

**ECOLOGICAL EPIDEMIOLOGY OF AN INVASIVE HOST GENERALIST PARASITE,
DICROCOELIUM DENDRITICUM, IN CYPRESS HILLS INTERPROVINCIAL PARK,
ALBERTA**

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B.Sc., University of Alberta, 2009

A Thesis
Submitted to the School of Graduate Studies
of the University of Lethbridge
In Partial Fulfillment of the
Requirements for the Degree

DOCTOR OF PHILOSOPHY

Department of Biological Sciences
University of Lethbridge
LETHBRIDGE, ALBERTA, CANADA

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For my grandfather, William Norman Jackson.

A source of eternal love and support throughout my life, and lives of all my family.

1934-2011

ABSTRACT

Innate variability in parasite transmission is one of the hallmarks of the phenomenon of parasitism. Empirical research aimed at quantifying these differences is limited, particularly for generalist parasites that utilize a broad range of sympatric hosts. Using an ecological epidemiological approach, I characterized variability in transmission of an emerging host generalist parasite, *Dicrocoelium dendriticum*, in Cypress Hills Interprovincial Park, Alberta. ‘Hotspots’ for ant-to-ungulate transmission were characterized by the presence of aspen (*Populus tremuloides*) trees on moderately graded, south, or east facing slopes at elevation > 1300m. Individual fluke performance and *per capita* fecundity were approximately equal among naturally-infected elk and experimentally-infected sheep and cattle. However, when these data were combined with host population size and host residency time in CHP, the sub-population of roughly 4000 cow/calves that are pastured in CHP contribute approximately 80% of the estimated 300 billion eggs that contaminate pasture each year.

**“The wisest mind has something yet to learn.”
- George Santayana -**

ACKNOWLEDGMENTS

I would like to express my utmost gratitude to my supervisor, Dr. Cameron Goater. His enthusiasm for parasitology is what first ignited my interest in this area of research and remained infectious throughout my graduate career. His mentorship, support, and guidance have contributed greatly to the many successes I have enjoyed during my tenure. I have no doubt that the essential research values he has instilled will continue to aid me in the future. Thank you also to Drs. Douglas Colwell and Stefan Kienzle. Through the mentorship of Dr. Colwell I developed an appreciation for, and skills related to, veterinary parasitology and laboratory diagnostics. Dr. Kienzle's endless support and incredible teaching skill provided an introduction to GIS, the potential applications of which continued to excite me throughout my academic career.

Thanks are also owed to my colleagues at the University of Lethbridge, especially Kim Dohms, Brad van Paridon, Stephanie Crowshoe, and Scott Seward. Your camaraderie, support and an open ear made this entire process more enjoyable. I would also like to express my gratitude to Dawn Gray, Mark Mueller, Tim Anderson, Jay Gedir and Andrea McMillan for their assistance in data collection and analysis.

A special thank you is owed to my parents and grandparents: Fred Thomson, Beverly Thomson, Betty Jackson, Colin Thomson and Eleanor Thomson. You have all played a significant role in who I have become and in all of my successes. Lastly, I owe an eternal thank you to my best friend and partner in life, Cole Beck, for his unwavering love and support through times of frustration and triumph.

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CHAPTER 1: GENERAL INTRODUCTION

1.1 GENERAL OVERVIEW

The international movement of livestock, landscape modification associated with modern agricultural practices, and translocation of wildlife for conservation, agricultural and hunting purposes brings together novel combinations of indigenous and non-indigenous species (NIS) (Daszak *et al.*, 2000; Hatcher *et al.*, 2012). A significant, albeit often neglected, concern associated with the introduction of NIS is the risk of parasite and pathogen ‘spill-over’ into previously unexposed populations of hosts. Examples of parasite spill-over are common across the globe. In Great Britain, the presence of bovine tuberculosis in the European badger (*Meles meles*) represents a considerable threat to domesticated cattle (Brooks-Pollock & Wood, 2015). The lungworm nematode (*Protostrongylus stiles*), common in wild sheep (*Ovis* spp.), has recently emerged in muskoxen (*Ovibos moschatus moschatus*) with the reintroduction of this host to its historical range in the Canadian north (Kutz *et al.*, 2004). These emerging novel host-parasite interactions can be highly pathogenic to host individuals and can significantly affect population health and fitness. Consequently, parasite spill-over is associated with important economic and conservation implications, particularly for endangered host species (Daszak *et al.*, 2000; Hoberg & Brooks, 2008). An understanding of the factors that amplify or constrain transmission is therefore necessary for effective management of invasive parasites and host populations (Hoberg & Brooks, 2015).

Risk factors for parasite spill-over and subsequent emergence in novel areas are complex. Most simply, the rate and direction of spread are dependent on the contact or overlap in distribution of infected and uninfected hosts. However, successful invasion is

also determined by a combination of traits intrinsic to the parasite (e.g. degree of specificity, reproductive output) and to the range of host species (e.g. variable host competence), as well as environmental factors extrinsic to the hosts (Ostfeld & Keesing, 2000; Hatcher *et al.*, 2012). These factors may act in a species-specific and context-dependent manner (e.g. May & Anderson, 1978; Stromberg, 1997; Barger, 1999), often complicating our understanding of where, when, and in which hosts' risk of transmission is highest. A better understanding of the connections and heterogeneities among parasites, their hosts and the landscape may enhance our ability to identify transmission "hot spots" and hosts that contribute disproportionately to parasite transmission (Ostfeld & Keesing, 2000; Wilson *et al.*, 2002; Paull & Johnson, 2011). These data may in turn facilitate forecasts of the likelihood and rate at which parasites will disperse into new geographic regions and successfully establish in novel host species. However, few empirical studies exist that are designed to evaluate the rate and direction of spread of parasites in multi-host systems. The paucity of accurate epidemiological data is paramount for parasites dependent upon obligate vectors or intermediate hosts for at least one stage of development. Not surprisingly, studies have predominantly focused on single parasite/single host species interactions for domestic hosts. This limited focus pervades our understanding of the transmission dynamics of invasive, host generalist parasites that tend to infect a broad range of wild and domestic species of host (Cleaveland *et al.*, 2002).

1.2 ECOLOGICAL EPIDEMIOLOGY

The focus of epidemiological research has, in recent years, been extended by ecologists to include not only the study of human-specific disease dynamics, but also to encompass the dynamics of infectious pathogens in any species of host (Begon, 2009). Parasitologists

and epidemiologists recognize that parasite distributions vary enormously across space, among host species, and between individuals (Wilson *et al.*, 2002). Indeed, the notion of heterogeneity in parasite burdens within and among samples of hosts is one of the hallmarks of the phenomenon of parasitism (Goater *et al.*, 2014). Ecological epidemiology has advanced as a branch of epidemiological research that aims to understand the patterns and underlying processes associated with heterogeneity of occurrence of parasites and other infectious diseases (Begon, 2009). Using an ecological epidemiology approach to characterize heterogeneity in parasite transmission dynamics at three levels - environmental, host species, and individual hosts - a clearer understanding of the fundamental mechanisms promoting parasite colonization and persistence in novel environments and hosts is attainable.

1.2.1 Environmental heterogeneity and landscape epidemiology

The characterization of heterogeneity in parasite dynamics among hosts and vectors or intermediate host species across a landscape is a key area of emphasis in ecological epidemiology (Begon, 2009). This focus stems from the long-held recognition that rates of transmission among samples of hosts collected from different sites, even those that are adjacent and connected, can differ by several orders of magnitude (e.g. Malone, 2005). The environmental features of transmission “hot spots” amplify parasite survival or densities of hosts and are characterized by high parasite prevalence (percentage of infected hosts), high infection intensity (number of parasites per infected host), or an increased rate of transmission. As a result, these hot spots also represent a point source for parasite dispersal into novel hosts and geographic regions (Paull & Johnson, 2011).

For direct lifecycle parasites of wild and domestic ungulates, (i.e. exposure via direct contact or through ingestion of infective larvae on pasture), variability in host habitat selection and use influences the timing and location of parasite-seeded pasture (e.g. Altizer *et al.*, 2003). The distribution, rate of development, and survival of free-living larvae is then affected by the prevailing environmental conditions (e.g. Ng'ang'a *et al.*, 2004; Beck *et al.*, 2015). However, the influence of environmental heterogeneity may be more pronounced among indirectly transmitted parasites. The intersection of high parasite, vector/intermediate host, and final host densities characterize hot spots. Transmission of infective stages for these types of parasites may therefore be limited to more isolated patches across the landscape (Paull & Johnson, 2011).

Quantitative description of spatial heterogeneity over a diverse landscape is dependent upon the ability to spatially link parasite data with environmental and topographical features (Reisen, 2010). Classic studies often involved painstaking field surveys, in combination with complex laboratory studies. The development of the Ollerenshaw Index, which combined parasite prevalence in livestock from individual ranches with data from local weather stations, is a prime example. This index continues to provide a basis for short-term forecasts of *Fasciola hepatica* (Trematoda) outbreaks on English rangelands (Ollerenshaw & Rowlands, 1959). However, the validity and accuracy of this and other early models, which were based on collating, mapping and analyzing prevalence data, is limited. The use of modern geographic information systems (GIS) tools and spatial statistics has greatly enhanced epidemiological research for a number of common parasitic infections of humans, domestic animals and wildlife, contributing to an improved understanding of parasite transmission biology (Hay *et al.*, 2013). Using a GIS-based approach researchers have shown that regional temperature and precipitation patterns account for a significant

proportion of the total variability in transmission of *Plasmodium*-causing malaria (Sehgal *et al.*, 2010) and tick-borne encephalitis (Merler *et al.*, 1996). With epidemiological factors influencing parasite transmission over a range of spatial and temporal scales, these tools have become invaluable for landscape epidemiologists (Brooker *et al.*, 2002). A GIS allows researchers to acquire, store and visualize spatial data, but also to analyze, synthesize and statistically model environmental data to highlight epidemiological trends (Reisen, 2010). The development of GIS-based distribution models using Bayesian methodologies is now recognized as a powerful tool for the identification of key habitat characteristics underlying the distribution of parasites at the landscape scale (Yang *et al.*, 2005).

Broad-scale GIS-based epidemiological models have improved our understanding of parasite ecology and have been successfully applied to direct intervention strategies (Pullan *et al.*, 2011). A key example includes the establishment of the Schistosomiasis Control Initiative in sub-Saharan Africa (Brooker, 2007). Additionally, GIS and remote sensing-based analyses have guided the control of schistosomiasis in cattle, water buffalo, and humans in China (Yang *et al.*, 2005). However, these broad-scale approaches often underestimate the spatial heterogeneity in host-parasite contact on a local-scale (Malone, 2005; Musella *et al.*, 2010). Comprehensive epidemiological data are rare, with fine-scale data available for only select parasites. Studies have therefore focused on regional and continental variability in parasite occurrence. Further, anthropogenically-significant parasites and pathogens continue to dominate epidemiological literature with limited focus on a number of subclinical parasites (Musella *et al.*, 2010). As a result, the ecological determinants of fine-scale heterogeneity in transmission remain poorly understood for a broad range of parasites. These neglected parasites may represent a considerable conservation and economic concern.

High-resolution geo-spatial models of transmission can provide accurate depictions of local and regional parasite distribution patterns, many of which are of high human, veterinary, and conservation importance. In the analysis of spatial heterogeneity in transmission of *Fasciola hepatica*, Malone (2005) found that regional climate variables could account for a significant proportion of inter-annual variation in transmission between snails and cattle. However, this broad scale approach failed to account for local scale variation in risk. A 100-fold difference in *F. hepatica* transmission risk was documented between adjacent cattle farms owing to heterogeneity in local ecological characteristics. Evaluation of fine-scale variation in risk of transmission across the landscape is a key priority for understanding the complex transmission dynamics and environmental determinants for indirect life-cycle parasites, which utilize a broad range of intermediate and final host species.

1.2.2 Intraspecific and interspecific heterogeneity

In addition to heterogeneity at the landscape scale, interspecific and intraspecific variability in parasite transmission can significantly influence the rate and direction of spread for invasive parasites. When spatial heterogeneity in the distribution of infective parasite stages is controlled, even small differences in host suitability and susceptibility can produce high variability in mean abundance and parasite prevalence within a population of hosts (Anderson & May, 1978). In an elegant set of laboratory experiments, Keymer and Anderson (1979) manipulated the distribution of eggs of the tapeworm *Hymenolepis diminuta*. Even when the spatial distribution of eggs was approximately uniform, parasite distributions were highly aggregated in the flour beetles (*Tribolium confusum*) populations. Parasites are often found to exhibit a highly aggregated distribution within a population of

hosts, with the majority of individuals harbouring low numbers of parasites (Shaw & Dobson, 1995). From this overarching pattern, Woolhouse *et al.* (1997) proposed the “20/80” rule where 80% of transmission events can be attributed to 20% of hosts. The key implication here is that a small number of highly infected ‘superspreading’ individuals and ‘superspreader’ subgroups are disproportionately responsible for amplifying parasite transmission and play a significant role in the spread of infection into new hosts and to new geographical areas (Lloyd-Smith *et al.*, 2005; Cross *et al.*, 2009).

Among naturally infected animals, opportunistic sampling has demonstrated that variability in parasite transmission can be attributed to differences in age, gender, condition, behaviour and genetics (Wilson *et al.*, 2002). In the absence of effective immunity, parasite loads may increase with chronic exposure to a plateau in older animals. Alternatively, among hosts that acquire an active immune response, the aggregation of parasites may decline with age (e.g. Beck *et al.*, 2014). Adaptations of classic single host-single parasite models (e.g. Anderson & May, 1978; May & Anderson, 1978) have been used to evaluate the relative significance of these mechanisms, as well as the importance of interactions between them in amplifying differences in parasite burden among individuals (Wilson *et al.*, 2002; Lloyd-Smith *et al.*, 2005; Begon, 2009). However, patterns of infection and the drivers of transmission heterogeneity are highly variable among species of host and parasite. Therefore, a combination of intensive field surveys and laboratory analyses will often be required to determine the host-specific and parasite-specific determinates of transmission heterogeneity.

For parasites that can infect multiple species of host (i.e. generalists), interspecific variability in host-parasite contact, susceptibility, and host competence can also lead to species-specific differences in parasite transmission (Haydon *et al.*, 2002). It is presumed

that a true generalist has evolved more general host recognition and tolerance strategies that allow individuals to transmit and become reproductively mature in a broad range of hosts. However, not all parasites are equal, nor are their hosts (Agosta et al., 2010). Parasites can be classified along a host specialist - host generalist continuum. The magnitude of asymmetries in parasite performance (i.e. recruitment, growth, reproduction, and development) and transmission among host species is influenced by the extent to which a parasite is a host specialist or host generalist (Combes, 2001; Poulin, 2007). Therefore, the degree of host specificity may significantly impact a parasite's host finding ability and overall transmission success (Hatcher *et al.*, 2012). For highly specialized parasites, transmission and dispersal is limited by the interactions between one or a few suitable host species (Hatcher *et al.*, 2012). For these specialists, spill-over is only epidemiologically significant if parasites are able to reach reproductive maturity in a novel host. In these cases, the determination of key transmission rate parameters (i.e. parasite fecundity, rate of transmission) and the underlying risk factors for emergence of specialist parasites is relatively less complicated. In contrast, extreme host generalists that successfully utilize a broad range of host species represent a complex 'nightmare' for applied and theoretical epidemiologists (Grenfell *et al.*, 2002).

Due to the innate complexity of transmission patterns for multi-host parasites, accurate comparison of parasite performance are rare. The best evidence comes from experimentally infected species of host, where confounding factors such as host age, immune status, parasite genetics and infectivity are controlled. In a classic study, Hairston (1962) showed that the intensity, prevalence, morphology and *per capita* reproduction of the trematode *Schistosoma japonicum* varied extensively among sympatric humans, dogs, pigs and rats. In this early example, species-related differences in parasite transmission were

incorporated into simple models to demonstrate that targeted control of the rate population effectively reduced transmission. These disparities in parasite performance and host contributions to total parasite transmission are likely to be common among generalist parasites. However, such heterogeneity in transmission cannot be established or assumed *a priori* (Shaw & Dobson, 1995; Lloyd-Smith *et al.*, 2005).

The basic reproductive number (R_0) has been employed as a mathematical approximation for the ‘transmission threshold’ of a parasite. R_0 represents the number of reproductively mature offspring produced over the lifetime of a single adult parasite (Anderson & May, 1991; Streicker *et al.*, 2013), where R_0 must be ≥ 1 for infection to spread. However, for generalist parasites with multiple hosts and multiple developmental/transmission stages, calculation of this value is particularly complex. Alternatively, measurement of the relative contribution of each host species to the total number of infective stages (Streicker *et al.*, 2013) or infected vectors (Kilpatrick *et al.*, 2006) can be used in the estimation of host-related differences in parasite fitness. Although these data can provide evidence of species-specific variability in the shedding of parasite eggs, comparisons also necessitates the evaluation of relevant population characteristics, such as host density. In a comparison of gastrointestinal parasite transmission among small mammal communities across the eastern United States, Streicker *et al.* (2013) identified that two distinct, but not mutually exclusive features were responsible for transmission asymmetries among sympatric species of hosts: heterogeneity in host population abundance and relative parasite performance. In addition to providing key insights into the identification of superspreading hosts, these data may also provide direction for intervention strategies. For example, targeted treatment of highly abundant domestic dogs has alleviated transmission of the rabies virus in wild carnivores in the Serengeti (Lembo *et al.*, 2007).

The gold standard for evaluating interspecific and intraspecific variability in parasite performance and the contribution to transmission requires detection of parasites from a large number of equally sampled hosts from populations of known size, where movement of hosts can be estimated (Poulin, 2007). Ideally, through post-mortem examination, reliable estimates of parasite prevalence, distribution and transmission parameters (e.g. exposure, rate of encounter, patency, fecundity) can be determined (Wobeser, 2007; Altizer *et al.*, 2013). Unfortunately, where transmission involves large wildlife, accurate data are often unavailable due to logistical, financial and ethical constraints. As an alternative, indirect diagnostic measures of parasite abundance (e.g. coprological egg detection methods, anti-parasite antibody detection via ELISA's) are commonly used. However, the sensitivity of these methods can be low. Additionally, accurate approximations of host population size are also rare. As a result, the dynamics of emerging parasites in natural multi-host systems are often poorly understood (Shaw & Dobson, 1995).

1.3 MODEL SYSTEM

Dicrocoelium dendriticum provides a unique model for evaluating transmission heterogeneity of a host generalist parasite across space, species and individuals. With historical introductions outside of its' home range in central Europe via movement of infected livestock and wildlife, this fluke is now present throughout Europe, Asia, north Africa and is found in patches in North America (Soulsby, 1982; review by Otranto & Traversa, 2002). Characterized as a host generalist, adult flukes have been documented in the bile ducts and gall bladder of a wide range of mammalian hosts, principally including domestic and wild ruminants. However, infection has also been documented in camelids, rabbits, pigs, dogs, and horses (review by Otranto & Traversa, 2002). Among naturally- and

experimentally-infected hosts, cholangitis, liver fibrosis, anaemia and reduced weight gain have been documented (review by Manga-González *et al.*, 2001), with changes in liver function (i.e. increased production of bilirubin, albumen, hepatic enzymes) above burdens of ~4000 adult flukes (Manga-González & González-Lanza, 2005). However, hosts are normally asymptomatic, with no clear economic significance associated with infection.

Over 90 species of terrestrial molluscs have been found to act as a suitable first intermediate host for the development of *D. dendriticum* larvae. Following ingestion of embryonated eggs from host feces, miracidia hatch, migrate through the gut wall and settle into the adjacent vascular connective tissue, where they become mother sporocysts. The sporocysts migrate to the digestive gland and produce several daughter sporocysts. Cercariae are produced asexually inside each daughter sporocyst. Cercariae migrate to the respiration chamber and are released onto pasture in mucus clusters ('slime balls') of up to 5000 individuals and are then ingested by ants (*Formica* spp.). Metacercariae develop in the ant abdomen, becoming infective to hosts 1-2 months post exposure (Manga-González & Ferreras, 2014). One or two individual cercariae remain unencysted on the subesophageal ganglion. The presence of this parasite in the brain is associated with modified behaviour, with larval infected ants attaching themselves to vegetation surrounding the nest and entering into a state of torpor. Subsequent infection of definitive hosts can then occur through the ingestion of these 'zombie' ants (review by Otranto & Traversa, 2002; Waldner *et al.*, 2004). Adult flukes are then reproductively mature 49-79 days post infection (Campo *et al.*, 2000).

Although *D. dendriticum* has been present in North America since the 1930's (Mapes & Baker, 1950), this fluke was not detected in Alberta, Canada until 1988. The lancet fluke was found in <1 % of hunter-shot wapiti (*Cervus canadensis*), moose (*Alces*

alces), white-tailed deer (*Odocoileus virginianus*), and mule deer (*O. hemionus*) during a province-wide survey (Pybus, 1990), with no evidence of the fluke found during previous parasitological surveys (e.g. Kingscote *et al.*, 1987). Infected hosts (N = 4) were localized in or around the Cypress Hills Interprovincial Park (CHP), a conservation preserve located approximately 100 km north of the Canadian/United States border and extending from the south-eastern corner of Alberta into the south-western corner of Saskatchewan (49°40'N, 110°115'W). With an approximate total upland area of 2590 km², maximum elevation of CHP varies from 1,466m above sea level at the western end in Alberta to 1,067 m in Saskatchewan. Embedded within the extensive grassland natural region (Downing & Pettapiece, 2006), the top of the plateau is dominated by plains rough fescue (*Festuca* sp.), western porcupine grass (*Stipa curtiseta*), sedges, and various forbs. The slopes and valleys of this island park are characteristic of the montane natural subregion, with grasses and forbs interspersed with stands of lodgepole pine (*Pinus contorta*), white spruce (*Picea glauca*), trembling aspen (*Populus tremuloides*) and balsam poplar (*P. balsamifera*).

The life-cycle of *D. dendriticum* requires spatial overlap in distribution of suitable invertebrate intermediate (terrestrial molluscs and *Formica* spp. ants) and definitive hosts (reviews by Manga-González *et al.*, 2001; Otranto & Traversa, 2002). Thus, transmission is clearly tied to landscape characteristics. The emergence of *D. dendriticum* in CHP therefore provides an opportunity to investigate local-scale ecological covariates for the geographic colonization and establishment of this invasive parasite, as well as the potential for subsequent range expansion. Typically, infection in livestock is associated with lowland or mountain pastures with dry, calcareous alkaline soils (review by Manga-González *et al.*, 2001) and a high percentage of wood, rocks, and arable soil with sparse trees (Musella *et al.*, 2010). Presumably, these characteristics provide suitable microhabitat for terrestrial

gastropods and *Formica* spp. ants. However, the use of these generalized landscape characteristics in predicting risk of transmission is likely to result in inaccurate projections. The specific microhabitat characteristics that define the overlap in distribution of intermediate and final hosts species is likely to vary on a local scale relative to the range of hosts utilized in a specific geographic region. Since *D. dendriticum* was first detected within CHP, a new intermediate host snail species (*Oreohelix* spp.) has been identified (B.J. van Paridon, Ph.D. Thesis, unpublished observations). Highlighting the fine-scale ecological covariates of *D. dendriticum* intermediate host distribution may therefore provide key insights for the emergence of this parasite in CHP and the risk of parasite dispersal beyond the current distributional range.

The emergence of *D. dendriticum* in CHP is also a powerful model system for evaluation of circulation and transmission patterns among a range of suitable hosts. Despite this parasite having been rare prior to 1990, subsequent opportunistic sampling has indicated that prevalence now ranges from 40-90% in the community of large sympatric herbivores on the Alberta side of the CHP (Goater & Colwell, 2007). This is among the highest reported globally (review by Ducháček & Lamka, 2003). In CHP, prevalence of infection and parasite abundance are highly variable (Goater & Colwell, 2007).

Accurate comparisons of individual and host species differences in relative contribution to total parasite transmission are rare (Poulin, 2007). Host-related differences in the parasite abundance and circulation have been documented among freshwater fish populations (e.g. Aho & Kennedy, 1987; Baldwin & Goater, 2003). However, the calculation of species-related differences in parasite circulation failed to incorporate a measure of host population size and density. The accuracy of these comparisons is therefore limited. The integration of host density and assessments of parasite distribution

patterns is necessary to more reliably estimate heterogeneity in parasitism and the relative contribution of different populations of sympatric hosts to overall transmission. Such data are often unavailable for multi-host parasites shared among large mammals (Wilson *et al.*, 2002; Hatcher *et al.*, 2012).

1.4 THESIS OBJECTIVES

Geographic information systems (GIS) tools are employed in Chapter 2 to evaluate fine-scale variation (1m²) in risk of *D. dendriticum* transmission to sympatric ungulate hosts in CHP. Little is known about the ecological drivers of local scale variation in risk of transmission, with previous studies focusing on more broad scale distribution patterns of infected hosts. In this study, I define ‘hot spots’ for parasite transmission by the presence/absence of infected intermediate hosts at 100 randomly selected sites in CHP. GIS-based Bayesian multivariate spatial statistical analyses are then used to determine locally specific habitat covariates for risk of *D. dendriticum* transmission from larval infected ants to a suitable grazing host.

In chapter 3 I present an evaluation of individual heterogeneity in parasite abundance among two naturally infected host species. Here, I specifically evaluate variability in *D. dendriticum* abundance relative to host age in wapiti and cattle. Individual elk and cattle were aged and necropsied to test the hypothesis that 0.5-2 year old elk are most at risk of exposure to larval *D. dendriticum*.

In Chapter 4, data from experimentally- and naturally-infected hosts are combined to evaluate heterogeneity in key performance characteristics of parasites between individual hosts and between host species. The relative performance of *D. dendriticum* was compared among naïve cattle and sheep experimentally exposed to known numbers of *D.*

dendriticum metacercariae. Faecal egg counts were collected over the course of a 120-day experimental infection program for comparison of the time to egg shedding. With collection and incubation of live flukes, I evaluated variability in percent recruitment, fluke morphology, and estimates of daily fecundity. Flukes were also collected from naturally-infected elk for comparison of per capita performance relative to livestock hosts.

In Chapter 5, I evaluate the annual relative contribution of sympatric ungulate host species (cattle, wapiti, mule deer, and white-tailed deer) to the infective pool of *D. dendriticum* eggs onto pasture. Combining estimates of *per capita* worm fecundity, parasite and host abundance, parasite prevalence, and host residency time in CHP, I estimated the relative contribution of each resident host species to the contamination of CHP pasture with *D. dendriticum* eggs.

Together, these data allow me to identify hosts and environments that contribute disproportionately to transmission, with significant implications for understanding risk of parasite emergence in new geographic regions beyond CHP. Exploring the transmission patterns of this generalist parasite may also provide broader insights parasite ecology and patterns of emergence locally, regionally and across the globe.

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CHAPTER 2

Where are the zombies? Landscape epidemiology of the invasive liver fluke,
Dicrocoelium dendriticum in their ant intermediate hosts

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Prepared as a manuscript for submission

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2.1 ABSTRACT

Ecological influences on the transmission stages of parasites are many and varied, particularly for species whose life-cycles require transmission to and from a series of obligate hosts. A shift from broad-scale (100's to 1000's of km²) geospatial evaluations towards local-scale assessments is necessary to yield insights regarding the ecological conditions required for successful transmission. We evaluated site-specific variation in ant-to-ungulate transmission of the invasive trematode, *Dicrocoelium dendriticum*, in Cypress Hills Interprovincial Park in Alberta, Canada. Cross-sectional field data collected at over 100 randomly selected sites were used to construct hierarchical logistic regression models to identify local-scale topographical and ecological covariates of *D. dendriticum* transmission, as measured by the presence/absence of larvae-infected ants. 'Clinging' metacercariae-infected formicid ants were detected at ~63% of aspen-dominant sites and were not present under other canopy types, in riparian zones, or on grasslands. All infected ants contained at least one unencysted parasite in the brain and mean metacercarial intensity varied significantly between sites (1-250 larvae/host). Statistical models indicated that "hot spots" for ant-to-ungulate transmission were characterized by pure or mixed stands of aspen (*Populus tremuloïdes*) on moderately graded, south or east facing slopes found at elevation > 1300m. Additional sites (N = 35) were surveyed for validation of this model. Clinging ants were detected at 73% of high- risk sites and at 20% of intermediate-risk sites, indicating excellent discriminatory model performance. These results provide one of the first fine-scale (1m² resolution) GIS-based risk maps for an emerging parasite in North America.

2.2 INTRODUCTION

The survival, reproduction, and dispersal of hosts and their associated parasites vary enormously across a landscape. This heterogeneity is largely determined by a diverse, often fragmented abiotic and biotic environment (Hess *et al.*, 2002). The identification of ecological features that give rise to parasite transmission opportunities is therefore fundamental to understanding the epidemiology of parasite transmission. This knowledge is also central to the direction of targeted management programs. However, a paucity of accurate and comprehensive epidemiological data continues to limit our understanding of the ecological limits on parasite distribution, rates of transmission, and on the likelihood of ‘spill-over’ into novel geographic regions and species of host (Cleaveland *et al.* 2002; Cross *et al.*, 2009). Early studies aimed at describing spatial heterogeneity in parasite prevalence or burden across an ecologically diverse landscape often involved a combination of painstaking field surveys and complex laboratory studies. In a classic study, Ollerenshaw & Rowlands (1959) combined meteorological and parasite prevalence data from individual ranches to forecast *Fasciola hepatica* (Trematoda) outbreaks on English rangelands. The Ollerenshaw Index continues to provide short-term forecasts for risk of fascioliasis transmission and direct treatment on English rangelands.

Increased accessibility and use of Geographic Information Systems (GIS), in combination with flexible and powerful statistical tools (i.e. Bayesian geostatistical modelling), has allowed for the visualization, analysis and synthesis of both biologically- and statistically-relevant models that connect landscape, climate and risk of parasite transmission at regional and continental scales (Reisen, 2010). In the case of important anthropogenic diseases, including schistosomiasis (Yang *et al.*, 2005; Brooker, 2007) and tick-borne encephalitis (Merler *et al.*, 1996), habitat and climate features have proven to be strong

predictors of parasite prevalence and intensity. For example, spatial models incorporating regional patterns in temperature and humidity accounted for 90% of the variation in *Plasmodium* spp. prevalence among non-migratory birds in Central Africa (Sehgal *et al.*, 2010). Similarly, geospatial models for complex lifecycle trematodes (e.g. *F. hepatica*) and soil-transmitted helminths have shown that broad-scale climatological and topographical features can be used to predict transmission across ecologically diverse landscapes (Kantzoura *et al.*, 2011; Pullan *et al.*, 2011).

These models have contributed to an improved understanding of parasite transmission biology and have also proven useful in directing intervention strategies (Pullan *et al.*, 2011). However, these broad-scale approaches, which typically surmise regional and continental variability in parasite prevalence, continue to underestimate the tremendous complexity and variability in the distribution of parasites and their hosts on a local-scale (Malone, 2005; Musella *et al.*, 2010). Further, anthropogenically-significant parasites and pathogens continue to dominate inquiry in landscape epidemiology, leading to the neglect of subclinical parasites that may represent threats to the health of domestic stock and wildlife conservation. As a result, the ecology of fine-scale transmission patterns remains poorly understood for a broad range of parasites. This is particularly limiting within the context of invasive or emerging parasites, where a lack of epidemiological data restricts the ability to forecast future outbreaks.

