, the parasite becomes diploid, but it remains unclear when this nuclear event occurs.

Developmentally, the engulfed nuclear material divides within larger cell or trophamnion which serves as a nurse cell supporting its development. As the nuclear material of the small "cell" divides, it forms blastomeres which eventually forms a cluster embryoes within the trophamnion. Embryoes elongate, each eventually undergoing gastrulation, while the nucleus of trophamnion gradually becomes reticulate (undergoes senescence). In northern latitudes, in late May to late July of the year prior to spawning, when oocytes accumulate yolk, yielding a planula-type larva having two distinct epithelial layers forms. Infected eggs become darkly pigmented and larger in size than uninfected eggs, and the nucleus of the host egg is damaged as the parasite persists. Larvae of the parasite elongate and undergo multiple budding to form stolons in August as it

grows. Stolons continue to grow and elongate through the end of summer, when they become convoluted, typically possessing 30 to 40 buds. Buds continue to develop, and the typical

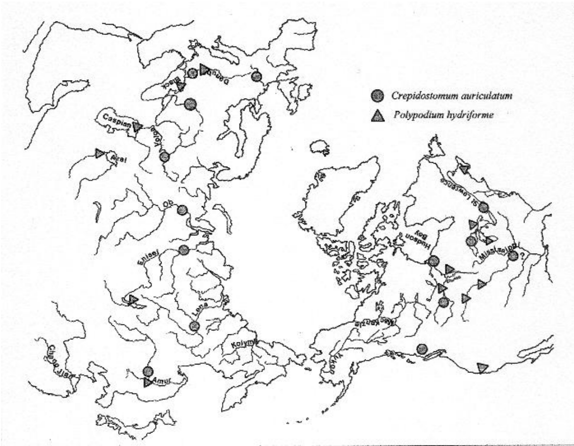

Figure 8. Circumpolar occurrences of *P. hydriforme* indicated by filled triangles (from Choudhury and Dick 2001).

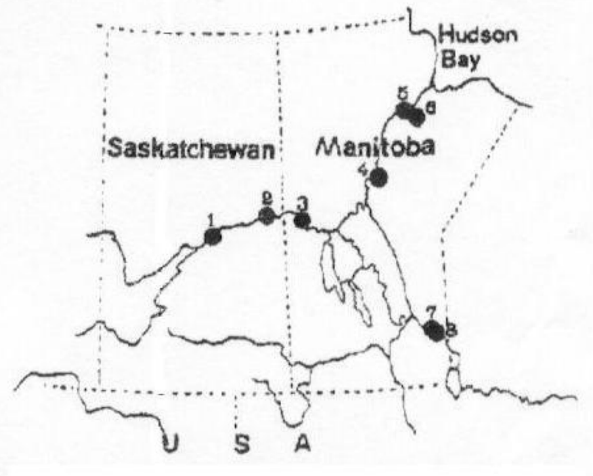

Figure 9. Sampling locations and occurrences in Hudson drainage at sites 2, 4, and 5 (see Dick et al 1991).

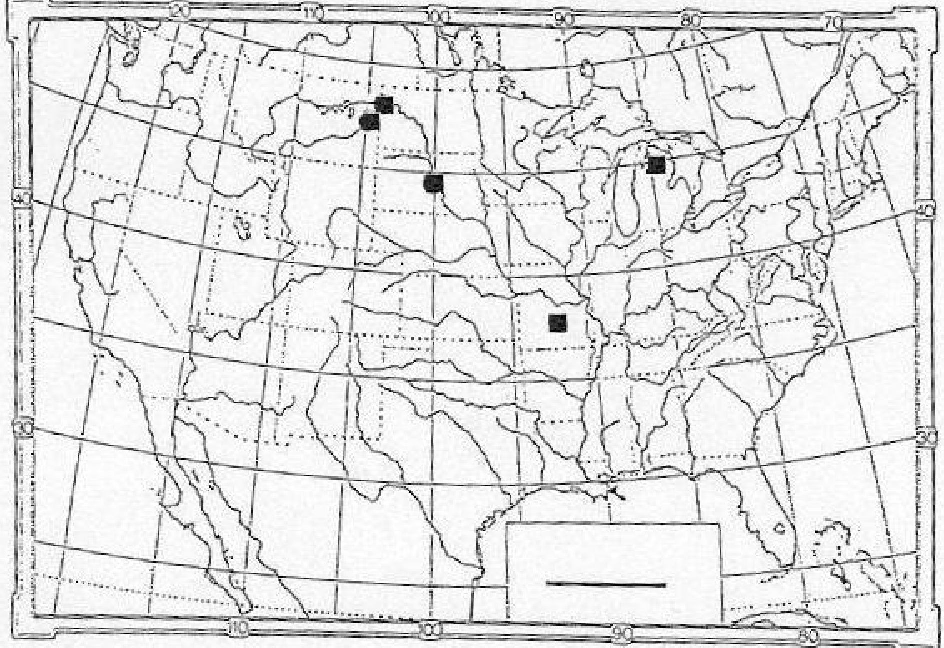

Figure 10. Occurrences of *P. hydriforme* in US indicated by filled circles and boxes (modified from Holloway et al 1991).

hydrozoan tentacles begin to form and eventually acquire nematocysts, generally by end of October. During winter, the parasite's development is arrested, but between March and May of following year, buds on stolons begin increase in size and tentacles elongate. Before the host spawns, stolons invert (turn inside out) and are released to the oviduct when the infected egg ruptures during spawning. Stolons with tentacles are subsequently released directly to the water, and once in water, stolons break into pieces. Fragmentation of stolons continues throughout summer until organisms with 6 tentacles or less predominate. As yolk reserves in stolons are depleted, each forms a typical hydrozoan mouth and feeds on small invertebrates, and growth of the individual continues. Growth results in more tentacles being developed, and the "free-living" stage of the organism reproduces by binary fission. Locomotion occurs in the typical hydrozoan fashion, e.g., "walking" about substrate using tentacles.

In August, male and female gonads form, generally with oogonia formed first. It is unclear whether reproduction involves self-fertilization or whether the organisms are parthenogenic. It is currently thought the free-living, sexual *Polypodium* spp. deposit gametophores onto larval fish where the parasites become attached to the yolk sac, head, body, tail, and fin fold of young sturgeon, and the parasite life-cycle is renewed (see review articles; Raikova 1994, 2002).

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Waterborne Diseases of Terrestrial and Wetland Vertebrates

Many diseases of terrestrial, avian and aquatic life are zoonotic, i.e., transmissible between humans and animals, causing infection in both species (Figure 11; see, e.g., Krauss et al 2003, Hugh-Jones et al 2000, Friend et al 1999, and http://www.vetmed.wisc.edu/pbs/zoonoses/ last accessed December 14, 2004). While humans are frequently involved as hosts in zoonotic disease processes, their inclusion in the current work was not reflective of an effort targeted on human health risks, but rather a recognition that terrestrial vertebrates, including humans, are

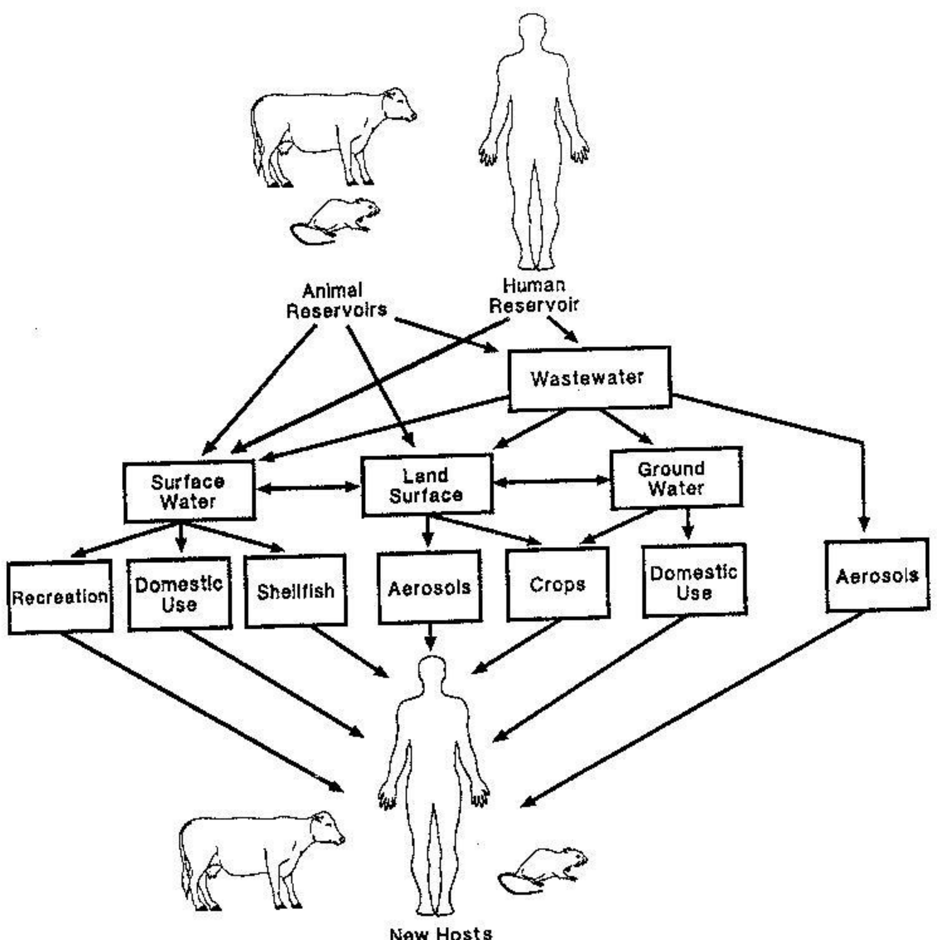

Figure 11. Illustration of interrelationships between hosts (here, illustrated by mammals) and pathogen sources in typical zoonotic diseases.

representative "targets" of disease agents potentially transferred collateral with interbasin water

diversions. The following groups of microorganisms have been linked with the occurrence of waterborne disease, and these broad categories have been used to summarize risks associated with representative biota of concern identified by Reclamation and Technical Team. All can be found in raw water or finished water, especially if the water is characterized by high turbidity. Broadly, these groups are:

- cyanobacteria
- bacteria
- fungi
- viruses
- protozoa

Cyanobacteria. While cyanobacteria have been linked to fish mortalities in the wild, these biota of concern for the current investigation are considered here, primarily for convenience. Cyanobacteria, especially when populations increase to problematic levels in ponds, lakes, and wetland habitats, are not predisposed to impact one group of receptors over another, e.g., cyanobacterial toxins are equally active with fishes, wildlife, and humans.

Bacteria. Bacteria are the most widely distributed life forms. Pathogenic bacteria range in length from approximately 0.4 to 14 micrometers and 0.2 to 1.2 micrometers in width. Some of the more common strains of bacteria that are associated with waterborne disease outbreaks are Legionella, Salmonella typhi and Cholera. These bacteria can be killed with proper disinfection practices associated with current water treatment techniques.

Fungi. Fungi can cause a variety of diseases in plants and animals, yet none were identified as biota of concern in this current investigation. Fungal diseases of animals can be categorized at infections or altered host responses consequent to exposure, most often manifested as allergies or toxicity reactions (e.g., to fungal toxins). Fungal allergies and toxic reactions are important concerns in agriculture and other industries where fungal contamination is common, but only

fungal infections are highlighted briefly given the primary focus of potential agents transferred as a consequence of an interbasin water diversion.

Fungal infections, or mycoses, result from invasion of living tissue by a fungus. Mycoses represent the most common form of fungal disease, yet of the more than 200,000 species of fungi, fewer than 200 are known to infect humans and other terrestrial vertebrates. As zoonotic infections, mycoses of terrestrial vertebrates may be categorized as superficial mycoses, cutaneous mycoses, subcutaneous mycoses, or systemic mycoses.

Viruses. Viruses are inactive when living outside of a host cell. Viruses linked to waterborne diseases range in size from 0.02 to 0.09 micrometers and have protein coats that provide protection from environmental hazards. Unlike bacteria, fungi, and protozoa, viruses contain only one type of nucleic acid (RNA or DNA). Key waterborne viral pathogens such as rotavirus and Norwalk virus among others, or those associated with water sources owing to vectors relying on surface water habitats common to their life cycle include emerging diseases such as West Nile virus, are problematic for water resource managers. Viruses can be killed with proper disinfection practices associated, with current water treatment techniques, and may be removed from finished water using certain filtration technologies.

Protozoa. Protozoa common in open bodies of water are much larger than bacteria and viruses. To survive harsh environmental conditions, some species can secrete a protective covering and form a resting stage called a “cyst.” Encystment can protect protozoa from drinking water disinfection efforts and facilitate the spread of disease.

Each of these broad categories will be considered in in the following synoptic life history overviews, which will focus on attributes presented by biota of concern that are primarily related to their being disease agents.

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Cyanobacteria[8]

Periodic blooms of algae, including true algae, dinoflagellates, and cyanobacteria or blue-green algae have been re-ported in marine and freshwater bodies throughout the world. Although many blooms are merely an aesthetic nuisance,some species of algae produce toxins that kill fish, shellfish, humans, livestock and wildlife. Proliferations of freshwater toxin-producing cyanobacteria are simply called “cyanobacterial blooms” or “toxic algal blooms.” Cyanobacterial blooms initially appear green and may later turn blue, sometimes forming a scum in the water. Although algal blooms historically have been considered a natural phenomenon, the frequency of

[8]Developed from: Creekmore, L.H., 1999, Algal toxins, In M. Friend, J.C. Franson (Technical editors), and E.A. Ciganovich (Editor), 1999, Field Manual of Wildlife Diseases: General Field Procedures and Diseases of Birds, Biological Resources Division, Information and Technology Report 1999–001, USGS, Biological Resources Division National Wildlife Health Center, Madison, Wisconsin, pp. 263-266.

occurrence of harmful algae appears to have increased in recent years. Agricultural runoff and other pollutants of freshwater wetlands and water bodies have resulted in increased nutrient loading of phosphorus and nitrogen, thus providing conditions favorable to the growth of potentially toxic algae. The detrimental impact of cyanobacterial blooms on wetland, shore, and pelagic species has long been suspected but not often been substantiated because information on the effects of these toxins in fish and wildlife species is lacking and diagnostic tools are limited.

Causative agents. Some dinoflagellates (in marine and estuarine habitats, especially) and cyanobacteria produce toxins that can affect domestic animals and humans. Some of these toxins such as domoic acid, saxitoxin (paralytic shellfishpoisoning or PSP toxin), brevetoxin, and cyanobacterial toxins (including anatoxins, microcystins, and nodularins) have been suspected, but they have rarely been documented, as the cause of wildlife mortality (Table 2).

Table 2. Documented instances of wildlife mortality caused by algal toxins in surface waters (modified from Creekmore 1999).

Toxin	Algal species	Toxin type(s)
Cyanobacterial	Microcystis spp. Anabaena spp. Aphanizomenon spp. Nodularia spp. Oscillatoria spp.	Heptatoxins (microcystins and nodularin) Neurotoxins (anatoxin-a and anatoxin-a(s))

Cyanobacterial toxins adversely affect wetland and terrestrial species such as amphibians, reptiles, birds, and mammals (including humans), as well as fish and other aquatic organisms exposed to these toxins (see, e.g., , Briand et al 2003, Meyer and Barclay 1990, Friend et al 1999). Many bird and mammal species can be affected by cyanobacterial toxins, most often noted as increased mortality in birds and wildlife ("die-offs") that occur in conjunction with a cyanobacterial bloom. Cyanobacterial toxins may be found in wildlife food items, but there have been very few instances in which the algal or cyanobacterial toxin has been isolated from the ingesta or tissues of affected

birds. For example, although exposure likely occurs through multiple pathways, cyanobacterial toxicosis has been suspected in mortalities of free-ranging ducks, geese, eared grebes, gulls, and songbirds.

Distribution. Many of the organisms responsible for algal and cyanobacterials blooms are widely distributed and not limited to either Missouri River or Red River watersheds. In recent years, the organisms or the occurrence of signs of toxicosis appear to be increasing in distribution, or at least in the occurrence of signs and symptoms of cyanobacterial toxicosis. Natural weather-related events can aid dispersal of these organisms, and it is suspected that some organisms may be transported long distances in waters of “fish wells” or other human-aided transport mechanisms. Increased nutrient loading in surface waters has also been strongly associated with the increased occurrence of algal and cyanbacterial blooms and toxicoses.

