

MANAGEMENT OF *PSOROPTES* IN FREE RANGING BIGHORN SHEEP

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By

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ABSTRACT

The overall objective of this thesis is to address gaps in knowledge and provide tools that will aid in the management of the psoroptic mange outbreak in bighorn sheep (*Ovis canadensis*) of the Okanagan region of Canada. A multi-pronged approach was taken including outbreak investigation, development of disease detection tools, investigation of treatment options, and finally review of management action approaches. Based on comparison of *Psoroptes* morphology and mitochondrial gene sequencing, the most likely source of the outbreak was determined to be a cross-species transmission event from rabbits. This transmission likely occurred on the Okanagan Game Farm, closed in 1999, and therefore new exogenous sources of disease in the area are unlikely. A commercially available enzyme linked immunosorbent assay (ELISA) marketed for *Psoroptes* detection in domestic sheep was optimized for use with bighorn sheep serum with a test sensitivity of 98.7% and specificity of 94.0%. A treatment trial conducted on *Psoroptes*-infested Canadian bighorn sheep found that both the injection of extended-release eprinomectin and topical application of fluralaner were unsuccessful in eliminating mite infestations; meanwhile, orally administered fluralaner cleared the infestations and greatly improved clinical signs following a single treatment when used at either 5mg/kg or 25mg/kg dosages. The fence and handling systems used in the treatment trial for housing and control of bighorn sheep movement were reviewed and critical weaknesses in design and construction were identified to address a gap in literature on bighorn sheep fencing requirements. The complexity of population-level application of new knowledge often involves ongoing uncertainty leading to delays in management action. While *Psoroptes* has rarely been managed in wild sheep, respiratory disease has received considerably more attention. Interviews conducted with 13 wildlife professionals involved in the management of bighorn sheep respiratory disease were used to help identify common challenges and opportunities in the management of North American bighorn sheep. Investigation of strategies aimed at preventing pathogen introduction into naïve herds is essential before eradication efforts can be considered. A more systematic approach to addressing sources of disease introduction is a necessary step in the development of management options for both respiratory disease and *Psoroptes*. Four main types of barriers that impede management action were identified; social and political challenges, resource limitations, knowledge barriers, and physical/landscape barriers. Some of these are beyond the control of wildlife managers while strategies to address others are discussed. Increased attention to the

human dimensions of wildlife management and the application of an adaptive management approach is needed. The tools developed throughout this thesis and the lessons learned through these interviews are valuable resources for wildlife managers to use when engaging stakeholders in developing an adaptive management plan for *Psoroptes* in British Columbia.

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DEDICATION

This thesis is dedicated to the animals who temporarily gave their freedom to make this project possible and to all those who generously gave their time, resources, energy and passion to this project. Your generosity and enthusiasm inspire me to continue working to protect this planet and all of its inhabitants.

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CHAPTER 1: OVERVIEW OF BIGHORN SHEEP DISEASE MANAGEMENT IN NORTH AMERICA

1.1 Canadian Bighorn Sheep

Bighorn sheep (*Ovis canadensis*) are a highly prized, charismatic species of mountain ungulate found throughout the western portions of North America ranging from southern British Columbia and Alberta in the north down to Mexico in the south (Buechner 1960). The division of the species into different subspecies throughout their range has been debated in the literature but the three subspecies that are currently recognized are California bighorn sheep (*O. c. californiana*), Desert bighorn sheep (*O. c. nelsoni*), and Rocky Mountain bighorn sheep (*O. c. canadensis*) (Wehausen and Ramey 2005; Buchalski et al. 2016). Some Canadian biologists refer to bighorn sheep in the southern interior of British Columbia as California bighorn sheep (*O. c. californiana*), but according to morphometric and genetic evidence all Canadian bighorn sheep are Rocky Mountain bighorn sheep (*O. c. canadensis*) (Demarchi 2004; Wehausen and Ramey 2005).

Bighorn sheep are cherished for their aesthetic value by wildlife enthusiasts of all types. Okanagan First Nation folklore relates to bighorn sheep as beloved relatives that look out for the needs of their human cousins; wildlife observers revel at their agility on rocky escape terrain and impressive sparring during the rut; and hunters revere them for their impressive and beautiful curled horns and the remote terrain in which they are found. All are concerned with their conservation and determined to ensure their ongoing presence on the North American mountain landscape. Despite this prized position in North American culture bighorn sheep have experienced dramatic declines in both range and abundance since the European colonization of western North America. It is estimated that populations once numbered in the one to two million range prior to European colonization and decreased to the low tens of thousands in the early 1900s as a result of overharvesting and the introduction of exotic disease with domestic sheep (Buechner 1960). Since the 1960s, conservation efforts have been successful in enabling bighorn populations to rebound back to the current estimates of over eighty five thousand animals (Wild Sheep Foundation 2017). Although numerous stakeholder groups place high value on bighorn

sheep, it is usually rooted in different cultural contexts and consequently it is not surprising that perspectives and opinions on bighorn management are divergent and difficult to reconcile.

The field of wildlife management aims to identify, understand, modify, prevent, or counteract the circumstances that contribute to population declines. Numerous factors drive these population trends as a result of anthropogenic and environmental influences including habitat destruction, hunting, predation, competition for forage, and wildlife disease (Miller et al. 2012). Two such diseases of significance to Canadian bighorn sheep are psoroptic mange and respiratory disease. Both of these diseases are thought to have been transplanted to North America with domestic sheep and both have caused severe population declines in at least some of the affected bighorn populations (Sweatman 1958; Buechner 1960; Miller et al. 2012).

Management of diseases in wildlife must be done from an epidemiologic point of view, considering both individual animal-level factors and broader population-level factors. The objective of this thesis is to provide tools and understanding for the management of wildlife disease in Canadian bighorn sheep. This thesis primarily focuses on aspects of psoroptic mange that require further clarity for management of this disease in Canada and addresses key gaps in knowledge. Due to a lack of management activity directed at *Psoroptes* in North American bighorn sheep, the final chapter of this thesis pertaining to the use and implementation of the adaptive management approach, focuses on efforts currently directed at respiratory disease management in bighorn sheep. Discussion of these findings is carried out with regard to their relevance to respiratory disease management as well as important lessons to be applied in the development of *Psoroptes* management plans in the future.

1.2 Overview of Psoroptic Mange

Mites of the genus *Psoroptes* are a non-burrowing, ectoparasitic, mange-causing mite known to parasitize numerous domestic and wild mammalian hosts including horses, sheep, goats, cattle, rabbits, deer, wapiti, water buffalo and bighorn sheep (Sweatman 1958; Zahler et al. 1998; Bates 1999; Zahler et al. 2000; Amer et al. 2015). The mite has been found throughout Europe as well as on hosts in New Zealand, USA, Canada, Mexico, Egypt, South Africa, Uganda and Chile (Zahler et al. 1998; Zahler et al. 2000; Pegler et al. 2005; Amer et al. 2015). Infestations are highly contagious between conspecific hosts (Bates 1999; Pegler et al. 2005) and affected animals generally exhibit highly pruritic, exudative, alopecic lesions, however subclinical infestations also occur (Bates 1996; Bates 1999). Lesions tend to localize to the ears

of species such as rabbits, bighorn sheep, and goats, while they tend to be generalized in other host species including domestic sheep, equids, and cattle (Zahler et al. 2000; OIE 2013). *Psoroptes* infestations pose an important welfare threat and have significant economic ramifications when present in domestic populations (Losson 2012a; Amer et al. 2015). The disease was the focus of intensive management resulting in *Psoroptes* eradication from domestic species in some countries including Australia, New Zealand, Canada and the USA, while eradication efforts in other countries continue to be unsuccessful (van den Broek and Huntley 2003). Despite the eradication from domestic sheep in North America, *Psoroptes* has been documented in select American bighorn sheep populations throughout the 19th and 20th centuries, and in some cases, it has been associated with significant population declines (Boyce and Weisenberger 2005; Miller et al. 2012).

The lifecycle of *Psoroptes spp.* was meticulously described by Sweatman in order to elucidate potential differences between them (1958). The lifecycle takes place entirely on the host and involves five life stages; egg, larva, protonymph, deutonymph, and adult (Sweatman 1958). Male and female mites are indistinguishable at the egg and larval stages after which time sexual dimorphism develops. Female deutonymphs and adults are referred to as pubescent females and ovigerous females respectively. *Psoroptes* mites are barely visible to the naked eye, ranging in size from almost 200um at the egg stage to about 550um in body length for adult males and about 750um in body length for ovigerous females excluding their setae or legs (Sweatman 1958).

The time required for *Psoroptes* to complete its lifecycle is heavily dependent on environmental conditions, specifically temperature and humidity, but under optimal conditions the egg to egg lifecycle is reported to take between 14 and 21 days, with each life stage taking longer under suboptimal conditions (Sweatman 1958). Meanwhile, off the host, mites can survive and retain infectivity for a period of up to 15 days under realistically simulated environmental conditions (O'Brien et al. 1994). The key distinguishing attribute of the *Psoroptes* genus is the presence of a relatively long, segmented caruncle (sucker) at the end of the first, second, and fourth legs of adult females and the first, second, and third legs of adult males, where other genera in the psoroptidae family have short unsegmented caruncles (Sweatman 1958; Pegler et al. 2005; Wall and Kolbe 2006). It is only in the mature male life stage that species differentiation based on phenotypic characteristics was considered possible. Species identification was performed by measuring the length of a hair-like structure on the male opisthosomal lobe called

the outer opisthosomal setae (OOS) from numerous male mites and calculating the average OOS length in a population. However, wide ranges of OOS length were reported within each proposed species and significant overlaps in published OOS ranges of each species often made differentiation challenging and sometimes uncertain (Sweatman 1958; Zahler et al. 1998; Bates 1999; Zahler et al. 2000). The division of the *Psoroptes* genus into species has been the topic of much discussion and debate since it was first described by Viborg in 1813 (Zahler et al. 1998; Bates 1999; Zahler et al. 2000; Wall and Kolbe 2006; OIE 2013; Amer et al. 2015; OConnor and Klimov 2015).

The genus was historically divided into between 5 and 9 different species based on host preference, and site of infestation on the host, with the OOS length used as the main distinguishing morphological criterion between them (Sweatman 1958; Pegler et al. 2005). The validity of these distinctions has been thoroughly explored since the original Sweatman description because cross infection trials indicate that host specificity is not absolute (Wright et al. 1981; Foreyt 1997). Furthermore, mites originating from different host species, categorized as different mite species based on OOS length, were able to interbreed to produce viable, fertile offspring capable of colonizing either of the originating host species demonstrating a lack of “reproductive isolation” between the species (Wright et al. 1983). Pegler et al (2005) proposed that the morphologic differences observed may be a result of phenotypic plasticity or the ability of strains of mites to adapt to their primary host thus disputing the validity of the species differentiation based on OOS length. Despite this species unification, cross infection trials of mites found on different host species are only sometimes successful and unique mite variants have been shown to exhibit dissimilar properties including virulence or rate of lesion development when infesting a particular host (Wright et al. 1981; Bates 1999). The reasons for differences in mite virulence, host susceptibility and infestation severity remain unclear (Zahler et al. 2000; Siegfried et al. 2004; Sarre et al. 2015).

With the rise of polymerase chain reaction (PCR) and DNA sequencing technologies, molecular techniques have increasingly been applied to clarify questions around *Psoroptes* taxonomy (Gu et al. 2014). DNA sequence analyses have been performed on DNA microsatellite markers, mitochondrial DNA and ribosomal DNA of *Psoroptes* mites from different host species and parts of the world (Ochs et al. 1999; Evans et al. 2003; Wang et al. 2012; Gu et al. 2014; Amer et al. 2015; Juan et al. 2015; OConnor and Klimov 2015). Variation observed in these

regions do not always correspond to the host from which the mites were obtained and thus molecular studies generally further support the assertion that the distinction between disparate species of mites based on host origin is invalid (Zahler et al. 1998; Ochs et al. 1999; Pegler et al. 2005). According to some authors, *Psoroptes natalensis*, found on water buffalo in South Africa, may be an exception as it shows enough consistent genetic divergence from the strains of *Psoroptes* found in most hosts worldwide, to justify categorization as a unique species (Wang et al. 2012; Amer et al. 2015). *P. natalensis* also shows morphologic variation in that the OOS of most *Psoroptes* strains are narrow and hair-like, while the OOS of *P. natalensis* is flattened or “spatulated” (Sweatman 1958; Amer et al. 2015). Nevertheless, with the possible exception of *P. natalensis*, *Psoroptes* is currently regarded as a single genotypically and morphologically diverse species united under the first described name, *Psoroptes ovis*, also referred to as *Psoroptes spp.* (Wall and Kolbe 2006; OIE 2013) and will therefore be referred to as simply *Psoroptes* throughout this dissertation.

Treatment and eradication of *Psoroptes* mites require a strategic and coordinated effort due to numerous problematic characteristics of the disease. Therapeutic measures must be applied to all members of a population simultaneously in order to interrupt the infection cycle because animals do not develop immunity following treatment. Many drugs have no residual activity, and some have no effect on the mite eggs, necessitating multiple sequential treatments (Ortega-Mora et al. 1998; O’Brien 1999). Some previously relied upon treatments contained organochlorines such as dichlorodiphenyltrichloroethane (DDT) and benzene hexachloride (BHC) which had this desirable residual activity but were found to be highly toxic to the environment and to exposed humans prompting their restriction (O’Brien 1999; van den Broek and Huntley 2003).

Due to its global nature and profound impact on domestic species, *Psoroptes* has been the subject of a substantial amount of research in domestic species; however, significantly less attention has been paid to its impact and management in bighorn sheep. In Canada in particular, the impact of psoroptic mange in bighorn sheep is a new consideration. Psoroptic mange has been documented in bighorn sheep throughout much of their American range including Arizona, California, Colorado, Idaho, Montana, Nevada, New Mexico, Oregon, Washington and Wyoming, dating back to the mid 1800s in some cases (Lange et al. 1980; Welsh and Bunch 1983; Foreyt et al. 1990; Muschenheim et al. 1990; Mazet et al. 1992; Miller et al. 2012). Reports of Canadian bighorn sheep with mange-like symptoms were first received by government

biologists in 2003 and *Psoroptes* was officially identified in 2011 (Reid 2011; Scott et al. 2013). Variable effects have been observed in *Psoroptes* infested US bighorn populations ranging from persistent and undulating infestations with minimal effects on herd abundance (Foreyt et al. 1990; Muschenheim et al. 1990) to large scale population declines and at least one near-extirpation event (Lange et al. 1980; Foreyt et al. 1990; Boyce and Weisenberger 2005).

