

A ONE HEALTH APPROACH TO *ECHINOCOCCUS CANADENSIS* AND OTHER
PARASITIC ZOOSES IN REMOTE, RURAL AND INDIGENOUS COMMUNITIES

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ABSTRACT

In Canada, parasitism in people and well-managed animal populations is less common now than a century ago, likely due to accessible anthelmintics, heightened public awareness, and improved sanitation. Some zoonotic parasites, such as *Echinococcus canadensis* are now rarely diagnosed in people, but persist mainly in northern populations where diagnostic services are limited.

Veterinary services are also limited in these areas, and as a result, human and animal incidence data does not exist, is outdated, or underestimates the true incidence. We closed this knowledge gap in certain areas of western Canada by determining the prevalence of *E. canadensis* and other zoonotic parasites in wildlife (wolves [*Canis lupus*] and ungulates; Chapters 2 and 3), domestic dogs (*Canis familiaris*; Chapters 4 and 5), and people (Chapters 6-8). Using a One Health framework, we also explored parasite control practices and potential policy solutions for rural and remote communities (Chapters 8 and 9).

During post-mortem examination, we observed *E. canadensis* in approximately 11% (11/105) of elk [*Cervus canadensis*], and 21% (34/165) of wolves. Our examination of historical post-mortem reports of ungulates demonstrated that *E. canadensis* is distributed throughout Canada, except for the high Arctic islands, the Maritime provinces, and the island of Newfoundland. Our analysis of dog feces collected throughout Saskatchewan suggested that patent taeniid (*Taenia* or *Echinococcus* spp.) infection was rare (0-4%), and that rural and northern dogs had higher endoparasitism than urban dogs. Sero-surveillance for four zoonoses (*E. canadensis*, *Toxoplasma gondii*, *Trichinella*, and *Toxocara canis*) by enzyme-linked immunosorbent assay indicated similar results - that people in northern SK (65% of 201) had higher exposure to one or more parasites than those in southern SK (12% of 113). Using patient health records, we reported annual incidence rates for clinical illness for the following zoonotic parasites: echinococcosis – 1.4/1 000 000; toxoplasmosis- 1.7/1 000 000; and toxocariasis-0.06/1 000 000. In the final chapter we compared the cost of treating human echinococcosis cases with a prevention program based on dosing dogs with praziquantel at 6 week intervals in the Kelsey Trail region, where human incidence is highest. Based on direct healthcare costs, such a program is not currently cost saving, but could become so if echinococcosis incidence increased. Preventative programs should be considered for high risk communities, which are often economically marginalized and lack appropriate resources to effectively control zoonotic parasitism. Putting One Health into action may require integrated human-animal healthcare

services, introduction of community-based animal health workers, and increased transdisciplinary research to improve access to and uptake of preventative healthcare services for parasitic zoonoses in northern and remote communities.

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LIST OF ABBREVIATIONS

AB – Alberta

AE – Alveolar Echinococcosis

BC – British Columbia

bp - base pair

bw – body weight

CA - Census Agglomeration

CADTH - Canadian Agency for Drugs and Technologies in Health

CAHW – Community-based Animal Health Worker

CCWHC – Canadian Cooperative Wildlife Health Centre

CE – Cystic Echinococcosis

CIHI – Canadian Institute for Health Information

CMA - Census Metropolitan Area

Cox1 - Cytochrome *c* oxidase subunit 1

DAD – Discharge Abstract Database

DALY – Disability Adjusted Life Year

DDT – Dichlorodiphenyltrichloroethane

DNA – Deoxyribonucleic acid

EHO – Environmental Health Officer

EID – Emerging Infectious Disease

ELISA - Enzyme-linked Immunosorbent Assay

HIV – Human Immunodeficiency Virus

HS – Humane Society

ICUR - Incremental Cost-Utility Ratio

MB – Manitoba

MBUN – Meaningless But Unique Number

NACRS – National Ambulatory Care Reporting System

NAD1 - NADH dehydrogenase subunit 1

NB – New Brunswick

NCR – North Central Region

NL – Newfoundland and Labrador

NS – Nova Scotia
NRCP – National Reference Centre for Parasitology
NT – Northwest Territories
NTZ – North Tundra Zone
NU - Nunavut
NSR- North Slave Region (NT)
ON – Ontario
PCR – Polymerase Chain Reaction
PE – Prince Albert Island
PZQ - Praziquantel
QALY – Quality Adjusted Life Year
QC – Quebec
RMNP – Riding Mountain National Park (Manitoba)
SK – Saskatchewan
SPCA – Society for the Prevention of Cruelty to Animals
SSR – South Slave Region (NT)
TCPS: CORE – Tri-Council Policy Statement: Course on Research Ethics
YT – Yukon Territories

CHAPTER 1 - INTRODUCTION

Parasite surveillance in a One Health context

One Health overview

“Between animal and human medicine there is no dividing line -nor should there be.”

~ Rudolph Virchow (1821-1902)

In both veterinary and human medicine, trends pertaining to research, diagnostics and treatment tend to appear and disappear. One such trend, ‘One Medicine’, was introduced approximately one hundred years ago, centered around a centuries old belief that supported holistic and inclusive strategies for studying human and veterinary medicine (1,2). Over the last century, the two disciplines diverged, and appeared to undergo a silo effect, each becoming increasingly specialized. Research in these fields became increasingly reductionist, and focused on understanding medicine at the molecular level (3). Recently, ‘One Medicine’ re-appeared under the banner of ‘One Health’ (2,4). This concept recognizes the health links between people, animals, and environment, and strives to re-unite those working in related fields at all levels of research and government (2). This regained interest might be due to the marked increase in emerging infectious disease (EID) events, of which 60% are of non-human animal origin (zoonotic) (5). These events often have drivers that relate to the environment (deforestation, human encroachments, climate change); animals (intensification of animal production, wildlife/livestock translocation); and economics (trade globalization, poverty) (5,6). Emerging infectious diseases can cause catastrophic loss of human and/or animal life, as demonstrated by the 2014 Ebola outbreak in Africa, and are one of the greatest challenges currently facing wildlife conservation efforts (7,8). As well, EIDs can cause severe economic losses, especially if livestock culls are imposed, trade barriers are implemented, or if the direct and indirect costs of treating human patients are high (2,5).

Zoonotic disease is a frequent focus of One Health related research; however, many other human-animal connections also affect health (4). Animals can inflict non-infectious injuries on people, including bites, kicks or scratches, and people can inflict poor welfare conditions on livestock and companion animals, loss of habitat on wildlife, and widespread environment contamination that affects everyone (eg. DDT). On a more positive note, people benefit greatly from laboratory animals, livestock, working animals (equids, bovids, dogs [*Canis lupus*

familiaris], etc), wildlife, and guide animals. ‘Zooeyia’ is a term recently coined to represent the health benefits that people experience by interacting with animals, pets in particular (9). For example, people who own dogs appear to exercise more frequently, regardless of season or gender, and this in turn positively influences risk factors for obesity, heart disease, and joint pain (9). Pets can also improve mental health and feelings of social connectedness, among other psychosocial benefits (9). ‘Zoobiquity’ is also a relatively new term for comparative medicine, and describes the physical and behavioural health issues shared by people and animals (10). Zoobiquity proponents investigate shared human/animal health problems by studying phylogenetics and shared environmental conditions. The overall objective is to improve diagnostic capabilities and treatment options for people and animals by building collaborative relationships between human and animal health providers. Similarly, the term ‘syndemic’, characterized as multiple afflictions that interact to cause greater than expected disease burden, was coined in 1994 to recognize the value of integrating social science into the One Health equation (11,12). Proponents urged policy makers to take social determinants of health into consideration when planning and implementing preventative health policies. For example, they argued that the high levels of substance abuse, HIV/AIDS, and violence concomitantly observed in urban core areas in the United States were inextricably linked, forming clusters due to underlying causes such as low socioeconomic status (11,12). Preventative healthcare strategies are only likely to succeed if these underlying causes are identified and resolved. In another example, patients co-infected with HIV and *Mycobacterium bovis* had lower survival than those with HIV alone, highlighting the increased zoonotic risk for those patients with compromised immunity who come in contact with animals or infected animal products (11). Rock et al. (2009) proposed modifying the definition of syndemic to include human-animal interactions:

Two or more afflictions that interact synergistically within the context of specific physical and social environments, especially as a result of inequality within and between human populations, to produce excess disease burdens in a human population, an animal population, or multiple such populations (11).

Although One Health discussions often focus on animal-human health, environmental and economic drivers are important to take into consideration. Environments in flux,

such as those experiencing deforestation, urbanization and climate change, strongly influence species distributions, abundance, and even survival. Climate change, for example, is thought to have contributed to a large proportion (29%) of vector-borne EID events over the last decade (5). Environmental factors also contribute to human-wildlife conflicts, including infectious disease transmission, crop damage, and physical injury, especially when important resources (food, water, space) become limited. As well, environments can act as reservoirs for infectious pathogens (eg. anthrax), and influence psychological wellbeing.

Environmental degradation has many drivers, and economic wellbeing may be the most important. Low socio-economic status is a major risk factor for health disparity and EID events, as well as an incentive for engaging in high risk employment activities that may have long term consequences (and costs) in the One Health context (5). Human health disparities caused by the connection between economy and environment are considered ‘wicked’ problems, or more simply, social messes, and are unlikely to be resolved soon, if ever (13). This is especially true when a powerful group generates profits at the expense of the environment, while another group, usually marginalized or poor, suffers the consequences. In Canada, petroleum extraction from the bituminous sands in northern Canada is extremely contentious because it has a high economic reward for the country, but is also high risk for habitat disruption, water contamination, and adverse health effects for people/animals in an area inhabited primarily by those of Indigenous descent (14).

Human and animal adverse health events can inflict large economic burdens, both on individuals and the government. As a result, public policy relating to public health and livestock health are strongly influenced by economic arguments (cost-benefit or cost-efficacy). Therefore, managers of programs intending to improve community health by prevention or treatment must take economic drivers, costs, and potential consequences into account, in addition to factors such as feasibility, efficacy, and acceptability.

Population level research in rural, remote, and Indigenous communities in Canada

In Canada, and around the world, people residing in rural regions have poorer overall health, and shorter life expectancy, than those residing in urban settings (15). According to Health Canada, social and physical environments as well as socio-economic status are the most relevant determinants of health impacting rural communities (16). Other factors include gender, culture, education/literacy, access to health services, employment conditions, and support networks (16). The definition of 'rural' has at least six widely accepted definitions in Canada, and this can lead to confused communication between researchers and policy makers (15,17–19). The current Statistics Canada definition classifies rural residents as those residing in areas with a population less than 1000 persons and with a population density less than 400 persons per square kilometer (20). Two other descriptors exist under the umbrella of rural population: 'remote' and 'northern'. Respectively, these terms describe areas where a community is physically separated (eg. no road access year-round), or at the southern limit of discontinuous permafrost. Both remote and northern communities experience a degree of physical isolation beyond that of rural communities, and in Canada, many struggle with issues relating to food security, high cost of living, housing shortages, employment, and lack of services (medical, veterinary, tele-communication) (21). Perhaps in response to these challenges, the proportion of Canadians residing in rural regions has plummeted over the last century (from 87% in 1851 to 19% in 2011), leaving behind a high demographic proportion of aging seniors and young children (18,22). Rural health practitioners face many challenges, such as professional and social isolation, that motivate a strong culture of collaboration, creativity in using and procuring human and physical resources, and flexibility in navigating technological challenges and climate extremes (23). As a result, many rural practitioners may already be well-equipped to operate in a One Health framework.

Indigenous groups, including those who self-identify as First Nations, Metis, Inuit, and Aboriginal, currently make up 4% of Canadians, and are the fastest growing segment of the population (24). Approximately half of all Indigenous people in Canada reside in rural, remote and northern regions, and similar to Indigenous groups elsewhere in the world, have poorer health outcomes, a lower life expectancy, and a higher burden of disease than non-Indigenous groups (19,25–27). In particular, Indigenous groups in Canada are over-represented for health

issues pertaining to high infant/child mortality, high incidence of infectious disease (eg. HIV/AIDS, hepatitis, tuberculosis), malnutrition, poor maternal health, addiction to alcohol and banned substances, lifestyle diseases (eg. obesity, diabetes), and interpersonal violence (27,28). Indigenous communities in the far north can face significant challenges in obtaining medical services, which may include limited access to diagnostic technology, lack of patient confidentiality, long distances to medical centres, high turnover of medical professionals, and lack of cultural awareness from health providers (15,29). These communities are vulnerable to impacts of climate change and might have the greatest need for integrated One Health surveillance, where human, animal and environmental data are collected and analyzed simultaneously (3,30). One reason for their vulnerability is that many Indigenous groups remain strongly connected to the land and to wildlife for spiritual, cultural, and nutritional reasons. This puts some people at higher risk of exposure to parasites of animal origin (eg. trichinellosis, toxoplasmosis); in particular, those who process animal carcasses or ingest the raw meat of game animals (31–33).

Despite the potential value of participatory community-based research for both communities and researchers, some Indigenous communities are reluctant to engage. This is partially due to researchers who behaved unethically in the past, resulting in strained relationships with academic institutions, lost trust, and a reluctance to collaborate with new researchers (34). New standards called ‘OCAP (Ownership, Control, Access, Possession) principles’ have become the gold standard for ensuring fair dealings in community participatory research settings (34). These guidelines recommend involving residents at all research levels (proposal, data collection, data analysis, dissemination of results, policy), using culturally appropriate behaviour/language, and ensuring that risks and/or benefits are shared (34). Advantages to this approach are the improvement in local capacity, and the ability for communities to conduct self-driven research programs. Holistic health and beliefs of inter-connectedness among people, animals and the environment are concepts shared among many Indigenous cultural groups. These concepts are well aligned with One Health, and may be readily accepted by communities wishing to participate in One Health related research.

Parasitology surveillance in a One Health context

Perhaps one of the greatest challenges to the paradigm shift from current health practices to an integrative One Health approach is persuading individual researchers, health practitioners, and government agencies of various disciplines to work collaboratively. Barriers to interdisciplinary collaboration include role/turf rivalries (competing for responsibilities), physician dominance of decision-making, language (eg. different meanings of the same acronym, discipline specific jargon), data separation (eg. segregating human and animal data), misaligned goals, and the difficulty in extrapolating health outcomes in one species to another (35,36). Despite these barriers, collaboration among multiple disciplines is valuable, and lack of collaboration between veterinarians and physicians is a missed opportunity for early detection of zoonotic disease outbreaks. For example, West Nile Virus first emerged in birds in New York city (USA) in 1999, and Minamata disease (a neurological illness associated with mercury) first emerged in Japanese cats in 1956; neither event was communicated with human health authorities (2,36–38). Joint human-animal health surveillance and services are critical for translating the theory of One Health into practice (3). This requires new tools, such as secure methods of collecting and sharing surveillance data between agencies, the creation of aggregated human-animal surveillance databases, and strategies for conducting community-level surveillance in developing countries (3,36). It also requires funding that is specifically designated for integrative health projects (2).

The use of animals as sentinels for shared environmental threats or emerging diseases is not new, and recent work in remote regions of British Columbia and the Northwest Territories highlights the roles of dogs as potential sources and sentinels for human and wildlife infection with zoonotic pathogens (38–40). Some of the key requirements for sentinel hosts are that they are highly exposed, highly sensitive/susceptible, they rapidly exhibit clinical signs or detectable immune or molecular responses, and are not endangered species (41). Ideally, they are not an infection source for people or an amplifying host for the agent or pathogen. Dogs in northern and remote environments may be at higher risk of endoparasitism than urban dogs due to lack of veterinary services and more frequent exposure to wildlife parasites. In turn, they may act as bridging hosts (Figure 1-1), enabling the indirect transfer of zoonotic parasites between people and wildlife. When people feed dogs infected meat or when dogs scavenge wildlife carcasses on

then own, they can become infected and then shed infective parasite stages into areas inhabited by people. Parasite transmission can also occur in the opposite direction (eg. *Giardia* transmission from people to dogs to wildlife). Canine parasite surveillance for the purposes of improved public health has many advantages. These include reductions in cost, in-situ environmental pathogen exposure levels, decreased concern for ethics/privacy in comparison with human sampling, and the non-invasive nature of fecal and blood analysis. In Canada, domestic dogs could act as sentinels for a wide range of zoonotic pathogens, including *Echinococcus* spp., *Toxocara canis*, *Giardia*, *Diphyllobothrium*, and *Borrelia burgdorferi* (Lyme disease).

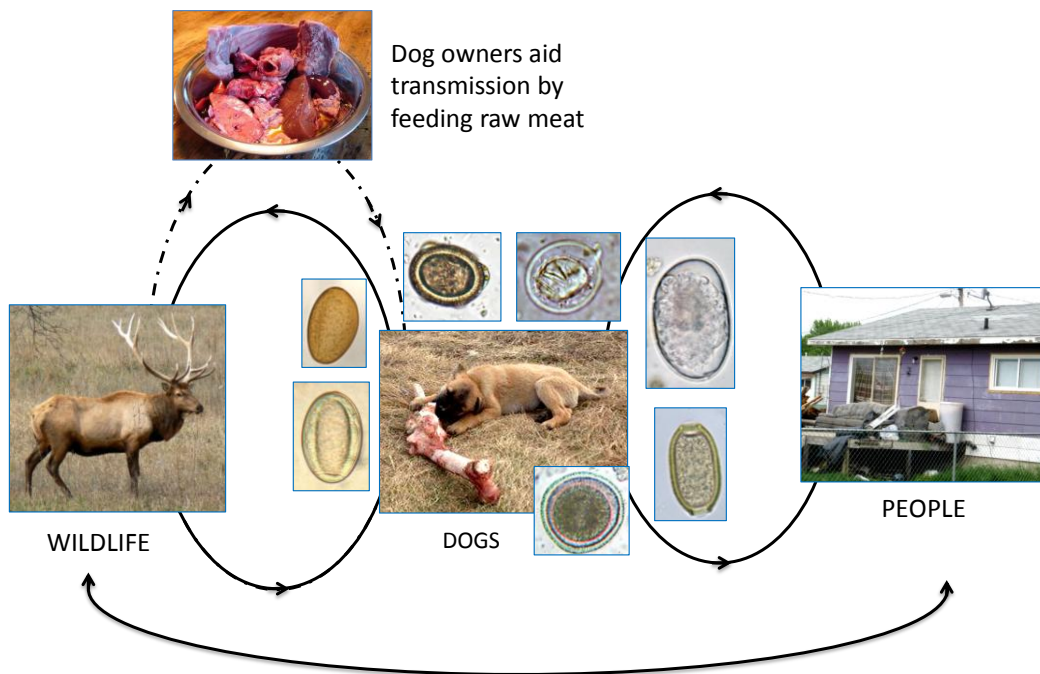


Figure 1-1: Schematic of domestic dogs as bridging hosts between wildlife and people (arrows signify pathogen transmission routes).

To fully embrace a One Health or ‘shared risk’ approach to zoonotic parasite management, researchers must move beyond the simple use of animals as sentinels, and conduct human and animal surveillance in communities concurrently (3,36,42). This approach would ideally be participatory in nature, allowing community members to collect, analyze, and disseminate data;

and also be multi-disciplinary, including social scientists and anthropologists in addition to veterinarians and physicians (3,11,34,42). To date, joint human-animal parasite surveillance has occurred infrequently in Canada, with the exception of testing harvested wildlife in concert with human incidence of exposure or disease (33,43).

Human hydatid disease, caused by *Echinococcus canadensis* (the cervid strain of *E. granulosus*), was historically most prevalent in northern and Indigenous areas of Canada, especially where sled dogs were the predominant mode of transportation (44,45). Infected canids contaminate the environment by shedding eggs that are infective to cervids and people, and although the lifecycle is primarily sylvatic, domestic dogs can act as bridging hosts for *E. canadensis* transmission. Although the incidence of this disease decreased dramatically in the past century, northern and Indigenous residents are still at higher risk of infection than non-Indigenous Canadians residing in the south (46). In 2008, a 6-year old child from northern Saskatchewan (SK) was diagnosed with a cerebral hydatid cyst (47). Subsequent serological surveillance within the child's community demonstrated that 11% (12 of 106) of residents had been previously exposed to the parasite and that 6% (9 of 155) of environmentally collected canine fecal samples contained eggs of *E. canadensis* (47). In this case, the opportunity to conduct community level human and animal sampling simultaneously offered many advantages, such as identifying dogs as an infection source for people and assessing risk factors for human exposure. *Echinococcus canadensis* is an ideal pathogen to study using a One Health framework because this parasite circulates among people, animals (wild and domestic), and the environment, in addition to causing serious global economic losses (48). It is unlikely to ever be fully eradicated in Canada due to the presence of wildlife reservoir hosts, and, in certain regions, might benefit from changing environmental factors such as climate change (49). Long term echinococcosis control, especially in high risk communities, will require support from the veterinary community and public health officials. The public is best equipped to prevent echinococcosis infection when residents understand *E. canadensis* transmission routes and control strategies, and when they have access to drugs required to treat patent infection in the definitive host.

The work in this thesis expands the current understanding of *E. canadensis* prevalence and distribution in western Canada, and builds on previous work exploring transmission of zoonotic pathogens among people, animals, and the environment (47,50).

Overview of *Echinococcus canadensis* in Canada, Alaska, and Greenland

Echinococcus granulosus is a species complex of taeniid cestodes responsible for cystic hydatid disease in people world-wide. According to the World Health Organization, hydatid disease is one of the most expensive parasitic zoonoses to treat and prevent world-wide (48). Life cycles of *E. granulosus* involve carnivore definitive hosts and herbivore intermediate hosts, and various species/strains utilize different assemblages of domestic livestock, wildlife, and people (51). Adult cestodes, which are quite small (2-7 mm), reside in the small intestines of definitive hosts and shed eggs that are immediately infective for intermediate hosts. Ingested eggs release oncospheres that penetrate the intestinal wall of the new host, undergo tissue migration, and eventually create unilocular cysts containing larval protoscolices in organ tissue (most often liver or lung). People are considered accidental hosts, in which cysts form but may not develop fertile protoscolices (45).

Species and strains

There are at least 10 genotypes of the *E. granulosus* species complex that circulate in different host assemblages worldwide (52). The pastoral strains (G1-3, *E. granulosus sensu stricto*) which circulate among domestic livestock and dogs, are not thought to be present in northern North America, although they have been introduced into sheep rearing regions of the western continental USA (45). In northern Canada, two genotypes (G8 and G10) circulate in largely sylvatic cycles involving cervids and wild canids (Figure 1-2); only the G8 genotype has been reported in Alaska (52,53). It is possible that one or both of these genotypes may have been introduced into the North American Arctic along with infected reindeer imported from Siberia and Fennoscandia in the early part of the twentieth century (45,52); both G8 and G10 strains have been identified in Fennoscandia (54).

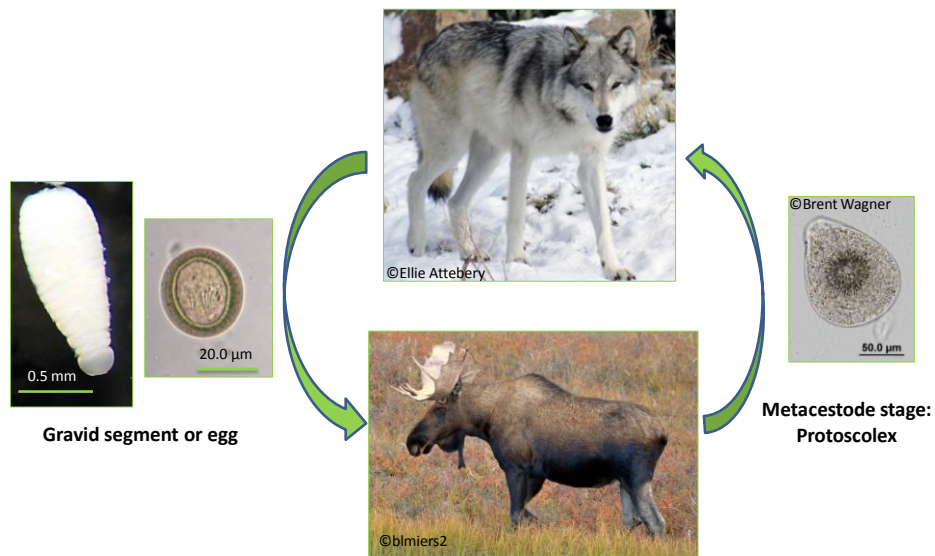


Figure 1-2: Sylvatic lifecycle of *Echinococcus canadensis*.

The taxonomic status of the *E. granulosus* species complex is somewhat controversial. Initial phylogenetic analyses based on mitochondrial DNA suggested that the G6-G10 genotypes be unified as the species *E. canadensis* (52,55). However, more recent phylogenetic analyses based on nuclear DNA suggest that only the G8 and G10 cervid strains be unified under the name *E. canadensis*, and the G6, G7 and G9 strains (in camels, pigs, and people, respectively) be unified as *E. intermedius* (54). Therefore, in this thesis, *E. canadensis* will be used when referring to the cervid strain(s) present in North America, differentiated into the G8 and G10 genotypes where relevant.

Geographic distribution

Echinococcus canadensis is present across northern Canada and Alaska but is not established in Greenland (45). In Canada, *E. canadensis* is present in all provinces and territories with the exception of the East Coast (provinces of Nova Scotia, Prince Edward Island, New Brunswick and the island of Newfoundland) where wolves (*Canis lupus*) have been historically absent (44). Indeed, *E. canadensis* remains common in northern North America wherever wolves and ungulates co-exist (44°N – 68°N), however it may be absent in the High Arctic islands due to the

low year-round density of ungulate intermediate hosts (44,49,56). This parasite is not present on the island of Newfoundland due to the extirpation of wolves in the early part of the twentieth century, even though ungulate intermediate hosts (moose, *Alces alces*, and caribou, *Rangifer tarandus*) are now abundant. However, recent colonization by coyotes (*Canis latrans*) may enable local transmission of *E. canadensis*.

Transmission, prevalence, and animal health impact

The sylvatic strains of *E. canadensis* cycle primarily between canid definitive hosts and cervid intermediate hosts via sympatric predator-prey relationships. In Canada and Alaska, the larval cysts are detected most commonly in moose and caribou/reindeer; however, other ungulate intermediate hosts have been reported, including wapiti (*Cervus elaphus*), muskoxen (*Ovibos moschatus*), mountain goats (*Oreamnos americanus*), American bison (*Bison bison*) and black-tailed deer (*Odocoileus hemionus*) (57–62). Cysts in microtine rodents, reported earlier, are most likely to be *E. multilocularis* (63); gray squirrels (*Sciurus carolinensis*) have been experimentally infected with *E. granulosus* (61). Adult cestodes have been reported in the intestinal contents of wolves, coyotes and domestic dogs (44,49,64,65). According to Rausch (66) historical reports of red fox (*Vulpes vulpes*) and arctic fox (*V. lagopus*) infected by *E. granulosus* in Alaska and the Northwest Territories were misclassified (*E. multilocularis* was a more likely candidate). Foxes are no longer considered to be natural hosts of *E. granulosus* (66).

In sub-Arctic and Arctic regions of North America, the prevalence of infected animals is highly variable among different host species and between locations, and comparisons are difficult due to a range in detection effort and methods. In intermediate hosts, moose in northern Alaska were reported to have a higher prevalence of infection than those in the south (24% and 4%, respectively), and 0.5 to 6% of caribou were infected (59,67,68). Between 3% and 5% of Alaskan reindeer were reported infected with *E. canadensis* (45,61). In Canada, moose in the Yukon Territories were infected at high prevalence (43%), as were caribou/reindeer in the Northwest Territories (20-35%). In sub-Arctic and temperate regions of Canada, elk were infected at a somewhat lower prevalence (6-21%) (61). Sporadic cases in ungulates in northern regions of SK, Manitoba and Labrador have also been reported (57,69,70).

Detection of *Echinococcus* spp. infection in definitive hosts has historically relied on collection of adult cestodes from the intestines during necropsy. Identification of *Echinococcus* eggs detected in feces is complex as eggs are indistinguishable from other taeniid species; therefore, molecular methods are required for identification, which have only recently been applied in North America. Using these methods, 6% of fecal samples from free-ranging dogs in one community in northern SK contained eggs of *E. canadensis* (G10) (47), but in other communities, only eggs of *Taenia* spp. were detected (71). Several *Taenia* spp. have distributions in wild canids that overlap *E. canadensis* in the North, including *T. crassiceps*, *T. pisiformis* and *T. polyacantha* (65). Taeniid eggs were detected in 5% of 423 wolves in Greenland, but were not identified further (72).

In Alaska, based on recovery of adult cestodes at necropsy, 30% of 200 wolves were infected with adult cestodes of *Echinococcus* spp., with sled dogs (10-22%) also reported as common hosts (45,59,67). A similar prevalence (also based on necropsy) is reported in wolves in the Yukon Territory (22%; N=89) as well as in the Northwest Territories (24%; N=21) (73). One survey of dogs culled from eight towns in the Northwest Territories reported a 12% prevalence (4/33) of *E. canadensis* (56).

The overall impact of *E. canadensis* infection on intermediate hosts is unknown, but varies according to parasite load, cyst location and host species. In moose intermediate hosts, hydatid cysts (metacestodes) are present in various organs (lung, liver, spleen, heart, kidneys), while in wild reindeer, cysts are generally restricted to the lungs (45,74). High intensities of hydatid cysts in moose are thought to increase the likelihood of predation by wolves or human hunters, possibly due to decreased stamina and pulmonary function as a result of space-occupying lung lesions (75, 76). Infected canid definitive hosts do not appear to be at risk of increased morbidity or mortality, and generally experience no adverse effects.

Transmission, prevalence, and public health impact

People are exposed to *E. canadensis* through the accidental ingestion of eggs passed in the feces of definitive hosts (wolves, coyotes, and dogs). These eggs are immediately infective once they have passed into the environment, and may adhere to the coat of an animal and a wide variety of

surfaces (77). Theoretically, hunters and trappers of wild carnivores could be at risk due to their close contact with carnivore hides, feces and intestines. Dog owners who reside in endemic areas could also be at risk. Although the relative significance of these exposure routes is currently unknown, it is likely that people are predominantly infected through ingestion of contaminated surface water, produce, and soil. The eggs of *Echinococcus* spp. and related taeniids are extremely resistant to extremes of temperature and humidity and can persist in the environment for several years. Dogs are considered important “bridging hosts” between people and wildlife due to their non-selective diet and their close contact with people (45). Subsistence hunting within a community, where dogs have access to offal and carcasses, is also considered an important risk factor for human exposure to *E. canadensis* (47). However, people are not infected through consumption of meat or organs from wild game but instead through contact with feces of dogs that have scavenged carcasses or been fed offal.