Field signs of algal and cyanobacterial blooms and toxicosis. While abiotic factors such as temperature, precipitation, and nutrient loading are highly likely to influence the development of algal and cyanobacterial blooms, there have not been enough confirmed instances of bird or wildlife mortality caused by cyanobacterial blooms to establish seasonal patterns of occurrence. Field signs reported are variable and they depend on the toxin involved. For example, cyanobacterial poisoning of birds and mammals leads to caused neurologic signs that include muscle tremors, a characteristic side-to-side head movement, pouch scratching, awkward flight in birds and abnormal gate in mammals, toe clenching, twisting of the head over the back, vomiting in mammals, and loss of the righting reflex just before death. Display of signs and symptoms is highly species dependent.

Characteristic gross lesions observed on necropsy are few, and none are particularly diagnostic. Many of the toxins, particularly the neurotoxins, do not produce a grossly observable lesion although the behavioral signs clearly indicate compromised nervous system function. For cyanobacterial toxins targeting liver function, e.g., birds exposed to toxic blooms of *Microcystis*, notable lesions of necrosis or tissue death and hemorrhage in the liver may be observed. Such

lesions have been reported in domestic mammals and birds, including ducks, that have died as a result of exposure to a toxic *Microcystis* bloom or that were experimentally dosed with microcystin.

Diagnosis. Definitive diagnosis of algal and cyanobacterial toxicosis is technically difficult, and reliance on circumstantial evidence such as the occurrence of a freshwater cyanobacterial bloom in conjunction with a die-off, supported by clinical and pathologic findings (e.g., evidence of neurological dysfunction or at worse, lack of evidence of the presence of other types of toxins or infectious disease) must frequently influence a presumptive diagnosis. In fish and wildlife, analysis of the upper gastrointestinal tract contents or tissues of affected individuals for toxins is possible but not widely practiced, owing to limited "off the shelf" tests. Even when levels of particular toxins can be measured it may be difficult to assess their significance. There are presently no established toxic thresholds for wildlife species. Recently developed methods using enzyme linked immunosorbent assay (ELISA) technologies permit detection of microcystins in animal tissues and gastrointestinal contents. Samples of suspect waters may be collected in the field, and may be diagnostic if collected at the time of the die-off event. Generally, collection of field samples, be those dead or dying animals or environmental samples such as surface water should be the routine practice.

Control and prevention. Because it is difficult to definitively identify algal and cyanobacterial toxins as the causeof wildlife mortalities, few control measures have been instituted. Currently, more interest has been expressed in the role that algal and cyanobacterial toxins play as threats to human water and food supplies (see, e.g., Chorus and Bartram 1999). Outside these public health settings which emphasize water treatment or source water controls to prevent problem blooms, identifying and characterizing the conditions that trigger harmful algal and cyanobacterial blooms may aid in developing strategies to prevent fish and wildlife mortality associated with these events. And, these control measures would benefit the problems more directly related to human health issues associated with algal and cyanobacterial blooms.

Regardless of whether receptors of concern are humans, domestic livestock, or fish and wildlife, controlling nutrient loading through reduced fertilizer use, improved animal waste control, and improved sewage treatment may reduce the number, or likely locations, of toxic algal or cyanobacterial blooms. Careful monitoring and early detection of potentially toxic blooms could allow time to initiate actions to prevent or reduce increased morbidity or mortality in populations associated with water sources displaying or tending to display algal or cyanobacterial population growth linked to toxin production. For humans, most toxic freshwater cyanobacteria are not problematic unless waterborne toxin is ingested. Some organisms irritate the skin and others release toxic compounds into the water and, if aerosolized by wave action, these compounds may cause problems when people inhale them. Similar exposures unlikely occur with wildlife, but are poorly documented. As in the investigation of all wildlife mortality events, wear rubber or latex gloves when handling carcasses.

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Legionella: Links between public health risks and ecological risks

Legionella pneumophila, the causative agent of Legionnaires’ disease and related respiratory ailments (e.g., Pontiac fever), is a facultative intracellular pathogen, and represents but one species of bacteria whose role in public health is often dominated by proximal expressions of the disease while being poorly understood within the context of the species’ life history (see, e.g.,

Fields 1996, 2002, Golovlev 2000, Segal and Shuman 1999, Steinert et al 2002). For many health professionals and the lay-public, the organism is known only as a well characterized public health problem. As a causative agent of human disease, route of exposure is predominately via inhalation of aerosols. Once inhaled, the organism is ingested by human macrophages where the it effectively evades microbicidal defenses of phagocytes by maintaining a vacuolar pH near neutrality, thus preventing phagosome-lysosome fusion. The organism in effect becomes an intracellular parasite, growing grows exponentially within a specialized vacuole borne within the phagocyte (see, e.g., Harb et al 2000, Fields 2002). These events are similar to those characteristic of other intracellular parasites such as *Toxoplasma gondii, Leishmania donovani,* and *Mycobacterium tuberculosis* (see, e.g., Roberts and Janovy 2005), but in contrast to these organisms, *L. pneumophila* can be cultivated in the laboratory using standard microbiological media and techniques (Fields 2002). Although Legionnaires disease and other forms of legionellosis have probably existed in the past, the clinical entities were not recognized until 1976 when several cases of Legionnaires disease occurred at a national convention of the American Legion in Philadelphia, Pennsylvania. In that initial outbreak and in subsequent occurrences of the disease, man-made devices such as air-conditioner cooling towers, showers, respirators, and other equipment that generate aerosols of standing water contributed to the spread of the organism from the environment and caused outbreaks of *Legionella* pneumonia (see, e.g., Lin et al 1998, Murga et al 2001).

Occurrence in natural and man-made environments. While the focus of most public health professionals resolves on proximal sources as roots of infection, the species of *Legionella* afford an opportunity to considered the ecology of an infectious disease agent within the context of source waters, e.g., potentially stemming from interbasin water diversions. *L. pneumophila* and related species has been isolated from almost any type of freshwater sample (see, e.g., Dutka and Ewan 1983, Atlas 1999), and it is quite evident that *Legionella* spp. are common in natural waters, especially those of high nutrient content and thermally polluted (Figure 12 and Figure 13). Waters of this type appear to serve as a reservoir of *Legionella* spp. from which the bacterium enters and colonizes water cooling systems, humidifiers, hot water systems, and similar structures. Some of these human-engineered systems provide a very favorable environment for *Legionella* in

terms of temperature, level of aeration, organic nutrients and iron concentration, yet these "habitats" afford similar conditions found in open-waters.

In natural or artificial systems the organism generally develops as a member of a biofilm (see, e.g., Armon et al 1997, Harb et al 2000, Marrão et al 1993, Murga et al 2001) but there is nearly always an association between *L. pneumophila* and the free-living protozoa *Acanthoameba polyphaga* (see, e.g., Cirillo et al 1994, Harf and Monteil 1988, Harb et al 2000, Kwalk et al 1998, Newsome et al 1998). In many respects, the intracellular mechanisms that assure survival of *L. pneumophilia* in human phagocytes are identical to those mechansisms that allow for an intracellular existence with its free-living protozoan "host." This involves the intracelluar growth of *L. pneumophila* inside amoebic trophozoites, an adaptation that enables *L. pneumophila* to survive chlorination of cooling water and similar systems. Aerial distribution inside amoebae, and as fragments of biofilm or as large droplets from natural or man-made systems, may represent a means by which the bacterium bypasses defense mechanisms in the lung. From a broader environmental viewpoint, intracellular growth is probably beneficial to *L. pneumophila* in preventing predation by other protozoa, ensuring a supply of nutrients, including iron, and protecting against UV irradiation to which *Legionella* is highly sensitive.

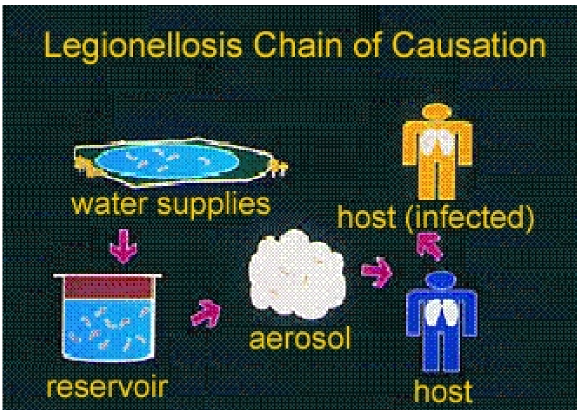

Figure 12. Conceptual model linking human host with *L. pneumophilia* (from CDC public domain sources).

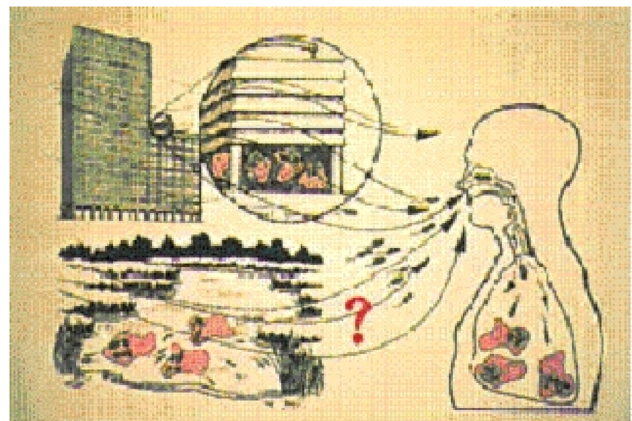

Figure 13. Graphic illustration linking human hosts with man-made devices that mediate exposure and presumptive links directly with native habitats of *L. pneumophilia* (from CDC public domain sources).

Frequently *Legionella* spp. are found in close association with algae and protozoa. Whether members of the Family Legionellaeae are capable of free-living is speculative, but several lines of evidence strongly suggest that legionellae grow exclusively within other organisms. Many amoebae have *Legionella*-like organisms growing intracellularly, and some protozoa, e.g., *Hartmanella vermiformis*, have been identified in several outbreaks of Legionnaires disease. *L. pneumophila* can reproduce within amoebae and ciliated protozoa such as *Tetrahymena pyriformis*, so mechanisms are clearly available for long-term populations in the wild.

Legionellosis is the direct consequence of the ability of *Legionella* spp. to gain access to human lungs by dispersal in aerosols. This occurs in a variety of ways, all of which involve a man-made device. Perhaps the most common device that has been responsible for introducing the organism into the human environment is the air-conditioning cooling tower. In this device, water returning from an air-conditioning system is allowed to evaporate. Due to the increased temperature and exposure to the external environment, water that contains legionellae can be co-colonized by protozoan hosts that under some conditions may facilitate explosive growth of the bacteria. Either bacteria or protozoa infected with bacteria are then aerosolized throughout the air-conditioning

system. Experiments in susceptible animals have shown that co-infection of *L. pneumophila* and *H. vermiformis* produces more-acute disease than infection with *L. pneumophila* alone.

Water distribution systems (public and private) and standard plumbing devices such as shower heads, pipes, and heat-exchange bumpers have also been shown to harbor the organism (see, e.g. Lin et al 1998), and in these structures, the role of biofilms is similar. Biofilms within these devices allow consortia of organisms to thrive, and it is likely that protozoan members of biofilms serve as hosts for *Legionella* spp. Other devices such as spas and whirlpools, respiratory therapy devices, and even grocery store produce misters have been shown to be a source of *Legionella* spp., diversity of habitats capturing the range of possibilities similar to those in the wild. Across all these habitats, however, the potential for legionellosis is only realized when water containing legionellae alone or in combination with a protozoan is aerosolized, then inhaled by a susceptible individual. Legionellae are frequently present in potable water supplies but pose a health threat only when conditions favor replication of the organism to large enough numbers, conditions generally captured by the formation of biofilms that contain a susceptible host, temperature, and the absence of added biocides.

Disease occurrence. Legionnaires' disease has been an emergent disease since the 1970's. In the last few years, the increased use of a test for detecting urinary antigen associated with *L. pneumophila*, Serogroup 1 in individuals presenting with pneumonia has facilitated diagnosis of legionellosis. Transmission of the disease agent continues to be dominated by exposure to aerosols, and evidence of *Legionella* in aerosols derived from cooling towers has been provided in various studies, although disputes in the epidemiological literature indicate that other sources may be acting as sources of the bacteria.

Legionnaires' disease is normally acquired by inhalation or aspiration of legionellae from a contaminated environmental source. Relatively little is known about sporadically occurring cases of community-acquired legionellosis, which accounts for most infections, although correlation analyses suggest that a substantial proportion of these cases may be residentially acquired and

associated with bacteria in hot water distribution systems. In households and other man-made systems (e.g., water handling systems within buildings, municipal water distribution systems), *Legionella* spp. have been isolated from water with a temperature as high as 63˚C, and the contamination is associated with other bacteria and protozoa. Biofilm formation can provide a means for survival and dissemination of *L. pneumophila*, interfering with efforts to eradicate bacteria from water systems. The accumulation of microorganisms on the pipeline surfaces and the formation of biofilms are influenced by many factors, such as surface materials, concentration and quality of nutrients and disinfectants, temperature and hydraulics of the system, and pipe surface roughness.

Figure 14 through Figure 16 illustrate data available from public health agencies (see, e.g., http://www.cdc.gov/mmwr/preview/mmwrhtml/mm4753a1.htm and similar summaries for other disease agents) that would serve as baseline data for evaluating risks potentially associated with interbasin water diversions. While state-wide data routinely reported would differ within a spatial context relative to our focus on Missouri River and Red River basins, if such assessment and monitoring efforts were identified for focused analysis, disease incidence data could be apportioned by HUCs and relative changes in incidence of disease could be monitored as baseline data and (potentially) post-diversion data. Data summarized as illustrations in Figure 14 through Figure 16 suggest apparent baseline for, e.g., Minnesota, North Dakota, and Manitoba across years is comparable with respect to variability, and upon collaboration with respective agencies within-state and within-province, data should be available for a river basin-based analysis.

In 1998, the overall reported rate of legionellosis in the United States was 0.51 cases per 100,000 population. However, data from prospective, population-based studies of persons with pneumonia indicate that the actual rate of legionellosis is more than 10-fold this number.

Figure 14. Typical summary data available from CDC through compilation of state-wide records.

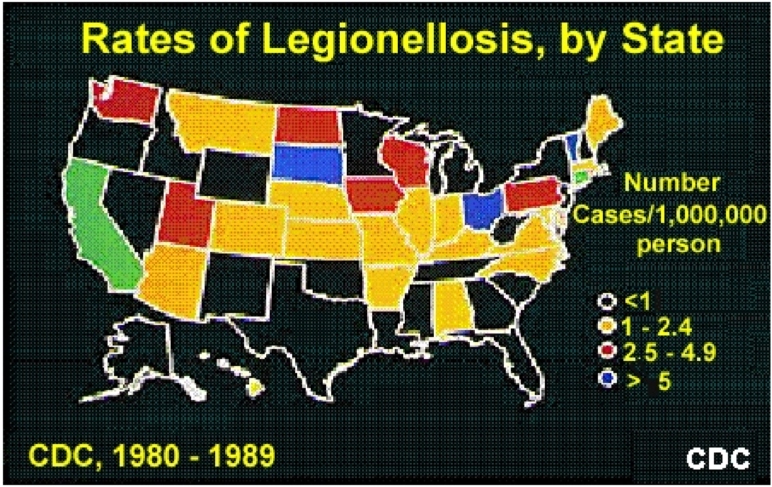

Figure 15. Illustration of disease rates derived by state, which could serve as preliminary data for evaluating baseline and "post-diversion" monitoring data focused on potential changes in disease rates associated with interbasin water diversions.

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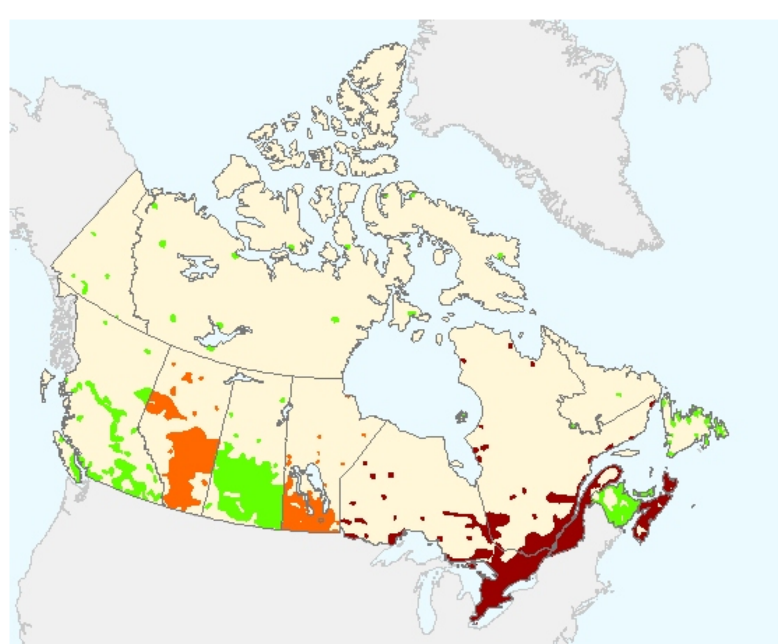
Figure 16. Province-wide data compiled and report for legionellosis (both sexes combined, [including not specified], all ages [Incl. Not Specified] for 2000).