The effects of *Psoroptes* can be difficult to identify because infestation alone is rarely a direct cause of death, and yet infestation may increase the risk of death by predation through hearing loss (Norrix et al. 1995). Therefore, even when population declines are closely associated with *Psoroptes* outbreaks, managers may be reluctant to call the parasite the "cause" of decline. Nonetheless, it is clear that *Psoroptes* is a negative contributor to the cumulative effects that drive population trends (Miller et al. 2012). More holistic conceptual models of causation are useful in this regard. While *Psoroptes* infestation is not likely to independently result in death in bighorn sheep, it increases the likelihood of mortality, particularly in combination with other factors, and should therefore be considered at least a "component-cause" in population declines. The reason for the wide variability in observed population level effects of *Psoroptes* in bighorn sheep remains uncertain; however, it is clear that the appearance of this disease in BC has been associated with a significant decline in the affected population while comparable nearby unaffected herds are not experiencing the same population reductions (Reid 2013a). Possible theories to explain the variable outcomes observed include differences in host susceptibility or underlying health, predator abundance, mite strain virulence, environmental conditions, or some combination of these factors.

Inconsistency in treatment efficacy, cost and risk of wildlife capture, and minimal long term benefits make the decision to undertake wildlife treatment using currently available methods difficult to justify (Kinzer et al. 1983; van den Broek and Huntley 2003; Lekimme et al. 2010). The challenging logistics of herd-wide capture and treatment, especially in situations of interconnected metapopulations with broader home ranges, adds further challenge to management of this disease in wildlife. The recent discovery of macrocyclic lactone resistance in *Psoroptes* collected from domestic sheep in the UK adds to the necessity of discriminate use of anthelmintics and provides additional incentive for the investigation of new antiparasitic drugs and approaches to *Psoroptes* treatment in *Ovidae* (Doherty et al. 2018).

Research into field-worthy treatment approaches for *Psoroptes* in bighorn sheep has been attempted with mixed success. Strategies attempted include ivermectin medicated feed (Foreyt 1993), ivermectin sustained release implants (Boyce et al. 1992), 5% coumaphos dustbags suspended over salt licks (Lange et al. 1980), aimtraz-impregnated collars, and cyfluthrin impregnated ear tags (Bleich et al. 2015). Despite these varied approaches, the only report of a successful *Psoroptes* eradication effort in free ranging bighorn sheep occurred in the San Andres Mountains of New Mexico between 1999-2002 (Boyce and Weisenberger 2005). The San Andres Mountain population dwindled to a single individual who was caught and treated for her *Psoroptes* infestation. After using a radio-collared sentinel ram to confirm that no other infected sheep were in the area, the sole survivor was re-released and the population was augmented. Numerous gaps in knowledge regarding *Psoroptes* in Canadian bighorns need attention before meaningful management action can be considered. To ameliorate these gaps in knowledge, this thesis aims to:

- improve understanding of the source of the Canadian *Psoroptes* outbreak,
- improve detection of potential subclinical carriers in bighorn sheep,
- and provide additional preliminary investigation into wildlife-appropriate treatment options for this parasite.

1.3 Overview of Bighorn Sheep Respiratory Disease

Unlike *Psoroptes*, respiratory disease is a well-documented, well-researched, and well-accepted driver of population decline in bighorn sheep herds throughout both the United States and Canada (Besser, Highland, et al. 2012; Kuzyk et al. 2012; Besser et al. 2013; Plowright et al. 2013; WAFWA Wild Sheep Working Group 2017). Respiratory disease has been blamed for 175 separate epizootic mortality incidents in bighorn sheep populations between 1970 and 2014 (WAFWA, 2017). It has affected 17 of the 20 regions in North America that harbour bighorn sheep and resulted in the loss of over 14,000 adult bighorn sheep individuals (WAFWA, 2017).

Respiratory disease refers to a contagious pneumonia affecting bighorn sheep at the population level and is considered to be one of the most important factors affecting bighorn sheep populations across North America (Cassirer and Sinclair 2007; Brewer et al. 2014). Respiratory disease epizootics in bighorn sheep are characterized by all-age mortality events followed by years or decades of enzootic pneumonia in lambs resulting in poor lamb recruitment and long term population declines (Besser et al. 2012). Outbreaks have been associated with a variety of

pathogens such as *Mycoplasma ovipneumoniae* and several Pasteurellaceae, including *Pasteurella multocida*, *Mannheimia haemolytica*, and *Bibersteinia trehalosi* (Besser et al. 2012). Current research suggests that while pneumonia infections are frequently polymicrobial in nature, *Mycoplasma ovipneumoniae* is the key predisposing factor (Besser et al. 2012). In the absence of *M. ovipneumoniae*, pneumonia related mortalities may occur but epizootic die-offs and chronic poor lamb recruitment will not (Besser et al. 2013; Butler et al. 2018).

These pathogens are directly transmitted between hosts through contact or aerosol transmission (Besser et al. 2014). The management of respiratory disease in bighorn sheep is complicated by the fact that domestic sheep and goats act as asymptomatic reservoir hosts for these pathogens (Besser et al. 2012) and therefore human movement of domestic sheep can introduce profound risk to naïve bighorn sheep in an area. Supportive and preventative care for bighorn sheep including vaccination, mineral supplementation, anthelmintic treatment, and supplemental feeding have been relatively ineffective in changing the outcome for pneumonia-affected bighorn sheep (Wood et al. 2017; Cassirer et al. 2018). While the theory that puts *M. ovipneumoniae* at the center of attention is not accepted by all wildlife professionals, almost all agree that domestic sheep and goats constitute a significant risk to wild sheep populations (Butler et al. 2017; Drew and Weiser 2017; Wood et al. 2017). Controversy remains over whether *M. ovipneumoniae* is present in wildlife outside of the Caprinae subfamily (Highland et al. 2018) and this doubt regarding the major potential routes of disease exposure for wild sheep may delay action aimed at reducing the risk that domestic sheep and goats pose.

Progress regarding the role of *M. ovipneumoniae* in the epidemiology of respiratory disease outbreaks has enabled new pathogen-specific research and management approaches aimed specifically at detecting and eradicating *M. ovipneumoniae* in domestic and wild sheep populations. It is currently thought that *M. ovipneumoniae* persists in a population via chronically shedding bighorns that survived the initial epizootic mortality events and that these individuals perpetuate disease outbreaks in wild herds year after year (Plowright et al. 2017; Cassirer et al. 2018; Garwood 2018). This information, in combination with the discovery that serologic exposure to *M. ovipneumoniae* (assessed via antibody detection) is far more common than detection of *M. ovipneumoniae* bacteria (via PCR) suggests that not all *M. ovipneumoniae* exposed animals act as maintenance hosts to perpetuate infection in affected herds (Cassirer et al. 2018). This picture is further complicated by the fact that some animals shed the bacteria

intermittently and it is unclear whether those “intermittent shedders” maintain infection and only shed the bacteria intermittently or if they are reinfected by other persistently infected animals before shedding again (Plowright et al. 2017). If only a subset of animals, the “chronic shedders”, act as maintenance hosts and they can be detected and removed from a population, it is hypothesized that *M. ovipneumoniae* and the associated lamb die-off cycle could be interrupted and eliminated from a population without necessitating a complete herd depopulation. This strategy has been implemented in South Dakota, where one of the first test and cull programs directed at *M. ovipneumoniae* eradication was carried out (Garwood 2018).

South Dakota’s *M. ovipneumoniae* test and cull program involved the capture and GPS collaring of every single animal in a herd, with a target of three capture and test events per animal within one year in order to detect all intermittent shedders. Animals that produced positive PCR test results at all capture events were removed from the population. Initial effects include improved lamb recruitment rates (Garwood 2018). Because of the necessary logistics, this approach is only feasible for small isolated herds in highly accessible terrain and is expensive on a per-animal basis. Nevertheless, this presents a promising new management option for the control and elimination of respiratory disease caused epizootic mortality events in some populations and a similar operation is planned in a *M. ovipneumoniae* positive herd of bighorn sheep in British Columbia.

As a result of a relative lack of success achieved in the management of respiratory disease in wild bighorn sheep populations, the Wild Sheep Working Group (WSWG) of the Western Association of Fish and Wildlife Agencies (WAFWA) established the Wild Sheep Disease Management Venture (DMV) in 2015. The DMV aims to “assist jurisdictions to evaluate, validate and implement adaptive management actions that may prevent infection, clear pathogens and improve herd performance” (WAFWA, 2016). The lack of management success with this disease despite an abundance of research and attention devoted to this topic is a testament to the obstacles that wildlife disease management encounters. Respiratory disease management in bighorn sheep also exemplifies a situation where the challenge of wildlife disease management extends beyond understanding the natural sciences and spills over into the social sciences. The role that private citizens can play in driving this disease complex in bighorn sheep necessitates an appreciation and understanding of the multi-faceted human dimensions of wildlife management. This thesis therefore does not intend to expand the body of knowledge on the natural sciences

aspects of respiratory disease. Rather it investigates the difficulties faced in implementing management action for respiratory disease across North America. In doing so, this thesis hopes to gain further insight into the process of wildlife disease management and highlight important challenges and lessons that will improve the effective use of wildlife disease knowledge for respiratory disease and other comparable wildlife diseases.

1.4 Overview of Wildlife Disease Management

While basic principles of wildlife disease management are often conserved across diseases, species, or locations, each wildlife disease management situation involves a unique set of challenges as a result of the particular conditions in which that disease occurs (Nishi et al. 2004). These circumstances are the product of what is known as the epidemiological triad. Made up of the host, the pathogen, and the environment, the triad represents the understanding that numerous factors interact to create the circumstances in which disease occurs. The effect of any disease must be considered with reference to the context in which it exists and is not necessarily consistent in impact between different herds (Dohoo et al. 2003). This lesson is important in considering why *Psoroptes* may have different impacts on different bighorn populations, or why interventions that are successful in mitigating the effects of respiratory disease in one herd may be impossible or ineffective in another. The term “environment” should be considered more broadly than the immediate ecosystem in which an animal or population lives to consider the larger system in which wildlife disease management must operate. This includes the human and social factors that must be considered when attempting wildlife disease management as well. Interventions must be specific to each location, often requiring adaptation, implementation, and evaluation of management knowledge at a population specific level.

Wildlife management in Canada is guided by a set of seven core principles collectively referred to as the “North American Model of Wildlife Conservation” (Organ et al. 2001). These are:

- 1) Wildlife resources are a public trust
- 2) Markets for game are eliminated
- 3) Allocation of wildlife is by law
- 4) Wildlife can be killed only for a legitimate purpose
- 5) Wildlife is considered an international resource
- 6) Science is the proper tool to discharge wildlife policy

7) Democracy of hunting is standard

Under this model, the care of wildlife is the responsibility of provincial and federal agencies on behalf of the public interest, and the decision to undertake action as well as the selection of which actions to take are based in decisions that must be driven by science and social factors (Organ et al. 2001). The decision to undertake action aimed at addressing an issue of disease in a wildlife population is therefore driven by public interest and conservation. In this setting, the management of disease in wildlife is generally carried out for one of three reasons; zoonotic risk of a wildlife disease resulting in human health implications, impact of the wildlife disease on domestic livestock resulting in economic implications; or the impact of disease on a wildlife population with its own intrinsic value (Wobeser 2002).

Management of disease in wildlife is an iterative process where research and intervention take place continuously throughout the progression from disease outbreak investigation to disease stabilization or eradication. It involves a diverse range of fields from mathematical modelling to the philosophical and sociological study of human behaviour (Delahay et al. 2009). While the vast diversity within these topics is beyond the scope of any single piece of work, modern wildlife disease management plans must consider all these aspects in order to be strategic and effective in defining and achieving their goals. Wildlife disease management is made challenging by the circumstances in which the disease occurs with regard to both the natural sciences and epidemiologic aspects, and the social sciences and human dimensions complications (Delahay et al. 2009). Each management challenge is therefore unique to the area in which it is being applied.

Initially, it is often useful to consider infectious disease management and response with reference to a set of standardized steps that must take place in order to effectively manage a disease outbreak. These steps, described as response interventions by the World Health Organization are: anticipation, early detection, containment, control and mitigation, and elimination or eradication (World Health Organization 2018). The broad range of knowledge encompassed by these topics necessitates the involvement of numerous experts and many years of work in the development of wildlife health knowledge and in the development and implementation of wildlife disease management plans. While the pursuit of this wildlife health knowledge can be a never-ending process, limited resources and time sensitive constraints such as declining populations often force managers to take action despite ongoing uncertainty (Chadès et al. 2017). In order to address the need for continued learning and research while

simultaneously initiating management action, the concept of adaptive management has been proposed. Adaptive management involves the intentional structuring of management action in such a manner as to allow wildlife managers to compare the effects of different management actions and the ability of those action to achieve predetermined management goals (Enck et al. 2006; Lauber and Decker 2012). This allows the act of wildlife management to also be a tool for learning. In the final chapters of this thesis the utility of an adaptive management approach to the management of respiratory disease in bighorn sheep is investigated and important barriers and lessons to be learned from its implementation that can be applied to comparable wildlife disease scenarios, in this case the Canadian *Psoroptes* outbreak, are discussed.

1.5 Objectives of Research

This thesis aims to address key gaps in knowledge necessary for the management of psoroptic mange in Canadian bighorn sheep and uses lessons learned through a review of respiratory disease management to provide suggestions for the development of *Psoroptes* management plans. The discussion proceeds through the steps of outbreak management focusing on the natural science components in need of attention for *Psoroptes* including determining the outbreak source, validating detection tools, addressing gaps in literature around bighorn sheep movement control, evaluating new treatment options and investigation into social science aspects of bighorn sheep management. Figure 1.1 outlines the research questions addressed in these five primary research chapters as they relate to important gaps in knowledge for effective wildlife disease management.

Through this exploration of the steps of disease management and intervention, this thesis aspires to be a useful and applicable resource for wildlife managers in their efforts to mitigate the effects of these diseases and promote conservation of this iconic species in North America.