Rate of parasite transmission can vary by several orders of magnitude on a fine-scale, even across contiguous and connected landscapes. For instance, Malone (2005) showed a 100-fold difference in *F. hepatica* prevalence between adjacent cattle farms owing to heterogeneity in local ecological characteristics. At this local scale, the likelihood and rate of host-parasite contact can be influenced by a variety of parasite-, host- and/or environment-

specific factors (Altizer *et al.*, 2003; Ostfeld *et al.*, 2005; Vanderwaal *et al.*, 2015). The influence of environmental heterogeneity has been shown to be paramount for indirect lifecycle parasites where successful transmission is limited by the microhabitat preferences and environmental thresholds for obligate vectors, intermediate hosts and/or definitive hosts (Vanderwaal *et al.*, 2015). Evaluation of local heterogeneity in distribution of infected vectors or intermediate hosts is therefore needed to define the specific ecological barriers to parasite transmission and dispersal. This then holds key implications for defining risk of host-parasite contact, understanding historical introduction pathways and contemporary distribution patterns, and highlighting opportunities for parasite emergence. However, fine-scale geospatial studies remain underutilized, with resolution of data often limited to 10's of kilometers (Malone, 2005; Musella *et al.*, 2010).

The emergence of the lancet liver fluke, *Dicrocoelium dendriticum* in Cypress Hills Interprovincial Park, Alberta (CHP) presents a unique ecological model for studying site-specific variation in parasite transmission from intermediate-to-final-host species. This generalist trematode is found in the liver and gall bladder of domestic and wild ruminants, but also in camelids, rabbits, pigs, dogs, horses, and humans (review by Otranto & Traversa, 2002). Through the movement and introduction of infected domestic livestock and wildlife, the distribution of this cosmopolitan parasite has expanded beyond its historical home range in Europe. The lancet fluke is now present throughout Europe, Asia, North Africa, and in isolated patches across North America. Although *D. dendriticum* was absent in CHP prior to approximately 1990 (Goater & Colwell, 2007), prevalence of infection among sympatric hosts is among the highest reported globally (review by Ducháček & Lamka, 2003; Goater & Colwell, 2007; Beck *et al.*, 2014; Beck *et al.*, 2015). The results of recent field surveys have found that 40-90% of all co-grazing white-tailed deer (*Odocoileus*

virginianus), mule deer (*O. hemionus*), elk (*Cervus canadensis*), and domestic beef cattle now harbour *D. dendriticum* in CHP (Beck *et al.*, 2014; Beck *et al.*, 2015; Goater & Colwell, 2007). This amplified rate of parasite transmission may be attributed to a range of host and environmental characteristics. For example, Beck *et al.* (2015) suggest that annual immigration of high densities of naïve and immunologically incompetent cattle each year contribute to *D. dendriticum* infective pool (i.e. egg shedding). However, despite the wide distribution of this generalist trematode, the fine-scale ecological requirements for transmission remain poorly understood.

Transmission of this parasite is clearly tied to the landscape where the distributions of suitable definitive and invertebrate intermediate hosts overlap (reviews by Manga-González *et al.*, 2001; Otranto & Traversa, 2002). Evaluation of infected intermediate host distribution patterns in CHP therefore provides an opportunity to identify fine-scale ecological covariates of *D. dendriticum* transmission. Using hierarchical logistic regression models we combined high-resolution land surface and vegetation characteristics with cross-sectional field data to evaluate the fine-scale (1m²) ecological characteristics that may influence spatial heterogeneity in risk of ant-to-final host transmission.

2.3 MATERIALS AND METHODS

2.3.1 Cypress Hills Interprovincial Park, Alberta, Canada

The Cypress Hills Interprovincial Park straddles the southern border of Alberta and Saskatchewan (49°30' N, 110°0'W). Rising approximately 200m above the surrounding prairie (1,050-1,470m above sea level), the plateau of this island-like park is dominated by plants characteristic of the grassland natural ecoregion (Downing and Pettapiece, 2006), such as rough fescue (*Festuca campestris*) and shrubby cinquefoil (*Potentilla fruticosa*).

The forest cover dominating the slopes down to the surrounding grasslands is a mosaic of trembling aspen (*Populus tremuloides*), balsam poplar (*Populus balsamifera*), lodgepole pine (*Pinus contorta*) and white spruce (*Picea glauca*) (Hildebrant & Hubner, 1994). CHP is characterized by a cool and moist climate. Mean annual precipitation (607 mm) and mean daily maximum and minimum temperatures (17.5 °C and 6.1 °C) are characteristic of the cool, moist climate of this ecoregion (Environment Canada, 2014). In winter, temperatures on average range from -14.3 °C to -2.5 °C. Maximum lows often exceed -25 °C. Further details on the natural history of CHP are discussed in Chapter 1 and in Beck *et al.* (2014).

2.3.2 Mapping data

ArcGIS version 10.1 was used for study design and for all mapping analyses. Spatial analyses of the ecological covariates of ant-to-ungulate transmission required the following digital data sources: aerial photographs (Spatial Data Warehouse Ltd., 2006), existing road network, water features such as streams and lakes, CHP boundary, digital elevation model (DEM), and generalized land cover (GeoBase, 2012). For sampling point design, CHP was delineated into grassland and forest land-cover types. A 1m resolution DEM, used to calculate slope (terrain gradient in %) and aspect (direction of slope), was created by resampling and smoothing the original 30m resolution DEM.

2.3.3 Sampling design

For the purposes of this study, we assume that a grazing mammal's risk of exposure to metacercariae will be highest where the numbers of ants clinging to vegetation is highest. Thus, rates of metacercariae-to-ungulate transmission will be highest where clinging ants are

most common. Sites where infected ants were detected were termed ‘positive’ and sites where they were absent were termed ‘negative’. ArcMap was used to randomly select a total of 110 sites within the boundaries of CHP (Fig. 2.1). One hundred points were randomly selected within treed areas and another 10 were selected from within the fescue-dominant grasslands that occur on the flat plateau. We focused on regions that were dominated by forest stands (aspen, poplar, spruce, or pine) as preliminary surveys indicated that clinging ants were never observed on grasslands (C.P. Goater, unpublished data).

For each sampling point, three quadrats (15 X 20m) were surveyed from late May to late August of 2010 and 2011. The first quadrat was centered on the GPS coordinate of each randomly allocated sample point. The second and third quadrats were located 20 meters upslope and 20 meters downslope, respectively, from the central point. This 3-quadrat approach was used to evaluate within-site variation. Within each quadrat we assessed ecological characteristics coinciding with the presence/absence of *D. dendriticum* infected intermediate hosts. Sampling periods were targeted to early mornings, when temperatures were below 20°C. Results from field studies indicate that beyond this maximum threshold temperature, infected ants detach from vegetation (C.P. Goater, unpublished data).

A systematic grid survey was conducted within each quadrat. Elk and deer populations move freely within CHP, while cattle subpopulations are restricted in their movement by fencing. Therefore, while access to elk and deer is assumed to be ubiquitous across the park, cattle occurrence was determined by the presence/absence of faecal pats in the quadrats. Ant nest density (nest/m²) was estimated within each quadrat by counting each visible nest. Terrestrial snail density was estimated from counts of three randomly placed 1 m² sub-quadrats following Forsyth (2004). A subsample of each snail genera present

(minimum of $N = 5$ individuals) was dissected in the lab to determine *D. dendriticum* presence. The total number of infected ants clinging to plants were counted in each quadrat during grid survey. A subsample of ants were collected directly from ant hills and from the collection of those in tetania attached to plants (minimum of $N = 10$), preserved in 90% ethanol and dissected to determine mean metacercariae intensity for each quadrat. Cover type classifications were determined as grass and shrubland-dominant, aspen-dominant, spruce-dominant, pine dominant, or mixed forest. Sites with at least 33% deciduous tree cover were considered mixed deciduous-conifer forest (Faber-Langendoen *et al.*, 2007).

2.3.4 Evaluation of spatial and temporal variation in the occurrence of infected ants

Our preliminary analyses in 2010 showed that the presence of infected ants was associated with proximity to aspen. Additional randomly generated sampling points were surveyed in 2011 to evaluate the spatial and temporal consistency in the presence of clinging ants relative to the distribution of aspen. The locations of aspen stands were extracted from pan-chromatic (grey-scale) aerial photographs (1 m² resolution) taken for the Alberta and Saskatchewan blocks of CHP by selecting pixel values indicative of aspen reflectance. A distance gradient was then calculated from the location of aspen stands (Fig. 2.2). Sampling points were then randomly generated within aspen-dominant, coniferous dominant, and fescue-dominant areas, respectively. This survey included: (1) 30 previously un-sampled aspen-dominated sites in CHP, Alberta; and (2) 30 additional sampling points within aspen and 10 points in fescue or coniferous dominant areas in CHP, Saskatchewan.

At each sampling point I evaluated the co-location of aspen and infected clinging ants. I also evaluated the change in number of clinging ants, mean metacercariae intensity, and density of ant nests with increasing distance from aspen (m) via 25m transects beginning at

the exterior SE corner of each quadrat. Lastly, 10 positive sites were randomly selected from the 2010 survey and revisited in 2011 to evaluate whether the presence of infected ants varied from year to year.

2.3.5 Statistical analysis and hierarchical modelling

To further evaluate the landscape epidemiology of infected intermediate hosts we assessed a range of potential ecological covariates. These included: site survey data from the initial 110 sampling points (i.e. presence/absence of clinging ant, ant nest density, presence/absence of cattle, canopy type), distance to aspen, slope grade, elevation (m), and aspect. Site characteristics were expressed as mean \pm SEM, pooling data from the three sampled quadrats surveyed for each geo-referenced sampling point. Average values were compared with Kruskal-Wallis tests. Slope values were summarized into three categories for site comparison: gentle ($<7.5^\circ$), intermediate (7.5° to 15°), and steep ($>15^\circ$) (Osumi *et al.*, 2003). The proportions of positive or negative sites that are characterized by specific ecological characteristics were calculated for site comparison. Proportions were compared using Fisher's exact tests. 95% confidence intervals (CI) are calculated for proportions (p) using the Wald method (Vollset, 1993; $p \pm z\sqrt{pq/n}$, where $z = 1 - \alpha/2$ of the standard normal distribution and $q=1-p$). Non-parametric univariate correlations were used to evaluate the relationships between the presence/absence of infected ants and potential ecological covariates. Covariates with regression coefficients with $p < 0.05$ were included in subsequent multivariate regression analyses.

Bayesian inference was used to construct hierarchical binary response logistic regression models in OpenBUGS, version 3.2.2 (Speigelhalter *et al.*, 2012) to determine the ecological covariates influencing the likelihood that infected ants were present (1=

infected ants present; 0 = infected ants absent). This approach allowed for the evaluation of the likelihood of models constructed using potential ecological covariates individually and in combination. A total of seven ecological characteristics were evaluated: ant nest density, presence/absence of specific canopy types, presence/absence of cattle, distance to aspen, slope grade, elevation, and aspect. These factors were: (1) categorized if data were binary (e.g. cattle: present = 1; absent = 0); or (2) standardized by subtracting the mean and dividing by 2 standard deviations if data were continuous. By standardizing continuous variables, we account for inconsistency in dimension and variance, which improves the efficiency of the sampling algorithm, and has no effect on the resulting model (Gelman & Hill, 2007). Aspect was the one exception, as its value represents an angle, where 0 or 360 degrees represent North, 90 represents East, and 180 represents South. Aspect was linearly transformed as the cosine (northness) or the sine (eastness) of the aspect with values ranging from -1 to 1 (McDermid & Smith, 2008).

As the likelihood of ant-to-final host transmission is characterized by the presence or absence of infected ants, we assigned a Bernoulli distribution for all models (values ranging from 0 to 1). Models were run as a linear function on a *logit* scale. An uninformative normal prior distribution was assigned to regression coefficients (mean = 0, tau = $1/\sigma^2 = 1.0 \times 10^{-4}$). All models also included date of sampling as a random effect. We assumed that the date of sampling, coded for month (June = 1; July=2; August = 3), to follow a uniform normal distribution.

The first 260,000 Markov chain iterations were discarded for all models and an additional 100,000 iterations were stored for parameter estimation. This initial burn-in was required to ensure that the Markov chains converged and that the parameter space was correctly explored (McCarthy, 2007). Competing models were ranked based on their

deviance information criterion (DIC), following standard methods (Spiegelhalter *et al.*, 2002; see also Burnham & Anderson, 2002). From a set of models, the best model is the one with the lowest DIC value (i.e. the model that retains the most amount of information, best fits the data, and contains the fewest number of parameters). Differences between the DIC_i value of each model from the DIC_i of the best model ($\min DIC_i$) were calculated ($\Delta DIC = DIC_i - \min DIC$) for model comparison. Models within two ΔDIC units of the best performing model were considered to have strong support, four to seven DIC units having considerably less support, and greater than ten, no support (McCarthy, 2007).

Risk of ant-to-ungulate transmission was calculated for each of the models within two ΔDIC of the top-performing model and was projected onto our study area. Prior to inclusion in risk calculation, the continuous variables (distance from aspen, elevation and slope) were standardized and aspect was linearly transformed as the cosine (northness) or the sine (eastness) of the aspect as above. Model outputs were averaged to account for model uncertainty (McCarthy, 2007). With the likelihood of ant-to-ungulate transmission ranging from 0 (no risk) to 1 (highest risk), we assigned values for low (< 0.3), intermediate (0.3-0.6) and high (0.7+) risk of transmission (McCarthy, 2007).

2.3.6 Model validation

To validate our model for risk of ant-to-ungulate transmission, 35 additional sites were randomly selected in 2014 within the low (10 sites), intermediate (10 sites) and high-risk (15 sites) zones in accordance with the mean model projection. The proportion of sites containing infected ants (\pm 95% CI) for each risk category was then determined. For positive sites, the following were also determined to evaluate consistency with the earlier dataset: distance from aspen, slope grade, aspect and elevation. The receiver operating

characteristic (ROC) statistic was used to test model specificity and sensitivity (Pullan, 2011). Overall diagnostic accuracy of the ROC curve was measured by the area under the curve (AUC), where an AUC value < 0.7 was considered to indicate poor discriminatory performance, 0.7 - 0.8 acceptable, 0.8 - 0.9 excellent and > 0.9 outstanding discriminatory performance (McCarthy, 2007).

2.4 RESULTS

2.4.1 Survey data and ecological covariates

A range of mollusc species were identified at the surveyed sites based on morphological characteristics (Forsyth 2004), including: *Cionella lubrica*, *Discus shimckii*, *Discus whitneyi*, *Vitrina* sp., *Zonitoides* sp., and several *Oreohelix* spp. The sporocysts and cercariae of *D. dendriticum* were only found in *Oreohelix* spp. snails. However, due to variability in moisture conditions over the length of the collection season (May to August) comparisons of snail density and *D. dendriticum* prevalence in naturally infected snails were not obtained. Clinging ants were detected at 19 of the 110 sites surveyed in 2010 (17.3%: 95% CI: 13.8 - 20.8 %). Of these positive sites, 95% (95% CI: 90.1 - 99.9 %) had either an aspen-dominant or mixed canopy (aspen and lodge pole pine and/or white spruce). Of all sites that contained aspen, 63.3% (95% CI: 54.8 - 71.9 %) had infected ants. All metacercariae-infected ants belonged to the *Formicidae* family (primarily *Formica sanguinea*, with some *F. subaenescens* and *F. fusca*; B.J. van Paridon, unpublished data). Clinging *Formica* spp. ants always contained encysted metacercariae and were found attached to various plants, including *Lupinus pusillus*, *Thermopsis rhombifolia*, *Vicia americana*, *Amelanchier alnifolia*, and *Taraxacum officinale*. Ants collected from the same

nests, but not found clinging to vegetation, were never infected. These findings allowed us to classify risk of infection based on the presence/absence of infected ants.

Initial association analyses showed that mean metacercariae intensity at a site was strongly and positively correlated with the number of infected ants (Table 2.1). The number of clinging ants, metacercariae intensity and ant nest density all significantly decreased with increasing distance away from aspen. Ant nest density was significantly higher at positive ($\bar{x} = 0.16 \pm 0.02$ nest/m²) versus negative ($\bar{x} = 0.10 \pm 0.01$ nest/m²) sites ($p < 0.001$). We also detected significant spatial and temporal variation in the numbers of clinging ants ($p < 0.001$), with counts ranging from 1 to 350 individuals/quadrat and peak numbers found clinging to plants in July ($\bar{x} = 35.5 \pm 13.6$).

The significance of aspen was found to be both temporally and spatially consistent, with 70.4 % (95% CI: 61.6 - 79.2%) and 68.2% (95% CI: 58.3 - 78.1 %) of aspen sites surveyed in 2011 positive in the Alberta and Saskatchewan subsample locations, respectively. Mean metacercariae intensity (ranging from 1 - 250 larvae/host) did not significantly vary over time ($p > 0.5$) with averages of 40.5 ± 26.1 , 27.6 ± 1.8 , and 33.8 ± 8.3 documented in June, July, and August, respectively. Although the total number of clinging ants varied between sites ($p < 0.001$) and over time ($p < 0.01$), the presence of infected intermediate hosts was temporally consistent with clinging ants detected at 100% of the sites surveyed in consecutive years.

2.4.2 Spatial variation in risk of ant-to-ungulate transmission

The original 110 survey sites were used exclusively in model development. Of the positive aspen sites, 60% (95% CI: 50.3 - 69.8 %) had a south to east facing aspect. In contrast, negative sites were found at locations ranging from north to south and east to west

facing. On average, grade of slope varied significantly between positive and negative sites. 77.5% (95% CI: 71.1 - 83.9%) of positive sites were found on intermediate (7.5° to 15°) to steep (>15°) slopes. In contrast, 85% (95% CI: 79.5 - 90.5%) of negatives fell on a gentle slope (< 7.5°). Although the distribution of positive versus negative sites did not differ significantly relative to site elevation ($p > 0.05$), 72.5% (95% CI: 64.2 - 80.7%) of positive sites were found at elevations >1300 m above sea level.

When the presence/absence of clinging ants was modelled, proximity to aspen, slope grade, and slope aspect (eastness or northness) were found to be useful predictors (Table 2.2). Inclusion of site elevation and date of sampling also significantly improved model performance. Various combinations of these parameters were included in the 4 top performing models. Risk of ant-to-ungulate transmission was calculated using each of these models (Table 2.3), and then averaged to account for model uncertainty and projected across CHP, Alberta (Fig. 2.3). Risk was consistently higher in aspen-dominant areas falling on south/east facing slopes with a moderate to high grade, with risk higher at sites above 1300m in elevation.

2.4.3 Model validation

Clinging ants were not detected within the low risk sites. In contrast, 73.3% (95% CI: 51 - 95.7%) of the 15 high-risk sites were positive, with an average of 12.7 ± 6.3 ants found clinging. Of these positive sites, 90% (95% CI: 71.4 - 100%) were found in aspen dominant areas or within 10 m of aspen, with clinging ants detected no more than 8.0 ± 2.9 m from aspen. Further, these sites were consistent with data collected for model development, with 70% (95% CI: 41.6 - 98.4%) of positive sites falling on moderate to high-grade slopes, and 65% (95% CI: 35.4 - 94.6%) on south/east facing slopes, 70% (95% CI: 41.6 - 98.4%) of all

positive sites found above 1300m. Clinging ants were detected at 20% (95% CI: 0 - 44.8%) of the intermediate risk sites surveyed. These two sites were on average 24.0 ± 2.0 m from aspen falling on moderate to high grade, north-east facing slopes. The area under the ROC curve (AUC) was >0.8 (95% CI: 0.7 - 1.0), indicating excellent discriminatory performance to classify areas of risk.

2.5 DISCUSSION

Past geospatial research has predominantly focused on characterizing the ecological covariates for transmission of anthropogenically-significant parasites and diseases (e.g. Malone, 2005; Sehgal *et al.*, 2010). Thus, the fine-scale ecological covariates of many complex lifecycle parasites remain poorly understood. This study presents the first high-resolution (1m^2) analysis aimed at predicting *D. dendriticum* ant-to-ungulate transmission. The limited dispersal and ecological thresholds for the first intermediate host species of *D. dendriticum* in CHP, *Oreohelix* spp. (review by Manga-González *et al.*, 2001; Weaver *et al.*, 2006), along with the conspicuous ‘clinging’ behaviour of infected Formicid ant second intermediate host (review by Manga-González *et al.*, 2001) allowed for the detection of local variation in intermediate host occurrence. Our top models therefore reveal the key ecological covariates of infected intermediate species distribution patterns, allowing for accurate predictions for risk of ant-to-ungulate transmission across a highly complex boreal/grassland habitat.

Our results suggest that the presence of aspen is positively associated with the likelihood of ant-to-ungulate transmission. My data cannot pinpoint the suite of microhabitat conditions that underlie high rates of *D. dendriticum* transmission into ants under aspen canopies. But it is at these sites where the densities of both intermediate hosts

tend to be highest, where they tend to co-occur, and where, presumably, the opportunity for transmission of cercariae into ants is relatively high. Although formicid ants were present in a wide range of habitat types (e.g. fescue grasslands, spruce forests, lodgepole pine forests), the density of ant nests and the numbers of clinging ants increased with their proximity to stands of aspen-dominated forest. Puntilla (1996) has shown that formicid ants require a relatively open canopy for colony establishment and optimal development. Similarly, the diversity and density of terrestrial molluscs, especially the occurrence of *Oreohelix* spp., is associated with forest stands dominated by deciduous trees (Kralka, 1986; Boag & Wishart, 1982; Hendricks, 2003). Aspen-dominant areas are commonly co-located with a dense and complex understory, providing greater foraging opportunities, predator protection, and the availability of micronutrients such as calcium. The presence of this canopy and understory may also provide optimal ambient and soil temperatures, moisture and humidity conditions for the establishment and survival of the intermediate host species of *D. dendriticum* (Kralka, 1986; Weaver *et al.*, 2006; Calder *et al.*, 2011).

My follow-up study in 2011 showed that habitat characteristics within aspen stands are also important predictors of *D. dendriticum* transmission into ants. The two most important are slope aspect and grade. This result was unexpected, but it is consistent with what is known about the micro-distribution of *Oreohelix* spp. Members of this species complex typically occupy south-facing slopes at higher elevations (Hendricks, 2003). Infected *Formica polyctena* have also been documented on southward-facing slopes in southwest Germany (Spindler *et al.*, 1986). Additionally, the prevalence of *D. dendriticum* in grazing beef cattle in NW Spain was also significantly higher among cattle grazing on pastures with a high (>25%) slope grade (Diaz *et al.*, 2007). As sites that are too wet are also not ideal for intermediate host species of *D. dendriticum* (Kralka, 1986), these moderate-

to high-grade slopes provide a well-drained habitat, particularly during heavy rains and during snow-melt. Although significant differences in elevation were not detected between positive and negative sites in my study, the inclusion of elevation did increase the performance of the model. These data are also consistent with the relatively broad elevation range (1,350 -2100m above sea level) that *Oreohelix* spp. are found to occur in the Black Hills of Wyoming and South Dakota (Anderson *et al.*, 2007).

The ecological covariates of the presence of infected zombie ants may also represent the overlap in habitat selection with sympatric definitive hosts known to graze in CHP. Fecal deposition patterns for *Parelaphostrongylus tenuis* and *Fascioloides magna* infected deer indicated a selection of upland deciduous forest (Vanderwaal *et al.*, 2014). In CHP, signs of elk (i.e. pellet groupings) were also lower in areas with medium to heavy conifer, spruce, and shrub cover, (especially shrubby cinquefoil), very light grass cover, and heavy deadfall (Lee, 1970). The incidence of pellet groupings was also low at sites more than 200m from canopy cover and at sites with slopes greater than 20 degrees and north-facing. Although habitat preference studies reveal that elk have a higher preference for forested habitat and steeper slopes than cattle, significant overlap in diet and habitat use has been documented (Lee, 1970). These observations are important because they indicate that while well-drained, aspen-dominated stands provide appropriate conditions for cercariae-ant transmission, they are likely also hot spots for the contamination of the substrate with parasite eggs. In other words, well-drained, south-facing aspen stands appear to provide optimal conditions for transmission into intermediate hosts: a source of worm eggs from a range of definitive hosts, high densities of *Oreohelix* spp., and high densities of formicid ants.

The presence of aspen is the top factor in the spatial models presented here. Given the overriding importance of this component, it follows that factors that determine the distribution and density of this tree species are important in determining the introduction and subsequent spread of this invasive parasite. Aspen establishment in CHP has increased exponentially since 1980, with a 4.4% annual increase and a sharp peak in the mid-1990's (Widenmaier & Strong, 2010). Peak aspen encroachment in the park approximately coincides with the timing of *D. dendriticum* emergence in sympatric elk, deer, and cattle (Goater and Colwell, 2007). Although it would be very difficult to assign direct causation between aspen encroachment and the emergence of *D. dendriticum*, the coincident timing is striking. The sharp increase in aspen establishment is likely a consequence of a long-term response to a 70-year fire-suppression program, although there has also been a general increase in number of frost-free days in the park (~6 days/decade; Curforth *et al.*, 2004), an increase in summer precipitation by 3.9 mm/decade, and cooler average summer temperatures (Widenmaier & Strong, 2010). Aspen encroachment and more favourable climate conditions may therefore have cumulatively provided ample 'vacant' habitat for ant and snail intermediate hosts that in turn influenced the frequency of contact (Cleaveland *et al.* 2007) between *D. dendriticum* infective stages and susceptible ungulate hosts. Forest composition and structure, resulting from forest management, has also significantly affected the distribution and prevalence of the meningeal worm, *Parelaphostrongylus tenuis*, in white-tailed deer (Vanderwaal *et al.*, 2015). These changes impacted transmission opportunities by influencing intermediate host abundance and modifying the thermal conditions and vegetation composition of the microenvironment. Overall, these results provide an excellent example of the profound role that anthropogenic habitat modification (in this case, fire suppression) plays in determining key epidemiological rate parameters.

The results also highlight how anthropogenic factors can impact the rate of introduction of parasites outside their native ranges, and potentially, further spread into new habitats.

The *D. dendriticum* transmission risk model may also have important management implications. Among naturally and experimentally infected animals, the presence of infection has resulted in changes in liver function (i.e. increased production of bilirubin, albumen, hepatic enzymes, etc.), liver cholangitis and fibrosis, anaemia and reduced weight gain (review by Manga-González *et al.*, 2001). Chemical treatment for this parasitic infection is, however, ineffective in final hosts (review by Otranto & Traversa, 2002; Manga-González *et al.*, 2005). Additionally, the use of pesticides and molluscicides to limit transmission is inconceivable. The extrapolation of the model presented here within CHP, as well as in to other ecologically similar habitats, spatially delineates where risk of transmission is greatest to cattle and other definitive hosts. Projections of this model could therefore be useful in directing targeting grazing practices and in selecting specific sites for grazing exclusion fencing to reduce risk of exposure.

I expect that the extrapolation of my model will likely have greatest significance within the distributional range of the *Oreohelix* spp. complex, including regions throughout the Rocky Mountains and the Black Hills of Wyoming and South Dakota (Weaver *et al.*, 2006). However, wider application of this model on a global scale may be limited. Given the cosmopolitan distribution of this fluke, and the broad spectrum of intermediate and final host species that can be utilized, considerable species-specific variation in environmental tolerance ranges can be expected (Brooker *et al.*, 2002). This is likely to be a common issue for the extrapolation of geospatial models at both broad and local scales (e.g. Brooker, 2007). *D. dendriticum* has now been reported in North America from sites in Newfoundland to Canada's far west coast (B.J. van Paridon, Ph. D. thesis, unpublished

observations). *Oreohelix* spp. are not present at either location. Further, high intensities of *D. dendriticum* (~10,000 flukes per liver) have been documented among grazing sheep on the island of Coll, Scotland (Sargison *et al.*, personal communication), evidence of a high rate of snail-to-ant-to-ungulate transmission despite the distinct absence of treed areas. Thus, future research would need to account for differences in ecological zones and microhabitat requirements of acting intermediate hosts in each respective area. Future studies would also benefit from the incorporation of parasite transmission complexities, such as the density of intermediate and final host species and variability in parasite intensity among hosts.

Elucidation of the fine-scale heterogeneity of the presence of *D. dendriticum* infected zombie ants provided valuable insights into the epidemiology of this invasive trematode. The spatial model presented here describes the microhabitat preferences of *D. dendriticum* infected ant and snail intermediate hosts and, in turn, spatially delineated where risk of intermediate host-parasite contact is amplified - i.e. in aspen dominant sites with calcareous, alkaline soils on a well-drained, south or east facing slope. These patterns can be used to project risk of parasite emergence beyond the contemporary distributional range. The local-scale approach depicted here may also be used to test the mechanisms by which heterogeneity in local environment influences other indirectly transmitted parasites. An improved understanding of parasite ecology will likely become increasingly important as opportunities for the biological introduction of non-indigenous species and the spill-over of novel parasites into naïve hosts will be amplified by impending global climate change and the continued encroachment on wildlife habitat (Cross *et al.*, 2009; Hoberg, 2010; Hatcher *et al.*, 2012).

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Table 2.1: Summary statistics of pair-wise correlations for selected habitat characteristics and infection characteristics in ants infected with larval *D. dendriticum*. Values above the diagonal are Spearman rho correlations. Significance values are below the diagonal.

	Distance from aspen (m)	Number of clinging ants	Mean no. parasites/ant	Nest density
Distance from aspen (m)		-0.667	-0.675	-0.320
Number of clinging ants	< 0.001		0.963	0.375
Mean no. parasites/ant	< 0.001	< 0.001		0.386
Nest Density	0.010	0.002	0.002	

Table 2.2: Summary of top multivariate hierarchical models for risk of *D. dendriticum* transmission from ants to definitive hosts. Models are ranked based on the Deviance Information Criterion (DIC). Month of sampling is included in all models as a fixed effect.

Rank	Model Parameters	DIC	Δ DIC
1	Distance from aspen (m), Elevation (m), Eastness, Northness, Slope (%)	51.6	0
2	Distance from aspen (m), Elevation (m), Eastness, Slope (%)	51.9	0.2
3	Distance from aspen (m), Eastness (m), Slope (%)	53.3	1.6
4	Distance from aspen (m), Elevation (m), Eastness, Northness	53.3	1.6
Null model	-	129.1	77.5

Table 2.3: Model coefficient values for local ecological covariates of the presence of *D. dendriticum*-infected ants.