Rate per 100,000 population	**0.00 - < 0.01**	0.01 - < 0.02	0.02 - < 0.07	0.07 - < 0.11	0.11 - 0.36

Province	Rate per 100,000
Newfoundland	0.00
Prince Edward Island	0.00
Nova Scotia	0.11
New Brunswick	0.00
Quebec	0.16
Ontario	0.36
Manitoba	0.09

Province	Rate per 100,000
Saskatchewan	0.00
Alberta	0.07
British Columbia	0.00
Yukon	0.00
Northwest Territories	0.00
Nunavut	0.00

Data updated: 2003

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Enteric bacteria: *Escherichia coli* and *Salmonella* spp.

Escherichia coli and relationships to waterborne disease

Bacteria occur across a wide range of habitats, yet their ecology and interrelationships with disease processes are difficult to characterize and generally relies on indirect observations and measurement. Typically, the colons of mammals and birds have been regarded as natural habitat for *E. coli*; *E. coli* is one of the first bacterial species to colonize mammals after birth. Densities of *E. coli* in the large intestine of mammals (and birds) has been estimated from 1 million to 10 million cells per gram of colon (see, e.g., Hurst 2002, Quinn et al 2002). These population levels make *E. coli* a minor component of the microbiota of the colon, since this section of the intestine is largely anaerobic and has a total bacterial density calculated at some 100 billion cells per gram of colon. In the gut, *E. coli* passes through a single one cell division daily in contrast to growth in laboratory media which generally attains doublings six times a day or more (e.g., for *E. coli* K-12). Vertebrates other than mammals harbor *E. coli* as part of their normal gut flora, but generally occur as different strains or combinations of strains from those in birds and mammals and at lower population levels (see Quinn et al 2002). These observations suggest the species and its various serotypes are widespread beyond the often studied representatives considered in strictly a public health investigation.

Owing to the focus of our present-day diagnostic tools, when *E. coli* causes increase morbidity (or mortality), fecal contamination of water or food is commonly suspected, and environmental microbiologists, including public health and veterinary health professionals, primarily focus on characterizing pathways that link *E. coli* originating from vertebrate sources, usually mammalian or avian colon, to its introduction into a receptor's gastrointestinal system where the bacteria can reproduce. If pathogenic, the bacteria may present through signs and symptoms of disease. Strains of *E. coli* found in aquatic environments (natural or anthropogenic in character, e.g., water distribution systems) are generally more diverse than strains obtained directly from hosts. A number of studies have found that aquatic and soil bacterial populations of *E. coli* can increase

population density over time, indicating that these bacteria grow and survive in these external environments outside their host's gut. These studies suggest that *E. coli* infections can develop from sources other than fecal contamination of surface water (as well as groundwater and food).

E. coli in a nutrient-poor environment such as water or mud differs in many respects to its habitat in the vertebrate gut, e.g., bacteria in nutrient-poor environments divide at around 10% the rate achieved in the laboratory, and thrive via commensal relations with host animals (see, e.g., Rosenberg 1999). Generally, one strain of *E. coli* will dominant within a specific microhabitat (e.g., opportunistic host or microsite in aquatic or sediment habitat), but new genotypes appear to develop relatively quickly through the genetic drift that occurs under varying environmental conditions. Hence, dominance by any given strain is transitory.

As a member of the Enterobacteriaceae, molecular phylogeny studies with *E. coli* indicate that it is closely related to some other pathogens of vertebrates, including *Shigella* and *Salmonella*, *Vibrio cholera*, and *Haemophilus* spp. Enterobacteria are characterized by their capacity for facultative respiration, and are highly diverse in habitats supporting their populations, e.g., many members are free-living. Others live in commensal relationships with animals or plants. Strains of *E. coli* range from the relatively harmless K-12 serotype widely used in experimental laboratories to enterohemorrhagic *E. coli* such as O157:H7 which appears to have acquired many of its genes by horizontal transfer since diverging from K-12. Horizontal or lateral gene transfer is an attribute of bacteria that facilitates exchange of DNA within or across species lines. Such "gene-swapping" takes place through bacterial conjugation; through the intervention of bacteriophages; or through transformation, wherein bacteria acquire "loose" DNA from their environment.

E. coli O157:H7 follows a range of pathways in acquiring hosts, and it is becoming increasingly recognized as a waterborne pathogen, e.g., outbreaks involving drinking water supply or recreational water exposure illustrate the role of water in transmission (see, e.g., Mara and Horan 2003). Contaminated drinking water and recreational water have been associated with outbreaks of hemorrhagic colitis caused by E. coli O157:H7 in the areas of concern, but conventional water

treatment (e.g., chlorination) ensures that potable water and water used in recreational settings, if treated, are free of microbial pathogens. Studies focused on chlorine resistance of E. coli O157:H7 compared to wild-type E. coli suggests both pathogenic and nonpathogenic strains are significantly reduced within 1 minute of exposure to free chlorine, and that chlorine levels typically maintained in water systems are sufficient to inactivate these organisms (See Appendix 11 and Appendix 12).

Serotype O157:H7 has been the causative agent of several recent fatal outbreaks, with the more commonly recorded events linked to foodborne disease (see, e.g., NRC 2004). Pathogenicity is a function of virulence factors that *E. coli* K-12 lacks. These virulence factors influence metabolic pathways and prophages of the serotype, and enable DNA elements to move around on a chromosome which enables the enterobacteria to undergo genetic recombination more frequently than in other organisms. Lateral or horizontal gene transfer creates bacterial genomes that are mosaics of genes with different evolutionary histories, which in part enable the wide range of habitats the *E. coli* can occupy. "Pathogenic islands" are characterized as regions that present high rates of recombination; these regions are locations on the genome that confer pathogenicity to the organism (Lawrence and Ochman 1998 as cited in Souza et al 2002).

Plasmids and environmental plasticity. Bacteria carry some of their genetic information in the form of extrachromosomal elements known as plasmids, which are highly dynamic, circular DNA molecules that readily move between strains within species and (potentially) between species. Plasmids are common in *E. coli*, although bacteria can survive without a plasmid. The role of plasmids in bacterial life history varies, however, and some bacteria store a high percentage of its genome in these mobile genetic structures. Over 300 plasmids have been described in *E. coli* and confer genetic information for assimilating rare sugars and for producing colicins (substances that kill possible competitors of the same species); resistance to antibiotics and heavy metals; immunity against bacteria-targeting viruses and colicins; genes that code for genetic exchange; and filaments related to pathogenesis and the production of toxins. In general the distribution of a plasmid depends not only on its range of bacterial hosts, but also on a complex system of incompatibility

among plasmids of the same type. Conjugative plasmids rely on conjugation to mediate movement between bacteria and contain genes necessary for bacterium-to-bacterium recognition. Conjugative plasmids convey their genetic material between bacteria via formation of pili that allow for transfer of DNA, while nonconjugative plasmids may be transferred collaterally when conjugation.

Many plasmids are capable of transfers among different species, although the mechanisms mediating such interspecies transfers are incompletely characterized. Some plasmids readily undergo interspecies exchanges of genetic material and may be regarded as promiscuous. These plasmids tend to be over represented in bacterial populations, and have been referred to as "epidemic plasmids," since they often allow bacteria to acquire virulence factors or resistance to antibiotics by horizontal transfer. Promiscuous plasmids potentially contribute to coevolution, since the movement of extrachromosomal genomes of interacting species may evolve in parallel. In addition to these promiscuous or epidemic plasmids, there are clonal plasmids that are only transferred from "parent" to "child" as part of asexual reproduction, and plasmids that are transferred only between individuals of a species that have specific base sequences within their genome.

Ecological and population genetics implications of genomic plasticity. *E. coli* reflects genomic plasticity common to bacteria (and viruses) which confer on these organisms an extraordinary ecological plasticity. In bacteria, reproduction is not limited to sexual processes, since bacteria divide by binary fission to produce clones. Following a process of asexual reproduction through binary fission, genetic variation arises solely by way of mutations passed along to clones. Horizontal transfer, which involves exchange of genetic material between individuals through a parasexual process, also serves as a source of variability in populations, and in bacterial population, the balance between these two processes is called the degree of clonality, e.g., a highly clonal species is one reliant on binary fission and is distinguished by a collection of independently evolved lineages where adaptation to environmental factors relies primiarly on selection of complete lineages, most often manifested by genetic drift. In contrast, if a species

exhibits high rates of recombination, populations display attributes of randomly mating, "sexual" populations. Degrees of sexuality and clonality of bacterial populations vary, but the current characterization of *E. coli* reveals high levels of genetic variation within its populations, although research findings suggest that recombination is a rare phenomenon in *E. coli* associated with humans or domesticated animals. The high genetic diversity observed from *E. coli* probably stems from periodic selection where a genotype selectively displaces others present in the population, viz., in an asexual population, once a favored mutation spreads by natural selection, it replaces not just the gene involved, but a complete genotype. Such a mechanism would assure adaptation to particular niches, and the process would yield genetic diversity through the collection of very different strains, each adapted to a different environment.

E. coli is characterized by great genetic and ecological diversity, which reflects its high level of genetic recombination and exchange. These genetic mechanisms generate of a large quantity of genotypes, although they may not be expressed in each generation. Recombination of plasmids and gene fragments of genes also contribute to the species ability to invade "new" environmental niches and new hosts, and the spread of new variants of *E. coli* enable the species to be highly competitive across a range of environments, e.g., serotype O157:H7 was identified as a pathogen in 1982 (see, e.g., http://www.cdc.gov/ncidod/dbmd/diseaseinfo/escherichiacoli_g.htm). Given its genetic potential, *E. coli* has generated structured populations of various ecotypes or pathogenic strains that can live in a large number of environments that previously were thought secondary or atypical for the species.

As a representative disease agent potentially transferred between Missouri River and Red River watershed, *E. coli* has displayed abilities to rapidly adapt to a wide range of environmental conditions, e.g., existing as a free-living organism, in commensal mutualism in the colons of mammals and birds, and as a pathogen capable of infecting a wide range of hosts. As a pathogen, *E. coli* extends its ecological plasticity to invade other "niches" successfully, e.g., different tissues or organs across a range of species may be targets for infection beyond its normal occurrence as member of the gastrointestinal microbiota (see, e.g., Quinn et al 2002).

Occurrence of disease associated with pathogenic strains of *E. coli.* While the focus of the current investigation was not public health, the role of disease agents in the landscape was considered appropriate to the evaluation of risks of biota transfer associated with interbasin water diversions. As a disease agent, various strains of *E. coli* are infective for a wide range of fish and wildlife, and the species is not limited to those diseases tracked by federal, state, and county health offices. However, the available spatial and quantitative data for addressing disease occurrence is best developed for those serotypes presenting adverse effects in human populations. As with many health-related agencies, the majority of cases investigated and subsequently linked to E. coli O157:H7 exposure are food-related. Similarly, *Salmonella* occurrence in Minnesota over the same 3-year period was predominately food-related (see following section). Figure 17 and Figure 18 illustrate data typically available to baseline assessment or “post-diversion” monitoring. Again, while state-wide data routinely reported would differ within a spatial context relative to our focus on Missouri River and Red River basins, if such assessment and monitoring efforts were identified for focused analysis, disease incidence data could be apportioned by HUCs and relative changes in incidence of disease could be monitored as baseline data and (potentially) post-diversion data. Data summarized as illustrations in Figure 17 and Figure 18 (e.g., for Minnesota and Manitoba, respectively) suggest data are available to evaluate basin-wide incidence of disease, if collaboration with respective agencies within-state and within-province would be identified as data needs for monitoring programs.

Figure 17. *Escherichia coli* O157:H7 occurrence in Minnesota over a 3-year period (2000-2002; source, Minnesota Department of Health).

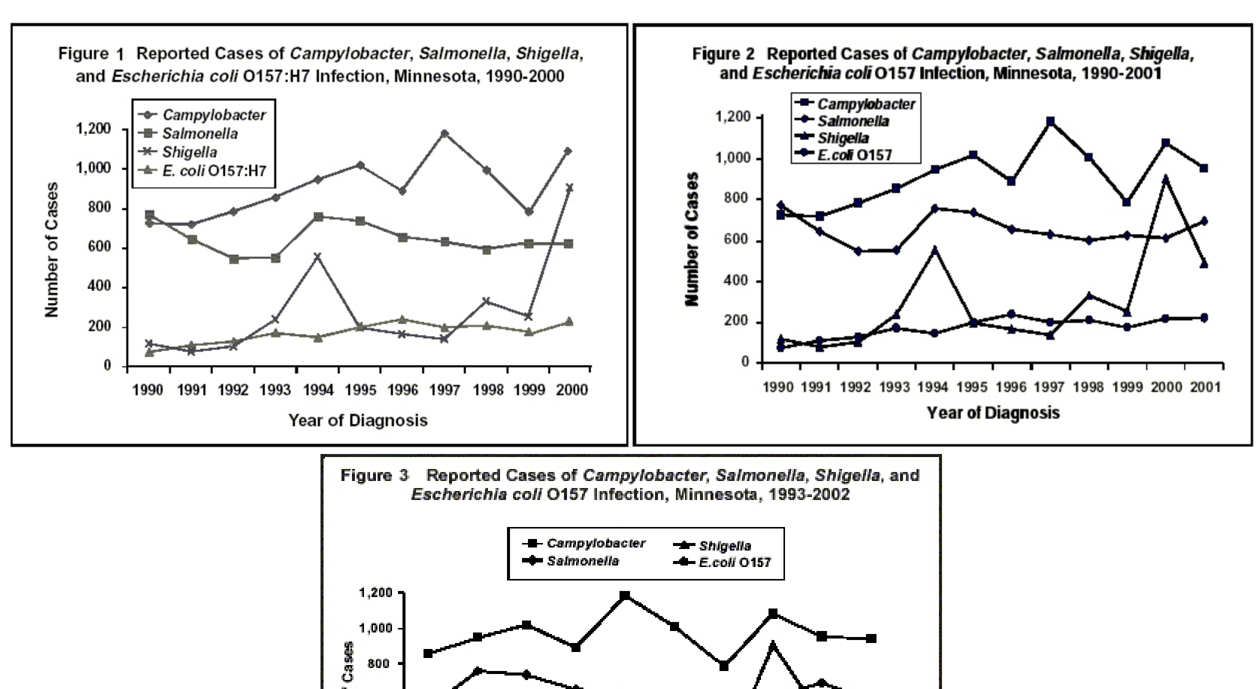

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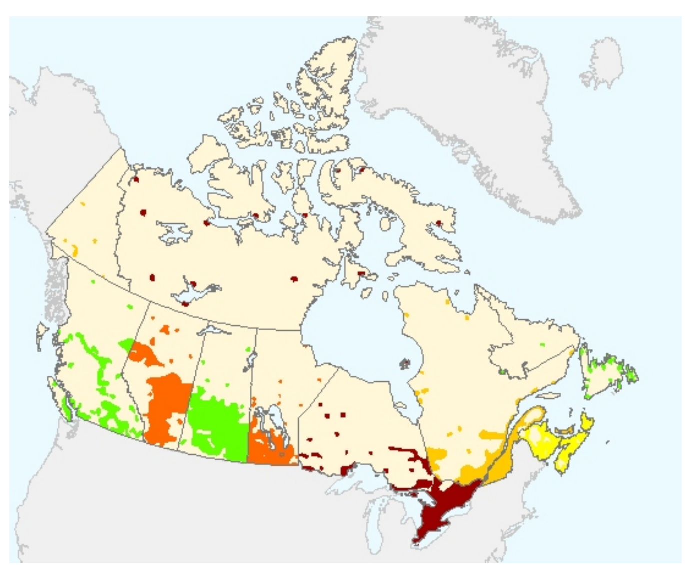
Figure 18. Province-wide incidence of "Verotoxigenic" *E. coli* in Canada (sexes combined, including not specified; all ages, including not specified), 2000.



Rate per 100,000 population	0.55 - < 4.50	4.50 - < 6.51	6.51 - < 7.59	7.59 - < 12.20	12.20 - 134.53

Province	Rate per 100,000
Newfoundland	0.56
Prince Edward Island	6.51
Nova Scotia	4.99
New Brunswick	4.50
Quebec	7.26
Ontario	12.20
Manitoba	7.59
Saskatchewan	4.11
Alberta	10.80

Province	Rate per 100,000
British Columbia	3.92
Yukon	6.54
Northwest Territories	14.69
Nunavut	134.53

Data updated: 2003

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***Salmonella* spp. as bacteria related to waterborne disease**

Salmonellosis outbreaks in human populations generally result from ingestion of foods of animal origin that, but an increasing number of events have been associated with surface water exposures, consumption of fresh produce, person-to-person transmission, and commensal-to-human transmission (e.g., pets being the source of infectious agent). In part, these reports reflect sporadic infections rather than widespread outbreaks. For example, in the US from 1993 to 1997, the number of reported culture-confirmed *Salmonella* infections was approximately six-fold higher than the number of cases of salmonellosis reported associated with outbreaks (see CDC 2000).