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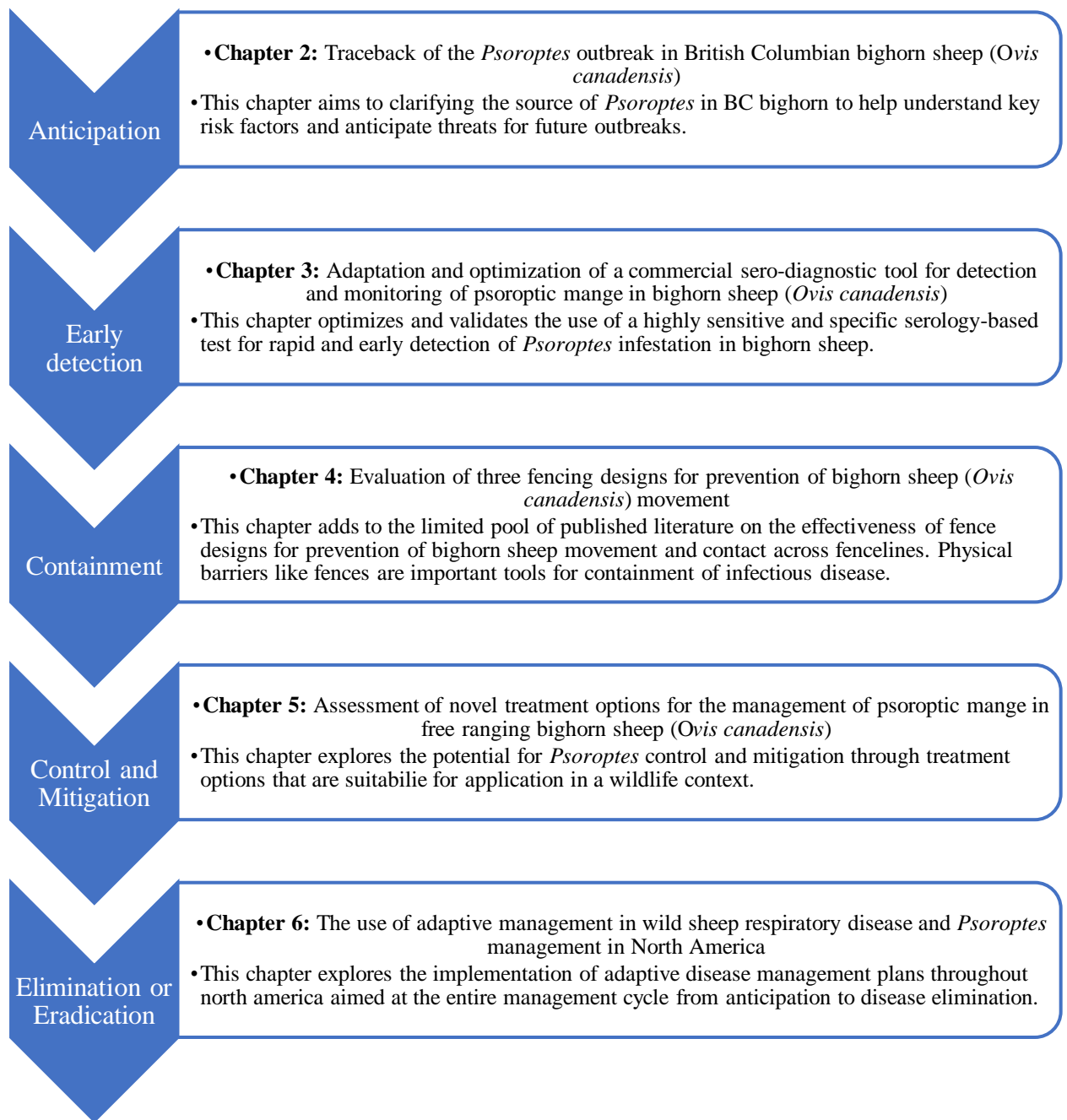


Figure 1-1: The focus of the chapters of this thesis in addressing the steps of disease management and intervention

CHAPTER 2: TRACEBACK OF THE *PSOROPTES* OUTBREAK IN BIGHORN SHEEP (*OVIS CANADENSIS*) IN BRITISH COLUMBIA

The research described in chapter 2 investigates the source of the Psoroptes outbreak in Canadian bighorn sheep. Determining the source of the outbreak is an essential piece of information for wildlife managers before proceeding to management action for this parasite. This manuscript is intended for submission to the International Journal for Parasitology: Parasites and Wildlife.

Hering conducted all morphologic and morphometric data collection and analysis. Chilton and his lab completed the DNA sequencing and collaborated with Hering in the design and interpretation of the genetic component of this study.

2.1 Introduction

Psoroptic mange, caused by non-burrowing mites of the genus *Psoroptes*, is a newly recognized disease in British Columbia (BC) bighorn sheep. While the disease has been reported in bighorn sheep populations in the United States dating back throughout the late 19th and 20th century (Lange et al. 1980), its presence had not been documented in Canadian herds before it was identified in 2011 (Harper et al. 2002; Scott et al. 2013). The outbreak of *Psoroptes* in Canadian bighorn sheep was first detected in the Similkameen region of southern BC within approximately 50 km of the USA border and was identified in the corresponding Sinlahekin population of bighorn sheep in Washington state in 2013 (Scott et al. 2013; Harris et al. 2018). Declines of up to 40% of the affected Canadian herds have been observed and declines are suspected in Washington state as well (Reid 2013a; Harris et al. 2018). The appearance of *Psoroptes* in this transborder metapopulation of bighorn sheep has puzzled biologists because it is not known to be connected with other American bighorn populations through any natural migratory routes. It is separated from the closest known *Psoroptes* infested bighorn herds by over 250 kilometers and several large water ways, making natural ram dispersal an unlikely source of introduction (Cassirer 2005; Borg et al. 2017; Harris et al. 2018). Identifying the source of the *Psoroptes* outbreak in this bighorn sheep population is an essential step in the management

of this disease that will enable managers from BC and throughout North America to improve understanding of *Psoroptes* transmission and mitigate risk of disease outbreak in naïve herds.

Prior to European colonization in North America, local First Nations had not observed psoroptic mange in bighorn sheep (Buechner 1960). It was postulated that *Psoroptes* was brought to North America on infested domestic sheep in the 1800s and that the parasites were transmitted to wild sheep through contact between the two species (Sweatman 1958; Buechner 1960). By the late 1800s to early 1900s, severe infestations had been observed in bighorn sheep populations from California to Oregon and Wyoming. In the late 1800s, high bighorn herd range contiguity in the western portions of the United States combined with persistence of the parasite in domestic sheep likely facilitated *Psoroptes* transmission throughout much of the bighorn range. By 1960, large scale population declines led to range contraction and large areas of bighorn extirpation (Buechner 1960; Borg et al. 2017).

In 1973 *Psoroptes* was eradicated from domestic sheep in the United States (van den Broek and Huntley 2003), and this, combined with bighorn sheep habitat fragmentation likely slowed the spread of *Psoroptes*. Efforts to recover and re-establish the historical range of bighorn sheep through translocations and reintroductions were well underway and despite attempts to treat *Psoroptes* infested animals used in translocations, these human-assisted animal movements were a cause of at least some of the continued spread of the disease (Foreyt et al. 1990). This challenge has been largely rectified since that time by the implementation of standardized herd health assessment protocols for source stock used in bighorn translocation events (Western Association of Fish & Wildlife Agencies - Wildlife Health Committee 2015).

While *Psoroptes* was considered eradicated from Canadian domestic sheep in 1924 (van den Broek and Huntley 2003), it continues to be observed parasitizing other host species such as pet rabbits in Canada. It was once believed that different species of *Psoroptes* infested different hosts with *Psoroptes ovis* infesting sheep while rabbits were infested with *Psoroptes cuniculi* (Sweatman 1958). The parasites that were found on bighorn sheep throughout the USA matched the morphologic description of *Psoroptes ovis* (Boyce et al. 1990). In recent years, taxonomic re-examination challenged the previously held belief of heterospecificity of *Psoroptes* mites (Zahler et al. 2000; Pegler et al. 2005). *Psoroptes* species identification was traditionally done on the basis of host species, location of infestation on the host, and measurement of the outer opisthosomal setae length of the adult male *Psoroptes* mite (Sweatman 1958). However, some

cross-species infestation trials have demonstrated mite transmission between host species as well as interbreeding of mites previously categorized as heterospecific, supporting the taxonomic amalgamation of the *Psoroptes* species (Bates 1999). Genetic evaluation of *Psoroptes* mites from different hosts and locations using common genetic targets has included investigation of microsatellite markers, the second internal transcriber sequence of the rDNA gene (ITS2), and the mitochondrial gene cytochrome oxidase subunit I (COI) (Zahler et al. 1998; Evans et al. 2003; Pegler et al. 2005; Amer et al. 2015; Juan et al. 2015). This molecular work has provided additional support for the conspecificity of these mites. While no clear rules on the molecular differentiation of species exist, most authors agree that all mites of the *Psoroptes* genus with the possible exception of *Psoroptes natalensis* in water buffalo (Amer et al. 2015) should be unified under the name of priority, *Psoroptes ovis*. It is common in the literature to refer to them simply by the genus name *Psoroptes* and they will therefore be referred to as such throughout this work (Zahler et al. 1998; Ochs et al. 1999; Evans et al. 2003; Pegler et al. 2005; Juan et al. 2015; OConnor and Klimov 2015). While results from cross-species transmission studies are inconsistent, the possibility that Canadian bighorn sheep could have been exposed to the mite from another *Psoroptes* susceptible host species such as rabbit, horse, or deer (Wright et al. 1983; Bates 1999) is a necessary consideration.

In this study, *Psoroptes* mites collected from the BC bighorn sheep, were compared with *Psoroptes* mites collected from domestic rabbits, and from wild bighorn sheep throughout the USA. The relatedness of these different mite populations was investigated using molecular and morphometric data to identify the likely source of the BC bighorn sheep *Psoroptes* outbreak.

2.2 Materials and Methods

2.2.1 Sources of Mite Isolates

Mite samples (n=4) were collected from the ears of naturally infested bighorn sheep of the Penticton Indian Band herd of British Columbia (BC) that were captured for the treatment trial in 2017 (Chapter 5). Two additional samples associated with this outbreak were collected from animals found dead in the Sinlahekin herd of the USA, just south of the Canada-USA border (Harris et al. 2018). Rabbit samples (n=3) were collected from privately owned domestic rabbits in 2018 and 2019 in Edmonton, AB (n=1) and Maple Ridge, BC (n=1), and an archived *Psoroptes* sample from a rabbit host (1988) was obtained from the Wyoming State Veterinary Lab; however, no location of origin was available for this sample. Samples from endemic bighorn

Psoroptes infestations were opportunistically collected from naturally infested bighorn sheep in Nevada (n=2), Oregon (n=2), and Idaho (n=5) during routine wildlife captures and mortality investigations between 2007 and 2019. Unfortunately, mites collected from the Asotin herd of the Hells Canyon metapopulation in the southeastern portion of Washington State (the closest previously known *Psoroptes* infested herd in the USA) were available in extremely limited quantities, were highly desiccated, and DNA replication attempts were therefore unsuccessful. As a result, mites from this herd were not used in analysis; however, 3 of the Idaho samples, and both of the Oregon samples were obtained from other herds that are part of the Hells Canyon metapopulation and it is therefore presumed that these mites would be highly similar.

Throughout the analysis mites were grouped based on geographic location and species of origin with one group composed of mites collected from Canadian and Sinlahekin (USA) bighorn sheep, a second group composed of mites collected from the remaining USA bighorn sheep, and a third group composed of mites collected from rabbit hosts. Morphologic, morphometric and molecular comparisons investigated differences within and between these groups.

Psoroptes mites were collected by extracting cerumen and hyperkeratotic skin crusting from the ear lesions and within ear canals of the host using cotton tipped swabs or forceps. Samples were then stored dry or in ethanol until they could be transported for inspection, mite isolation, and analysis. Mites were extricated from the cerumen and crust under a stereoscopic dissecting microscope and identified as *Psoroptes* based on the presence of cone shaped suckers (pulvilli) on the end of their characteristically long jointed pretarsi (peduncles) (Sweetman 1958; Bates 1999; Pegler et al. 2005; Amer et al. 2015) (Figure 2-1).

2.2.2 Morphologic Inspection and Morphometric Setae Measurement

Subsamples of adult male mites were removed from each sample, placed on a microscope slide, flattened with a cover slide and examined under a computer and camera-enabled compound microscope. Micrographs of the opisthosomal lobes and setae were taken under 25x and 40x magnification and the 4th setae, known as the outer opisthosomal setae (OOS), was measured using the free-hand measurement tool, in the SPOT Basic Image Capture software (SPOT Imaging) following calibration using a standardized 0.01µm stage micrometer.

When visible and intact, both the left and right OOS were measured from each mite and the longer of the two measurements was used in data analysis. In cases where one of the OOS was broken or not able to be distinguished from the other opisthosomal setae microscopically, the

remaining OOS was used in data analysis. Outer opisthosomal setae lengths were analyzed using a Kruskal-Wallis non-parametric test to compare OOS lengths between individuals and between populations and a Dunn's multiple comparison test was used for post-hoc pairwise comparisons. All statistics were completed in R Studio (Version 1.1.423 – © 2009-2018 RStudio, Inc) using the FSA: Fisheries Stock Analysis R package for the Dunn's test (Ogle et al. 2019).

2.2.3 DNA Extraction

Pooled samples of 20 or more mites from a single host (when available) were placed in uniquely identified vials in 60% ethanol for DNA extraction, replication and sequencing. DNA was extracted from each of the pooled groups of mites without homogenization using the DNeasy tissue kit™ (Qiagen) following the method described by Dergousoff and Chilton (2007). Briefly, pools of mites were removed from the ethanol, and combined with 180 µl of ATL Buffer (Qiagen), 20 µl of Proteinase K (15µg/µl) and left to incubate overnight at 55°C. Next, 200µl of AL buffer (Qiagen) was added and the sample was vortexed and incubated at 70°C for 10 minutes. Two hundred microliters of ethanol (100%) was then added and the solution was passed through a spin column. Rinsing was performed using wash buffers AW1 and AW2 (Qiagen), and the DNA was eluted with 100 µl AE buffer (Qiagen) and stored at -70 °C until PCR could be completed.