Model	Variable	Parameter Mean	SD	B	SE B
1	Intercept	-	-	-27.7	0.2
	Distance from aspen	108.7	310.7	-171.2	0.3
	Eastness	-	-	2.2	<0.1
	Elevation	1360.9	67.1	-1.6	<0.1
	Northness	-	-	0.9	<0.1
	Slope	9.7	6.5	1.8	<0.1
2	Intercept	-	-	-24.9	0.2
	Distance from aspen	108.7	310.7	-151.8	0.3
	Eastness	-	-	1.9	<0.1
	Elevation	1360.9	67.1	-1.8	<0.1
	Slope	9.7	6.5	1.9	<0.1
3	Intercept	-	-	-23.7	0.2
	Distance from aspen	108.7	310.7	-145.1	0.2
	Eastness	-	-	1.9	<0.1
	Slope	9.7	6.5	2.0	<0.1
4	Intercept	-	-	-26.3	0.5
	Distance from aspen	108.7	310.7	-158.6	0.6
	Eastness	-	-	1.8	<0.1
	Elevation	1360.9	67.1	-1.7	<0.1
	Northness	-	-	0.9	<0.1

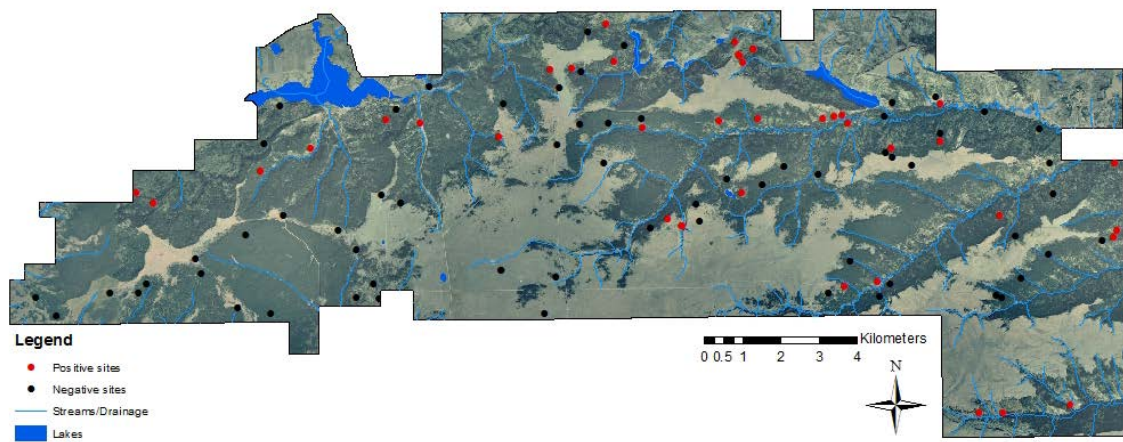


Figure 2.1: Distribution of 110 study sites in Cypress Hills Interprovincial Park, Alberta sampled in snow-free months of 2010 and 2011.

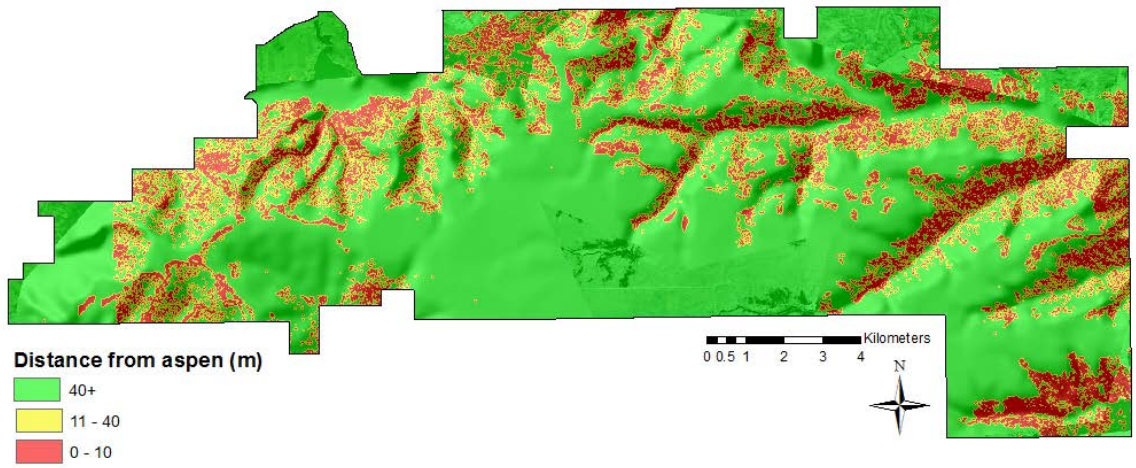


Figure 2.2: A distance model for Cypress Hills Interprovincial Park, Alberta depicting the calculated distance (m) from aspen-dominant canopy.

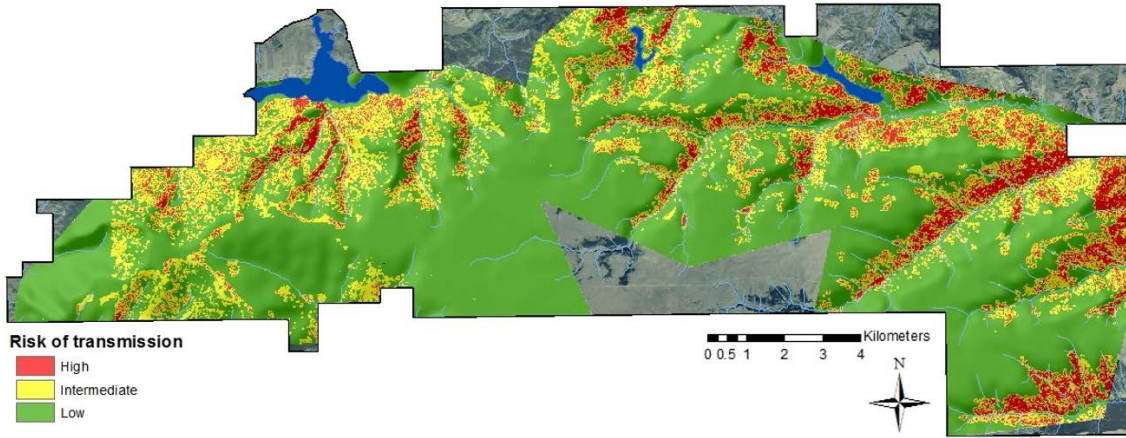


Figure 2.3: Expected risk of ant-to-ungulate transmission for *D. dendriticum* in Cypress Hills Interprovincial Park, Alberta. Risk of infection ranged from 0 (no risk) to 1 (highest risk); low risk < 0.3, intermediate risk = 0.3-0.6, high risk \geq 0.7.

CHAPTER 3

Fluke abundance versus host age for an invasive trematode (*Dicrocoelium dendriticum*) of sympatric elk and beef cattle in southeastern Alberta, Canada

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This chapter has been published in the *International Journal for Parasitology: Parasites and Wildlife* (2014, 3: 263 - 268)

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3.1 ABSTRACT

Epidemiological parameters such as transmission rate, rate of parasite-induced host mortality, and rate of development of host defenses can be assessed indirectly by characterizing the manner in which parasite burdens change with host age. For parasites that are host generalists, estimates of these important parameters may be host-species dependent. In a cross-sectional study, we determined age-abundance profiles of infection in samples of sympatric free-ranging elk and domestic cattle infected with the lancet liver fluke, *Dicrocoelium dendriticum*. This parasite was introduced into Cypress Hills Interprovincial Park in southeastern Alberta, Canada in the mid-1990s, and now occurs in 60-90% of co-grazing elk and beef cattle examined at necropsy. The livers of 173 elk were made available by hunters during the 1997- 2011 hunting seasons and livers from 35 cattle were purchased from ranchers. In elk, median worm abundance peaked in 6-24 month-olds (median = 72, range = 0-1006) then significantly declined to <10 worms/host in 10-16 year olds. The decline in fluke burden with age is not consistent with an age-related decline in exposure to metacercariae in intermediate hosts and high rates of fluke-induced host mortality are unlikely. Rather, the pattern of peak fluke burdens in elk calves and juveniles, followed by a decline in older animals is consistent with the development of a protective immune response in older hosts. There was no pattern of worm accumulation or decline in sympatric cattle, although statistical power to detect a significant effect was low. These results highlight the complexity and context-dependent nature of epidemiological processes in multi-host systems.

3.2 INTRODUCTION

Heterogeneity in parasite burdens within and among host populations is one of the hallmarks of ecological epidemiology. Even under laboratory conditions where features such as parasite exposure rate, host immune status, host condition, and environmental characteristics are tightly controlled, heterogeneity in parasite burdens between hosts is often very high (Hudson & Dobson, 1995; Wilson *et al.*, 2002; Tompkins *et al.*, 2011). Not surprisingly, when among-host heterogeneity is evaluated within naturally occurring host-parasite interactions, differences between hosts can vary by several orders of magnitude. In these complex, natural systems, epidemiological parameters such as transmission rate, rate of parasite-induced host mortality, and rate of development of host defenses can be assessed indirectly by characterizing the manner in which parasite burdens change with host age. Thus, the determination of the relationship between host age and parasite abundance has important epidemiological and management implications (Hudson & Dobson, 1995; Boag *et al.*, 2001). These implications can be expected to be especially important for generalist parasites that are shared among sympatric host species, especially for those species of parasite that have been introduced outside their native range. Yet our understanding of general epidemiological processes, including the relationships between host age and parasite burden, remains limited for generalist parasites in multi-host systems (Wilson *et al.*, 2002; reviews by Tompkins *et al.*, 2011).

The lancet liver fluke, *Dicrocoelium dendriticum* (Trematoda: Dicrocoeliidae), is a parasite of the bile ducts of grazing mammals. Evidence from experimental exposures of domestic stock to metacercariae (Manga-González & González-Lanza, 2005), together with molecular sequencing of worms collected from several species of domestic and wildlife hosts (B.J. van Paridon *et al.*, unpublished Ph.D. Thesis), have confirmed that *D.*

dendriticum is a host generalist. This species has been widely introduced outside its native range in mainland Europe, including into various locations in western and eastern North America (Lewis, 1974; Goater & Colwell, 2007). In Cypress Hills Interprovincial Park (CHP) in south-eastern Alberta, where *D. dendriticum* was introduced in approximately the mid 1990s, prevalence now ranges from 60% to 90% in sympatric deer, elk, and beef cattle each year, with intensities frequently exceeding 1000 worms per host (Goater & Colwell, 2007). In samples of hosts from this location, the opportunity exists to evaluate the association between host age and worm abundance in different host species within a region of known sympatry. The purpose of this study is to evaluate relationship between host age and *D. dendriticum* abundance in sympatric free-ranging elk and cattle in CHP.

3.3 MATERIALS AND METHODS

3.3.1. Cypress Hills Interprovincial Park

The Park straddles the southern Alberta-Saskatchewan border in southwestern Canada (49°37.5'N, 110°W). It is a complex habitat mosaic that encompasses two distinct ecological sub-regions embedded within the extensive 'Grassland Natural Region' (Downing and Pettapiece, 2006). Vegetation characteristic of the mixed grass natural sub-region is dominant in sites below approximately 1100 m in CHP, including extensive stands of plains rough fescue, western porcupine grass, sedges, and various forbs. At elevations >1100 m, vegetation characteristic of the montane natural subregion dominates, with characteristic grasses and forbs, interspersed with stands of lodgepole pine (*Pinus contorta*), white spruce (*Picea glauca*), trembling aspen (*Populus tremuloides*), and balsam poplar (*P. balsamifera*). Together, the combination of these two sub-regions that co-occur within the relatively narrow confines of the Park (531,000 hectares) creates an island-like habitat that is

distinctive from the adjacent and highly cultivated lowland grasslands (the extensive dry mixed grass natural subregion that is characteristically known as ‘prairie’). Regions of the park above approximately 1250 m were unglaciated by the Wisconsin Laurentide Ice Sheet (Stalker, 1965).

The community of sympatric large herbivores on the Alberta side of CHP includes approximately 700 elk, 300 mule deer (*Odocoileus hemionus*), 300 white-tailed deer (*Odocoileus virginianus*), and 4000 beef cattle (Alberta Tourism, Parks and Recreation, 2013). Elk (*Cervus canadensis*) were re-introduced into the Park in 1938. Annual fall hunts have been regulated and monitored by Park’s personnel since 1978 to establish a density of 350–700 resident elk. Beef cattle have grazed within almost all regions of the Park since 1918, typically grazing cow–calf pairs or yearlings that are introduced in early June and removed in late October (Hegel *et al.*, 2009). Upon removal from the Park cattle receive a dose of broad-spectrum antiparasitic drugs (i.e. ivermectin, moxidectin) which are ineffective against *D. dendriticum* infection (Ballweber & Baeten, 2012).

3.3.2. Fluke counts

Opportunistic collections of whole elk livers from hunters began in autumn 1997 and continued haphazardly until 2005. Collections occurred annually, each fall, from 2009 to 2011. During each hunting season, data on kill site, gender, and approximate age (calves \leq 6 months, juveniles = 6 – 24 months, adults \geq 24 months) were collected by Park’s personnel for all elk shot within the Park. Calf, juvenile, and adult cattle were purchased opportunistically from two of the three CHP stock associations between 2003 and 2013. All sampled cattle grazed within the park from early June to late October each year since birth. Data on host age and sex were made available from stockowners.

Livers from elk were harvested within 4 h of host death and frozen whole; cattle livers were examined immediately after slaughter. The dissection of individual livers for enumeration of worms followed methods described by Goater and Colwell (2007). Each liver was thawed, weighed, and cut into approximately 5-mm-wide strips. The sections were placed into saline-filled pans and gently palpated to dislodge flukes from the hepatic and bile ducts. Once all sections were processed, the pans were examined for the presence of flukes. Flukes were enumerated using a dissection microscope and then preserved in ethanol.

3.3.3. Analyses

We follow the terminology of Bush *et al.* (1997) to describe parasitological parameters. Prevalence is the proportion of infected hosts within a sample of hosts and abundance is the total number of parasites in an infected host, including those that were uninfected. We used mean abundance to describe the central tendency of worm counts within a sample of hosts. Due to limited sample sizes associated with the opportunistic sampling program, analyses involved data pooled between years. The distribution of worms within a sample of hosts was estimated by exponent k of the negative binomial distribution (NBD) using Quantitative Parasitology 3.0 (Reiczigel & Rozsa, 2005). The overall distributions of worms within the samples of elk and cattle were highly aggregated, and worm counts could not be transformed to normality. Thus, for comparison of worm counts between pairs of samples, we used non-parametric Mann-Whitney U tests. Kruskal-Wallis tests were used to evaluate differences in medians between multiple samples.

The initial analysis of the association between host age, liver weight, and fluke abundance focused on 61 elk livers collected during the 2009–2011 hunting seasons. These

hosts were aged by evaluation of the cementum of a single I-1-lower incisor tooth per animal (Matson, 1981). Previous studies have confirmed that this method is the most accurate for ageing elk and other grazing ungulates (Hamlin *et al.*, 2000). Ageing was completed in a commercial laboratory (Matson Laboratories Inc., Missoula, Montana) by two independent technicians that were blind relative to the source and identity of each animal. There was no significant difference in median abundance in relation to host gender (number of males = 115, number of females = 51; $p = 0.71$) so the genders were pooled for all subsequent analyses unless stated otherwise.

For analyses, data on the relationship between host age and worm counts were fitted to a generalized linear model with an underlying negative binomial error distribution using maximum likelihood in R (R Core Team, 2014). This analysis is appropriate for count data that are highly aggregated and for data sets that involve unequal and small sample sizes (Pacala & Dobson, 1988). Associations between elk liver weight and worm abundance for each gender were also evaluated using maximum likelihood methods.

We analyzed a second sample of 112 elk livers collected opportunistically during the 1997–2005 hunting seasons to complement our assessment of age-related effects. Elk collected during this period were not aged via analysis of the cementum and therefore cannot be fitted to the age–infection profile used in the previous analysis. Rather, count data from individual hosts collected during this period were pooled with data from cementum-aged hosts (total $n = 173$), and then assigned into the broad age categories described in Goater and Colwell (2007): calf (~6 months old); juvenile (~6 – 24 months old); and adult (24+ months old). Confidence intervals for prevalence values were calculated using the Wald method (Vollset, 1993; $p \pm z\sqrt{pq/n}$, where $z = 1 - \alpha/2$ of the standard normal distribution and $q=1-p$).

Variation in median worm abundance with cattle age was assessed from a sample of 35 cattle that had grazed from early June to end of October in CHP for consecutive years. Differences in median intensities were assessed using non-parametric Mann-Whitney U tests. Data on the relationship between host age and worm abundance were fitted to a generalized linear model with an underlying NBD using maximum likelihood following the approach described earlier.

3.4 RESULTS

The overall distribution of *D. dendriticum* within the sample of 61 elk aged via cementum analyses did not differ significantly from the theoretical model of NBD ($X^2 = 12.7$, $p > 0.05$; $k = 0.19$). For the sample of 61 elk of known age, worm prevalence did not significantly differ ($X^2 = 2.7$, $p = 0.14$) between juveniles (76%, 95% CI: 60 - 91%) and adults (65%, 95% CI: 49 - 82%). However, worm abundance varied significantly with host age ($X^2 = 5.3$, $p < 0.01$), with data fitting the NBD ($\ln(Y_i) = 5.18 - 0.24X_i$; $D_i = -539.2$; $p < 0.001$; Fig. 3.1). The results also showed that some individuals were exposed to metacercariae within their first grazing period at approximately 3-6 months of age (Fig. 3.1). Worm counts were highly variable within 0.5 - 2-year olds (Fig. 3.1), with some individuals accumulating up to 1000 worms. Beyond 2 years of age, there was a sharp decrease in worm numbers, such that by approximately age 5, few hosts contained greater than 100 worms.

For data pooled from 1997 to 2011, median worm abundance was significantly higher in calves and juveniles ($n = 73$) compared to adults ($n = 100$, $p < 0.001$; Table 3.1). Approximately 70% (95% CI: 62 - 76%) of the total sample of hosts contained less than 100 worms; 5% (95% CI: 3 - 9%) contained greater than 1000 adult worms (Fig. 3.2). Of

the nine animals harboring over 1000 worms, five were calves and three were juveniles, with four of the five calves containing greater than 2000 worms. In contrast, 90 of the 120 animals containing less than 1000 worms were adults.

The distribution of worm counts within cattle did not differ significantly from the theoretical model of NBD ($X^2 = 11.9$, $p > 0.05$; $k = 0.39$). Median worm abundance did not significantly differ between males and females (number of males = 6, number of females = 29, $p = 0.31$). Approximately 84% (95% CI: 71-98%) of cattle under the age of 2 are infected with *D. dendriticum* while all adult cattle included in our sample were infected. Worm abundance did not significantly vary with cattle age ($\ln(Y_j) = 5.3 + 0.1X_j$; $D_j = -436$; $p > 0.05$; Fig. 3.3). The power (Cohen, 1992) to detect the effect of age in cattle was very low (power: 0.24, $n = 35$, $p < 0.01$) as a result of low sample sizes and highly variable worm counts, particularly for >2 year old hosts. Fluke intensities were highly variable in cattle regardless of age (Fig. 3.3); ranging from 9 to 983 worms in calves and juveniles and from 1 to 1490 in adults. The highest counts (1490 worms) occurred in a 4-year old cow.

Adult elk had significantly higher median liver weights than calves/juveniles, for both males ($n = 76$, $p < 0.001$) and females ($n = 28$, $p < 0.01$). Liver weight in cows increased non-linearly with age ($Y_j = 3.7 - 2.54 \times \exp(-0.42 \times X_j)$; $R^2 = 0.82$, $df = 10$, $SSE_R = 0.36$), with livers tending toward an asymptote in adults older than 6 years (Fig. 3.4). Livers were not available for bulls older than 6 years of age. For <6 year old bulls, liver weight increased linearly with age ($Y_j = 2.75 + 0.33X_j$; $R^2 = 0.54$, $F_{1,42} = 34.22$, $p < 0.001$). For bulls, worm abundance significantly declined as liver weight increased ($\ln(Y_j) = 9.27 - 1.30X_j$; $D_j = 36.47$; $p < 0.05$). This association was not statistically significant for cows ($D_j = 14.62$; $p > 0.05$).

3.5 DISCUSSION

Results from the analyses of the two elk data sets indicate that the general pattern of worm counts with host age involves a peak at approximately 2 years of age, followed by a decline. These results are consistent with the earlier observations by Goater and Colwell (2007) on a restricted sample of elk collected from the same region. The results are also consistent with those from longitudinal field studies involving *D. dendriticum* in domestic cattle (González-Lanza *et al.*, 1993) and sheep (Manga-González *et al.*, 1991) in which fecal egg counts were higher in calves and juveniles compared to adults. These results indicate that, for elk, there is a rapid increase in worm numbers during the first 1–2 years, followed by a significant decline thereafter. In contrast, the clear age-related pattern in elk was absent in cattle that co-grazed on the same pasture. These contrasting results in elk versus cattle highlight the context-dependent nature of key epidemiological processes for generalist parasites in multi-host systems.

Our analysis of the cementum-aged elk provides the strongest evidence for a significant age versus abundance relationship. These results indicate a rapid rise in worm abundance in 1–2 year old elk, with the accumulation of worms following single or multiple exposures to metacercariae between June and September. Unpublished data from our laboratory indicate that the density of metacercariae infected ants in CHP peaks in June and early July, coinciding approximately with the initiation of grazing that occurs 2–3 months after birth (Robbins *et al.*, 1981). The density of infected ants on vegetation then declines on pasture toward the end of summer (C.P. Goater, unpublished data). On average, individual ants collected clinging to plants in the same areas of CHP as the hosts necropsied in this study harbour from 1 to 255 metacercariae ($x = 27 \pm 22$, $n = 143$; C.P. Goater, unpublished data). Thus, the high variation in fluke abundance that was observed in naive calves and also

yearling elk is best explained by chance ingestion of infected ants that contain variable numbers of metacercariae. This pattern of stochastic encounter with infected ants likely repeats during their second spring/summer, when prevalence reaches 90% in 2-year-olds.

Several mechanisms have been proposed to explain peaked patterns of abundance with host age (Anderson & Gordon, 1982; Duerr *et al.*, 2003). The simplest explanation for the observed pattern in elk lies in the stochastic encounter of 2–3 month old calves with metacercariae in a small number of infected ants, followed by worm senescence at 1–2 years. Experimental studies involving sheep exposed to known numbers of metacercariae show that adult worms live for at least 270 days (Hohorst & Lanmler, 1962). Similar studies in our laboratory involving sheep and cattle show that 150-day-old worms continue to produce eggs (D.D. Colwell, unpublished data). Thus, it is conceivable that a single period of exposure of calves to metacercaria during their first grazing season, followed by worm senescence at 1–2 years could explain the peaked pattern of fluke abundance in elk.

There are two reasons why this simple exposure/death scenario is unlikely in this system. First, there is no supportive evidence for an age-related change in habitat or diet that could lead to reduced rates of exposure to metacercariae in ants. Results from field studies on individual elk, including those in Cypress Hills Interprovincial Park (Lee, 1979), have shown that grasses and forbs dominate the diets of both juvenile and adult elk (Baker & Hobbs, 1982; Cook *et al.*, 1996). Further, Wickstrom *et al.* (1984) showed that elk increase their intake of grasses and forbs as they age. Second, experimental exposures of sheep and cattle to known numbers of metacercariae have consistently shown rates of recovery between 10% and 20% (Campo *et al.*, 2000; D.D. Colwell, unpublished data). Such low recovery of adults implies that many metacercariae within individual ants are not infective, or that the rate of metacercariae mortality during ingestion/

migration/establishment is high. These results mean that single, or at least restricted, encounters with infected ants are unlikely to lead to the intensities of infection that we observed in elk. Rather, these hosts are probably frequently exposed to metacercariae throughout each grazing season, and rates of exposure increase due to increased vegetation intake as hosts age.

Fluke-induced host mortality, concentrated in older hosts, could also explain peaked age-abundance patterns (Anderson & Gordon, 1982; Duerr *et al.*, 2003). There exists no evidence that *D. dendriticum* contributes to host mortality, although the key experiments have not been done in wildlife. Experimental studies in sheep indicate that mortality is rare in infected hosts where fluke counts are significantly higher than observed in CHP (Sargison *et al.*, 2012). Subtle and chronic effects range from minor irritation of the mucosal lining of the bile ducts, to mass fibrosis and cholangitis of liver tissue (Manga-González & González-Lanza, 2005). In extreme cases, hepatobiliary changes in infected sheep cause reductions in weight gain and milk production, and can cause necrosis of the skin and other pathologies associated with general inflammatory defenses (Sargison *et al.*, 2012). Although cases of mortality associated with *D. dendriticum* appear to be rare, interactions with other forms of mortality such as predation, hunting, malnutrition, other parasites and pathogens, and extreme weather could result in the removal of heavily infected hosts. These interactions would have to occur consistently within almost all elk >2 years of age to produce the decline in abundance observed in our data. Nonetheless, a key implication of the age-abundance pattern observed in elk is that studies aimed to understand the effect of *D. dendriticum* on wildlife should initially focus on calves, where peak-intensities coincide with small liver sizes.

Exposure-dependent acquired immunity could also explain the decline in worm

abundance at 2 years in elk. Exposure-dependent immunity of this type has been invoked to explain age-related declines for macroparasites of humans, domestic stock, and laboratory animals (Woolhouse, 1998). The results of experimental studies indicate that established macroparasites often promote the production of anti-parasite antibodies, leading to protection against subsequent exposures (Srisawangwong *et al.*, 2011). In natural systems, a delayed antibody-based response to nematodes has been documented in wood mice, *Apodemus sylvaticus* (Quinnell, 1992), and Soay sheep, *Ovis aries* (Gulland & Fox, 1992). In both of these cases, a delayed immune response best explained convexity in the age-abundance pattern in field-collected hosts. Although anti- *D. dendriticum* antibodies have been detected in experimentally and naturally infected sheep (Manga-González & González-Lanza, 2005; Revilla-Nuín *et al.*, 2005) and cattle (Wedrychowicz *et al.*, 1995; Colwell & Goater, 2010), the protective nature of these antibody responses and their role in determining liver fluke burdens requires further research, particularly within wildlife hosts.

The lack of an association between age and fluke burdens in beef cattle must be interpreted with caution due to low statistical power. But despite the low numbers of >2 year old cattle in our sample, it is striking that two of these hosts were among the most heavily infected animals in the overall sample of hosts. The most heavily-infected cow was a 4-year-old that had grazed within the Park since birth. Likewise, the heavily-infected 10-year-old cow had also grazed in the Park since birth. These anecdotal observations on a few hosts contrast with the pattern observed in elk in which only 1 of 32 hosts that was >2 years of age contained more than 100 flukes. One explanation for these results is that these older, heavily-infected cattle were not exposed to metacercariae during earlier grazing seasons. Given the high prevalence in our sample of 1- and 2-year olds, this is unlikely. An alternative explanation is that in contrast to elk, some cattle accumulate worms as they age,

despite the presence of anti-*D. dendriticum* antibodies (Colwell & Goater, 2010). This interpretation is consistent with the results of studies involving domestic stock exposed to *Fasciola hepatica*, in which the presence of anti-fluke antibodies was not associated with protection from further infection (Cleary *et al.*, 1996; Bossaert *et al.*, 2000).

As expected, the distribution of liver flukes was highly aggregated within the samples of cattle and elk. This characteristic pattern means that a restricted proportion of both populations of hosts is responsible for the contamination of pasture in CHP with liver fluke eggs. Our results indicate that host age, at least for elk, contributes to this pattern of aggregation. Crude calculations that take into account our data on worm prevalence, worm abundance, and estimates of host population size can be used to estimate the relative roles of the cattle population versus the elk population in the dissemination of eggs onto pasture. Winter aerial surveys in CHP indicate a fairly stable population of approximately 800 elk (Hegel *et al.*, 2009). Based on studies completed on other well-studied elk populations, calves comprise approximately 20% of a total herd (Bender & Piasecke, 2010). Thus, in CHP, the subpopulation of calf elk (about 160 hosts) harbour about 60% of the ~60,000 worms in the total worm population. In contrast, for cattle, approximately 2000 calves and their 2000 mothers introduced into CHP each spring will harbour approximately 1.1×10^6 worms a few months later, with both age classes contributing roughly similar numbers of fluke eggs onto pasture. These crude calculations imply that the cattle population, on account of its larger size and the absence of a reduction in worm counts with host age, contributes roughly 95% of the total population of fluke eggs that are produced from these two species of hosts. Further empirical studies involving more species of potential definitive host, together with the application of epidemiological models, could increase our precision regarding the relative roles of different species of host in disseminating infective stages of

generalist parasites.

3.6 ACKNOWLEDGEMENTS

We appreciate the generous cooperation of the stockowners, big-game hunters, ranchers, Parks personnel, and Provincial wildlife authorities of Cypress Hills Provincial Park, Alberta, Canada without which this project would not have been possible. Funding from The Alberta Conservation Association Grant in Biodiversity, the Beef Cattle Research Council (Grant no. 2008F064), and Agriculture Funding Consortium (Grant no. AB-0450) is gratefully acknowledged. To the best of our knowledge the content of this paper is presented with no possible conflicts of interest for the contributing authors.

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Table 3.1: Prevalence and abundance of *D. dendriticum* in hunter-shot elk from Cypress Hills Interprovincial Park, Alberta between 1997-2011.

Age	Years	N	Prevalence (\pm 95% CI)	Mean		
				Abundance (\pm SD)	Median	Range
All	1997-2000	17	94 \pm 11	91 \pm 169	17	0-653
	2003-2005	95	79 \pm 19	428 \pm 769	37	0-4343
	2009-2011	61	71 \pm 22	136 \pm 239	10	0-1006
Calves and Juveniles (\leq 2 years of age)	1997-2000	5	80 \pm 19	277 \pm 270	194	0-653
	2003-2005	39	90 \pm 14	774 \pm 988	278	0-4343
	2009-2011	29	76 \pm 20	237 \pm 300	38	0-1006
	Pooled	73	84 \pm 8	548 \pm 811	192	0-4343
Adults ($>$ 2 years of age)	1997-2000	12	100	29 \pm 47	14	1-167
	2003-2005	56	71 \pm 22	126 \pm 264	11	0-1102
	2009-2011	32	66 \pm 23	31 \pm 53	4	0-243
	Pooled	100	73 \pm 0.9	82 \pm 203	35	0-1102

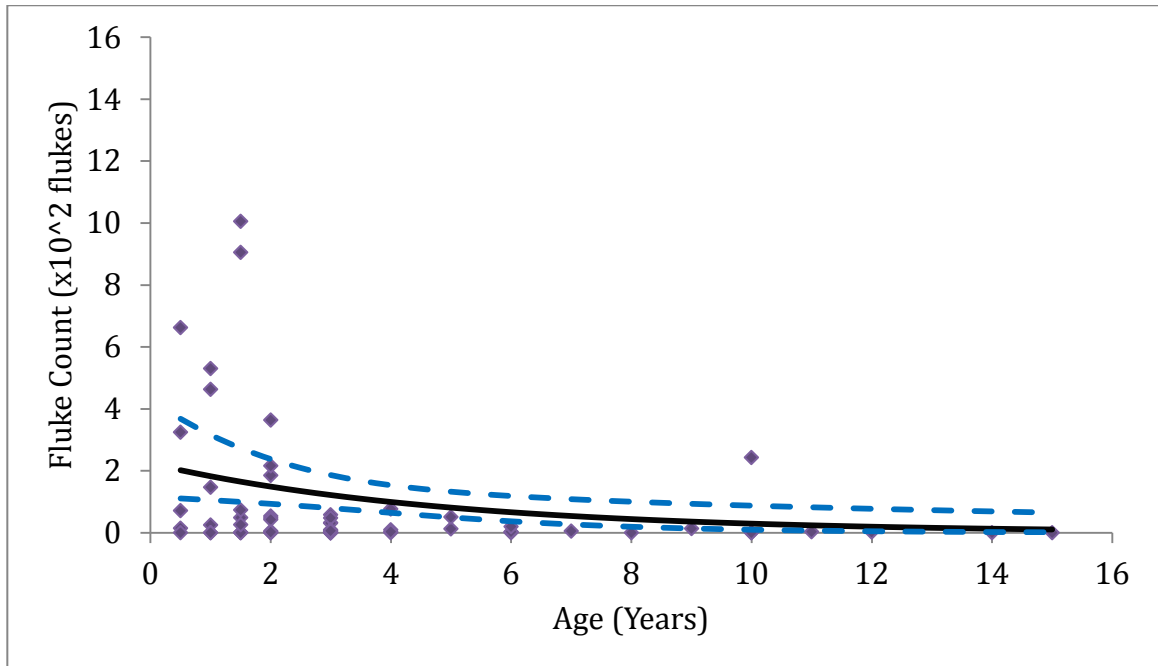


Figure 3.1: Age-abundance profiles for the trematode, *D. dendriticum* in a population of elk sampled from 2009 to 2011 from Cypress Hills Interprovincial Park, Alberta. The solid line represents the negative binomial distribution model fit using maximum likelihood; the dashed lines represent the 95% confidence intervals.