As with many of the biota of concern considered in this current investigation, the nomenclature of the genus *Salmonella* has undergone considerable change in recent years, many of those changes directly reflecting the increasing use of genomic libraries to characterize phylogenetic relationships within a taxonomic system (CDC 2000, Hurst 2002). For example, based on DNA hybridization and other taxonomic studies, there are currently two recognized species in the genus *Salmonella* – *S. enterica and S. bongori* (Brenner et al 2000). *S. enterica* includes 6 subspecies: *enterica, salamae, arizonae, diarizonae, houtenae,* and *indica*, each with numerous serotypes (serotype names are written in Roman (not italicized) letters, and the first letter of the serotype name is capitalized; e.g., *Salmonella* serotype [ser.] Typhimurium or *Salmonella* Typhimurium). Over 2,400 serotypes have been identified, approximately 60% of which belong to *S. enterica* subsp. *enterica*.. Serotyping provides a consistent subtyping scheme that has changed little over time, permitting analysis of trends in *Salmonella* surveillance data. For example, infection with *Salmonella* Typhi (typhoid fever) was common in the United States in the late nineteenth and early twentieth centuries, but with widespread chlorination of drinking water supplies and improved sewage disposal practices, the incidence of *S. typhi* has declined dramatically (Tauxe 1996). Coincident with this decline has been the rise in the incidence of non-typhoidal salmonellosis, usually attributed to waterborne disease agents or foods of animal origin such as eggs, meats, and poultry.

A full summary of Salmonella serotypes and their association with disease occurrences (as sporadic infections or outbreaks) through 1998 is presented in CDC (2000), and for the current investigation we have presented selected serotypes that most closely match those specifications related to exposures associated with biota that may be transferred consequent to water diversions between the Missouri River and Red River basin. These epidemiologic summaries focus on graphical displays of data for common *Salmonella* serotypes reported in the United States during the 1968-1998 reporting period, and represent the type of data, when updated, that would be available to intensive baseline assessment or “post-diversion” monitoring activity.

Occurrence of salmonellosis. Figure 19 and Figure 20 summarize non-human sources of S. Typhimurium and S. Enterica, respectively, and Table 3 (CDC 2000) tallies *Salmonella* serotypes by record of occurrence throughout the US. These compiled data summarized by CDC represent input from states, generally reporting county-level data. For example, for state records, compiled data from Minnesota for 2002 (Table 4) illustrates available summary data for incidence of reported diseases aggregated by county into state regions, which could be applicable to future studies focused on river comparisons of disease incidence. Similarly, Manitoba presents summary maps and numeric data that could be similarly applied to subsequent analysis, if desired.

Trends in disease incidence. As in the large works from which these graphic summaries are derived (e.g., CDC 2000), it is beyond the scope of this narrative summary to comment on specific data for each serotype. General trends, however, are worth noting, particularly as those related to waterborne disease occurrence that might related to the current investigation’s focus on interbasin biota transfers. Throughout the US, S. Typhimurium, S. Enteritidis, and S. Heidelberg were the three most frequently reported serotypes from 1968 to 1998 and accounted for over 50% of all reported *Salmonella* isolations in the United States during the period. While the rate of reported S. Typhimurium has recently been relatively stable, there has been a general increase in S. Enteritidis infections in the United States up until 1995 (Hargrett-Bean et al 1988, Rodrigue et al 1990, Angulo et al 1999).

Figure 18. Non-human sources of S. Typhimurium (1968-1998).

Table 3. Summary of occurrence of *Salmonella* serotypes as percent of total cases in US.

Top 100 reported isolates of *Salmonella* serotypes
Ranking
United States, 1968-1998

Rank	Serotype	Count	Percent	Rank	Serotype	Count	Percent
1	*S.* Typhimurium	301548	29.4072	51	*S.* Miami	1901	0.1854
2	*S.* Enteritidis	145405	14.18	52	*S.* Hartford	1855	0.1809
3	*S.* Hejdelberg	82442	8.0398	53	*S.* Havana	1730	0.1687
4	*S.* Newport	60983	5.9471	54	*S.* Rubislaw	1659	0.1618
5	*S.* Infantis	32392	3.1589	55	*S.* Worthington	1652	0.1611
6	*S.* Agona	27414	2.6734	56	*S.* Paratyphi A	1644	0.1603
7	*S.* Saintpaul	21646	2.1109	57	*S.* Adelaide	1633	0.1593
8	*S.* Hadar	20715	2.0201	58	*S.* Virchow	1630	0.159
9	*S.* Montevideo	19928	1.9434	59	*S.* Norwich	1596	0.1556
10	*S.* Group B	18991	1.852	60	*S.* Cubana	1477	0.144
11	*S.* Thompson	17858	1.7415	61	*S.* Johannesburg	1442	0.1406
12	*S.* Javiana	16622	1.621	62	*S.* Kentucky	1379	0.13448
13	*S.* Oranienburg	16339	1.5934	63	*S.* Bovismorbificans	137	0.1339
14	*S.* Typhi	16333	1.5928	64	*S.* Kottbus	1317	0.12843
15	*S.* Muenchen	14495	1.4136	65	*S.* Alachua	1282	0.12502
16	*S.* Derby	10680	1.0415	66	*S.* Haardt	1255	0.12239
17	*S.* Braenderup	10452	1.0193	67	*S.* Albany	1122	0.10942
18	*S.* Blockley	9734	0.9493	68	*S.* Gaminara	1054	0.10279
19	*S.* Anatum	7983	0.7785	69	*S.* Livingstone	1027	0.10015
20	*S.* Panama	7026	0.6852	70	*S.* Meleagridis	961	0.09372
21	*S.* Java	6865	0.6695	71	*S.* Newington	906	0.08835
22	*S.* Poona	5766	0.5623	72	*S.* Urbana	847	0.0826
23	*S.* Berta	5579	0.5441	73	*S.* Subspecies IIIA/IIIB	773	0.07538
24	*S.* Group D1	4862	0.4741	74	*S.* Oslo	769	0.07499
25	*S.* Manhattan	4852	0.4732	75	*S.* Siegburg	746	0.07275
26	*S.* Reading	4615	0.4501	76	*S.* Group E1	706	0.06885
27	*S.* Litchfield	456	0.445	77	*S.* Minnesota	673	0.06563
28	*S.* Schwarzengrund	4435	0.4325	78	*S.* Uganda	539	0.05256
29	*S.* Mississippi	4279	0.4173	79	*S.* Group G	530	0.05169
30	*S.* Paratyphi B	4262	0.4156	80	*S.* Drypool	516	0.05032
31	*S.* Bredeney	4230	0.4125	81	*S.* Duesseldorf	512	0.04993
32	*S.* Senftenberg	4090	0.3989	82	*S.* Inverness	448	0.04369
33	*S.* Group C1	4012	0.3913	83	*S.* Saphra	436	0.04252
34	*S.* Ohio	3887	0.3791	84	*S.* Ibadan	434	0.04232
35	*S.* Group C2	3613	0.3523	85	*S.* Newbrunswick	427	0.04164
36	*S.* Sandiego	3524	0.3437	86	*S.* Marina	377	0.03677
37	*S.* Mbandaka	3291	0.3209	87	*S.* Glostrup	369	0.03599
38	*S.* Bareilly	3243	0.3163	88	*S.* California	342	0.03335
39	*S.* Brandenburg	3055	0.2979	89	*S.* Eastbourne	337	0.03286
40	*S.* Weltevreden	2972	0.2898	90	*S.* Flint	321	0.0313
41	*S.* Give	2871	0.28	91	*S.* Hvittingfoss	303	0.02955
42	*S.* Tennessee	2847	0.2776	92	*S.* Stanleyville	291	0.02838
43	*S.* Stanley	2524	0.2461	93	*S.* Pomona	268	0.02614
44	*S.* London	2519	0.2457	94	*S.* Lindenburg	262	0.02555
45	*S.* Dublin	2503	0.2441	95	*S.* Irumu	256	0.02497
46	*S.* Cerro	2502	0.244	96	*S.* Istanbul	238	0.02321
47	*S.* Chester	2462	0.2401	97	*S.* Bardo	236	0.02301
48	*S.* Choleraesuis	2250	0.2194	98	*S.* Lomalinda	219	0.02136
49	*S.* Muenster	2063	0.2012	99	*S.* Madelia	216	0.02106
50	*S.* Indiana	1946	0.1898	100	*S.* Subspecies IIIA	212	0.02067

Note: *S.* Typhimurium includes var. copenhagen
S. Choleraesuis includes var. kunzendorf

Figure 19. Non-human sources of S. Enteritidis (1968-1998).

Figure 20. Summary maps for S. Typhimurium by county.

Figure 9 - Age standardized rates per 100,000 of reported isolates by County

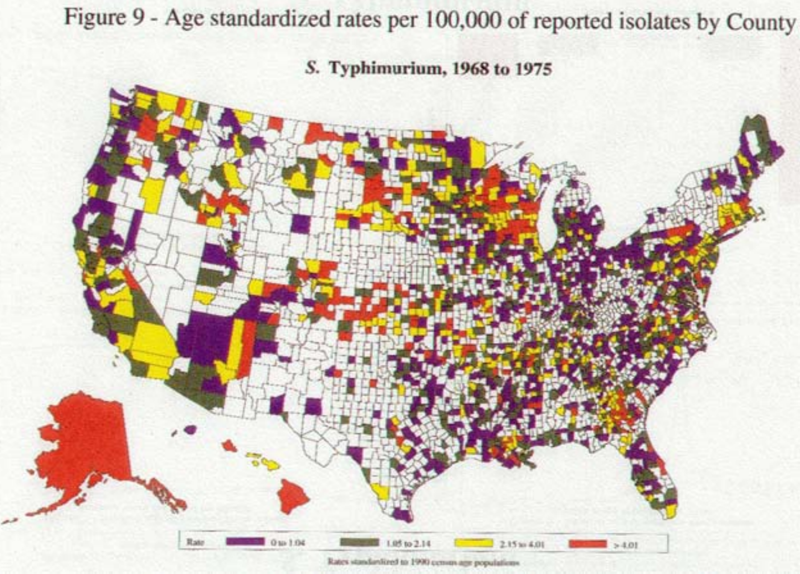


Figure 10 - Age standardized rates per 100,000 of reported isolates by County

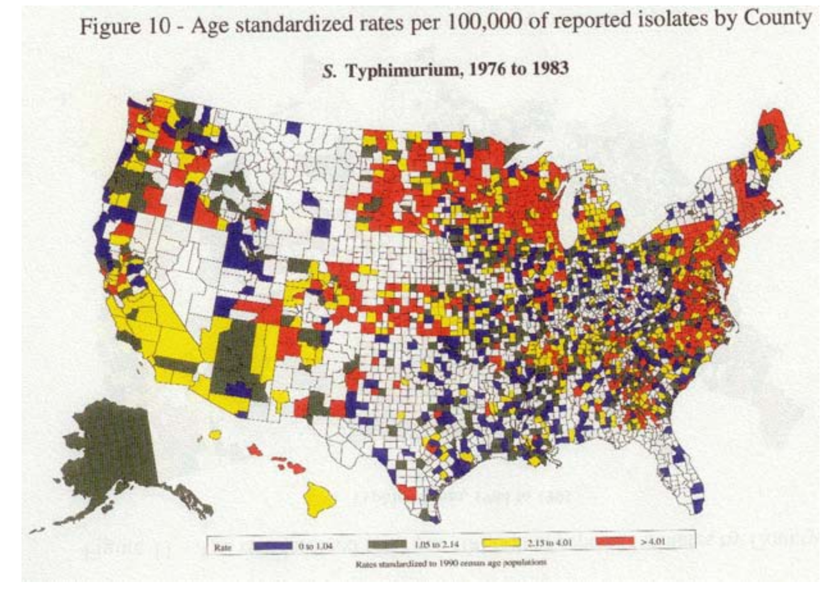


Figure 11 - Age standardized rates per 100,000 of reported isolates by County

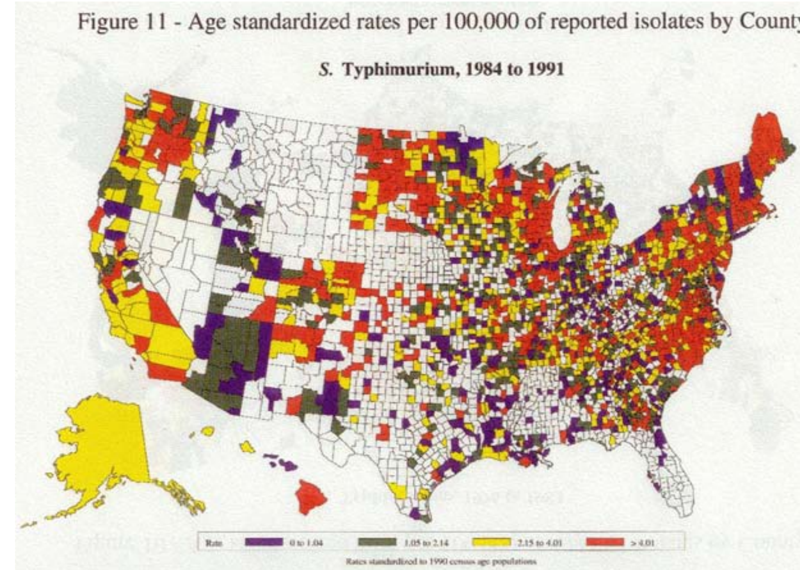


Figure 12 - Age standardized rates per 100,000 of reported isolates by County

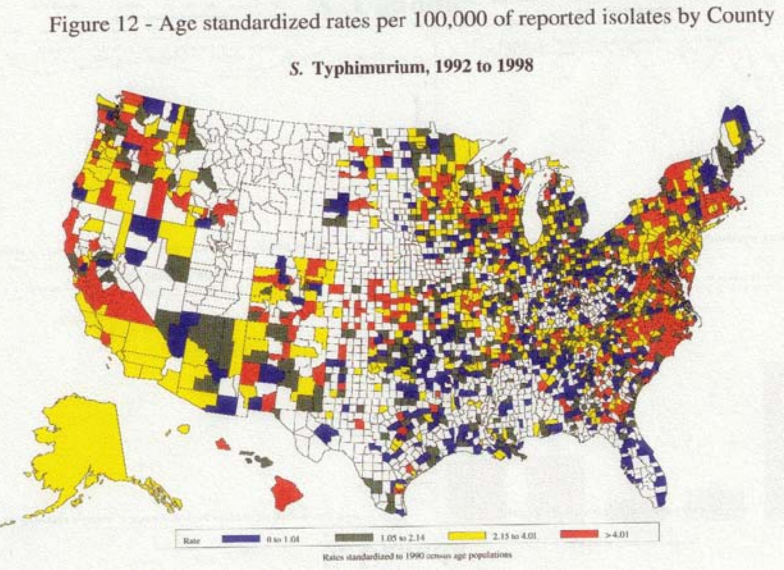


Figure 21. Summary maps for S. Enteritidis by county.