Cystic hydatid disease in people is most often characterized by unilocular fluid-filled cysts in the liver and lungs, although aberrant locations such as the brain have also been documented (47,78,79). Symptoms can include coughing, anorexia, fever, shortness of breath, chest or abdominal pain, and functional neurological deficits if cysts are associated with the brain, nerves or spinal cord (79,80). *Echinococcus canadensis* has been considered to be less likely to cause anaphylactic shock and secondary seeding than the pastoral strain(s) of *E. granulosus* (81). Autochthonous cases of cystic hydatid disease in Canada and Alaska do not commonly result in fatality; however, infection with the G8 strain has been known to cause severe clinical disease, and most recently, death (53,81). Treatment options for hydatid disease include surgical removal, benzimidazole chemotherapy and PAIR (puncture-aspiration-injection-reaspiration) therapy (82). In ideal circumstances a physician might use a “watch and wait” strategy to monitor and treat this disease; however, the limited availability of medical imaging equipment and geographic barriers to accessing medical care may make this approach impractical in northern communities (82–84).

Human cystic hydatid disease did not appear in Canadian literature until 1883, and until the 1950s, most cases were detected in immigrants from Iceland, an area historically endemic for the pastoral variant of *E. granulosus* (60). In Alaska, the first human case was recorded in 1941

(85,86). Human hydatid disease is reportable to Alaskan state public health authorities; peak numbers of cases were detected from 1953 to 1973, nearly all of which were Indigenous people (45). Cases of autochthonous hydatid infection were reported with increasing frequency in Canadian Indigenous populations in the latter half of the twentieth century, mainly due to incidental observation of cysts during tuberculosis screening (84). Similar to Alaska, 99% of 141 cystic hydatid cases in Canada in the 1950's occurred in Indigenous people (56).

In 1952, Indian Health Services and the Institute of Parasitology in Canada initiated efforts to determine the prevalence of infection in Indigenous communities using the Casoni skin test. Initial efforts used antigen obtained from Australian sheep cysts; this was soon replaced by antigen obtained from reindeer in Aklavik (YT), resulting in greater test sensitivity (60,78). Between 1954 and 1957, positive Casoni skin test results were found in 6 to 52% of people across northern Canada (N=3,429). Prevalence was lower in Alaska, where the skin test employed did not use antigens of *E. canadensis* (87). Cultural practices including food preparation, acquisition of locally acquired foods, outdoor food storage, and the presence of large working dog populations may have significantly increased the risk of hydatid infection in the middle of the twentieth century.

Today, imported and autochthonous cases of cystic hydatid disease occur infrequently in North America, in part due to the eradication of *E. granulosus* in Iceland, global efforts to control the disease, and the gradual phasing out of sled dogs as a method of transportation in the North. In Alaska, where human cases are reportable, zero to three cases per year in Indigenous and other residents have been reported since 1973. In Canada, neither human nor animal cases are nationally reportable (although laboratory confirmed cases in animals are annually notifiable to the World Organization for Animal Health), so surveillance is limited to case reviews and serosurveillance.

A review of hospital records in Edmonton hospitals, which act as referral centres for northern Alberta and the Northwest Territories, identified 42 cases of suspected or confirmed cystic hydatid disease between 1991 and 2001 (79). Indigenous patients were over-represented in this group, as 41% self-identified as Indigenous, compared with the 5% of Albertans and 3% of

Canadians who self-identified as Indigenous in 2001. These results are supported by Gilbert et al. (46), who reviewed hospital cases using ICD codes for *Echinococcus*/hydatid disease across Canada between 2001 and 2005 (N=108). In this review, people living north of the 55th parallel were 4.88 times (95% CI 2.52-9.44) more likely to be hospitalized for echinococcosis than the average Canadian (2.9 cases versus 0.72 cases per 1,000,000 people annually). Hospital records also indicate that women may be at higher risk of developing hydatid disease than men (RR 1.92, 95% CI 1.29-2.87) (46,79,88).

Casoni skin tests have been replaced with serological testing, based on IgG ELISA, in northern and Indigenous communities (89). Seroprevalence in Inuit and James Bay Cree in Quebec (0.1-8.3%) and First Nations in Saskatchewan (11%) indicate that northern and Indigenous populations, especially in western Canada, continue to be at risk for exposure to *E. canadensis* (47,90–92). However, the association between positive serology and clinical cystic hydatid disease is unclear. As well, this disease is under-diagnosed due to a variety of factors including many asymptomatic cases, non-specific symptoms, the long progression of the disease, and waning awareness in the medical community. Therefore, these factors should be considered when interpreting the apparent decline in prevalence of cystic hydatid disease in human populations in North America.

Future impact of climate and landscape change

The worldwide distribution of strains in the *E. granulosus* species complex demonstrates that this group of cestodes transmits well in different climates and in a wide variety of hosts (93); however, species and genotypes may vary in hardiness (48,49). *Echinococcus canadensis* is particularly well adapted to cold northern climates. Eggs passed in the feces of definitive hosts can survive in the environment for several years before infecting a new host; eggs and cysts may survive even longer if encased within a protective barrier (eg. snow, feces, sewage, or a host carcass) (48). Temperatures above +35°C or below -30°C can damage eggs, while temperatures above 60°C or below -70°C completely inactivate them (93–95). Regardless of temperature, *Echinococcus* eggs are sensitive to desiccation at low humidity and are inactivated within 1 day at 0% relative humidity (RH) and within 4 days at 25% RH (48,95). Diker et al. (96) tested the viability of hydatid cysts from sheep (*E. granulosus*) at a variety of temperature and RH

combinations in the laboratory, and estimated environmental survival times for cysts in discarded carcasses: 3 to 36 days in winter (-10 to 0°C), 12 to 28 days in spring/autumn (10 to 20°C) and 3 to 4 days in summer (30 to 40°C) (96). This has limited application to northern North America, where winter air temperatures drop far below -10°C and the species present is *E. canadensis*. Egg and cyst viability experiments demonstrate that the duration of cold and freeze-thaw cycles are more important to inactivation than the magnitude of cold (87).

Density increases in moose populations are associated with increasingly aggregated distributions and increases in prevalence of *E. canadensis* (76). The northern distribution of moose in Canada and Alaska is thought to be limited by snow cover and vegetation; however, warming trends that increase food availability could allow moose to move further north (97). Woodland and barren-ground caribou populations are currently decreasing, as a result of anthropogenic and natural environmental changes, but could potentially be replaced by other ungulate hosts suitable for harbouring *E. canadensis*, such as moose and wapiti (98,99). If snow cover decreases by 10 to 20%, as predicted, the High Arctic could become more supportive of densely populated predator-prey food webs and might become a new area for emergence of *E. canadensis* (100).

Arctic temperatures over the last two thousand years were warmest in the period between 1950 and 2000, despite a previous cooling period (101). Mean annual precipitation in the Canadian Arctic has increased 2 to 25% over the last 62 years (102). With regard to effects on transmission of *E. canadensis* in the future, novel weather patterns may alter sympatric territories of predator-prey systems or egg survival in the environment, possibly resulting in the emergence of hydatid disease in new areas, as well as retreat from warming areas (eggs have decreased survival in warmer temperatures). Warming winter temperatures could increase the window of opportunity for hydatid cysts (which are freeze susceptible) to survive before infecting a new definitive host. Increased precipitation in the north is protective for eggs against desiccation, but could also limit the accessibility of eggs on vegetation for ingestion by intermediate hosts (49). Climate change could cause breakdowns in sanitation infrastructure, potentially reducing access to clean water through events such as water contamination with eggs of *Echinococcus* (1,103). Finally, emergence of a related species (*E. multilocularis*) as a result of increased globalization, climate and landscape change, and altered interfaces with wildlife reservoirs serves as an important

reminder about the versatility of these cestodes (49,93,104,105).

Conclusions

In Canada, the incidence of cystic echinococcosis in people is low; however, this disease remains over-represented in vulnerable populations, including those residing in northern and Indigenous communities. The recent arrival of molecular tools to differentiate between *Echinococcus* species and genotypes at all life stages is an important step forward for differentiating strain virulence and documenting emergence or control in geographic locales. These tools have yet to be fully employed in Canada, but have important applications in 1) estimating human risk, 2) documenting distribution changes of *Echinococcus* spp. in people, pets and wildlife, and 3) supporting taxonomic changes to the genus *Echinococcus*, which might encourage changes to animal import laws that would reduce the risk of new strains emerging in Canada. Decreasing echinococcosis incidence further will require a One Health approach where both human and animal health practitioners collaborate to implement and evaluate control programs in high risk communities.

Thesis Objectives

This thesis focuses on zoonotic endoparasites, and specifically *E. canadensis*, which affect people, wildlife and companion animals in Saskatchewan, Canada. Its objectives are as follows:

- (1) To measure the prevalence of *E. canadensis* and other zoonotic endoparasites in:
 - a) wildlife
 - b) dogs
 - c) people
- (2) To review strategies for *Echinococcus* control in people and pets (dogs and cats).

Graphical Abstract

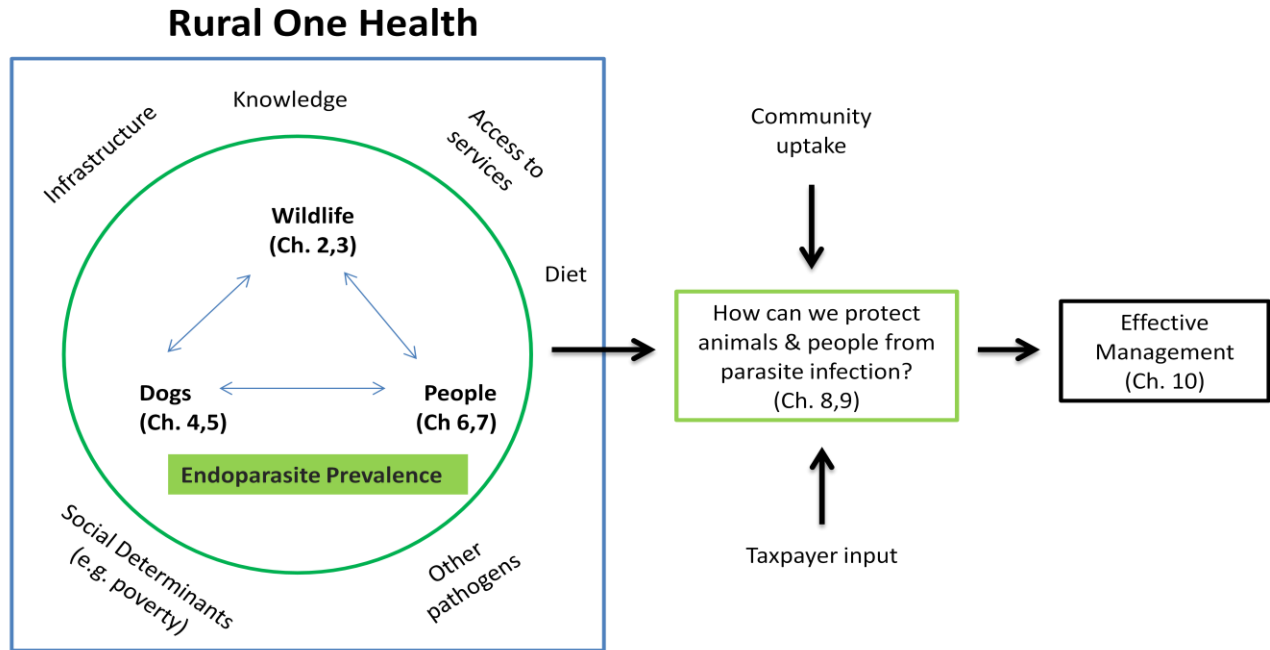


Figure 1-3: Graphical abstract – a theoretical framework for exploring zoonotic parasites in rural communities using a One Health approach.

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CHAPTER 2 - Surveillance for *Echinococcus granulosus* (*E. canadensis*) genotypes in Canadian ungulates

Citation

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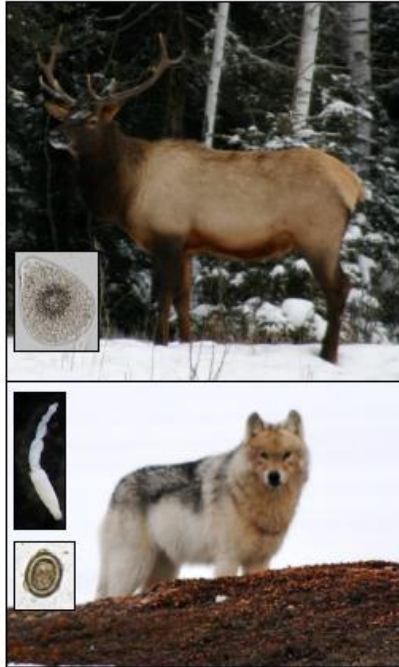
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Graphical Abstract

Abstract

The geographic and host distribution, prevalence and genotypes of *Echinococcus granulosus* (*E. canadensis*) in wild ungulates in Canada are described to better understand the significance for wildlife and public health. We observed *E. granulosus* in 10.5% (11/105) of wild elk (wapiti; *Cervus canadensis*) in Riding Mountain National Park, Manitoba, examined at necropsy, over two consecutive years (2010-2011). Molecular characterization of hydatid cyst material from these elk, as well as three other intermediate wildlife host species, was based on sequence of a 470 bp region of the NADH dehydrogenase subunit 1 (NAD1) mitochondrial gene. In moose (*Alces alces*), elk, and caribou (*Rangifer tarandus*) from northwestern Canada, the G10 genotype was the only one present, and the G8 genotype was detected in a muskox (*Ovibos moschatus*) from northeastern Canada. On a search of the national wildlife health database (1992-2010), cervids with hydatid cysts were reported in all provinces and territories except the Atlantic provinces, from which wolves (*Canis lupus*) are historically absent. Of the 93 cervids with evidence of hydatid cysts, 42% were elk, 37% were moose, 14% were caribou, and 6% were white-tailed and mule deer (*Odocoileus virginianus* and *Odocoileus hemonius*). In these animals, 83% had cysts in lungs only, 8% in both lungs and liver, 3% in liver alone, and 6% in other organs. These observations can help target surveillance programs and contribute to a better understanding of the *E. granulosus* species complex in Canadian wildlife.

Keywords: *Echinococcus granulosus*, wildlife, Canada, Cervid, Cystic, Echinococcosis

Introduction

Echinococcus granulosus is a species complex of cyclophyllid cestodes belonging to the Taeniidae family, consisting of at least 10 distinct genotypes (G1-G10), each of which circulates in unique host assemblages (1). Only the sylvatic genotypes (G8 and G10) occur in Canada (1) and have little veterinary significance; however, the livestock genotypes (G1-G3), which have a global distribution, are responsible for extensive economic damage due to livestock production losses and human illness (2). Molecular evidence and older morphological studies suggest that the G8 and G10 genotypes would best be re-classified as a separate species (*Echinococcus canadensis*) (1,3–5). *Echinococcus canadensis* is thought to be distributed across Canada, except in the Maritime Provinces and the island of Newfoundland where wolves were extirpated (6). It is also unlikely to occur in the High Arctic Islands where harsh climate conditions and a low density of intermediate hosts provide natural barriers for successful transmission between wolves [*Canis lupus*] and ungulates (7).

The North American sylvatic genotypes cycle between definitive canid hosts (such as wolves and coyotes [*Canis latrans*]) and cervid intermediate hosts (mainly caribou [*Rangifer tarandus*], moose [*Alces alces*], and elk [aka. wapiti; *Cervus canadensis*])(6). Domestic dogs with access to raw viscera from infected cervids can also act as definitive hosts (8) and, because of their close proximity to people, should be regularly de-wormed or denied viscera to avoid zoonotic transmission. Infected canids harbour adult tapeworms in their small intestine, shedding gravid proglottids and infective eggs into the environment in feces. These eggs, once ingested by a suitable ungulate intermediate host, penetrate the walls of the small intestine as oncospheres, and eventually develop into unilocular larval cysts in various organs (most often lungs (6,9,10)). Neither the definitive nor intermediate wildlife hosts appear to suffer serious adverse effects (10,11); however, heavily infected cervids are more likely to succumb to predation, either by wolves or by people (12,13). This may be a result of decreased pulmonary function, or in the case of intense disseminated infection, poor body condition and decreased stamina.

Surveillance for *E. canadensis* in Canadian wildlife is most often conducted opportunistically when animals are found dead, as part of community hunts, or when a large cull is undertaken.

Hydatid cysts recovered from Canadian wildlife and farmed ungulates are often dismissed as incidental findings, and even those recovered from human infections are seldom characterized at the molecular level. Thus, limited information is available regarding the geographical distribution and pathogenicity of the G8 and G10 genotypes. In this paper we present results from a genotypic analysis of hydatid cysts recovered from elk, caribou, moose, and muskox (*Ovibos moschatus*) in Canada, as well as a cross-Canada overview of hydatid cysts detected by pathologists in wild ungulates at the various nodes of the Canadian Cooperative Wildlife Health Centre (CCWHC; www.ccwhc.ca)(14) .

Materials and Methods

Origin of ungulate tissues

Elk (N=105) removed from Riding Mountain National Park (RMNP), Manitoba (MB) as a part of a *Mycobacterium bovis* control program were examined for presence of hydatid cysts in 2010 and 2011 (15). Cysts were also obtained from one adult male moose found dead in RMNP in 2011, as well as hunted animals including one elk from RMNP, one caribou from Tasiujaq, Quebec and one muskox from Kugluktuk, Nunavut. Cysts from RMNP animals were recovered by visual inspection and systematic palpation of organ tissue, followed by excision of all cyst-like masses. They were bagged separately, labelled, and stored chilled (~5°C) for 1 day until they could be transported to the University of Saskatchewan for identification. The caribou and muskox samples were stored at -20°C prior to shipping. Each hydatid cyst was pierced using a 22 GA needle and drained. One drop of fluid from each specimen was placed on a slide with a cover-slip in order to identify protoscolices under a light microscope. Presence of protoscolices and flame cell activity was used to determine fertility and viability, respectively (16). The hydatid liquid and remaining cyst material were placed in 70% ethanol and stored at room temperature.

Molecular characterization

To confirm that the cysts collected were indeed hydatid, DNA was extracted from 200µL of hydatid fluid using the DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA). Primers were used to amplify a 470 bp segment of the NADH dehydrogenase subunit 1 (NAD1) mitochondrial gene as previously described (17,18). PCR products were resolved using electrophoresis (110 V,

30 min) on ethidium bromide stained 1.5% agarose gels, and products were visualized under UV light. PCR products that produced positive bands were purified using ExoSAP-IT (Affymetrix Inc., Santa Clara, CA), and sent for sequencing at the National Research Council Plant Biotechnology Institute (Saskatoon, SK). Sequences were aligned using the Staden Software Package (Pregap 4, Gap 4) and compared to other sequences stored in GenBank™ (National Center for Biotechnology Information).

CCWHC search

The CCWHC maintains a national database of wildlife disease occurrences investigated by staff at the five Canadian veterinary colleges (AB, SK, ON, QC, PEI) and the Animal Health Centre (BC Ministry of Agriculture. In general, whole carcasses or animal tissues were submitted for diagnostic examination to the CCWHC by biologists, conservation officers, and hunters. In 2011, we searched the database using the terms ‘Echinoc’, ‘granulosus’, and ‘hydati’, (to include both English and French phrases) in any of the comments or morphological diagnosis fields. The results were limited to ungulates and the years 1992 to 2010.

Statistical analysis

Data were entered into a Microsoft™ Excel spreadsheet, checked for errors and analyzed using SPSS (version 19; Chicago, Illinois, USA). Dichotomous outcome variables were entered into 2 x 2 contingency tables, and the statistical significance between proportions were determined by Fisher’s Exact Test at the 5% level.

Results

Occurrence

The overall prevalence of hydatid cysts in the sample of elk from RMNP was 10.5% (11/105) (Table 2-1). The prevalence did not differ significantly by sex with 14.6% in the 48 adult males collected in 2010, and 7% in 57 adult females in 2011 (p-value = 0.338). The number of cysts per infected elk ranged from 1 to 4; all cysts were found in lung tissue, except 1 in spleen (Table 2-2). There were very few intact protoscolices in the hydatid fluid; half of the animals were infected with sterile cysts, and no protoscolices exhibited flame activity. In contrast, protoscolex density was high in the 7 cysts present in the moose from RMNP.

Table 2-1: Reported prevalence of *Echinococcus canadensis* in moose (*Alces alces*), caribou (*Rangifer tarandus*), elk (*Cervus canadensis*) and deer (*Odocoileus* spp.) from Canada.

Prevalence % (N)	Location	Province	Citation
<i>Moose</i>			
30 (96)	Moose Mountain PP ^a , Prince Albert- Nipawin area, Hudson Bay	SK	(34)
68 (100)	Wells Gray PP	BC	(9)
71 (17)	Lake of the Woods	ON	(3)
43 (14)	Kapuskasing	ON	
40 (43)	N/A	SK, AB	
17 (53)	Elk Island NP ^b	AB	
52 (62)	Northern/western AB	AB	(21)
0 (22)	Cypress Hills PP		
15 (39)	Elk Island NP		
67 (54)	Chapleau Crown Game Preserve	ON	(10)
58 (45)	Mont Tremblant NP	QC	(12)
42 (114)	Reserve La Verendrye	QC	(35)
32 (76)	Reserve des Laurentides	QC	
0 (50)	Reserve Matane	QC	
44 (580)	Southwest QC	QC	(36)
0 (16)	Eastern ON	ON	(37)
47 (224)	Southwest QC	QC	(25)
73 (51)	N/A	AB	(23)
<i>Caribou</i>			
21 (14)	Wholdaia Lake, Northern SK	SK, NT	(34)
21 (14)	N/A	SK	(3)
20 (517)	Reindeer Depot	NT	
1 (159)	Northern Labrador	NL	(22)
4 (488)	North-central Canada	MB, SK, NT	(24)
<i>Elk</i>			

16 (280)	Elk Island NP	AB	(3)
11 (359)	Cascade and Red Deer valleys	AB	(20)
16 (949)	Bow Valley		
38 (193)	Jasper NP		
21 (94)	N/A	AB	(23)
11 (105)	Riding Mountain NP	MB	This study
<i>Deer (White-tailed)</i>			
0.3 (353)	Sleeping Giant PP	ON	(6)
7 (28)	Moose Mountain PP	SK	(34)
44 (9)	Lake of the Woods	ON	(3)
28 (7)	Moose Mountain PP	SK	
0 (147)	N/A	AB	(23)
<i>Deer (Mule)</i>			
0 (263)	N/A	AB	(23)

^aPP = Provincial Park; ^bNP = National Park

Table 2-2: Description of hydatid cysts found in the lung tissue of wild elk (*Cervus canadensis*), muskox (*Ovibos moschatus*), moose (*Alces alces*) and caribou (*Rangifer tarandus*) in Canada.

Elk and moose were from Riding Mountain National Park (Manitoba).

ID #	Year	Host Species	# cysts	Protoscolex Density ^a	Genotype	Accession # (GenBank)
1281	2010	Elk	1	0	G10	KC505418
1300	2010	Elk	4	0	G10	KC505419
1235	2010	Elk	4	0	G10	KC505417
1284	2010	Elk	1	0	G10	KC505416
1394	2010	Elk	1	0	G10	KC505415
1037	2010	Elk	1	0	G10	NS
1156	2010	Elk	2	A -0 B- 2	G10	KC520777
101	2011	Elk	1	0	G10	KC520779

1435	2011	Elk	1 ^b	0	G10	NS
1444	2011	Elk	1	0	G10	KC520780
1292	2011	Elk	1	10	G10	KC520776
1325	2011	Elk	2	A -0 B- 1	G10	KC520778
OM-06	2010	Muskox ^c	2	0	G8	NS ^e
76095	2011	Moose	7	20-200 (range)	G10	KC520775
102	2011	Caribou ^d	1	0	G10	KC520781

^aNumber of protoscolices/mL hydatid fluid (A,B used in the case of multiple cysts per animal);

^bSpleen tissue; ^cKugluktuk, Nunuvut; ^dTasiujaq, Quebec; ^eNS = Not submitted.

Molecular characterization

PCR products of the NAD 1 locus were successfully amplified and sequenced in all 15 samples. The samples recovered from all cervids were identified as the *E. granulosus* G10 genotype, most closely related to GenBank accession nos. AF525297.1 (19) and DQ144041.1 (1). The cyst recovered from the muskox was most closely related to the G8 genotype (GenBank accession no. EU151429.1) (5).

Historical records

In total, 93 reports of cystic hydatid infection were retrieved from the CCWHC database. These included 39 elk, 34 moose, 13 caribou, 3 white-tailed deer (*Odocoileus virginianus*), 3 mule deer (*Odocoileus hemionus*) and 1 unknown species. The majority of positive tissue submissions originated from the western prairies (Saskatchewan 33%, Alberta 18%, Manitoba 2%), followed by the northern territories (Northwest Territories 15%, Yukon Territories 5%, Nunavut 1%), central Canada (Ontario 18%, Quebec 3%), and finally British Columbia 4% in the Pacific west (Figure 2-1). There were no reports from Atlantic Canada. Reports spanned a geographic range between 44°N to 68°N and 73°W to 135°W (Figure 2-1). The majority of infected animals had hydatid cysts in lung only (83%), followed by 3% in the liver only, 2% in the kidney, and 1% each in the spleen and skeletal muscle. Nine of 93 animals had cysts in more than one site (lung and liver [N=7]; lung, liver, kidney and spleen [N=1]; lung and heart [N=1]). Only moose and

caribou harboured cysts in organs other than the liver and lungs, and moose had the highest number of cysts per animal.

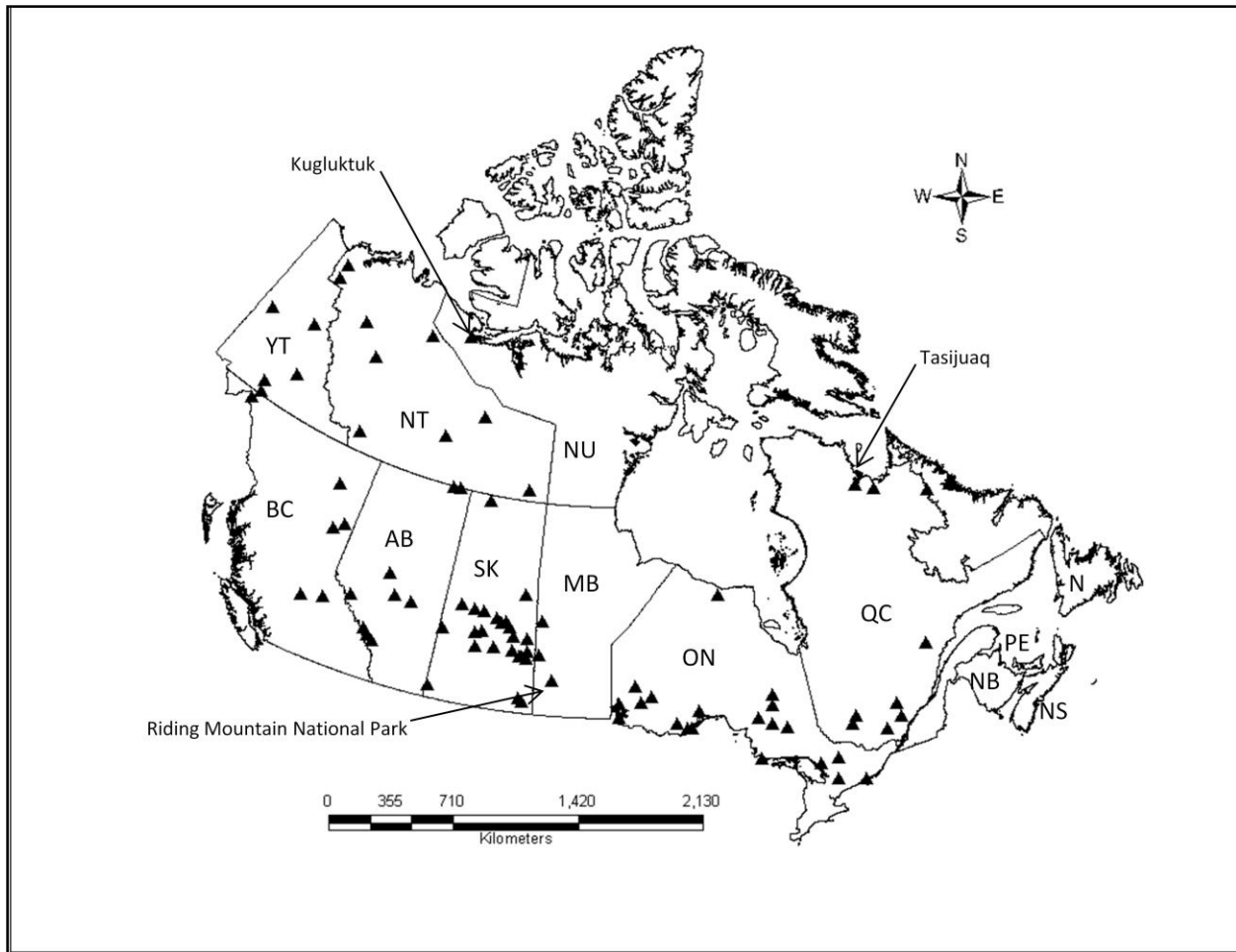


Figure 2-1: Geographic locations where *Echinococcus canadensis* cysts were recovered from wild ungulate hosts as reported in the literature (1952-present), the CCWHC database (1992-2010), and this study [YK: Yukon Territories; NT: Northwest Territories; NU: Nunavut; BC: British Columbia; AB: Alberta; SK: Saskatchewan; MB: Manitoba; ON: Ontario; QC: Quebec; NB: New Brunswick; PE: Prince Edward Island; NS: Nova Scotia; NL: Newfoundland and Labrador].

Discussion

Occurrence

A review of Canadian literature (1952-present) suggests that ungulate species are not equally suitable as intermediate hosts for *E. canadensis*. Intensity of infection appears to be low in

muskoxen, white-tailed deer, and mule deer whereas moose, elk, and caribou appear to be highly competent hosts, with higher prevalence and intensity of infection in areas co-inhabited by coyotes or wolves (3,6,20–24). In moose and caribou the intensity of infection, as defined by the number and size of cysts, is positively correlated with age (9,12,21,25), and possibly gender (females>males) (20,24). The overall apparent prevalence of cystic hydatid infection in the RMNP elk was similar to that reported in elk by other studies conducted in Western Canada (Table 2-1). The scarcity of protoscolices in RMNP elk support previous suggestions that elk are less suitable as intermediate hosts of *E. canadensis* than moose (3). Our results do not support previous reports of higher infection in females than males; however, our sample size was small.

Molecular characterization

We did not detect the livestock genotypes (G1-3) of *E. granulosus* in wildlife, which could support the belief that these genotypes are not present in Canada. Alternately, ungulates are likely poor hosts for these genotypes, but so few cervid isolates have been characterized genetically that it is difficult to draw conclusions at the present time. Our results failed to identify mixed infection in the animals sampled; however, we did not amplify DNA from all individual cysts from each animal. Molecular identification is important for sterile cysts as the differences in viability among genotypes in different host species is not yet known. It also serves as a method of definitive diagnosis, as identification based on gross or histological examination in the absence of protoscolices is difficult. No immediate conclusions can be drawn from these data regarding the proposed new taxonomic status of *E. canadensis*; however, this molecular characterization of hydatid cysts serves as a point of comparison for future biogeographical studies of hydatid infection in intermediate hosts.