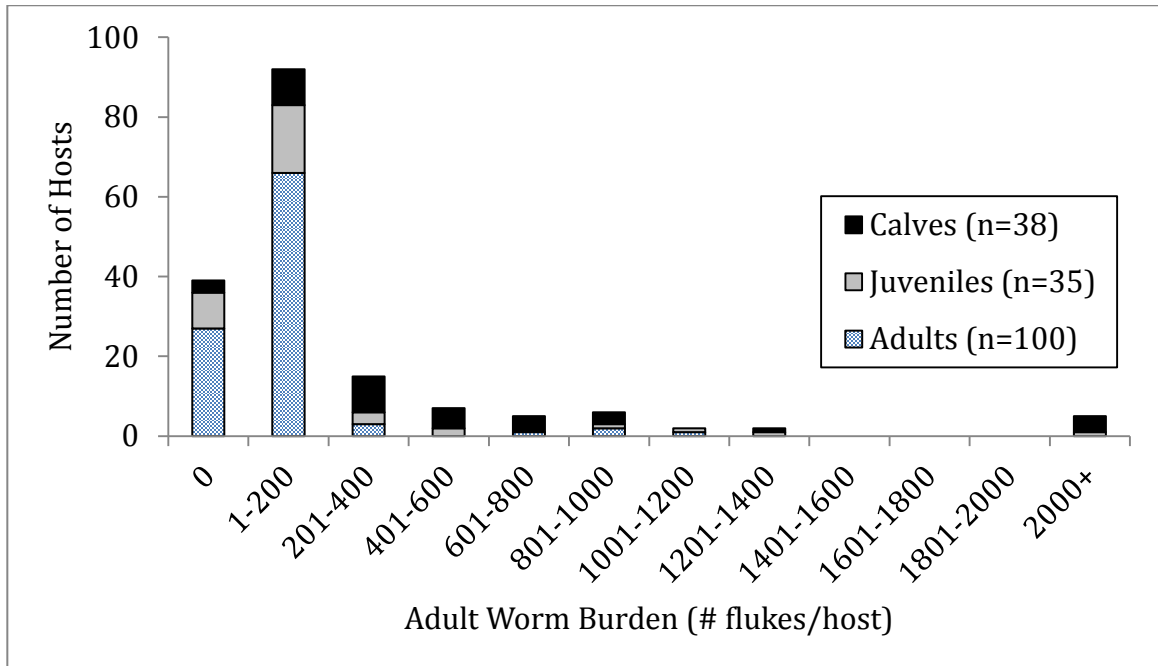


Figure 3.2: Stacked frequency distribution of adult *D. dendriticum* in calf, juvenile, and adult elk collected between 1997 and 2011 from Cypress Hills Interprovincial Park, Alberta.

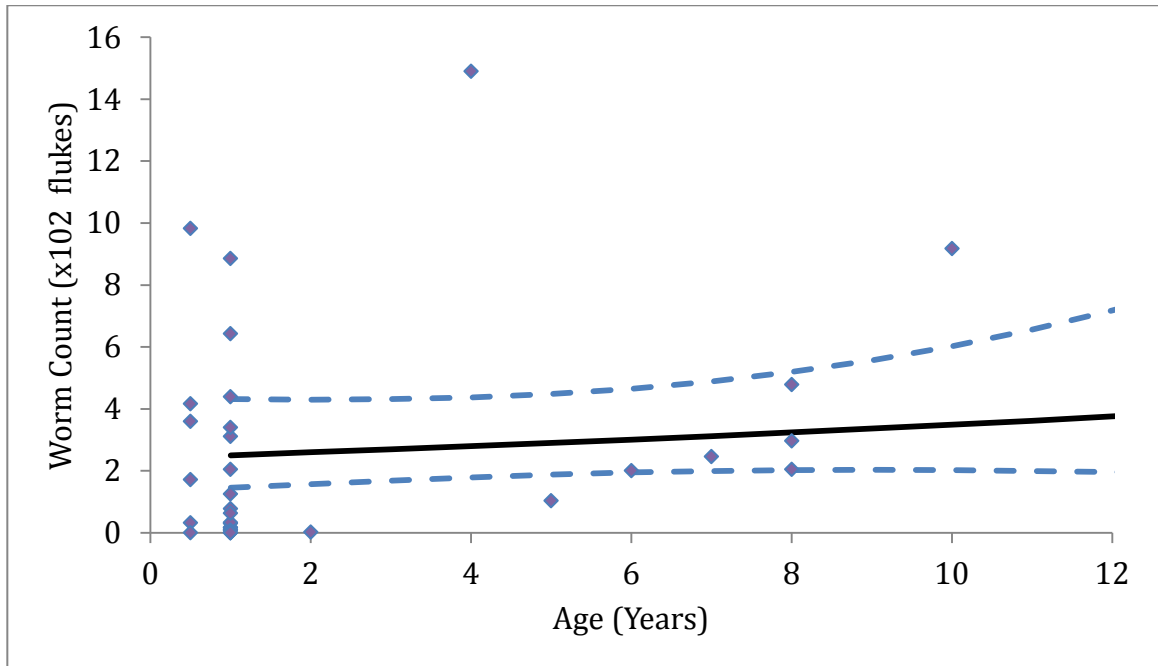


Figure 3.3: Age-abundance profile of infection for the invasive trematode, *D. dendriticum* in beef cattle sampled from 2003 to 2013 from Cypress Hills Interprovincial Park, Alberta. The solid line represents the negative binomial distribution model fit using maximum likelihood; the dashed lines represent the 95% confidence interval.

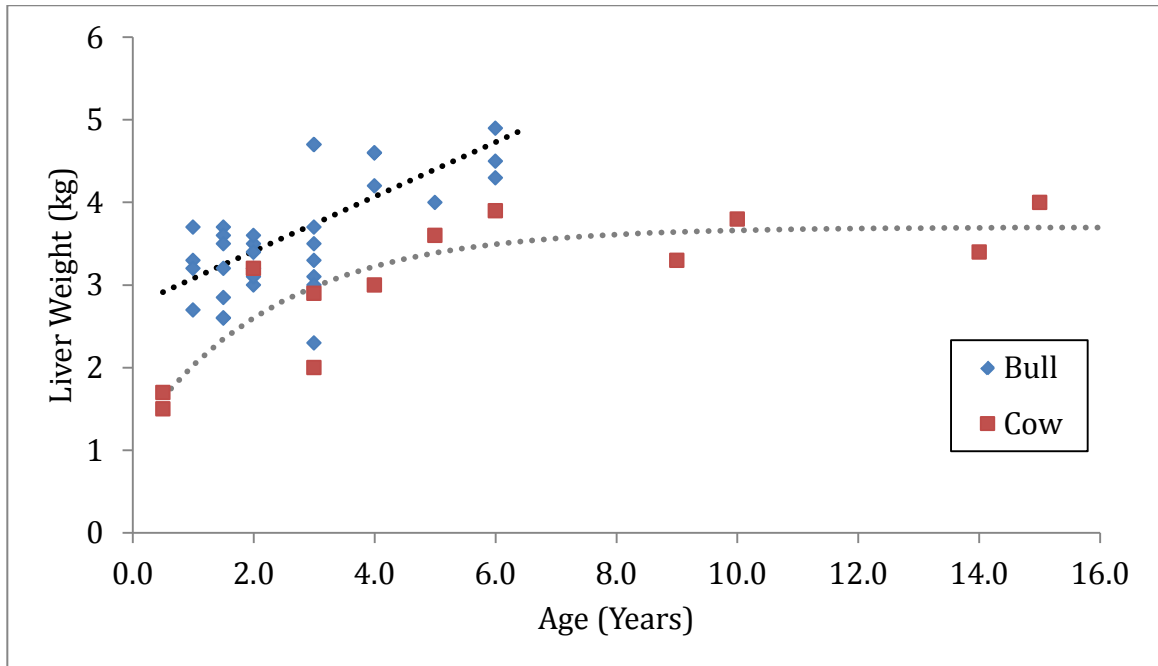


Figure 3.4: Relationship between liver weight and host age for elk sampled from 2009 to 2011 from Cypress Hills Interprovincial Park, Alberta. Regression lines are maximum likelihood estimates.

CHAPTER 4

Comparative recruitment, morphology and reproduction of a generalist trematode,
Dicrocoelium dendriticum, in three species of host

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Short title: Relative performance of *Dicrocoelium dendriticum* in three definitive hosts

This chapter has been published in *Parasitology* (2015, 142: 1297-1305)

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4.1 ABSTRACT

Epidemiological rate parameters of host generalist parasites are difficult to estimate, especially in cases where variation in parasite performance can be attributed to host species. Such cases are likely common for generalist parasites of sympatric grazing mammals. In this study, we combined data from experimental exposures in cattle and sheep and natural infections in elk to compare the recruitment, morphology and reproduction of adult *Dicrocoelium dendriticum*, a generalist trematode that has emerged in sympatric grazing hosts in Cypress Hills Interprovincial Park, Alberta. Overall, there were no significant differences in the recruitment of metacercariae and in the pre-patency period of adults in experimentally-exposed cattle and sheep. All flukes reached reproductive maturity and the degree of reproductive inequality between individual flukes within each infrapopulation was moderate and approximately equal among the three host species. Neither fluke size nor *per capita* fecundity was constrained by density-dependence. Thus, fitness parameters associated with growth and reproduction were approximately equivalent among at least three species of definitive host, two of which were sympatric on pastures in this Park. The generalist life-history strategy of this trematode, which is known to extend to other stages of its life-cycle, has likely contributed to its invasion history outside its native range in Europe.

4.3 KEY FINDINGS

- Rates of adult fluke recruitment and time to egg shedding were similar in experimentally-infected sheep and cattle
- All adult flukes, regardless of host species, contained eggs *in utero*
- Reproductive inequalities were moderate within fluke infrapopulations and approximately equal among host species

- Fluke growth and reproduction was not density-dependent in any host species
- Adult *Dicrocoelium dendriticum* is a broad host generalist

4.3 INTRODUCTION

Parasitologists have long recognized that a parasite's position along the host specialist - host generalist continuum influences fundamental aspects of host/parasite biology. Thus, the extent to which a parasite is a host specialist or host generalist impacts the nature of host immunological responses, the magnitude of transmission asymmetries among host species, and also influences key components of parasite fitness, such as growth, reproduction, and development (Combes, 2001; Poulin, 2007). The direction and magnitude of parasite-mediated natural selection and parasite-host co-evolution can also be expected to differ for host specialists versus generalists (e.g. Richner, 1998). Perhaps less well recognized is the notion that the degree of host specialization also has important implications for applied and theoretical epidemiologists. For parasites that are host specialists, key rate parameters such as transmission, growth, reproduction, senescence, and parasite-induced host mortality can be estimated from experiments involving a single (or few) species of host. It follows that control strategies will be much simpler in cases involving single species of parasite in a single species of host. But the epidemiological picture becomes much more complex for generalist parasites that have the potential to infect multiple species of sympatric host (Grenfell *et al.* 2002). In an early example, Hairston (1962) showed that the trematode *Schistosoma japonicum* developed in sympatric humans, dogs, pigs, and rats. Within each of these hosts, parameters such as prevalence, fluke intensity, fluke size and *per capita* fluke reproduction varied extensively. He then used simple models to demonstrate that effective control was only realistic if intervention was targeted at the rat host population. This example illustrates that for generalist parasites in multiple species of host, the relative performance of individual parasites in different hosts is a key epidemiological factor.

The relative performance of generalist parasites within multi-host communities is particularly important in the context of parasites that have been introduced outside their native host ranges. The rate of parasite ‘spill-over’ between hosts that have invaded a new habitat and sympatric species of host may be high (Cleaveland *et al.* 2002; Cross *et al.* 2009), but it is only epidemiologically important if the invasive parasites develop to maturity within their new hosts. Further, the extent to which the presence of one or more species of host within a multi-host community either dilutes or enhances (Keesing *et al.* 2006) overall rates of transmission into the host community will depend on parasite establishment and performance in different species of host. This means that the epidemiological consequences of invasion into a multi-host community will be determined by the rate of transmission of infective stages into different species of hosts, and also by variability in relative rates of parasite development among sympatric host species. The problem is that for generalist parasites in multi-host systems, including those that involve invasive parasites, information on relative fluke performance in different sympatric species of host is rarely available. For the few exceptions involving invasive parasites or hosts (e.g. the trematode *Schistosoma japonicum* in 4 species of mammal (Hairston, 1962); the acanthocephalan *Metechinorhynchus salmonis* in 10 species of fish (Holmes *et al.* 1977)), transmission dynamics within multi-host communities was strongly influenced by the relative performance of parasites within different species of host.

The liver fluke, *Dicrocoelium dendriticum* (Trematoda: Dicrocoeliidae) has been widely introduced outside its homeland in central Europe, most likely through the movement of infected livestock (especially sheep) across international borders. The fluke is now common in northern and southern Europe, in northern Africa, and in isolated pockets in western and eastern North America (review by Otranto & Traversa, 2002; Rojo-Vásquez

et al. 2012). Evidence from the host survey literature indicates that the adult stage of *D. dendriticum* is a host generalist within and outside its native range, found in a wide array of cervine, ovine, and bovine hosts. Furthermore, evidence from experimental infections in sheep, beef cattle, and laboratory hamsters, together with faecal analyses for the characteristic eggs, has shown that at least some flukes reach reproductive maturity in all of these hosts (e.g. Manga-González *et al.* 1991; Campo *et al.* 2000; Sánchez-Campos *et al.* 2000; Manga-González & González-Lanza, 2005). Once absent in Alberta, Canada, the fluke is now common within a range of co-grazing ungulates in Cypress Hills Interprovincial Park (CHP), a conservation reserve located in the southeastern corner of the province. Goater and Colwell (2007) showed that 60-90% of resident elk (*Cervus canadensis*) and beef cattle (*Bos taurus*) are infected from year to year and all infected hosts contain gravid flukes.

The broad host spectrum of this fluke and its recent introduction into CHP provides an opportunity to evaluate relative performance of a generalist parasite within a multi-host system. In this study, we used experimental exposures in sheep and cattle to compare relative patterns of fluke recovery and intensity and to obtain comparative data on *per capita* fluke size and reproduction. Opportunistic collections of hunter-shot elk provided comparable data for infected wildlife collected from CHP. Our overall aim was to evaluate the performance of flukes collected from alternative hosts to provide a foundation for subsequent assessment of the roles of alternative host species in the overall transmission of *D. dendriticum* eggs onto shared pasture.

4.4 MATERIALS AND METHODS

4.4.1 *Cypress Hills Interprovincial Park*

CHP is a 531, 000 ha plateau rising approximately 200 m above the surrounding prairie (1,050-1,470 meters above sea level) in southeastern Alberta and southwestern Saskatchewan, Canada. The southern perimeter of the park is located approximately 100 km north of the Canada/United States border (49°37.5'N, 110°W). Sympatric elk (*Cervus canadensis*), mule deer (*Odocoileus hemionus*), white-tailed deer (*O. virginianus*) and beef cattle graze within the park. A managed elk hunt, initiated in 1978, runs annually each fall to maintain a density of 350-700 animals (Hegel *et al.* 2009). Further details regarding the natural and cultural history of CHP are in Hildebrant and Hubner (1994).

4.4.2 *Experimental infections*

A total of eighteen (2 control and 16 experimental) weaned Canadian Arcott sheep (ages: 6 mo. to 9 years old) were selected from a research flock maintained at the Agriculture and Agri-Food Canada Lethbridge Research Centre (LRC) in Lethbridge, Alberta. Twelve Holstein cattle (ages: 6 mo. to 2 years old) were purchased from a dealer and housed at the LRC for the duration of the infection trials (2 control and 10 experimental). The steers and sheep were housed separately in a feedlot and fed a basic diet of hay/barley silage. All animals were handled and maintained under the guidelines specified by the Canada Council for Animal Care (LRC Animal Care Committee protocol numbers 08233, 0925 and 1044).

Prior to exposure to metacercariae, sheep and cattle were treated once with a standard application of Ivomec® (Merial, Baie-D'Urfe, Quebec) to eliminate pre-existing helminth infections. For each infection trial, metacercariae were dissected from naturally-infected

formicid ants (*Formica sanguinea*, *F. subaenescens* and *F. fusca*; B.J. van Paridon & C.P. Goater, *unpublished observations*.) obtained from CHP in June 2011-2013 and packaged into gel capsules just prior to administration *per os*. Two sheep and two calves were assigned to Group A, the sham control, receiving capsules but no metacercariae. Group B (4 sheep and 4 calves) were orally inoculated with 625 metacercariae in 2011. Twelve sheep and six calves (Group C) were inoculated with ~1000 metacercariae, half in 2012 and the others in a third trial in 2013 (Table 1).

Rectal faecal samples were collected approximately every two weeks for up to 22 weeks post infection (p.i.) to determine the approximate onset of fluke reproduction in individual hosts. Faecal samples were labelled and frozen prior to analyses. Faecal egg counts were conducted using the modified Wisconsin method as outlined by Zajac and Conboy (2005) with saturated zinc sulfate solution (specific gravity = 1.36).

Animals were selected at random for slaughter at 110-150 days p.i. Live flukes were collected from sheep and cattle using standard methods described in Goater and Colwell (2007). Each liver was weighed and then cut into approximately 5mm-wide strips. Each strip was mechanically palpated into saline to dislodge the flukes from the bile duct. Live adult flukes were counted under a dissecting microscope and collected for subsequent analyses. These procedures allowed us to compare patterns of adult fluke recovery, mean fluke intensity, and fluke fecundity (Bush *et al.* 1997) in the two species of host.

4.4.3 Natural infections in elk

Logistical constraints prevented experimental exposures in wildlife. However, we collected data on fluke intensity and individual fluke fecundity (see below) in elk during the 2009-2013 hunting seasons to provide a general comparison with data on adult flukes

collected at approximately the same time from sheep and cattle. Where possible, live adult flukes were collected for subsequent analyses in the laboratory.

4.4.4 Morphology and reproduction of adult flukes

Data on *per capita* fluke size and reproduction came from live adult flukes collected at necropsy from 5 randomly selected sheep and cattle, and 3 elk harvested in 2013. Flukes from sheep and cattle were between 110-150 days old. The ages of flukes originating from elk are unknown, but were assumed to be equal to, or greater than, their 60-day pre-patency period (review by Otranto & Traversa, 2002). All intact flukes from each host were removed and washed with RPMI 1640 culture media (Sigma-Aldrich Canada Co., Oakville, Ontario) at pH 7.4. A subset of adult flukes was randomly selected from each host, isolated, and then transferred immediately into 24-well tissue culture plates. Each well was filled with 3 mL of RPMI 1640 and the plates were incubated at 37°C for 24 hrs to simulate host body temperature. This procedure is standard for the maintenance of live parasites for prolonged periods (Geary *et al.* 1993). Adult *D. dendriticum* continually shed eggs for up to 4 days within this media (M.A. Beck & C.P. Goater, unpublished observations). Following a 24 hr incubation period, the flukes were fixed in heated aceto-formal-alcohol (AFA) under light coverslip pressure.

These incubation and preservation procedures provided flukes of excellent quality. Morphological data on body length (BL), body surface area (BA), and uterus area (UA) were obtained from digital images (resolution of 250 pixels mm) using ImageJ software (Abramoff *et al.* 2004) for flukes from sheep (N = 5-18 flukes/host, Total N = 80), cattle (N = 7-11 flukes/host, Total N = 47) and elk (N = 6-25 flukes/host, Total N = 39) following procedures adapted from Valero *et al.* (1999) for liver flukes (*Fasciola hepatica*) originating

from naturally-infected sheep. The fecundity of individual flukes was determined by counting the numbers of eggs at the bottom of each well for sheep (N = 85), cattle (N = 51) and elk (N = 54). Following these counts, flukes and eggs were preserved in 90% ethanol. To estimate total reproductive output/day, *ex vivo* egg counts over 24 hours were averaged for each host species and multiplied by fluke intensity to estimate total eggs shed/24hrs.

4.4.5 Statistical Analyses

Parasitological terms such as intensity and infrapopulation follow definitions in Bush *et al.* (1997). Parametric tests involving intensity and egg count data were used with assumptions of normality met using raw or square root (n+1) transformed data. Differences in mean values were evaluated using independent t-tests or ANOVAs with Tukey's post hoc analyses. Pairwise comparisons of proportions used chi-square tests. 95% confidence intervals (CI) were calculated for proportions (p) using the Wald method (Vollset, 1993; $p \pm z\sqrt{pq/n}$, where $z = 1 - \alpha/2$ of the standard normal distribution and $q=1-p$).

Differences in rates of maturation and fluke recovery in experimentally infected cattle and sheep were evaluated using data on mean time to egg shedding and proportion of recovered flukes. Proportion of recovered flukes refers to the numbers of recovered adult flukes relative to the numbers of metacercariae administered. Assessment of density-dependence in fluke growth and reproduction within each host species was evaluated with standard parametric regressions involving fluke intensity, fluke body surface area (BA), and *ex vivo* egg counts. Morphometric data were evaluated with a nested ANOVA to apportion variation in fluke BA into between-species, between individual hosts, and between individual fluke effects. The percentage contribution by host species and individual hosts were calculated as a percentage of total variance (Rowe *et al.* 2009).

Comparisons of fluke reproduction included parametric comparisons of parasite eggs per gram (EPG) in host faeces on the date of necropsy and *ex vivo* egg counts from individual live flukes obtained during necropsy. A nested ANOVA design was used to apportion observed variation into between-species, between individual hosts, and between individual fluke effects. Percentage contribution of each main effect was calculated as a percentage of total variance. Lorenz curves and Gini coefficients, adjusted for sample sizes, were then used to describe inequality in *ex vivo* fluke reproduction within infrapopulations of the three different definitive hosts according to methods described in Dobson (1986). Applied examples included Shostak and Dick (1987) and Poulin and Latham (2002). Prior to the estimation of reproductive inequalities, *ex vivo* egg counts were ranked from lowest to highest for each host species. Lorenz curves are then calculated by plotting cumulative percent (%) of daily reproductive output against the cumulative number of individual flukes.

4.5 RESULTS

4.5.1 Rates of maturation and fluke recovery in sheep and cattle

Fluke eggs were first detected in lamb faeces at 7 to 9 weeks p.i., whereas eggs were first detected in cattle faeces at 8 to 12 weeks p.i. Although these data indicate earlier egg release in sheep, there was no significant difference in the timing of initial egg release between the two species of host ($t_{20} = -1.11$, $p = 0.28$). Flukes were present within the livers of all exposed hosts at necropsy (Table 1). Intensities ranged from 46-283 flukes in sheep and 1-324 in cattle. The proportion of recovered adult flukes (sheep: 18.4%; 95% CI = 0.0 - 42.4%; cattle: 12.8%; 95% CI = 0.0 - 33.5%) did not significantly differ between hosts ($\chi^2 = 0.95$, d.f. = 1, $p = 0.33$).

4.5.2 Density-dependent fluke performance

The association between size of individual flukes, measured as BA, and *per capita* fluke fecundity was non-significant in sheep ($r = 0.03$, $F_{1,75} = 0.05$, $p = 0.82$) and elk ($r = 0.05$, $F_{1,37} = 0.08$, $p = 0.78$). This association was significantly positive for flukes from cattle ($r = 0.34$, $F_{1,45} = 5.88$, $p = 0.02$), indicating that larger flukes tended to produced more eggs. BA varied significantly with intensity in cattle ($r = 0.38$, $F_{1,45} = 7.35$, $p < 0.01$) but not in sheep ($r = 0.05$, $F_{1,78} = 0.18$, $p = 0.67$). *Ex vivo* egg counts did not vary significantly with fluke intensity in cattle ($r = 0.08$, $F_{1,49} = 0.35$, $p = 0.56$) or sheep ($r = 0.18$, $F_{1,81} = 2.77$, $p = 0.10$). Analyses comparing *ex vivo* egg counts could not be completed for elk because fluke intensity data were only available for two hosts.

4.5.3 Comparative morphology of adult flukes in cattle, sheep, and elk

Morphological data on fluke BA, uterus area (UA), and body length (BL) were highly positively and significantly inter-correlated for flukes assessed from each host species, particularly for data involving BA and UA (Table 2; Range in R^2 values : BA vs UA= 0.48 - 0.88; BA vs UL= 0.62 - 0.88; BL vs UA= 0.41 - 0.58). Due to the magnitude of these significant inter-correlations, and for consistency with the literature involving other flukes, we focused our comparative analyses on BA.

The frequency distributions of BA data for the total sample of flukes from each host species indicated that a high proportion of flukes from elk (0.77 ± 0.08) and from sheep (0.86 ± 0.09) were $< 8 \text{ mm}^2$. In comparison, a higher proportion (0.38 ± 0.14) of flukes had a $\text{BA} \geq 9 \text{ mm}^2$ in cattle (Fig. 1). Overall, there was a significant difference in mean fluke BA between the three species of host (Table 2). Post-hoc comparisons showed that flukes in cattle were on average, 14% larger than those in sheep and elk. However, results from a

nested ANOVA (Table 3) showed that host species only accounted for 10.7% of the overall variation in fluke BA, while most of the variation was due to differences between host individuals (44.6%; $F_{2,152} = 19.7$, $p < 0.001$) and between individual flukes (44.7%).

4.5.4 Comparative fluke reproduction

Mean EPG for samples collected on the date of necropsy differed significantly ($t_{20} = 3.36$, $p < 0.01$) among sheep ($x = 38.5 \pm 6.4$) and cattle ($x = 8.2 \pm 3.3$), with values approximately 80% higher in sheep.

All recovered flukes were gravid. Each fluke contained hundreds to thousands of eggs *in utero*. Variation in egg output over the 24 hr incubation period (Fig. 2a) spanned 3 orders of magnitude (range = 1 - 5820 eggs/day/fluke). In elk, approximately 72% (95% CI: 60 - 84%) of flukes shed fewer than 1000 eggs in 24 hours, of which 14% (95% CI: 5 - 24%) shed fewer than 100 eggs. In contrast, only 44% (95% CI: 34 - 55%) and 45% (95% CI: 31 - 59%) of flukes from sheep and cattle, respectively, shed fewer than 1000 eggs over the 24 hr incubation period. Approximately 14% (95% CI: 5 - 23%) of flukes from sheep and cattle shed more than 3000 eggs in a day, while less than 2% (95% CI: 0 - 5%) of flukes harvested from elk shed such high numbers of eggs.

Estimates of mean egg production/day varied significantly among host species for flukes harvested from sheep ($x = 1530.0 \pm 147.3$), cattle ($x = 1561.1 \pm 173.7$), and elk ($x = 701.4 \pm 98.6$). Tukey's post-hoc comparisons showed that mean *ex vivo* egg counts did not significantly differ among sheep and cattle ($p_{cs} = 0.94$), while counts for flukes harvested from elk were significantly lower ($p_{ce} < 0.001$; $p_{se} < 0.001$). However, results from a nested ANOVA (Table 3) showed that host species and host individual only accounted for 19.8%

and 15.2% of the variation in egg counts, respectively, while 65.0% of the variation could be explained by differences between individual flukes ($F_{12,175} = 3.9$, $p < 0.001$).

Since only a small proportion of the overall variation in fluke fecundity could be attributed to host species and host individuals, it is likely that inherent variation among individual flukes plays a significant role. Overall, reproductive inequalities were similar among fluke infrapopulations in cattle ($G'_{RC} = 0.44$, 95% CI = 0.37 - 0.52), sheep ($G'_{RS} = 0.47$, 95% CI = 0.39 - 0.52) and elk ($G'_{RE} = 0.53$, 95% CI = 0.44 - 0.59) with no apparent differences between host species (Fig. 2b). These results indicate that inequalities in reproduction arise from a small number of highly fecund flukes within each infrapopulation. If these data are assumed to represent random samples of flukes from the three host species, the Lorenz curves indicate that approximately 10% of flukes in any infrapopulation contribute about 30-35% of all eggs shed by a particular host. Lastly, estimates of total daily egg output using *ex vivo* egg counts (sheep: $26.4 \pm 8.5 \times 10^4$ eggs/day; cattle: $23.6 \pm 8.1 \times 10^4$ eggs/day; elk: $16.4 \pm 5.9 \times 10^4$ eggs/day) did not significantly differ among host species ($F_{2,15} = 0.23$, $p = 0.79$).

4.6 DISCUSSION

Our data show that rates of recruitment of adult *D. dendriticum* from metacercariae in ants were approximately equivalent between at least two species of sympatric host. The rates of fluke development and time to reproductive maturity were also approximately equal between species of host. Virtually all flukes, in all hosts, reached maturity, although rates of *per capita* fecundity were highly variable. The evidence for relatively equivalent fluke performance among hosts is strongest for flukes assessed from experimentally infected sheep and cattle, where confounding factors such as dose and fluke age were

controlled. The similarities in overall fluke performance were also consistent in naturally infected elk, with heterogeneity among individual flukes accounting for >50% of the total variation in reproductive performance and morphology among the fluke infrapopulations. Host-related differences were also absent in comparisons of estimated total daily egg output calculated using *ex vivo* egg counts. Taken together, these data show that *D. dendriticum* performance is approximately equivalent in all three species of hosts, two of which (cattle and elk) are sympatric within a known site of introduction in Alberta, Canada (Goater & Colwell, 2007; Beck *et al.* 2014).

Our results from the experimental infections confirm those from prior experimental work involving other domestic hosts. Manga-González and González-Lanza (2005) exposed lambs to 1000-3000 metacercariae and then monitored rates of fluke recruitment, growth, and faecal egg production. Our results involving lambs and beef cattle were consistent with the results of this study relative to fluke recruitment, pre-patency period, and fluke size. Similarly, results indicating relatively equal performance among host species are consistent with data from field studies documenting similarities in fluke burden and fecal egg counts in sheep and goats (Jithendran & Bhat, 1996). These results indicate consistency in fluke performance between host species that are amenable to experimental exposures (e.g. sheep and cattle). While our data from flukes collected from naturally infected elk are less conclusive, patterns of overall fluke size and *per capita* reproduction are consistent with those from the two domestic hosts.

Our analyses also indicate that fluke performance was not density-dependent. The growth and survival of adult *D. dendriticum* was independent of the numbers of flukes in individual hosts. There was no detectable increase in *per capita* fecundity with fluke size in experimentally-infected sheep or elk, although this relationship was moderately positive in

cattle. However, fluke performance did not decrease with an increase in fluke intensity in any of the three host species. These results were unexpected, particularly when overall variation in intensity spanned four orders of magnitude. Density-dependence is a frequent outcome within many host-parasite interactions (Shostak & Scott, 1993), including for the trematode *Fasciola hepatica* in rats (Valero et al. 2006) and for the nematode *Trichostrongylus columbriformis* in sheep (Dobson et al. 1990). The absence of density-dependence may be related to the small size and hence, minimal nutrient requirements of individual flukes. The minor hepatocellular damage associated with infection may also not be sufficient to activate density-dependent immunity. Density-dependent fluke performance may be evident at the higher intensities observed within some hosts (especially deer) sampled in Cypress Hills Interprovincial Park (up to 5000 per liver (Goater & Colwell, 2007) and other geographic regions (up to 10,000 per sheep on the Isle of Coll, Scotland (Sargison et al. personal communication)). Threshold density-dependent dynamics have been documented for *T. columbriformis* in sheep, in which decreased parasite fecundity was restricted to sheep that harboured upwards of three thousand individuals (Dobson et al. 1990).