Figure 10 - Age standardized rates per 100,000 of reported isolates by County

S. Enteritidis, 1976 to 1983

Figure 9 - Age standardized rates per 100,000 of reported isolates by County

S. Typhimurium, 1968 to 1975

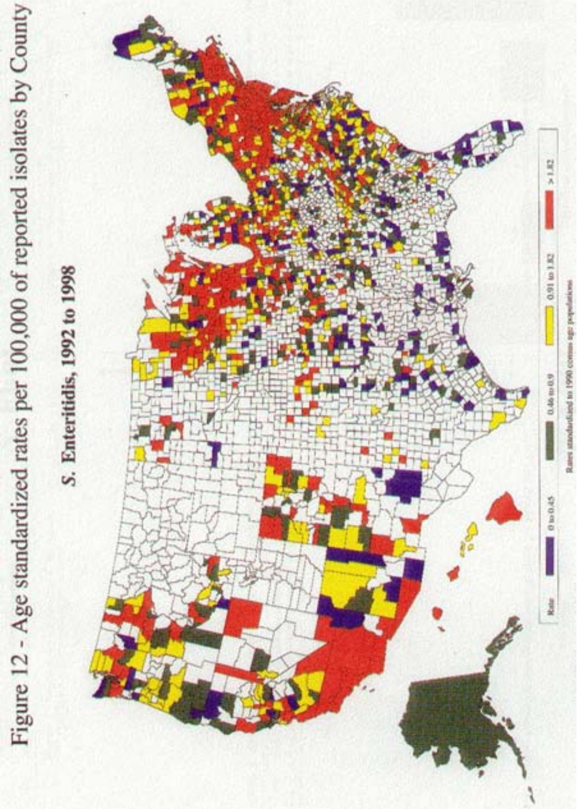


Figure 12 - Age standardized rates per 100,000 of reported isolates by County

S. Enteritidis, 1992 to 1998

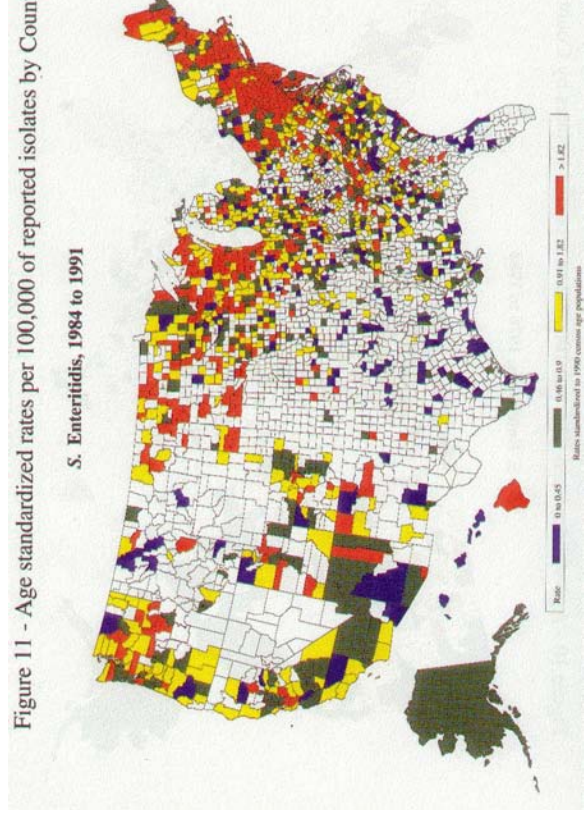


Figure 11 - Age standardized rates per 100,000 of reported isolates by County

S. Enteritidis, 1984 to 1991

Table 4. Example of Year, 2002 report from Minnesota illustrating available data for baseline and "post-diversion" monitoring.

Cases of Selected Communicable Diseases Reported to the Minnesota Department of Health, by District of Residence, 2002

District* (population per U.S. Census 2000)

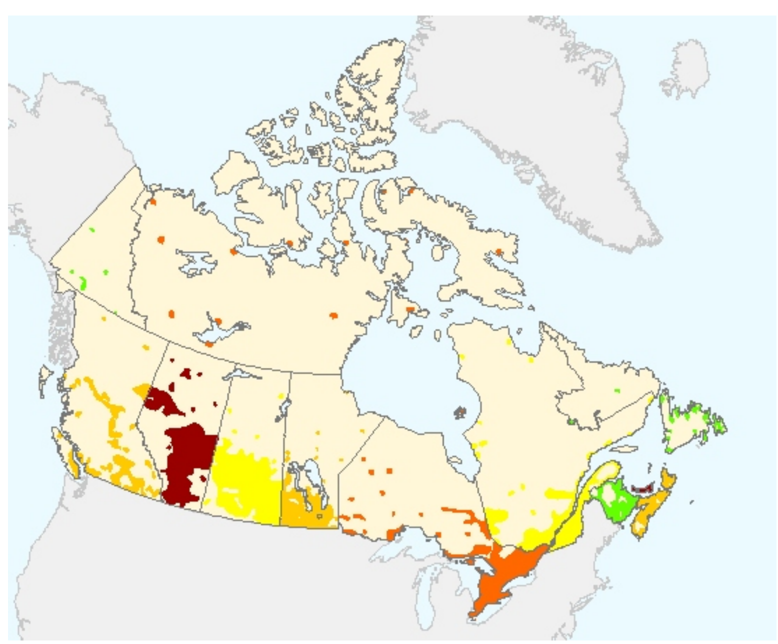
Disease	Metropolitan (2,642,056)	Northwestern (152,001)	Northeastern (248,425)	Central (683,787)	West Central (222,691)	South Central (280,332)	Southeastern (460,102)	Southwestern (230,085)	Unknown Residence	Total (4,919,479)
Campylobacteriosis	480	22	37	127	52	50	104	69	0	941
Cryptosporidiosis	44	2	11	38	37	7	49	18	0	206
Ehrlichiosis	31	0	3	109	0	1	4	1	0	149
Encephalitis - viral										
LaCrosse	8	0	0	0	0	2	3	0	0	13
West Nile	13	4	0	4	10	4	5	8	0	48
Escherichia coli O157 infection	51	5	14	27	14	5	31	13	0	160
Hemolytic Uremic Syndrome	3	0	2	4	1	0	1	0	0	11
Giardiasis	533	13	32	133	23	59	125	64	0	982
Haemophilus influenzae invasive disease	24	3	4	6	4	2	7	2	0	52
HIV infection other than AIDS	189	1	4	8	1	2	5	0	1	211
AIDS (cases diagnosed in 2002)	135	1	2	7	1	1	3	0	1	151
Legionnaires' disease	12	0	5	0	0	0	1	0	0	18
Listeriosis	1	0	0	2	0	0	1	0	0	4
Lyme disease	401	11	22	358	5	14	52	4	0	867
Measles	2	0	0	0	0	0	0	0	0	2
Mumps	5	0	0	0	0	0	0	0	0	5
Neisseria meningitidis invasive disease	8	3	6	3	1	4	8	3	0	36
Pertussis	287	6	22	49	3	1	8	53	0	429
Rubella	0	0	0	0	0	0	0	0	0	0
Salmonellosis	349	14	31	61	27	26	54	31	0	593
Sexually transmitted diseases*	10,229	163	476	886	181	348	736	285	0	13,304
Chlamydia trachomatis - genital infections	7,402	152	423	755	158	314	640	263	0	10,107
Gonorrhea	2,697	10	53	123	22	34	91	19	0	3,049
Syphilis, total	130	1	0	8	1	0	5	3	0	148
primary/secondary	55	0	0	2	0	0	1	1	0	59
early latent**	23	0	0	0	0	0	0	0	0	23
late latent***	50	1	0	6	1	0	4	2	0	64
congenital	1	0	0	0	0	0	0	0	0	1
other	1	0	0	0	0	0	0	0	0	1
Chancroid	0	0	0	0	0	0	0	0	0	0
Shigellosis	169	10	1	13	14	5	6	4	0	222
Streptococcus pneumoniae invasive disease	300	22	38	88	31	38	56	25	0	598
Streptococcal invasive disease - Group A	82	4	10	14	4	5	23	5	0	147
Streptococcal invasive disease - Group B	187	7	26	31	10	10	31	9	0	311
Tuberculosis	184	0	5	10	5	7	20	6	0	237
Viral hepatitis, type A	39	0	3	2	2	1	6	0	0	53
Viral hepatitis, type B (acute infections only, not perinatal)	36	0	4	5	2	1	2	2	0	52
Viral hepatitis, type C (acute infections only)	2	0	6	5	1	0	0	0	0	14
Yersiniosis	7	1	1	5	1	1	2	1	0	19

*Cases for which the patient's residence is unknown are assigned the geographic location of the reporting clinic.
**Duration ≤1 year
***Duration >1 year

County Distribution within Districts
Metropolitan - Anoka, Carver, Dakota, Hennepin, Ramsey, Scott, Washington
Northwestern - Beltrami, Clearwater, Hubbard, Kittson, Lake of the Woods, Marshall, Pennington, Polk, Red Lake, Roseau
Northeastern - Aitkin, Carlton, Cook, Itasca, Koochiching, Lake, St. Louis
Central - Benton, Cass, Chisago, Crow Wing, Isanti, Kanabec, Mille Lacs, Morrison, Pine, Sherburne, Stearns, Todd, Wadena, Wright
West Central - Becker, Clay, Douglas, Grant, Mahnomen, Norman, Otter Tail, Pope, Stevens, Traverse, Wilkin
South Central - Blue Earth, Brown, Faribault, LeSueur, McLeod, Martin, Meeker, Nicollet, Sibley, Waseca, Watonwan
Southeastern - Dodge, Fillmore, Freeborn, Goodhue, Houston, Mower, Olmsted, Rice, Steele, Wabasha, Winona
Southwestern - Big Stone, Chippewa, Cottonwood, Jackson, Kandiyohi, Lac Qui Parle, Lincoln, Lyon, Murray, Nobles, Pipestone, Redwood, Renville, Rock, Swift, Yellow Medicine

Population and Public Health Branch (PPHB)

Figure 22. Province-wide records for Salmonellosis (both sexes combined, including not specified); all ages, including not specified), 2000.



Rate per 100,000 population	6.53 - < 14.62	14.62 - < 16.75	16.75 - < 19.58	19.58 - < 23.85	23.85 - 65.45

Province	Rate/100,000
Newfoundland	7.62
Prince Edward Island	23.85
Nova Scotia	18.25
New Brunswick	13.63
Quebec	14.62
Ontario	19.96
Manitoba	16.75
Saskatchewan	16.15

Alberta	26.94
British Columbia	17.14
Yukon	6.54
Northwest Territories	19.58
Nunavut	65.45

Data last updated: 2003

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Protozoan infectious agents and waterborne disease. Key protozoa being considered as representative agents of waterborne disease in this investigation include:

- Cryptosporidium
- Giardia

[9]**Cryptosporidium parvum**. The current taxonomic status of *Cryptosporidium* aligns the genus with other coccidia, although recent molecular studies have shown that members of the genus are more closely related to the gregarines than to eimerians or even the malaria. The current taxonomic position of *Cryptosporidium* places the genus in the Phylum Apicomplexa, Class: Conoidasida (see Roberts and Janovy 2005).

Cryptosporidium parvum is a parasitic protozoan about 5 microns in diameter and spherical in shape. Members of the genus infect epithelial surfaces, especially those along the gut, and can be found in a wide range of vertebrates. *C. parvum* is predominately a parasite of neonate animals, and older animals generally develop milder infections, even when unexposed previously to this parasite.

Life-cycle. The life cycle of *C. parvum* is depicted in Figure 23 and begins with ingestion of the sporulated oocyst, the resistant stage found in the environment. The vertebrate intestine provides good “habitat” for these protozoan parasites, yet the number of oocysts needed to establish an infection in humans varies. For example, one study suggested that the 50% infectious dose in humans was around 132 oocysts, although one volunteer was infected with as few as 30 oocysts. In contrast, another study using a more aggressive isolate suggests that even lower numbers of oocysts (nine) can sometimes initiate infections and cause disease (see, e.g., Haas et al 1996, Gale

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[9]Original material developed by Steve J. Upton, Ph.D., Division of Biology, Ackert Hall. Kansas State University, Manhattan, Kansas; http://www.ksu.edu/parasitology/basicbio; revised and updated June, 2004 through November, 2004.

2000 and references therein, Teunis et al 2002 and references therein). Vertebrates present various degrees of susceptibility to the parasite, and the effective doses varies between individuals and among isolates.

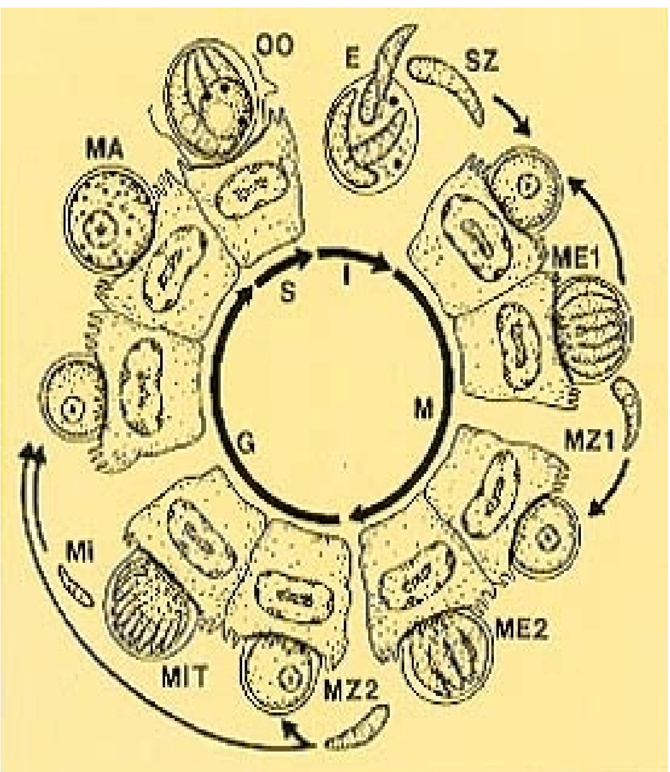


Figure 23. Life cycle of *Cryptosporidium parvum*. Abbreviations: (E) Excystation (either as thick-walled oocyst from environment or via thin-walled oocyst excysting in situ), resulting in release of 4 sporozoites through suture in wall; (G) Gamogony; (I) Infective phase; (M) Merogony; (ME1) Type I meront containing 8 merozoites; (ME2) Type II meront containing 4 merozoites; (MA) Macrogamete, containing wall forming bodies; (Mi) Microgamete; (MiT) Microgametocyte with 16 non-flagellated microgametes; (MZ1) Type I merozoite; (MZ2) Type II merozoite; (OO) oocyst; (S) Sporogony; (SZ) sporozoite. See 1986, J Protozool 33: 98-108.

Development of *Cryptosporidium* occurs rapidly, and each generation can develop and mature in as little as 12-14 hours. Due to the rapidity of the life cycle, plus the autoinfective cycles, huge numbers of organisms can colonize the intestinal tract in several days. The ileum is the primary target of infection, with secondary sites being the duodenum and large intestine. In individuals that are immunosuppressed (e.g., under medication or not immunocompetent), parasites can sometimes be found in the stomach, biliary and pancreatic ducts, and respiratory tract. Diarrhea, weight loss, and abdominal cramping are clinical signs of the disease and in immunosuppressed individuals electrolyte imbalance may occur. The prepatent period, which is the interval between infection and the first appearance of oocysts in the feces, is generally 4 days (3 days in heavy infections) in animals infected experimentally. In human outbreaks where lower numbers of oocysts are probably ingested, 4-6 days is probably typical. Patency, which is the length of time

oocysts are shed in the feces, generally lasts 6-18 days (4-10 days of diarrhea) in immunocompetent individuals but may be prolonged in immunosuppressed patients. Some individuals shed oocysts but appear asymptomatic.

Epidemiology. Oocysts of *Cryptosporidium* are widespread in the environment and can be found in freshwater surface waters such as rivers and streams, and lakes and ponds. Depending on type and location of freshwater habitat, *Cryptosporidium* oocysts may occur in high numbers. For example, surface waters receiving runoff from livestock operations characteristically present high oocysts counts, while relatively "pristine" areas may have very few oocysts. The extent of oocyst contamination, however, does not necessarily lessen the infectivity of a given "dose" of infective agent. The occurrence of *Cryptosporidium* in the environment also varies with season, e.g., *Cryptosporidium* becomes a problem in surface waters in most areas of North America is generally March-June, when spring rains increase run-off and many neonate animals are present in the environment to amplify oocyst numbers. Despite the seasonal variation in disease occurrence, studies suggest that many adult animals produce low levels of oocysts on a regular basis, which enhances the environmental load and serves as a source of infection year around. Ruminants, cervids, swine, cats, dogs, and other mammals may all contribute to numbers of *Cryptosporidium* oocysts in the environment both in rural and urban areas.

Failures or overloaded public water utilities have occasionally resulted in community outbreaks of cryptosporidiosis. In other cases, infections have been acquired from swimming pools and water parks because of fecal accidents. In most cases, various degrees of diarrhea, some weight loss and abdominal cramping were the extent of illness. In some individuals, however, specifically young children, the elderly, and immunosuppressed patients, cryptosporidiosis became chronic and life-threatening. It should be noted that it is nearly impossible to determine the origin of many individual cases of cryptosporidiosis.

Recent studies have shown that *C. parvum* exists as no less than two distinct species. Genotype 1 (or genotype H for human) is now termed *Cryptosporidium hominis* and is almost exclusively a

parasite of humans (with a few minor exceptions), while Genotype 2 (or genotype C for calf) is considered the traditional *Cryptosporidium parvum* and occurs in a wide range of animals, including humans. The former species tends to be more aggressive in humans, with a patent period nearly doubling that of genotype 2 and averaging just under 2 weeks. Rarely, both species can be found infecting the same person. Genetic markers on different chromosomes reveal there is little or no mixing between the two (i.e. isolates are not found that are composed of mixed genotypes), strongly supporting the notion that two distinct (but morphologically identical) species exist. Either species may cause an outbreak.