2.2.4 Primers and PCR Conditions

The mitochondrial gene cytochrome oxidase subunit I (COI) was amplified using primers COF14 (5'-GGTCAACAAATCATAAAGATATTGG-3'), and COR72 (5'-TAAACTTCAGGGTGACCAAAAAATC-3') (Wang et al. 2012). The COI PCR was performed in 25 µl volumes containing 200µM of dNTP (Bio-Rad), 3mM MgCl₂, 0.75µM of each primer, 1.25U DNA polymerase (Phusion HotStart II), 5µl 5X Buffer Phusion Green HF buffer, 17.1µl H₂O, and 1µL of template DNA using a thermocycler with the following conditions: 95°C for 5 min (initial denaturation), 35 cycles of 95°C for 1 min (denaturation), 40°C for 1 min (annealing), and 72°C for 30s (extension); followed by 72°C for 5 min (final extension). Negative control (i.e. no gDNA template) samples were included in each set of PCRs.

The mitochondrial gene cytochrome B (Cyt B) was amplified using the forward primer (5'-TGTGAGAATAACTCCAATTCTAG -3'), and reverse primer (5'-GGTGAAAGATACTACCCCACT-3') which were designed for this study based on previously

published mitochondrial sequences (Gu et al. 2014). The Cyt B PCR was performed in 25 μ l volumes containing 200 μ M of each dNTP (Bio-Rad), 1.5mM MgCl₂, 0.75 μ M of each primer, 1.25U DNA Phusion High-Fidelity DNA polymerase, 5 μ l 5X Buffer Phusion Green HF buffer, 15.9 μ l H₂O, and 2 μ L of template DNA using a thermocycler with the following conditions: 98°C for 30s (initial denaturation), 35 cycles of 98°C for 10s (denaturation), 50°C for 30s (annealing), and 72°C for 1 min (extension); followed by 72°C for 5 min (final extension). Negative control (i.e. no gDNA template) samples were included in each set of PCRs.

Amplicons (5 μ l) were subjected to 1.5% agarose gel electrophoresis. All amplicons of the expected size were purified (as described by Krakowetz et al. 2014) prior to automated DNA sequencing using the same primers used for the PCR (i.e., in separate reactions).

2.2.5 Phylogenetic Analysis

Sequences were manually aligned and compared with the previously published sequence of *Psoroptes cuniculi* mitochondrial DNA on GenBank (accession number KJ957822) (Gu et al. 2014). Comparison was performed in a pairwise manner to highlight the similarities and differences between sequences. Percent sequence similarity (S) was calculated between the different mite samples using the formula $S=M/L$ where M is the number of alignment positions a base is shared between two alignments, and L is the number of alignment positions compared (Dergousoff and Chilton 2007). DNA sequences were translated into amino acid sequences using the invertebrate mitochondrial genetic code

2.3 Results

2.3.1 Samples Analyzed

Morphometric analysis was performed on subsamples from the same host for which DNA sequencing was successful except for two samples where no DNA sequence was obtained. Sequence data was also obtained for another three samples for which morphometric examination was not performed. Morphologic inspection and morphometric measurements were performed on a total of 131 mites collected from 17 different host animals. A total of 22 DNA sequences (13 CytB and 9 COI) were obtained from 15 unique hosts. The make-up of these samples and sequences are summarized in Table 2-1 below.

The USA bighorn mites had opisthosomal lobes containing three prominent opisthosomal setae (2nd or inner, 3rd or middle, and 4th or outer) with a well-defined angle at the outer edge of

the opisthosomal lobe (Figure 2-2a) while mites from Canadian bighorn sheep and rabbit hosts both had consistently less prominent OOS, and a less distinct outer opisthosomal lobe edge (figure 2-2b). The USA bighorn origin mite OOS lengths had a median of 150 μ m (range = 114 μ m -193 μ m), Canadian bighorn mite OOS lengths had a median of 81 μ m (range = 62 μ m - 101 μ m) and rabbit mite OOS lengths had a median of 85 μ m (range = 57 μ m -142 μ m) (Figure 2-3). Kruskal-Wallis analysis for differences in the OOS length of the mites matched the morphologic observations of the opisthosomal lobe shape. Outer opisthosomal setae lengths were significantly different between the different host group (i.e. rabbit vs. Canadian bighorn, vs USA bighorn) (Kruskal-Wallis chi-squared = 96.04, df = 2, P<0.001). Post-hoc testing using a Dunn's test for pairwise comparison found that Canadian bighorn sheep mites were not significantly different from rabbit mites (p=0.28) but were significantly different from USA bighorn mites (p<0.001). Similarly, rabbit mites were also significantly different than USA bighorn mites (p<0.001).

When blocked by host animal rather than host group, the null hypothesis was also rejected (Kruskal-Wallis chi-squared = 104.33, df = 16, P<0.001). Pairwise comparisons using the Dunn's test revealed no significant differences between the different individuals within any of the three host groups. Pairwise comparisons between individuals of the Canadian host group with the other two host groups found 1 out of 15 pairwise comparisons with rabbit hosts that were significantly different, and 43 out of 48 pairwise comparisons with the USA bighorn mites that were significantly different. All five of the pairwise comparisons that were not significantly different between Canadian and USA bighorn sheep involved the sample "BHS_CAN6-WA" for which only one male mite was present in the sample.

2.3.2 Cytochrome B sequencing

DNA sequencing of Cyt B (341 bp) was successful for 13 different samples (Table 2-1), encompassing all three mite host groups. Analysis was performed over a 307 BP section which was complete for 12/13 recovered sequences. Complete (100%) similarity was observed among the four Canadian bighorn samples and also among the seven USA bighorn samples. A single base pair substitution was observed between the sequences of the two rabbit origin samples. Seven point mutations made up of four purine transitions, one pyrimidine transition, and two transversions were discovered between the USA and Canadian bighorn samples (97.7% homology). These nucleotide differences corresponded to one mutational difference in the amino

acid sequence. Two samples of rabbit origin were successfully sequenced, one of which was 100% similar to the Canadian bighorn sequences, and the second contained one pyrimidine transition (99.7% homology) resulting in an amino acid substitution (Appendix 1).

Comparison of the Canadian Cyt B sequence with the published *Psoroptes cuniculi* sequence on GenBank (Accession # KJ957822, collected from an infested New Zealand white rabbit in China) reveals 12 point mutations (96.1% homology) including three purine transitions, six pyrimidine transitions, and four transversions (Gu et al. 2014). These corresponded to three differences in amino acid sequence. Comparison of the same published sequence with the USA bighorn sequence revealed thirteen nucleotide mutations representing three purine transitions, six pyrimidine transitions, four transversions, and four mutations in amino acid sequences. Percent similarity of haplotypes of mites from different host origins is displayed in Table 2-2.

2.3.3 Cytochrome C oxidase subunit I Sequencing Results

DNA sequences were successfully acquired from 9 different samples (Table 2-1), including two Canadian bighorn samples and 7 USA bighorn samples. None of the rabbit origin samples were successfully sequenced at this locus. A maximum of 660 base pairs were sequenced however most samples produced only partial sequences. Analysis was performed over a 305 BP section which was complete for 8/9 recovered sequences. Similarity was 99.7% within the Canadian bighorn and USA bighorn groups with each displaying one silent pyrimidine transition within their groups. Between groups, nine point mutations were found (97.0% similarity) made up of four pyrimidine transitions, four purine transitions, and one transversion resulting in two amino acid substitutions. Comparison with the published amino acid sequence of “*Psoroptes cuniculi*” from Gu et al. (2014), revealed seven point mutations composed of four purine transitions and three pyrimidine transitions, resulting in one amino acid substitution relative to the sequence of *Psoroptes* from Canadian bighorn. When comparing this *P. cuniculi* sequence to the USA bighorn sequences at this locus ten point mutations were observed, composed of four purine transitions, five pyrimidine transitions and one transversion resulting in three amino acid substitutions (Appendix 2). No deletions, insertions or changes resulting in non-sense mutations were observed in any sequences.

2.4 Discussion

Evidence of two genetically and phenotypically separate groups of mites was apparent based on the morphologic, morphometric and molecular analyses. The distinctly different shape

of the opisthosomal lobes and the different lengths of OOS of the Canadian bighorn and rabbit samples relative to the USA bighorn samples shows a clear distinction between these two groups. These different phenotypes were reported by Boyce et al (1990) who found that mites collected from bighorn hosts had slightly longer “length of lateral margin of opisthosomal knob” than mites collected from rabbit hosts as well as significantly longer OOS lengths. The Canadian bighorn outbreak is the first report of mites that resemble the rabbit phenotype causing natural infestation in a bighorn sheep population. Despite the small number of rabbit mites that were successfully sequenced, the very high percent similarity found between the Canadian bighorn sheep and rabbit mites relative to the USA bighorn samples adds important supportive evidence. It shows that these differences are the result of shared ancestry between the mites of rabbits and those found on Canadian bighorn sheep rather than the possibility of convergent evolution of this phenotype. This indicates that the mite infestation that is present within the Canadian bighorn herds did not come from a disease spillover event originating from nearby infested bighorn populations in the USA, but rather it likely represents a species barrier jump where the ecotype that is generally found on rabbits began to infest bighorn sheep.

Samples of wild rabbits or hares from BC were solicited for this study to assess *Psoroptes* infestation level and perform morphometric and molecular comparisons but no samples could be located. While *Psoroptes* is known to survive in the environment for a period of 10-14 days, and therefore cross-species transmission events are theoretically possible without direct contact between rabbits and wild bighorn sheep; it begs the questions of why there, and why then? A look at the history of the area reveals a likely possibility in the form of a wildlife park that was located in the area called the Okanagan Game Farm.

In 1999, twelve years prior to the official detection of *Psoroptes* in Canadian bighorns, the Okanagan Game Farm, located in what is now considered the epicenter of the *Psoroptes* outbreak, closed (Horton 2007). The facility held a number of exotic and native species. Among them was a herd of bighorn sheep from locally caught and imported stock from the USA (C. Lacey, personal communication, January 22, 2016). The facility also had a domestic rabbit colony used for feeding the carnivores which was reported to be infested with mites and not treated but rather heavily infested animals were euthanized for feed (H. Schwantje, personal communication, June 20, 2019). At the time of closure, bighorn sheep were captured and individually inspected by provincial and federal veterinarians for export to a variety of captive

and free ranging locations in the USA, at which time none of the sheep showed clinical symptoms of *Psoroptes* infestation (H. Schwantje, personal communication, June 20, 2019). A game farm employee, as well as a private veterinarian who did work for the game farm, reported inspecting and unsuccessfully treating bighorn sheep for mange-like symptoms during its operation (C. Lacey, personal communication, January 22, 2016; D. Ward, June 17, 2019). The etiologic cause of those symptoms was never confirmed.

It is unclear whether the infestation started in the Okanagan Game Farm or whether imported animals brought the infestation with them but according to Lacey's old notes, the outbreak began with two symptomatic bighorn sheep in 1991, many years after the opening of the park, and continued until the park shut down in 1999 (C. Lacey, personal communication, January 22, 2016). Additionally, the absence of reports of the rabbit ecotype of *Psoroptes* infesting bighorn sheep elsewhere suggests that the infestation was likely not introduced by infested source stock at the founding of the Okanagan Game Farm bighorn population (Boyce et al. 1990). This captive situation would have artificially put bighorn sheep in close proximity to infested rabbits and could have been a prime opportunity for the disease transfer from rabbits to bighorn sheep through contaminated equipment, feed, or direct contact between the animals.

While the bighorn sheep were reportedly asymptomatic at the time of the Okanagan Game Farm closure, and were sent to USA collections and not released, it is reported that during its operation bighorn sheep did escape the confines of the Okanagan Game Farm and founded the "Kruger Hill Subpopulation" (Harper et al. 2002). Thus it seems highly likely that the Okanagan Game Farm facilitated a host-species jump by bringing these host-species in unnaturally close proximity, creating an opportunity for direct or indirect transmission. These bighorn sheep escapees would then have been the likely source of *Psoroptes* infestation in the free-ranging Canadian bighorn herds, though it is unclear why there was such a long period of time between this disease introduction and the detection of disease in surrounding bighorn populations. It is possible that relative separation of bighorn herds and infrequent inter-herd migrations contributed to the slow spread of the infestation during this time.

2.5 Conclusion

Improved understanding of the source of the Canadian *Psoroptes* outbreak is important for wildlife managers to be able to mitigate the risk of new *Psoroptes* outbreaks in bighorn sheep. The findings presented here show strong evidence that the *Psoroptes* mites infesting BC bighorn

sheep did not move into Canada through natural bighorn movement from other infested bighorn populations but rather, they likely crossed the species barrier from rabbits. Based on the history, it seems likely that this species jump was facilitated by artificial housing conditions in the Okanagan Game Farm and may still be unlikely to occur in free ranging situations. The difference in these mite strains may be a reason why Canadian bighorn populations have been more severely impacted by *Psoroptes* than many American bighorn populations are; however, a population decline was caused in New Mexico by mites matching the USA *Psoroptes* descriptions as well, so mite strain differences are unlikely to fully explain the variability observed in the population-level impacts of this disease (Boyce et al. 1990).

These findings also provide an important timeline for when *Psoroptes* may have first been introduced to Canadian bighorn sheep, which is important for traceback and impact analysis. For example, Bighorn sheep were transplanted from the Keremeos area of BC (now endemic with *Psoroptes*) into the Okanagan Mountain Park (outside of the current *Psoroptes* infested area) in 2007, before the confirmation of the *Psoroptes* outbreak in the Okanagan, but after its introduction (Reid 2012). While *Psoroptes* has not been reported in the Okanagan Mountain Park animals at this time, continued monitoring of those animals as well as serological testing for exposure would be worthwhile to ensure that *Psoroptes* was not translocated with any of those animals in 2007.

This study provides a real-world example of the lack of host specificity of the *Psoroptes* mite that has been reported in cross-infection trials (Wright et al. 1983; Bates 1999). It is the first report of a natural infestation of bighorn sheep with mites that are morphologically and genetically associated with rabbits (formerly known as *Psoroptes cuniculi*). It raises questions about the potential for difference in the virulence of this strain of *Psoroptes* mites when infesting bighorn sheep. It also suggests that further assessment of the *Psoroptes* infestation status of other competent host species in the Okanagan area such as rabbits, elk, deer, or horses is worthwhile if a bighorn sheep *Psoroptes* eradication effort is to be entertained. Finally, this outbreak demonstrates the necessity of further research into several key areas that could have helped prevent the outbreak in the first place. These include improved detection of disease (Chapter 3), improved treatment of *Psoroptes* in bighorn sheep that could have been used while they were still in captivity (chapter 5), or improved fencing and enclosure design to prevent accidental escapes

of captive bighorn sheep (Chapter 4). These gaps in knowledge are addressed throughout this thesis.