Further evidence for the absence of strong density-dependent constraints on development and survival comes from the observation that virtually all flukes, within all infrapopulations, were gravid. All flukes from the three host species that were sampled for morphological assessments contained eggs *in utero* and all randomly selected individuals that were incubated within growth media produced eggs over 24 hours. This pattern of reproduction was consistent for infrapopulations in sheep and cattle, where factors such as fluke age and dose were controlled and also for infrapopulations in naturally-infected elk. Although our data show that the numbers of eggs released by individual flukes was highly

variable within and between individual hosts, the onset of egg release 7-12 weeks p.i., and the probability of reaching reproductive maturity, was not. These results are in striking contrast to the patterns of reproduction that are reported in other vertebrate/helminth interactions, in which reproductive inequalities within infrapopulations tend to be much higher (Keymer *et al.* 1983; review by Dobson, 1986). Shostak and Dick (1987) showed that, on average, only 33% of individual cestodes (*Triaenophorus crassus*) collected from infrapopulations in pike (*Esox lucius*) were gravid, and only a fraction of these shed eggs *ex vivo*. Overall, 10% of these gravid flukes produced 85% of the total eggs shed. Similar examples involving other host/parasite interactions are described in Dobson (1986). In contrast, the most fecund 10% of *D. dendriticum* individuals sampled from the three hosts produced only 30-35% of the total eggs shed, and all flukes contributed eggs. These results suggest that the rates of development, growth, and reproduction of *D. dendriticum* in the biliary system of its definitive hosts are less constrained by factors such as inter- and intraspecific competition for host resources and/or by host responses. The implications of this contrasting pattern of fluke development, and especially reproduction, to patterns of population genetic structure and evolutionary potential provide interesting follow-up opportunities.

Taken together, the general transmission strategy of adult *D. dendriticum* is one of low host specificity, approximately equivalent *per capita* fluke performance in different hosts, density-independent growth and reproduction, and modest reproductive inequality between individual flukes. In effect, this means that almost every metacercariae that is recruited into the liver of a wide range of host species will develop to maturity and release large (albeit variable) numbers of viable eggs onto shared pasture. Why might natural selection favour a generalist life-history strategy for *D. dendriticum*? One possibility is that low host specificity

optimizes overall rates of transmission for trematodes such as *D. dendriticum* that complete all of their life-cycle stages within terrestrial habitats. Thus, optimal rates of host exploitation for flukes that are recruited within the livers of several potential hosts may be one solution to offset the low probability of transmission during the various stages of the *D. dendriticum* life-cycle (Poulin, 2007). Comparable examples involving other terrestrial or semi-terrestrial parasites (e.g. fasciolid trematodes, trichinellid nematodes, and some taeniid cestodes) tend also to be broad host generalists during their adult stages (Goater *et al.* 2014).

An alternative explanation is that exploitation by these small flukes within the bile ducts of large livers provides optimal quality and quantity of host resources to support high fluke fecundity, unencumbered by inter-specific or intra-specific competition (Poulin, 2007). This scenario may occur among sympatric grazing mammals in CHP where parasite intensities tend to be relatively low, and there are no co-occurring parasites in the livers (Goater & Colwell, 2007; Beck *et al.* 2014). A related possibility is that the liver of these ungulates may provide a relatively immuno-privileged microhabitat that does not constrain the growth and fecundity of *D. dendriticum* in potential definitive hosts. Evidence from serological assays involving experimentally-infected mammals has shown that anti-*D. dendriticum* antibodies are detectable at approximately 60 days post-infection in sheep (González-Lanza *et al.* 2000; Ferreras-Estrada *et al.* 2007). However, this immunological response may not provide adequate protection, as is apparent among sheep chronically exposed to *F. hepatica* (Pérez *et al.* 2002) and cattle experimentally infected with *F. gigantica* (Molina & Skerratt, 2005). Analyses of age-intensity patterns of infection in cattle sampled from CHP are also consistent with a lack of effective host immunity (Beck *et al.* 2014). These results indicate that effective host defences are minimal, absent, or at least slow acting for many potential hosts of *D. dendriticum*. Given the role of host defence in

selecting for host specificity across a range of host/parasite interactions (Combes, 2001; Poulin, 2007), the absence of effective anti-*D. dendriticum* immunity may best explain the patterns of host utilization and parasite performance observed in this study.

The ability of *D. dendriticum* to attain approximately equal fitness within a range of definitive hosts likely enhances opportunities for host encounter and for host switching, and likely also increases overall rates of dispersal (Combes, 2001; Hoberg & Brooks, 2008). Since this fluke utilizes a notoriously high number of species of terrestrial snail and formicid ants as first and second intermediate hosts (review by Manga-González *et al.* 2001) respectively, such opportunities likely extend through the entire life-cycle. Ultimately, a generalist strategy of this kind - one that extends throughout the various life-cycle stages - will influence rates of colonization, including into novel geographical areas and naïve host populations. This is consistent with historical and contemporary patterns of host switching and the translocation of *D. dendriticum* outside its native range in Europe into areas in North and South America, northern Europe, north Africa and the Middle East (review by Otranto & Traversa, 2002; Goater & Colwell, 2007; Rojo-Vásquez *et al.* 2012). Combined with rapid environmental change and increased mixing of wildlife and domestic stock as a result of anthropogenic changes in landscape use (review by Agosta *et al.* 2010), the reproductive strategy of *D. dendriticum* may increasingly facilitate the formation of novel host-parasite associations.

4.7 ACKNOWLEDGMENTS

We thank staff of the Lethbridge Research Center, especially Dawn Gray, for providing excellent technical expertise. We also appreciate the generous cooperation of the big-game hunters and Park personnel of Cypress Hills Provincial Park, Alberta, Canada.

4.8 FINANCIAL SUPPORT

This research was supported by grants from the National Sciences and Engineering Research Council of Canada (CPG. Grant no. 40130); and the Beef Cattle Research Council (Grant no. 2008F064).

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Table 4.1: Summary of the number of adult *D. dendriticum* recovered from the livers of experimentally-infected cattle and sheep with their associated faecal egg counts and number of eggs shed by flukes over for a 24 hour period. All values are mean +/- SEM.

Group	Infection Dose	Host	N	Fluke Intensity	Eggs/g faeces	Mean Number of Egg/24 hrs
A	Control	Sheep	2	0	0	0
		Cattle	2	0	0	0
B	625	Sheep	4	137.3 ± 18.0	46.8 ± 7.8	1093.4 ± 204.7
		Cattle	4	42.8 ± 30.2	3.8 ± 2.8	1043.2 ± 206.5
C	1127 ± 186.1	Sheep	12	183.3 ± 27.7	35.8 ± 8.1	2256.0 ± 261.9
		Cattle	6	198.7 ± 47.5	11.2 ± 4.9	1986.6 ± 241.8

Table 4.2: Morphometric data for adult *D. dendriticum* from experimentally-infected sheep and cattle and naturally infected elk from Cypress Hills Interprovincial Park, Alberta. Values shown are mean \pm SEM and range.

	Sheep (N = 80)	Cattle (N = 47)	Elk (N = 39)	ANOVA <i>p</i> -value
Body area, BA (mm ²)	6.6 \pm 0.1 3.9 - 10.6	7.8 \pm 0.3 4.5 - 11.4	6.7 \pm 0.3 3.6 - 9.4	< 0.001
Uterus area, UA (mm ²)	2.8 \pm 0.1 1.1 - 5.0	3.0 \pm 0.1 1.7 - 4.4	2.4 \pm 0.1 1.1 - 4.3	0.002
Body length, BL (mm)	5.7 \pm 0.1 4.2 - 7.6	6.5 \pm 0.1 4.5 - 8.5	5.8 \pm 0.1 4.3 - 7.2	< 0.001

Table 4.3: Nested ANOVA results for the effects of host species and individual animal combinations on variation in fluke body area (mm²) and *ex vivo* egg count for flukes incubated for a 24-hour period.

	Source	df	MS	F value	<i>p</i> -value	Variance Component	%
BA	Species	2	25.0	19.7	<0.001	0.3	10.7
	Individual hosts(Species)	10	17.3	13.7	<0.001	1.3	44.6
	Residual	152	1.3			1.3	44.7
	Total	164					
<i>Ex vivo</i> Egg Count	Species	2	1.3X10 ⁷	11.5	<0.001	3.6X10 ⁵	19.8
	Individual hosts(Species)	12	4.6X10 ⁶	3.9	<0.001	2.7X10 ⁵	15.2
	Residual	172	1.2X10 ⁶			1.2X10 ⁶	65.0
	Total	186					

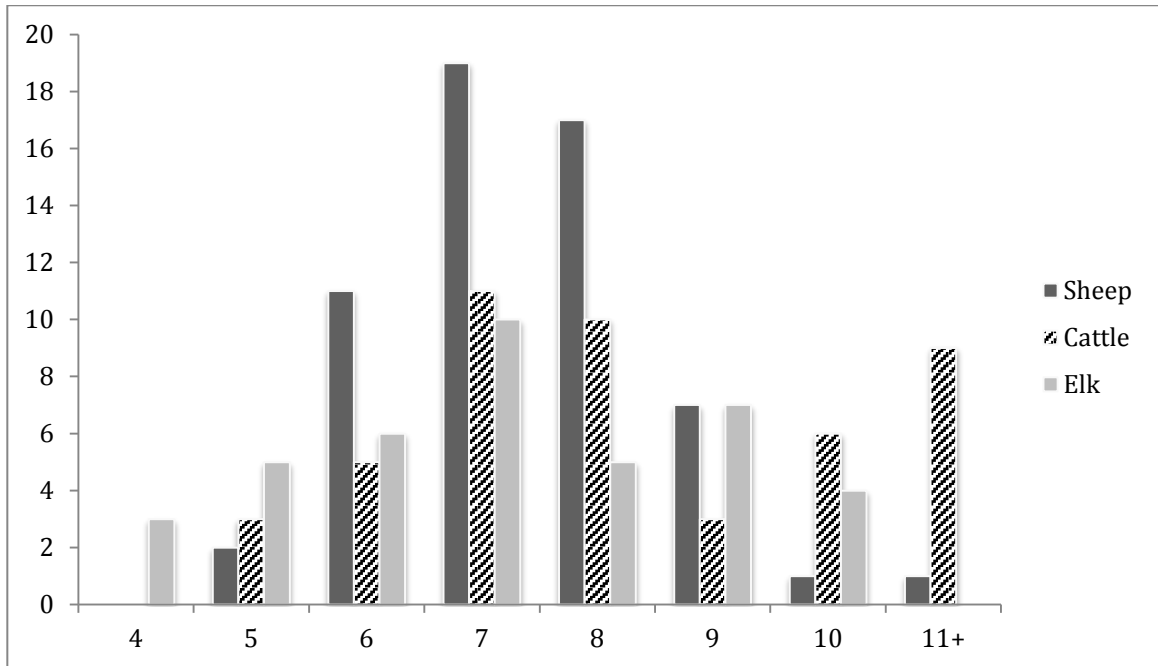


Figure 4.1: Frequency distribution of body surface area for individual *D. dendriticum* from experimentally infected sheep (N = 80), cattle (N = 47) and elk (N = 39).

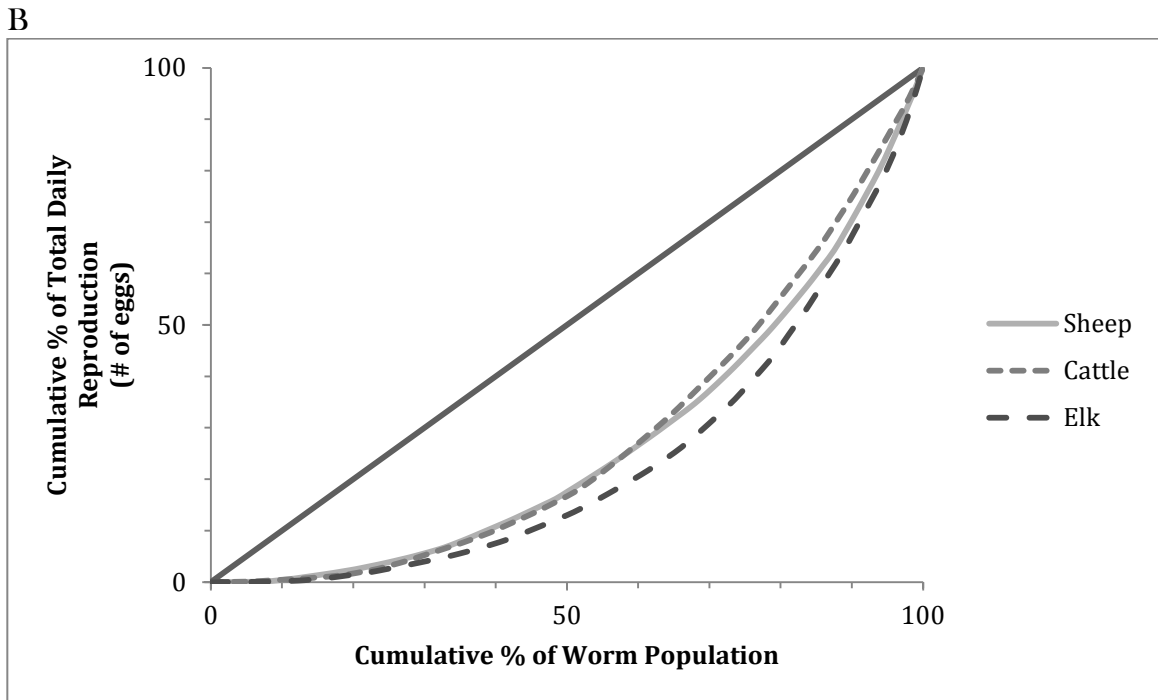
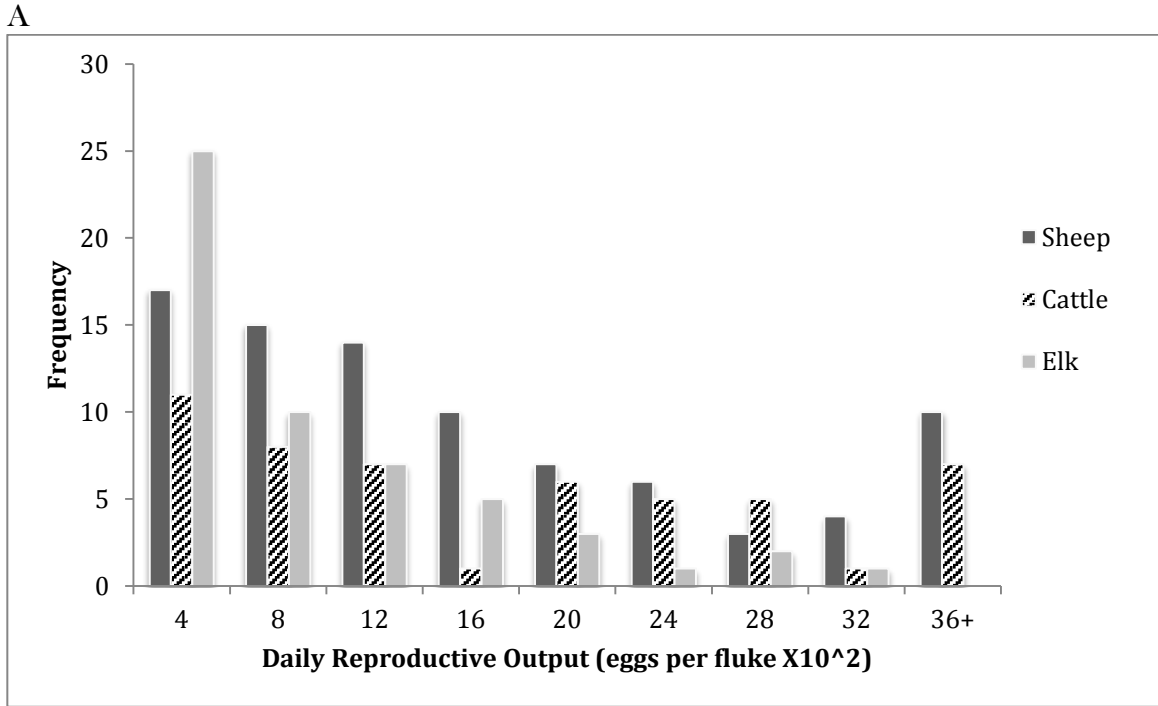


Figure 2: (A) Frequency distributions of daily reproductive output for individual *D. dendriticum* collected from experimentally infected sheep (n = 86), cattle (n = 51) and elk (n = 54); (B) Lorenz curves for cumulative percent (%) of daily reproductive output of flukes plotted against the cumulative number of individual flukes. Pairing of the cumulative percent of the fluke population responsible for a cumulative percent of total daily

reproduction in each species of hosts is achieved after ranking the flukes from least to most fecund. The solid line represents the line of equality ($G_R = 0$).

CHAPTER 5

Circulation of an invasive liver fluke (*Dicrocoelium dendriticum*) among sympatric ungulate hosts in Alberta, Canada

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Prepared as a manuscript for submission

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5.1 ABSTRACT

The emergence of parasites and pathogens outside their normal host and geographic range is a globally-recognized concern. A key shortcoming of models that attempt to forecast the spread and impact of emerging parasites, especially host generalists, is that little data exist on the relative contribution of sympatric host species to the transmission of infective stages. In this study, we estimated relative rates of egg-to-pasture transmission for an emerging generalist trematode, *Dicrocoelium dendriticum*, among sympatric ungulates in Cypress Hills Interprovincial Park, Alberta (CHP). We combined estimates of host population size, prevalence, mean fluke abundance, *per capita* fluke fecundity, and host residency time to estimate the relative annual contribution of each host species to contamination of pasture with *D. dendriticum* eggs. Worm prevalence and abundance were significantly higher in samples of yearling and adult beef cattle and in juvenile elk than in adult elk and subpopulations of white-tailed and mule deer. Overall, the sub-population of roughly 4000 cow/calves that are pastured in CHP contribute approximately 80% of the estimated 300 billion eggs that contaminate pasture each year. Because transmission into snail intermediate hosts is known to occur in regions of CHP where beef cattle are excluded, the much smaller sub-populations of calf and yearling deer and elk also play a role in disseminating eggs within the region. These results emphasize the complex and interactive roles of domestic host density, relative immuno-competence, and host community structure, in understanding transmission dynamics of emerging, multi-host parasites. In addition to providing some of the first estimates of egg-to-pasture transmission rates for a multi-host parasite, these results can also inform management strategies for this and other emerging multi-host parasites.

5.2 INTRODUCTION

A significant, but often neglected, concern associated with the translocation of domestic stock and wildlife, or the introduction of non-indigenous host species, is the ‘spill-over’ of parasites to and from infected hosts (Cleaveland *et al.* 2002; Cross *et al.*, 2009). The emergence of these novel host-parasite interactions represents a significant threat from both economic and conservation perspectives (Keeling & Gilligan, 2000; Ostfeld & Keeling, 2000; Tompkins *et al.*, 2011). The epidemiological picture is particularly complex for generalist parasites that can infect several host species (Cleaveland *et al.*, 2001; Streicker *et al.*, 2013). Heterogeneities in relative host abundances, exposure risk, compatibility and susceptibility can translate to high variability in rates of parasite transmission (e.g. Haydon *et al.*, 2002; Streicker *et al.*, 2013). For these parasites, the epidemiological characterization of so-called ‘superspreaders’ or ‘superspreading groups’ of hosts (Cross *et al.*, 2009; Morgan *et al.*, 2006) becomes increasingly complicated, and intractable, as the complexity and structure of a host community increases.

A parasite’s basic reproductive number (R_0), can be used to quantify and characterize the extent to which host species heterogeneity impacts parasite transmission dynamics. For macroparasites, R_0 represents the number of reproductively mature offspring recruited over the lifetime of a single adult parasite (Anderson & May, 1991; Streicker *et al.*, 2013) and can be used as a mathematical approximation for the ‘transmission threshold’ of a parasite (Goater *et al.*, 2014). However, estimation of R_0 is particularly complicated for parasites with multiple hosts and multiple developmental/ transmission stages. The impacts of host species heterogeneity on rate of transmission will vary relative to the degree of specificity of a parasite (Haydon *et al.*, 2002; Streicker *et al.*, 2013) and can therefore not be

defined *a priori* among a range of sympatric hosts. Alternatively, for complex lifecycle generalist parasites, measures of the relative contribution of each host species to the total number of infective stages (Streicker *et al.*, 2013) or infected vectors (Kilpatrick *et al.*, 2006) can be used to estimate a host's relative contribution to transmission. This approach has been primarily limited to direct lifecycle pathogens and parasites (e.g. gastrointestinal nematodes) in small mammals or domesticated stock. Ideally, robust estimates of relative host contribution require invasive sampling of large numbers of equally sampled, sympatric hosts, along with accurate data on parasite fecundity and the relative abundance of hosts and their parasites (Poulin, 2007). Opportunities to collect these data are rare, particularly for parasites shared across the wildlife-domestic stock interface. As a result, we have a poor understanding of the extent to which the degree of host specificity influences the likelihood of parasite spill-over into naïve hosts and geographic regions for parasites shared among large sympatric mammals.

The lancet liver fluke, *Dicrocoelium dendriticum*, is an emerging trematode that infects a range of species of large ungulate hosts in Cypress Hills Interprovincial Park, Alberta. Originating in central Europe, this host generalist is now present throughout Europe, Asia, north Africa and in isolated patches in North America (review by Otranto & Traversa, 2002; Rojo-Vásquez *et al.*, 2012). While this parasite was rare in the small, island-like Cypress Hills Interprovincial Park (CHP) in Alberta, Canada in the early 1990's, this fluke is now present in 40-90% of all resident elk (*Cervus canadensis*), mule (*Odocoileus hemionus*), white-tailed deer (*O. virginianus*) domestic cattle (Goater & Colwell, 2007; Beck *et al.*, 2014). With high prevalence and mean abundance among a range of CHP sympatric hosts, there is significant risk of parasite spill-over into new geographic regions and host populations beyond CHP through the translocation of infected definitive and intermediate

hosts. Despite evidence of relatively equal performance of individual flukes (recruitment, morphology, and fecundity) among experimentally-infected hosts (Beck *et al.*, 2015), parasite distribution patterns differ relative to host age in elk and cattle, with evidence of protective immunity limiting parasite recruitment only in older elk (Beck *et al.*, 2014). However, the implications of these patterns on overall parasite transmission at the host community level remain poorly understood, particularly in relation to differences in host abundance and the transient cattle population that only graze in CHP during the snow-free months (Hegel *et al.*, 2009; Alberta: Tourism, Parks and Recreation, 2013).

Determining which hosts and host-specific characteristics amplify parasite transmission would allow for the identification of superspreading individuals or subgroups for transmission of this generalist parasite. The first aim of the present study is to characterize patterns of circulation of *D. dendriticum* among sympatric ungulate host species (cattle, elk, mule deer, and white-tailed deer) in CHP. Combining data on the prevalence of infection in different species of host, relative parasite abundance in each species of host, estimates of parasite fecundity (Beck *et al.*, 2015), host abundance and host residency time in CHP, we also evaluated the relative roles of each host species, and sub-populations of hosts, to the transmission of eggs onto pasture.

5.3 MATERIALS AND METHODS

5.3.1 Cypress Hills Interprovincial Park, Alberta, Canada

The parasite survey was conducted in CHP, an interprovincial conservation preserve extending from the south-eastern corner of Alberta into south-western Saskatchewan (see Chapter 1). A managed annual elk hunt, initiated in 1978, occurs each fall to maintain a density of 350-700 resident animals in the Park. An annual fall deer hunt also occurs within

the wildlife management units (WMU) surrounding the Park. Beef cattle have grazed within almost all regions of the Park since 1918, with cow-calf pairs and yearlings introduced in early June and removed in late October (Hegel *et al.*, 2009). Further information on the history and ecology of CHP is presented in Chapter 1 and in Beck *et al.* (2014) and Beck *et al.* (2015).

5.3.2 Data collection

Whole elk livers were collected opportunistically from 2003-2005 during the fall hunting seasons (N = 119). An additional 113 livers were harvested during the 2009-2013 fall hunting seasons. Mule deer (N = 22) and white-tailed deer (N = 16) livers were also collected during the 2009-2013 fall hunting seasons from the wildlife management units surrounding the Park (WMU 116, 118, and 119), an area >5700 square kilometers (Government of Alberta, Provincial Base Map, 2012). Host gender for elk and deer was established at time of collection and host age was approximated (calves: < 6 months, juveniles: 6-24 months, adults: > 24 months; Goater & Colwell, 2007; Beck *et al.*, 2014). Known-aged beef cattle were purchased opportunistically between 2003-2014 from the three CHP stock associations. All cattle included in our survey had grazed within the park during the snow-free months (June-October) each year since birth.

Intact livers were either frozen within four hours of host death or processed immediately when feasible following methods described in Goater and Colwell (2007) and Beck *et al.* (2014). Each liver was thawed, weighed, cut into approximately 5mm-wide strips and palpated to dislodge the parasites. Adult flukes were identified by morphological characteristics (Soulsby, 1982), assessed for reproductive maturity, and enumerated, using a dissection microscope.

Estimates of *per capita* parasite fecundity were based on data presented in Beck *et al.*, (2015). Subsamples of live flukes were randomly selected from naturally infected elk (n = 6-25 flukes/host, Total N = 53) and experimentally infected cattle (n = 7-11 flukes/host, Total N = 51) and sheep (n = 5-18 flukes/host, Total N = 85). The parasites were incubated in RPMI 1640 media for a period of 24 hours to obtain estimates of daily egg production. As live flukes were not available from white-tailed deer or mule deer, fecundity estimates for flukes from experimentally-infected sheep were used in subsequent calculations for these hosts given their roughly similar liver size, physiology, and diet (M.A. Beck, unpublished data; Lee, 1979; Spaulding *et al.*, 2009).

Host abundance for elk and deer populations were estimated from annual winter aerial surveys completed over four consecutive years (AB Sustainable Resources, 2011). CHP is home to large resident populations of sympatric elk (N = 800), mule deer (N = 300), and white-tailed deer (N = 300) (Hegel *et al.*, 2004; Alberta. Tourism, Parks and Recreation, 2013). The age structure of wildlife host populations was estimated based on a study in northwestern New Mexico where elk calves comprise approximately 20% of the annual population total (Bender & Piasecke, 2010). Cattle population abundance and population age structure is based on data from the CHP stock associations with approximately 2000 calves and their 2000 mothers introduced to CHP each spring (Rangeland Conservation Service Ltd, AB Environment).

For the purposes of estimating relative contribution to transmission per annum we assume that wildlife hosts (elk and deer) reside in CHP 365 days of the year. The residency period for cow-calf pairs and yearlings is approximately 90 days each year (Hegel *et al.*, 2009). Adult cows, exposed to *D. dendriticum* during previous grazing seasons, likely begin shedding eggs when returned to CHP pastures each year. However, relative contribution to

transmission per annum is abbreviated for all hosts in the first year of grazing. Based on experimental data from Campo *et al.* (2000), adult *D. dendriticum* do not shed eggs until 49 - 79 days post infection ($\bar{x} = 59.0 \pm 1.6$). Therefore, length of the prepatency period is subtracted off the annual host residency times to estimate total eggs shed per annum for hosts in their first grazing year.

5.2.3 Analyses

We follow the parasitological terminology in Bush *et al.* (1997). Prevalence of infection is the proportion of hosts infected within a population of hosts and abundance is the total number of adult flukes present in an infected host, including zeros. All analyses were completed using data pooled between years unless otherwise stated. The distribution of adult flukes within samples of hosts was estimated using the exponent k of the negative binomial distribution (NBD) using Quantitative Parasitology 3.0 (Reiczigel & Rozsa, 2005). As fluke counts were highly aggregated, worm counts could not be transformed to normality. Median abundances were compared between samples with Mann-Whitney U tests and Kruskal-Wallis tests (for comparison of multiple medians). Confidence intervals for proportions were calculated using the Wald method (Volset, 1993; $p \pm z\sqrt{pq/n}$), where $z = 1-\alpha/2$ of the standard Normal distribution and $q = 1-p$), with values compared using Chi-square tests.

Following the definition in Baldwin and Goater (2003) we use the term ‘circulation’ to describe the rate of exchange of parasites among sympatric hosts. In the evaluation of *D. dendriticum* circulation patterns we follow some of the basic assumptions outlined by Aho and Kennedy (1987). These include the following: (1) CHP is a closed ecological system with movement of host populations limited to within the Park boundary; (2) all eggs shed by

individual flukes are viable and retain their infectivity for one year; and (3) density-dependence does not restrict the establishment, survival, growth and fecundity of individual flukes. Assumption 3 is supported by the results in Beck *et al.*, (2015), where comparison of fluke performance illustrated that fluke performance did not vary with host liver size. Additionally, 100% of flukes collected from naturally- infected elk and experimentally- infected cattle and sheep were gravid at any given time.

We estimated relative rates of egg-to-pasture transmission in two ways following the methods in Baldwin & Goater (2003). Circulation was first estimated as p , the proportion of infected hosts (I/H_i), where I is the number of infected individuals and H_i is the total number of infected and uninfected animals sampled of host species i . The second estimate of circulation (C) was calculated as the proportion of all parasites in N host species found in host species i using the following equation:

$$C_i = \frac{H_i A_i p_i}{\sum_{j=1}^N H_j A_j p_j} \quad (1)$$

where A_i is the mean parasite abundance for host species i . H_i is the estimated population size (infected and uninfected) of host species i . The total number of individual parasites found in host species i is $c_i = H_i A_i p_i$ and the total number of parasites in N host species is $c = \sum_{j=1}^N H_j A_j p_j$.

The relative contribution of different host species to total egg-to-pasture transmission (T_i) is calculated as the number of eggs released by all flukes within each sympatric host population per annum, based on the following equation adapted from Streicker *et al.*, (2013):

$$T_{1i} = \frac{H_i A_i p_i \lambda_i r_i}{\sum_{j=1}^N H_j A_j p_j \lambda_j r_j} \quad (2)$$

where, λ_i is *per capita* daily parasite reproduction for host species i with the calculation weighted by the host residency time, r_i , within the park. The total number of eggs shed onto pasture per annum by host species i is $t_i = H_i A_i p_i \lambda_i r_i$ and the total contribution of N host species in the community to the contamination of pastures is $t = \sum_{j=1}^N H_j A_j p_j \lambda_j r_j$. Egg shedding estimates were then adjusted as the relative proportion of the total reproductive output from all hosts.

Patterns of adult *D. dendriticum* infection in CHP are variable relative to host age (Beck *et al.*, 2014). To account for this we completed additional calculations among aged elk and cattle for (i) parasite circulation (p_i and C_i calculations as above); and (ii) relative contribution to total transmission (T_{2i}) using the following equation:

$$T_{2i} = \frac{H_{ai} A_{ai} p_{ai} \lambda_{ai} r_{ai} + H_{yi} A_{yi} p_{yi} \lambda_{yi} (r_{yi} - l_{yi})}{\sum_{j=1}^N H_j P_j p_j \lambda_j r_j} \quad (3)$$

where t_i is separated into eggs shed by the subpopulations of adult (a) hosts ($t_{ai} = H_{ai} A_{ai} p_{ai} \lambda_{ai} r_{ai}$) and hosts < 2 years of age ($t_{yi} = H_{yi} A_{yi} p_{yi} \lambda_{yi} (r_{yi} - l_{yi})$). Parameter definitions are consistent with those presented in Equations 1 and 2 for adult hosts and for hosts < 2 years of age (y). The additional parameter l_i is the length of the prepatency period of *D. dendriticum* for hosts of species i in their first year of exposure, which is subtracted off an estimated annual host residency time to estimate total contribution for eggs shed per annum. Again, egg-shedding estimates were adjusted as the relative proportion of the total

reproductive output attributed to these two host species. Differences in parasite performance in relation to host age have not been established for white-tailed deer and mule deer.