Cryptosporidiosis occurs throughout North America, and North Dakota, Minnesota, and Manitoba have documented cases of the disease (see Figure 24 and Figure 25). As illustrated by Figure 24 and Figure 25, epidemiologic data for *C. parvum* are available, but “as is,” the data must be recompiled within the context of river basins in order to address intensive baseline

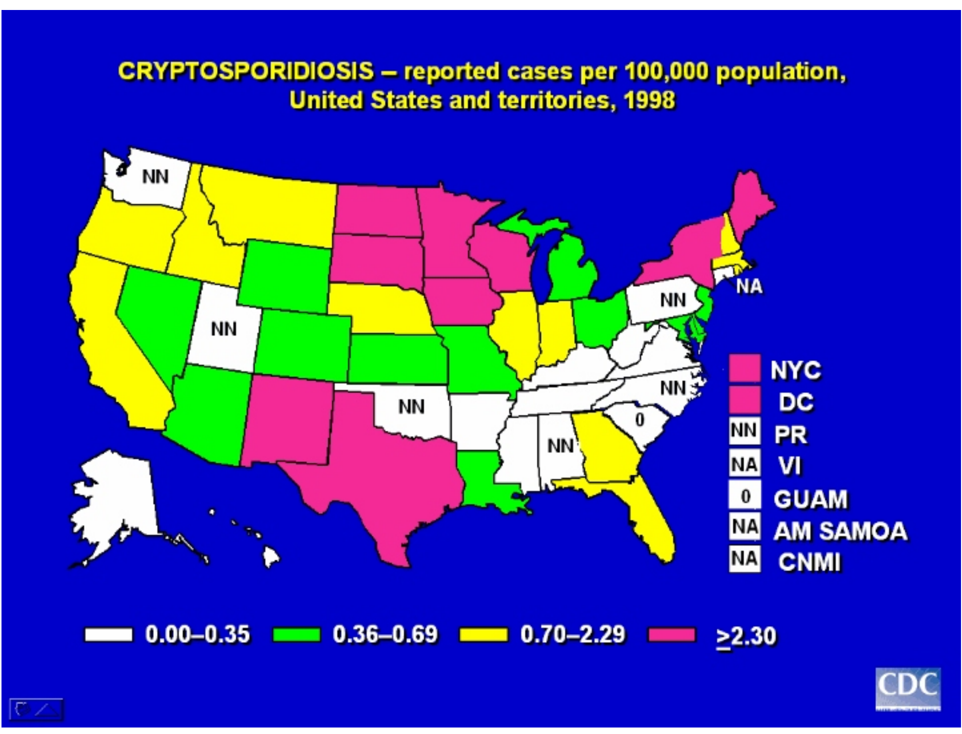


Figure 24. Incidence of cryptosporidiosis as compiled by CDC for 1998.

assessment or monitoring activity, as those relate to biota transfers that might manifest as disease

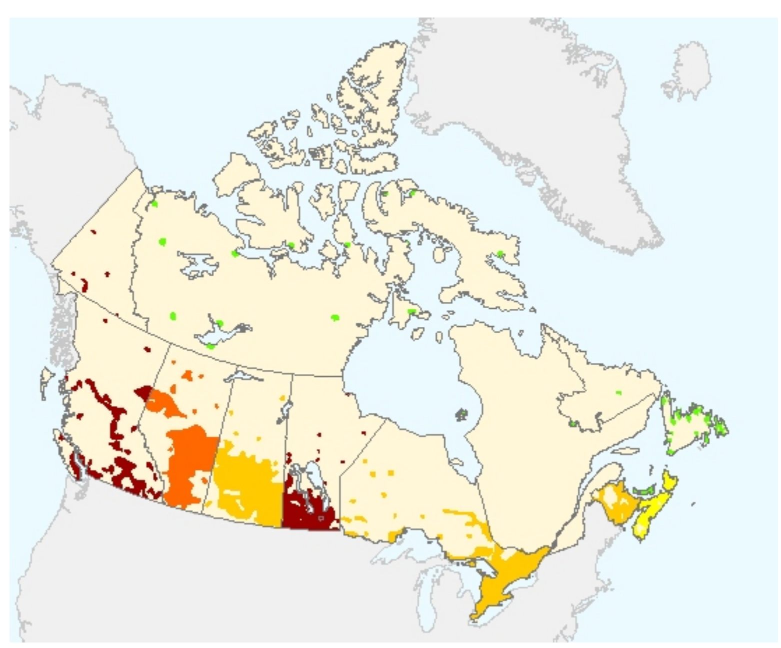
outbreaks associated with agents in source waters. Currently, available data across years suggest that incidence withing those states is similar, although statistic comparisons among states and provinces requires time-series data that presently were not incorporated into the current investigation.

Prevention and control. See Appendix 12 for overview of water treatment control technologies targeted on prevention and control of cryptosporidiosis. Because all *Cryptosporidium* infections are initiated though ingestion of environmentally resistant oocysts, control of this stage is the single most important factor in limiting the spread of the disease. Infected animals and humans will continue to contaminate the environment, and elimination of these sources is virtually impossible.

Numbers of *Cryptosporidium* oocysts reported by various groups from public water samples are highly variable. Concentration techniques for oocysts in environmental samples are relatively poor and detection methods often cross- react with algae or other debris. Hence, evaluation of disinfection is challenging. Numerous other species of *Cryptosporidium* incapable of infecting humans occur in the environment and may also cross-react in diagnostic tests. In addition, many oocysts detected are probably not viable due to age, freezing, or UV radiation. Current regulatory efforts, in part, focus on water treatment technologies targeted on preventing cryptosporidiosis (see Appendix 12).

On-Line Population and Public Health Branch (PPHB)

Figure 25. Province-wide incidence of cryptosporidiosis (both sexes combined, including not specified; all ages, including not specified), 2000.



Rate per 100,000 population	0.00 - < 0.01	0.01 - < 1.86	1.86 - < 3.19	3.19 - < 4.16	4.16 - 16.34

Province	Rate per100,000
Newfoundland	0.00
Prince Edward Island	0.00
Nova Scotia	0.64
New Brunswick	2.65
Quebec	
Ontario	1.86
Manitoba	5.76
Saskatchewan	3.13

Province	Rate per100,000
Alberta	3.19
British Columbia	4.16
Yukon	16.34
Northwest Territories	0.00
Nunavut	3.64

Data updated: 2003

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Giardia. Giardia is one of the most common protozoan parasites in vertebrates, including humans. In vertebrates, the passage of *Giardia* species from one host to another occurs predominately via a fecal-to-oral route, most frequently through ingestion of contaminated water (e.g., drinking water or water ingested with foods washed with contaminated water). *Giardia* spp. display alternation of generations and exists in two distinct life forms, the trophozoite and the cyst. The flagellated trophozoite moves about the host's gastrointestinal tract where it makes use of its adhesive disk to attach to intestinal cells lining the gut and feeds on cellular secretions of the small intestine. In contrast, the cyst stage is a thin-walled, but resistant stage of the life cycle capable of surviving in the external environment after passing from host. Both life stages can be shed by an infected host, but the trophozoite is not capable of surviving environmental conditions harsher than those characteristic of its host internal environment. Cysts can be transferred to new hosts by direct or indirect contact with the infected-host feces.

Under the Interim Enhanced Surface Water Treatment Rule (IESWTR; see guidance from EPA http://www.epa.gov/OGWDW/mdbp/ieswtr.html last accessed December 11, 2004), potable waters from water treatment facilities must meet performance criteria intended to eliminate threats of *Giardia* infections (See Text Box 1 summarizing drinking water regulatory guidance). Groundwater sourced by drinking-water wells and not be affected by leaking septic tanks or surface waters not adversely affected by land-use practices, e.g., pasture runoff, would not be expected to be contaminated with *Giardia* spp. or *Cryptosporidium* spp. Under IESWTR removal of *Giardia* should be assured, given the specified performance criteria targeted on *Cryptosporidium* which is much smaller then *Giardia*, and filtration practices intended to remove *Cryptosporidium* should remove *Giardia* cysts.

Text Box 1. Summary of EPA regulations regarding drinking water, including guidance pertinent to *Giardia* spp. and *Cryptosporidium* spp. disinfection.

Environmental Protection Agency (EPA) regulations regarding drinking water, 1974–2003

Regulation/date	Description
Safe Drinking Water Act/1974 and 1986 and 1996 amendments	Authorizes EPA to set national standards to protect drinking water and its sources
Total Coliform Rule (TCR)/and Maximum Contaminant Level (MCL)/1989	Requires routine monitoring for total coliforms of all public water systems plus periodic on-site inspections for systems that take <5 samples/month to evaluate and document treatment, storage, distribution network, operation and maintenance, and overall management. Systems that collect ≥40 samples/month (i.e., typically, systems that serve >33,000 persons) violate MCL if >5.0% of the samples collected during each month are positive for total coliforms; systems that collect <40 samples/month violate MCL if two samples during the month are positive for total coliforms. If a system has a total coliform-positive sample, then 1) that sample must be tested for the presence of fecal coliforms or *Escherichia coli*, and 2) three repeat samples must be collected (four, if the system collects ≤1 routine sample/month) within 24 hours and analyzed for total coliforms. If positive, the sample must be analyzed for fecal coliforms or *Es. coli*. In addition, ≥5 routine samples must be collected during the next month of sampling, regardless of system size. For any size system, if two consecutive total coliform-positive samples occur at one site during a month, and one of these samples is also fecal coliform-positive or *Es. coli*-positive, the system has an acute violation of the Maximum Contaminant Level and must notify the state and the public immediately.
Surface Water Treatment Rule (SWTR)/1989	Covers all water systems that use surface water or groundwater under the direct influence of surface water; all systems must disinfect their water, and the majority of systems must filter their water also, unless they meet EPA-specified filter-avoidance criteria that define high-quality source water. Specific requirements include • a combined filter-effluent–performance standard for turbidity (i.e., for rapid granular filters, 0.5 nephelometric turbidity unit [NTU] maximum for 95% of measurements [taken every 4 hours] during a month) and no single NTU reading >5.0; • watershed protection, redundant disinfection capability, and other requirements for unfiltered systems; • a 0.2-mg/L disinfectant residual entering the distribution system; and • maintenance of a detectable disinfectant residual in all parts of the distribution system. This rule requires that all such systems reduce the level of *Giardia* by 99.9% (3-log reduction) and viruses by 99.99% (4-log reduction) through a combination of removal (filtration) and inactivation (disinfection).
Information Collection Rule/ 1996–1998	Requires systems serving ≥100,000 persons to provide treatment data and monitor disinfection byproducts and source water quality parameters. Surface water systems are also required to monitor *Cryptosporidium*, *Giardia*, total culturable viruses, and total and fecal coliforms or *Es. coli* ≥1 time/month for 18 months. Results provided information to facilitate development of the Long Term 2 Enhanced SWTR, which is intended to protect against microbial risks by targeting those systems with suboptimal quality source water and to balance the health risks associated with disinfection byproducts and the anticipated Stage 2 Disinfection Byproduct Rule.
Interim Enhanced Surface Water Treatment Rule (IESWTR)/1998	Follow-up to SWTR that covers all public systems using surface water or groundwater under the direct influence of surface water and serving ≥10,000 persons. Key provisions include • a 2-log *Cryptosporidium*-removal requirement for filtered systems; • strengthened combined filter-effluent–turbidity performance standards for systems using conventional filtration treatment or direct filtration (0.3 NTU maximum for 95% of measurements during a month and no single NTU reading >1.0); • individual filter turbidity monitoring provisions;

Text Box 1 (continued). Summary of EPA regulations regarding drinking water, including guidance pertinent to *Giardia* spp. and *Cryptosporidium* spp. disinfection.

(*Continued*). Environmental Protection Agency (EPA) regulations regarding drinking water, 1974–2003

Regulation/date	Description
	• disinfection profile and benchmark provisions to ensure continued levels of microbial protection while facilities take necessary steps to comply with new disinfection byproduct standards; • revision of the definition of groundwater under the influence of surface water and the watershed-control requirements for unfiltered public water systems to include detection of *Cryptosporidium*; • requirements for covers on newly finished water reservoirs; • sanitary surveys for all surface water systems regardless of size; and • an MCL goal of zero oocysts for *Cryptosporidium*.
Lead and Copper Rule/2000 changes	Streamlines requirements, promotes consistent national implementation, and reduces the burden for water systems.
Long Term 1 Enhanced SWTR (LT1ESWTR)/2002 and the Filter Backwash Recycling Rule (FBRR)/2001	Companion regulations for IESWTR; LT1ESWTR applies to public water systems that use surface water or groundwater under the direct influence of surface water and that serve <10,000 persons. FBRR regulates how treatment plants recycle water that has been used to backwash a filter or that has been extracted from treatment plant sludge. FBRR regulates the point in the treatment plant at which the contaminated recycle water may be introduced, assuring that the water is subject to the entire particle and *Cryptosporidium parvum* removal process.
Long Term 2 Enhanced SWTR (LT2ESWTR)/expected in 2003	Applies to all systems using surface water or groundwater under the influence of surface water; will provide additional protection against *Cryptosporidium*. Systems will be assigned to a treatment category on the basis of their source-water *Cryptosporidium* levels; the category then determines how much additional treatment is required.
Stage 2 Disinfection Byproduct Rule (DBPR)/expected in 2003	Will apply to community water systems and nontransient noncommunity water systems that use an alternative to ultraviolet disinfection or deliver disinfected water; systems will be required to monitor for total trihalomethanes and the sum of five haloacetic acids and comply with MCLs at each monitoring location as a locational running annual average.
Ground Water Rule (GWR) (1996 amendment to EPA's Safe Drinking Water Act)/expected to be finalized in 2003	Applies to public groundwater systems (i.e., systems that have ≥15 service connections, or regularly serve ≥25 persons daily for ≥60 days/year) or any system that mixes surface and groundwater if the groundwater is added directly to the distribution system and provided to consumers without treatment. Establishes multiple barriers to protect against bacteria and viruses in drinking water from groundwater sources; establishes targeted strategy to identify groundwater systems at high risk for fecal contamination. Key areas include • system sanitary surveys; • hydrogeologic sensitivity assessments for nondisinfected systems; • source-water microbial monitoring by systems that do not disinfect and that draw from hydrogeologically sensitive aquifers or have detected fecal indicators within the system's distribution system; • corrective action by any system with substantial deficiencies or positive microbial samples indicating fecal contamination; and • compliance monitoring for systems that disinfect to ensure that they reliably achieve 4-log (99.99%) inactivation or removal of viruses. GWR does not apply to privately owned wells that serve <25 persons (e.g., individual homeowner wells).

[10]**Life history of *Giardia lamblia*.** A typical *G. lamblia* life cycle is illustrated in Figure 26. Infection occurs by the ingestion of cysts in contaminated water or food, and in the small intestine excystation releases trophozoites that multiply by longitudinal binary fission. The trophozoites

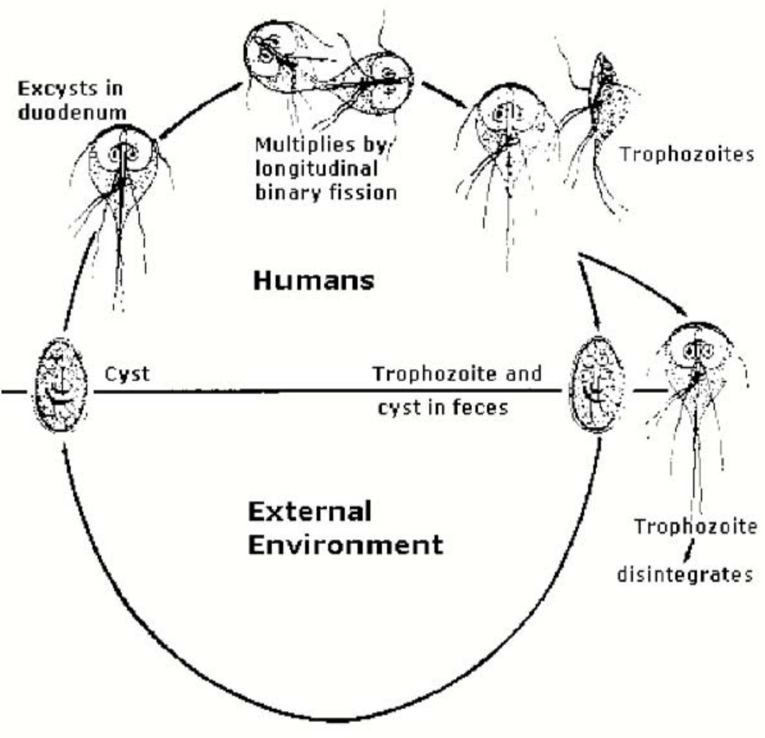

Figure 26. Life cycle of *Giardia lamblia*.

remain in the lumen of the proximal small bowel where they can be free or attached to the mucosa by a ventral sucking disk. Encystation occurs when the parasites transit toward the colon, and cysts are released to the environment with normal (non-diarrheal) feces. The cysts are hardy, can survive several months in cold water, and are responsible for transmission. Because the cysts are infectious when passed in the stool or shortly afterward, person-to-person transmission is possible. While animals are infected with *Giardia*, their importance as a reservoir is unclear, especially as that relates to the disease being a zoonosis.