Historical records

The records retrieved from the CCWHC database over the last few decades confirm the ongoing transmission of *E. canadensis* in most of northern, western, and central Canada. They also identified geographic locations in northern Ontario, British Columbia, Nunavut and the Northwest Territories where *E. granulosus* had not previously been reported by the literature. Many factors influence the prevalence and distribution of sylvatic parasites over time, including

climatic variation, migration or extirpation of host species and land use changes. To our knowledge, no data have been published in the last several decades that adequately describe the distribution of *E. canadensis* in Canadian ungulates. Surveillance to detect emergence is especially important in areas of newly established host assemblages, such as the island of Newfoundland, where coyotes and moose now intermingle (26). Our results based on passive surveillance suggest that *E. granulosus* transmission is not occurring in Atlantic Canada; however active surveillance is needed to confirm this conclusion. The historical data indicate that the majority of hydatid cysts found in ungulates were located in lungs, which supports the findings of previous reports (6,9,10), suggesting that surveillance could focus on examining lung tissues.

Several factors presented limitations in this study. We have no reports of *E. canadensis* in domestic livestock, as the database focuses on wildlife surveillance; however it is thought that this parasite rarely infects domestic ungulates (with the exception of farmed reindeer and elk) (1,3,27). The data contained in each report is limited by the quality of tissue submitted, whether the pathologist had access to the entire carcass, and whether the pathologist reported the cysts, often considered as incidental findings. Most CCWHC records did not state the infection intensity (# of cysts per animal), fertility, or viability. We are unable to report the prevalence of hydatid disease in Canadian wildlife, as the number carcasses examined by CCWHC during the study period is unknown. As well, sampling bias, as previously described, and low sample size numbers would make any estimate unreliable. The absence of reports from Nunavut and northern Labrador could be due the lack of proximity and the relative difficulty in transporting tissue samples to any of the CCWHC nodes.

Conclusions

This is the first cross-Canada review of *E. granulosus* in wild ungulates to be published since 1963 (3). Human echinococcosis remains a public health concern at northern latitudes and in some Indigenous communities where people live in close proximity to areas co-habited by moose and wolves (8,28). Hydatid infection in people was historically endemic to certain Canadian populations (29); however, changing risk factors, the advent of widespread anthelmintic use in domestic dogs, and public health education have decreased the risk of

infection (30,31). Most recently, the annual overall incidence rate of echinococcosis in Canadians was estimated to be 0.72 cases per million people; however, this rate is likely to be an underestimate because definitive diagnosis of clinical cases is difficult (28). Historically, autochthonous cases of human echinococcosis in Canada were believed to be less serious than cases caused by the imported livestock genotype. However, this medical paradigm was challenged in 1999 when two Alaskan patients diagnosed with hepatic echinococcosis (G8 genotype) experienced severe sequelae, including one fatality (32,33). Historical records of cystic hydatid disease are useful for both veterinary and medical professionals as they help define the potential distribution, and the risk of infection. Transmission and distribution of *E. canadensis* may increase as a result of rapid climate and landscape change, in combination with increased globalization of travel and trade, suggesting that national surveillance of this parasite will continue to be important for both human and animal health (31). This is especially true for areas where canids and ungulates have only recently come into close proximity.

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CHAPTER 3 - *Echinococcus multilocularis* and *E. canadensis* in wolves from western Canada

Citation

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Performed the experiments: JMS, KG

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© Cambridge University Press 2013. This paper has been updated from its original form to include 72 wolves from Aklavik, Fort Macpherson, Inuvik, Paulatuk, and Tuktoyatuk (NT). It has been reformatted from the original version, modified to reflect the graduate committee's comments, and is reprinted with permission.

Transition Statement

Echinococcus canadensis has an indirect lifecycle that requires both ungulate intermediate hosts (Chapter 2) and canid definitive hosts. This chapter characterizes the prevalence and genetic make-up of *Echinococcus* species in wolves (*Canis lupus*) from western Canada. Because wolves shed a variety of potentially zoonotic pathogens, the prevalence of both *E. canadensis* and other endoparasites are reported.

Abstract

Wolves (*Canis lupus*) are large apex predators that eat a wide variety of prey species, have long migration routes, and could facilitate the spread of different helminth species from endemic areas to non-endemic areas. *Echinococcus* species are important parasites of wildlife, domestic animals, and people worldwide; however, little is known about the prevalence, intensity and genetic diversity of *Echinococcus* tapeworms in Canadian wildlife. Intestinal helminths, including *Echinococcus* tapeworms, were harvested from the intestines of 92 of 165 (56%) wolves from ten sampling regions in the Northwest Territories, Manitoba, and Saskatchewan, and identified to genus level by microscopic examination. Molecular methods, used to determine species and/or genotypes of cestodes, identified *Echinococcus canadensis* (34/165; 21%), *E. multilocularis* (12/165; 12%), *Taenia krabbei* (26/165; 16%), *T. multiceps* (3/165; 2%), *T. hydatigena* (7/165; 4%), and *Diphyllobothrium latum* (4/165; 2%). Mixed infections of *E. canadensis*/*E. multilocularis*, as well as of the G8/G10 genotypes of *E. canadensis* were observed. These findings suggest that wolves may be an important definitive host for both *E. canadensis* and *E. multilocularis* in western Canada. This is the first report of wolves naturally infected with *E. multilocularis* in North America, and of wolves harbouring mixed infections with multiple species and genotypes of *Echinococcus*. These observations provide important information regarding the distribution and diversity of zoonotic species of *Echinococcus* in western North America, and may be of interest from public health and wildlife conservation perspectives.

Keywords: *Echinococcus granulosus*; wolf; Canada; genotype; geographic distribution

Key Findings

- Wolves in western Canada were infected with *Echinococcus multilocularis* and *E. canadensis*
- Wolves harbored mixed infections of *E. multilocularis*/*E. canadensis* and *E. canadensis* G8/G10 genotypes
- Wolves may play a far greater role in transmitting *E. multilocularis* than previously thought

Introduction

In Canada, most wolves reside in Holarctic regions (30 000 – 60 000 animals), and food sources may include small mammals, large ungulates, reptiles, amphibians, and fish, depending on the region (1). This helps to explain the high parasite richness (number of parasite species) found in wolves (2). A meta-review of the literature identified 72 helminth species (27 cestode spp, 16 trematode spp, 28 nematode spp, and one acanthocephalan) observed by post-mortem examination of wolf intestines, coproantigen testing, muscle biopsy, or fecal analysis (2). Previous helminths identified in tundra wolves from the YK and NT include *Diphyllobothrium* spp., *Taenia* spp., *E. canadensis*, *Toxascaris leonina*, and *Spirocerca* spp. (3).

Echinococcus species are cestodes that cycle among domestic and sylvatic animals, with occasional spillover into people. Two zoonotic *Echinococcus* species are distributed in Canada – *Echinococcus canadensis* and *Echinococcus multilocularis*. Previously, *E. canadensis* was known as the sylvatic strain of *E. granulosus* (or the G8 and G10 cervid genotypes); however, recent molecular evidence based on mitochondrial genes support the nomenclature change (4–7). *Echinococcus canadensis* utilizes large canids (wolves, coyotes [*Canis latrans*] and dogs [*Canis familiaris*]) as definitive hosts; and ungulates, primarily cervids (moose [*Alces alces*], elk [*Cervus canadensis*], caribou [*Rangifer tarandus*], and deer [*Odocoileus* spp.]) as intermediate hosts (8). *Echinococcus canadensis* has a widespread distribution across Canada, and is found in every province and territory except the Maritime Provinces and the island of Newfoundland (8,9). *Echinococcus multilocularis* is reported only in western Canada and is thought to occur as two geographically and genetically segregated populations (ie. the Northern Tundra Zone and the North Central Region) (10). *Echinococcus multilocularis* predominantly utilizes smaller carnivores (coyotes, dogs, foxes [*Vulpes* spp], and domestic cats [*Felix catus*]) as definitive hosts and a wide variety of small mammals as intermediate hosts (eg. tundra voles [*Microtus oeconomus*], deer mice [*Peromyscus maniculatus*], and brown lemmings [*Lemmus trimucronatus*]), although aberrant intermediate hosts (such as people, domestic dogs, etc.) do occasionally occur (11–13).

Wolves have long been considered the most important definitive host for *E. canadensis*, but with the exception of one experimentally infected animal, wolves infected with *E. multilocularis* have

not been reported in North America (2,11,14–16). This could be explained by a variety of factors, including the difficulty in harvesting and identifying adult cestodes of *Echinococcus* species (in part due to zoonotic risk), wolf predation preferences, and variable host specificity. The recent advent of molecular tools has facilitated species level differentiation, as well as identification of genotypic variations. The objectives of this study are to report the occurrence and identity of *Echinococcus* cestodes harvested from Canadian wolves, and to better define the geographic and host distribution of these and other intestinal parasites.

Materials and Methods

Wolves were harvested by trappers, hunters, and wildlife personnel from Saskatchewan (SK), Manitoba (MB) and the Northwest Territories (NT) for other purposes (2009 to 2012), and intestines were submitted for examination under University of Saskatchewan animal care research ethics approval protocol 20090126. The small intestines were ligated, excised, and frozen at -80°C for a minimum of 3 days prior to processing, in order to inactivate eggs of *Echinococcus* infective for people (10). Intestinal helminths, including *Echinococcus* strobilate adults, were harvested from the intestines of wolves using the scraping, filtration, and counting technique (17), morphologically identified to genus level, and stored in 70% ethanol. Intensity was estimated for *Echinococcus* spp. cestodes by counting the number of individuals in two 10% aliquots, and calculating the average number of cestodes per wolf. Freezing and ethanol fixation precluded definitive morphological identification of the adult cestodes to species level. To identify *Echinococcus* spp. cestodes, two to nine individual, intact, adult cestodes were selected from each wolf, and DNA was extracted from individual cestodes (18). PCR analysis of each worm was conducted using taeniid specific primers to amplify a 470 bp region of the NADH dehydrogenase subunit 1 (NAD1) mitochondrial gene of *E. canadensis* (19). A 395 bp region of NAD1 of *E. multilocularis* was amplified using species-specific primers for any samples that did not amplify using the primers for *E. canadensis* (20). To identify *Taenia* and *Diphyllobothrium* spp., one or two individuals of each species were selected from each infected wolf, and DNA was extracted from approximately 0.1 g of tissue using the DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA). Two different regions of the cytochrome c oxidase subunit 1 (CO1) mitochondrial gene were amplified: a 446 bp amplicon for *Taenia* spp. and a 437 bp amplicon for *Diphyllobothrium* spp. (21,22). PCR products were resolved by electrophoresis

(110V, 30 mins) on a 1.5% agarose gel stained by RedSafe nucleic acid staining solution (ChemBio Ltd, Hertfordshire, UK), and viewed under UV light. PCR products with positive bands were purified using QIAquick PCR Purification Kit (Qiagen Inc., Valencia, CA), and sent for sequencing (Macrogen Inc., Seoul, Korea). Forward and reverse DNA sequences were aligned using a Staden Software Package (Pregap 4, Gap 4). The aligned sequences were entered into GenBank™ (National Center for Biotechnology Information) and compared by BLASTn search to previously published sequences for identification.

Results

We examined 165 wolves (72 female, 92 male, 1 unknown), of which 15 were juvenile, 104 were adult, and 46 were of unknown age. One or more adult helminth species were detected in 92 of 165 wolves (prevalence of 56%). Intestinal parasite prevalence (based on morphological identification) was as follows: *Taenia* spp. (46/165; 28%), *Toxascaris leonina* (28/165; 17%), *Diphyllobothrium* spp. (5/165; 3%), *Alaria* spp. (8/165; 5%), *Uncinaria stenocephala* (1/165; 0.6%), and *Echinococcus* spp. (48/165; 29%) (Table 3-1). The median intensity of *Echinococcus* infection was approximately 2200 worms (range: 5, 24250).

Table 3-1: Location and occurrence of *Echinococcus* species infection in wolves examined by necropsy (Canada).

Province ^a	Sample size (N)	Location/region	Infection Prevalence ^b		
			<i>Echinococcus</i>	<i>E. canadensis</i> ^c	<i>E. multilocularis</i> ^c
NT	25	Southern Slave Region	8 (32)	4 (16)	1 (4)
NT	19	Northern Slave Region	3 (16)	3 (16)	1 (5)
NT	29	Sahtu Region	12 (41)	6 (21)	4 (14)
NT	8	Fort Macpherson	2 (25)	2 (25)	0 (0)
NT	17	Aklavik	1 (6)	1 (6)	0 (0)
NT	24	Inuvik	3 (13)	3 (13)	0 (0)
NT	17	Tuktoyatuk	1 (6)	1 (6)	0 (0)
NT	6	Paulatuk	2 (33)	2 (33)	0 (0)
SK	5	Prince Albert National Park	3 (60)	3 (60)	1 (20)
SK	1	Key Lake	0 (0)	-	-
SK	11	Unknown	11 (100)	8 (73)	3 (27)
MB	3	Riding Mountain National Park	2 (67)	1 (33)	2 (67)
Total	165		48 (29)	34 (21)	12 (7)

^a NT – Northwest Territories; SK – Saskatchewan; MB - Manitoba

^b Based on morphological identification

^c Based on molecular identification of selected cestodes (2-9 per wolf); samples from 9 wolves could not be identified beyond the genus level

Echinococcus cestodes were successfully characterized using molecular techniques in 39 of the 48 infected wolves. Based on NAD1 sequence data, *E. multilocularis* was identified in 12 wolves, representing a minimum prevalence of 7% (12 of 165 wolves). Seven of the *E.*

multilocularis positive wolves were co-infected with *E. canadensis* G10. The minimum prevalence of *E. canadensis* in these wolves was 21% (34 of 165). Of these, 20% (33 of 165) had *E. canadensis* G10, 4% (6 of 165) had *E. canadensis* G8 (5 had both genotypes). Other helminths identified using CO1 sequence data were as follows: *T. krabbei* (26/165; 16%), *T. multiceps* (3/165; 2%), *T. hydatigena* (7/165; 4%), and *Diphyllobothrium latum* (4/165; 2%). Co-infection by *Echinococcus* spp. and *Taenia* spp. was observed in 17 of 165 (10%) wolves.

Sequences from the G10 genotype of *E. canadensis* were most similar (99% identical) to a reindeer isolate from Finland (accession no. AF525297.1), and sequences from the G8 genotype were most similar (99% identical) to a moose isolate from the USA (AB235848.1). Sequences from *E. multilocularis* cestodes were most similar (99-100% identical) to a human liver cyst from Poland (JX266826.1), an M2 European genotype (AJ237640.1), and a European-type haplotype found in a domestic dog from British Columbia, Canada (JF751034.1). Sequences of suitable length and quality were submitted to GenbankTM and assigned accession nos. as follows: *E. canadensis* G8 KC848478-KC848483; *E. canadensis* G10 KC848484-KC848493; *E. multilocularis* KC848462-848477.

Only *E. canadensis* G10 was found in all ten of the sample regions (Figure 3-1). *Echinococcus multilocularis* was found in the five most southern regions of MB, SK and the NT, while *E. canadensis* G8 was found in Prince Albert National Park (PANP) in SK, and the South Slave, Sahtu, and Inuvik regions of the NT. Mixed infections of *E. multilocularis* and *E. canadensis* G10 were observed in Riding Mountain National Park (RMNP) in MB, PANP, and in the Sahtu region.

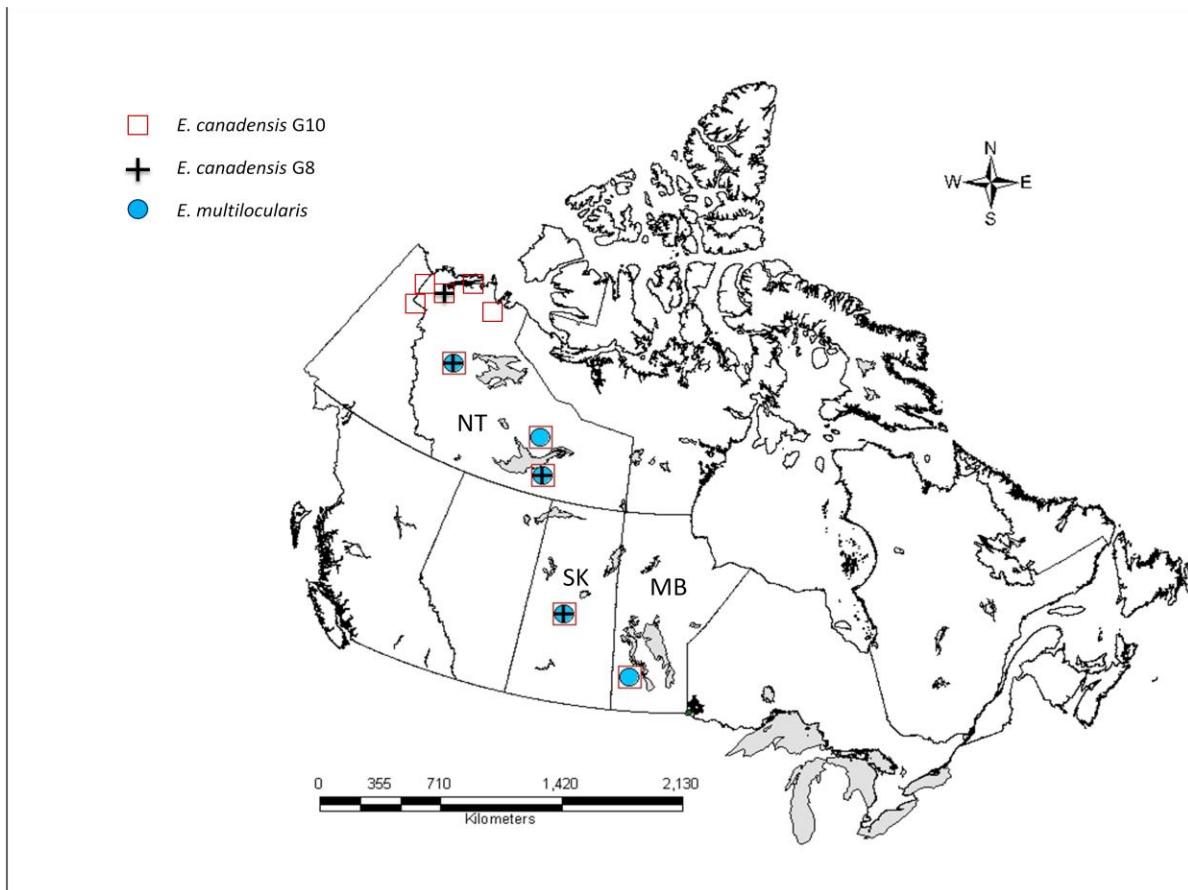


Figure 3-1: The occurrence of *Echinococcus multilocularis* and *E. canadensis* (genotypes G8 and G10) in wolves across ten sampling regions in Canada (N=165; NT – Northwest Territories, SK – Saskatchewan, MB – Manitoba).

Discussion

To our knowledge, this is the first report of *E. multilocularis* in naturally infected North American wolves (2,11,15,16). Wolves infected by *E. multilocularis* have previously been reported in Europe, Russia and China (2,11,23). Our study suggests that the significance of wolves for sylvatic transmission of this cestode in North America may be significantly underestimated. Previously, the northern distribution of this parasite was thought to track that of the Arctic fox (*Vulpes lagopus*), which was considered to be the most important definitive host at Arctic latitudes (16). We found a minimum infection prevalence of 7% in 165 wolves; similarly the prevalence of *E. multilocularis* in Arctic foxes in mainland regions of Alaska and the western Canadian Arctic is 2-9% (16). Compared to canids from RMNP (24), our reported median infection intensity of adult *Echinococcus* cestodes is higher than that previously reported in

wolves, and is far higher than that of red foxes. Wolves travel long distances and could contribute to range expansion of *E. multilocularis* (25). Wolves are known to consume a wide variety of prey species, including ungulates and rodents (26). Tundra wolves utilize rodents as a greater part of their diet than timber wolves, and would be expected to encounter *E. multilocularis* with higher frequency (3,26,27), but our results suggest the opposite – that timber wolves residing in southern regions of western Canada also encounter *E. multilocularis*. This most likely reflects a high prevalence of infection in rodent intermediate hosts as well as maintenance in other definitive hosts, including coyotes, red foxes, dogs, and cats.

This is the first demonstration of wolves naturally infected with multiple species and genotypes of *Echinococcus*, although mixed infections have previously been reported in dogs (28–30). Mixed infections of *Echinococcus* species and genotypes may be explained by the finding that exposure to larval stages of *Echinococcus* (hydatid cysts) by definitive hosts does not elicit a sufficient immune response to prevent a subsequent infection (31). Presumably, a definitive host could develop mixed infections through the consumption of various intermediate hosts harbouring different species and genotypes of *Echinococcus*. Mixed infections probably occur more frequently than suggested by our results, as this study was limited by the number of adult *Echinococcus* cestodes processed per wolf, and by the number of cestodes for which we successfully amplified DNA. We observed co-infection of wolves with *E. canadensis* G8/G10 genotypes in SK and NT, and co-infection with *E. multilocularis*/*E. canadensis* G10 in MB, SK and NT. Although we did not find *E. canadensis* G8 strain in a mixed infection with *E. multilocularis*, this likely reflects the relative rarity of this genotype as well as the need for more widespread geographic sampling. Mixed infections with *Taenia* spp. were observed in 17 of 165 (10%) wolves sampled, suggesting that cross-protective immunity does not occur.

The *Taenia* species observed in the NT wolves have distributions that span several continents, and utilize different host assemblages that depend on regional availability of definitive and intermediate hosts (32). Possible intermediate hosts for these species in Canada include moose, caribou, deer, elk, or muskoxen for *T. hydatigena*; big horn sheep (*Ovis canadensis*), Dall sheep (*O. dalli*), moose, deer, or caribou for *T. krabbei*; and snowshoe hare (*Lepus americanus*), or caribou for *T. multiceps*. Only caribou, muskoxen, moose, and snowshoe hare would be likely to

be found in the northern region of the NT where our samples were collected. The finding of *D. latum* is consistent with previous surveillance of wolves in the NT (3), and is predictable based on the close vicinity of the sampling locations to the Beaufort Sea as well as multiple lakes. Wolves become infected by ingesting infected tissues of marine or freshwater fish, such as pike (*Esox lucius*), yellow perch (*Perca flavescens*) or walleye (*Sander vitreus*) (33). *Toxascaris* and *Uncinaria* were the only nematode species observed in these wolves, similar to other parasite surveillance work in NT and the Yukon Territories, where *Spirocerca* was the only other nematode reported (3). This result may support previous observations that *Toxascaris* and *Uncinaria* are more cold adapted than other closely related nematode species (eg. *Toxocara canis* and *Ancylostoma caninum*). Other helminths that may have been missed due to the sampling technique include worms found in the stomach (eg. *Physaloptera*), cecum (eg. *Trichuris*), and other locations (eg. *Trichinella*).

Our findings of *Echinococcus* in wildlife may cause concern in both the animal and public health sectors. *Echinococcus* infection does not cause significant pathology in definitive hosts, and although hydatid cyst growth in ungulate hosts is usually asymptomatic, pulmonary infections may restrict vital capacity and endurance. Limited evidence is available to demonstrate that infected ungulates are more likely to be removed from herds by hunters or natural predators (34,35). In contrast, rodents are seriously compromised by their role as intermediate hosts of *E. multilocularis* (36). *Echinococcus* species are zoonotic, and although people are aberrant dead-end hosts, infection can cause severe long-term health consequences, including death (37). Cystic hydatid disease associated with *E. canadensis* is thought to be less pathogenic than that associated with the pastoral species in the *E. granulosus* species complex; however, severe clinical disease has been reported in people infected with the G8 strain in Alaska (38). Alveolar echinococcosis caused by *E. multilocularis* is especially dangerous for people, and the western coast of Alaska has been considered a highly endemic focus. This may in part reflect the unique ecology of the disease (especially on islands in the Bering Strait) as well as the possibility of Asian strains of this parasite (39). More work is needed to determine the significance of finding European-type strain(s) of *E. multilocularis* in wolves in northern and western Canada, and their relationship to strains elsewhere in the circumpolar North. The observation of *E. multilocularis*

in wolves is an important finding for wildlife managers, veterinarians, and public health personnel in western Canada.

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CHAPTER 4 - Sentinel surveillance for zoonotic parasites in companion animals in Indigenous communities of Saskatchewan

Citation

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Transition Statement

Dogs (*Canis familiaris*) are infected with many of the same endoparasites as wolves, including *E. canadensis*, and can be important sources of zoonotic parasite transmission by bridging the gap between people and wildlife. Dogs are infected with *E. canadensis* and other endoparasites by hunting and scavenging, or when they are fed raw offal, especially in rural and remote areas where dogs have easy access to wildlife. Historically in Canada, Indigenous people were at higher risk of exposure to *E. canadensis* than European settlers. This chapter examines the prevalence of endoparasites in rural and remote Indigenous communities in Saskatchewan, Canada, where parasite control methods are limited and dogs often roam freely.

Abstract

Indigenous communities may have increased risk of exposure to zoonotic parasites including *Echinococcus granulosus*, *Toxocara canis*, *Toxoplasma gondii*, *Diphyllobothrium* spp., and *Giardia duodenalis*, for which dogs may serve as sentinels for, or sources of, human infection. Fecal samples were collected from dogs and the environment in 5 Indigenous communities across Saskatchewan and Alberta (N=58, 62, 43, 66 and 25). Parasites in individual fecal samples were quantified using fecal flotation and a commercial immunofluorescent antibody test for *Giardia* and *Cryptosporidium*. Overall, the prevalence of intestinal parasitic infection was 20-71%, which is 5-16 times higher in Indigenous communities than in a nearby urban centre in Saskatchewan. The overall prevalence of *T. canis*, *Diphyllobothrium* and taeniid eggs in dog feces was, respectively, 11.8%, 4.9% and 1.2% in our study, compared with 0-0.2% in urban dogs. *Giardia* cysts present in 21% of samples were identified as zoonotic genotype Assemblage A.

Introduction

Parasitic infections acquired by zoonotic transmission can cause serious illnesses in people, and can financially burden healthcare systems (1,2). In 1882 (3), Canadian data demonstrated that cystic hydatid disease, caused by the zoonotic parasite *Echinococcus granulosus*, was over-represented in Indigenous populations compared to non-Indigenous Canadians, and this trend has not changed (4,5). More recently, surveillance of people residing in northern Indigenous communities across Canada has raised concerns regarding the sero-prevalence of exposure to parasitic zoonoses, including *Echinococcus*, *Toxoplasma*, *Trichinella*, and *Toxocara* (2,6–11).

Dogs act as bridging hosts between wildlife and people and can serve as sources of human infection with *Echinococcus* spp., *Toxocara canis*, and zoonotic genotypes of *Giardia* through shedding infective parasite eggs and cysts in feces (12–14). For other parasites, such as *Toxoplasma*, *Trichinella*, and *Diphyllobothrium*, dogs may serve as sentinels of shared environmental risks for humans consuming the same wild game or fish (15). The widespread use of anthelmintics has greatly decreased the risk of dogs developing patent parasitic infections in areas where veterinary services are available; however, many northern and remote areas of Canada do not have access to these services or products (15).

Currently, a knowledge gap exists in our understanding of the prevalence and significance of zoonotic parasites in people, wildlife and domestic animals in northern and Indigenous communities in Saskatchewan. Research in other areas of northern Canada (Nunavut, northern Ontario and Nunavik) indicates that people residing in these areas may be at higher risk of exposure to parasitic zoonoses because of a combination of unique risk factors. Large free-roaming dog populations, a reliance on locally acquired food, limited veterinary and/or medical services, and contaminated water sources are all factors that increase risk of parasite exposure (12,14,15).

Surveillance of dogs in remote Indigenous communities has identified a broad range of potentially zoonotic parasites, including: nematodes (*Uncinaria* and *Trichuris*), cestodes (*Diphyllobothrium*, *Dipylidium*, and *Echinococcus*), trematodes (*Metorchis*) and protozoa (*Giardia* and *Cryptosporidium*) (12,14–17). In the 1970's, surveillance demonstrated that dogs in 2 remote areas were disproportionately parasitized, as compared to those in 5 urban areas of SK. Parasites with zoonotic potential (*T. canis*, *Metorchis*, taeniids, *Diphyllobothrium* and *Uncinaria*) had 5-52 times greater overall prevalence in dog feces from remote communities than urban communities (14,17). More recently, dog fecal samples collected from one Saskatchewan reserve in 2008 were 85, 153, and 8 times more likely to be infected with *T. canis*, *Giardia*, and *Cryptosporidium*, respectively, than dog feces collected in Saskatoon in 2008-2009 (18,19). In addition, both humans and dogs on this reserve were infected with *E. granulosus*, the cause of cystic hydatid disease in people (8). In the current study, we examined feces from dogs in Indigenous areas of the Canadian Prairies to measure the prevalence of zoonotic parasites.

Materials and Methods

Dog feces

Between 2009 and 2011, dog feces were collected from 5 Indigenous rural or remote communities from public health regions in Saskatchewan (SK; Sunrise, Mamawetan Churchill River [MCR-A and B], and Keewatin Yatthe [KY]) and Alberta (AB; Chinook Health [CH]) under University of Saskatchewan animal care research ethics approval 2009-0126. Fecal samples were obtained directly from the rectum (N = 135) of dogs brought to mobile veterinary

clinics, or from the ground (N = 124). In 4 communities samples were simultaneously collected at the clinic and from the ground along major thoroughfares, on school properties, from the yards of consenting dog owners, at parks and playgrounds, and at the local landfill. Fecal samples were rejected if they appeared grey or white in colour (an indicator of age of sample). All samples were sealed in labelled plastic bags and kept cool for the duration of the sampling period (1-2 days). Fecal samples were then stored at -80°C for at least 5 days to inactivate eggs of *Echinococcus* spp. Parasite eggs were quantified in approximately 5 grams (wet weight) of feces from each sample using a modified Wisconsin fecal flotation and light microscopy to identify to the family or genus level (20). Approximately 1 g of feces from each sample was screened for *Giardia* cysts and *Cryptosporidium* oocysts, using a sucrose gradient flotation followed by a commercially available antibody fluorescence assay (Waterborne Inc.; New Orleans, LA) (21). In cases where a sufficient amount of fecal matter was available for only one assay, the Wisconsin test was prioritized.

Giardia genotyping

Molecular methods were used to identify the genotypic assemblages of *Giardia* cysts in individual fecal samples from MCR-A (SK) and CH (AB) regions (number of positive samples =15 and 21, respectively). DNA was extracted from cysts using the DNeasy Blood and Tissue Kit (QIAGEN Inc., Valencia, CA). A 511-bp segment of the β -giardin gene was amplified using a two-step nested PCR procedure (22). PCR products were resolved using ethidium bromide stained 1.5% agarose gels, and products were visualized under ultraviolet UV light. PCR products were purified using the QIAquick gel extraction kit (QIAGEN Inc., Valencia, CA) before DNA sequencing with the secondary PCR primers. DNA sequencing was performed at the National Research Council Plant Biotechnology Institute (Saskatoon, SK).