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Psoroptes samples needed to complete this investigation were collected by numerous biologists and veterinarians. Thanks to Katherine Bardsley of the Wyoming State Veterinary lab and Dr. Mani Lejeune of the Canadian Wildlife Health Cooperative who taught me to isolate and identify *Psoroptes* mites. Thanks to the many people who collected and submitted samples for this research: Drs. Peregrine Wolff, Mark Drew, and Frances Cassirer, collected many of the American bighorn sheep *Psoroptes* samples; Jeff Heinlen collected samples from the USA side of the Canadian bighorn outbreak; Andrew Walker, Craig McLean, Aaron Reid, and Helen Schwantje who helped me collect the Canadian bighorn sheep samples; Arbutus West animal clinic and Dr. Eryn Hanak who collected the Canadian rabbit samples. Thanks also to Heather Down, Dr. John Taylor and the University of Victoria for providing lab space and the use of microscopes and computers that allowed for the mite isolation and morphometric measurement. A huge thank you to Dr. Neil Chilton and his lab at the University of Victoria who performed the DNA extraction, and PCR for the purposes of this research. Thank you to Drs Ilya Blum and Tasha Epp for guidance on statistical analysis of the morphometric data. Finally, thank you to Dr. Helen Schwantje for proposing the concept of doing a disease outbreak investigation and considering rabbits as a possible source of disease.

2.7 Literature Cited

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Host origin	Morphologic and Morphometric Sample Size			DNA Sample Size	
	# of mites inspected	# hosts represented	% mites with both OOS measured	Cyt B	COI
USA bighorn	67	8	82%	7	7
Can bighorn	49	6	96%	4	2
Rabbit	15	3	80%	2	0
Total	131	21	86%	13	9

Table 2-1: Herd and host origin of mites inspected, and DNA successfully sequenced. % mites with both OOS measured = the proportion of mites for which measurement of the outer opisthosomal setae (OOS) was possible from both the left and right OOS of the mite in which case the longer of the two was included in data analysis. Cyt B = number of pools of mites for which the cytochrome B (Cyt B) mitochondrial gene was successfully sequenced (where each pool represents multiple mites from a single host), COI = number of pools of mites for which the cytochrome oxidase subunit 1 (COI) mitochondrial gene was successfully sequenced (where each pool represents multiple mites from a single host).

	Cytochrome B		Cytochrome c oxidase subunit I	
	Base pair Similarity (%)	Amino Acid Similarity (%)	Base pair Similarity (%)	Amino Acid Similarity (%)
CAN BHS x Rabbit	99.7%-100%	99.0%-100%	N/A	N/A
CAN BHS x USA BHS	97.7%	99.0%	97.0%	98.0%
CAN BHS x <i>P. Cuniculi</i>	96.1%	97.1%	97.7%	99.0%
USA BHS x <i>P. cuniculi</i>	95.8%	96.1%	96.7%	97.0%

Table 2-2: Genetic Similarity between *Psoroptes* mites of differing host origins including samples collected from Canadian Bighorn sheep (CAN BHS), American bighorn sheep (USA BHS), North Canadian pet rabbits (Rabbit), and a published GenBank sequence for *P. cuniculi*



Figure 2-1: Characteristic long segmented peduncle (blue circle) that differentiates the genus of Psoroptes spp. from other psoroptidae that have relatively short unsegmented peduncles.

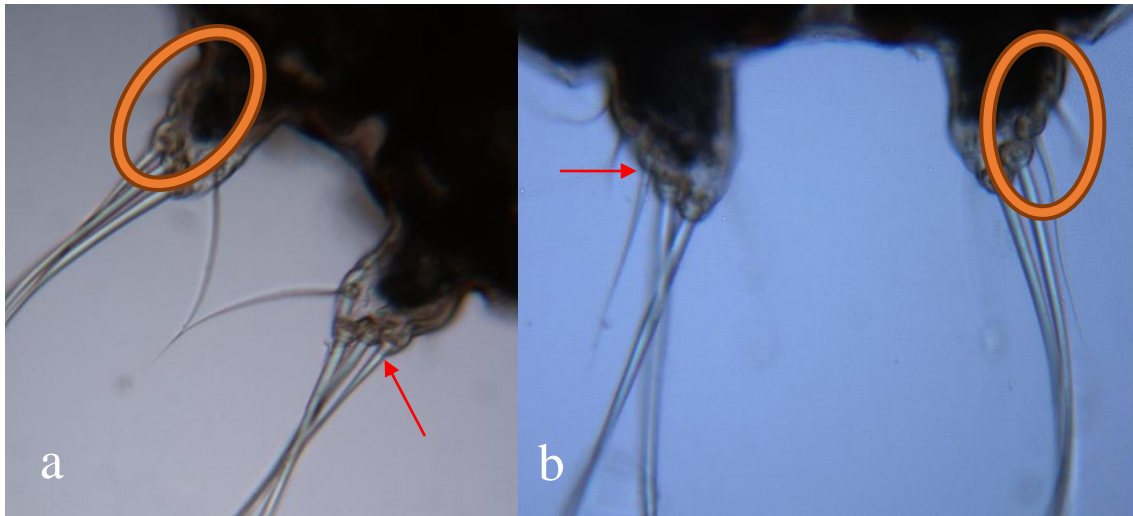


Figure 2-2: (a) Micrograph of a characteristic opisthosomal lobe of a USA bighorn mites. The photographed mite was collected from a bighorn sheep in the Hells Canyon metapopulation. Note the more prominent outer opisthosomal lobe edge (orange circle) and broad base to the OOS (red arrow)

(b) Micrograph of a characteristic opisthosomal lobe of BC bighorn and rabbit origin mites. This mite was collected from a bighorn in the Okanagan region of BC. Note the less distinct outer opisthosomal edge (orange circle) and the relatively less prominent base of the OOS (red arrow).

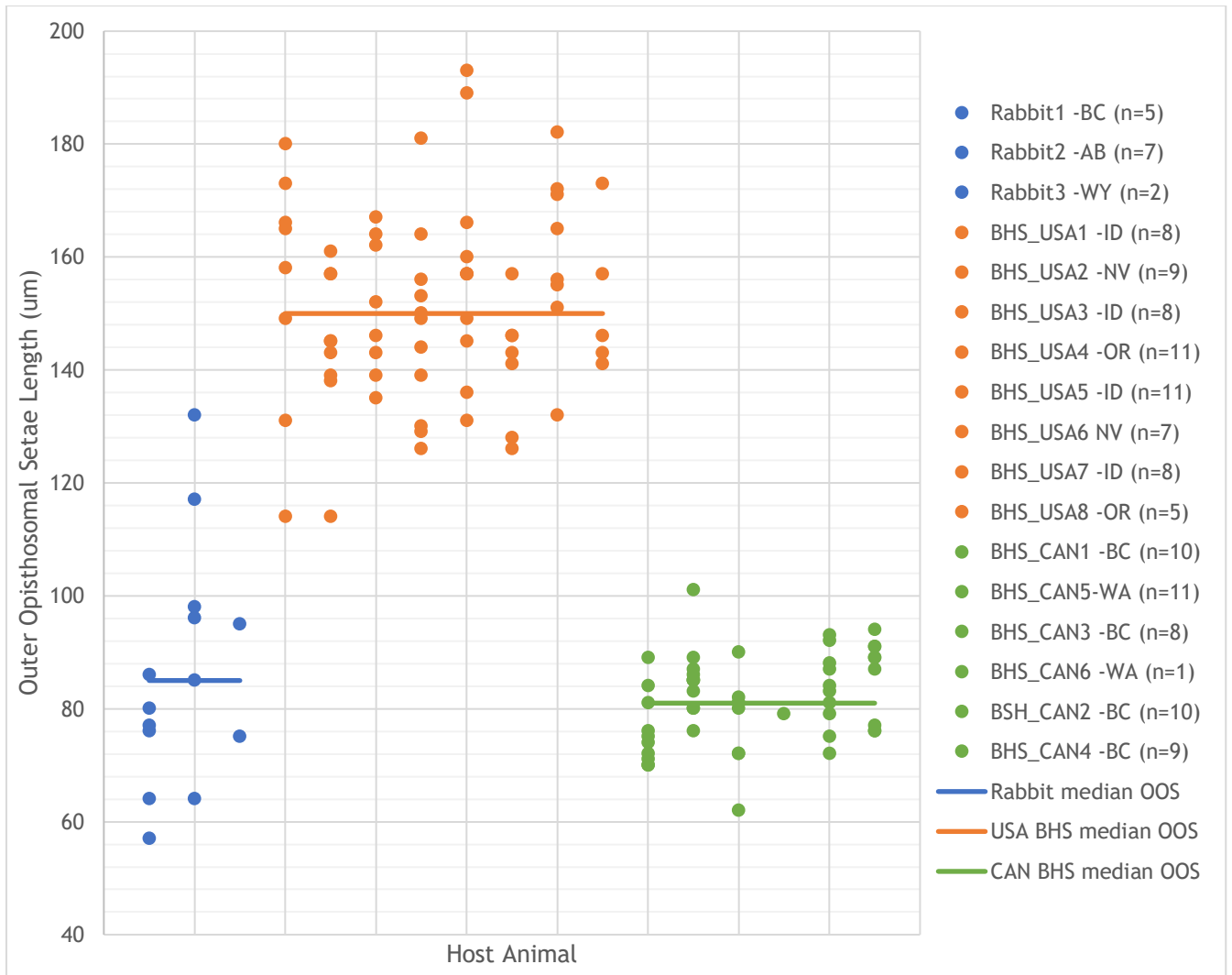


Figure 2-3: Distribution of outer opisthosomal setae (OOS) lengths of *Psoroptes* mites collected from rabbits (labelled Rabbit), USA bighorn sheep (labelled BHS_USA), and Canada-outbreak associated bighorn sheep (labelled BHS_CAN). Each is marked with the province or state of host origin. Horizontal lines represent median OOS of each host grouping.

CHAPTER 3: ADAPTATION AND OPTIMIZATION OF A COMMERCIAL SERO-DIAGNOSTIC TOOL FOR DETECTION AND MONITORING OF PSOROPTIC MANGE IN BIGHORN SHEEP (*OVIS CANADENSIS*)

The research described in Chapter 3 aims to improve detection of Psoroptes mites using a commercially available ELISA that is available to any lab interested in performing the test. This ELISA can allow increased access to analysis of archived samples and can further clarify the current and historic distribution of the mite. It should also help address the challenge of detecting subclinical carriers of the mites and improve herd-level disease profiling. This manuscript is intended to be submitted to the Journal of Veterinary Parasitology.

Archived blood samples were collected from biologists and veterinarians across North America. Hering completed all sample and data collection, as well as all laboratory work, and data analysis included in this chapter. Pilot testing of the ELISA (not included in this manuscript), guidance, and troubleshooting on the use of the ELISA was provided by its creators, Drs. Stewart Burgess and Francesca Nunn (Moredun Research Institute).

3.1 Introduction

Psoroptic mange infestations have been reported in some wild sheep populations of the United States of America since the 19th century (Sweatman 1958; Lange et al. 1980). *Psoroptes* mites have significant welfare and aesthetic effects on their hosts and may predispose wild sheep to predation through hearing loss (Norrix et al. 1995). Infestations can be a significant contributor to wild sheep mortality (Boyce and Weisenberger 2005; Miller et al. 2012) but mixed outcomes have been reported in affected herds, ranging from relatively benign transient infestations (Muschenheim et al. 1990) to rapid and severe population declines (Boyce and Weisenberger 2005).

While *Psoroptes* have been present in USA populations of bighorn sheep for many years, their presence in free ranging Canadian bighorn sheep is a relatively new development with the first animals with confirmed infestation identified in 2011 in British Columbia (Scott et al. 2013). *Psoroptes* has since spread to all bighorn herds on the west side of the southern Okanagan and Similkameen area of British Columbia, but has not been observed in any other bighorn populations because highways and fences form barriers to animal movement.

Subclinical infestations with *Psoroptes* may represent an important contributor to the persistence and spread of this parasite in wild sheep as it does with domestics (Burgess et al. 2012). Blood-based testing methods can improve disease detection, enable improved surveillance through retrospective testing of archived serum, and act as confirmatory testing for suspect microscopy results or clinical signs. This study tested the versatility of the *LilliTest* Sheep Scab enzyme-linked immunosorbent assay (ELISA) test kit (Lillidale Diagnostics 2016), in the detection of *Psoroptes* antibodies in bighorn sheep. The *LilliTest* Sheep Scab ELISA is a commercially available ELISA test kit marketed for *Psoroptes* detection in domestic sheep. It was evaluated for its ability to detect host antibodies against the mite antigen, Pso o 2, in bighorn sheep under several conditions including existing infestations, newly developing infestations in lambs, and the reduction in antibodies following successful treatment of infested animals. The use of this test as a herd-level disease surveillance tool was also explored.

3.2 Materials and Methods

The ELISA conditions were first optimized using known positive and negative bighorn sheep serum after which time the ELISA test performance was calculated using archived serum samples from known infested and disease-free bighorn sheep. The ELISA was then conducted on serum from naïve bighorn lambs with new developing infestations, on serum from animals with resolving infestations following treatment with anthelmintics, and on archived serum samples from a herd of uncertain *Psoroptes* status with suspect clinical signs.

3.2.1 Study Population

Positive and negative control stock was made from pooled bighorn sheep serum of known infestation status for use in the ELISA optimization. Aliquots of these same pooled sera samples were frozen in numerous cryovials and acted as positive and negative ELISA plate controls throughout the study to allow inter-plate comparison and quality control.