5.4 RESULTS

5.4.1 Infection patterns

Parasite abundance was highly variable in all species of host (Fig. 5.1), with the overall distribution of flukes not differing significantly from the theoretical NBD model in elk ($\chi^2 = 27.6$, $p > 0.05$; $k = 0.159$), cattle ($\chi^2 = 10.6$, $p > 0.05$; $k = 0.377$), mule deer ($\chi^2 = 6.1$, $p > 0.05$; $k = 0.124$) and white-tailed deer ($\chi^2 = 4.1$, $p > 0.05$; $k = 0.07$). Median abundance varied significantly among host species ($p < 0.001$) with the highest value in cattle (Table 5.1). The majority of wildlife hosts harboured low numbers of flukes, with 75.0% (95% CI: 69.4 - 80.6%) of elk, 95.5% (95% CI: 86.8 - 100.0%) of mule deer, and 75.0% (95% CI: 53.8 - 96.2%) of white-tailed deer infected with fewer than 100 flukes. In contrast, only 34.1% (95% CI: 19.6 - 48.7%) of cattle harboured similarly low parasite counts. Only 3.9% (95% CI: 1.3 - 6.4%) of elk, 9.8% (95% CI: 0.0 - 18.8%) of cattle and 6.3% (95% CI: 0.0 - 18.8%) of white-tailed deer harboured more than 1000 flukes. The highest parasite intensities were found in a fawn white-tailed deer (3942 flukes) and a calf elk (4343 flukes).

5.4.2 Host species differences in prevalence and egg-to-pasture transmission

Estimates of p varied significantly among host species ($\chi^2 = 23.3$, $df = 3$, $p = 0.001$) with 72.4% (95% CI: 66.7 - 78.2%) of elk, 92.7% (95% CI: 84.7 - 100.0%) of cattle, 36.4% (95%

CI: 16.3 - 56.5%) of mule deer and 62.5% (95% CI: 38.8 - 86.2) of white-tailed deer harboured gravid flukes.

Combining data on prevalence, host abundance and mean fluke abundance we estimated that a total 1.8×10^6 flukes are shared among the four resident host species in a given year. Overall, the cattle population harboured 90.6% (95% CI: 80.7 - 99.2%) of all flukes. In contrast, the populations of mule deer, white-tailed deer and elk harboured <1.0% (95% CI: 0.0 - 1.5%), 3.4% (95% CI: 0.0 - 12.4%), and 5.8% (95% CI: 2.8 - 8.8%) of the total number of flukes, respectively.

The proportionate contribution to the total production of *D. dendriticum* eggs onto pasture varied among host species. The cattle population shed a total of approximately 232.4×10^9 eggs onto pasture each grazing season, thus contributing 78.7% (95% CI: 66.6 - 90.8%) of all eggs shed onto pasture per annum in CHP (Table 5.2). Mule deer, white-tailed deer, and elk contributed approximately 0.4% (95% CI: 0.0 - 3.0%), 11.7% (95% CI: 0.0 - 27.4%), and 9.2% (95% CI: 5.5 - 12.9%) of the eggs released onto pasture, respectively. Although the predominant role of cattle in *D. dendriticum* circulation is surprising given their relatively short residency time in CHP each year, this suitable host is a numerically dominant feature on the landscape representing 73.2% (95% CI: 72.1 - 74.4%) of all suitable hosts.

3.3 Host age and egg-to-pasture transmission

Median abundance varied significantly with age in elk ($p < 0.001$) but not in cattle ($p > 0.05$; Table 5.3). Estimates of C relative to age class differed between host species. With calves comprising approximately 20% of the total herd (Bender & Piasecke, 2010), of which approximately 84% were infected, the subpopulation of infected calf elk (about 121 hosts)

were estimated to contain about 69.6% (95% CI: 37.8- 80.2%) of the approximately 105,000 worms in the total worm population and shed 65.8% (95% CI: 59.7- 71.8%) of the 23.7×10^9 eggs shed by elk per annum. In contrast, the 2000 bovine calves and their 2000 mothers introduced into CHP each spring are estimated to harbour approximately 1.1×10^6 worms a few months later, with adults harbouring 66.7% (95% CI: 26.9 - 64.4%) of all *D. dendriticum* found in cattle. These crude calculations, combined with estimates of fluke fecundity (Beck *et al.*, 2015) and host residency time, imply that the large subpopulation of adult cattle contributes roughly 71.8% (95% CI: 53.4 - 90.2%) of all fluke eggs that are produced from these two species of hosts. The total contribution by elk and bovine calves was small in comparison (elk: $10.5 \pm 15\%$; cattle: $12.3 \pm 15\%$).

5.5 DISCUSSION

Quantifying between- and within-species differences in rate of parasite transmission is key to understanding risk of dispersal and in designing targeted intervention strategies for emerging multi-host parasites. Our results indicate that *D. dendriticum* utilizes 4 sympatric host species in CHP. This is consistent with the other parasite survey data, where *D. dendriticum* has been documented in a broad range of domestic and wild ruminant species, as well as rabbits, pigs and horses in Europe (Goater & Colwell, 2007; review by Otranto & Traversa, 2002; Rojo- Vásquez *et al.*, 2012). In CHP approximately 60-90% of cattle, elk and white-tailed deer are infected at any given time, with lower parasite prevalence in mule deer (~40%). Although variable, this pattern of occurrence is high relative to other geographic locations, with 32%, 54%, 21% and 27 to 63% of surveyed hosts found infected in Germany (1975), Slovakia (1984-1986), Hungary (1993) and northern Spain (1991-1993), respectively (review by Ducháček & Lamka, 2003).

Despite significant circulation among sympatric CHP ungulates, disparity in mean abundance and prevalence was apparent among species and hosts of differential age. This is a common epidemiological pattern in natural systems (Hudson & Dobson, 1995), where even small differences in host body size, behaviour and immune efficacy can significantly impact parasite transmission (Arneberg, 2002; Goater *et al.*, 2014). As the infective parasite stages of *D. dendriticum* are found in discrete patches across the landscape (see Chapter 2), we expect that variation in host species distribution and diet could translate to differences in rate or frequency of exposure. However, the habitat and diet composition of sympatric wild and domestic definitive host species in CHP overlap significantly (Lee, 1979). Indeed, our data suggest that nearly all animals under the age of 2 are exposed to infective metacercariae at the initiation of grazing post-weaning. This is consistent with the age-infection profiles presented in Beck *et al.* (2014) and the generalist reproductive strategy presented in Beck *et al.*, (2015). Alternatively, host-specific differences in immuno-competence could account for differences in patterns of infection between sympatric host populations. While average fluke abundance has been shown to decrease with age in elk, indicative of an acquired protective immunological response, such activity was not apparent in cattle (Beck *et al.*, 2014). Ineffective immunological activity following repeated exposure to metacercariae is consistent with the higher observed overall prevalence and abundance of infection in cattle, with susceptible hosts continuing to accumulate flukes over time. The low parasite success in mule deer provides further support that heterogeneity in immune efficacy may be an underlying driver of *D. dendriticum* transmission dynamics. Although other environmental and species-specific barriers to transmission cannot be ruled out, low recruitment of *D. dendriticum* in mule deer may be indicative of an innate resistance to infection. Innate anti-parasite resistance is also thought to explain the absence of *Fascioloides magna* in Alberta

mule deer (Pybus *et al.*, 2015). However, given the detection of anti-*D. dendriticum* antibodies in experimentally infected lambs (Gonzalez-Lanza *et al.*, 2000) and cattle (Colwell & Goater, 2010), further studies are needed to evaluate how heterogeneities in host immuno-competence affect rate of parasite transmission at the host population and host community levels.

Prior to this study, the relative contribution of each sympatric hosts species to the overall transmission of *D. dendriticum* within CHP was unknown (Goater and Colwell, 2007). Combining estimates of host population size, liver worm burdens, egg production rates, and host residency time within CHP we determined that cattle contribute approximately 80% of the 295 billion eggs that contaminate pasture each year. The predominant role of cattle in *D. dendriticum* transmission is somewhat surprising given the transitory presence of this host species in CHP each year. However, cattle are a numerically dominant feature on the landscape representing approximately ~73% of the total number of hosts on pasture during the snow-free months. Of this total contribution, cattle >2 years of age are responsible for ~85% of all eggs shed onto pasture by the total cattle population. Thus, the population of cattle, confined to CHP grazing pastures, is disproportionately responsible for the majority of eggs shed onto pasture.

Taken together, these data are consistent with the idea that host abundance and host community composition are key epidemiological features affecting overall rate of *D. dendriticum* transmission. It is typically assumed that for host generalist parasites, cumulative rates of transmission decrease in the presence of multiple, highly abundant species of hosts due to variability in host competence and susceptibility (i.e. dilution)(Dobson, 2004; Keesing *et al.*, 2006; Ostfeld & Keesing, 2000). For example, a broad range of species can act as suitable reservoirs for *Borrelia burgdorferi*, the causative

bacterium of Lyme disease. However, host competence in the ability to amplify or transmit the virus varies significantly across a community of mammalian hosts (Ostfeld *et al.*, 2006). However, if parasite performance is relatively equivalent among a broad range of hosts, the rate of transmission and risk of dispersal are amplified, rather than diluted, in the presence of a diverse and highly abundant assemblage of hosts. In such cases, the host species with the greatest population density is disproportionately responsible for parasite transmission (Dobson, 2004; Enzema, 2003). This is also consistent with classic epidemiological models for single host - single parasite interactions where the frequency of host-parasite contact and rate of transmission increases with density of hosts (Anderson and May, 1978; May and Anderson, 1978). Clear evidence of this pattern among naturally infected sympatric populations has, until now, remained limited.

Although our data provide a suitable approximation and insight into the circulation and relative contribution of naturally infected sympatric hosts to the contamination of pasture, further sampling is necessary. As our empirical study relied on data collected from small samples of experimentally-infected hosts and opportunistically sampled wildlife, the detection of superspreading individuals is problematic (Streicker *et al.*, 2013). Further, the assumption that all eggs shed by individual flukes are viable and retain their infectivity for one year may overestimate the relative contribution of cattle to the contamination of CHP pasture. Egg shedding in experimentally infected cattle and sheep increased to a peak in late fall and early winter (Manga-González & Ferreras, 2014; Manga-González & González-Lanza, 2005). If the kinetics of *D. dendriticum* egg deposition is applied to CHP, peak egg shedding likely occurs at the end of the snow-free-grazing season and continues after cattle have been removed from the Park. Further, controlled field studies have documented resistance of *D. dendriticum* eggs to low temperatures (<-20°C), while egg mortality peaks

(up to 100%) with summer temperatures high in July and August (Manga-González & Ferreras, 2014; Manga-González & González-Lanza, 2005). Together this may limit the relative contribution of cattle to the accumulation of viable parasite eggs on pasture. Future work should therefore aim to weight estimates according to fluctuations in host and intermediate host richness and abundance, as well as heterogeneity in parasite distribution patterns (i.e. measure of aggregation within a host population) and temporal variability in parasite transmission and reproduction (e.g. Dobson, 2004; Morgan *et al.*, 2006).

The data presented here show that a large proportion of sympatric ungulates are infected in CHP in a given year. The translocation of domestic stock and wild ungulate populations therefore represents a significant risk for parasite spill-over to naïve species of host. Further, the low host specificity and associated broad range of hosts utilized by this generalist parasite (Ducháček & Lamka, 2003; Beck *et al.*, 2015) increases the likelihood of emergence beyond CHP (Hatcher *et al.*, 2012). This presents a significant management challenge. Where transmission is predominantly influenced by the abundance of hosts, as is the case for *D. dendriticum* in CHP, management and intervention may be effective if focused on a single species (Streicker *et al.*, 2013). However, the targeting of a single species of host will likely be insufficient. With peak intensities and high prevalence of infection in white-tailed deer and young elk, subgroups of these host species represent potential ‘superspreaders’ for *D. dendriticum* transmission. The relatively free movement of these wildlife hosts within and outside the park offers significant risk for the geographical translocation and spill-over of *D. dendriticum* into novel host populations. For example, white-tailed deer population density and distribution range continues to expand at northern peripheries due to changes in climate and land use (Vanderwaal *et al.*, 2015), presenting a significant risk for transmission of this and other parasites (e.g. *Parelaphostrongylus tenuis*

and *Fascioloides magna*) to new geographic regions. As such, effective management of this and other invasive parasites may require a combined targeting of select species and specific ‘super-infected’ or ‘super-shedding’ individuals (Streicker *et al.*, 2013).

The likelihood of parasite emergence is determined by a combination of traits intrinsic to the parasite (e.g. degree of specificity, reproductive output) and the range of host species (e.g. variable host competence), as well as factors extrinsic to the final hosts (e.g. suitable environment, vector/intermediate host abundance) (Hatcher *et al.*, 2012; Ostfeld & Keesing, 2000). However, assessing risk of parasite transmission across the wildlife-livestock boundary is usually extremely difficult. Accurate data are often scarce and open to bias, with large and equal sample sizes needed to detect heterogeneities in patterns of infection among and within a range of suitable hosts (Poulin, 2007). The data presented here allow us to conclude that for extreme host generalists, that possess the ability to transmit and perform equally well in a broad range of host species, host abundance and community composition significantly affect rate of transmission. As human development and livestock grazing continues to encroach on shrinking wildlife habitat, the incidence of emerging novel host-parasite interactions is likely to increase alongside the availability of higher densities of suitable hosts (Daszak, 2000). A greater understanding of how individual host species and host community structure affect the transmission and establishment of parasites should therefore become a priority in parasite epidemiology (Lafferty *et al.*, 2006; Johnson *et al.*, 2008). The data and analysis framework presented here are therefore an important step in the investigation of transmission risk of multi-host generalist parasites. Combining the real data presented here in multi-host transmission models, which incorporate the complex dynamics of transmission, may therefore provide key insights into the trajectories of parasite

dispersal, the underlying drivers of invasion, and the likelihood of emergence in key host populations.

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Table 5.1: Adult *D. dendriticum* infection summary in livers of beef cattle and hunter shot elk, mule deer, and white-tailed deer in, and adjacent to, Cypress Hills Interprovincial Park, Alberta, Canada.

Species	N	<i>A</i> (Mean ± SEM)	Median	Range	<i>p</i> (95% CI)
Elk	232	184.7 ± 32.1	13	0-4343	72.4 (66.7 - 78.2)
Cattle	41	444.6 ± 98.8	266	0-117	92.7 (84.7 - 100.0)
Mule Deer	22	19.6 ± 9.3	0	0-181	36.4 (16.3 - 56.5)
Whitetail Deer	16	290.2 ± 230.3	7	0-3942	62.5 (38.8 - 86.2)

A : mean worm abundance

p : proportion of infected hosts

Table 5.2: Relative percent contribution of potential definitive host species to annual contamination of *D. dendriticum* eggs onto pasture in Cypress Hills Interprovincial Park, Alberta, Canada. Contribution is calculated as % of total eggs shed per annum by all surveyed species of hosts.

Host	N	λ_i	H_i	A_i	p_i	t_i	t_i ($\times 10^9$)	T_i (95% CI)
Elk	232	701	800	184.7	0.7	365	27.2	9.2 (5.5-12.9)
Cattle	41	1561	4000	444.6	0.9	90	232.4	78.7 (66.6-90.8)
Mule Deer	22	1494	316	19.6	0.4	365	1.2	0.4 (0.0-3.0)
White-tailed Deer	16	1494	346	290.2	0.6	365	34.5	11.7 (0.0-27.4)

λ_i : *per capita* parasite fecundity; based on *ex vivo* counts in Beck *et al.* (2014) with values for deer based on experimentally infected sheep

H_i : estimate of host population size

A_i : mean worm abundance

p_i : proportion of infected hosts

t_i : estimate of time spent in the park annually (days)

t_i : estimate of total eggs shed on pasture annually

T_i : relative contribution to total transmission annually (percent of total eggs shed to pasture annually)

Table 5.3: Relative percent contribution of calf and adult elk and cattle to annual contamination of *D. dendriticum* eggs onto pasture in Cypress Hills Interprovincial Park, Alberta, Canada. Contribution is calculated as % of total eggs shed per annum by all surveyed hosts.

Host	Age (years)	λ_i	H_i	A_i	p_i	t_i	t_i ($\times 10^9$)	T_i (95% CI)
Elk	Calves (<2)	701	160	597.4	0.8	306	15.6	10.5 (6.5-14.4)
	Adults (>2)	701	640	76.2	0.7	365	8.1	5.4 (2.5-8.4)
Cattle	Calves (<2)	1561	2000	224.9	0.8	31	18.3	12.3 (2.2-22.3)
	Adults (2+)	1561	2000	380.0	1.0	90	106.8	71.8 (58.0-85.6)

λ_i : *per capita* parasite fecundity; based on *ex vivo* counts in Beck *et al.* (2014)

H_i : estimate of host population size

A_i : mean worm abundance

p_i : proportion of infected hosts

t_i : estimate of time spent in the park annually (days)

t_i : estimate of total eggs shed on pasture annually

T_i : relative contribution to total transmission annually (percent of total eggs shed to pasture annually)

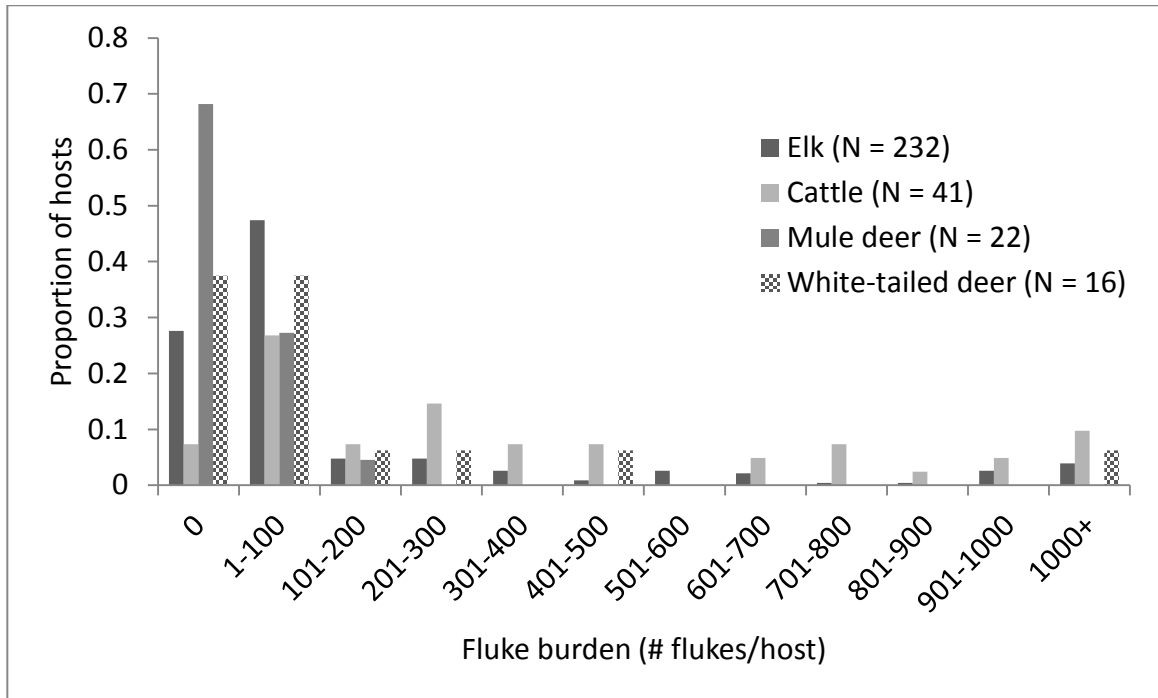


Figure 5.1: Frequency distributions for *D. dendriticum* in elk, mule deer, white-tailed deer, and cattle in Cypress Hills Interprovincial Park, Alberta, Canada.

CHAPTER 6: GENERAL DISCUSSION

6.1 CHAPTER SYNTHESIS

A broad understanding of the drivers for parasite emergence is dependent, in part, on an appreciation of why a parasite occurs in a particular region and in particular groups of hosts. These factors contribute to defining the trajectories for spread and the underlying drivers for parasite invasion (Hoberg, 2010; Hoberg & Brooks, 2015). However, a paucity of robust epidemiological data often precludes reliable estimates of parasite distribution and rates of transmission, particularly for emerging, indirectly-transmitted host generalist parasites (Morgan *et al.*, 2006; Hoberg & Brooks, 2008). Insufficient data on heterogeneity in transmission over space, between species of host, and among individuals continues to limit the development and implementation of evidence-based surveillance and intervention strategies for a large number of parasites and pathogens (Wilson *et al.*, 2002). The emergence of *Dicrocoelium dendriticum* in Cypress Hills Interprovincial Park (CHP) has provided a unique opportunity to evaluate the environmental and host-specific factors that generate, maintain, and constrain parasite distribution and likely influence the opportunity for parasite spill-over into novel hosts and geographic regions.

In Chapter 2, I presented the first high-resolution (1m²), GIS-based analysis aimed at predicting hot spots of *D. dendriticum* ant-to-ungulate transmission within CHP. As expected, ant-to-ungulate *D. dendriticum* transmission varied significantly over space. A suite of fine-scale ecological covariates can accurately predict spatial variation in risk of ant-to-ungulate transmission – i.e. aspen dominated sites on well-drained, south or east-facing slopes, where temperature and moisture retention is presumably moderated by the presence of local vegetation. These habitat features were consistent with the microhabitat

requirements of the first intermediate host, the *Oreohelix* spp. mollusc (Boag & Wishart, 1982; Kralka, 1986; review by Manga- González *et al.*, 2001; Hendricks, 2003) and also aligned with increased densities of clinging ants, higher ant nest density, and the conditions required for Formicid ant colony establishment (Punttila, 1996). The models presented in Chapter 2 therefore provided key insights into the ecology of *D. dendriticum* transmission. GIS-based projections of this model can be used to characterize spatial variability in risk of ant-to-ungulate transmission and for extrapolating risk of parasite emergence beyond its' contemporary distributional range.

Although characterizing spatial heterogeneity in transmission is essential to understanding historical and contemporary patterns of transmission, forecasts of parasite dispersal that fail to incorporate the host component are likely to be inaccurate. In a particularly elegant set of experiments, the distribution of the tapeworm *Hymenolepis diminuta* was highly aggregated among experimentally exposed flour beetles (*Tribolium confusum*) even when infective stages were evenly distributed over space (Keymer & Anderson, 1979). Indeed parasite success and rate of transmission are significantly influenced by heterogeneities within and among populations of hosts (Haydon *et al.*, 2002; Wilson *et al.*, 2002). My results in Chapter 3 indicated that there are cases where the rate of parasite transmission varies relative to individual heterogeneity and species-specific differences in host competence. Average *D. dendriticum* abundance peaked in 6-24 months old CHP elk and sharply declined with host age (Beck *et al.*, 2014). The most parsimonious explanation for this pattern was the development of a protective immune response among chronically exposed adult elk (>2 years of age). In comparison, fluke abundance did not vary significantly with age in free-ranging cattle. Although patterns of infection varied among adult hosts, peak parasite counts among naturally exposed calf elk

and cattle suggest that all animals are highly susceptible to infection within their first two years of life (Goater & Colwell, 2007; Beck *et al.*, 2014). Relatively equivalent parasite performance (i.e. recruitment, morphology and reproduction) was also found among parasite naïve hosts that were both naturally (elk) and experimentally (sheep and cattle) exposed to *D. dendriticum* (Beck *et al.*, 2015a). It is therefore expected that hosts <2 years of age shed relatively high numbers of eggs onto pasture in each successive grazing season. The relative contribution to transmission by the subpopulations of adult hosts of each species then varies according to host immuno-competence following repeated exposure, with subpopulations of highly infected hosts responsible for the majority of egg-to-pasture transmission (Beck *et al.*, 2014).

It is not until we combine estimates of host population size, fluke burden, egg production rates, and host residency time within CHP that host species-related differences in transmission can be illuminated (Chapter 5). The comparison of relative contributions to the contamination of CHP pasture in Chapter 5 established a high *per capita* transmission rate among cattle. This population of domestic hosts was estimated to contribute approximately 80% of the 300 billion eggs that contaminate pasture each year. This result is not unexpected given the 8-fold difference in cattle population density within CHP compared to other species of sympatric ungulates. However, as final host-to-snail transmission is known to occur in regions of CHP where beef cattle are excluded, the much smaller sub-populations of young elk (< 2 years old) and white-tailed deer must also play a role in disseminating eggs within the region.

Taken together, the data presented in this thesis improve our understanding of the drivers of the emergence and establishment of *D. dendriticum* in CHP. Since *D. dendriticum* was introduced to CHP in the late 1980's, the prevalence of *D. dendriticum*

has risen significantly in all sympatric grazing hosts (Goater & Colwell, 2007). Currently, 40-80% of all elk, white-tailed deer, mule deer and cattle are infected (Goater & Colwell, 2007; Beck *et al.*, 2014). As a key variable in the ant-to-ungulate transmission risk model, the presence of aspen-dominated habitat appears to have provided optimal conditions for obligate arthropod and molluscan intermediate hosts (Chapter 2). Further, the concentration of high densities of sympatric hosts (Chapter 5) and the low host specificity of *D. dendriticum* (Beck *et al.*, 2015a) may have allowed this host generalist to maximize its' transmission opportunities. Low host specificity boosts parasite transmission success by effectively increasing the density of suitable hosts, amplifying the opportunity and rate of host-parasite contact (Combes, 2001; Hoberg & Brooks, 2008).

The translocation of high densities of infected cattle and select subgroups of sympatric wildlife (i.e. young elk and deer) represent a significant risk for the spill-over of *D. dendriticum* into novel geographic regions and species of host (Combes, 2001; Hoberg & Brooks, 2008). The expanded distribution of *D. dendriticum* is a concern to livestock producers and conservationists as *D. dendriticum* may negatively impact host health. Cholangitis, liver fibrosis, anaemia and reduced weight gain (review by Manga-González *et al.*, 2001), along with changes in liver function (i.e. increased production of bilirubin, albumen, hepatic enzymes) (Theodoridis *et al.*, 1991; Manga-González & González-Lanza, 2005), have been documented in naturally- and experimentally-infected hosts. The models and data presented in this thesis may therefore be important tools for accurately predicting risk of *D. dendriticum* emergence and directing evidence-based management programs. Further, the spatial models that I developed could conceivably be used to predict where risk of ant-to-ungulate transmission is highest, allowing for targeted intervention or exclusion of hosts from areas where host-parasite contact is most likely. Additionally,

comparisons of parasite performance and reproductive output allowed for the identification of supershedding individuals and subpopulations of hosts. Together, these data allow for the determination of where, when and in which hosts, risk of transmission is the greatest.

6.2 METHODOLOGY/ADVANCES

6.2.1 *GIS and Bayesian geostatistical analyses*

Although the use and accessibility of GIS tools has increased over the last two decades (Reisen, 2010), they remain underutilized in many areas of epidemiological research. Contemporary research has predominantly focused on deciphering broad-scale patterns of disease and parasite prevalence in relation to generalized ecological and climate covariates. This region-based approach is useful in evaluating generalized patterns in parasite transmission (e.g. Beck *et al.*, 2015b) and in directing broad-scale management strategies (Pullan *et al.*, 2011; Hay *et al.*, 2013). However, as conditions can vary drastically on regional and local scales, even between adjacent and connected patches of land, this low-resolution approach underestimates local heterogeneity (Malone, 2005; Musella *et al.*, 2010). The high-resolution (1m²) GIS-based modelling approach presented in Chapter 2 is rare for landscape epidemiological studies. The evaluation of fine-scale covariates of parasite transmission allowed me to define a suite of specific microhabitat conditions and environmental limitations that characterize the overlap in distribution of parasite, intermediate, and final host species (Malone, 2005; Musella *et al.*, 2010). Thus, these data provided insights into the transmission ecology of this parasite on a fine-scale, with key implications for forecasting the risk of parasite dispersal. The local-scale approach demonstrated in Chapter 2 may also be useful for understanding spatial variability in rates of transmission for other indirectly transmitted generalist parasites.

Accurate occurrence data for most parasites are sparse. Spatial statistical analyses aim to provide detailed predictions of distributions by relating presence or prevalence of infection to specific environmental predictors (Elith and Leathwick, 2009). My modelling approach made use of an Information Theoretic approach within a Bayesian framework where *a priori* assumptions about parasite biology and transmission were not required. Ecologists and epidemiologists are increasingly making use of Bayesian inference to model the role of selected covariates and their effects on the spread or risk of disease. Bayesian methods are of particular interest to epidemiologists because they can be used to make probabilistic predictions (i.e. what is the probability of the hypotheses being true given the observed data?), while conventional statistics are restricted to statements about long-run averages obtained from hypothetical replicates of sample data (McCarthy, 2007). The flexibility of the Bayesian framework can accommodate both continuous and binary data (e.g. presence/absence of infection), providing ways to account for the many sources of variation inherent in parasite epidemiology. This includes variation in the predictors themselves (e.g. long range climate data), unobserved processes, and spatial and temporal autocorrelation, even when sample sizes are small (Barker, 2008). Model outputs provide assessments of relative model performance in regards to: (1) how well the different models fit the data (i.e. describing central tendency and variance of the data), predictions which are both unbiased and precise; and (2) parsimony, i.e. all else being equal, a simple model is better than a more complicated model. The best model is the one that explains the maximum level of detail in the simplest possible way (McCarthy, 2007). Overall, the results I present in Chapter 2 and in Beck *et al.* (2015b) demonstrate the applicability of this approach at both local and regional scales.

Regardless of the modelling method chosen, validation is an important, and yet often neglected step. On the rare occasions when model validation has been completed, analyses often rely on the cross-validation of data that are withheld from model development (i.e. data splitting). Model accuracy is then assessed based on how well models predict the withheld data (Elith and Leathwick, 2009). Where presence or abundance data are sparse and/or inconsistent, such withheld data are unlikely to provide a reliable test of model accuracy. Alternatively, evaluations of model performance should aim to use independent, well-structured presence-absence, prevalence or abundance datasets for validation. This approach was utilized in the validation of the *D. dendriticum* ant-to-ungulate transmission risk model (Chapter 2) and in the regional-risk assessment for spatial variability in gastro-intestinal nematode (GIN) transmission (Beck *et al.*, 2015b). Such datasets have rarely been used to evaluate model-based predictions. Ideally, models should be developed and tested in iterative cycles that take account of the desired uses of the model, investigate the ecological rationality of the modelled responses, and explore errors in predictions (Elith and Leathwick, 2009; Hay *et al.*, 2013).

6.2.2 Diagnostics

The opportunistic sampling of sympatric grazing ungulates in CHP (cattle, elk, mule deer, and white-tailed deer) allowed for the direct comparison of actual parasite counts and the collection of adult flukes for subsequent comparisons of parasite performance (Chapters 3-5). This gold standard is rare, particularly for a host generalist with wild hosts utilized during at least one developmental stage in the parasite lifecycle (Wilson *et al.*, 2002). These data provided a unique opportunity to compare intra- and interspecific variability in parasite circulation, recruitment, development, *per capita* fecundity, and relative

contributions to overall transmission among a range of sympatric hosts. However, this approach was constrained by sampling heterogeneity and low host sample sizes.