Taeniid egg speciation

In the CH region community, taeniid eggs from canine feces were identified to species level using PCR followed by DNA sequencing. DNA was extracted from eggs using the DNeasy Blood and Tissue Kit (QIAGEN Inc., Valencia, CA). A segment of the nicotinamide adenine dinucleotide (NADH) dehydrogenase subunit 1 (NAD1) gene was amplified using primers for an approximately 500-bp region of this mitochondrial gene (JB11 5'-AGA TTC GTA AGG GGC

CTA ATA-3' and JB12 5'-ACC ACT AAC TAA TTC ACT TTC-3'). PCR was run according to the following sequence: initial denaturation (94°C for 3 minutes), 40 amplification cycles (94°C for 15 seconds, 50°C for 30 seconds, 72°C for 30 seconds), final extension (72°C for 1 minute). Ethidium bromide stained agarose gel electrophoresis was used to visualize the PCR products, followed by PCR product purification and DNA sequencing as described above.

Results

In the 5 communities sampled, 20-71% of fecal samples from client owned dogs and/or the environment contained eggs or cysts of at least one species of parasite, and approximately 45% of these positive samples contained multiple parasite species (Table 4-1). Free-roaming dogs did not have significantly higher odds of shedding parasites than client-owned dogs in MCR-A, MCR-B or KY at the 95% confidence level, although a trend was apparent (Table 4-2). Overall, nematode infections were most common, with *T. canis*, *Toxascaris leonina*, and *Uncinaria stenocephala* infection in 12%, 16% and 8% of 254 samples, respectively. *Giardia* cysts and *Cryptosporidium* oocysts were identified in 21% and 4% of 231 samples, respectively, while tapeworms [taeniids (1%), *Diphyllobothrium* (5%)] and coccidia [*Isospora* (6%)] were less common.

Table 4-1: Prevalence of intestinal parasite eggs, cysts and oocysts in canine feces collected in Indigenous communities across Alberta and Saskatchewan public health regions, as identified through quantitative sucrose flotation and immunofluorescent assay.

	Chinook Health	Mamawetan Churchill River A	Mamawetan Churchill River B	Sunrise	Keewatin Yatthe
Community ID	CH	MCR-A	MCR-B	SR	KY
Month/Year	09/2009	09/2011	06/2010	11/2011	05/2010
<i>Toxocara</i>	9/62 (15%)	9/58 (16%)	7/66 (11%)	2/25 (8%)	2/43 (5%)
<i>Toxascaris</i>	25/62 (40%)	8/58 (14%)	3/66 (5%)	1/25 (4%)	3/43 (7%)
<i>Uncinaria</i>	0/62 (0%)	20/58 (34%)	0/66 (0%)	0/25 (0%)	0/43 (0%)
Taeniid	2/62 (3%)	0/58 (0%)	1/66 (2%)	0/25 (0%)	0/43 (0%)
<i>Diphyllobothrium</i>	0/62 (0%)	10/58 (17%)	1/66 (2%)	0/25 (0%)	1/43 (2%)
<i>Isospora</i>	3/62 (5%)	2/58 (3%)	3/66 (5%)	0/25 (0%)	7/43 (5%)
<i>Giardia</i>	13/40 (22%)	21/57 (37%)	5/66 (8%)	1/25 (4%)	0/43 (0%)
<i>Cryptosporidium</i>	4/40 (10%)	1/57 (2%)	1/66 (2%)	1/25 (4%)	1/43 (2%)
*Overall	38/62 (61%)	41/58 (71%)	15/66 (23%)	5/25 (20%)	11/43 (26%)

*Overall prevalence measured as the number of samples containing at least one parasite species divided by the total number of samples

Table 4-2: Comparison of parasite prevalence in feces collected from dogs brought to remote animal health clinics (client owned) versus feces collected off the ground (environmental) in three Indigenous Saskatchewan communities

Public Health Region Sampling Site	Client Owned	Environmental	Odds Ratio	95% Confidence Interval
MCR-A	25/40 (63%)	16/18 (89%)	4.8	1.0-23.9
MCR-B	4/16 (25%)	11/55 (20%)	1.0	0.3-3.7
KY	3/17 (18%)	8/26 (31%)	2.1	0.5-9.3

Giardia genotyping was successful in 90% (19/21) and 87% (13/15) of samples from CH and MCR-A, respectively. All were zoonotic genotype Assemblage A (GenBank Accession nos. JQ978656--JQ978688). These sequences all contained a cytosine at position 606 of the β -giardin gene (numbered relative to *G. duodenalis* Portland I, X85958), consistent with their identification as subassemblage AI within assemblage A (23).

NAD1 sequence from taeniid eggs in a fecal sample from the CH region was similar to *Taenia pisiformis* (88% identical over 491 nucleotides to AJ239109) (GenBank Accession no. JQ917875). The remaining sample had a low egg count (5 eggs per gram) and did not amplify on PCR.

Discussion

Sources and sentinels

Our study suggest that dogs in remote and rural areas can act as both sources and sentinels for human exposure to zoonotic parasites, as reported previously (15,24,25). Parasites of known public health concern were found in all communities. For some of these parasites, pets are a potential source of human exposure. For example, people can become accidental hosts for larvae of the roundworm *Toxocara* spp. when they ingest eggs passed in pet feces, or possibly, larvae encysted in paratenic hosts (24). Toxocariasis can cause ocular and visceral larval migrans, and is the most frequent parasitic zoonoses passed from pets to people in the United States (14,26–30). Clinical toxocariasis may be less common in Canada, with toddlers at highest risk (31). Dog

ownership was not an important risk factor for seropositivity for *T. canis* in Canada, emphasizing the importance of environmental contamination by free-ranging dogs for transmission of this zoonosis (31,32). Canids, including domestic dogs, are a definitive host for tapeworms in the Taeniidae family, and pass eggs infective to people in their feces. At the microscopic level, all Taeniid eggs appear alike, and molecular techniques are needed to identify species (8). We found non-zoonotic *T. pisiformis* in southern Alberta, but other species, including *Taenia crassiceps* and *E. granulosus*, are potentially present, and mixed infections would not necessarily have been detected by the techniques that we used.

Dogs may be a source of human infection, but also a potential recipient of infection from human sewage (33). *Giardia duodenalis* has been identified in a variety of wildlife species and companion animals in Saskatchewan, including dogs, coyotes and beavers (*Castor canadensis*; 18,19,34,35). It is most often spread by direct contact or via contaminated food and water sources (36). In our study, 2 to 37% of dog fecal samples from four communities were positive for *G. duodenalis*, and the zoonotic genotype A was confirmed in dogs in two communities. Himsworth *et al* (18) found a prevalence of 61% in dog feces from another Indigenous community in SK, while Gaunt *et al* (19) found a prevalence of 0.4% in dogs from an urban centre in SK. However, prevalence of *Giardia* is generally underestimated in surveillance studies due to the sporadic shedding of cysts, poor sensitivity of flotation assays, and the potential for subclinical infection (36). *Giardia duodenalis* Assemblage A has been identified in dogs in SK, AB and the Northwest Territories, and is considered zoonotic due to its lack of host specificity (hosts include dogs, cats and people) (15,18,37). Assemblage A is known to be more virulent in people than other genotypes, and should be considered a potential risk to public health in the CH and MCR regions where we identified infection in dogs (34,38).

For other parasites, dogs are not direct sources of human infection but instead serve as sentinels for parasites that are acquired by both people and dogs through common routes of exposure. For example, cestodes in the genus *Diphyllobothrium* (most likely *D. latum* or *D. dendriticum*) cannot be transmitted directly from dogs to people. Instead, both are infected through the consumption of infective plerocercoids in raw or undercooked fish (38). Dogs may also serve to amplify the abundance of this cestode in the environment. For animal sentinels to be useful for

public health purposes, they must be highly susceptible, highly exposed, and show a detectable response. Since dogs in remote and Indigenous communities are often free-ranging, have access to human food and garbage, and carcasses of local fish and wildlife, they are highly exposed and thus make excellent sentinels.

Disparities and community-specific parasite profiles

Dogs in Indigenous and northern communities in western Canada had a markedly higher prevalence of parasite shedding than dogs in a nearby urban centre (19). This finding is most likely attributable to factors such as age, reproductive status, gender, housing, geographic region, diet, and access to veterinary care (13,39). For example, dogs brought to mobile veterinary clinics in Indigenous communities are quite young (mean age = 1-2 years), likely because older animals have already been sterilized. Free ranging dogs in these communities are also young, possibly as a result of dog management practices (eg. dog shoot days) and high natural mortality in many communities. Juvenile dogs are more likely than adults to shed parasites, which may in part account for the high prevalence that we observed.

Our study revealed a distinct profile of parasite shedding and exposure in each community, even those in relatively close proximity; likely the result of variation in risk factors such as access to harvested wildlife, human garbage, clean water, and veterinary services (28). However, it is also important to note that parasite shedding is affected by season, which varied among the sample collections. One possible explanation for the low prevalence of *Uncinaria* infection was freezing at -80°C, which may have rendered the eggs unidentifiable. As well, some fecal samples collected from the ground may have originated from the same animal, causing the population prevalence to be over or under-estimated depending on whether the animal was shedding parasite eggs. Sample collectors were unable to distinguish dog feces from the feces of wild canids, such as wolves or coyotes; however, these wildlife are considered unlikely within the communities.

Identifying local risk factors and developing community specific parasite profiles can significantly aid veterinarians and health professionals in introducing locally effective animal and human health interventions. Key messaging in knowledge translation includes administering a broad-spectrum dewormer to companion animals regularly (at least once a year), removing and

disposing of animal waste regularly, cooking meat and fish consumed by both people and pets, and washing hands prior to eating and after handling animals or animal waste (28). Population control of free-roaming dogs, and preventative healthcare for owned dogs are crucial components to decreasing environmental contamination; many parasite eggs and cysts can survive months to years in the environment and are resistant to commonly available disinfectants. This will require improved access to veterinary products and services currently unavailable in the entire northern one-half of SK. Finally, our work suggests that surveillance of parasites in companion animals is a potential tool for detection of zoonotic risks for people, and could be used to evaluate the efficacy of animal and public health interventions. Using sentinels in this way could benefit communities by producing rapid, discrete, and economical estimates of human health risk, and simultaneously improving both animal and public health. Additional investigation into this application for animal sentinels requires exploring the relationship of prevalence levels and parasite species between people and companion animals.

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CHAPTER 5 - Rural origin, age and endoparasite fecal prevalence in dogs surrendered to the Regina Humane Society, 2013

Citation

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Performed the experiments: JMS, LD

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Transition Statement

Chapter 4 demonstrated that dogs in rural and remote Indigenous communities have a higher level of endoparasitism than that reported in owned dogs in Saskatoon. It remains unclear whether Saskatchewan (SK) dogs that are in closer proximity to veterinary clinics (urban and southern rural communities) also have high levels of endoparasitism. We did not detect *Echinococcus canadensis* infection in any of the dogs reported in Chapter 4, but we did observe such infections during a national study of Canadian companion animal shelters in 2009-2010 (Villeneuve *et al*, in prep). Regina Humane Society (RHS) is one of the largest animal shelters in SK, and it re-homes companion animals that originated in urban and rural communities across

the southern half of the province. This chapter reports the results of fecal parasite testing conducted for RHS dogs, including molecular work to differentiate between infection by *Taenia* spp. and *E. canadensis*. As well, we present an analysis of risk factors, such as geographic origin, associated with endoparasitism.

Abstract

We report the results of fecal parasite surveillance in dogs surrendered to the Regina Humane Society, Saskatchewan, Canada, between May and November 2013. Overall, 23% of 231 dogs were infected with at least 1 intestinal parasite. Endoparasite infection was positively associated with rural origin ($P=0.002$) and age (<12 months; $P<0.001$).

Introduction

Considerable differences exist in endoparasite prevalence and species diversity for pet populations at national, state, and regional levels, underscoring the importance of determining region-specific parasite profiles (1). These parasite profiles change over time due to factors such as parasite emergence and pet population structure and dynamics, and should be re-assessed regularly. Prevalence of canine parasites is often higher in rural or remote communities than city centres, due to limited or absent veterinary services, feeding non-commercial food to pets, access to untreated water, and/or the presence of large free-roaming dog populations (2–5). Companion animals, dogs in particular, can act as bridging hosts between wildlife or livestock and people for numerous zoonotic parasites. Regular surveillance is needed to develop regionally-appropriate anthelmintic treatment regimens in order to reduce parasite transmission among dogs, and from dogs to people.

The Regina Humane Society (RHS) is located in Regina, Saskatchewan (SK; 50.4547° N, 104.6067° W), a major population centre in southern Saskatchewan. It accepts dogs surrendered from all areas of the province. Dogs are treated with anthelmintic (pyrantel pamoate) upon entry to the shelter to minimize the risk of parasite transmission among animals, and from dogs to people. Ideally, a fecal examination would be performed on each dog at intake to determine correct anti-parasitic treatment; however, limitations in human and financial resources make this unfeasible. The goals of this study were to 1) determine the prevalence and intensity of parasites

in dogs at intake to help guide initial deworming protocols at the (RHS); and 2) determine if a dog's geographic origin (rural or urban), age, reproductive status and/or gender are associated with fecal parasite status.

Materials and Methods

Fresh fecal samples were collected from dogs upon admission to the RHS (May-November 2013), and within a day of anthelmintic treatment. Samples were stored at 4°C for up to 5 d prior to shipping to the University of Saskatchewan where processing occurred. Eggs, cysts, and oocysts were identified to family or genus level according to morphology and morphometrics by an experienced observer (for example, eggs of *Uncinaria* were measured to ensure that they were distinguished from those of *Ancylostoma*). Fecal egg counts (FEC) were performed on 4 g wet weight (ww) of sample using a double centrifugation sucrose modified Stoll flotation (6).

Giardia spp. cysts were isolated from a subset of samples using quantitative sucrose gradient centrifugation followed by an immunofluorescence assay on 4 g (ww) of feces (Giardi-a-Glo, Waterborne, New Orleans, Louisiana, USA) (7).

To identify taeniid eggs to the species level, DNA was extracted from feces using the E.Z.N.A. Stool DNA Kit (Omega Bio-tek, Norcross, Georgia, USA), and amplified using a 446 nucleotide segment of the cytochrome *c* oxidase subunit 1 (*cox1*) mitochondrial gene (8). Amplicons were purified using the QIAquick PCR Purification Kit (Qiagen, Valencia, California, USA), sent for sequencing (Macrogen, Seoul, Korea), and entered into GenBank™ (National Center for Biotechnology Information) for comparison to previously published sequences.

Geographic origin (rural versus urban), age, gender, and reproductive status (intact, spayed/neutered) were recorded for all dogs. Rural origin was defined using the Statistics Canada definition for the 2011 Census (ie. an area with a human population less than 1000, and a population density <400 persons/km²) (9). Data were entered into a spreadsheet and checked for errors. Fisher's exact test was used to identify associations between overall fecal parasite status (positive for 1 or more parasite species) and demographic data (gender, reproductive status, rural/urban, age) (SPSS version 20; IBM Corporation, Armonk, New York, USA). Associations between individual parasite infections and descriptors, and between rural location and

reproductive status were also explored. Statistical significance was assessed using 2-sided *P*-values at the 5% level.

Results

Two hundred and thirty-one dogs (118 male, 112 female, 1 unknown) were surrendered to the RHS during the study period, from 38 communities mainly located in southern Saskatchewan (Figure 5-1). Of these dogs, 18% (42 of 231) had been de-sexed (25% of males and 13% of females), and 31% (73 of 230) were under the age of 1 y. Seventy-five percent (173 of 230) came from 1 of 5 urban population centres (Regina, White City, Lumsden, Moose Jaw or Balgonie), while the remainder came from 1 of 33 rural communities (Figure 5-1). Origin and gender data were missing for 1 dog. Overall, 23% (52/231) of dogs were positive for at least 1 parasite species, including nematodes (*Toxocara canis*, *Toxascaris leonina*, *Uncinaria stenocephala*), cestodes (*Diphyllobothrium* spp., *Taenia pisiformis*), and protozoans (*Giardia* spp., *Isospora* spp., *Sarcocystis* spp.) (Table 5-1). Overall infection prevalence was 3.5 times higher in puppies (43% of 73) than adults (12% of 157), and approximately 2 times higher in dogs from rural areas compared to dogs from urban centers (Table 5-1). Both age ($P < 0.001$) and rural origin ($P = 0.002$) were significantly associated with infection, while gender ($P = 0.177$) and reproductive status ($p = 0.100$) were not.

Table 5-1. Parasite prevalence and intensity in urban and rural dogs surrendered to the Regina Humane Society May-November 2013 (N=231).

	Urban N (%)	Rural N (%)	Total N (%)	Overall Intensity (eggs/cysts/oocysts/g) Mean, Median, Minimum-Maximum
Sample size	172 (75)	58 (25)	231 ^a	
Cestodes				
<i>Diphyllbothrium</i>	1 (0.6)	2 (3)	3 (1.3)	381, 575, 10-588
<i>Taenia pisiformis</i>	1 (0.6)	0	1 (0.4)	3 ^b
Nematodes				
<i>Diocotophyma renale</i>	1 (0.6)	0	1 (0.4)	1 ^b
<i>Toxascaris</i>	3 (2)	2 (3)	5 (2)	656, 128, 5-3000
<i>Toxocara canis</i>	7 (4)	12 (21)	19 (8)	648, 372, 2.5-5188
<i>Trichuris</i>	0	1 (2)	1 (0.4)	3 ^b
<i>Uncinaria</i>	0	1 (2)	1 (0.4)	48 ^b
Trematodes				
<i>Alaria</i>	4 (2)	3 (5)	7 (3)	313, 25, 5-1875
<i>Metorchis</i>	1 (0.6)	0	1 (0.4)	56 ^b
Protozoans				
<i>Isospora</i>	7 (4)	2 (3)	9 (4)	2769, 233, 3-11680
<i>Sarcocystis</i>	4 (2)	5 (9)	9 (4)	886, 153, 3-5540
<i>Giardia</i> ^c	7 (9.5)	5 (16)	12 (11)	2339, 767, 17-12450
Overall ^d	30 (17)	22 (38)	52 (23)	

^aLocation data missing for 1 sample

^bIntensity data based on 1 value

^c*Giardia* test conducted on subset of total samples (75 urban, 31 rural, 105 total)

^dOverall prevalence was calculated as the number of dogs infected with at least 1 parasite type divided by the total sample number

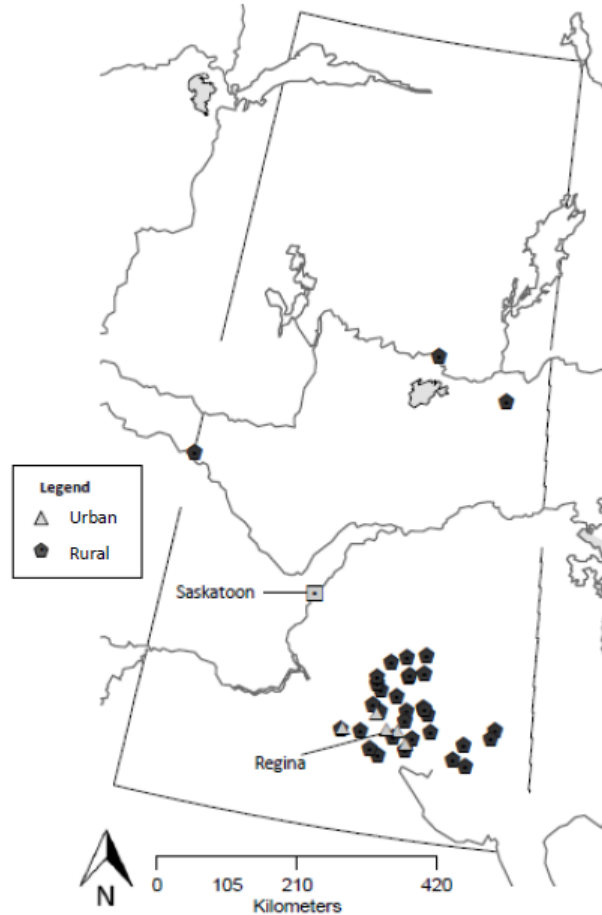


Figure 5-1. Geographic origin of dogs surrendered to the Regina Humane Society (Regina, Saskatchewan, Canada) in May-November 2013 ($n=231$).

Only *T. canis*, *Sarcocystis* spp., *Giardia* spp., and *Isoospora* spp. occurred with sufficient frequency to be assessed for associations with descriptors. Eggs of *T. canis* were associated with young dogs ($P<0.001$), rural origin ($P<0.001$), and intact reproductive status ($P=0.028$). The presence of *Sarcocystis* was positively associated with rural origin ($P=0.047$); *Isoospora* and *Giardia* were both positively associated with puppies ($P=0.005$ and $P=0.025$, respectively). Geographic origin was not significantly associated with reproductive status ($P=0.430$).

Discussion

Dogs surrendered to the RHS in the summer of 2013 had a prevalence of fecal endoparasite shedding (23%) that was almost 6 times higher than that of dogs from the closest city (Saskatoon) of similar size (10). Infection prevalence was almost twice that reported from an

American national survey of dogs brought to veterinary clinics (1). Helminth and protozoan fecal parasite shedding, in particular *T. canis*, *Isospora* and *Giardia* were most common in juvenile dogs. Young animals are more susceptible to parasitic infections probably due to behavior and/or reduced immunocompetency. This is consistent with other surveillance studies conducted in both Canada and the United States (1,11,12). Good husbandry practices are important to prevent both prenatal transmission of *T. canis* and neonatal transmission of *Giardia* and *Isospora* in these young animals. Endoparasitism and fecal shedding of *T. canis* and *Sarcocystis* occurred more frequently in rural dogs than urban dogs.

Of the 5 dogs that originated in rural central Saskatchewan (Otter Lake, Pelican Narrows and Onion Lake), 4 were infected with at least 1 parasite, and all were sexually intact. This may reflect the relative difficulty of accessing veterinary services in these locations, and is consistent with a previous study demonstrating that endoparasitism in dogs from rural indigenous communities was 5-16 times higher than in dogs residing in a nearby urban centre (5,10). For some parasites, the primary route of infection is through predation or scavenging of wild fish and game, such as taeniid and *Diphyllobothrium* tapeworms, the intestinal trematodes *Alaria* and *Metorchis*, the giant kidney worm *Dioctophyma renale*, and the protozoan *Sarcocystis*. This suggests that prevalence of these parasites should be higher in rural animals; however, these parasites were not significantly more common in fecal samples from rural origin dogs than urban origin dogs in this study. This could be explained by limitations in intake data for dogs, such as detailed travel history, dietary information, past living conditions (eg. indoor/outdoor), and prior access to regular veterinary care. As well, some rural communities were close to urban centres and those dogs might have had risk factors more similar to their urban counterparts. Likewise, many urban communities in western Canada have significant green spaces that are shared with wildlife, and off-leash areas often have access to local rivers and lakes.

We observed *Giardia* spp. prevalence (11%) in RHS dogs that was almost 3 times higher than the American national estimate, but only slightly higher than that found in smaller-scale Canadian surveys (~7%) (1,11,13). *Giardia* spp. infection is known to occur more frequently in young dogs, and conditions of crowding (eg., shelters, kennels) (11,13). This is because cysts are environmentally resistant and immediately infective when passed in the feces (14). The

prevalence of this parasite was not significantly higher in rural dogs than urban dogs, demonstrating the widespread distribution of this parasite, and the tendency of dogs to drink available surface waters.

The majority of dogs in this study came from Regina and small surrounding communities where veterinary care is available within a reasonable driving distance. In spite of this, endoparasite prevalence in rural dogs was high, suggesting several possible scenarios: 1) rural dogs were more likely to be stray/free-roaming, 2) rural dogs are more exposed (ie. higher levels of environmental contamination or increased access) or more susceptible (ie. younger demographics), or 3) deworming attitudes and compliance differ between urban and rural pet owners. Rural dogs were as likely to be de-sexed as urban dogs, demonstrating that rural pet owners in southern SK have access to veterinary care for their animals. Our finding that sexually intact dogs were more likely to shed *T. canis* than non-intact dogs is supported by a large study (N=1 213 061) in the USA where intact dogs had a higher risk of shedding *T. canis*, *Ancylostoma* spp., and *Trichuris* spp. (15). Possible explanations include the young age of most sexually intact dogs, sexually intact dogs encountering parasites more frequently through roaming behaviour, reactivation of somatic *Toxocara* larvae during pregnancy, and spay/neuter status acting as an indicator for improved animal care (including prophylactic use of dewormers, and feeding a commercial diet) (15). Increased focus on client education regarding regular anthelmintic use and parasite prevention measures may be appropriate for rural dog owners.

Pyrantel pamoate, the anthelmintic currently in use at RHS, is indicated for treatment of *T. canis*, *T. leonina*, *Ancylostoma caninum*, and *U. stenocephala* in dogs (16). When administered according to manufacturers' instructions, prophylaxis with pyrantel pamoate at intake should have successfully treated 77% (178 of 231) of dogs surrendered to the shelter. The RHS and other shelters could consider running fecal flotations on high risk dogs (puppies and those from remote locations), or switching to a broad-spectrum anthelmintic (one that would also target cestodes and possibly trematodes) to maximize parasite removal. Unless molecular methods are used, it is not possible to distinguish eggs of zoonotic species of *Echinococcus* (including *E. canadensis* and *E. multilocularis* in this region) from non-zoonotic *Taenia* spp., such as *T. pisiformis*. Therefore, shelter animals shedding taeniid eggs should be treated with praziquantel

or an effective cestocide prior to adoption to minimize risks to public health. Specific treatments for protozoan parasites are probably not indicated, given the asymptomatic nature of infection, with the exception of *Isospora*, which can cause clinical outbreaks of coccidiosis in kennels, and zoonotic genotypes of *Giardia*, especially for dogs adopted into high-risk households (those with children under 5, or people with chronic medical conditions or immunosuppression).

Conclusions

These findings emphasize the increased potential for parasitic infection in and zoonotic transmission from shelter dogs, especially those of young age and rural origin. Simple measures, such as deworming, limiting pet access to wildlife and domestic livestock, and prompt removal of animal waste from kennels, are all cost-effective methods of limiting the risk of animal and human illness due to parasites.

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CHAPTER 6 - Parasitic Zoonoses: One Health Surveillance in Northern Saskatchewan

Citation

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Transition Statement

People residing in remote and northern communities may be at increased risk of exposure to zoonotic parasites due to high levels of endoparasitism in free-roaming dogs, limitations to water infrastructure, traditional food preparation methods, and a close reliance on country foods. In previous chapters we examined wolves (Chapter 3) and dogs (Chapters 4 and 5) as potential

sources of *Echinococcus canadensis* infection. Chapter 6 uses a One Health approach to determine if people in a remote and northern Indigenous community have high exposure levels to *E. canadensis* and other parasitic zoonoses. This was accomplished by measuring human seroprevalence of 4 zoonotic parasites, identifying risk factors associated with exposure, and determining endoparasite levels in dogs.

Abstract

We report the results of a joint human-animal health investigation in a Dene community in northern Saskatchewan, where residents harvest wildlife (including moose, bear, elk, and fish), live in close contact with free roaming dogs, and lack access to permanent veterinary services. Fecal analysis of owned and free-roaming dogs over two consecutive years (N=92, 103) identified several parasites of public health concern, including *Toxocara canis*, *Diphyllobothrium* spp., *Echinococcus/Taenia*, *Cryptosporidium* spp. and *Giardia* spp. Administration of pyrantel pamoate to a subset of dogs (N=122) in the community in the first year was followed by reduced shedding of *T. canis* and other roundworms in the second year, demonstrating the potential utility of canine de-worming as a public health intervention. Using direct agglutination tests with confirmatory indirect fluorescent antibody test, 21% of 47 dogs were sero-positive for exposure to *Toxoplasma gondii*. Using enzyme-linked immunosorbent assay (ELISA) seroprevalence rates in 201 human volunteers were as follows: *Toxoplasma gondii* (14%), *Echinococcus granulosus* (48%), *Toxocara canis* (13%) and *Trichinella* spp. (16%). Overall 65% of participants were sero-positive for at least one parasite. A survey administered to volunteers indicated few associations between widely accepted risk factors for parasite exposure and serological status, emphasizing the importance of environmental transmission of these parasites through soil, food, and waterborne routes.

Author Summary

Parasites are ubiquitous, and while some parasitize only one host, others are capable of crossing species barriers. Zoonotic parasites move between animals and people, and in some cases cause significant veterinary, medical and/or public health problems. Such parasites may be more prevalent in areas where veterinary and medical services are scarce, and especially if sanitation infrastructure is suboptimal. Additional risk factors include reliance on country foods, proximity

to pets that come in contact with wildlife, and eating undercooked or raw fish and game. We visited one northern Indigenous community over two consecutive years to determine the prevalence of internal parasites in dogs, as well as to demonstrate the effect of selective deworming on reducing environmental contamination by zoonotic parasites. In addition, we collected blood samples and administered surveys to human volunteers in order to explore the relationship between exposure to four zoonotic parasites and several widely accepted risk factors for exposure (eg. pet ownership). Our findings indicate that levels of parasite exposure in this community were higher than similar studies conducted in other Canadian Indigenous communities. Public health interventions that utilize a one health strategy by integrating medical, veterinary and environmental expertise may be the most effective approach in reducing human and animal exposure to parasites in this community.

Introduction

Northern Indigenous peoples have recently been identified as being at high risk for acquiring parasitic zoonoses due to socioeconomic factors and a close relationship with the land (1). Hunting and fishing are common activities in northern Saskatchewan where consumption of country foods is an integral part of a traditional Dene diet and a very important contribution to food security in regions where commercial foods are often expensive, unavailable, and nutritionally inadequate (2). Free-roaming dogs continue to play important roles in Indigenous communities as wildlife deterrents, security, companion animals, and occasionally transport (3). Human exposure to zoonotic parasites might be above average in these communities if free-roaming dogs have access to raw game or fish and subsequently shed infective stages of parasites in areas frequented by people. Other risk factors for exposure to zoonotic parasites include contaminated or inadequately treated drinking water, handling and consumption of locally caught and inadequately cooked game or fish, challenges of waste disposal in remote environments, and/or absence of veterinary services (4–6).

Recently, the prevalence of intestinal parasitic infection in dogs was reported to be as high as 71% in northern Saskatchewan (7). Several genera of zoonotic parasites have been identified in dog populations including *Echinococcus/Taenia*, *Giardia*, *Cryptosporidium*, *Toxocara* and *Diphyllbothrium*, for which dogs may serve as sources or sentinels for human exposure (6,7).