The positive control pool (PC) consisted of archived hyper-immune sera, pooled from naturally infested bighorn sheep from a British Columbia herd with clinically severe ear lesions (n=14). Severe ear lesions were those ranking 4/4 according to the severity scoring rubric described by Pan et al.'s (2006) characterized by hyperkeratotic exudative debris encompassing the entire auricle of the ear. These clinical signs were coupled with abundant live mites visualized on microscopic examination of ear exudate collected from those individuals. The duration of infestation of these free-ranging individuals was unclear; however, reports of

symptomatic animals in the area from which these samples were collected go back at least 9 years prior to capture and sample collection. The negative control pooled sera (NC) consisted of archived bighorn sheep sera from Canadian bighorn sheep that were free of clinical signs of psoroptic mange and located in areas never before reported to be infested with *Psoroptes* (n=24). Specifically, 20 of the negative samples included in the NC pool were collected in 2009 from bighorn sheep in the Elk Valley Provincial Park of BC, over 300 km east of the *Psoroptes*-infested Canada bighorn herd. The remaining 4 samples used in the NC pool came from bighorn sheep in the Tranquille Ecological Reserve in 2014, about 150 km north of the *Psoroptes*-infested Canadian bighorn herd.

Following optimization, the ELISA's test accuracy was determined using serum collected from known *Psoroptes* infested (positive) and *Psoroptes* free (negative) bighorn sheep. Positive samples came from free ranging bighorn sheep from the USA (n=44) and Canada (n=33) while negative samples came only from Canada (n=33). Positive Canadian samples were composed of archived serum from previously captured bighorn sheep from the Okanagan and Similkameen area of British Columbia (n=14) in addition to serum from infested bighorn sheep captured for initiation of a *Psoroptes* treatment trial in the same area (n=19) (Chapter 5). Positive USA samples were collected at the time of routine wildlife captures from *Psoroptes* positive herds in Oregon, Washington, Idaho, and Nevada. Ear swab microscopy was not routinely performed to confirm diagnosis in *Psoroptes* infested herds, because it is thought to have poor sensitivity in cases where infestations are less severe or subclinical (Bates 1996). Instead, positive cases were defined as any individual from which *Psoroptes* mites were identified on the ear swab microscopy of that individual or any herd-mate from their source herd within one year of the time of blood collection.

Due to the possibility of subclinical disease, only samples collected from asymptomatic animals in historically *Psoroptes*-free areas of British Columbia were used as known negative samples (n=33). Thirteen of these negative samples came from the bighorn sheep in Tranquille Ecological Reserve while the remaining 20 samples came from the Elk Valley bighorn sheep. Archived positive and negative samples were collected by a variety of wildlife biologists and vets so consistent lesion severity scoring was not available for all the positive samples. Among the positive Canadian samples, all animals displayed gross clinical signs of infestation. Among the 44 serum samples of known positive herds from the USA, 3 sheep displayed no symptoms of

Psoroptes infestation, 8 displayed mild suspect symptoms of *Psoroptes* infestation (mild hair loss in the ear but no skin crusting), 25 displayed overt evidence of *Psoroptes* infestation (including skin crusting in the ears), and the remaining 8 had unreported *Psoroptes* infestation severities but were located in herds with microscopy-confirmed infestations.

Analysis of the serology associated with new developing infestations was performed on 11 bighorn sheep lambs born in captivity during the *Psoroptes* treatment trial (Chapter 5) and naturally exposed to *Psoroptes* infestation over the first 6 months of life. Lambs were handled once monthly for sample collection during captivity. One lamb was lost from the sampling pool after three months due to mortality. Three additional lambs were removed from the sampling pool because they were administered treatment for psoroptic mange as part of the treatment trial.

Analysis of the antibody response to treatment was performed using serum collected from infested bighorn sheep at the time of treatment and 28 days after oral administration of 25mg/kg of fluralaner. These bighorn sheep were heavily symptomatic at the time of treatment with abundant live mites found in their ear swab microscopy. Twenty eight days after treatment, during follow-up inspection and sample collection, they were free of live mites and exhibited profound reductions in gross ear lesion severity (Chapter 5). No additional antibody testing was performed after that date because the animals were released from captivity immediately following the 28 day post-treatment examinations.

Finally, a real-world application of the ELISA test was performed on a herd of unknown *Psoroptes* infestation status. Archived serum samples from two neighboring herds, the Wenaha and Mountain View herds of the Hells Canyon metapopulation, collected in 2010 (n=15 and 4 respectively) were tested using the ELISA. Wenaha animals had microscopy confirmed *Psoroptes* infestations in 2003 and 2008. By 2010, no overt symptoms of infestation were observed in 5 out of the 15 animals that were captured, and the remaining 10 animals had only minor suspect symptoms such as areas of hair loss, but no areas of skin crusting. In the Mountain View herd, only 1/4 animals were reported to have suspect symptoms (hair loss in the ears) with the remaining 3 animals showing no signs of infestation. No mites were discovered in the ear microscopy of any of the samples collected in 2010 (Hells Canyon Bighorn Sheep Restoration Committee 2010; F. Cassirer, E-mail communication, January 9, 2019). The population estimate of these two herds at that time were reported as a combined sum of 120 animals (Hells Canyon Bighorn Sheep Restoration Committee 2010). These serum samples were assayed using the

protocol described here and the results are discussed with reference to herd level interpretation of infestation status and management implications.

3.2.2 ELISA Optimization

The *LilliTest* Sheep Scab ELISA kit is a commercially available ELISA test kit developed by Moredun Research Institute for detection of *Psoroptes* in domestic sheep (Nunn et al. 2011; Burgess et al. 2012). It is an indirect ELISA aimed at the detection of IgG antibodies specific to the *Psoroptes* mite protein Pso o 2 in the serum of exposed sheep (Nunn et al. 2011).

A checkerboard titration protocol published by Fitzgerald industries international (International Atomic Energy Agency, Accessed March 28, 2017) was used for optimizing the indirect ELISA conditions using bighorn sheep serum. Optimal test conditions were determined by titrating serial antigen dilutions against serial 2-fold dilutions of pooled positive and negative bighorn sheep serum. The stock recombinant Pso o 2 antigen was diluted in distilled/deionized water (at 2 µg/ml, 1µg/ml, and 0.5µg/ml concentrations) and the ovine serum was diluted from 1:100 to 1:3200 in a blocking buffer (BB: phosphate buffered saline [PBS] with Tween 80® [0.5%] and NaCl [0.5M]). The test conditions producing the highest PC:NC ratio was selected as optimal.

3.2.3 ELISA Test Protocol

The optimal ELISA protocol used throughout the trial closely resembles that established for domestic sheep by Nunn et al. (2011). The ELISA procedure used is as follows:

- 1) Fifty microliters of the antigen were diluted to 2 µg/ml in distilled/deionized H₂O and added to each well of a 96-well microplate (Greiner medium-binding Microlon ® 96 well microplate).
- 2) The plate was covered with a plate sealer and left overnight at room temperature to incubate.
- 3) The plate contents were discarded, and the plate was blotted dry on paper towel. The plate was washed 5 or more times using a wash buffer and blotted dry between each wash. Wash buffer was composed of PBS solution and Tween® 20 (0.05%).
- 4) Each well was loaded with 100 µl of blocking buffer composed of PBS, Tween® 80 (0.5% v/v), and NaCl (0.5M). The plate was then sealed with a plate sealer and incubated for 60 minutes at 37°C.

5) The plate contents were discarded, and the plate was blotted dry on paper towel. It was then washed 5 or more times using a wash buffer and blotted dry between each wash.

6) Test serum, positive control, and negative control serum was diluted 1/800 v/v in blocking buffer and 50 µl was loaded into each well in addition to 4 wells of blocking buffer as a blank control according to the test kit instructions. Each sample was assayed in duplicate and the controls were assayed in quadruplicate with duplicate controls placed and loaded at the beginning and end of each plate. The plate was then sealed with a plate sealer and incubated for 60 minutes at 37°C.

7) The plate contents were discarded, and the plate was blotted dry on paper towel. The plate was washed 5 or more times using a wash buffer and blotted dry between each wash.

8) Each well was then loaded with 50 µl of the rabbit anti-sheep IgG Horseradish Peroxidase-labelled conjugate, diluted 1/2000 v/v in blocking buffer. The plate was then sealed with a plate sealer and incubated for 60 minutes at 37°C.

9) The plate contents were discarded, and the plate was blotted dry on paper towel. The plate was washed 5 or more times using a wash buffer, ensuring that the wash buffer was retained in wells for >1 minute on washes 4 and 5.

10) The wells were each loaded with 50 µl of tetra-methylbenzidine (TMB) substrate and the plate was incubated at room temperature for 5 minutes.

11) Fifty microliters of TMB stop solution were added to each well to stop the reaction and the optical density (OD) of the colour change was read using a spectrophotometer at OD_{450nm}.

All ELISA iterations were run in duplicate on the same microplate during the checkerboard optimization and throughout the trial. Plates were rerun if the plate average PC OD was <1.0, or the NC OD was >0.35. Samples were rerun if the inter-well coefficient of variation (CV) was >10. The same batch of PC and NC pools were used on all ELISA plates to allow for inter-plate standardization.

3.2.4 Assay Analysis

Once optimal test conditions were established, archived serum of known infestation status bighorn sheep was assayed in duplicate to select an optimal positive/negative cut-off. In order to account for inter-plate variability, the cutoff was selected in relation to the average NC serum

pool OD of each plate and sample ODs were adjusted by dividing the average sample OD on a plate by the average NC OD on that plate. This cut-off was used throughout the following analyses of developing infestations and treated infestations.

3.3 Results

3.3.1 ELISA Optimization

Optimal conditions for the ELISA were achieved using an antigen dilution of 2.0 µg/ml and a serum dilution of 1:800 v/v in BB with a development time of 5 minutes (Figure 3-1). At the highest antigen concentration (2 µg/mL) and positive serum concentrations (1:100 and 1:200), precipitation occurred following addition of the TMB stop solution.

3.3.2 ELISA Test Performance on Archived Serum

Archived positive and negative serum samples tested under the same conditions showed a range of NC adjusted ODs (Table 3-1). Test specificity and sensitivity parameters as they relate to different positive/negative cut-offs are displayed in Figure 3-2. An optimal cutoff of 1.5 times the average OD of a plate's four negative controls (1.5*NC) was selected as the value providing the maximum combined test sensitivity and specificity resulting in an overall test specificity of 94.0% (31/33 negative sera tested negative). This cutoff produced accurate positive results in all 33 of the Canadian bighorn sera and in 43/44 of the USA positive samples giving a test sensitivity of 98.7%.

All 3 animals that were free of clinical signs from known infested herds from the USA tested positive on the ELISA using the established cutoff. The one false negative test result from the USA herds (NC adjusted OD =1.25) was described as having “ears mostly clean of scabies” and was categorized among the 8 samples with “suspect clinical signs” that were collected from known infested herds. Alternative ELISA cutoffs could be selected to prioritize either test sensitivity or specificity. A cutoff of 1.2*NC would obtain 100% sensitivity and a specificity of 85.7% using the set of archived samples available for this study, while a cutoff of 2.9*NC was required to obtain a 100% specificity resulting in a 90.9% sensitivity.

3.3.3 Antibody Response to Developing *Psoroptes* Infestations in Lambs

Serum OD_{450nm} values were generally higher in the lambs during the first month of life than in either of the following two months. Using the established ELISA OD_{450nm} cutoff, 9/11 lambs tested positive at one month of age but none tested positive at two months of age. By 3

months of age 3/11 tested positive and by 4 months all 10/10¹ remaining lambs tested positive. After 4 months 3 lambs were removed from the pool for the treatment trial and the 7 remaining lambs continued to test positive each month for the following 3 months until all animals were treated with anti-parasite medication in preparation for their release from captivity at the conclusion of the treatment trial (Figure 3-4).

Grossly, none of the 11 lambs had any detectable sign of *Psoroptes* infestation at 1 month of age, but 10/11 displayed clinical signs of *Psoroptes* infestation in at least one ear by two months of age and all showed symptom of infestation by 3 months of age and for the remainder of the study period until treatment.

3.3.4 Antibody Response Before and One Month after Treatment

ELISAs performed on serum collected before and after treatment showed no significant reduction in negative adjusted OD_{450nm} over that 4-week period (Paired-sample T-test P=0.27). At the time of treatment average negative adjusted OD_{450nm} values among treatment trial animals was 9.37 (+/-3.35), and 28 days later the average negative adjusted ELISA score was 8.40 (+/- 3.11). None of the animals seroconverted to ELISA negative within 4 weeks following treatment (Table 3-1).

3.3.5 ELISA Results in a Herd of Unknown Infestation Status

All 4 of the Mountain View samples and 14/15 Wenaha herd samples tested negative using the 1.5*NC cutoff. The fifteenth Wenaha sample gave a weak positive result of 1.54*NC. The range of negative adjusted optical densities obtained from these archived serum samples was 0.55 to 1.54 with a mean of 0.91 and a standard deviation of 0.27 (Figure 3-3).

3.4 Discussion

3.4.1 Test Sensitivity and Specificity

Following optimization, this ELISA test demonstrated high test sensitivity and specificity for the detection of *Psoroptes* exposure in bighorn sheep. Given that a key strength of antibody-based testing is the potential ability to detect antibodies in subclinically infested animals, it would be beneficial for the ELISA to be applied to more asymptomatic animals from known infested herds. That asymptomatic animals, as well as the majority of animals with suspect lesions, all tested ELISA positive using the selected cutoff, is strong evidence that the ELISA is capable of

¹ One lamb was lost to mortality between the 3rd and 4th month of observation.

detecting exposure and/or subclinical states of infestations. However, the sample size included in this experiment is not sufficient to describe testing accuracy in this portion of the population specifically.

Adjusting the cutoff to improve either test sensitivity or test specificity while sacrificing the other, as applied by Boyce et al. (1991), can allow adaptation of the test to different ecological contexts. This approach alone does not remove uncertainty regarding how to interpret the “weak positive” results that fall within this overlapping OD range such as the one weak positive result obtained from the Wenaha herd. Interpretation of these results should be done with consideration of the ecological context and the potential consequence of incorrect test results. For example, a false negative test result for an infested animal that is preparing to be translocated has a high ecological cost and therefore the lower test positive threshold might be selected to increase test sensitivity and guard against the higher cost of a false negative. Meanwhile, using a low threshold when performing general health assessments would result in an erroneously high prevalence estimate due to poor test specificity.