Use of alternative diagnostic tools may present a unique opportunity for accurate evaluation of spatial patterns for the transmission of a broad range of parasites, especially when host sacrifice is not a feasible option. The results in Beck *et al.* (2015b) provide a good example, demonstrating the pairing of a modern diagnostic measure of parasite status (antibody detection in serum by ELISA), with local and regional environmental characteristics to evaluate spatio-temporal heterogeneity in GIN transmission. I anticipate that the development and use of antigen-capture assays will increasingly be utilized as a non-invasive diagnostic alternative. This diagnostic technique has been used as a proxy for intensity for the trematode *F. hepatica* in lambs (Mezo *et al.*, 2004) and the cestode *Anoplocephala perfoliata* in horses (Skotarek *et al.*, 2010). Following the development of coproantigen detection methods, experimental comparison of diagnostic techniques will be necessary. Comparative assessment of suitable diagnostic techniques, including faecal egg count detection, antibody detection in serum by ELISA, and coproantigen detection by ELISA against parasite counts at necropsy as the gold standard against which these indirect diagnostic techniques are measured is the logical next step (Skotarek *et al.*, 2010).

6.3 STUDY LIMITATIONS

6.3.1 Local-scale mapping

The applicability of the ant-to-ungulate transmission risk model presented in Chapter 2 is likely to be limited to ecological similar landscapes. Given the cosmopolitan distribution of this host generalist fluke, and the broad range of intermediate and final host species that can be utilized (reviews by Manga-González *et al.* 2001; Otranto & Traversa, 2002), species-

specific differences in environmental tolerances should be expected (Brooker *et al.*, 2002). Further, fine-scale studies may not account for the influence of broad-scale climate patterns on parasite transmission (e.g. Beck *et al.*, 2015b) and host distribution (e.g. Brooker *et al.*, 2006). This is likely a common issue in epidemiological studies. Future research involving *D. dendriticum* at the landscape scale must therefore account for differences in ecological requirements for the intermediate hosts in each geographic region and aim to evaluate the significance of broad-scale ecological covariates.

6.3.2 Opportunistic sampling

Epidemiological studies often rely on the opportunistic collection of data. This typically results in sampling heterogeneities that in turn, constrain analyses and inference accuracy. As a result, mean parasite intensities and the degree of parasite aggregation may be underestimated (Shaw & Dobson, 1995; Woolhouse *et al.*, 1997). This sampling ‘bias’ is common across different age categories, with a decline in sample sizes as hosts’ age (Wilson *et al.*, 2002). This was shown to be a limiting issue in our evaluation of the distribution of *D. dendriticum* among individual elk and cattle with respect to host age (Beck *et al.*, 2014). Statistically, there is much debate over how to best capture the degree of aggregation within a sample of hosts. In their comparison of a number of aggregation indices, Gregory and Woolhouse (1993) found that this overdispersed distribution is best described by the negative binomial distribution exponent k (Anderson & May 1978; May & Anderson 1978; Shaw & Dobson, 1995). Sample size issues can be minimized when using maximum-likelihood estimates of k (Pacala & Dobson, 1988; Gregory & Woolhouse, 1993). Application of this index allowed for the comparison of age-abundance patterns in CHP elk and domestic cattle, despite decreasing sampling sizes in older animals (Beck *et al.*, 2014).

However, such indices may still underestimate parasite aggregation, particularly when the parasite population is highly aggregated within the host population(s). Future research must therefore aim to follow a stratified sampling approach relative to population demographics, with large, equal samples of individuals belonging to the broad range of host species (Wilson *et al.*, 2002; Poulin, 2007).

Reliance on opportunistic sampling in a small geographic area also limited our ability to compare the performance of *D. dendriticum* across a broad range of host species. All three ruminant hosts in our study belong to the order Artiodactyla (Spaulding *et al.*, 2009). Phylogenetic relatedness of these host species (domestic cattle and sheep, wild elk), and in turn the conservation of host traits, could lend itself to equal host utilization and similarities in parasite performance (Poulin, 2007; Hoberg and Brooks, 2008). Low taxonomic distinctiveness plays a significant role in determining host breadth for helminth parasites of freshwater fish, with low specificity documented in parasites of a speciose host family (Poulin, 1992). However, reproductively mature *D. dendriticum* have also been documented in camels, rabbits, pigs, dogs, horses, humans and experimentally-infected laboratory hamsters (Manga-González *et al.* 1991; Campo *et al.* 2000; Sánchez-Campos *et al.* 2000; review by Otranto & Traversa, 2002; Manga-González & González-Lanza, 2005). Future research is therefore needed to evaluate potential differences in parasite performance among more distantly related taxa with focus on physiological, immunological, and genetic variability among a diverse array of host species.

6.3.3 *Ex vivo* comparisons of fluke performance

Accurate data on variability in individual parasite fecundity are exceedingly rare. The opportunistic collection and incubation of individual *D. dendriticum* allowed for

comparisons of daily fluke fecundity *ex vivo*. However, this measure of fecundity may not be representative of individual performance *in vivo* with flukes disconnected from the original size of the infrapopulation. Individual fluke fecundity was measured while each fluke was maintained alone in wells of culture plates, in an artificial medium, with ample food and no competitors. Further research is therefore needed to assess host-related patterns in parasite performance and to validate whether our conclusions of equal performance and the absence of density-dependent parasite performance (Beck *et al.*, 2015a) remain valid *in vivo*.

6.4 PROSPECTS FOR FUTURE RESEARCH

6.4.1 GIS application and integration

The utility of the *D. dendriticum* risk model for ant-to-ungulate transmission may be limited to similar ecological areas. However, *D. dendriticum* has been reported across Europe (review by Otranto & Traversa, 2002) and North America (B.J. van Paridon, Ph.D. thesis, unpublished observations). The fine-scale model presented in Chapter 2 does not account for the species-specific ecological preferences of the broad range of intermediate host species utilized across the cosmopolitan distribution of this invasive parasite. Perhaps the way forward is to combine assessments on multiple geospatial scales, effectively scaling up and scaling down to cover similar geographic areas. A similar approach was taken to evaluate the broad and fine-scale ecological covariates for the distribution and abundance of *Fasciola hepatica* in Columbia (Valencia-Lopez *et al.*, 2012). Notably, this approach would allow for the determination of local scale variation in host-parasite contact, as well as defining the broad-scale ecological and climatological covariates of host movement and host density. The resulting models would provide biologically and statistically accurate forecasts

for parasite transmission patterns and risk of emergence under various climatic regimes and different climate change projections.

6.4.2 Modelling transmission and forecasting emergence

Formalizing our understanding of *D. dendriticum* transmission dynamics in mathematical models could accommodate forecasts of the direction and rate of spread for this invasive parasite. Such models have application in the development of evidence-based intervention strategies. Models have been used successfully in this way for several important livestock diseases (e.g. MacKenzie & Bishop, 2001; Keeling, 2005). However, additional complexities in wildlife systems limit the applicability of livestock-based models. Patterns of host movement, host population size, density and contact rates are likely to vary considerably among species of host, with key implications for the transmission of complex lifecycle, multi-host parasites across the wildlife-livestock boundary (Morgan *et al.*, 2006). Focusing on the likely drivers of transmission for directly transmitted pathogens, Morgan *et al.* (2006) provided model-based predictions for the roles of wildlife and livestock in the epidemiology of pathogen transmission. Extensions of these models may provide key insights into parasite epidemiology. However, the parameterization, exploration and validation of transmission models of complex life-cycle, multi-host parasites are likely to be far more complex than those presented for direct lifecycle generalists. The epidemiological patterns of *D. dendriticum* presented in Chapters 2-5 of this thesis, however, present an important first step.

Future model-based examination of intra- and interspecific data must address some of the key areas of data scarcity identified throughout the data chapters of this thesis. Data on variation in host abundance, home range size, and geographic overlap among suitable

hosts may be useful in understanding the circulation of parasites among a range of hosts and the relative contribution of each host species to overall parasite transmission. Incorporation of the determinants of heterogeneity in intermediate and final host species spatial distributions and habitat use will allow for projections of biologically relevant transmission opportunities (Morgan *et al.*, 2006). Models must also aim to account for the role that physiological, immunological, and genetic heterogeneity across the host range play in determining host suitability and susceptibility when the spatial opportunity for transmission arises (Lafferty and Holt 2003).

6.4.3 Dicrocoelium dendriticum as an extreme host generalist

It is generally assumed that natural selection favours the development of high host specificity in parasites, with functional trade-offs limiting the fitness of generalists (Combes, 2001). Indeed, generalists tend to be found at lower abundance and lower community richness in comparison with specialist parasites (e.g. Vázquez *et al.*, 2005). However, *D. dendriticum* appears to possess the ability to perform equally well in a broad range of hosts (Beck *et al.*, 2015a; Chapter 5). This low host specificity also extends throughout the life-cycle of this parasite. *D. dendriticum* utilizes over 90 spp. of terrestrial snail and a diverse number formicid ant spp. as first and second intermediate hosts, respectively (review by Manga-González *et al.* 2001). A generalist strategy of this sort may facilitate the introduction, establishment and emergence of novel parasites in new hosts and geographic regions. However, the mechanisms allowing this parasite to adapt and exploit a broad host complex remain poorly understood. Such research could provide key insights into the ecological epidemiology of this invasive parasite and for understanding the generalist parasite phenomenon itself.

6.5 IMPLICATIONS

Anthropogenically-accelerated climate warming and increasing pressure on the landscape are expected to significantly alter the distribution and structure of contemporary host-parasite assemblages and may ultimately increase the likelihood of parasite spill-over into novel hosts and new geographic regions (Cleaveland *et al.*, 2002; Kutz *et al.*, 2004; Agosta *et al.*, 2010). Generalist parasites represent the greatest risk, with transmission into novel hosts likely to increase with biotic mixing. The data presented in this thesis demonstrate that *D. dendriticum* possesses the ability to attain approximately equal fitness in a broad range of hosts. This fluke also utilizes a notoriously high number of species of terrestrial snail and formicid ants as first and second intermediate hosts (review by Manga-González *et al.* 2001), respectively. A generalist strategy of this sort likely enhances opportunities for host encounter and host switching, and increases overall rates of parasite dispersal (Combes, 2001; Hoberg and Brooks, 2008).

However, as the results of my analyses of the ecological epidemiology of *D. dendriticum* transmission demonstrate, patterns of emergence are likely to vary significantly over space, between populations, and among individuals. Indeed, climate and development-induced changes in intermediate and final host distribution patterns may disrupt the transmission patterns of complex lifecycle parasites (Rogers & Randolph, 2006). In some instances, transmission may be accelerated, while in others, mismatch in environmental tolerance may limit intermediate-to-final host transmission (Pickles *et al.*, 2013). Ultimately, an increased focus of research on the identification and monitoring of individuals, populations and locations that amplify rate of transmission is necessary to understand the complexities of parasite transmission and patterns of emergence (Hoberg & Brooks, 2015).

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APPENDIX I

Where's the risk? Landscape epidemiology of gastrointestinal parasitism in Alberta beef cattle

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This manuscript has been published in the journal *Parasites & Vectors* (2015, 8: 434-447)

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1.1 ABSTRACT

Gastrointestinal nematodes (GIN) present a serious challenge to the health and productivity of grazing stock around the globe. However, the epidemiology of GIN transmission remains poorly understood in northern climates. Combining use of serological diagnostics, GIS mapping technology, and geospatial statistics, we evaluated ecological covariates of spatial and temporal variability in GIN transmission among bovine calves pastured in Alberta, Canada. Sera were collected from 1000 beef calves across Alberta, Canada over three consecutive years (2008-2010) and analyzed for presence of anti-GIN antibodies using the SVANOVIR *Ostertagia osteragi*-Ab ELISA kit. Using a GIS and Bayesian multivariate spatial statistics we evaluated the degree to which variation in specific environmental covariates (e.g. moisture, humidity, temperature) was associated with variation in spatial and temporal heterogeneity in exposure to GIN (*Nematodirus* and other trichostrongyles, primarily *Ostertagia* and *Cooperia*). Variation in growing degree days above a base temperature of 5°C, humidity, air temperature, and accumulated precipitation were found to be significant predictors of broad-scale spatial and temporal variation in serum antibody concentrations. Risk model projections identified that while transmission in cattle from southeastern and northwestern Alberta was relatively low in all years, rate of GIN transmission is generally higher in the central region of Alberta. The spatial variability in risk is attributed to higher average humidity, precipitation and moderate temperatures in the central region of Alberta in comparison with the hot, dry southeastern corner of the province and the cool, dry northwestern corner. Although more targeted sampling is needed to improve model accuracy, our projections represent an important step towards tying treatment recommendations to actual risk of infection.

1.2 KEY WORDS

Gastrointestinal nematodes, GIS, Bayesian, Multivariate hierarchical models, Cattle.

1.3 INTRODUCTION

The distribution, occurrence, and intensity of parasites varies enormously between samples of hosts from different sites, seasons, and years, in part due to interspecific sensitivity of infective stages to variable environmental conditions (Wilson *et al.*, 2002). However, an incomplete understanding of the epidemiology of many direct and indirect-lifecycle parasites continues to limit the identification of high-risk locations and peak transmission periods. To address these key knowledge gaps, landscape epidemiologists seek to characterize variability in rates of parasite transmission in the context of changing climatic and landscape characteristics that arise naturally or through anthropogenic modification (Reisen, 2010). Key advances in this area have often involved the use of modern Geographical Information Systems (GIS) tools, statistical modelling, and improved diagnostic techniques. Evaluation of spatial patterns for a number of vector-borne and other parasitic infections, including schistosomiasis (e.g. Clements *et al.*, 2008) and malaria (e.g. Seghal *et al.*, 2010), have facilitated the prediction of transmission risk in unsurveyed areas, have directed large-scale intervention programs (Pullan *et al.*, 2011), and have helped predict future outbreaks relative to climate warming projections (Fox *et al.*, 2011). Despite advances in the use of these spatial tools (e.g. Bennema *et al.*, 2009), major gaps central to understanding spatial heterogeneity in gastro-intestinal nematode (GIN) transmission remain. The lack of accurate epidemiological data is especially acute in northern latitudes involving domestic stock as hosts (Hoberg *et al.*, 2008).

GIN occur globally in grazing mammals, representing a significant threat to the sustainability of livestock production (Morgan *et al.*, 2013). Infection is a common cause of reduced weight gain, intestinal dysfunction, dysentery, anorexia, and anaemia (Hoberg *et al.*, 2001). In Canada, livestock operations represent a significant component of the agrarian economy. As elsewhere, GIN control programs continue to rely on intensive anthelmintic use aimed at preventing the accumulation of parasite burdens over successive grazing seasons. This approach has been based on observed increases in host productivity following the application of anthelmintics (MacGregor *et al.*, 2001; Reinhardt *et al.*, 2006). Macrocytic lactone dosage for roundworm and ectoparasite control is associated with an estimated saving of \$7.04 per head in calves and \$4.2 per head in yearling cattle compared with control of ectoparasites alone (Bauck *et al.*, 1989; Kim Jee *et al.*, 1992). Despite these clear production and health benefits, the blanket treatment of animals can result in the overuse of anti-parasitics. This gives rise to the threat of anthelmintic resistance affecting the ability to control these parasites and is associated with high costs to producers (Sutherland & Leathwick, 2011; Morgan *et al.*, 2013).

An improved understanding of the influence of climatic characteristics on GIN transmission can aid in the development and implementation of evidence-based parasite control programs aimed at reducing this economic burden and reducing the risk of anthelmintic resistance. Each species of GIN has critical temperature and moisture requirements for optimal development, beyond which development slows and the likelihood of larval survival declines (Sutherland & Scott, 2010). Suboptimal environmental conditions, such as temperature and moisture extremes, that impacts the distribution and survival of free-living larval stages (e.g. Ng'ang'a *et al.*, 2004; Wang *et al.*, 2014), likely result in variability in transmission. At present, little information is available regarding the broad-scale environmental factors that influence the availability of GIN larvae on pasture in northern latitudes. The use of GIS for the development of broad-scale statistical models is therefore valuable for prediction of risk of GIN transmission and in providing an ecologically grounded baseline for management.

Here we focus on improving our understanding of heterogeneity in risk of GIN in domestic beef cattle at a province-wide scale. Our objectives were to: 1) define the temporal and spatial variability in GIN transmission across the province of Alberta, Canada; 2) use a GIS-based approach to evaluate the broad-scale environmental covariates of spatial and temporal heterogeneity in transmission; and 3) create a model to predict risk of infection. We combined standard indirect measures of parasite transmission (ELISA detection of anti-GIN antibody concentrations) with GIS technology to characterize variability in GIN exposure over three consecutive years in bovine calves. Bayesian inference was used to model variability in parasite exposure in relation to key environmental characteristics.

1.4 METHODS

1.4.1 Study area

The province of Alberta extends from 49° to 60° latitude north, with an area of approximately 661,848 square km. The province has three major biogeographical divisions ranging from west to east which vary in elevation and associated climate: the mountains, the foothills, and the plains (Bailey *et al.*, 2010). Our study area is focused on the 79, 000 square km plains region where grazing on native rangelands, Crown land and community pastures is most extensive (Fig. 1) (Bailey *et al.*, 2010). The plains region comprises the majority of the total area of the province, with elevation varying from 800 m along the

eastern border of the province to approximately 1800 m along the foothills belt in the west (GeoBase, 2000). The southeastern corner of this region has an average annual precipitation (1971-2000) of 331 mm (CV: 8.4 %), and an annual maximum temperature (1971-2000) of 21.7°C (CV: 0.3%) during peak grazing season (Jun to Oct) that is associated with a high rate of evapotranspiration, frequent hot dry winds, and prolonged periods of low precipitation. Further north, the annual precipitation increases to about 515 mm (CV: 7%) in the centre of this zone, and then decreases to 475 mm (CV: 6.0%) in the far northwest and 487 mm (CV: 13.0%) in the northeast. Evapotranspiration likely decreases with a maximum annual temperature of 16.8°C (CV: 0.2%) during the grazing season in the northwest of this region and 18°C (CV: 0.2%) in the northeast. Average precipitation also increases markedly from east to west, with approximately 368 mm (CV: 7.7%) of rain along the eastern boarder of the province to as much as 467 mm (CV: 8.5%) on the edge of the foothills (Daly, 2010).

1.4.2 Hosts

In fall (November through December) of 2008-2010, 1000 cross-bred (Angus cows X Hereford bulls) and purebred Angus calves were sampled from a total of 26 auction markets across Alberta. Auction markets were distributed throughout the study region and were opportunistically sampled. Calves were sampled by feedlot staff working in conjunction with Feedlot Health Management Services and researchers from Agriculture and Agri-food Canada. We targeted beef calves coming off their first year on pasture to minimize variation in egg counts due to host age and immunity. We also restricted the sampling window to a 6-wk period each fall to minimize heterogeneity due to inherent seasonal variation. Calves were born in April-May of each year and were maintained on pasture with their dams until weaning in November through December. Calves are transitioned to a finishing diet upon entry into a feedlot or go into a feeding program to prepare them for grazing in their second year (Waldner *et al.*, 2004). All cattle were sampled prior to anthelmintic treatment, with a 10% random sample (Waldner *et al.*, 2004) of calves selected from each sampled “lot” (auction market). Animals were handled under the guidelines of the Canadian Council for Animal Care (Animal Care Committee protocol # 08233, 0925 and 1044).

1.4.3 Faecal egg counts

The presence of eggs in samples of faeces was used to identify the GIN spp. present in each host. Faeces were collected by rectal palpation, stored in individual labelled bags, and frozen prior to analysis. A modified Wisconsin technique with a sugar solution (Zajac & Conboy *et al.*, 2012) was used to process faecal samples. Parasite eggs were identified to genus (*Nematodirus* spp.) according to descriptions in Olsen (1974). Due to similar egg morphologies, all trichostrongyle genera were pooled and termed ‘trichostrongyles’.

1.4.4 Serum antibody concentrations

Blood was collected by jugular venipuncture into vacutainer tubes with serum separators (BD-Canada Inc., ON) from each calf, analyzed using SVANOVIR® *Ostertagia ostertagi*- Ab ELISA kits (Boehringer Ingelheim SVANOVA, Uppsala, Sweden). The reference sera were diluted 1:140 (Colwell *et al.*, 2014). Optical density values read at 405 nm were standardized as an optical density ratio (ODR) using negative and positive control sera samples included on each plate.

1.4.5 Mapping and Meteorological data

All GIS-based mapping analyses were completed in ArcGIS, version 10.1 (Source: ESRI). Spatial analysis required the following digital data sources: digital elevation model (DEM, source: Geobase), generalized land cover map (source: DB Geoservices Inc.), road network (source: ESRI), geo-referenced auction market locations (Fig. 1), and climate data (source: Alberta Agriculture and Rural Development:

<http://agriculture.alberta.ca/acis/alberta-weather-data-viewer.jsp>). For visualization, the Alberta base map was obtained from free sourced data made available in joint by National Geographic, Esri, De Lorme, NAVTEQ, UNEP-WCMC, USGS, NASA, ESA, METI, NRCAN, GEBCO, NOAA, and IPC.

Precise coordinates for grazing pastures were not available. Thus, we calculated the likely service area for each individual lot. These service area polygons were created using the existing road network around each georeferenced lot location, making the assumption that producers select an auction market based upon minimum driving distance. We assumed that unknown sources of error, including lot preferences of producers, cancel each other out. Areas in the province where grazing is not common were excluded based on elevation (over 1250m), land cover type (e.g. coniferous forests, lakes), and presence of urban development (Fig. 1).

Meteorological data were averaged from all geo-referenced climate stations falling within each polygon. The environmental variables considered in the study, especially those associated with temperature and moisture availability, were selected based upon their known role in determining nematode viability and infectivity (Stromber, 1997; Barger, 1999; Ng'ang'a *et al.*, 2004). We only used same-year environmental data, as overwinter larval survival and development of eggs is unlikely (Ranjan *et al.*, 1992). It is therefore assumed that GIN exposure is related to the seeding of pasture in the spring by dams infected during the previous grazing season(s).

Environmental data were collected from May to October to represent the growing season prior to the collection of faecal and serum data at sacrifice (Vanderstichel *et al.*, 2012). This temporal period represents the development period of larvae shed when adult cattle are returned to pasture in May of each year, typically followed by peak GIN intensities in cattle and on grazing pasture during the summer months (Charlier *et al.*, 2007). Data were obtained for the following periods: May-September, June-September, July-September, August-September, May-October, June-October, July-October, and August-October. These data included: (i) total accumulated precipitation (mm), (ii) average daily accumulated precipitation (mm); (iii) average, minimum, and maximum air temperature (°C), (iv) average, minimum, and maximum relative humidity (%), (v) total accumulated growing degree days (GDD) with a base 5°C, and (vi) average daily growing degree days (GDD) with a base 5°C. Relative humidity is a dimensionless ratio, expressed in percent, of the amount of atmospheric moisture present relative to the amount that would be present if the air were saturated. Since the latter amount is dependent on temperature, relative humidity is a function of both moisture content and temperature. Accumulated GDDs were calculated as the accumulation of days with an average daily temperature exceeding 5°C for each of the stated temporal periods. Mean daily GDD is an average of the daily increase in GDD with a base temperature of 5°C for each weather station.

1.4.5 Statistical analyses

ODR data were normalized by log (n+1) transformation. Due to cross antigenicity, *O. ostertagi* ELISA kits are indicative of exposure to a number of GIN genera (Bennema *et al.*, 2009). Chi-squared statistics were used to compare prevalence (p) between samples, with 95% confidence intervals (CI) calculated using the Wald method (Vollset, 1993; $p \pm z\sqrt{pq/n}$), where $z = 1 - \alpha/2$ of the standard normal distribution and $q=1-p$). Mean ODR (\pm SEM) values for each polygon were evaluated using ANOVA with Tukey's post hoc comparisons for each sampling year.

Environmental data were paired with mean ODR values from each polygon. Variables were standardized by subtracting the mean and dividing by two standard deviations (Gleman & Hill, 2007). This conversion accounts for differences in dimension and variance, improves the efficiency of the sampling algorithm, and has no effect on the resulting model.

Bayesian inference was used to construct hierarchical logistic regression models in OpenBUGS version 3.2.2 (Spiegelhalter *et al.*, 2012) testing each environmental variable separately, and in combination, for each of the eight temporal periods. The main advantage of the Bayesian approach is that parameter uncertainty is fully accounted for when performing prediction and inference, even when sample sizes are small. With a hierarchical Bayesian approach we obtain a full accounting of variability among individual polygons, years of sampling, and other environmental covariates, together with estimates of observation errors (Burnham & Anderson, 2002; Elith & Leathwick, 2009). Risk of transmission was modelled as a linear function on a log scale. A non-informative prior distribution (mean = 0, tau = 1.0×10^{-4}) was assigned to the regression coefficients. Sampling year, assumed to follow a uniform normal distribution was included in all models as a random effect.

For all models, we discarded the first 60,000 iterations, with another subsequent 40,000 iterations used to estimate model parameters. This initial burn-in was required to ensure that the model chains converged and that the parameter space has been correctly explored (Elith & Leathwick, 2009). Competing models were ranked by their deviance information criterion (DIC), which is a measure of model fit to the data. The best model is that with the lowest DIC value. To compare models, difference between the DIC_i of each model and the DIC_i of the best fit model ($\text{min}DIC_i$) was calculated for each model:

$$\Delta DIC = DIC_i - \text{min}DIC_i \quad (1)$$

Models within two ΔDIC units of the top performing model were considered to have strong support, within four to seven ΔDIC units to have considerably less support, and greater than ten, no support (Burnham & Anderson, 2002; McCarthy, 2007).

Expected ODR values (i.e. estimated risk of transmission) were calculated for each individual polygon using all the models within two ΔDIC of the top-performing model. Values were then averaged to obtain a mean expected ODR value for each polygon for each of the three sampling years. Using this approach, we account for model uncertainty (McCarthy, 2007). Average annual expected transmission risk was assigned values corresponding to low, moderate and high mean ODR values of <0.3 , 0.3 to 0.5 , and > 0.5 , respectively. 'High risk' (ODR >0.5) was considered indicative of high rates of GIN exposure (Charlier *et al.*, 2005).

Temporal variability in environmental covariates was evaluated using ANOVA with Tukey's post-hoc comparisons for polygons where risk of high GIN exposure varied between years. To assess the spatial accuracy of our model predictions, we compared model projected ODR values with observed ODR values for each polygon using Chi-squared analyses. Parametric correlation coefficients were obtained comparing observed mean ODR values and model projected ODR using data pooled for all three years. Mean square error was then calculated to assess model accuracy.

1.4.7 Model Validation

To validate the GIN transmission risk model, we targeted two polygons for follow-up analyses in 2013. Based on our model predictions, one polygon had consistently low risk of GIN transmission, whereas a second had moderate to high risk of economically significant parasite transmission. The Agriculture and Agri-Food Canada, Lethbridge Research Centre (LRC) field station located at One Four, Alberta (49.4° N, 110.7° W) was selected as the low risk site. A ranch near Stettler, Alberta (52.3° N, 112.7° W) was selected as the moderate to high-risk site. Blood was collected from each calf by jugular venipuncture and analyzed as outlined above for a total of 167 cross-bred calves from the LRC field station and 75 calves from the Stettler ranch.

For initial comparison of parasite transmission differences between the two ranches we calculated: (1) mean ODR (\pm SEM) for each ranch; (2) proportion of samples with bootstrapped 95% CI that were parasite negative (ODR < 0.0); and (3) proportion of calves with bootstrapped 95% CI with high intensity infections (ODR > 0.5) (Charlier *et al.* 2005). Mean values were compared using parametric t-tests, and differences in prevalence values were evaluated using Chi-squared.

Climate data for 2013 were obtained from the Alberta Agriculture and Rural Development. Using these environmental data, mean (\pm SEM) expected ODR values were calculated using the top performing models. We compared model projections based on data obtained from the closest climate station to each respective ranch using Euclidean straight-line distances. We then validated our model using parametric t-tests to compare observed and expected ODR values. Parametric t-tests are also used to compare environmental means used in calculating model projections of exposure risk between these two sites.

1.5 RESULTS

1.5.1 Infection patterns

97.2% (95% CI: 96.2-98.3%) of the 1000 calves sampled from 2008-2010 were sera-positive for GIN. Although the overall proportion of sera-positive animals remained consistent between years, and estimated prevalence did not significantly vary among individual polygons in 2008 ($\chi^2=3.1$, $p = 0.86$) and 2010 ($\chi^2= 5.7$, $p = 0.86$), spatial variation in mean ODR was significant in all three years (Fig. 2; 2008: $F_{3,281} = 24.4$, $p < 0.001$; 2009: $F_{9,321} = 2.4$, $p < 0.05$; 2010: $F_{7,237} = 5.7$, $p < 0.001$). The proportion of infected hosts varied significantly among polygons in 2009 ($\chi^2= 35.2$, $p < 0.01$), however, this variation can be accounted for by an increase in the number of sera-negative animals ($n = 20$) from the LRC ranch. Data from five polygons that were sampled in all three years also showed significant annual variation in transmission. These include data from the following five auction markets presented in Fig. 1: High River, Innisfail, Lethbridge, Lloydminster, and OneFour, Alberta. Colwell *et al.* (2014) reported significant variation in ODR between years for cattle

sampled from the LRC ranch at OneFour, Alberta (N 50.5° W -113.4°), with values significantly higher in 2009 in comparison with 2008 and 2010. Despite significant variation, risk of infection was consistently low (ODR < 0.35) in this southeastern corner of the study area (Fig. 2). Annual variation in ODR for cattle from the High River area paralleled results observed at the LRC ranch, while in 2009 ODR values were significantly lower in the Lloydminster polygon in comparison with other sampling years. In contrast, annual differences in mean ODR were not detected for a polygon along the southern border of the province (Lethbridge), nor were annual differences detected in the center of our survey area (Innisfail).

Of the > 1000 models run, three additional models were within two Δ DIC of the top-performing model. Various combinations of the following variables were found to be significant predictors of transmission risk in these models (Table 1): minimum air temperature, average daily growing degree days with a base of 5°C, accumulated precipitation, daily average accumulated precipitation, and minimum humidity. These top models all relied on environmental data collected from July to October of each respective grazing year. Models calculated using environmental data for the other seven temporal periods did not perform equally as well.

Expected ODR was calculated for each of the 4 models (Table 2), averaged to account for model uncertainty (McCarthy, 2007) and projected across the study area for all three years (Fig. 3). Model projected risk was consistently low (ODR < 0.35) in the far southeast. The total area where risk of economically significant infection was high increased in 2010 in comparison with 2008. This change may be attributed to a general increase in accumulated precipitation, fewer total GDDs, lower average maximum temperature, and higher minimum average temperature (Table 3). In comparison, these same polygons had increased total number of GDD in 2009, with maximum temperature ranges similar to that of 2008.

When data from all three years were pooled, a significant correlation was detected between mean observed ODR values and model projected ODR values ($R = 0.46$, $df = 26$, $p < 0.05$), with 18% of the variance in ODR explained and a root mean square error of 0.082. Model predictions were more consistently accurate for polygons with intermediate risk on average, in comparison with polygons with a more extreme low or high mean ODR. The polygons with 'extreme' low or high ODR relative to the rest of the study area are characterized by higher variance in ODR values. Our top four models all include daily average GDD with a base temperature of 5°C and minimum air temperature.