Few studies have simultaneously sampled people as well as free-roaming dog populations to determine their role as sources or sentinels for human infection with parasitic zoonoses (8). A number of human sero-prevalence studies have been conducted in northern and predominantly Indigenous regions of Canada; however, none of these has focused on Dene communities in northwestern Canada, which share many of the same socioeconomic and public health concerns as Inuit in Nunavut, and Inuit and Cree in Nunavik and the James Bay region of northern Quebec (9–16). Zoonotic infectious such as echinococcosis and trichinellosis occur more frequently in northern and Indigenous populations; however, incidence rates of other zoonotic parasites are currently unknown for northern Saskatchewan (17).

We conducted research relating to veterinary public health in one Indigenous community in the Keewatin Yatthé (KY) health authority over a two year period (2010, 2011). The KY region is located in the northwestern part of Saskatchewan, and is one of three public health regions that encompass northern Saskatchewan (14). Approximately 10 600 people reside in this area, of which 94% self-identify as Indigenous (primarily Métis, Dene and Cree); a proportion similar to that seen in James Bay and Nunavik, Quebec. Social determinants of health significantly contribute to health inequities in this population, and include the high cost of food, housing shortages, low income, and high unemployment. People in this health region have shorter life expectancy, higher all-cause mortality, and higher rates of chronic and communicable disease (including diarrheal outbreaks, tuberculosis, hepatitis C and HIV/AIDS) than the provincial average (14).

In this paper we study canine endoparasitism and human exposure to four parasites of medical concern: *Echinococcus granulosus*, *Trichinella*, *Toxocara canis* and *Toxoplasma gondi*. Social and behavioural risk factors for exposure to these parasites are also explored.

Materials and Methods

Participants

In 2011, we visited one community in northern Saskatchewan with an approximate population of 2400 people and, primarily through word of mouth, recruited 201 volunteers over the age of 4 years (female N=77; male N=124). In addition, we sampled dog feces collected from the ground

and samples from client-owned dogs brought to a veterinary service clinic in the community in 2010 and 2011.

Human serology and risk factor assessment

Approximately 5 mL of whole blood was collected directly into serum-separator tubes (BD; Franklin Lakes, NJ) and kept refrigerated. Tubes were centrifuged at 3000 rpm for 10 minutes within 8 hours of collection, and sera transferred to snap-top mini centrifuge tubes. Serum samples were sent to the National Reference Centre for Parasitology (McGill University, Montreal, QC) and tested for IgG antibodies against *T. gondii* (Diagnostic Automation/Cortez Diagnostics, Inc, Calabasas, CA), *Trichinella* spp., *Toxocara canis* and *E. granulosus* by using an in-house developed IgG and IVD Research (Carlsbad, CA) enzyme-linked immunosorbent assay (ELISA). Criteria for interpretation of serology results are provided in Table 6-1. Equivocal results were designated as sero-negative. Each participant was also asked to respond to a survey (Appendix C) pertaining to risk factors for parasite exposure. Questions addressed pet ownership, feeding practices, barriers to veterinary care, hunting, fishing and personal consumption of country foods. Not all participants completed the surveys in entirety, and some small children were grouped under their parents' surveys.

Table 6-1: Results of serological analyses and criteria for sero-status in people.

Parasite	Measurement	Criteria and this study's results		
		Negative	Equivocal	Positive
<i>Toxocara canis</i>	Optical Density	<0.25	≥0.25 to <0.35	≥0.35
Number Samples		164/201	10/201	27/201
<i>Trichinella</i>	Optical Density	<0.25	≥0.25 to <0.35	≥0.35
Number Samples		149/201	19/201	33/201
<i>Echinococcus granulosus</i>	Optical Density	<0.35	≥0.35 to <0.45	≥0.45
Number Samples		77/201	27/201	97/201
<i>Toxoplasma gondii</i>	Units IgG (IU/mL)	<1	NA	≥1
Number Samples		173/201	-	28/201

Canine fecal surveillance

Approximately 300-400 dogs were estimated to reside in this community. We conducted canine fecal collection and analysis in this community during the month of June over two consecutive years (2010: N=92; 2011: N=103) to test the effectiveness of anthelmintic administration as a public health intervention. Fecal samples were obtained by rectal collection from client-owned dogs brought to a mobile veterinary service clinic (2010: N=31; 2011: N=34), as well as by ground collection throughout the community (2010: N=61; 2011: N=69) as a measure of environmental contamination. All dogs (N=122) brought to the mobile clinic in 2010 were treated with pyrantel pamoate (Pyran; Vetroquinol, Lavaltrie, QC) as per label dose, and owners were given additional medication along with instructions to repeat the treatment after 7-10 days. The ratio of male to female dogs brought to the clinic was approximately one to one, and all intact animals were desexed. Approximately half of the clinic animals were within one year of age.

For feces obtained from the ground around the community, fresh fecal samples were collected but older samples (grey or white) were not collected. Samples were stored in sealed plastic bags and kept in coolers with ice during the collection period (1-2 days). Feces were transported to the University of Saskatchewan (Saskatoon, SK) and stored at -80 degrees Celsius for five days to inactivate taeniid eggs. A quantitative sucrose centrifugation flotation was used to quantify and identify parasite eggs and cysts from approximately 5 grams wet weight of feces (modified from (18)). *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts were identified using a sucrose gradient flotation and a commercial immunofluorescent assay (Waterborne Inc.; New Orleans, LA) on approximately 1 gram wet weight of feces (19).

Canine sero-surveillance

We conducted sero-surveillance of *Toxoplasma gondii* for dogs brought to the mobile veterinary service clinic in this community in 2011 (N=47). Approximately 3 mL of whole blood was collected directly into serum-separator tubes (BD; Franklin Lakes, NJ), and chilled on ice. Sera were collected as described for the human study. Sera were analyzed for the presence of antibodies to *T. gondii* at the University of Saskatchewan (Saskatoon, SK) using a modified direct agglutination test (Biomerieux Toxo-Screen DA kit; Montreal, QC) at a 1:40 dilution. Samples with equivocal results on this test were re--tested using an indirect fluorescent antibody test (IFAT; VMRD, Pullman, WA).

Ethics

All participants provided written informed consent and those under the age of 18 provided written consent from a parent or guardian to participate. Individual serology results were mailed back to the participant and/or their primary care physician. The human study was reviewed and approved by the University of Saskatchewan Biomedical Research Ethics Board (REB 11-07), as well as by the Keewatin Yatthé Health Region and the community leader. The animal fecal and serology studies were reviewed and approved by the University of Saskatchewan Animal Research Ethics Board (2009-0126 and 2010-0159, respectively), which adheres to the Canadian Council on Animal Care (CCAC) standards. Dog owners provided consent for their animals to be sampled, while consent for ground collection of dog feces was provided by the community leader.

Statistical methods

Human serology and survey data were entered into a spreadsheet and analysed using logistic regression to identify associations between outcomes (sero-status) and risk factors (SPSS, Chicago, Illinois, USA). The strength of association between an outcome and variables was reported as an odds ratio (OR) with 95% confidence intervals (CI) (OpenEpi version 2.3.1, Atlanta, GA, USA). Risk factors were tested for statistical significance in a multivariate model using manual backward elimination. Risk factors were considered confounders if their inclusion or exclusion changed the effect estimate of another risk factor by more than 10%. In the case of correlated risk factors, only one was included in the final model. A chi-square test was used to determine if proportions were significantly different (p-value < 0.05).

Results

Human serology and risk factor assessment

Of 77 women and 124 men (N=201) sampled, 65% had been exposed to at least one of four zoonotic parasites (Table 6-2). The participation rate was approximately 8%; however, a number of potential volunteers were turned away due to limited phlebotomy supplies. The prevalence of diagnostically relevant titres was as follows: *E. granulosus* 47.8% (96/201), *T. canis* 13.4% (27/201), *Trichinella* 16.4% (33/201) and *T. gondii* 13.9% (28/201). Of those who were seropositive, 24% had been exposed to 2 parasites, and 8% had been exposed to 3; no person had been exposed to all 4 zoonoses. Co-exposure occurred most commonly between *E. granulosus* and *Trichinella* (19/201; 9.5%), with similar proportions between *E. granulosus* and the remaining parasites: *T. canis* (17/201; 8.5%) and *T. gondii* (14/201; 7%).

Table 6-2: Sero-surveillance for *Echinococcus granulosus*, *Trichinella*, *Toxocara canis* and *Toxoplasma gondii* in northern Indigenous regions (Canada) (9–13,15,16). All studies were conducted by a single laboratory using the same tests except Tanner et al (10).

Reference	Location	Sample Size (N)	<i>Toxoplasma gondii</i>	<i>Echinococcus granulosus</i>	<i>Toxocara canis</i>	<i>Trichinella</i> spp
Sero-prevalence (%)						
(11)	James Bay, QC	250	5	4	3	1
(12)	James Bay, QC	267	9	0.7	4	0
(13)	Inuvialuit, NT	362	4	0.6	0.6	0.6
(14)	Nunatsiavut, NU	310	7	0.3	1	1
(16)	Nunavik, QC	917	60	8	4	1
(10)	Northern QC	1195	30	2	10	2
(15)	Mistissini, QC	50	10	0	4	0
This study	Northern SK	201	14	48	13	16

The survey (Appendix C) identified several practices that could potentially expose people to zoonotic parasites (Table 6-3). Nearly all participants ate locally acquired foods including meat, fish, mushrooms and berries. Popular methods of wild game and fish preparation included drying, smoking or cooking; while raw foods were rarely consumed. Of dog and cat owners, 74% fed raw meat and 70% fed fish to their pets on a regular basis. Participants aged 5-17 had higher odds of exposure to *T. canis* (OR 3.4 95% CI 1.2-10) than those over the age of seventeen; and feeding dogs non-commercial dog food increased the odds of exposure by 15 times (95% CI 1.8-126). Increased odds of exposure to *T. gondii* were observed in participants

older than fifty (OR 9.4 95% CI 1.1-77) and those who did not own pets (OR 3.8 95% CI 1.3-11.3); however, gender and hunting/trapping are probable confounders for pet ownership.

Table 6-3: Potential risk factors for exposure to four zoonotic parasites in a northwestern Saskatchewan community.

Risk Factor	Odds Ratios (95% Confidence Interval)				
	Sample Size (N)	<i>Toxoplasma gondii</i>	<i>Echinococcus granulosus</i>	<i>Toxocara canis</i>	<i>Trichinella</i>
Gender (male)	201	2.2 (0.9-5.3)	1.2 (0.7-2.1)	1.9 (0.8-4.8)	1.5 (0.7-3.4)
Does not hunt/trap	188	1.5 (0.7-3.6)	1.9 (1.1-3.4)	1.2 (0.5-2.8)	0.5 (0.3-1.2)
Wild game consumption	196	0.5 (0-4.9)	0.3 (0-2.9)	0.5 (0-4.5)	0.2 (0-1.4)
Does not own a pet	199	3.8 (1.3-11.3)	1.2 (0.7-2.1)	1.3 (0.5-3.1)	1.8 (0.8-4.2)
Non-commercial pet diet	73	0.4 (0.4-3.8)	1.9 (0.7-5.0)	15 (1.8-126)	1.0 (0.2-4.0)
Age 5-17*	174	0.2 (0-2)	1.8 (0.7-4.6)	3.4 (1.2-10)	2.0 (0.7-5.8)
Age over 50**	68	9.4 (1.1-77)	0.3 (0.1-0.8)	0.4 (0.1-1.3)	0.4 (0.1-1.5)

*compared with all other ages

**compared with 5-17 age group

Canine feces and serum

Examination of canine feces identified five parasite genera of relevant zoonotic potential in this community, including *Diphyllobothrium*, *Toxocara*, *Echinococcus/Taenia*, *Cryptosporidium* and *Giardia*. Ground collected fecal samples had more parasites (2010: 51% 31/61; 2011: 35%, 24/69) than fecal samples of dogs brought to the clinic (2010: 48%, 13/31; 15%, 5/34). Chi-squared analysis indicates that the decrease in overall prevalence of endoparasitism from 2010 (48%; 42/92) to 2011 (28%; 29/103) is statistically significant (p-value 0.005) (Table 6-4).

During this time period overall decreases were noted in roundworms (*Toxocara* 9%, *Toxascaris* 5%, *Uncinaria* 11%); while the prevalence of tapeworms increased (Taeniid 4%,

Diphyllbothrium 13%). Examination of client-owned dogs in this region in 2011 demonstrated an exposure prevalence of 21% (10/47) to *T. gondii*.

Table 6-4: Prevalence of canine intestinal parasites identified through quantitative sucrose flotation and immunofluorescent assay.

Community ID	Prevalence (%)		Intensity Mean, Median, Minimum-Maximum (eggs per gram)	
	KY-2010	KY-2011	KY-2010	KY-2011
<i>Toxocara</i>	8/92 (9%)	0/103 (0%)	77, 70, 10-230	0
<i>Toxascaris</i>	10/92 (11%)	6/103 (6%)	2316, 31, 3-22500	1652, 64, 5-9660
<i>Uncinaria</i>	10/92 (11%)	0/103 (0%)	174, 34, 3-1005	0
Taeniid	0/92 (0%)	4/103 (4%)	0	124, 123, 3-248
<i>Diphyllbothrium</i>	2/92 (2%)	16/103 (15%)	586, 586, 8-1165	1795, 23, 3-15000
<i>Isospora</i>	1/91 (1%)	0/103 (0%)	*470, 470	0
<i>Giardia</i>	11/89 (12%)	2/95 (2%)	185, 100, 33-733	183, 183, 33-333
<i>Cryptosporidium</i>	14/98 (14%)	4/95 (4%)	83, 50, 33-200	417, 250, 133-1033
*Overall	42/92 (48%)	29/103 (28%)		

*Overall prevalence was calculated as the number of samples with at least one parasite type divided by the total sample number.

Discussion

This study shows that the prevalence of exposure to zoonotic parasites for residents of northwestern Saskatchewan is higher than previously reported in other Canadian sero-prevalence studies. As well, dogs residing in this area appear to encounter and be infected by potentially zoonotic parasites at higher levels than dogs residing in Saskatoon (a provincial urban centre) (20). Exposure to *T. canis*, *T. gondii* and possibly *E. granulosus* was observed in both people and dogs, indicating that dogs may act as sources and sentinels for human infections. Wild meat consumption, pet ownership and hunting/trapping are generally considered to increase the risk of exposure to zoonotic parasites; however, our analysis indicated that there might be a slight overall protective effect. This demonstrates the complexity of parasite transmission routes and the possibility of protective immunity and /or traditional knowledge regarding harvesting and preparation of wild foods.

Echinococcus granulosus is a cyclophyllid cestode with a worldwide distribution, causing serious veterinary, medical and economic concerns for highly endemic regions (21). Human infection with *E. granulosus* causes hydatid disease, or echinococcosis, which is generally characterized as the formation of larval cysts in the liver and lungs. The average annual incidence rate of hydatid disease in Canada is 0.72 cases per million people, and is higher in women than men (RR 1.92, 95% CI 1.29-2.87) and north of the 55th parallel (RR 4.88, 95% CI 2.52-9.44) (17). Hospital records in both Canada and the United States show Indigenous people to be at higher risk of infection (22,23). In another recent study conducted in a Saskatchewan Indigenous community, 11% of 103 people were sero-positive for *E. granulosus*, and at least two cases of hydatid disease were identified (8; S. Skinner, unpubl. data). The sero-prevalence of 48% to *E. granulosus* in the current study is substantially higher than the 0-4% reported in other Indigenous communities of similar northern latitude, analysed using the same test and by the same laboratory, the National Reference Centre for Parasitology (9–13,15,16). We are not aware of any clinical cases in this community at the current time; however, there is no formal surveillance for this parasite in Canada. There is a strong possibility that the unexpected level of exposure is due to cross-reactions with other helminths. Diphyllobothriasis cases are relatively common in this region [J. Irvine, unpubl. data], and other possibilities include the liver fluke, *Metorchis conjunctus*, and various *Taenia* species. We know that *Diphyllobothrium* is present

in dogs in this community (Table 6-4), and *Metorchis* has historically been reported in dogs, wolves and people in SK (5,24–26).

Trichinella nematodes have long been associated with consumption of undercooked pork; however, livestock production practices have virtually removed this parasite from the domestic Canadian swine herd (27,28). North American wildlife may be infected with one of five zoonotic genotypes of *Trichinella*, and consumption of these animals has been the primary cause of Canadian trichinellosis outbreaks since the 1970s (27–32). In northern Saskatchewan, exposure is most commonly attributed to the consumption of wild bear meat (*Ursus americanus*); while in Inuit regions of Nunavut and Nunavik, exposure is associated with consumption of marine mammals such as walrus (*Odobenus rosmarus*;29,32–36). The national annual incidence rate of trichinellosis is only 0.09 cases per million people; however, rates are significantly higher in Nunavut and Nunavik (42 cases per million people)(17). In Canadian northern and Indigenous communities the sero-prevalence for *Trichinella* in people ranges between 0 - 5.5%, which is far lower than our reported exposure prevalence of 16.4%. Antibodies to this parasite can persist up to 19 years, making it difficult to detect recent changes in exposure frequency (37).

Toxocara canis is an ascarid nematode that cycles primarily among canids, and commonly infects domestic dogs in Canada and around the world. People may become exposed through accidental ingestion of eggs shed in dog feces, or by ingestion of tissue cysts in the undercooked meat of paratenic hosts. Toxocariasis, characterized by visceral or ocular larval migrans, is not commonly reported in Canada, but may cause serious health effects. In our study youth were more likely to be exposed than adults, consistent with observations that children are at highest risk for infection when they play in sand or soil contaminated by dog feces, or due to pica (38–40). We found that dog ownership was not a risk factor for exposure to *T. canis*, similar to one other study in Canada (41), thus supporting the importance of environmental (versus direct) transmission of this parasite. Feeding non-commercial diets to family dogs significantly increased the odds of human exposure to *T. canis*. This may be due to increased transmission to dogs via the paratenic host route, followed by human contact with eggs shed in dog feces. Alternatively, feeding non-commercial pet diets may correlate with other variables, such as

poverty and occupational exposures to soil, that put people at risk of exposure (42,43). Sero-prevalence for *Toxocara* was between 0.7-4% in recent studies in Inuit and Cree communities in northern Canada (10–13,15,16). Our reported prevalence of 13.4% is therefore much higher than that observed in Canadian communities north of the 60th parallel, consistent with observations of restricted survival of *T. canis* eggs at colder temperatures (40,44–46). It is on par with the 13.9% reported in the general population of the United States between 1988 and 1994, although this was dominated by samples from the southern USA where this parasite may have increased levels of transmission (42). Reducing risk of exposure to this parasite could focus on regular deworming of dogs, timely disposal of feces (the eggs are not immediately infective), and preventing dogs from defecating in areas where children play.

Toxoplasma gondii has a global distribution, and is one of the most important parasites in the Canadian North (1). This protozoan has a complex lifecycle involving felids as definitive hosts and a wide variety of vertebrate species as intermediate hosts. In our study population, routes for dog exposure include feeding raw meat to dogs, ingestion of garbage and wildlife. As well, sero-positive status in dogs is associated with age, diet, hospitalization, and health status; a sample of young, stray dogs had the lowest level of sero-positivity (47,48). We observed a lower level of exposure to *T. gondii* in our population (21%) than dogs tested in Alberta, the Northwest Territories and Ontario (33- 63%) (4,48), which may be due to the relatively young population sampled.

Dogs are not known to spread *T. gondii* to people, however, our finding suggest that people in the community may be at risk due to shared exposure routes. People become infected by ingesting or handling raw meat, ingesting contaminated drinking water, handling infective cat feces, or by congenital transmission, blood transfusion or organ transplant (49). We report a sero-prevalence of 13.9%, which is comparable to the NHANES estimate of 10.8% in the United States (50), and generally higher than that reported elsewhere in Canada using the same test in the last 6 years (5-10%). Inuit in Nunavik, Quebec have one of the highest sero-prevalences reported (30-60%), and are thought to have a unique constellation of risk factors including gender (female>male), drinking water sources, regular disinfection of water reservoirs, and limited education (9–13,15,16,51). Exposure to *T. gondii* in the Keewatin Yatthé region was

statistically higher with age (>50 years), and with those who did not own a pet; however, confounding variables might nullify the effect of pet ownership on sero-status.

Saskatchewan currently has the highest incidence rate of Human Immunodeficiency Virus (HIV) in Canada, at double the national average. Indigenous patients are disproportionately affected, and represented 79% of HIV/AIDS cases in 2009 (52,53). HIV/AIDS is a serious risk factor for development of clinical toxoplasmosis. Mortality attributed to toxoplasmosis in AIDS cases in Europe and the United States is estimated to be 30% and 10%, respectively (49,54). The higher proportion of immune-compromised individuals in northern Saskatchewan combined with limited veterinary services, frequent contact with wildlife, and lifting of previously restrictive climate conditions, may lead to emergence of previously uncommon zoonotic pathogens (eg. *T. gondii* and *Cryptosporidium*) as public health concerns.

The prevalence of endoparasitism in client-owned dogs from this community was similar to levels previously found in remote areas of Saskatchewan (5,55). Ground-collected fecal samples did not represent the true parasite prevalence in this community as multiple samples may have originated from the same animal. However, this method is an effective tool for estimating the overall level of environmental contamination as well as for identifying local parasites of zoonotic concern; in this case *T. canis*, taeniid tapeworms, *Diphyllobothrium*, *Giardia*, and *Cryptosporidium*. The voluntary nature of human and canine recruitment was another limitation of this study; however, we considered this strategy as crucial in building trust with the community. The purpose of blood testing was not revealed during recruitment, and only 17% of participants were aware that pathogens could move between animals and people. Thus, people with concerns of parasite exposure were not more likely to participate. Sampling of client-owned animals was biased towards pets with owners who considered veterinary services important. However, we considered this effect minimal, because all dog owners permitted blood and/or fecal collection and all veterinary services were cost-free. Shedding of roundworm eggs (*T. canis*, *Toxascaris* and *Uncinaria*) decreased in 2011, following administration of pyrantel pamoate to dogs brought to the mobile veterinary service unit in 2010. This could reflect drug effectiveness, decreased transplacental and transmammary transmission of *T. canis* due to spaying female dogs, and/or the effect of having fewer puppies, which are the primary source of

environmental contamination. Alternately, the observed concomitant decreases in protozoa, which are not affected by pyrantel pamoate, suggest that changes in parasite prevalence may result from factors such as annual climate variations and altered animal husbandry practices. Whatever the cause, the overall decrease of parasitism in dogs brought to the clinic and in environmental contamination is a benefit to public health; however, the increased prevalence of cestode eggs demonstrates the additional need for cestocidal treatment to reduce risks to human health. Finally, this study reinforces that surveillance and management of zoonoses in remote areas requires a One Health approach incorporating both veterinary and public health interventions, tailored to concerns at the local level.

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CHAPTER 7 - People, pets, and parasites: One Health surveillance in southeastern Saskatchewan

Citation

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Conceived and designed the experiments: JMS, MN, EJJ.

Contributed reagents/samples/analysis tools: JMS, SAE, MN, EJJ.

Performed the experiments: JMS, MN, SAE.

Analyzed the data: JMS.

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Transition Statement

Chapter 6 found that one Dene group had a far higher risk of exposure to *Echinococcus canadensis* than the general Canadian population. Food preparation methods, cultural practices, dietary preferences, and access to game differ between Indigenous groups, and between geographic locations. The purpose of the following chapter was to compare endoparasite exposure levels in a vastly different human population - one that is rural, southern, and Saulteaux.

Abstract

Residents of remote and Indigenous communities might experience higher exposure to some zoonotic parasites than the general North American population. Human sero-surveillance conducted in 2 Saulteaux communities found 113 volunteers exposed as follows: *Trichinella* (2.7%), *Toxocara canis* (4.4%), *Echinococcus* (4.4%), and *Toxoplasma gondii* (1.8%). In dogs, 41% of 51 fecal samples were positive for at least one intestinal parasite, 3% of 77 were seropositive for *Borrelia burgdorferi*, and 21% of 78 for *T. gondii*. *Echinococcus* exposure was more likely to occur in non-dog owners (odds ratio [OR]:11.4, 95% confidence interval [CI]:1.2-107, P=0.03); while *T. canis* was more likely to occur in children (ages 4-17) (OR:49, 95% CI:3.9-624; P=0.003), and those with a history of dog bites (OR:13.5, 95% CI:1.02-179; P=0.048). Our results emphasize the utility of dogs as sentinels for emerging pathogens such as Lyme disease, and the need for targeted surveillance and intervention programs tailored for parasite species, cultural groups and communities.

Introduction

Zoonotic parasites are ubiquitous, and challenge public health systems in both urban and rural environments, even within developed countries in North America. Waterborne outbreaks of *Cryptosporidium*, *Giardia* and *Toxoplasma gondii* have all occurred in Canadian cities in recent years, accompanied by extensive public health messaging to help protect urban residents (1,2). As compared to urban residents, rural, remote, and northern residents may encounter parasites more frequently, and by mechanisms that are covert, due to alternative water sources, reliance on wild game/fish, and closer relationships with wildlife, livestock, and the land. Consumption of undercooked or raw meat by people has been linked to food-borne outbreaks, including trichinellosis in northern Saskatchewan and toxoplasmosis in northern Quebec (3,4). Companion animals, dogs in particular, can facilitate zoonotic transmission of parasites by acting as a source of infection for people, and as a bridge between wildlife and people. *Echinococcus* and *Toxocara* spp. are two such examples, and are acquired by people through accidental ingestion of eggs shed in dog feces. On the other hand, surveillance of dogs can play a critical role in preventing human illness, serving as sentinels for infection when they are exposed at higher levels and earlier than people during vector range expansion or disease emergence in a region.

Indigenous peoples of Canada are reported to be at higher risk of exposure to some zoonotic parasites than non-Indigenous peoples, with potentially life-threatening consequences (5–7). Seropositivity is an indicator of exposure to a pathogen, and requires diagnostic follow-up testing to determine if an individual is actively infected. Some zoonoses, such as echinococcosis and toxocariasis, are likely under-detected and under-reported due to non-specific or asymptomatic case presentation, imperfect detection methods, and the prolonged period between infection and illness. Because most Canadian sero-prevalence studies are conducted in northern and/or remote Indigenous communities, sparse information is available for the general Canadian population or for southern Indigenous groups, even though the risk factors for parasite exposure may be similar. Sauteaux Ojibway reside in communities scattered across British Columbia, Alberta, Saskatchewan, Manitoba and Ontario. This project was conducted in collaboration with two Sauteaux Treaty 4 communities in southeastern Saskatchewan, where country foods are frequently consumed, even though the residents live within a one hour driving distance of an urban centre (~100 km). The goal of this paper is to explore levels of human and canine exposure to parasites in a southern, rural and Indigenous area of Saskatchewan. We chose to measure human exposure to *Trichinella*, *Toxoplasma gondii*, *Echinococcus*, and *Toxocara canis*, as these pathogens have been studied in several other Canadian Indigenous communities, and offer a good basis for comparison.

Materials and Methods

Human participants

This study was conducted in collaboration with members of two neighboring Sauteaux communities located geographically in the Sunrise Health Region of southeastern Saskatchewan. These rural communities house approximately 423 and 757 residents, and are surrounded by agricultural lands used primarily for cash-crop farming (8). Planning and implementation of this project occurred in collaboration with key community members at a community camp-out and while working together on a digital storytelling project. We recruited participants >4 years of age by word of mouth and by posters displayed in community gathering spaces. Sample collection occurred during two community events: 1) a low cost pet health clinic organized by the research team, and 2) the annual Treaty Days celebration.

Human serology and risk factor assessment

Each adult participant first completed a survey pertaining to risk factors for parasite exposure, including dietary habits, pet ownership, use of veterinary services, history of dog bites, and hunting practices. Parents were asked to complete surveys on behalf of their children.

Approximately 3-5 mL of blood was then collected from each participant into serum separator tubes (BD, Franklin Lakes, NJ) and refrigerated overnight. Samples were centrifuged at 3500 rpm for 10 minutes, and sera were pipetted into snap-top micro-centrifuge tubes. Sera were frozen at -20°C until transported to the National Reference Centre for Parasitology (McGill University, Montreal, QC) and analyzed by in-house and IVD Research (Carlsbad, CA) developed enzyme-linked immunosorbent assays (ELISA) for immunoglobulin G antibodies against *Echinococcus*, *Toxocara canis*, *Trichinella*, and *Toxoplasma gondii*. ELISA results were interpreted according to criteria in Table 7-1, with equivocal results treated as negative.

Table 7-1: Criteria for serological evaluation of four zoonotic parasites and results of sero-surveillance in two Saulteaux communities in southeastern Saskatchewan (N=113).

Parasite	Measurement	Criteria and results		
		Negative	Equivocal	Positive
<i>Toxocara canis</i>	Optical Density	<0.25	0.25-0.35	>0.35
Number Samples		106	2	5
<i>Trichinella</i>	Optical Density	<0.25	0.25-0.35	>0.35
Number Samples		106	4	3
<i>Echinococcus granulosus</i>	Optical Density	<0.35	0.35-0.45	>0.45
Number Samples		107	1	5
<i>Toxoplasma gondii</i>	Units IgG (IU/mL)	<1	NA	≥1
Number Samples		111	NA	2

Dog serology

Blood samples were collected from dogs at their homes in November 2011 (n=32), and again in November 2012 (n=46) from dogs brought to a remote service veterinary clinic. If the dog became unduly stressed or fractious, we discontinued sampling. A standard veterinary history intake form was filled out for each animal brought to the remote clinic, including age, gender, vaccination and deworming history, and observations of ectoparasites. Approximately 3 mL of blood was collected from each dog into serum separator tubes, and samples were kept on ice during transport to the University of Saskatchewan. Tubes were spun at 3500 rpm for 10 minutes and sera were frozen at -20°C. Exposure to *T. gondii* was determined using an indirect fluorescent antibody test (IFAT; VMRD, Pullman, WA) at a serum dilution of 1:50. We evaluated exposure to four vector-borne pathogens (*Dirofilaria immitis*, *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, and *Ehrlichia canis*) using SNAP 4Dx Plus tests (IDEXX Laboratories, Inc.; Westbrook, Maine) according to manufacturer instructions. Both tests were validated for use in dogs.