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[10]Original material courtesy of the Division of Parasitic Diseases at the National Center for Infectious Diseases, Centers for Disease Control & Prevention; (accessed June, 2004 http://www.dpd.cdc.gov/DPDx/HTML/Giardiasis.htm).

Trophozoites of *G. lamblia* range from 12 to 15 µm in length and are pear-shaped. Two nuclei, median bodies, and four pairs of flagella are characteristic hallmarks of the species. Cysts of *Giardia* are the life form generally found in feces, and are ovoid, 6 to 12 µm long. Cysts contain two to four nuclei at one end and present prominent diagonal fibrils. Motile trophozoites live in the duodenum and jejunum and multiply by binary fission. As trophozoites transit the lower intestine, encystation occurs as the motile forms of the organisms enter the fecal stream, lose their motility, round up, and ultimately excreted as dormant, resistant cysts

Pathologically, the presence of intestinal trophozoites results in an increased turnover of intestinal epithelium, with replacement of mature cells by immature intestinal cells. The replacement of mature gastrointestinal epithelia with immature types reduces the gut's overall ability to digest and absorb fats and fat-soluble vitamins; hence, the diarrhea and steatorrhea observed during the course of the disease. Although antibodies are generally not produced in response to infections, both cellular and humoral host defenses are initiated consequent to infection.

Epidemiology. *Giardia* infection occurs worldwide, with an incidence usually ranging from 1.5 to 20 percent, with higher incidences generally occurring where sanitary standards are low. Although people of all ages may harbor these organisms, infants and children are more often infected than are adults. Carriers are probably more important in the spread of these organisms than symptomatic patients because cysts are less likely to be present in diarrheic stool. Recent outbreaks, some of epidemic proportions, have occurred in North America, generally linked to drinking contaminated water from community water supplies or directly from rivers and streams.

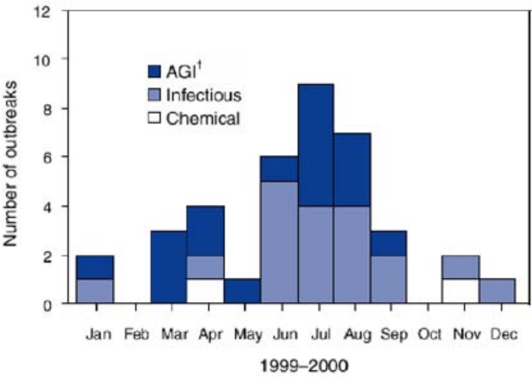
Many animals harbor *Giardia* organisms that are indistinguishable from *G. lamblia.* In the past, these isolates were assumed to be host-specific, but recent evidence suggests this is not always the case. At least some of the *Giardia* strains that parasitize animals may also infect humans and vice versa which complicates the problem of defining species in this genus. The role of animal reservoirs of *Giardia* and zoonotic mechanisms associated with human infections is also of increasing concern. For example, the finding of *Giardia*-infected animals in watersheds from

which humans acquired giardiasis, and the successful interspecies transfer of these organisms, strengthens the possibility that giardiasis is a zoonotic infection. In these settings, infected beavers are believed to be one source of water-borne giardiasis, since beaver *Giardia* isolates are capable of infecting dogs and humans. Hence, dogs may also be another source of human giardiasis. Many vertebrates harbor *Giardia* spp. indistinguishable from *G lamblia*, and there is evidence that *Giardia* strains isolated from domestic livestock or vertebrate wildlife (e.g., cattle, deer, beaver) may be infective for humans. Different strains of *G. lamblia* possibly vary in virulence. There are many sources of potentially infective agent; hence, fecal-oral transmission of disease can occur via drinking water and may be a problem wherever water treatment fails or is absent.

Multiple illustrations in Figure 27 summarize the national picture for waterborne diseases in the US for 1999-2000. Data compiled to yield graphic summaries in Figure 27 are given a regional focus on Minnesota, North Dakota, and Manitoba through summary Table 5through Table 9, and Figure 28 (with companion summary table). As such, available sources from, e.g., CDC and state, or provincial health agencies, indicate that data would be available for analysis of intensive baseline assessment or “post-diversion” monitoring studies, if interbasin water diversions are realized.

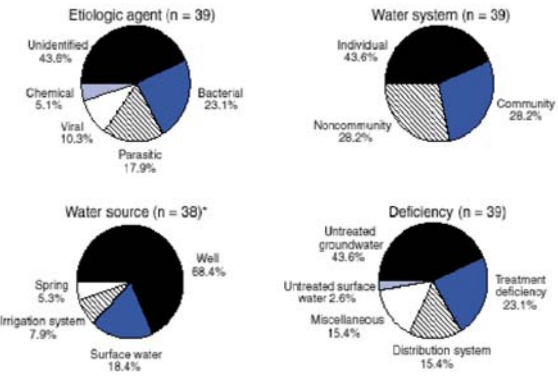
Figure 27. Multiple graphic summaries of waterborne disease occurrence in US and Canada (available from CDC at http://www.cdc.gov/ncidod/diseases/list_waterborne.htm last accessed December 14, 2004).

FIGURE 1. Number of waterborne-disease outbreaks associated with drinking water, by etiologic agent and month — United States, 1999–2000 (n = 38)*



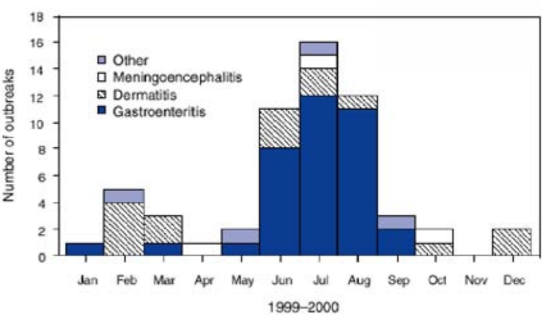
* One outbreak of *Salmonella* Bareilly was not included.
† Acute gastrointestinal illness of unknown etiology.

FIGURE 2. Waterborne-disease outbreaks associated with drinking water, by etiologic agent, water system, water source, and deficiency — United States, 1999–2000 (n = 39)



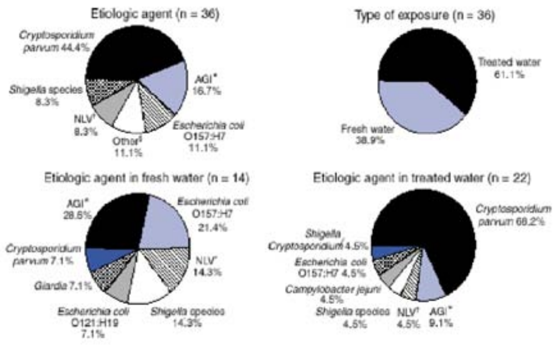
* One outbreak of *Salmonella* Bareilly was not included.

FIGURE 3. Number of waterborne-disease outbreaks associated with recreational water, by illness and month — United States, 1999–2000 (n = 58)*



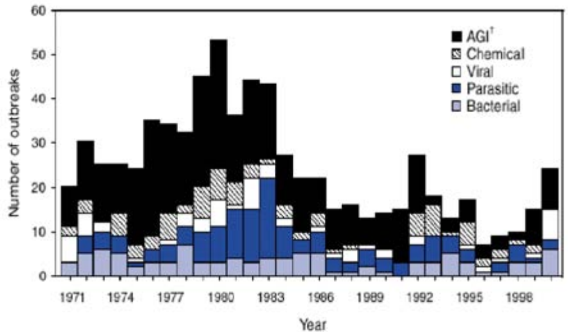
* Information regarding the month was not provided for one outbreak of meningoencephalitis.

FIGURE 4. Waterborne-disease outbreaks of gastroenteritis associated with recreational water, by etiologic agent and type of exposure — United States, 1999–2000



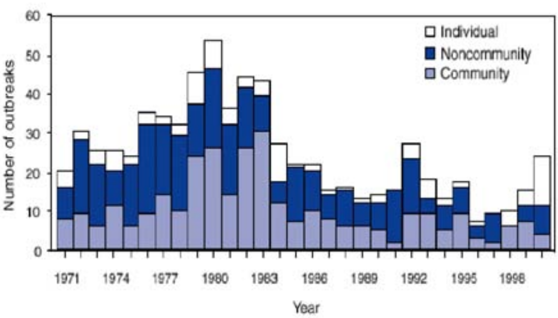
* Acute gastrointestinal illness of unknown etiology.
† Norwalk-like virus.
§ These included outbreaks of *Campylobacter jejuni*, *Giardia*, *Escherichia coli* O121:H19 and one mixed *Shigella/Cryptosporidium* outbreak.

FIGURE 5. Number of waterborne-disease outbreaks associated with drinking water, by year and etiologic agent — United States, 1971–2000 (n = 730)*



* The total from previous reports has been corrected from n = 691 to n = 688.
† Acute gastrointestinal illness of unknown etiology.

FIGURE 6. Number of waterborne-disease outbreaks associated with drinking water, by year and type of water system — United States, 1971–2000 (n = 730)*



* The total from previous reports has been corrected from n = 691 to n = 688.

Figure 27 (continued). Multiple graphic summaries of waterborne disease occurrence in US and Canada (available from CDC at http://www.cdc.gov/ncidod/diseases/list_waterborne.htm last accessed December 14, 2004).

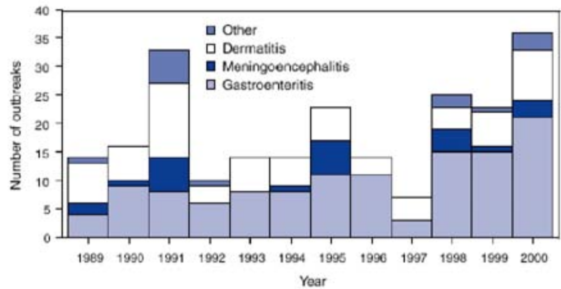


FIGURE 7. Number of waterborne-disease outbreaks associated with recreational water, by year and illness — United States, 1989–2000 (n = 229)*

* The total from previous reports has been corrected from n = 171 to n = 170.

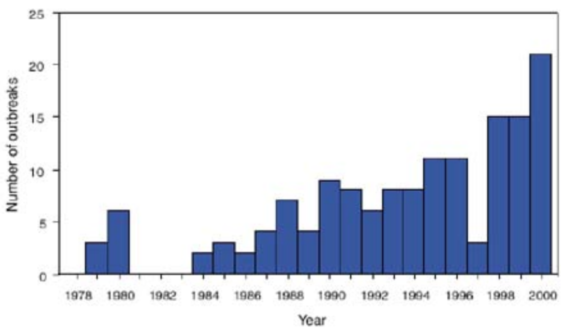


FIGURE 8. Number of outbreaks involving gastroenteritis associated with recreational water, by year and illness — United States, 1978–2000 (n = 146)

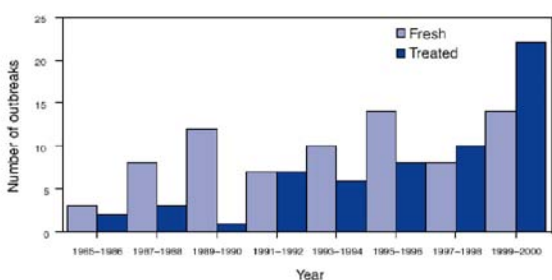


FIGURE 9. Number of outbreaks involving gastroenteritis associated with recreational water, by water type — United States, 1985–2000 (n = 135)

Table 5. State of Minnesota records of reportable communicable diseases, 2002.

Table 2. Cases of Selected Communicable Diseases Reported to the Minnesota Department of Health, by District of Residence, 2002

District* (population per U.S. Census 2000)

Disease	Metropolitan (2,642,056)	Northwestern (152,001)	Northeastern (248,425)	Central (683,787)	West Central (222,691)	South Central (280,332)	Southeastern (460,102)	Southwestern (230,085)	Unknown Residence	Total (4,919,479)
Campylobacteriosis	480	22	37	127	52	50	104	69	0	941
Cryptosporidiosis	44	2	11	38	37	7	49	18	0	206
Ehrlichiosis	31	0	3	109	0	1	4	1	0	149
Encephalitis - viral										
LaCrosse	8	0	0	0	0	2	3	0	0	13
West Nile	13	4	0	4	10	4	5	8	0	48
Escherichia coli O157 infection	51	5	14	27	14	5	31	13	0	160
Hemolytic Uremic Syndrome	3	0	2	4	1	0	1	0	0	11
Giardiasis	533	13	32	133	23	59	125	64	0	982
Haemophilus influenzae invasive disease	24	3	4	6	4	2	7	2	0	52
HIV infection other than AIDS	189	1	4	8	1	2	5	0	1	211
AIDS (cases diagnosed in 2002)	135	1	2	7	1	1	3	0	1	151
Legionnaires' disease	12	0	5	0	0	0	1	0	0	18
Listeriosis	1	0	0	2	0	0	1	0	0	4
Lyme disease	401	11	22	358	5	14	52	4	0	867
Measles	2	0	0	0	0	0	0	0	0	2
Mumps	5	0	0	0	0	0	0	0	0	5
Neisseria meningitidis invasive disease	8	3	6	3	1	4	8	3	0	36
Pertussis	287	6	22	49	3	1	8	53	0	429
Rubella	0	0	0	0	0	0	0	0	0	0
Salmonellosis	349	14	31	61	27	26	54	31	0	593
Sexually transmitted diseases*	10,229	163	476	886	181	348	736	285	0	13,304
Chlamydia trachomatis - genital infections	7,402	152	423	755	158	314	640	263	0	10,107
Gonorrhea	2,697	10	53	123	22	34	91	19	0	3,049
Syphilis, total	130	1	0	8	1	0	5	3	0	148
primary/secondary	55	0	0	2	0	0	1	1	0	59
early latent**	23	0	0	0	0	0	0	0	0	23
late latent***	50	1	0	6	1	0	4	2	0	64
congenital	1	0	0	0	0	0	0	0	0	1
other	1	0	0	0	0	0	0	0	0	1
Chancroid	0	0	0	0	0	0	0	0	0	0
Shigellosis	169	10	1	13	14	5	6	4	0	222
Streptococcus pneumoniae invasive disease	300	22	38	88	31	38	56	25	0	598
Streptococcal invasive disease - Group A	82	4	10	14	4	5	23	5	0	147
Streptococcal invasive disease - Group B	187	7	26	31	10	10	31	9	0	311
Tuberculosis	184	0	5	10	5	7	20	6	0	237
Viral hepatitis, type A	39	0	3	2	2	1	6	0	0	53
Viral hepatitis, type B (acute infections only, not perinatal)	36	0	4	5	2	1	2	2	0	52
Viral hepatitis, type C (acute infections only)	2	0	6	5	1	0	0	0	0	14
Yersiniosis	7	1	1	5	1	1	2	1	0	19

*Cases for which the patient's residence is unknown are assigned the geographic location of the reporting clinic.
**Duration ≤1 year
***Duration >1 year

County Distribution within Districts

Metropolitan - Anoka, Carver, Dakota, Hennepin, Ramsey, Scott, Washington
Northwestern - Beltrami, Clearwater, Hubbard, Kittson, Lake of the Woods, Marshall, Pennington, Polk, Red Lake, Roseau
Northeastern - Aitkin, Carlton, Cook, Itasca, Koochiching, Lake, St. Louis
Central - Benton, Cass, Chisago, Crow Wing, Isanti, Kanabec, Mille Lacs, Morrison, Pine, Sherburne, Stearns, Todd, Wadena, Wright
West Central - Becker, Clay, Douglas, Grant, Mahnomen, Norman, Otter Tail, Pope, Stevens, Traverse, Wilkin
South Central - Blue Earth, Brown, Faribault, LeSueur, McLeod, Martin, Meeker, Nicollet, Sibley, Waseca, Watonwan
Southeastern - Dodge, Fillmore, Freeborn, Goodhue, Houston, Mower, Olmsted, Rice, Steele, Wabasha, Winona
Southwestern - Big Stone, Chippewa, Cottonwood, Jackson, Kandiyohi, Lac Qui Parle, Lincoln, Lyon, Murray, Nobles, Pipestone, Redwood, Renville, Rock, Swift, Yellow Medicine

Table 6. State of Minnesota records of reportable communicable diseases, 2000.