3.4.2 Seroconversion in Newborn Lambs and Treated Animals

Since antibodies were not detected in the second month of sampling but were detected in the first, it is likely that the high ELISA positive rate in one month old lambs is the result of passive transfer of maternally derived IgG antibodies obtained in colostrum from their dams within the first hours of life (Khan and Ahmad 1994). Those antibodies would deteriorate resulting in negative ELISA results for the coming months until the lambs themselves became infested and began to produce their own *Psoroptes* specific antibodies. Most lambs did not seroconvert, based on the established cutoff value, until closer to 4 months of age despite the appearance of symptoms of infestation at 2-3 months of age. This lag period is not consistent with the early detection of disease prior to onset of clinical signs reported by Nunn et al with this ELISA in domestic sheep (2011). While these results indicate the age of onset of infestation in newborn lambs as well as the time period needed for lambs to develop antibodies to this parasite, they may not be representative of the amount of time needed for immunocompetent adult animals to develop antibodies to new infestations and therefore may not reflect the true performance of this ELISA in adult bighorn sheep.

Following oral treatment with fluralaner (25mg/kg), none of the animals became ELISA negative within the first month when sampling was performed. This finding likely reflects the

persistence of IgG antibodies in the host rather than the efficacy of treatment as these findings are consistent with other studies that suggest that decline in *Psoroptes* antibody levels will only occur 6-8 months following resolution of infestation (Mazet et al. 1992; Burgess et al. 2012). This ELISA is therefore not a useful tool in assessment of freedom from disease or treatment efficacy immediately following treatment.

3.4.3 Herd Level Diagnostics

Herd level approaches to infectious diseases like psoroptic mange are highly important since apparently disease-free animals could be subclinically infested or can become re-infested if cohabitating with other infested animals. Consequently, disease freedom should be assessed at the herd level. An individual animal's test result is therefore less relevant to managers than a herd level assessment of infestation status. Given that one of the Wenaha herd test results was weakly positive, this population could be considered suspect for *Psoroptes* infestation. Alternatively, a herd level sensitivity and specificity of the testing performed can be calculated using the test parameters obtained for this cutoff, the number of samples collected, the estimated population size and two scenario parameters: hypothetical disease prevalence, and minimum number of positive test results required to consider a herd positive. Since a population estimate for the adjacent Wenaha and Mountain View herds in 2010 was only available as a cumulative total, the results will be discussed as a single herd.

The reported population estimate of the two herds is 120 animals (Hells Canyon Bighorn Sheep Restoration Committee 2010) and nineteen samples were collected from this joint herd. Using the test sensitivity of 98.7%, specificity of 94.0%, and selecting a disease prevalence detection limit of $\geq 5\%$ with one positive test result as the cut point, a herd level specificity of 28.9% and a herd level sensitivity of 90.7% is obtained. Under these conditions there is therefore a 71.1% chance that a truly negative herd would test positive (AusVet 2019). Alternatively, increasing the cut point to 2 positive results required to consider a population disease positive (meaning the set of 19 samples included in this study gives a negative herd level test result instead of a positive result) offers a herd level sensitivity of only 64.8% (AusVet 2019). These calculations demonstrate how difficult it is to detect disease that is only present in a herd at a very low prevalence. While many *Psoroptes* infestations in wild bighorn sheep appear to be present at higher prevalences than 5% (Reid 2013a), there are reports of large bighorn herds with low seroprevalence to *Psoroptes* (Mazet et al. 1992) and so the possibility of a low seroprevalence in a

truly infested herd cannot be ruled out. These ELISA results suggest that the Wenaha and Mountain View population of bighorn sheep may have cleared themselves of their *Psoroptes* infestation, however the possibility that *Psoroptes* prevalence is low in those herds but the disease is still present cannot be ruled out. Further inspection and confirmation of these results is necessary to increase the confidence in this conclusion.

3.5 Conclusion

The difficulty in the detection of subclinical or early stage disease makes management of herds with unknown disease status challenging and poses a considerable risk to the spread of disease through natural animal movements or translocation events. The *LilliTest* Sheep Scab ELISA is a commercially available ELISA that can be easily adapted to a wildlife context and attains a high level of sensitivity and specificity with minimal optimization. This ELISA is unlikely to be accurate for disease detection in very young lambs with developing infestations or for confirming successful treatment of recently treated animals however interpretation at the herd level adds an extra layer of disease detection capability and would be a useful tool for wildlife professionals in the detection and monitoring of *Psoroptes* in herds of unknown or suspect disease status.

3.6 Acknowledgements

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Group	Number of samples	Mean NC-adjusted OD	Standard Deviation
Archived positive			
USA	44	4.68	1.74
Canada	33	5.31	1.52
Archived negative			
Canada	33	0.88	0.45
New lamb infestations			
1 month of age	11	2.56	1.23
2 months of age	11	0.55	0.17
3 months of age	11	2.30	2.80
4 months of age	10	10.13	3.34
Treated animals			
Treatment day	27	9.37	3.35
1 month post treatment	27	8.40	3.11
Unknown <i>Psoroptes</i> status			
Wenaha Herds	15	0.93	0.30
Mountain View Herd	4	0.81	0.12

*Table 3-1: LilliTest sheep scab ELISA optical densities (OD) relative to the negative control (NC). Samples include archived sera from bighorn sheep of known *Psoroptes* exposure status, developing lamb infestations, treated/resolving infestations, and samples from two Hells Canyon herds of unknown infestation status.*

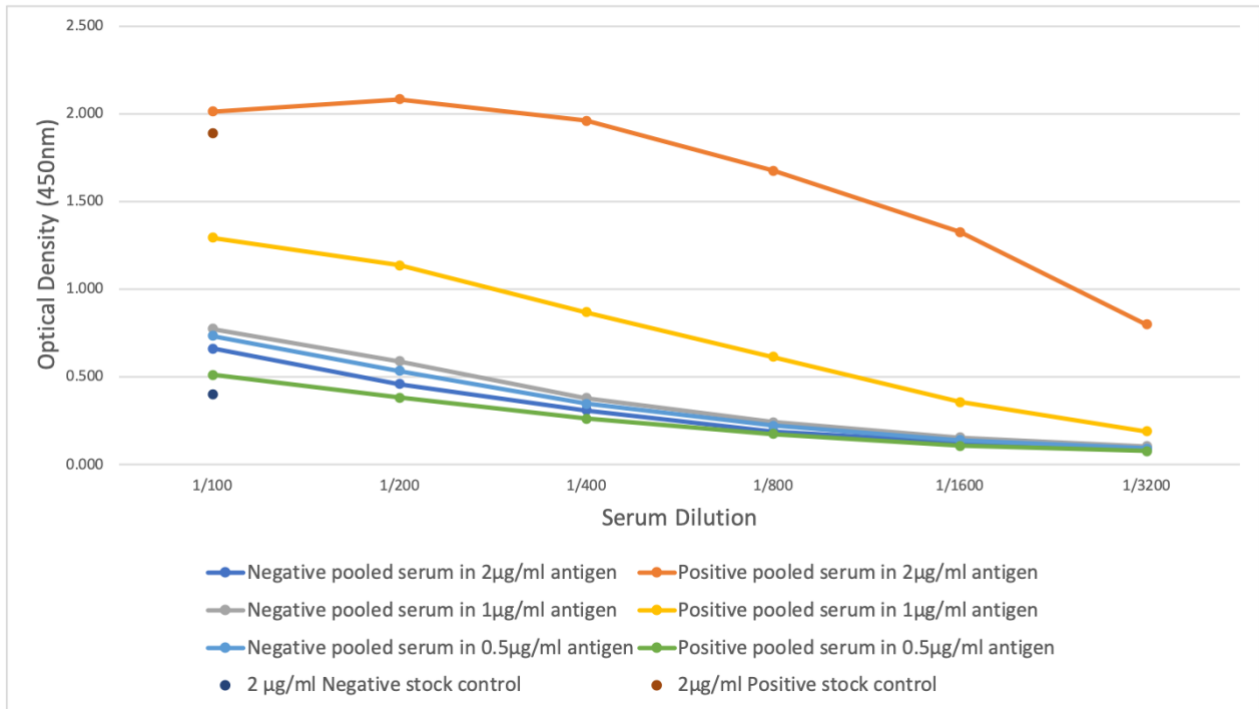


Figure 3-1: LilliTest Sheep Scab ELISA optical densities (OD_{450nm}) of serially diluted pooled positive (PC) and negative (NC) serum at multiple antigen concentrations.

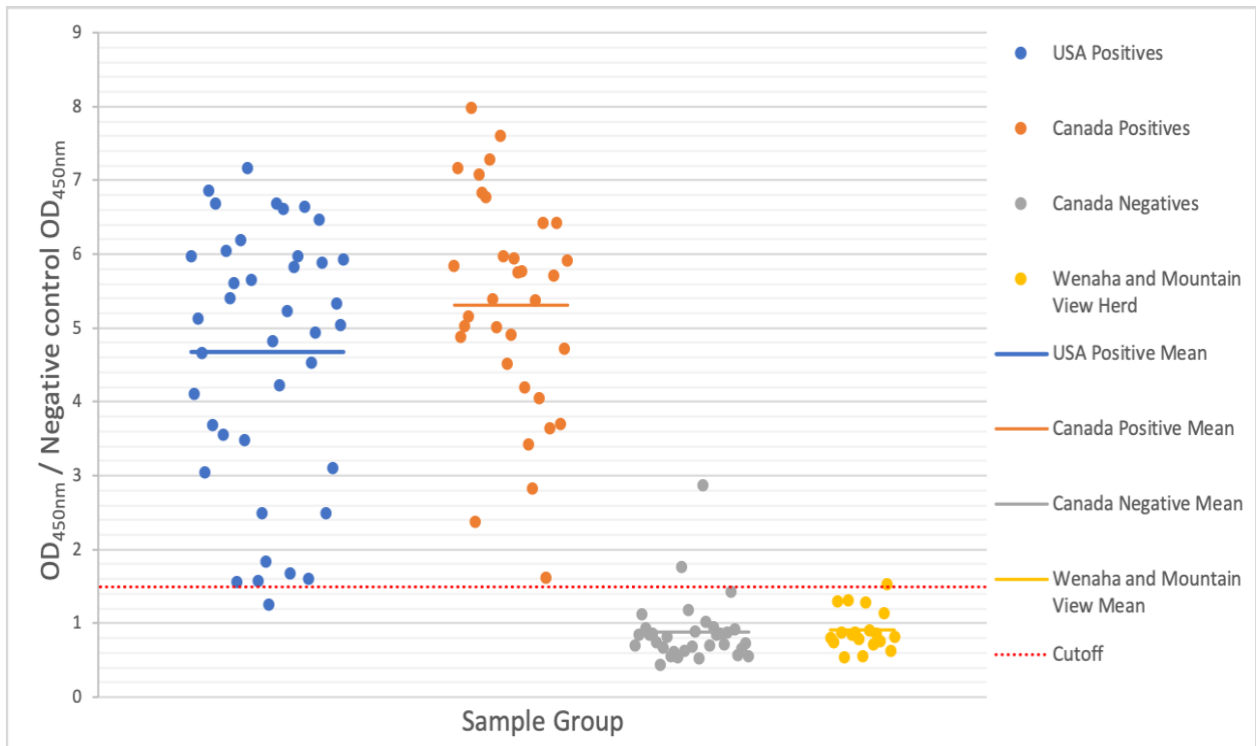


Figure 3-2: LilliTest Sheep Scab ELISA optical density (OD) scores divided by the negative control optical density (NC) of the ELISA plate. Negative control (NC) adjusted optical density distributions of each sample group and the group mean are displayed.

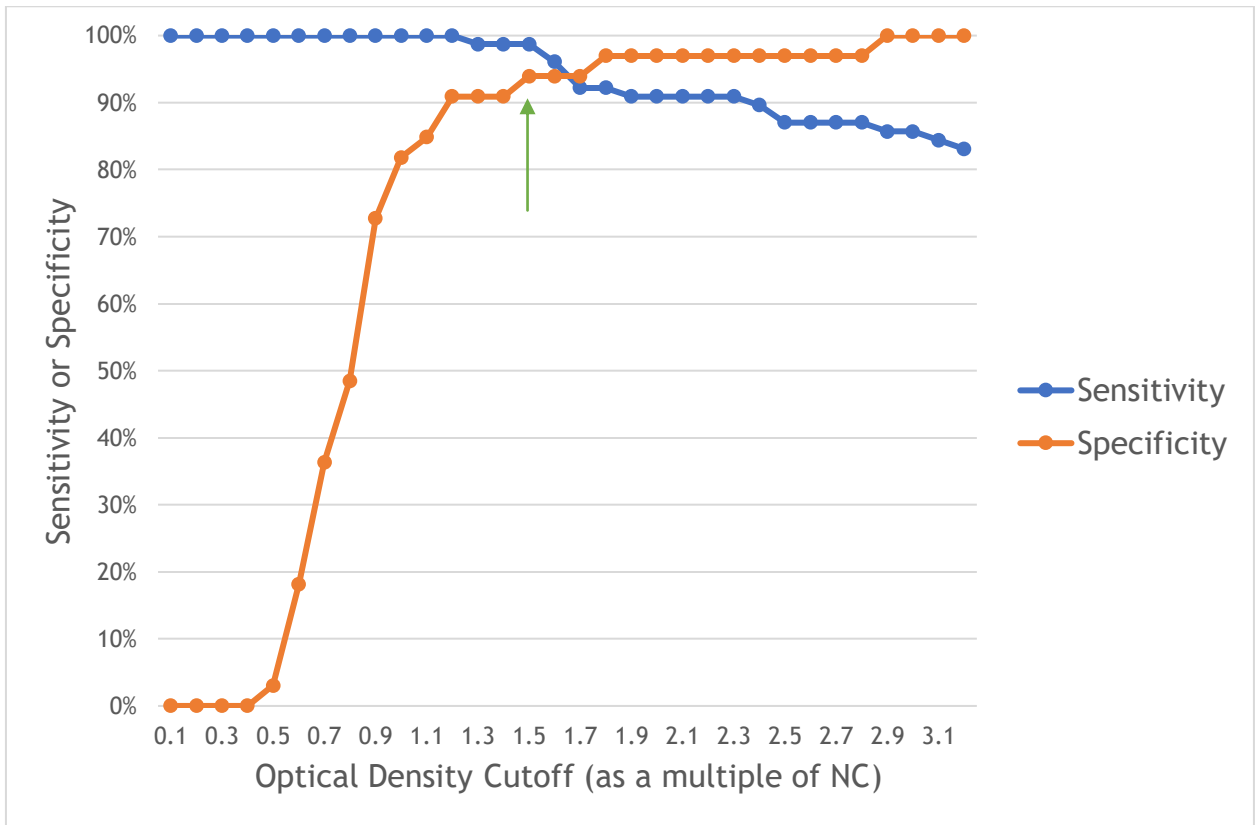


Figure 3-3: LilliTest Sheep Scab ELISA test sensitivity and specificity at different optical density (OD) cutoffs relative to the negative control pooled serum. Green arrow indicates optimal cutoff.