Canine fecal surveillance

Canine fecal samples were collected in November 2011 (N=25), and again in June 2013 (N=26) from dog owners' yards and roadways. Only one fecal sample was collected per property to avoid collecting multiple samples from the same dog. Fecal samples were individually bagged, stored on ice, and brought to the University of Saskatchewan Zoonotic Parasite Research Unit for processing. Following a 3-day freezing period at -80°C (to inactivate zoonotic *Echinococcus* eggs), samples were analysed for parasite eggs using a modified double centrifugation and quantitative sucrose Stoll flotation (9). Briefly, 4 grams wet weight (ww) of each sample was homogenized in 40 mL dH₂O and strained through a single layer of 40-60 weight cheesecloth, using a tongue depressor to squeeze out excess water. A 5 mL sterile syringe (BD; Franklin Lakes, NJ) was used to transfer a 10% aliquot of fecal slurry into a 15 mL test tube, which was then filled to the top with dH₂O. Test tubes were centrifuged (1500 rpm, 10 min) and the supernatant poured off. The pellet was re-suspended in Sheather's sucrose flotation solution (spp gravity 1.26) by vortexing (maximum speed), filled to the top with Sheather's, and a cover slip (22 x 22 mm) was applied. After a second period of centrifugation (1500 rpm, 10 min), the cover slip was placed on a labelled glass slide and viewed under a microscope at 10-40x

magnification. Helminth ova and cysts were counted for the entire slide, and used to calculate the total eggs or cysts per gram of feces. An additional sucrose gradient flotation and immunofluorescent assay was used to isolate *Giardia* cysts and *Cryptosporidium* oocysts (10). Briefly, 2-4 grams feces (ww) were homogenized in 8 mL sterile saline, strained through a double layer of cheesecloth, and transferred onto 5 mL methylene blue sucrose solution (specific gravity 1.13) in a sterile 15 mL Falcon tube (Thermo Fisher Scientific Inc. Waltham, MA). After centrifugation (1300 rpm, 5 min), the top layer of the sucrose gradient was pipetted into a second 15 mL Falcon tube, and centrifuged again (1300 rpm, 5 min). The supernatant was poured off, the fecal pellet was re-suspended in 1 mL saline solution by vortexing, and 15 μ L was pipette into the well of a fluorescent microscope slide (Thermo Scientific; Portsmouth, NH). The slide was dried at room temperature (30 min), and 20 μ L each of Giard-o-Glo and Crypt-o-Glo (Waterborne Inc.; New Orleans, LA) were added. Following an incubation period (37°C, 45 min), a cover slip was added, and the slide was viewed under a fluorescent microscope (40-100x magnification). Cysts and oocysts were counted for the whole slide, and then used to calculate cysts or oocysts per gram feces.

Ethics

The human component of this project was reviewed and approved by the University of Saskatchewan's Biomedical Research Ethics Board (REB 11-07). Each adult participant provided written informed consent, and those under the age of 18 provided written consent from a parent or guardian prior to participation. All results were kept confidential, and we informed each individual of their results by mail. We organized a follow-up meeting with community members to at the completion of the project to share the results, and to answer outstanding questions. Any person testing positive for exposure to *Echinococcus* and all children who tested positive for *T. canis* were encouraged to seek free follow-up testing with a human health provider. The canine component of this study was reviewed and approved by the University of Saskatchewan Animal Research Ethics Board (2009-0126 and 2010-0159), which adheres to Canadian Council on Animal Care (CCAC) standards. Consent to collect blood from individual dogs was provided by their owners, while canine feces around the community were collected with permission from community leaders.

Statistical methods

Bivariate analysis was used to identify correlations between survey responses and sero-status for individual parasites as well as overall parasite sero-status, with exposed and not exposed individuals coded as 1 and 0, respectively. Using a cut-off value of $p < 0.2$ to determine statistical significance, correlated variables were included by forward stepwise addition to build binary regression models, using the Likelihood Ratio Test to select the final model (SPSS version 20; IMB Corporation, Armonk, NY). The strength of association between independent variables and sero-status was assessed using an odds ratio (OR) with a 95% confidence interval (CI). All variables were treated as categorical. Confounding was assumed if the inclusion of one risk factor changed the effect estimate of another by more than 10%. A Pearson X^2 test was used to determine if canine sero-prevalence to *Toxoplasma gondii* was significantly different between the sampling years, using a 2-sided cut-off value of $p < 0.05$. The Wilson score interval corrected for population size was used to determine the statistical significance of differences in survey responses [(OpenEpi version 3.01; Atlanta, GA)].

Results

Human serology and risk factor assessment

The participation rate in these communities was approximately 11%: 113 volunteers (female N = 75; male N = 38) of 1000 residents > 4 years of age (Table 7-2) (8). Titres above the cut-off value were observed in 12% (13 of 113) of participants for at least one parasite of interest (Tables 7-1 and 7-3). Sero-prevalence for individual pathogens was observed as follows: *Echinococcus* 4.4% (5 of 113); *T. canis* 4.4% (5 of 113); *Trichinella* 2.7% (3 of 113); and *T. gondii* 1.8% (2 of 113). Co-exposure to *Echinococcus* and *Trichinella* was observed in 2% (2 of 113) of the study population.

Table 7-2: Subset of study population and risk factor variables examined in two Saulteaux communities.

Variable (N*)	n	%	95% Confidence Interval
Female gender (112)	75	66	58-75
Age (112)			
4-10	15	13	8-21
11-17	17	15	10-23
18-35	23	21	14-29
36-50	28	25	18-34
51-65	21	19	13-27
>65	8	7	4-13
Pet Ownership (113)			
Dog (yes)	81	72	63-79
Cat (yes)	34	30	22-39
Veterinary care (87)			
Pet ever de-wormed	44	51	40-61
Pet ever vaccinated	46	53	42-63
Does owner use veterinary services	34	40	30-50
Allow dog to roam (65)	45	69	57-79
Believes dogs cause problems in community (85)	59	69	59-78
Feed raw meat to dog (82)	15	18	11-28
Desexing (86)			
Pet is already desexed	23	27	19-37
Owner is against/unsure about desexing pets	28	33	24-43
Dog bite frequency (89)			
Never	56	63	53-72
Once	18	20	13-30
2-3 times	11	12	7-21
>3 times	4	4	2-11

Hunt/trap (107)	19	18	12-26
Eat wild meat (109)	82	76	66-82
Cooked	73	67	58-75
Dried	11	10	6-17
Smoked	8	7	4-14
Raw	0	0	0-3.4
Eat wild fish (107)	30	28	20-37
Cooked	28	26	19-35
Dried	4	4	1-9
Smoked	1	1	0.2-5
Raw	1	1	0.2-5

*N= number of participants who answered the question

Table 7-3: Sero-surveillance for four zoonotic parasites in Indigenous communities in Canada using the same enzyme-linked immunosorbent assays (ELISA) at the National Reference Centre for Parasitology.

Reference	Location	Sample Size (N)	<i>Toxoplasma gondii</i>	<i>Echinococcus granulosus</i>	<i>Toxocara canis</i>	<i>Trichinella</i>
Sero-prevalence (%)						
Cree						
(11)	Mistissini, QC	50	10	0	4	0
(12)	Eastern SK	110	NA	11	NA	NA
(13)	James Bay, QC	250	5	4	3	1
(14)	James Bay, QC	267	9	0.7	4	0
Inuit						
(15)	Inuvialuit, NT	362	4	0.6	0.6	0.6
(16)	Nunatsiavut, NU	310	7	0.3	1	1
(17)	Nunavik, QC	917	NA	8	4	NA
Dene						
(18)	Northwestern SK	201	14	48	13	16
Saulteaux						
This study	Southeastern SK	113	2	4	4	3

Bivariate analysis identified age (P=0.13), owning a cat (P=0.14), owning a dog (P=0.007) and feeding pets raw meat (P=0.007) as potential risk factors for *Echinococcus* exposure. Potential risk factors for the remaining parasites were as follows: *T. canis* - age (P=0.058) and history of

dog bites (P=0.007); *Trichinella* – age (P=0.14) and dog ownership (P=0.14); *T. gondii* – age (P=0.13) and history of not deworming pets (P=0.17). Only one variable was correlated to overall positive sero-status – feeding dogs raw meat (P= 0.11). Binary logistic analysis demonstrated that three variables were significantly associated with positive sero-status: (1) non-dog owners were more likely to be exposed to *Echinococcus* than dog owners (OR=11.4, 95% CI 1.2-107, P=0.03); (2) children (4-17 years) were more likely to be exposed to *T. canis* than adults (OR: 49, 95% CI: 3.9-624; P=0.003); and (3) individuals with prior dog bite experience (at least one time) were more likely to be exposed to *T. canis* than those who had never been bitten (OR:13.5, 95% CI: 1.02-179; P=0.048). No children under the age of 11 (N=15) showed evidence of exposure to *T. canis*.

Survey results including potential routes for parasite transmission and food preparation habits are described in Table 7-2. Community members owned more dogs than cats (P<0.001), consumed more wild caught meat than wild caught fish (P<0.001), and prepared meat by cooking rather than by drying, smoking, or consuming raw (P<0.001). Many believed that dogs caused problems in the community, with overpopulation, aggression, scavenging garbage bins, running loose, and disease transmission given as the main reasons. Approximately 60% of pet owners did not use veterinary care regularly (many dogs had received puppy vaccination/de-worming by the breeder/seller only), and reasons included cost, distance to a clinic (the nearest clinic is 20 km away), and lack of perceived need. Feeding raw meat to dogs, allowing them to roam freely in the community, and lack of de-worming were common practices.

Forty-nine pet owners, three of whom resided outside the communities, brought their animals to the remote clinic in November 2012. Additional dogs were treated at their homes by a mobile team, and several strays were brought in for treatment by community members. The mean age of owned dogs (n=64) brought to the remote clinic was 2.3 years, with 34 males, 26 females, and 4 for whom sex was not determined (1.3:1 male to female ratio). Of owned dogs, 33% were known to have visited a veterinarian in their lifetime, 50% had received only their first set of vaccines, and 6% were known to have been surgically sterilized. Thirty-four percent of dogs had been dewormed (in several cases by our team going door to door in Nov 2011), and 28% owners

reported observing ectoparasites on their dogs (14% ticks). There were also 19 cats brought to the clinic (ratio of 3 dogs:1 cat).

Canine serology and fecal analysis

Overall, 21% (16 of 78) of dogs were sero-positive to *T. gondii*; sero-positivity was significantly higher ($p=0.042$) in November 2012 than in November 2011 (28%, 13 of 46 and 9%, 3 of 32, respectively). For *B. burgdorferi*, 3% (2 of 77) of dogs were sero-positive overall, with 4% (2 of 46) of dogs sero-positive in 2012, and no sero-positives in 2011. *Cryptosporidium*, *Giardia*, *Alaria*, *T. canis*, *Toxascaris leonina*, *Uncinaria stenocephala*, and *Sarcocystis* species were detected in canine fecal samples (Table 7-4). The proportion of samples positive for at least one parasite was 62% (16 of 26) in June 2013 and 20% (5 of 25) in November 2011. Parasite richness (number of species) and median egg counts were higher in June 2013 than November 2011.

Table 7-4: Prevalence of eggs and cysts of endoparasites in canine fecal samples collected from the ground in November 2011 (N=25) and June 2012 (N=26).

Collection year	Prevalence (%)		Intensity Mean, Median, Minimum-Maximum (eggs per gram)	
	2011	2013	2011	2013
<i>Toxocara canis</i>	8	15	6, 6, 5-8	22, 22, 3-43
<i>Toxascaris leonina</i>	4	27	4670, NA, NA	36, 18, 8-65
<i>Uncinaria stenocephala</i>	0	8	0	28, 28, 5-50
<i>Alaria</i>	0	8	0	4, 4, 3-5
<i>Sarcocystis</i>	0	8	0	845, 845, 130-1560
<i>Giardia</i>	4	12	233, NA, NA	229, 250, 63-375
<i>Cryptosporidium</i>	4	8	133, NA, NA	906, 906, 875-938
*Overall	20	62		

*Overall prevalence was calculated as the number of samples with at least one parasite type divided by the total sample number.

Discussion

This joint animal and human (One Health) study offers valuable information on dietary preferences, risks and routes of parasite exposure, issues relating to dog ownership, and use of veterinary services in two Sauleaux Ojibway communities in western Canada (Tables 7-2 and 7-3). The overall sero-prevalence of four parasitic zoonoses was low (12%) in relation to previous studies conducted with Dene and Inuit communities in northern Canada. Sero-prevalence for *T. canis*, *T. gondii*, *Echinococcus*, and *Trichinella* were similar to levels observed in Cree communities in northcentral Canada, likely reflecting similar preferences for cooked meat (13,14). However, the exposure to *Echinococcus* in the Sauleaux population in the current study (4% of 113) was lower than in a nearby Cree community (11% of 110) where a clinical case was detected; although it should be noted that the latter study considered equivocal serological results as positive (12). As compared to the general North America population, sero-prevalence of *T. canis* and *T. gondii* in our study population was low. However, detection of exposure to *Echinococcus* and *Trichinella* would be considered unusual in the general North American population, suggesting that there is some level of exposure to these potentially serious pathogens in the study communities. Antibodies to *Trichinella* are thought to persist for 9-18 months, while those for *E. canadensis* and *T. canis* could be life-long (17).

We observed a variety of potentially zoonotic parasites in dog feces collected from the environment in the community, including *T. canis*, *Cryptosporidium*, and *Giardia*. Although the number of fecal samples obtained in 2011 and 2013 was low, the combined prevalence (21 of 51; 41%) is comparable to endoparasite levels in canine fecal samples collected from the ground in other Indigenous communities in Saskatchewan (19). The difference in prevalence between sampling years is likely due to seasonal and annual variation in climate and diet, with dogs shedding higher numbers of parasites in spring/summer than in fall/winter. Overall, the level of parasitism observed in canine samples from the study community was 10 times higher than levels observed in owned dogs in urban Saskatchewan (20), which likely reflects the relatively young age of the dogs in the population (~2 years was the mean age of dogs brought to the remote clinic), as well as the fact that most dogs in the study communities live outdoors, either exclusively or intermittently, and many are permitted to roam freely. Access to raw meat, lack

of deworming, and scavenging of wildlife and discarded offal, are likely routes of parasite infection.

We detected evidence of exposure to *B. burgdorferi*, the causative agent of Lyme disease, in the dog population of these communities. The sensitivity (99%, 95% CL: 94.3-99.9%) and specificity (99.9%, 95% CL: 97.4-99.9%) of this test are both high, suggesting a high level of confidence in our results (21). This tick-borne pathogen can cause serious illness in infected people, and public health officials should be aware of its presence in southeastern Saskatchewan. Approximately 14% of dog owners in the study communities reported finding ticks on their dogs in the past year. Although these were most likely adult ticks of *Dermacentor variabilis*, *D. andersoni*, or *Ixodes kingi*, this suggests that these dogs are at high risk of exposure to ticks, including adults and nymphs of *Ixodes scapularis*, the host for *B. burgdorferi*. Therefore, this may reflect a westward expansion of the tick *Ixodes scapularis* from the currently limited endemic region in southern Manitoba in western Canada, or adventitious ticks that have traveled from more southern areas with migratory birds. These serological findings precede diagnosis of human cases in this region of SK, further supporting the idea that dogs are highly suitable as sentinels for this emerging disease, given their higher level of exposure to ticks than people.

We report a relatively low human sero-prevalence for *T. canis* (4%) on par with several other northern studies (Table 7-3), but lower than the national American average (14%) reported by the Third National Health and Nutrition Examination Survey (22). This low human sero-prevalence may reflect the fact that many dogs defecated in surrounding bush areas, and thus were less likely to contaminate human environments through parasite eggs in their feces. As well, extremely cold winter conditions may also decrease the human risk of exposure to some infective parasite eggs from dog feces. For example, eggs of *T. canis* have reduced survival when frozen at temperatures of -20 to -30°C, which are normal winter temperatures in this region (23). This supports an observed latitudinal gradient in prevalence of *T. canis* in dogs and wild canids, with prevalence decreasing as one moves north and the parasite being relatively unknown at latitudes greater than 60°N in Canada (7). Our regression analysis did not identify dog ownership or feeding raw meat to dogs as significant risk factors for *T. canis* exposure. This finding is similar to two other Canadian studies (14,24), and suggests that contact with infective

eggs in the environment may be the primary exposure route for people, especially in communities where stray dogs are abundant. This study is consistent with previous findings that youth (ages 11-17) are more likely to be exposed to *T. canis* than adults or younger children, highlighting the importance of de-worming, and keeping dogs out of areas frequented by youth, such as schoolyards and sandboxes (18,25). Because this age group is more likely to develop ocular, rather than visceral, larval migrans, follow-up should include retinal examination (26). Our finding of dog bite history as a risk factor for *T. canis* exposure has not previously been reported. *Toxocara canis* is not transmitted through dog bites, suggesting that our finding is more reflective of frequent exposure to environments contaminated by dog feces.

The sero-prevalence of *Echinococcus* in these communities was similar to that observed in northern Quebec, but lower than levels reported in eastcentral and northwestern Saskatchewan (12,18). Although we did not find *E. canadensis* in dog feces, infected definitive and intermediate host species are present in the area (27). Our analysis identified dog ownership to be protective against exposure to this parasite; however, the wide confidence interval suggests that this finding be interpreted with caution. One possibility is that dog owners have higher awareness of the risks associated with contamination of the environment with dog feces, or higher awareness of the need for hand hygiene. Results from this study did not identify gender, age or hunting/trapping as important risk factors for *E. canadensis* exposure as reported previously (6,17,18), which could be due to the low sample size. However, our findings are similar to a related project conducted in a nearby SK community (12).

We observed evidence of human exposure to *T. gondii* at a level lower than the American average of approximately 11% in 1999-2004 (28). This may reflect dietary preferences for cooked meat as well as a relatively small felid population (the definitive host for *T. gondii*), but the estimate might be limited by sample size. The community exposure prevalence for *T. gondii* was higher in dogs than in people, which supports the premise that dogs are more highly exposed and therefore serve as sensitive sentinels for public health. The survey data for these Sauleaux communities identifies risk factors for *T. gondii* exposure as well as protective mechanisms. Commonly accepted risk factors for exposure include female gender, drinking contaminated water, contact with infected cat feces, having three or more kittens, and ingestion of raw meat,

milk or shellfish (29,30). In northern Canada, risk factors include female gender, increasing age and frequency of fishing, berry picking, bird handling, cleaning domestic water reservoirs, and consumption of marine mammals, fish, and birds (31). In our communities, we observed many cats living outdoors, where hunting and eating intermediate hosts (ie. rodents, birds) is a likely source of infection. Approximately one-fifth of human participants were involved with hunting/trapping and skinning/butchering activities, with approximately equal representation between males and females. No participants ate raw meat, and although smoked or dried meat could potentially contain infective tissue cysts, food-borne transmission does not appear to be a major risk factor for exposure in these communities.

Trichinellosis has been rare in southern Canada since the domestic swine herd was declared to be *Trichinella*-free. However, feral swine have recently become endemic to southern Saskatchewan, and could possibly act as a source of human infection, although the infection status of these animals is not yet clear (32). In northern areas, the relative risk of human infection is high, and outbreaks have been linked to the consumption of raw bear or walrus meat (4,33). Risk factors for hospitalization due to trichinellosis are male gender and age (≥ 21 years) (6). The sero-prevalence for *Trichinella* in the current study (3%) was low, similar to that reported in Inuit and Cree communities (0-1%) elsewhere in Canada, but lower than that reported in a Dene community in northwestern Saskatchewan (16%) (11,13,18,25). In other Dene communities in northern Saskatchewan, outbreaks of trichinellosis associated with consumption of black bear have been reported (4). Saualtaux cultural practices, which include cooking game and avoiding consumption of bear meat, are likely the primary reason for low *Trichinella* levels in this community.

Our survey of the general population participating in the human sero-surveillance study demonstrated that few people used veterinary services regularly, and that common practices such as de-worming, vaccination and surgical alteration to control reproduction, are not widely accepted. Although there were cultural barriers to utilizing vet services, cost and distance were also frequently stated as barriers to using veterinary services, and about 5% of the total population of the two communities brought pets to the remote, low-cost clinic that was based in the community. Interestingly, clinical history data taken from pet owners at a remote veterinary

clinic in November 2012 indicated even lower rates of deworming (34%), vaccination (31%), and surgical desexing (6%) in the 64 owned dogs brought to the clinic, although the ratio of dogs to cats brought to the clinic (3:1) was similar to that reported in the survey (2.4:1). In addition, the gender ratio of dogs brought to the clinic (1.3 male: 1 female) confirms anecdotal discussions with community members that male dogs are preferred to female dogs, because of the nuisance of female dogs in heat and the burden of raising puppies. Despite this, follow-up discussions with the community indicated that they did not wish to pursue surgical methods of dog population control at this time.

In contrast with more northern Indigenous populations (34), the overall risk of human exposure to zoonotic parasites appears to be low in these study communities located in southeastern SK. Although it is difficult to make direct comparisons, we interpreted our results in the context of similar studies using identical laboratory methods in other Canadian Indigenous communities. Community-level differences in parasite exposure and risk factors reflect the presence of important regional, cultural, and dietary differences, and highlights the importance of targeted surveillance and intervention programs tailored specifically for different cultural groups and communities. Finally, we suggest that a One Health approach must go beyond sero-surveillance studies to ensure that surveillance is linked to actions, such as providing reduced cost, culturally acceptable veterinary services to underserved regions, ensuring that study participants have access to follow up diagnostic testing and treatments, and that researchers need to work with community liaisons (or “brokers”) to ensure translation of the results to community members and leaders.

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CHAPTER 8 - Echinococcosis: An economic assessment of veterinary public health interventions in rural Canada

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Wrote the paper: JMS, ER

Author's note

This manuscript will be modified to include physician salary estimates and indirect (societal) costs of echinococcosis before it is submitted to a journal for publication.

Transition Statement

Elements of this thesis, such as the canine parasite surveillance component, were originally conceived when a First Nations child in a northern SK community was diagnosed with cystic hydatid disease, caused by *Echinococcus canadensis*. *Echinococcus* species are endemic to many countries, and some have implemented *Echinococcus* control programs, with varying degrees of success. This chapter examines incidence rates of human echinococcosis across health regions, and uses a public payer perspective to model the cost utility of introducing an *Echinococcus* control program in the highest risk health region (Kelsey Trail, SK) in Canada.

Abstract

Echinococcosis is a rare but endemic condition in people in Canada, caused by a zoonotic cestode for which the source of human infection is ingestion of eggs shed by canids. The objectives of this study were to identify risk factors associated with infection, and to measure the cost-utility of introducing an echinococcosis prevention program in a rural area. We analyzed human case reports submitted to the Canadian Institutes for Health Information between 2002 and 2011. Over this 10 year period, there were 48 cases associated with *E. granulosus*/*E. canadensis*, 16 with *E. multilocularis*, and 251 cases of echinococcosis for which species was not identified (total 315 cases). Nationally, annual incidence of echinococcosis was 0.14 cases per 100 000 people, which is likely an underestimate due to under-diagnosis and under-reporting. Risk factors for echinococcosis included female gender, age (>65 years), and residing in one of the northern territories (Nunavut, Yukon, or Northwest Territories). The average cost of treating a case of cystic echinococcosis in Canada was 10,668\$ CAD. Cost-utility analysis revealed that dosing dogs with praziquantel (a cestocide) at six week intervals to control cystic echinococcosis is not currently cost-effective, even in a health region with the highest incidence rate in Canada. Threshold analysis revealed that a relatively small increase (incidence= 8 per 100,000) of cystic echinococcosis or alveolar echinococcosis could result in a PZQ dosing program having a favourable cost-utility ratio. Our findings and a discussion of current gaps in echinococcosis surveillance and management may be of interest to veterinary and public health authorities in Canada and other nations in which echinococcosis remains a neglected disease.

Introduction

Echinococcosis in people, also known as hydatid disease, is a potentially fatal condition caused by zoonotic cestodes of the genus *Echinococcus*, and is widely distributed worldwide (1). In Canada, human cases are considered rare, resulting in approximately 0.72 hospitalizations per million people per year (2). Two species of this parasite are endemic to Canada: *E. multilocularis*, which causes alveolar echinococcosis (AE); and *E. canadensis* (formerly known as the G8 and G10 genotypes of *E. granulosus*), which causes cystic echinococcosis (CE) (1, 3,4). Autochthonous cases are thought to be caused almost exclusively by *E. canadensis*, and occur more commonly in northern latitudes (>55°), in women, and in groups of Indigenous descent (2,3,5,6). Foreign-acquired cases of echinococcosis could be caused by other species not

present in Canada (eg. *E. granulosus* sensu strictu). Echinococcosis is under-diagnosed in the human population due to prolonged disease progression, asymptomatic or nonspecific symptoms, and the difficulty of definitive diagnosis – especially in northern areas where medical imaging services are limited (1,7,8). It is also under-reported, as there is no formal requirement to report human cases to national public health authorities in Canada. Recent studies highlight the need to better determine the incidence and health care burden associated with human echinococcosis in Canada, especially in remote, northern, and Indigenous communities (2,9,10).

The lifecycle of *E. canadensis* is indirect, and utilizes wild cervids such as moose (*Alces alces*), elk (*Cervus canadensis*), and caribou (*Rangifer tarandus*) as intermediate hosts. Canids including wolves (*Canis lupus*), coyotes (*C. latrans*), and domestic dogs (*C. familiaris*) serve as definitive hosts (11–13). Neither intermediate hosts nor definitive hosts are thought to suffer serious adverse effects as a result of infection; however, intermediate hosts may be at higher risk of predation due to decreased pulmonary function (14,15). Similar to *E. canadensis*, *E. multilocularis* utilizes canid definitive hosts (eg. foxes [*Vulpes* spp.], wolves, coyotes and dogs), and a wide variety of small mammals as normal intermediate hosts (1). In contrast to *E. canadensis*, intermediate hosts of *E. multilocularis* experience serious pathological changes, and aberrant intermediate hosts, such as dogs and primates, occur more frequently (16). People are infected by accidentally ingesting eggs, and domestic dogs have been identified as high risk reservoirs for human exposure to both species of *Echinococcus*. This is especially true in areas where dogs eat offal or seek out wildlife as a food source, and where poverty is prevalent (1,17,18). *Echinococcus* eggs shed by infected dogs are identical to those of all other taeniid tapeworm species, making definitive identification costly and difficult.

Worldwide, echinococcosis affects 2-3 million people per year, at an estimated cost of \$750 million USD (19). In countries where *Echinococcus* is highly endemic, this disease represents a significant economic burden to healthcare systems, as well as to animal production systems (1,20,21). The direct costs of this disease vary between healthcare systems but are straightforward to calculate if good health records outlining diagnostic and treatment procedures are maintained (22). Calculating the indirect costs presents a far greater challenge, as infected individuals experience under-employment and long-term health consequences, even when they

have undergone treatment (20–22). For all forms of echinococcosis there is the possibility for recurrence and for long-term sequelae following treatment, further increasing the burden of disease (1,23).

Multiple countries have implemented programs to control and/or eradicate CE using a variety of methods to limit the transmission of the parasite between dogs, humans and sometimes livestock (1). The most effective strategy is 6-weekly cestocide treatment (praziquantel [PZQ]) of dogs in concert with human, canine and livestock surveillance (1). Some programs drastically reduced dog populations, implemented animal control legislation and/or introduced public health education. Currently there are no control programs in Canada, with status quo being simply to treat infected people. Only a few studies have calculated the cost effectiveness of various echinococcosis control programs and none have been done in Canada (24–26). The goals of this paper are to 1) report the incidence of echinococcosis based on existing national datasets, and 2) determine the cost-utility of using a preventative PZQ dog dosing strategy at 6 week intervals in comparison to status quo, for a high risk health region in Canada using a public pay perspective.

Materials and Methods

Database

We obtained case records for Canadians diagnosed with echinococcosis from the Discharge Abstract Database (DAD) and National Ambulatory Care Reporting System (NACRS) for 2002-2011 through the Canadian Institute for Health Information (CIHI). Nationally, all cases identified through day surgery, outpatient clinics and emergency department visits are reported to the NACRS database; while DAD captures hospital inpatient cases, including deaths, discharges and hospital transfers. Cases were coded using version 10 of the International Classification of Diseases (ICD-10) coding system of the World Health Organization. NACRS and DAD did not report data from one province (QC - Quebec) unless a resident was treated out of province, but did report data from all other provinces and territories (BC – British Columbia, AB – Alberta, SK – Saskatchewan, MB – Manitoba, ON – Ontario, NL – Newfoundland and Labrador, NS – Nova Scotia, NB – New Brunswick, YT – Yukon Territories, NT – Northwest Territories, NU – Nunavut). Other omissions included MB and NB data for 2002/2003 and NB data for 2003/2004 due to delays in transitioning from ICD-9 to ICD-10 coding. Due to the

small population in the 3 northern territories (YT, NT, NU), these cases were grouped together to avoid identifying patients or communities. Our dataset did differentiate between international patients and citizens, but did not report travel history or whether a person had recently immigrated to Canada. Cost and length of stay estimates were only available for 2009/2010 and 2010/2011, and did not include physician compensation.

Anonymized patient records from the NACRS and DAD databases were analyzed using SPSS statistical software (version 20; Chicago, Illinois, USA). Individual health card identification numbers (issued by provinces and territories to individual residents enabling free access to health care services) were assigned a Meaningless But Unique Number (MBUN), which we used to ensure that each individual was counted only once over the study period. Length of stay estimates and treatment costs of individuals hospitalized multiple times were combined for that individual. Rural/urban and neighbourhood quintile income classifications were based on an individual's postal code, and geographic location was reported by health region (according to a patient's health card). Rural/urban residence categories adhered to Statistics Canada definitions: (1) Rural (outside or fringe of Census Metropolitan Areas (CMAs) or Census Agglomerations (CAs)); (2) Urban Core (large urban area with $\geq 50\,000$ people for CMA or $\geq 10\,000$ people for CA); (3) Urban Fringe (small urban areas inside CMA or CA but separated from the urban core); (4) Urban areas outside CMAs/CAs (small towns with a population of 1000-10000 people and population density of ≥ 400 persons/km²) (27). Other variables included age (categorized as <14, 15-64 and >65 years), gender, province where treatment occurred, and discharge status (ie. a patient's health status or anticipated location after leaving the hospital). Population proportions of infections were compared between genders, age groups, and urban/rural location using the Z-test, with statistical significance reported at the $P < 0.05$ level. Only individuals over 14 years of age were included in the rural/urban comparison, and incidence was reported as the median rate over 10 years (28).

Economic evaluation

We conducted a cost-utility analysis comparing a strategy for CE prevention (PZQ dog dosing) with status quo (no prevention) to capture the costs associated with CE, along with its impact on quantity and quality of life. We modelled one cohort, representing the health region with the

highest incidence rate (Kelsey Trail, SK) and including residents of all ages, over the lifetime of the patient to fully represent the long term consequences of the disease and the possibility for recurrence. Consistent with the Canadian Agency for Drugs and Technologies in Health (CADTH) guidelines, a public payer perspective was chosen to represent the funders of both the prevention program and the health care system.

Modelling

We used decision analysis to construct a Markov cohort simulation model within Treeage[®] to determine incremental cost-utility. The model ran for 43 years, from average age of infection to average life expectancy, with Markov cycles occurring at one year iterations. For both PZQ treatment and status quo, the model considered the transition between five CE health states (Healthy, In Treatment, Sequelae, Fully Recovered, Dead), each with associated costs and utilities (Figure 8-1). Transition probabilities determined the likelihood of moving between states.