Table 2. Cases of Selected Communicable Diseases Reported to the Minnesota Department of Health by District of Residence, 2000

District* (population; 2000 Census data)

Disease	Metropolitan (2,642,056)	Northwestern (152,001)	Northeastern (248,425)	Central (683,787)	West Central (222,691)	South Central (280,332)	Southeastern (460,102)	Southwestern (230,085)	Unknown Residence	Total (4,919,479)
Campylobacteriosis	589	12	26	146	43	52	140	71	0	1079
Cryptosporidiosis	43	0	28	27	5	22	60	12	0	197
Ehrlichiosis	24	2	2	48	0	0	2	1	0	79
Encephalitis - viral										
LaCrosse	5	0	0	1	0	0	2	0	0	8
Western	0	0	0	0	0	0	0	0	0	0
Escherichia coli O157:H7 infection	109	6	1	29	7	16	31	17	0	216
Hemolytic Uremic Syndrome	6	0	0	2	0	2	1	2	0	13
Giardiasis	605	6	21	178	33	17	122	38	207	1227
Haemophilus influenzae invasive disease	31	2	4	9	1	2	1	2	0	52
HIV infection other than AIDS	216	1	3	6	2	2	6	3	1	240
AIDS cases (diagnosed in 2000)	142	0	2	8	1	1	3	4	0	161
Legionnaires' disease	8	0	0	2	0	2	3	2	0	17
Listeriosis	6	0	0	1	0	0	0	0	0	7
Lyme disease	249	3	9	165	2	3	31	3	0	465
Measles	0	0	1	0	0	0	0	0	0	1
Mumps	3	0	0	2	1	0	1	0	0	7
Neisseria meningitidis invasive disease	13	0	4	0	4	0	1	0	0	22
Pertussis	460	14	3	38	11	11	23	15	0	575
Rubella	1	0	0	0	0	0	0	0	0	1
Salmonellosis	364	7	14	72	25	30	68	32	0	612
Sexually transmitted diseases*										
Chlamydia trachomatis - genital infections	6166	203	294	503	148	198	464	126	0	8102
Gonorrhea	2843	14	90	99	19	31	44	20	0	3160
Syphilis total	63	0	1	9	1	0	4	1	0	79
primary/secondary	13	0	1	1	1	0	0	0	0	16
early latent**	16	0	0	2	0	0	0	0	0	18
late latent***	32	0	0	6	0	0	4	1	0	43
congenital	2	0	0	0	0	0	0	0	0	2
Chancroid	0	0	0	0	0	0	0	0	0	0
Shigellosis	766	11	16	50	7	15	15	23	1	904
Streptococcus pneumoniae invasive disease (Twin Cities only)	439	--	--	--	--	--	--	--	--	439
Streptococcal invasive disease - Group A	96	4	12	14	5	5	8	5	0	149
Streptococcal invasive disease - Group B	175	8	24	21	9	23	24	10	0	294
Tetanus	0	0	0	1	0	0	1	0	0	2
Tuberculosis	137	5	1	6	3	2	18	6	0	178
Viral hepatitis, type A	131	0	6	10	1	19	26	4	0	197
Viral hepatitis, type B (acute infections only)	46	0	1	4	0	2	5	0	0	58
Viral hepatitis, type C (acute infections only)	5	0	4	1	1	0	3	1	0	15
Yersiniosis	4	0	0	1	1	1	3	0	0	10

*Cases for which the patient's residence is unknown are assigned the geographic location of the reporting clinic.
**Duration ≤1 year
***Duration >1 year

County Distribution within Districts
Metropolitan = Anoka, Carver, Dakota, Hennepin, Ramsey, Scott, Washington
Northwestern = Beltrami, Clearwater, Hubbard, Kittson, Lake of the Woods, Marshall, Pennington, Polk, Red Lake, Roseau
Northeastern = Carlton, Cook, Lake, St. Louis
Central = Aitkin, Benton, Cass, Chisago, Crow Wing, Isanti, Itasca, Kanabec, Koochiching, Mille Lacs, Morrison, Pine, Sherburne, Stearns, Todd, Wadena, Wright
West Central = Becker, Clay, Douglas, Grant, Mahnomen, Norman, Otter Tail, Pope, Stevens, Traverse, Wilkin
South Central = Blue Earth, Brown, Faribault, LeSueur, McLeod, Martin, Meeker, Nicollet, Sibley, Waseca, Watonwan
Southeastern = Dodge, Fillmore, Freeborn, Goodhue, Houston, Mower, Olmsted, Rice, Steele, Wabasha, Winona
Southwestern = Big Stone, Chippewa, Cottonwood, Jackson, Kandiyohi, Lac Qui Parle, Lincoln, Lyon, Murray, Nobles, Pipestone, Redwood, Renville, Rock, Swift, Yellow Medicine

Table 7. State of Minnesota records of reportable communicable diseases, 1998.

Table 2. Cases of Selected Communicable Diseases Reported to the Minnesota Department of Health by District of Residence, 1998

	District (population; 1996 estimates*)									
Disease	**Metropolitan** (2,482,858)	**Northwestern** (149,731)	**Northeastern** (244,750)	**Central** (634,199)	**West Central** (219,312)	**South Central** (277,691)	**Southeastern** (440,013)	**Southwestern** (234,194)	**Unknown Residence**	**Total** (4,682,748)
Campylobacteriosis	546	23	24	112	28	46	173	54	0	1006
Cryptosporidiosis	40	4	1	20	8	16	64	20	0	173
Encephalitis - viral										
LaCrosse	1	0	0	0	0	0	3	0	0	4
Western	0	0	0	0	0	0	0	0	0	0
Escherichia coli 0157:H7 infection	130	0	4	44	4	2	20	5	0	209
Hemolytic Uremic Syndrome	9	0	0	2	2	0	6	1	0	20
Giardiasis	644	11	36	131	40	31	113	47	271	1324
Haemophilus influenzae invasive disease	47	3	3	8	5	3	6	2	0	77
HIV infection other than AIDS	193	0	1	7	0	6	7	2	10	226
AIDS cases (diagnosed in 1998)	167	1	0	5	1	2	5	0	0	181
Legionnaires' disease	4	1	3	0	0	0	1	3	0	12
Listeriosis	11	1	0	0	0	2	3	2	0	19
Lyme disease	154	0	5	79	0	7	15	1	0	261
Measles	0	0	0	0	0	0	0	0	0	0
Mumps	11	0	0	0	0	1	1	0	0	13
Neisseria meningitidis invasive disease	19	1	4	6	2	0	3	1	0	36
Pertussis	313	0	18	26	5	10	15	52	0	439
Salmonellosis	328	6	18	84	28	23	80	32	2	601
Sexually transmitted diseases**										
Chlamydia trachomatis - genital infections	5438	105	234	481	113	121	385	93	0	6970
Gonorrhea	2527	7	33	60	9	16	37	19	0	2708
Syphilis total	62	0	1	3	0	1	4	4	0	75
primary/secondary	9	0	0	0	0	0	0	0	0	9
early latent***	7	0	0	1	0	0	0	0	0	8
late latent****	46	0	1	2	0	1	4	4	0	58
Shigellosis	237	4	2	20	6	9	46	7	0	331
Streptococcus pneumoniae invasive disease (Twin Cities only)	506	--	--	--	--	--	--	--	--	506
Streptococcal invasive disease - Group A	105	6	10	19	3	7	18	5	0	173
Streptococcal invasive disease - Group B	145	6	8	21	9	17	12	11	0	229
Tetanus	0	0	0	0	0	0	0	0	0	0
Tuberculosis	123	2	7	2	1	3	21	2	0	161
Vancomycin Resistant Enterococci	105	0	7	11	4	7	13	2	0	149
Viral hepatitis, type A	76	4	9	19	9	16	5	7	0	145
Viral hepatitis, type B	57	0	3	4	1	1	5	0	0	71
Viral hepatitis, type C	9	0	1	7	0	1	1	0	0	19

*For sexually transmitted diseases, 1997 population estimates from the U.S. Census Bureau are used.
**Cases for which residence is unknown are assigned the geographic location of the reporting clinic.
***Duration <1 year
****Duration >1 year

County Distribution within Districts
Metropolitan = Anoka, Carver, Dakota, Hennepin, Ramsey, Scott, Washington
Northwestern = Beltrami, Clearwater, Hubbard, Kittson, Lake of the Woods, Marshall, Pennington, Polk, Red Lake, Roseau
Northeastern = Carlton, Cook, Lake, St. Louis
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Southeastern = Dodge, Fillmore, Freeborn, Goodhue, Houston, Mower, Olmsted, Rice, Steele, Wabasha, Winona
Southwestern = Big Stone, Chippewa, Cottonwood, Jackson, Kandiyohi, Lac Qui Parle, Lincoln, Lyon, Murray, Nobles, Pipestone, Redwood, Renville, Rock, Swift, Yellow Medicine

Table 8. Illustrative summary of disease incidence data available for North Dakota, 2000-2001.

Summary of Reportable Conditions
North Dakota, January - June 2001 and 2000

Reportable Condition	May-Jun 2001*	Jan-Jun 2001*	May-Jun 2000	Jan-Jun 2000
AIDS	0	1	2	3
Campylobacteriosis	11	37	28	42
Chlamydia	200	501	159	452
Cryptosporidiosis	1	3	3	8
E.coli O157:H7	0	1	3	8
Giardiasis	2	28	11	32
Gonorrhea	4	16	19	35
Haemophilus influenzae (invasive)	2	4	1	2
Hantavirus Pulmonary Syndrome	0	0	0	2
Hepatitis A	0	2	2	2
Hepatitis B	0	0	0	2
HIV (without AIDS diagnosis)	1	6	1	5
Legionellosis	0	1	0	0
Malaria	0	0	0	2
Measles (Rubeola)	0	0	0	0
Meningitis, Bacterial (non meningococcal)	0	2▲	1	3
Meningococcal disease	0	5	0	1
MRSA (invasive)	0	1	0	3
Mumps	0	0	2	1
Pertussis	0	0	1	2
Q fever	0	1	0	0
Rabies (animal)	6	24	34	83
Rubella	0	0	0	0
Salmonellosis	3	15	14	35
Shigellosis	1	13	2	4
•Streptococcal Disease, Group A (invasive)	0	7	1	4
•Streptococcal Disease, Group B (infant < 3 months of age)	0	0	1	2
•Streptococcal Disease, Group B (invasive†)	0	9	2	7
•Streptococcal pneumoniae, (invasive, children < 5 years of age)	0	7	1	1
•Streptococcal pneumoniae (invasive‡)	1	11	7	14
•Streptococcus pneumoniae, drug resistant	0	4	3	7
Toxic Shock Syndrome	0	0	0	1
Tuberculosis	0	3	2	2
Tularemia	0	0	0	2

*Provisional data

▲Meningitis caused by *Staphylococcus aureus* and *Streptococcus pneumoniae*.

•Includes invasive infections caused by streptococcal disease not including those classified as meningitis.

†Includes invasive infections of streptococcal disease, Group B, in persons ≥ 3 months of age.

‡Includes invasive infections caused by *Streptococcus pneumoniae* in persons ≥ 5 years of age.

Animal Rabies by Species
January – June 2001

Animal Species	Number
Badger	1
Cat	3
Cow	5
Dog	1
Horse	1
Skunk	13

Table 9. Illustrative summary of disease incidence data available for North Dakota, 1996-2001.

Summary of Selected Reportable Conditions
North Dakota, Division of Disease Control, 1996-2001

Reportable Condition	Jan-Feb 2001*	Jan-Feb 2000	Total 2000*	Total 1999	Total 1998	Total 1997	Total 1996	5-Year median 1996-2000
AIDS	1	0	8	9	5	11	13	9
Amebiasis		1	4	5	1	1	7	4
Anthrax (human)			1	0	0	0	0	0
Botulism, infant			0	1	0	0	0	0
Botulism, other			0	1	0	0	0	0
Campylobacteriosis	8	5	106	63	57	59	75	63
Chlamydia	163	155	909	934	1036	887	1022	934
Cryptosporidiosis		1	18	20	34	15	12	18
E.coli , shiga toxin positive (non-O157)			2	NR	NR	NR	NR	2
E.coli O157:H7		3	23	19	12	15	19	19
Giardiasis	17	10	65	104	82	135	148	104
Gonorrhea	5	7	73	81	80	73	42	73
Haemophilus influenzae, invasive		1	4	2	1	0	0	1
Hantavirus Pulmonary Syndrome			2	0	0	0	0	0
Hepatitis A			4	3	4	14	140	4
Hepatitis B		2	3	2	4	7	2	3
Hepatitis C			1	1	0	4	0	1
HIV (Without AIDS diagnosis)	3	1	8	6	9	10	4	8
Legionellosis			1	2	0	2	0	1
Listeriosis			2	NR	NR	NR	NR	2
Lyme Disease			2	1	0	0	2	1
Malaria		1	3	0	3	3	1	3
Meningitis, Bacterial (not meningococcal)	1		4	6	7	6	3	6
Meningococcal disease	2	2	2	4	5	2	5	4
MRSA - invasive	1		11	NR	NR	NR	NR	11
Mumps			1	1	2	0	2	1
Pertussis		1	5	22	17	2	1	5
Q fever			0	0	2	0	0	0
Rabies (animal)	14	10	117	147	155	91	77	117
Rocky Mountain Spotted Fever			0	0	2	0	0	0
Salmonellosis	1	11	73	58	68	69	63	68
Shigellosis	9	1	61	3	11	10	80	11
Streptococcal Disease, infantile, Group B	2	1	15	6	9	7	4	7
Streptococcal Disease, invasive Group A	4	2	9	8	8	8	1	8
Streptococcal Disease, invasive°			2	NR	NR	NR	NR	2
Streptococcus pneumoniae, drug resistant	2	2	5	5	2	0	3	3
Toxic Shock Syndrome		1	1	0	1	1	2	1
Tuberculosis		1	5	7	10	12	8	8
Tularemia		2	2	NR	NR	NR	NR	2

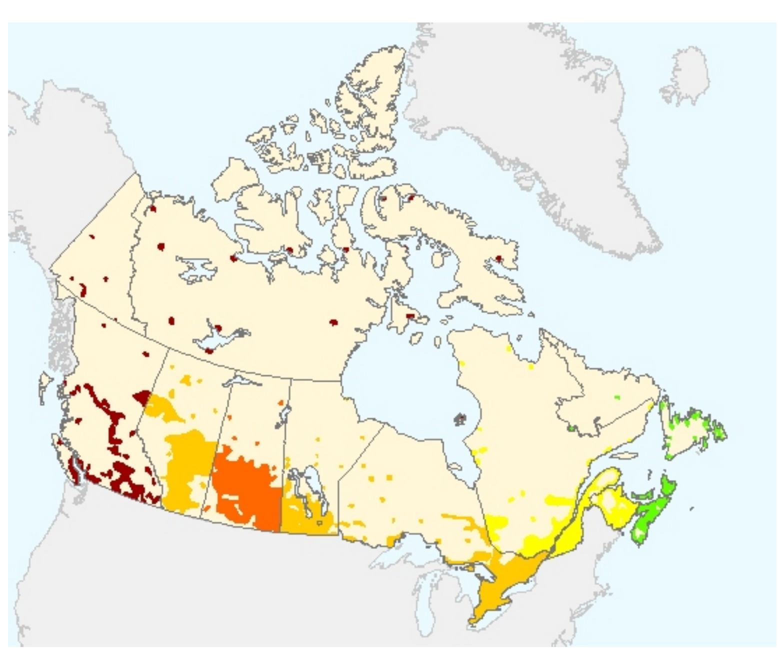
*Provisional data

NR: Not required to be reported during this year

° Includes invasive infections caused by all streptococcal organisms

On-Line Population and Public Health Branch (PPHB)

Figure 28. Province-wide incidence of giardiasis (both sexes combined, including not specified; all ages, including not specified), 2000.



Rate per 100,000 population	9.43 - < 11.51	11.51 - < 16.22	16.22 - < 20.35	20.35 - < 22.17	22.17 - 62.10

Province	Rate/100,000
Newfoundland	10.41
Prince Edward Island	10.84
Nova Scotia	9.44
New Brunswick	11.51
Quebec	12.61
Ontario	16.97
Manitoba	16.22
Saskatchewan	20.35
Alberta	17.24

Province	**Rate/100,000**
British Columbia	22.17
Yukon	62.10
Northwest Territories	34.27
Nunavut	21.82

Data updated: 2003

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