1.5.2 Model Validation

Mean ODR values differed significantly ($t_{10} = -3.84$, $p < 0.001$, $r = 0.67$) between the LRC ranch and the Stettler ranch (Table 4). The proportion of animals with ODR > 0.50 also significantly differed between sites ($\chi^2 = 11.79$, $p < 0.001$; Table 4) with higher ODR values on the Stettler ranch, indicative of an increased number of animals harbouring high parasite counts.

Despite higher average daily precipitation on the LRC ranch in 2013 (Table 5) mean ODR was significantly higher in Stettler than on the LRC ranch (Table 4; $t_6 = -7.6$, $p < 0.001$). These data are consistent with our comparison of model outputs for individual polygons with fewer GDDs, and higher minimum humidity associated with higher parasite intensities (Table 5). Mean expected ODR values did not significantly differ from observed mean ODR for the LRC ranch ($t_{69} = 1.647$, $p = 0.101$) and for the Stettler ranch ($t_7 = -1.536$,

$p=0.13$). However, model projections did underestimate mean ODR at both locations (Table 4).

1.6 DISCUSSION

Our results show that almost all cattle in Alberta are exposed to at least one species of GIN in their first year. These data are consistent with previous empirical studies and survey reports of calves sampled from pastures in other north-temperate locations (Sutherland & Scott, 2010; Colwell *et al.*, 2014). Despite this ubiquitous presence, the relative risk of GIN exposure, as measured by antibody concentrations in host sera, varied significantly between polygons and between years.

A suite of environmental variables, likely acting in concert, explained a significant proportion of the overall variation in risk of exposure to GIN. Results from empirical laboratory studies and experimental studies involving tracer animals have shown that a large number of factors influence larval transmission rates from pasture into cattle. Factors such as soil moisture, soil humidity and air temperature act at local scales in a species-specific and context-dependent manner (Stromberg *et al.*, 1997; Hoberg *et al.*, 2008; Sutherland & Scott, 2010). Local-scale variation of this sort explains the tremendous variation that is typically observed between herds, even in cases where herds are adjacent on a landscape. These local factors likely contributed to the approximately 80% unexplained variation in ODR values observed in this study. Yet despite this high level of background variation, the results of our study show that broad-scale variation in environmental factors, that operate at the scale of 10's or 100's of kilometers, explain a significant proportion of the overall variation in GIN infections in young cattle across Alberta.

The significance of the July to October temporal period in our top performing models is consistent with an increase in the availability of infective L3 on pasture throughout the grazing season (Sutherland & Scott, 2010). Distinct seasonality has also been detected in a number of pasture-based studies with an increase in egg shedding to a peak in late August to early October (Stromberg, 1997). This coincides with the peak in GIN spp. (e.g. *Ostertagia*, *Cooperia*, *Nematodirus*, and *Trichostrongylus* spp.) intensities detected in grazing tracer calves and dairy cattle in the fall and winter in northern temperate climates (Rickard & Zimmerman, 1992; Vanderstichel *et al.*, 2012). Given these patterns, it follows that variability in environmental conditions during the July to October period would significantly impact the availability of L3 larvae on pasture.

Relative to spatial heterogeneity in nematode transmission, the significance of the number of GDDs may represent a required minimum number of days above a threshold temperature for larval development. This minimum requirement is also consistent with the significance of minimum air temperature, with risk generally increasing with higher average low temperatures. In the case of *O. ostertagi*, optimal temperatures for development range between 20-25°C (Stromberg, 1997). Rates of development slow, or may cease completely, as temperatures declines below this optima. Thus, a minimum number of GDDs may be needed for development through one generation, with the number of potential generations increasing in a given year with the number of cumulative GDDs. This pattern has been demonstrated for the development of *F. hepatica* on pasture (Valencia-Lopez *et al.*, 2012). However, at the other extreme, rates of development decrease when temperatures exceed the maxima for larval survival (Stromberg, 1997). In a national survey of Canadian dairy cattle, Vanderstichel *et al.* (2012) documented higher exposure to GIN on farms in areas with lower average land surface temperatures. The decreased risk of transmission with

higher total GDDs may represent an increased number of consecutive days reaching beyond maximum threshold temperatures acting to limit parasite survival and development (Stromberg, 1997; Barger, 1999; Ng'ang'a *et al.*, 2004).

The significance of accumulated precipitation is also consistent with the transmission biology of infective 3rd stage larvae (L3) on pasture. While L3's can survive for long periods within desiccated faeces, they cannot migrate vertically onto surrounding herbage in the absence of sufficient rainfall (Wang *et al.*, 2014). For *Haemonchus contortus*, an average of 2mm daily rainfall failed to release substantial numbers of larvae (Wang *et al.*, 2014). In contrast, heavy rain has been found to yield high numbers of L3, with a daily average minimum of between 2 and 4 mm needed for larval migration on vegetation. Spatial variability in relative humidity can similarly affect the rate of desiccation of GIN eggs and free-living larval stages on pasture. These patterns are consistent with accounts of a cessation in larval development in the absence of sufficient moisture, regardless of the prevailing temperature (Stromberg, 1997). Combined with our data, these results show that relative humidity and the amount and temporal distribution of rainfall are important drivers for GIN transmission, with the number and survival of free-living L3 on pasture influenced by regional precipitation patterns. Similar results were found in the assessment of environmental covariates of GIN in dairy cattle across Canada (Vanderstichel *et al.*, 2012).

Trade-offs between relative moisture conditions, temperature, and number of GDDs can help explain the spatial and temporal trends described in our risk maps. Low risk of L3 transmission was consistent in the southeastern and northwestern corners of the province. Average annual precipitation is generally higher in the northwest and lowest in the southeast (Fig. 4). The reverse is apparent for 30-year average annual temperatures (Daly *et al.*, 2004) and number of GDDs for these regions. Data from the southeast are consistent with data from field and laboratory studies documenting that extremely arid conditions generally limit the development, survival and transmission of GIN. For example, the development of *O. ostertagi* L3 dropped from 30% to 5% following an increase in temperature from 25°C (optimal environmental conditions) to 32°C (Stromberg, 1997). In contrast, despite higher average annual precipitation in the northwest in comparison with the southeast, similarities in risk of transmission may be related to typically lower temperatures with fewer cumulative GDDs above a base temperature of 5°C delaying larval development and limiting the number of infective larvae available on pasture (Stromberg, 1997; Valencia-Lopez, 2012). Stromberg (1997) reports that *O. ostertagi* development rate is slowed at lower temperatures, taking up to 42 days at 5°C. Other studies suggest that development may not occur at or below 5°C (Stromberg, 1997). We can therefore assume that just as the excessively dry and warm south and east parts of the province are not conducive to high rates of transmission, neither are the wetter, colder regions that are characteristic of the north and west. In comparison with these two relative extremes, intermediate temperatures and moisture availability characterize the transmission hot spot identified in the centre region of our study area.

Temporal variability in the area of high risk for economically significant transmission can also be attributed to differences between regions in accumulated precipitation, cumulative GDDs, and average temperatures. The increased total area in 2010 in comparison with 2008 is consistent with reports that increased precipitation, along with fewer consecutive days above maximum threshold temperatures for parasite survival and development, promote increased parasite transmission (Stromberg, 1997; Barger, 1999; Ng'ang'a *et al.*, 2004). Similarly, the absence of high risk projections for 2009, along with

overall expansion of the total area where risk was low along the eastern border of the province, can be explained by lower average precipitation and the increased GDDs that characterized that year's unusually hot and dry summer.

Validation of our multivariate model demonstrated that projections at the regional level remained relevant in projecting ranch-specific risk. However, model projections did underestimate mean ODR values. This, as well as a large proportion of variation unaccounted for by our model, may be related to incidence of discrete rain events providing opportunity for drastic rise in availability in infective L3 (Wang *et al.*, 2014). These events were also presented as the most parsimonious explanation for high rates of L3 transmission in 2009 in an analysis of annual variation in serum antibody concentrations at the LRC ranch despite lower total accumulated precipitation (Colwell *et al.*, 2014).

The data for this study largely stemmed from regional-based parasite data. Information on the precise origin of host individuals, herd density and management strategies were not available. This limited the resolution and accuracy of our analyses. Additionally, our interpretations of risk were based on the assumption that detected antibodies (ODR) were indicative of exposure to a number of GI genera including *O. ostertagi* and *Trichostrongylus* spp. (see also Colwell *et al.*, 2014). This assumption is supported by data from tracer calves in Alberta (Stockdale & Harries, 1979; Kennedy, 1990). Future research may benefit from the use of alternative diagnostic techniques that allow for the differentiation and quantification of specific parasite species present in each host (Ai *et al.*, 2011). Comparisons of more local-scale variation in risk of transmission using higher resolution spatial data may provide further insight into the epidemiology of parasite transmission. These data are important given that, despite life-cycle similarities, species-specific sensitivity to environmental factors (e.g. tolerance of prolonged dry periods) is common (Wang *et al.*, 2014).

Despite the need to increase the overall robustness of model predictions, our model provides a baseline for evidence-based anthelmintic intervention. The data presented here demonstrate that in years with wetter- and warmer-than-average spring and summer conditions, we can expect higher rates of nematode transmission into yearlings in the fall, especially at sites in the center of our study area where transmission conditions appear to be optimal. Following further verification that links serum antibody concentrations to actual nematode burdens, these results can be used to guide future studies of GIN transmission biology and to maximize treatment efficiency (Hess *et al.*, 2002; Pullan *et al.*, 2011). The next step is to attain more accurate data on: 1) parasite species-specific variation in intensity; 2) animal origin, history, and pasture characteristics; and 3) species-specific climate thresholds for GIN transmission. Of significance here will also be the determination of whether GIN can overwinter on pasture in this region. Such information will provide a platform for explaining species-specific distributional patterns and allow for optimization of anthelmintic applications. Combined with projected changes in climate, increased pressure on the landscape to support a growing global population, and rising incidence of anthelmintic resistance, the ability to reliably define variability in risk of parasite transmission will be increasingly important (Hess *et al.*, 2002).

1.7 LIST OF ABBREVIATIONS

CV: Coefficient of variation

DIC: Deviance Information Criterion

ELISA: Enzyme-linked immunosorbent assay

ESA: European Space Agency

GEBCO: General Bathymetric Chart of the Oceans

GDD: Growing degree days

GIN: Gastrointestinal nematode

GIS: Geographic Information Systems

IPC: Integrated Food Security Phase Classification

LRC: Lethbridge Research Centre

METI: Maritime Environmental Training Institute

NASA: National Aeronautics and Space Administration

NOAA: National Oceanic and Atmospheric Administration

NRCAN: Natural Resources Canada

ODR: Optical density ratio

SEM: Standard error of the mean

UNEP-WCMC: United National Environmental Program - World Conservation Monitoring Centre

USGS: United States Geographical Survey

1.8 COMPETING INTERESTS

The authors declare that they have no competing interests.

1.9 AUTHOR'S CONTRIBUTIONS

MAB, CPG, and DDC conceived and developed the study. MAB performed the collection and analyses of the data and wrote the manuscript. SWK and MAB were instrumental in the design and implementation of GIS-based analyses. CPG and DDC clarified the manuscript. All authors read and approved the final manuscript.

1.10 ACKNOWLEDGEMENTS

We would like to thank Alberta stockowners, Feedlot Health Management Services, and the staff of the Lethbridge Research Centre at OneFour, Alberta, without which this project would not have been possible. We also appreciate the excellent technical help provided by Dawn Gray. This study was supported in part by the Beef Cattle Research Council (DDC. Grant no. 2008F064), Feedlot Health Management Services (DDC. Grant no. FHMS-1039), Alberta Livestock and Meat Agency Ltd. (CPG. Grant no. 2011R041R), the Agriculture Funding Consortium (CPG and DDC Grant no. AB-0450) and Agriculture and Agri-food Canada (DDC. Grant no. RBP-842). Additional funding support from Zoetis for the purchase of ELISA kits is gratefully acknowledged.

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Table 1: Summary of the top performing multivariate hierarchical models for risk of GIN transmission. Models are ranked based on the Deviance Information Criterion (DIC). Year is included in all models as a random fixed effect.

Rank	Model Parameters	DIC	Δ DIC
1	Daily average GDD (base 5°C), Minimum temperature (°C)	54.71	0.00
2	Daily average GDD (base 5°C), Minimum temperature (°C), Daily accumulated precipitation (mm/day)	56.02	1.31
3	Daily average GDD (base 5°C), Minimum temperature (°C), Total accumulated precipitation (mm)	56.11	1.40
4	Daily average GDD (base 5°C), Minimum temperature (°C), Minimum humidity (%)	56.27	1.56
Null Model	-	106.20	51.49

Table 2: Regression model for tests of associations between means of regional environmental data and serum antibody concentrations (ODR).

Model	Variable	Parameter Mean	SD	B	SE B
1	Intercept	-	-	-0.64	<0.01
	Daily Average GDD (base 5°C)	8.06	1.08	-0.40	<0.01
	Minimum Temperature (°C)	5.29	1.01	0.20	<0.01
2	Intercept	-	-	-0.64	<0.01
	Daily Average GDD (base 5°C)	8.06	1.08	-0.45	<0.01
	Accumulated Precipitation (mm)	153.31	40.49	-0.05	<0.01
	Minimum Temperature (°C)	5.29	1.01	0.22	<0.01
3	Intercept	-	-	-0.64	<0.01
	Daily Average GDD (base 5°C)	8.06	1.08	-0.05	<0.01
	Daily Accumulated Precipitation (mm)	1.25	0.33	-0.45	<0.01
	Minimum Temperature (°C)	5.29	1.01	0.22	<0.01
4	Intercept	-	-	-0.64	<0.01
	Daily Average GDD (base 5°C)	8.06	1.08	-0.45	<0.01
	Humidity Minimum	38.34	7.15	-0.05	<0.01
	Minimum Temperature (°C)	5.29	1.01	0.22	<0.01

SD Standard deviation, *B* Parameter coefficient, *SE B* Standard error of the coefficient

Table 3: Spatio-temporal patterns for the environmental covariates of variability in GIN transmission risk on Alberta pasture between 2008-2010.

Parameter	Polygon	Mean \pm SEM			ANOVA
		2008	2009	2010	
GDD Total (base 5°C)	8	867.5 \pm 10.0	979.5 \pm 46.5	865.1 \pm 35.0	F _{735,2} = 3.8
	10	814.5 \pm 31.7	852.4 \pm 28.2	803.2 \pm 12.4	F _{996,2} = 1.0
	12	795.8	799.6	726.2	-
	13	749.2	769.6	663.3	-
	14	865.7 \pm 21.0	902.9 \pm 28.1	804.9 \pm 15.6	F _{729,2} = 4.9
	15	937.6 \pm 18.6	950.4 \pm 18.0	855.0 \pm 15.4	F _{4788,2} = 8.9***
	18	881.8 \pm 25.0	892.2 \pm 22.7	812.1 \pm 23.1	F _{673,2} = 3.4
	19	875.4 \pm 17.5	893.6 \pm 13.2	803.2 \pm 12.4	F _{1104,2} = 10.8**
Acc. Precip. (mm)	8	98.6 \pm 8.0	204.4 \pm 32.7	158.8 \pm 28.3	F _{735,2} = 4.4
	10	181.4 \pm 10.3	137.2 \pm 12.0	178.2 \pm 13.9	F _{996,2} = 5.3*
	12	175.8	190.3	174.3	-
	13	181.3	206.6	285.2	-
	14	144.6 \pm 16.5	190.3 \pm 7.4	271.7 \pm 16.5	F _{729,2} = 20.7*
	15	127.46 \pm 7.5	183.4 \pm 7.1	217.3 \pm 8.5	F _{4788,2} = 34.4***
	18	113.3	124.1 \pm 9.8	208.0 \pm 21.6	-
	19	114.9 \pm 12.3	120.0 \pm 6.8	210.9 \pm 21.8	F _{1104,2} = 13.0**
Maximum Temp. (°C)	8	20.8 \pm 0.4	19.6 \pm 0.6	19.0 \pm 0.4	F _{735,2} = 3.5*
	10	19.3 \pm 0.32	18.5 \pm 0.5	17.8 \pm 0.3	F _{996,2} = 3.5*
	12	18.7 \pm 0.6	17.8 \pm 0.8	17.4 \pm 0.6	F _{366,2} = 1.0
	13	18.7 \pm 0.6	17.7 \pm 0.8	16.7 \pm 0.4	F _{489,2} = 3.6*
	14	18.9 \pm 0.4	17.8 \pm 0.6	17.1 \pm 0.4	F _{729,2} = 3.4*
	15	19.8 \pm 0.2	18.9 \pm 0.2	18.0 \pm 0.2	F _{4788,2} = 22.9***
	18	19.4 \pm 0.4	18.5 \pm 0.4	17.5 \pm 0.3	F _{673,2} = 5.4**
	19	19.2 \pm 0.3	18.5 \pm 0.4	17.5 \pm 0.3	F _{1104,2} = 6.6***
Minimum Temp. (°C)	8	4.1 \pm 0.4	4.6 \pm 0.4	4.5 \pm 0.3	F _{735,2} = 0.7
	10	3.2 \pm 0.3	3.0 \pm 0.3	3.3 \pm 0.3	F _{996,2} = 0.3
	12	3.3 \pm 0.5	2.8 \pm 0.5	3.4 \pm 0.5	F _{366,2} = 0.4
	13	2.3 \pm 0.5	2.9 \pm 0.5	3.0 \pm 0.3	F _{489,2} = 0.8
	14	4.6 \pm 0.4	4.8 \pm 0.4	5.2 \pm 0.3	F _{729,2} = 0.7
	15	4.5 \pm 0.1	4.8 \pm 0.2	5.2 \pm 0.1	F _{4788,2} = 6.5**
	18	4.0 \pm 0.4	4.7 \pm 0.3	5.4 \pm 0.2	F _{673,2} = 4.6**
	19	4.1 \pm 0.3	3.8 \pm 0.3	4.5 \pm 0.2	F _{1104,2} = 2.1

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 4: Comparison of observed and model-based projections of ODR in 2013. Projected values are based on data from the closest meteorological station in straight-line distance for ranches near One Four, Alberta and Stettler, Alberta.

Site	N	Observed			Model Projected ODR (mean \pm SEM)
		ODR (mean \pm SEM)	Proportion of calves with ODR <0.0 (95% CI)	Proportion of calves with ODR >0.5 (95% CI)	
LRC	16	0.36 \pm 0.02	0.05 (0.02-0.08)	0.25 (0.18-0.31)	0.16 \pm 0.01
Stettler	75	0.51 \pm 0.04	0.03 (0.01-0.05)	0.43 (0.35-0.50)	0.25 \pm 0.01

Table 5: Comparison of environmental data (July to October 2013) collected for model validation polygons. Environmental data are collected from the closest meteorological station by Euclidean straight-line distance for ranches near One Four, Alberta and Stettler, Alberta. Values are mean \pm SEM.

Environmental Parameters	LRC	Stettler	T-stat
Total Acc. Precip. (mm)	251.7	163.3	-
Daily Acc. Precip. (mm)	2.0 \pm 0.6	1.3 \pm 0.4	t ₂₄₄ = 9.5
GDD Total (base 5°C)	1231.9	1012.9	-
GDD Daily Average (base 5C)	10.0 \pm 0.6	8.2 \pm 0.5	t ₂₄₄ = 2.3*
Air Temperature Minimum (°C)	7.8 \pm 0.6	5.9 \pm 0.5	t ₂₄₄ = 2.3*
Humidity Minimum (%)	36.0 \pm 1.3	41.3 \pm 1.4	t ₂₄₄ = -2.7**

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

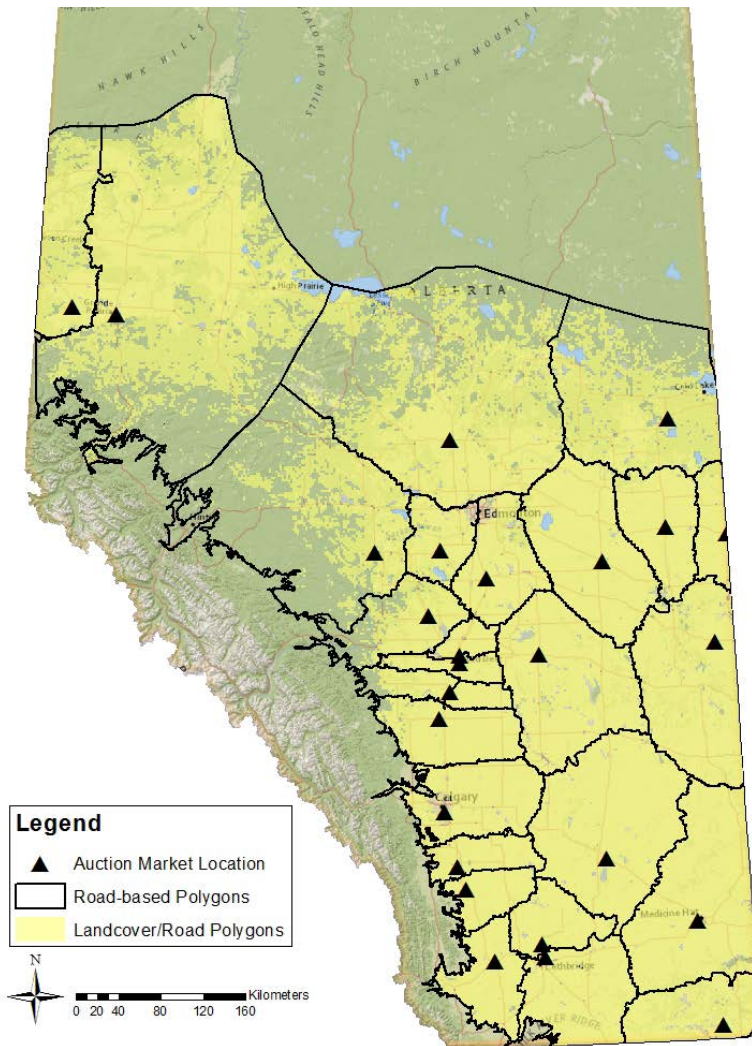


Figure 1: Sampling polygons for GIN survey in southern Alberta bovine calves. Southern Alberta was delineated into 26 service area polygons based on analyses of minimum driving distance to auction markets in accordance with the existing road network.

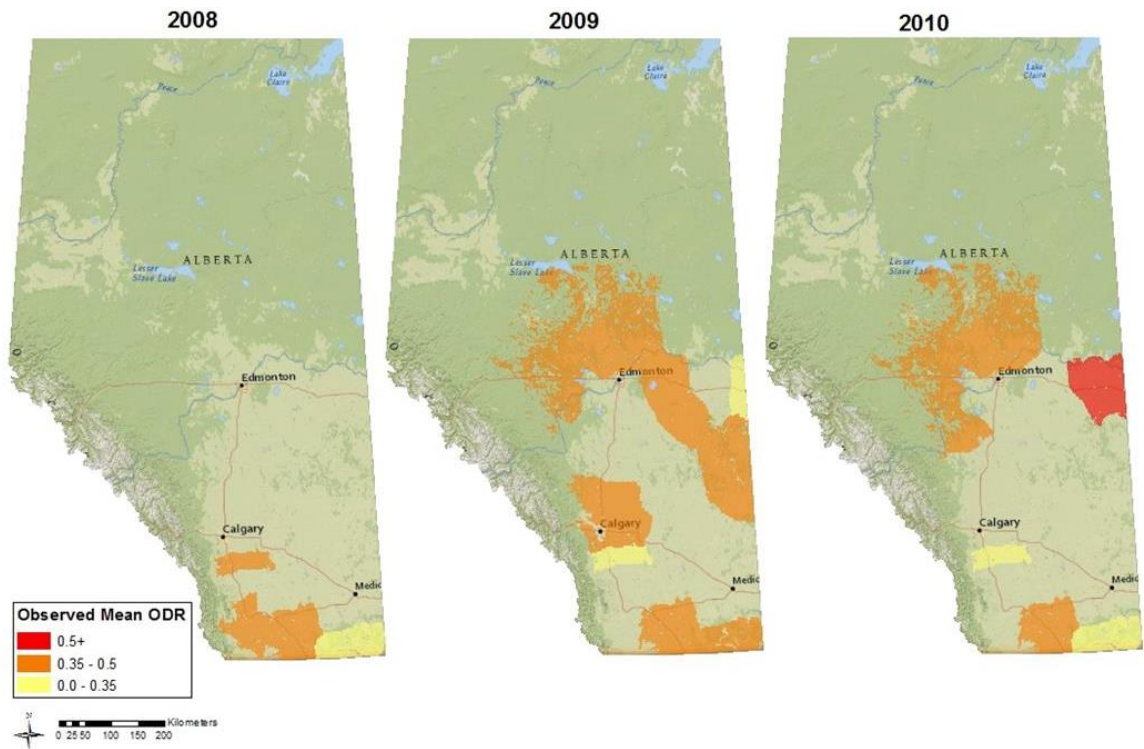


Figure 2: Observed mean antibody concentrations (ODR) in calves sampled at auction markets in Alberta from 2008 to 2010.

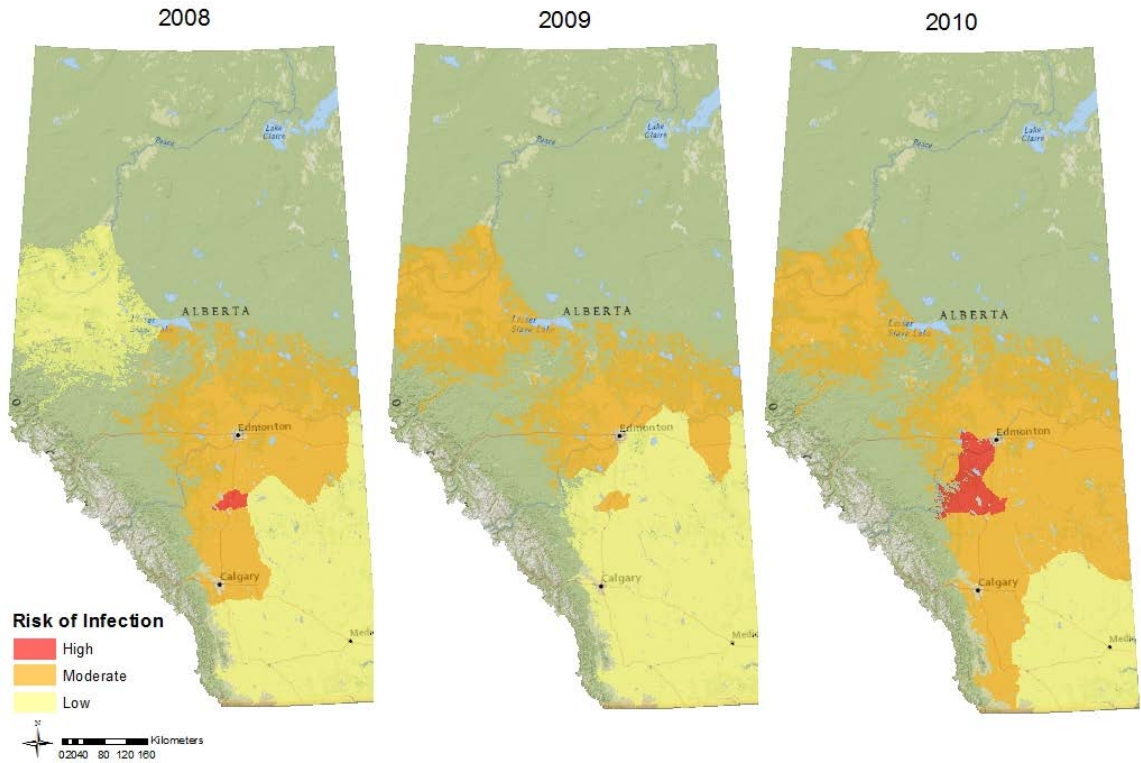


Figure 3: Model predicted spatial and temporal variation in risk of GIN transmission in Alberta bovine calves (2008-2010). Distribution of expected risk of nematode transmission calculated for each year using Bayesian inference to construct hierarchical binary response logistic regression models for ODR in cattle sampled at auction markets in southern Alberta from 2008-2010. Low, Moderate and High risk are differentiated according to mean regional optical density ratio values of <0.3 , $0.3-0.5$, and >0.5 , respectively, for cattle serum.

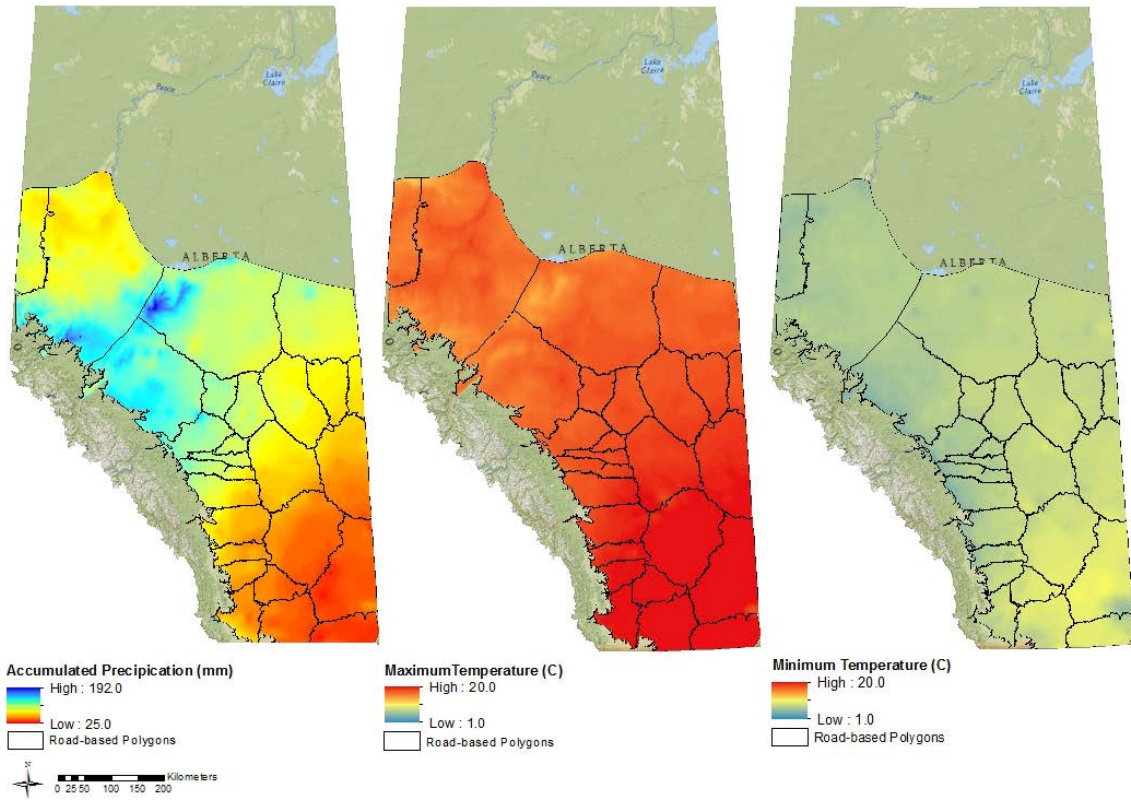


Figure 4: Alberta 30yr climate maps (1970-2000) for mean (i) accumulated precipitation (mm), (ii) maximum temperature (°C), and (iii) minimum temperature (°C) for July to October. Surface values were calculated as an average of monthly data created by Daly *et al.* (2010).