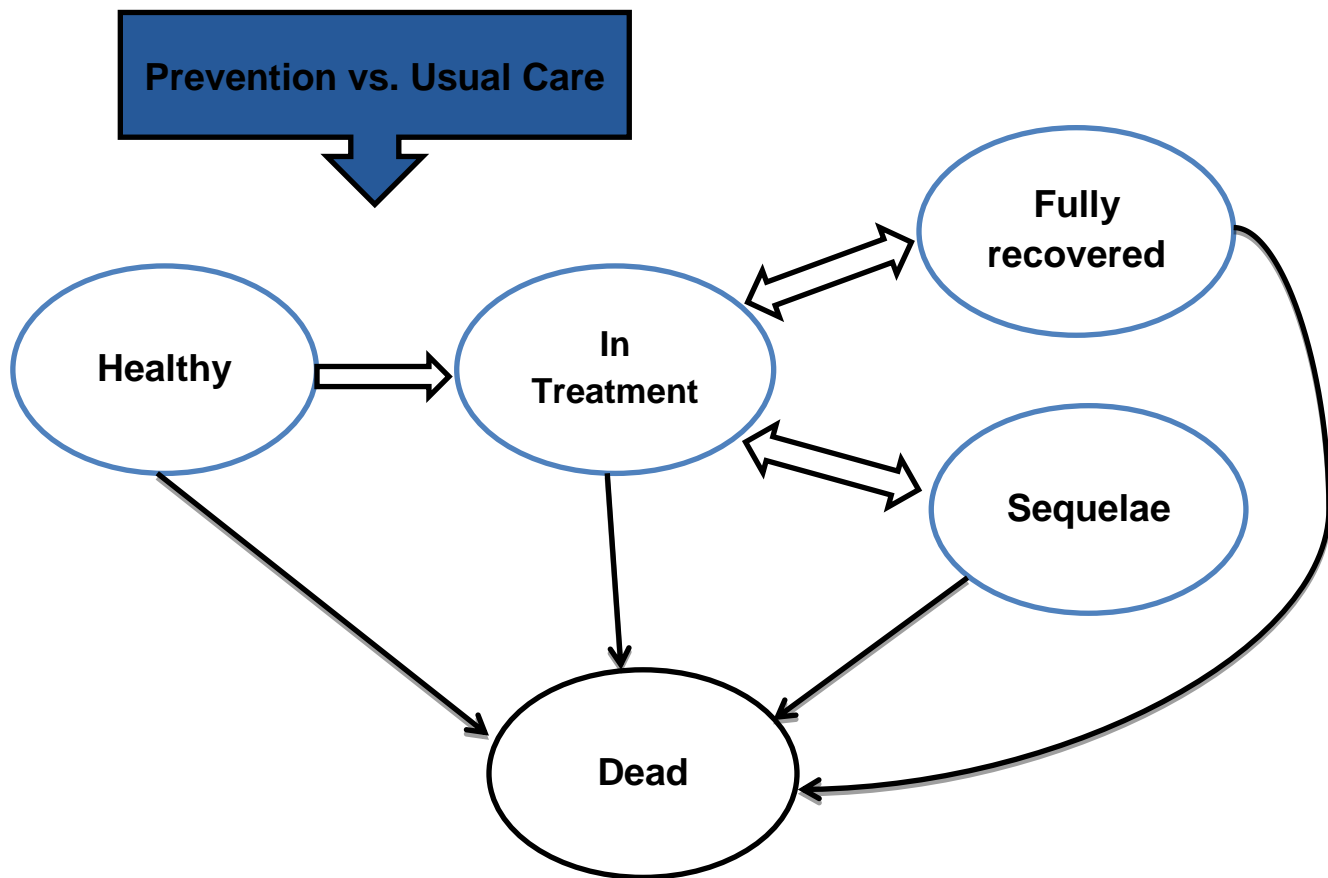


Figure 8-1: Markov model flow chart outlining the transition between health states of people infected with cystic echinococcosis.

Data inputs

We calculated the risk of developing CE in the Kelsey Trail Health Region using the incidence of hospitalization from the CIHI databases (AE cases were excluded from the model). The course of disease and the likelihood of different outcomes, including the risk of recurrent echinococcosis, risk of sequelae, fatality rates and all cause mortality rates were derived from the literature (1,22,23,29,30). Costs and utilities were both discounted at a rate of 5% as recommended in the CADTH guidelines, with sensitivity analysis at 0% and 3% discount levels (31). Base case estimates and sensitivity analyses are outlined in Table 8-1.

Table 8-1: Summary of data inputs, base estimates, and sensitivity analyses for cystic echinococcosis control in Canada.

Variable	Base estimate	Sensitivity analysis	References
<i>Utilities</i>			
Utility- Healthy	0.93	1-0.86	Mittmann et al. (1999)
Utility- in treatment	0.72	0.58-0.86	(33,34)
Utility- Treated permanent disease	0.89	0.8-0.93	(20)
Utility- Dead	0	0	N/A
<i>Costs</i>			
Cost Treatment	2182.69\$ per day in hospital*LOS (4.89)= 10,673.35\$	5093.09\$-13909.09\$	DAD/NACRS
Cost- PZQ program (total)	1.93	71,000-84,000	Expert opinion- Health Region
Cost- Dead	0	0	N/A
<i>Probabilities</i>			
Echinococcosis Risk	0.000016	0.00000707-0.000033	DAD/NACRS
Relative Risk	0.17 (after 10yrs)	0.4-0.05	(23)
Risk Recurrence	0.16	0.05-0.27	(1)
Risk Sequelae	0.075	0.02-0.15	(22,50)
Fatality Rate	0.03	0.01-0.05	(22,23,29)
Risk All-Cause Mortality	0.0057	0.0056-0.0059	(30)
Discount rate	0.05	0 & 0.03	(31)

We calculated the baseline relative risk of a PZQ dosing strategy versus status quo from CE incidence estimates in Chile before and after the implementation of a similar PZQ program (23). We chose this relative risk because it represented a similar pathogen (*E. granulosus sensu strictu*) to that observed in Canada, as well as being one of few successful programs conducted

on a continent rather than an island (23). We conducted sensitivity analysis using the variation in effectiveness in Chile, as well as estimates for the effectiveness of PZQ treatment of Alaskan dogs to eliminate *E. multilocularis* in a study population similar to the Kelsey Trail health region in Canada (32)

Utilities were used to value the outcomes observed in each health state, with dead having a utility of zero and the healthy average Canadian having a utility of 0.93. The treatment utility (0.72) was based on estimates for hepatic resection and liver cancer, as these illnesses have similar treatments and outcomes (33,34). Post treatment, those who fully recovered were assumed to return to the healthy state utility of 0.93, whereas those with sequelae had a slightly lower utility of 0.89. The sequelae utility was based on a SF-36 quality of life study of treated echinococcosis patients, which was then converted to a utility score using the Beaver Dam health outcomes study regression equation (35).

Two types of costs were considered: treatment costs of CE, and program costs of delivering PZQ. Treatment costs of CE provided by CIHI were only available for 2 years, and therefore we estimated average costs per case for the years 2002-2011 by calculating the mean cost per day times the length of stay (LOS) in the hospital. We are aware that treatment costs were underestimated because CIHI does not report physician costs. Program costs were provided by a SK health region director (H. Beatch, pers comm.), and included hiring an Environment Health Officer (EHO) or veterinarian, travel costs in and between communities, and the wholesale cost of treating all dogs in the Kelsey Trail Health Region with PZQ (2013 Associate Veterinary Purchasing Company Ltd.) at 6 week intervals. We estimated the average number of dogs by counting all dogs (owned and stray) in two SK rural Indigenous communities, and extrapolating our dog population estimate to Kelsey Trail (36). All costs were provided in 2011 Canadian dollars. We also conducted a budget impact analysis to determine the overall cost sustained and averted to the health region, and to more adequately represent the costs of the prevention program.

To ensure the validity of the model and the robustness of the findings we conducted one-way sensitivity analysis on all variables using plausible ranges. For the primary CIHI data, plausible

ranges were derived by calculating 95% confidence intervals for the risk of CE in Canada, and using the inter-quartile range for costs to account for non-normal distribution. For all other variables, the plausible range was drawn from the literature (Table 8-1). Finally, a threshold analysis was conducted to determine the level at which PZQ dosing would be cost-saving, and to identify the minimum incidence rate that would result in a cost-effectiveness of <\$20,000 per QALY (Quality Adjusted Life Year).

Ethics

This project was reviewed and approved by the University of Saskatchewan Biomedical Ethics Review Board (REB protocol number 13-51), which adheres to national standards set out by the Tri-Council for research involving humans. We report data at the level of the public health region to avoid inadvertently identifying individual patients or communities.

Results

Statistical analysis

Between 2002 and 2011, 384 discharge abstracts were submitted to the DAD and NACRS databases for patients under-going echinococcosis treatment. Of these, 69 abstracts were removed from descriptive analyses either because they were duplicates (the same individual obtaining medical care on multiple occasions), or because they lacked sufficient information to be assigned an MBUN. We report a median incidence rate of 0.14 cases per 100 000 people annually. The median age of an infected patient was 46 years. The highest frequency of cases was observed in females, those residing in an urban core, those aged 15-64, and those residing in neighbourhoods with the lowest income quintile ranking (Table 8-2). Relative to the female:male ratios reported by the 2006 Census (28), the proportion of female cases was significantly higher than the proportion of male cases at the national level (13:7, $P > 0.001$) and in three provinces (BC- 13:6, $P = 0.012$; AB- 25:6, $P = 0.032$, ON- 18:11, $P = 0.001$). The proportion of cases in the top age category (<65 years) was significantly higher than the other two categories at the national level ($P < 0.001$), and in AB, ON, and MB ($P = 0.001$, $P < 0.001$, and $P = 0.01$, respectively). In BC the proportion of cases in the top age category was significantly higher than the proportion of cases in the youngest age category ($P = 0.02$), but not the middle age group.

Table 8-2: Description of patients receiving care for echinococcosis in Canada from 2002-2011.

Descriptor	Frequency	% of cases ¹	Proportion x 10 ⁶	P-value
Gender				
Female	209	66.3	13	<0.001
Male	106	33.7	7	
Age (years)				
0-14	22	7.0	4	<0.001
15-64	207	65.7	5	<0.001
≥65	86	27.3	20	Reference
Urban/Rural				
Rural	29	11.2	9	0.27
Urban core	208	80.3	11	Reference
Urban fringe	5	1.9	5	0.11
Urban area outside CMA/CA ²	17	6.6	10	0.65
Missing	56	-	-	-
Neighbourhood Income Quintile³				
1- \$14 800	91	29.3	-	-
2 - \$25 800	65	20.9	-	-
3 - \$35 300	59	19.0	-	-
4 - \$46 900	50	16.1	-	-
5 - \$78 800	46	14.8	-	-
Missing	4	-	-	-
Province⁴				
BC	38	12.1	9	0.10
AB	51	16.2	15	0.72
SK	17	5.4	18	0.48
MB	17	5.4	15	0.97
ON	178	56.5	15	Reference
QC ⁵	1	0.3	-	-
NL	2	0.6	4	0.04
NS	3	1.0	3	<0.01

NB	2	0.6	3	<0.01
YK, NT, NU	6	1.9	59	<0.001

¹Percent of cases where descriptor data is available

² Only cases aged >14years used in rural/urban analysis; CMA-Census Metropolitan Area and CA-Census Agglomeration

³Average adjusted after-tax income for individuals calculated for 2006 in 2009 constant dollars (<http://www.statcan.gc.ca/pub/75-202-x/2009000/analysis-analyses-eng.htm#a2>)

⁴According to patient health card

⁵Only QC residents who received medical care out of province

We observed no significant differences in urban versus rural incidence among patients older than 14 years. The highest proportion of cases were observed in the territories (NU, NT, YK), while the lowest were observed in Atlantic Canada (NL, NS, PE, NB). The proportion of cases in these provinces and territories were all significantly different from the proportion of cases in ON. Our data suggests that the majority of echinococcosis patients were treated within their province of residence (311/323, 96%), except for those residing in the territories who were all treated elsewhere (NL, MB, or AB). At the regional level, annual incidence rates were highest in the Kelsey Trail Health Region (1.7 cases/100 000) in SK and the Norman Regional Health Authority (1.2 cases/100 000) in MB (Figure 8-2).

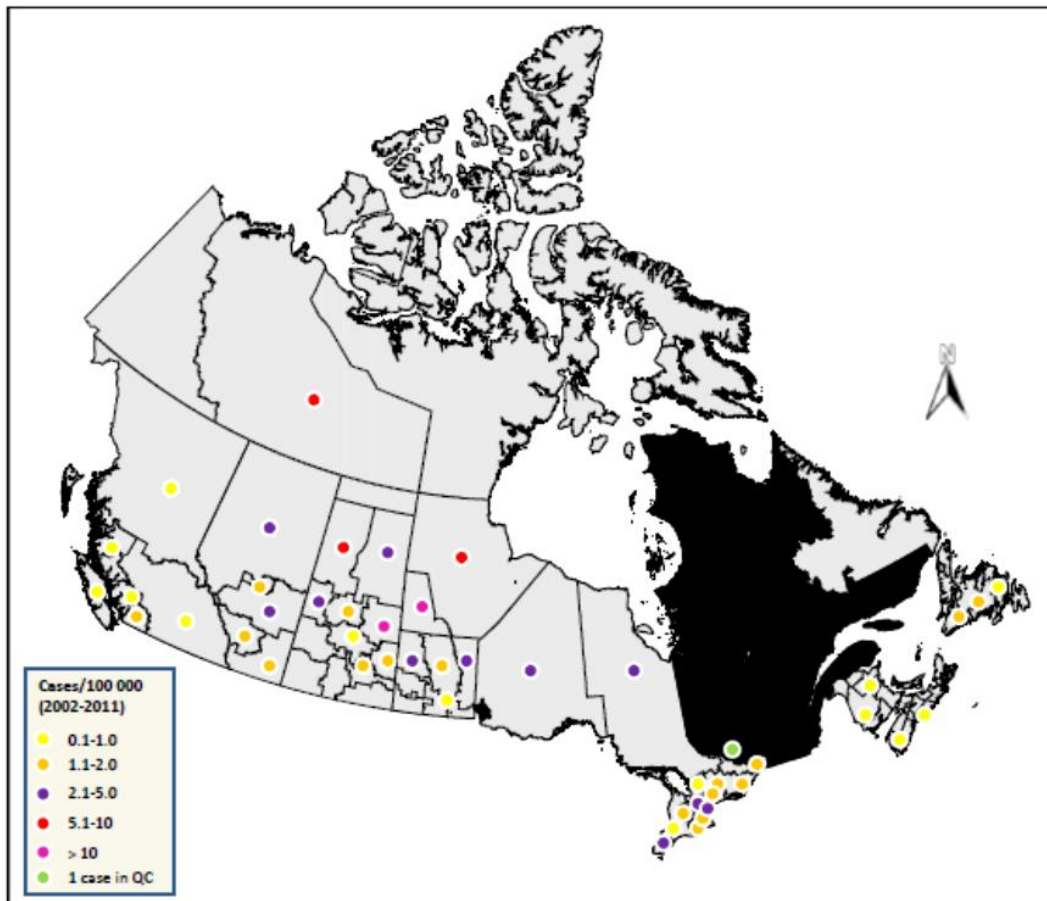


Figure 8-2: Total cumulative echinococcosis cases per 100 000 people for the years 2002-2011 reported by the Discharge Abstract Database and the National Ambulatory Care Reporting System and mapped by patient health region (except *QC).

Only 60% of case records reported cyst location within the body (188/315), and only 20% (64/315) reported *Echinococcus* species (CE versus AE; Table 8-3). For CE, the most commonly reported cyst location was lung, followed by liver, multiple sites, and bone; whereas liver and multiples sites were the most common descriptors for AE. Between 2002 and 2011, 2.3% of the 305 cases where discharge disposition was noted ended in fatality.

Table 8-3: Frequency of cyst locations and species of *Echinococcus* human cases in Canada (2002-2011).

ICD-10 code	Species	Cyst location	Frequency	% of cases
B67.0	<i>E. granulosus</i> ¹	Liver	16	5.1
B67.1	<i>E. granulosus</i>	Lung	21	6.7
B67.2	<i>E. granulosus</i>	Bone	2	0.6
B67.3	<i>E. granulosus</i>	Multiple sites	8	2.5
B67.4	<i>E. granulosus</i>	Unspecified site	1	0.3
B67.5	<i>E. multilocularis</i>	Liver	8	2.5
B67.6	<i>E. multilocularis</i>	Multiple sites	6	1.9
B67.7	<i>E. multilocularis</i>	Unspecified site	2	0.6
B67.8	<i>Echinococcus</i> ²	Liver	141	44.8
B67.9	<i>Echinococcus</i>	Unspecified site	110	34.9

¹ Presumably *E. canadensis* under new taxonomy

²Species unspecified

Economic evaluation

We based our model on the Kelsey Trail Health Region (SK), which had the highest CE incidence rate in Canada. The incremental cost-utility ratio (ICUR) for the base case was 185,484\$ per QALY gained. Even though the costs to treat someone with CE are quite high (10,668\$), the cost per person for ‘status quo’ was quite low, at 7.29\$ per person over the time horizon. The cost per person of the prevention program was also relatively low at 36.61\$ per person, signifying a small cost difference between the strategies at 29.31\$ per person. Therefore, the reasonably large ICUR for this prevention program is likely attributable to the very small utility from prevention compared to status quo (Incr. QALY= +0.0001582), with the outcome values in both strategies producing approximately 15.85 QALYs. The overall cost of running a prevention program in the Kelsey Trail Health Region, was approximately 71,327.93\$, which included total program costs (80,198.4\$) minus costs averted from not treating as many cases (8,8870.47\$; Table 8-4).

Table 8-4: Base case incremental cost per QALY and total costs (Can\$).

Program	Cost per year			Average costs	Average QALY	Incremental cost-utility ratio
	Total	Averted	Overall			
Status quo	10687.31		10687.31	7.2913	15.9379	
Prevention (PZQ)	80,198.4	8870.47	71327.93	36.6065	15.9381	
Incremental				+29.3152	+0.0001582	185, 284.07\$ per QALY

One-way sensitivity analysis demonstrated that none of the cost-utility ratios for any of the plausible variable ranges were under 80,000\$ per QALY. The best cost-utility ratio came at the high range of the plausible risk of CE (0.0000316 or incidence of 3.16 per 100,000) where the cost per QALY was approximately 82,258\$. The results also appeared to be sensitive to fatality rate, with an ICUR varying from 310,837.72-133,716\$ per QALY and discounting which, at a 0% discount rate, produced a cost per QALY of 90,487\$ (Table 8-5). Varying other data inputs did not significantly change the outcomes, most likely because the starting incidence was so low, thereby making other probabilities irrelevant. Sensitivity of the analysis to the risk of developing CE, prompted us to complete a threshold analysis to determine at what incidence the prevention program may be considered cost-effective. This analysis found at an incidence of 8 per 100,000 (risk=0.00008) the cost per QALY would be approximately 20,000\$, while at an incidence of 14 per 100,000 (risk=0.000014) the program would start becoming cost-saving (Table 8-5).

Table 8-5: Base case incremental cost per QALY and sensitivity analysis.

Variable	Cost per QALY (Can\$)
Base case	
Status quo vs. Prevention	185,294.07
Risk of disease	
Low (0.00000707)	208006.20
High (0.0000316)	82258.37
Threshold (0.00008)	20,000
Threshold (0.00014)	0
Fatality Rate	
Low (0.01)	310837.72
High (0.05)	133716.36
Discount	
3%	137506.49

Discussion

We report an echinococcosis incidence rate of 0.14 cases per 100 000 people annually, which is slightly higher than a previous Canadian estimate, likely because we included cases where echinococcosis was not the primary diagnosis. This is lower than CE incidence rates in other endemic countries including Spain, Portugal, Italy, Greece, and China; but is higher than countries such as New Zealand and Tasmania which are provisionally free following the success of control programs (1,23). As well, we believe that our incident rate under-estimates the true incidence as CE is not nationally notifiable, some cases were removed from analysis due to incomplete identifier information, and because up to 60% of CE cases are thought to be asymptomatic (1). Based on the best data currently available, we were not able to determine what proportion of cases were foreign-acquired; however, the universal nature of health care in Canada means that costs of treatment of foreign-acquired cases are still incurred.

Although the majority of case reports did not differentiate between CE and AE, highlighting another weakness in reporting, our findings suggest that most CE cases were likely to be

domestically-acquired. The highest incidence rates were in northern areas (Kelsey Trail and Keewatin Yatthe Health Regions [SK]; the territories [YK, NU and NT]; and Norman and Burntwood Regional Health Authorities [MB]) as opposed to regions where large metropolises are present. Of the top primary, secondary and tertiary immigration destinations in English-speaking Canada (Toronto, Vancouver, Calgary, Edmonton, Winnipeg, Hamilton, Ottawa, Saskatoon, Victoria, Regina, and Halifax), all had very low incidence rates (37). Sixteen individuals were diagnosed with AE over the ten year study period. In Canada, these are generally thought to be foreign-acquired, as no autochthonous cases have been reported in Canada since 1928 (38). However, six of these individuals resided in northern health regions (the territories [YK, NU, NT], North Zone [AB], Northeast Local Health Integration Network [ON], Northern Health [BC]), where immigration rates are low.

Our findings that echinococcosis was diagnosed most frequently in females and in older adults are supported by other Canadian studies (2,6). CE cases were most likely to have pulmonary or hepatic involvement, which is a common finding for the cervid strains in people, and these findings of gender, age and cyst tissue predilection are risk factors shared by wildlife cervid hosts (6,12,39). The highest frequency of CE cases occurred in low income neighborhoods but we were unable to determine if the proportion of cases relative to other income quintiles was significantly different. Low income individuals could be at higher risk of CE if they fed raw offal to pets and were unable to afford regular cestocidal dosing.

We report a CE treatment cost that is similar to that in the UK (10 215\$ USD), but far higher than that in other countries such as Jordan (524\$ USD) (22). Based on the base case analysis and one-way sensitivity analysis, the PZQ prevention program at an ICUR of 185,284\$ per QALY, is not cost-effective relative to other funded health care programs and current willingness to pay guidelines (31). Although the communities would have to incur costs for a few years while seeing little to no benefit, the overall costs of the PZQ strategy ($\approx 71,300\$$) are quite small in comparison to other prevention programs, such as rabies dog vaccination and HPV vaccination (40).

Several factors may impact the cost-utility and feasibility of the PZQ prevention program. First, veterinarians use PZQ to treat animals against a wide range of cestode species other than *E. canadensis*, including *Diphyllobothrium*, *Taenia*, *Dipylidium caninum*, and *Mesocestoides*, some of which can infect people and/or livestock. Other treatments to prevent zoonotic diseases in dogs, such as nematocides or rabies vaccination, could easily be added to the PZQ program infrastructure at a lower cost than administering all treatments separately. Second, the World Health Organization suggests the echinococcosis control go through multiple phases: 1) planning; 2) attack (costly and intensive control measures implemented); 3) consolidation (only high risk animals and people targeted); and 4) maintenance (1). While complete eradication is not feasible in Canada due to the presence of wildlife reservoir hosts, future prevention cost reductions are possible. For example, after echinococcosis rates in people and animals decreased, this low risk status could be maintained through cheaper methods (eg. education, less frequent owner-administered PZQ). Third, our threshold analysis demonstrated that an increased echinococcosis incidence could greatly impact the cost-utility of a prevention program. There is evidence that AE is expanding its range and emerging/re-emerging in many populations, both human and animal (41–44). In Canada, *E. multilocularis* has been documented in coyotes in several urban core areas, raising concerns of infection for domestic dogs, and possibly people (43). As well, dogs with European strain alveolar hydatid cysts have been documented in BC, AB, SK, and ON (45–47) (K. Gesy, pers comm.) demonstrating a widespread distribution for this emerging zoonosis. Range expansion of AE has been attributed to socio-economic factors, ecological disruptions, and potentially to climate changes, and is of particular concern as it generally results in worse health outcomes and significantly higher treatment costs than for CE (42,44,48). Fourth, this program would target often underserved and vulnerable populations that have poorer health outcomes, and therefore, the benefit of preventing disease in these risk groups may help to reduce health inequalities. Lastly, our results indicated that all YK, NU and NT patients travelled out of territory for treatment, which can be very expensive for the health care system. In NU, more than 25% of the operations budget is spent sending patients to southern referral centres to obtain care that is unavailable in the north, and this is a critical barrier to obtaining digital imaging results (required for CE diagnosis) in a timely manner (49).

Conclusions

Our study provides baseline human echinococcosis data at a time when European strain AE appears to be emerging in aberrant hosts across Canada and the risk of human exposure might be increasing. Improvements to echinococcosis surveillance could include developing serological tests that are optimal for Canadian strains (eg. *E. canadensis* G8 and G10), improved classification of echinococcosis by physicians (ie. to species level), increased awareness of echinococcosis among physicians in areas where this parasite is prevalent, and adding this parasite to the list of nationally notifiable pathogens. Improving surveillance would allow policy-makers and governments to make an informed decision about implementing a control program, with the knowledge that an increasing incidence greatly improves the cost-utility of an echinococcosis prevention program. Although PZQ dosing was not cost effective, it might still be warranted in high risk areas, especially as there are added benefits to people, pets and wildlife in controlling *Echinococcus* and other zoonotic cestodes. Other models using a less expensive control strategy over a smaller geographic area, and taking physician salary and indirect costs into account might indicate more favourable results for implementing CE prevention programs.

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CHAPTER 9 - CONCLUSIONS AND RECOMMENDATIONS

Prevalence and Geographic Distribution of *E. canadensis* in Canada

The work contained in this thesis found that infection with *E. canadensis* was more prevalent in wildlife (Chapters 2-3) than in domestic dogs (Chapter 4-5) or people (Chapters 6-8) in Saskatchewan. Since 1952, hydatid cysts have been reported in ungulates nationwide, except for the Maritime provinces and the high Arctic islands (1). Human echinococcosis cases have been reported from every province and territory with the highest and lowest incidence observed in the northern territories (NT, YK, NU) and Maritime provinces (NS, PE, NB), respectively. The national incidence rate for echinococcosis is 1.4 cases per 1 million per year; however, this is most likely an underestimate as this infection is both under-diagnosed and under-reported. Historic reports of *E. canadensis* infection in canids are less trustworthy, as adult cestodes are difficult to differentiate from those of *E. multilocularis*, and because different Taeniidae species shed in eggs canid feces that are morphologically identical. Molecular confirmation of *E. canadensis* (G8 and G10 genotypes) first occurred for elk and moose in 2006, and has since been reported in wild canids, stray dogs and other ungulates, but no hydatid material from people in Canada has ever been characterized (1–5). These reports show two distinct genotypes (G8 and G10) of *E. canadensis*, co-existing in sympatric distributions. To date, G8 has been observed in a muskoxen (NU), wolves (BC, AB, SK, and NT) and elk (AB); G10 has been observed in elk (AB, MB), moose (MB), caribou (QC), and wolves (BC, AB, SK, MB, NT, YK) (1,2,4,5). Co-infections of G8 and G10 have been observed in wolves from one province (SK) and one territory (NT), and adult cestodes are morphologically distinct (5,6). The work of this thesis (Chapters 2-3) found that G10 was more prevalent, and more widely distributed in ungulates and wild canids than G8. Although taxonomic revisions of the *Echinococcus granulosus* species complex are contentious, our evidence of sympatrically distributed genotypes supports the inclusion of species names *E. borealis* and *E. canadensis* to describe G8 and G10, respectively.

Prevalence and Geographic Distribution of Other Endoparasites in People and Animals in Western Canada

Our post-mortem examination of wolves from SK, MB and NT indicated that approximately half (56% of 165) were infected with one or more helminths. Cestode infection occurred most frequently (*Taenia*, *Echinococcus*, and *Diphyllobothrium*), followed by nematodes (*Toxascaris*

leonina and *Uncinaria stenocephala*) and flukes (*Alaria*). Our report of *E. multilocularis* in wolves was a new host record for this parasite. Presence of endoparasite ova in client-owned dogs and environmentally collected canine fecal samples varied greatly between communities in SK (20-83%), but was higher in northern, rural, and remote communities than urban population centres (7–12). Other high risk populations for endoparasitism included dogs surrendered for adoption and juveniles (<1 year old) (10). Overall, ascarid nematodes (*Toxocara canis* and *T. leonina*) and protozoa (*Giardia* and *Cryptosporidium*) were most prevalent in the dog study populations. Other parasites identified less frequently included *Uncinaria*, *Diphyllobothrium*, *Isospora*, *Sarcocystis*, *Alaria*, *Metorchis*, *Trichuris*, *Taenia*, and *Dioctophyma*. Approximately 19-21% of dog serum collected in three rural SK communities showed evidence of exposure to *Toxoplasma gondii*, which is lower than the canine sero-prevalence reported in AB, ON or NT (33-63%) (9,11–14). Each sampling site had a unique parasite profile, with varying parasite richness and prevalence of parasite species. These results indicate that dogs and wolves feed opportunistically on a wide array of terrestrial and marine wildlife, and that there are opportunities for sylvatic and domestic life cycles to overlap and amplify zoonoses.

Our assessment of human exposure to *T. gondii*, *Toxocara canis*, and *Trichinella* indicated that northern remote residents were exposed more often than southern rural residents in SK. Risk factors significantly correlated to parasite exposure in one community were not significantly correlated in the other community except for young age (<18 years), which was a risk factor for *Toxocara* exposure shared in both communities. It remains unclear why exposure levels were different in the two communities, but possible explanations include differences in food preparation, access to specific game species, knowledge of sanitation, and cultural practices. Similar to our work in Chapter 8, we obtained patient records (CIHI case reports) for individuals diagnosed with toxoplasmosis or toxocariasis between 2002 and 2011, and found that both conditions were rare in Canada (1.7 and 0.06 cases per 1 million persons per year, respectively). Human Immunodeficiency Virus (HIV) was a co-infection in 40% (203/523) of toxoplasmosis cases, suggesting that efforts to increase awareness of *T. gondii* transmission routes in immune-compromised individuals may be appropriate (Schurer *et al.*, unpublished data).

Limitations and Future Directions for *E. canadensis* Surveillance

Detection of *E. canadensis* in wildlife and domestic dogs is challenging, in part because infected hosts are often asymptomatic, and also because the tools required for definitive diagnosis are expensive or unavailable in remote regions (medical imaging or molecular methods). Post-mortem examination of intestinal contents is the most sensitive detection method for canids, but can be highly time consuming even for skilled technicians, and is unfeasible for domestic dogs ante-mortem (15). Fecal analysis is more cost-effective, but less sensitive because *E. canadensis* cestodes shed eggs sporadically, or may be pre-patent at the time of testing. As well, DNA must be extracted from taeniid eggs collected from feces in order to identify the species and/or genotypes, adding to the cost. Fecal analysis does provide important information regarding the level of environmental contamination by parasite ova. Coproantigen-ELISA testing is another option for *Echinococcus* detection in canid feces, and is steadily replacing arecoline purgation as a highly sensitive screening tool in *Echinococcus* control programs (15). Coproantigen-ELISA tests are now commercially available in Europe; however, further work is needed to determine the sensitivity of these tests for *E. canadensis* (G8 and G10 genotypes), distinct from other *Echinococcus* species and genotypes (15).

Diagnosis of cervid-strain CE in people is challenging for similar reasons. Infected individuals generally remain asymptomatic for many years or exhibit non-specific symptoms that are difficult to characterize. As well, infection may resolve without intervention, or may remain undetected, especially in remote locations where access to medical care is difficult. Currently, echinococcosis is not reportable at the national level, and prevalence estimates are limited to small community-based studies (3,9,11,16–22). The serological tests used in these studies are minimally invasive and less expensive than medical imaging, but only measure exposure to *E. canadensis*, and not actual infection. Cross-reactions (false positives) can occur when test subjects are infected with cestodes closely related to *E. canadensis*. Authors of these studies used different surveys to measure risk factors for exposure, and none reported follow-up testing results (ie. medical imaging) for sero-positive participants. As a result, there are disagreements in the literature regarding risk factors for echinococcosis. Most of these sero-surveillance projects are not correlated to *Echinococcus* surveillance in dogs or local canid populations, and therefore the source of human exposure is often unclear. Worldwide, dogs are considered the

most important source of human infection; however, we did not find dogs infected by *E. canadensis* at any of our study sites. Likewise, our regression models failed to identify dog ownership as a risk factor for infection, which could indicate low infection prevalence in dogs, or that free-roaming dogs spread eggs throughout the townsites, exposing survey respondents equally. We found wolves infected by *E. canadensis* at all study sites in SK, MB, and NWT suggesting that wild canids may be a more important source of *E. canadensis* infection for people than dogs. Future risk assessments should examine how people interact with wild canids and determine levels of soil contamination to resolve these questions.

The Canadian Institute of Health Information has collected CE hospitalization data since 1994 (23); however due to strict privacy laws, risk factor analysis is limited to a subset of information reported in patient medical records. During our analysis of CIHI data, we noted several issues that contribute to CE under-reporting in Canada. For example, CIHI reports case records as opposed to infected individuals, and when critical data such as individual health number and geographic location are missing, cases are deleted because they cannot be verified as unique. Some information, such as income and urban or rural residence, is based on postal codes and may exclude transient and homeless populations. Because case records provide all diagnostic ICD-10 codes at the time of examination, it is difficult to differentiate those undergoing treatment for CE from those being treated for a co-existing health condition. As well, physicians rarely differentiate between CE and AE in their diagnosis, or describe cyst location. This is a concern because eight cases of AE as an aberrant presentation in dogs have appeared in five provinces over the last five years, suggesting that highly virulent European strains of *E. multilocularis* with less intermediate host specificity are emerging in Canada (24–26; Gesy and Peregrine, unpublished data). With the current echinococcosis reporting flaws and lack of physician awareness with regards to differentiating AE from CE, it may be difficult to detect increases in AE human incidence. In addition, case records do not include travel or immigration history making it difficult to differentiate foreign from domestically acquired infections, in particular because echinococcosis has a long latency period. Moving forward, our understanding of CE risk factors and the location of high risk communities will be greatly enhanced by follow-up of sero-positive participants of surveillance studies, and by improved physician reporting. As well, hydatid cysts removed from people should be characterized genetically to determine

infection origin as well as differences in pathogenicity between species and genotypes of *Echinococcus*. This may prove challenging, as Canadian physicians often opt to follow a “watch and wait” approach in treating echinococcosis.

Parasite Control Strategies

Despite the fact that people can infect and be infected by their pets, strategies for parasite control in people and animals continue to focus on parasite treatment, rather than prevention (27,28). In the veterinary industry, this is likely because practitioner income is based on billed services and pharmaceutical sales. As well, there are few publically funded programs in operation that support veterinary public health services for companion animals. However, human activities often contribute to parasitic infections in animals, and education is an important tool for decreasing exposure. Research in developed and developing countries show the same results - pet owners have varying knowledge of parasites, and of transmission routes for zoonotic transmission (28,29). Although veterinarians are a trusted source of information, the widespread success of anthelmintics in decreasing companion animal parasitism has caused some practitioners to become complacent about client education (28). Likewise, some clients might become non-compliant with what they perceive as unnecessary, costly, and possibly deleterious treatments. Unfortunately, information provided to clients regarding parasite transmission is often incorrect, insufficient, or outdated, and deworming recommendations frequently do not match best practice (28–30). Our surveillance of companion animal shelters in Canada suggested that parasite control strategies were often developed with budget as a primary consideration (31). Pharmaceuticals were frequently used extra-label, drugs targeting cestodes and ecto-parasites were only distributed to a subset of animals, and fecal diagnostic testing was conducted only as needed (31).

Ironically, those who own pets may not be at highest risk of zoonotic parasitism, which could suggest increased awareness regarding the zoonotic potential of parasites. Dog ownership is not a risk factor for *Echinococcus* exposure in Canada, and because free-roaming dogs deposit feces haphazardly around human settlements, any person could be at risk of contact with infective eggs in the environment (9,11,20,22). Therefore, *Echinococcus* control measures require a One Health approach where interventions are provided at the community level rather than the

household level. This is supported by our surveys of northern and rural dog owners who reported that deworming dogs occurred rarely (if ever), even when veterinary services were nearby, and that cost was a primary barrier (11,12). Successfully eliminating *Echinococcus* from an area requires a highly organized, long-term, and inclusive community-wide approach that educates residents about transmission routes and dog population control, and legislates appropriate canine deworming (15,32). In particular, this includes educating pet owners about the risks associated with feeding their animals raw meat (Figure 1-1), and encouraging hunters to process carcasses ‘on the land’, where community dogs are unlikely to access offal. In communities where human echinococcosis incidence rates are high, surveillance of dogs and wild canids is needed to identify sources of infection. If dogs are shedding eggs, cestocides should be provided to all dog owners through subsidized veterinary public health programs to ensure that all animals are treated at 6 week intervals, regardless of an owner’s socioeconomic status. When wild canids are infected, control is far more challenging and may require praziquantel baiting or depopulation of canids in the surrounding areas. Although echinococcosis is currently considered a rare disease in Canada (33), our analysis indicates that a small increase in annual incidence, especially in a small community, would result in the *Echinococcus* control program being cost-saving. The cost to deworm dogs is low relative to the direct and indirect costs of treating human echinococcosis, not to mention the cost of human suffering and loss of productivity associated with morbidity and mortality (34). Additional benefits can be achieved by employing broad-spectrum anthelmintics that remove a wide array of parasites (eg. *T. canis*) that cause adverse health conditions in both dogs and people. Other preventative health measures, such as rabies lay vaccinator programs, can easily be added as needed, capitalizing on the infrastructure created by the deworming program (and vice-versa). Because echinococcosis, toxocariasis, and other zoonotic parasites occur more frequently in underserved populations (35,36), control programs should be considered even when the cost is not favourable from a public payer perspective.

Challenges and Recommendations for Community-Based Research

During my brief time working with rural and remote Indigenous communities I experienced or observed a variety of obstacles to producing high quality, ethically sound, and ultimately useful data. Despite recommendations set forward by the OCAP principles (37), research continues to

be driven largely by researchers, whose interest and expertise do not always align with community priorities. Researchers do not always communicate well with each other, and as a result, separate groups can work simultaneously in the same community on different projects without awareness of each others' presence. This is a lost opportunity for conducting higher level integrated data analysis, and contributes to the current paradigm of reductionist and “patchwork” research, as opposed to approaching health issues holistically.

Many rural and remote communities have limited resources for conducting self-driven research, and collaborations with outside investigators are key to addressing genuine problems that the community wants to address. However, academically based researchers often have different expectations relating to timelines, competing priorities, budgeting, data ownership, and privacy than community residents. Outsiders may be less aware of cultural sensitivities, experience difficulties identifying key stakeholders, and struggle to communicate with community members (especially if telecommunication services are limited, communities are inaccessible for parts of the year, or language barriers are present). Frequent changes to leadership administration and unaligned health priorities between leadership and residents can result in time set-backs and lost support.

Participatory research is now a gold standard for data collection involving Indigenous peoples, rural and remote communities, and groups formed by common interests (eg. occupation, disease status, age, sexual orientation, economic status, etc.) (38,39). It requires that investigators and community members collaborate on all aspects of research, including project planning, data collection and analysis, data interpretation, and communicating results to laypersons and experts. It also involves sharing risks (eg. project failure, costs), rewards (eg. financial profit, positive media attention), and responsibilities between participants. Participatory research is an important tool for achieving social justice in underserved and marginalized communities for two reasons: 1) it empowers participants to initiate positive change, and 2) results can be used to lobby policy makers for improved rights and services (37,39). Empowering communities to study and solve their own health problems is a critical element to improving overall health promotion and disease prevention (38). With zoonoses, taking a One Health approach allows us to go “upstream” of human cases to exercise prevention in animal and environmental reservoirs.

The following are my recommendations for improving participatory and integrated One Health research activities in those remote and/or Indigenous communities where veterinary and medical services are limited or do not exist.

1) Improve local capacity by involving local residents in research activities

Community residents, who may lack formal higher level education, are capable of collecting many types of data, and can be trained outside the school setting to become highly qualified technicians. This type of skills training increases local capacity for initiating self-driven research in the future, promotes equitable relationships through employment, and provides opportunities for long-term sample collection. As well, locals involved in research planning, data collection, and analysis are important allies for translating research goals and outcomes to community members (40,41). Residents who become involved in research activities during the initial planning stages provide critical information for culturally appropriate and logistically feasible sampling, have a vested interest in supporting research activities, and experience research successes first hand (40,41). Such individuals might be more likely to initiate or participate in research activities more readily when future questions arise.

2) Integrate preventative human and animal health services

Currently, medical and veterinary health services are delivered completely separately. Many veterinarians and physicians feel unqualified to discuss routes of zoonotic transmission, and as a result, the public is often left with erroneous information distributed by the mass media (28,30,42). In SK, permanent veterinary services are absent in the northern half of the province (north of Meadow Lake), and pet owners have no access to preventative pet care and little recourse when a pet is injured. As well, some remote communities have primarily free-roaming dog populations, in which dogs may have no owners, or ownership changes frequently. Anecdotal reports of nurses stitching up injured pets in their spare time demonstrate that human health workers are willing to stretch the confines of their formal job description to provide basic first aid to animals. Logistically, northern health regions could employ a veterinarian or animal health technician with public health training to complement the skills of human healthcare staff, or nurse practitioners could provide very basic preventative health services (eg. deworming, vaccination) to companion animals under the

remote supervision of a veterinarian. As Telehealth and other remote health services are becoming commonplace in northern Canada, the latter could capitalize on pre-existing infrastructure (43).

3) Provide legal and logistical support for veterinary paraprofessionals

Northern SK should look to other remote regions in the world where farmers have successfully introduced veterinary paraprofessionals, also known as Community-based Animal Health Workers (CAHWs), to solve veterinary service shortages (44). These CAHWs are trained by veterinarians to collect and report disease surveillance data, to educate animal owners, and to perform primary level tasks such as vaccination and deworming (44). Contrary to popular belief, they do not take business away from practicing veterinarians, but often increase demand for services by improving trust, and demonstrating the value of owning healthy animals. Frequently, they work in areas where vets are reticent to practice, due to political instability, insufficient client base and income, physical separation, or because the client-base is nomadic (44). In northern SK, Environmental Health Officers (EHOs), public health officials, or local residents could be trained to handle animals safely and perform community level vaccination and deworming programs for owned and free-roaming dogs, thus improving health for both people and animals. Barriers include privatized veterinary services, and legislation prohibiting unqualified workers from practicing veterinary medicine. These could be easily overcome, as the NT already permits non-veterinarians, who are trained in the technique by a veterinarian, to administer rabies vaccines to dogs, and because CAHW programming could be limited to areas where veterinarians already choose not to practice (45). In northern SK where employment options can be scarce (46), introducing CAHW training might be readily accepted, as it offers opportunities for year-round employment, mentorship, income generation without leaving the community, and involvement in activities that promote health and wellbeing.

4) Conduct human and animal disease surveillance simultaneously

An additional advantage to integrating human and animal health services is that disease incidence changes in either population can be detected and communicated among health workers. In essence, both people and animals become disease sentinels for each other,

depending on whether symptoms are more readily detected in one population or the other. As well, this allows management of zoonoses to become preventative rather than reactionary, fast-tracks collaboration between human and animal health professionals, and eliminates issues pertaining to jurisdiction and responsibility.

5) Promote interdisciplinary collaborations

Increasingly, funding institutions promote research teams that collaborate across disciplines (47–49). The extent to which these teams collaborate can be viewed on a continuum. Multidisciplinary collaborations, simply termed, are additive. Researchers from different disciplines study problems sequentially or with different goals, and with minimal interaction. Further along the spectrum, interdisciplinary research is interactive, involves shared goals, and generates new perspectives and disciplines of study (eg. bio-informatics, ecophilosophy) (47). Lastly, transdisciplinary collaborations cross the traditional boundaries of multiple disciplines to study complex problems with a dynamic and holistic approach (47). Specialists within the team work simultaneously, cohesively, and towards common goals, often teaching each other skills associated with their expertise and contributing to role expansion (47).

It is not unusual for a principal investigator to work on a project until his or her scope of expertise is exhausted, and then impart their findings in the literature or pass the project along to another group. In a community-based setting, this approach is inefficient because start-up efforts (eg. building trust, identifying key stakeholders, gaining familiarity with cultural norms) are time intensive and costly. This approach does not utilize the vast amount of qualitative or anecdotal information that a researcher might unconsciously obtain. To combat this segmented approach, community-based research should be transdisciplinary, and include front-line practitioners, community members, laboratory experts, and policy makers from start to finish. Multiple disciplines should be represented, including social science, anthropology, veterinary and human medicine, environmental science, economy, and public policy. Such teams are best equipped to study whole systems, and are able to assess perceived healthcare issues, identify cultural or community barriers, conduct surveillance, propose interventions, and assess attitudes towards and uptake of the pilot

intervention. They are also efficient because research designs and interventions that lack logistic feasibility, are too costly, or clash with local attitudes can immediately be discarded. Lastly, multiple disciplinary teams may have greater success in identifying common funding incentives, and maintaining long-term community presence.

Our research team experienced the benefits of transdisciplinary collaboration firsthand in a community where pet sterilization clinics were held following a fatal dog attack (12). The team included EHOs, nurses, veterinarians, community elders, a not-for-profit charitable organization, and an expert in policy and environmental sustainability. Through door-to-door visits and storytelling circles, we learned that rural dog populations impacted community health negatively when they were too large (eg. due to dog-related injuries, fear for disease transmission) or when they were too small (fear of wildlife, loneliness, lack of security). The impact was positive when dog populations were within a healthy range because residents participated in outdoor physical activities with less fear of dog packs or roaming wildlife, and because people felt more connected to their pets (Schurer *et al*, in prep). This information suggests that dog culls, which are a common reaction to dog-related injury, do not promote long-term health or wellness. From a funding perspective, those interested in sustainable dog population management could find common ground with those working on the growing prevalence of lifestyle diseases (obesity, diabetes) (50).

Just as the concept of One Health is not new, many of the ideas outlined above have been implemented in different countries and at different times. For transdisciplinary One Health research to reach its full potential, academics and large scale funding agencies will need to modify expectations with respect to productivity, timelines, data ownership, teamwork, and acknowledgement of success. Otherwise, principal investigators will have little motivation to step outside the comfort of their expertise, and take on the risks associated with highly collaborative teamwork. My own experiences in transdisciplinary work have been vastly educational, engaging, and ultimately contributed to a broad network of collaborators. This approach has also allowed me to understand syndemics in a real world context – by meeting those affected by unfavourable social determinants of health, as well as those working to alleviate human and animal healthcare issues at the individual and community levels. I strongly

believe that research would more often lead to successful healthcare interventions if scientists stayed involved from the benchtop to the policy level. I hope that the push for community-led, transdisciplinary, and conservation-focused research continues to grow as the world is faced with ever-faster disease emergence at the human-animal interface.

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2 messages

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Schurer J, Momar N, Quezenance H, Elmore S, Jenkins E. People, pets, and parasites: One Health surveillance in southeaster Saskatchewan. *Am J Trop Med Hyg.* 2014. In press.

Kind regards,

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Cathi Siegel <cbs15@case.edu>

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Chapter 5 Permissions

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Animal Research Ethics Board Certificates



Animal Research Ethics Board
Certificate of Approval

PRINCIPAL INVESTIGATOR
Dr. Emily Jenkins

DEPARTMENT/ORGANIZATION
Veterinary Microbiology

ANIMAL USE PROTOCOL #
20100159

TITLE
Seroepidemiology of parasites in domestic carnivores

SPONSORING AGENCIES
Saskatchewan Health Research Foundation (SHRF), Natural Sciences & Engineering Research Council of Canada (NSERC)

BIO SAFETY NUMBER
VMB-12

UNIFI FUND #
441316

APPROVAL DATE:
February 20, 2014

APPROVAL OF:
Renewal Animal Use Protocol

EXPIRY DATE:
February 28, 2015

Full Board Meeting

AREB Subcommittee

AREB Chair and
University Veterinarian

AREB Chair

CERTIFICATION

The University of Saskatchewan Animal Research Ethics Board reviewed the above-named research project. The proposal was found to be acceptable on ethical grounds. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this research project, and for ensuring that the authorized research is carried out according to the conditions outlined in the original protocol submitted for ethics review. This Certificate of Approval is valid for the above time period.

PROTOCOL MODIFICATIONS

Any modifications to this protocol must be approved by the UGACS AREB Chair prior to implementation, using the [AUE Modification Form](#).

ONGOING REVIEW REQUIREMENTS

Research programs that extend beyond one year must receive annual review. For the annual renewal, an annual review form (and progress report) must be submitted to the AREB within one month of the current expiry date each year the study remains open, and upon study completion. Please refer to the [Research Ethics Office website](#) for further instructions.

Michael Corcoran, Chair
Animal Research Ethics Board
University of Saskatchewan

February 21, 2014
Date Issued

Please send all correspondence to:

Research Ethics Office
University of Saskatchewan
Box 5000 RPO University, 1607-110 Gymnasium Place
Saskatoon SK S7N 4J6
Telephone: (306) 966-7126 Fax: (306) 966-2069 Email: ugacs.ethics@usask.ca



Animal Research Ethics Board
Certificate of Approval

PRINCIPAL INVESTIGATOR
Dr. Emily Jenkins

DEPARTMENT/ORGANIZATION
Veterinary Microbiology

ANIMAL USE PROTOCOL #
20090126

TITLE

Diagnostic tools for understanding the ecology of hydatid disease and other parasitic zoonoses in indigenous communities in north-central Saskatchewan

SPONSORING AGENCIES

Not Required

BIO SAFETY NUMBER
VMB-12

UNIFI FUND #

APPROVAL DATE:
July 21, 2014

APPROVAL OF:
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EXPIRY DATE:
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AREB Chair and
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Michael Corcoran, Chair
Animal Research Ethics Board
University of Saskatchewan

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Biomedical Research Ethics Board (Bio-REB)

Certificate of Approval

PRINCIPAL INVESTIGATOR: Emily Jenkins
DEPARTMENT: Veterinary Microbiology
Bio #: 11-07

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT
University of Saskatchewan
Saskatoon SK

SUB-INVESTIGATOR(S)
Stuart Skinner

STUDENT RESEARCHERS
Janna Schurer

SPONSORING AGENCIES
SASKATCHEWAN HEALTH RESEARCH FOUNDATION (SHRF)

TITLE
Parasite Surveillance in Northern Aboriginal Communities

ORIGINAL REVIEW DATE	APPROVED ON	APPROVAL OF	EXPIRY DATE
28-Feb-2011	08-Mar-2011	Researcher's Summary (05-Jan-2011) Information and Consent Form (04-Mar-2011) Questionnaire Acknowledgement of: Community consultation has been/will be undertaken	08-Mar-2012

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The study is acceptable on scientific and ethical grounds. The Bio-REB considered the requirements of section 29 under the Health Information Protection Act (HIPA) and is satisfied that this study meets the privacy considerations outlined therein. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this research study, and for ensuring that the authorized research is carried out according to governing law. This approval is valid for the specified period provided there is no change to the approved protocol or consent process.

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PRINCIPAL INVESTIGATOR Emily Jenkins	DEPARTMENT Veterinary Microbiology	Bio # 13-51
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Marwa Farag

STUDENT RESEARCHER(S)
Janna Schurer

FUNDER(S)
NATURAL SCIENCES & ENGINEERING RESEARCH
COUNCIL OF CANADA (NSERC)

TITLE
An Economic Assessment of Disease Burden Due to Parasitic Zoonoses (Echinococcosis, Toxoplasmosis, and Toxocariasis) in Canada

ORIGINAL REVIEW DATE 04-Mar-2013	APPROVED ON 12-Mar-2013	APPROVAL OF Research project as outlined in the application to access existing health data for research Part D: Data elements required for the research project	EXPIRY DATE 11-Mar-2014
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In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Part 4 of the Natural Health Products Regulations and Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. Members of the Bio-REB who are named as

Please send all correspondence to

Research Ethics Office
University of Saskatchewan
Box 5000 RPO University
1607 - 110 Gymnasium Place
Saskatoon, SK Canada S7N 4J8



Certificate of Completion

This document certifies that

Janna Schurer

*has completed the Tri-Council Policy Statement:
Ethical Conduct for Research Involving Humans
Course on Research Ethics (TCPS 2: CORE)*

Date of Issue: 6 June, 2014

Appendix C – Human Serology Surveys and Consent

INFORMATION AND CONSENT

Title of Study: "Parasite surveillance in northern Aboriginal communities"

Investigator:

Dr. Emily Jenkins, University of Saskatchewan, Phone: 306-966-2569

Sub-investigators:

Dr. Stuart Skinner, University of Saskatchewan, Phone: 306-655-1785

Janna Schurer, University of Saskatchewan, Phone: 306-966-7213

INTRODUCTION

This study aims to look at the amount of disease due to parasites transmissible between animals and people in northern and indigenous communities in Saskatchewan.

Your participation is entirely voluntary. It is up to you to decide whether or not to take part in this study. Before you decide, it is important for you to understand what the research involves. This consent form will tell you about the study, why the research is being done and the possible benefits, risks and discomforts.

If you wish to participate, you will be asked to sign this form. If you decide to take part in this study, you are still free to withdraw at any time and without giving any reasons for your decision. If you do not wish to participate, you do not have to provide any reason for your decision. You will not lose the benefit of any medical care to which you are entitled or are presently receiving.

Please read this form carefully and feel free to discuss it with your family, friends, nurse, or doctor before you decide.

NATURE AND PURPOSE OF STUDY

Parasites are known to be present in dogs and people in some northern and indigenous communities in Saskatchewan. The purpose of this study is to determine how much, if any, infection is present in the community in both humans and dogs. If there is infection we would like to determine how much is present and determine what puts people at risk. With this information, we can help provide advice on how to avoid becoming infected, and if necessary, on how to treat infections.

PROCEDURES TO BE FOLLOWED

If you agree to participate, then you will be asked to give one blood sample (about 5mL = 1 teaspoon) to determine if you have been exposed to parasitic infection. You have the option to submit a stool sample that would be tested for active parasitic infections (ie. eggs and cysts of common gastrointestinal parasites). You will also be asked to fill out a questionnaire asking about any health concerns that may be caused by parasites and activities that may put you in contact with them. It will require about 20 minutes to complete the questionnaire and to have your blood taken.

You will be informed of the results of your test by mail. The results will either inform you that you have been exposed to parasites (a positive test) or not exposed to parasites (a negative test). An accompanying letter will advise you whether or not you should discuss your results with your health care provider (family doctor or nurse practitioner). For some positive test results, public health will be notified. The test results will also be provided to the primary care physician or nurse practitioner who you identify, if you choose. Your primary care physician can provide follow up on positive results with further examination and/ or treatment. This may include ultrasound, x-rays, eye exams, or further blood or stool sampling.

DISCOMFORTS AND RISKS

There are no significant risks associated with this study. You may experience some temporary discomfort when the blood sample is taken. There is a small risk of bruising, infection or

swelling at the site where the needle is inserted; and some minor discomfort. Some people may feel faint and dizzy. If providing a stool sample, ensure that you wash your hands well after handling.

POTENTIAL BENEFITS

The study can tell us if you have been exposed to parasites, and a doctor can provide you with treatment, if necessary. As well, we hope to determine what puts people at risk for these infections so we can help keep people from being infected.

COST/REIMBURSEMENT

You will not be charged for the study or any research-related procedures. You will receive a gift card for participating in this study if you provide blood and/or stool samples and complete the questionnaire. In order to receive a gift card for themselves and/or their children, adults will need to provide a social insurance number.

WITHDRAWAL FROM THE STUDY

Participation in this study is voluntary and you can withdraw from the study at any time. You do not have to answer any of the questions on the questionnaire that you do not want to.

CONFIDENTIALITY

While absolute confidentiality cannot be guaranteed, every effort will be made to ensure that the information you provide for this study is kept entirely confidential. Your name will not be attached to any information, including your blood test, nor mentioned in any study report, nor be made available to anyone except the research team.

If you test positive, you will be informed by mail and you will be the only one who will be told of the test result, unless you indicate that you would like us to notify your physician/health care provider.

It is the intention of the research team to publish results of this research in scientific journals and to present the findings at related conferences and workshops, but your identity will not be revealed.

QUESTIONS

If you have any questions or concerns about this study, you should contact the investigators at the above phone numbers.

If you have any questions regarding your rights as a research subject or concerns about your experiences while participating in this study, you should contact the Chair of the Biomedical Research Ethics Board (Bio-REB), c/o, University of Saskatchewan at 306-966-4053.

STATEMENT OF SUBJECT

I have read, or had read to me, the above information before signing this consent form. The nature and procedures of the study have been fully explained to me. I understand the benefits and risks involved in taking part in this research study.

I have had time and opportunity to ask questions and all my questions have been answered. I have read all pages of this consent form and the risks described. If I do not participate or if I discontinue my participation in this study I will not be penalized, my future medical care will not be affected and I will not give up my legal rights. The issue of confidentiality has been explained to me and I understand who will have access to my medical records. I volunteer to take part in this study.

I understand I will receive a signed copy of this consent form.

Please check if you would like your healthcare provider to be informed of your results

Print name of subject

Name of healthcare provider

Signature of subject

Date (dated by subject)

Printed name of person explaining consent

Signature of person explaining consent

Date (dated by person explaining consent)

Mailing Address of Subject

Please initial if you have received an honorarium for your participation.

REB 11-07 Survey for Human Blood Collection clinics

1) What is your gender?

- a) Male b) Female

2) What is your age?

- a) 4-10 yrs b) 11-17 yrs c) 18-35 yrs d) 36-50 yrs e) 51-65 yrs f) >65 yrs

Do you have children under the age of 18 donating blood today?

Gender and ages of children: _____

3) How many dogs does your house have? a) 1 b) 2 c) 3 d) 4 e) 5 f) >5

4) How many cats does your house have? a) 1 b) 2 c) 3 d) 4 e) 5 f) >5

5) Have your animals ever been vaccinated (eg. rabies)? a) Yes b) No

6) Have your pet(s) ever been dewormed? a) Yes b) No

7) Do you regularly seek veterinary services for your pet(s)? a) Yes b) No

a. If no: why not? a) Cost b) Distance to vet clinic c) Not necessary d) Other (explain)

8) Are your pets spay/neutered? a) Yes b) No

9) Would you allow your pets to be spay/neutered? a) Yes b) No

a. If yes: would you be willing to pay for this service? a) Yes b) No

b. If no: why not? a) Cost b) Other (please explain_____)

- 10) Have you ever been bitten by a dog? a) Yes b) No
 If yes:
 a. How many times have you been bitten? a) 1x b) 2-3x c) >3 times
 b. For the most recent bite, was the dog your own, stray or somebody else's dog?
- 11) Do dogs cause problems in this community? a) Yes b) No
 a. If yes: what problems? _____)
- 12) How many times do you feed your pets per day?
 a) 1x b) 2x c) 3 d) >3x e) Other (explain)
- 13) What do you feed your pet(s)?
 a) Commercial mix b) Leftover food, hunted meat/fish) c) Other (please explain)
- 14) Do you ever feed your pet(s) raw meat or fish? a) Yes b) No
- 15) Do your pets live:
 a. Indoors always b. Outdoors always c. Both indoors and outdoors
- 16) When outside, are your pet(s):
 a) Always kept in an enclosed area (eg. chained or in a fenced yard)
 b) Sometimes free-roaming (less than two times per week)
 c) Always free-roaming
- 17) Do you hunt or trap? a) Yes b) No
 If yes, where do you dispose of the inedible portions?
 a) On the land b) At the dump c) Feed to pet(s) d) Other (please explain)
- 18) Do you skin or butcher animals? a) Yes b) No

19) Do you eat wild caught meat?

a) Yes b) No

If yes, how? (Circle all that apply)

a. Raw

b. Dried

c. Smoked

d. Cooked

e. Other (please describe) _____

20) Do you eat wild caught fish?

a) Yes b) No

If yes, how? (Circle all that apply)

a. Raw

b. Dried

c. Smoked

d. Cooked

e. Other (please describe) _____

Appendix D – Addenda to Previously Published Paper in Chapter 7

We initially used Pearson's chi-square test to determine if there were significant differences in dog exposure to *Toxoplasma gondii* between 2011 and 2012, and found that sero-prevalence differed at the 5% level ($P=0.042$). A more appropriate test would have been Fisher's exact test, which is optimized to detect prevalence differences when sample sizes are small. When applied to this dataset, this test yielded a non-significant result ($P=0.058$). In this case, it is appropriate to report the pooled estimate of dog sero-prevalence to *T. gondii* over the two years (21%, 16/78).

We reported a multivariate regression model for *Toxocara canis*, which included both age and history of dogbites as independent variables. This model was a better predictor of outcome (Likelihood Ratio Test: X^2 [df=1, alpha=0.05] = 13.261) than a model with age alone; however, the survey questions for dogbite history were not as reliably answered as age. As a result, we believe the model with age alone may better predict *T. canis* exposure status, and we report an odds ratio of 11 (95% CL: 1.2-105). This is also consistent with previous reports of young age as a risk factor for exposure to *T. canis*, and is biologically meaningful, since dog bites are not thought to directly transmit *T. canis* - they may indirectly indicate contact with dogs and a shared environment.

The sensitivity and specificity of the *Borrelia burgdorferi* serology test (IDEXX Laboratories, Inc.; Westbrook, Maine) were reported as 99%, 95% CL: 94.3-99.9% and 99.9%, 95% CL: 97.4-99.9%, respectively. These estimates are actually for heartworm (*Dirofilaria immitis*), which is one of the four pathogens tested for in the IDEXX 4Dx snap test. The correct estimates for *B. burgdorferi* are: sensitivity 94.1% 95% CL 88.3-97.6%, and specificity 96.5% 95% CL 92.9-98.3%. Although these estimates are slightly lower, we believe that the two sero-positive dogs were unlikely to be false positives given their close proximity to endemic areas in Manitoba and models forecasting the western spread of ticks that carry this pathogen.