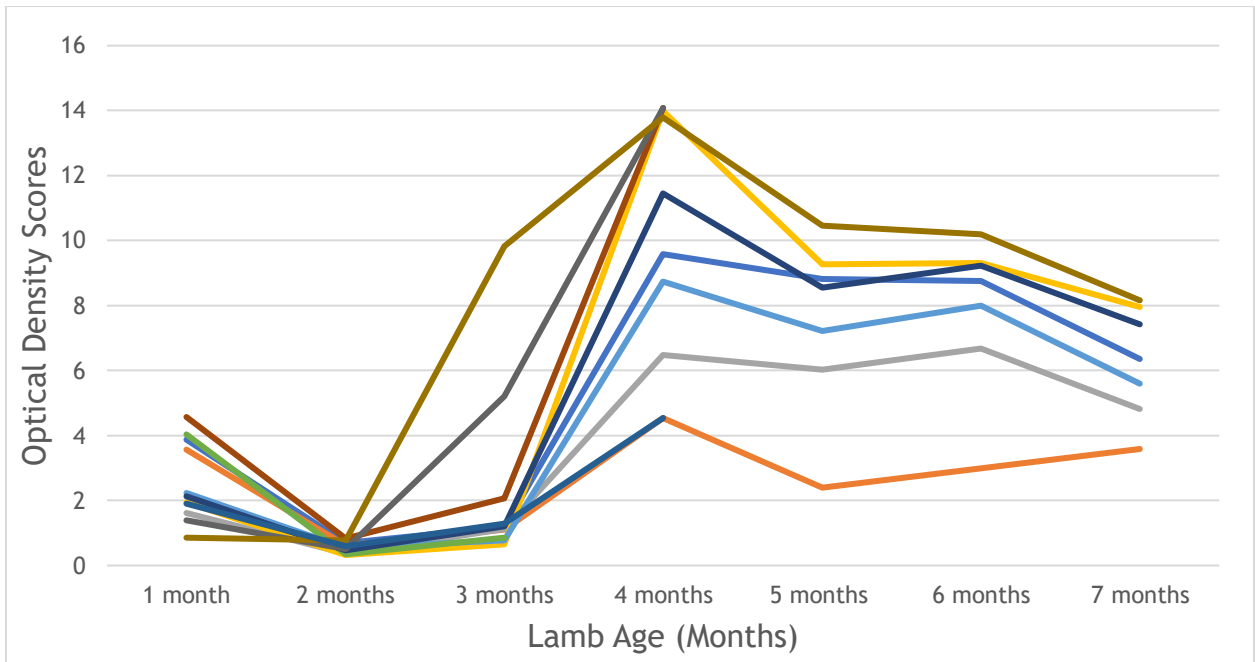


Figure 3-4: Progression of LilliTest Sheep Scab ELISA optical density (OD) scores in newborn lambs in the face of developing *Psoroptes* infestations. Optical density scores are divided by the mean plate negative control to adjust for inter-plate variability. Each colour represents one lamb's OD score change over time.

CHAPTER 4: EVALUATION OF THREE FENCING DESIGNS FOR PREVENTION OF BIGHORN SHEEP (*OVIS CANADENSIS*) MOVEMENT

*The research described in Chapter 4 addresses a gap in the published literature regarding bighorn sheep housing and fencing requirements. The need for this research to aid in preventing the escape of captive bighorn sheep was demonstrated by chapter 2 where the escape of captive bighorn sheep were identified as the likely source of the Canadian *Psoroptes* outbreak. This research provides additional information on potential points of weakness when attempting to prevent movement of bighorn sheep and has implications on the potential usefulness of these tools in facilitating separation of domestic and wild sheep for respiratory disease management.*

Research design, setup, data collection and analysis was performed by Hering. Matthew Jones, a summer student of the British Columbia Ministry of Forests Lands and Natural Resource Operations (FLNRO), assisted with camera maintenance and data collection, and used a subset of this data for his honours undergraduate thesis.

4.1 Introduction

Exposure to novel infectious agents is a key factor affecting the health of wild bighorn sheep (*Ovis canadensis*) in North America and has been associated with numerous population declines and mortality events (Miller et al. 2012). Important infectious diseases in bighorn sheep include respiratory disease, nasal sinus tumours, and psoroptic mange among others (Lange et al. 1980; Besser et al. 2012; Miller et al. 2012; Fox et al. 2016). Fencing is a fundamental tool used by wildlife managers to limit exposure to infectious agents (Wobeser 2002; Vercauteren et al. 2007a). According to the Wild Sheep Working Group (WSWG) of the Western Association of Fish and Wildlife Agencies (WAFWA) “Maintaining effective separation between bighorn sheep and domestic sheep or goats is presently the most effective tool available for minimizing risk of respiratory disease” (Brewer et al. 2014). The outbreak of psoroptic mange in Canadian bighorn sheep may have been prevented by adequate fencing of captive bighorn sheep on the Okanagan Game Farm (Chapter 2) and the spread of *Psoroptes* in Canada is limited at its eastern boundary by the presence of wildlife fencing along a highway (Reid 2013).

Fencing solutions have been studied as a method to achieve physical separation, prevent animal movement into undesired areas, and reduce disease transmission between wild and domestic

species (Vercauteren et al. 2007a; Vercauteren et al. 2007b; Fischer et al. 2011). The fencing requirements of different species vary based on size and athletic ability, but species-specific research is lacking for bighorn sheep. Fencing requirements will also vary based on the pathogen of concern. For example, *Psoroptes* can be transmitted directly between hosts or on other fomites like fence posts used for scratching so prevention of direct contact will likely eliminate most disease transmission (O'Brien 1999). Pathogens that cause respiratory disease on the other hand can be passed between animals through aerosol transmission (Besser et al. 2014). Specific separation requirements to prevent aerosol disease transmission are not possible because of the variability of important factors like environmental conditions, host behaviour, and pathogen dynamics (Morawska 2006); however, it is probable that disease spread is less frequent when direct contact is prevented. Therefore, the use of fencing to prevent nose to nose contact and increase separation distance would be a valuable first step in preventing respiratory disease spread as well (Besser et al. 2014).

Fences can form either psychological barriers, physical barriers, or a combination of both (Walter et al. 2010). Woven wire fencing is often effective in preventing the movement of ungulate species but it is also expensive and perhaps cost prohibitive (Castiov 1999). Electric fencing is a less expensive option but studies on the use of electric fencing for wildlife control show that different species respond differently to the same fencing structures and therefore research on the use of electric fencing must also be species-specific (Karhu and Anderson 2003). In this study the effectiveness of solid wood fencing, woven wire fencing (WWF) and electric fencing (EF) in preventing animal movement across these structures are described for the containment, housing, and separation of wild bighorn sheep.

4.2 Materials and Methods

4.2.1 Study Animals

Eighteen Rocky mountain bighorn sheep ewes and lambs were captured from the Okanagan region of British Columbia by a combination of net gunning and chemical immobilization and were transported to two purpose-built research enclosures within their natural range (Figure 4-1). Animals were placed in the enclosures between February and March of 2017 and were kept (12 in the east pen and 6 in the west pen) for a period of 14 months. The enclosed herd also included 11 lambs that were born in April and May of 2017 (8 in the east pen and 3 in the west pen). The remaining local wild herd consisted of approximately 40 individuals of unknown sex ratio who periodically visited their captive herd-mates.

4.2.2 Study Enclosure

Two adjacent 5-acre enclosures were constructed on hilly terrain near Penticton, British Columbia, Canada for the purpose of a *Psoroptes* drug treatment trial. Pens were located at an elevation of 450m above sea level and contained a 30m elevation change within the pens, with the highest elevation at the top inside corners and the lowest elevation at the bottom inside corners of the two pens (Figure 4-1). The terrain within the pens and fence lines consisted of gradual hills with vegetation characterized by primarily alfalfa grasses (*Medicago sativa*), sagebrush (*Artemisia tridentata*) meadow, and occasional lodgepole pine (*Pinus contorta*).

Three different physical barriers were used in the construction of the pens

- 1) Woven Wire Fence (WWF): The perimeter fences as well as a feeding pen that led into a crowding tub were constructed out of 2.4m tall, fixed knot, woven wire fencing. Because the fencing was donated, two different types of WWF were used. The feeding pens and some of the perimeter fence was constructed with 20-strand WWF (2096-6”) with 7.5cm x 15cm bottom segments that progressively increased in size to 17cm x 15cm top segments (Figure 4-2). The remaining perimeter fence was constructed using 17-strand WWF (1796-6) with 15cm x 15cm bottom segments that first narrowed to 15cm x 10cm segments and then expanded to 17cm x 15cm segments (Figure 4-2)(Tree Island Steel 2019). The entire perimeter fence was outfitted with a single strand of electric fence tape positioned 30cm above ground level and protruding from the outside of the wire fence by a 10cm outrigger to discourage predator entry into the enclosures. Additionally, all of the WWFs were outfitted with 0.9m overhangs protruding inwards at a 45° angle from horizontal from the top of the fence with 3 strands of high tensile wire spaced every 30cm from the top.
- 2) Electric Fence (EF): A secondary 1.4m tall, 4-strand, electric fence was assembled 1 meter outside the primary WWF of one of the two enclosures (referred to as “the east pen”) to create a three dimensional (3D) effect where multiple fences or barriers are used in combination to create an obstacle with both a vertical and a horizontal component (Paige 2012) as depicted in Figure 4-3. The EF consisted of 3 energized strands at heights of 0.35m, 1.15m and 1.4m, charged by a 10,000V solar powered energizer and a grounded strand at 0.7m. The EF was installed 6 months after introduction of the bighorn sheep to the enclosure during which time spatial use preferences of outside free-ranging sheep were opportunistically observed.

- 3) Solid walls: The handling/squeeze chute and crowding tub structures were built of solid 2.4m tall plywood walls with a 10cm slit between two 1.2m sheets of plywood for visualization and handling of animals from outside the tub and chute. The chute was enclosed on top by fencing but the crowding tub initially was not.

The circumference of each pen was about 575 meters. The 4-strand electric fence covered 420m of fence line which included all of the perimeter of one pen but not the area between the two pens (Figure 4-1).

4.2.3 Fencing Efficacy Evaluation

Qualitative and quantitative observations of the fencing successes in preventing animal crossings were made throughout the period of bighorn captivity through direct observation and remote camera recording. Bighorn sheep were moved through the handling system 12 times for examination, treatment, and *Psoroptes* sampling during their time in the enclosures. They were also observed without handling on a daily basis when being fed. Six motion-activated cameras were installed along the perimeter fence lines (Figure 4-1) to allow observation and comparison of free-ranging sheep behaviour outside of the pens in the presence or absence of the electric fence. Specific emphasis was placed on occasions when fence lines were crossed (i.e. “breached”) and the circumstances that enabled that to occur as those occurrences represented total or partial breakdown of these fencing barriers.

Three cameras were placed along the higher elevation fence line of each pen to monitor sites where free ranging sheep had been witnessed to visit the perimeter fence prior to the EF instalment. Cameras were mounted on wooden extensions from the WWF such that their line of sight was parallel to the fence line. Cameras used were a combination of Spypoint Force 10, and Bushnell Trophy Camera Essential E3 cameras set to capture pictures at 30 second intervals when activated by motion (Bushnell 2013; Spypoint 2019). Marking flags were used to demarcate an observation field of 15m along the fence line for each camera to allow comparison of visits between cameras and pens along an equivalent length of fence line. Flags were also used on the pen without the electric fence to indicate where it would have been placed and to show the area within which nose-to-nose contact would be possible.

Depleted camera batteries by windy conditions, dislodged cameras, and camera sensor or night-mode malfunctions resulted in significant data loss at some of the camera sites; therefore, the best functioning cameras were allocated to locations 3 and 5 (Figure 4-1) where signs of sheep

activity were greatest. Cameras were active from October 3, 2017 to April 3, 2018. Incomplete data sets where cameras were only operating correctly for portions of the observation period are highlighted in grey in Table 1-1.

The quantity of time spent by free ranging bighorn sheep in view of the cameras, referred to as “visitations”, are recorded as animal-hours. One animal-hour was defined as one animal being present in the photos of one camera for one hour or less. When multiple animals were present, each animal visible on film consisted of an animal-hour and animals present at one site for more than one hour were counted as multiple animal-hours based on the duration of their stay. When multiple pictures were taken by a single camera within an hour, they were assumed to be photos of the same animal unless distinguishing characteristics such as size, horn morphology, or sex, could provide confident differentiation of individuals so as to apply conservative estimates of visitation. Date, time, and where possible, the sex of the animal in each visit was recorded. Photos captured during incomplete data periods (marked in grey on table 1-1) are included in time of day, time of year, and sex ratio of visiting sheep data but not used in discussion of site selection or proximity to the WWF.

Visitations were divided into animal-hours spent less than 1 meter from the WWF (requiring an electric fence breach event for the east pen), and those spent more than 1 meter from the WWF. Visitations within 1 meter of the WWF represented opportunities for contact transmission rather than aerosol transmission of disease. Time of day, time of year and signalment of those animals considered to constitute the greatest risk of disease transfer are reported as well as implications about strengths and weaknesses of the fencing designs based on observed breach events.

4.3 Results

4.3.1 Animal-Hours Observed by Motion Activated Cameras

A total of 269 animal-hours of visitation were observed by the 5 functioning wildlife cameras over the course of 6 observed months. Preference of sheep for various areas along the observed fence line can be seen in the number of animal hours photographed by each camera during each of the monitoring periods (Table 4-1).

The two camera sites with complete data (3 and 6) show periods when visits were most abundant over the course of the 6-month observation period. More than 50% of total visiting animal-hours were observed by camera 3 despite representing no more than one third of the functioning observation area at any given time, and this site recorded more visitation hours than all other functioning sites combined during all time periods.

When grouped by proximity to the WWF, of the 218 animal-hours observed in the presence of electric fence, free-ranging animals spent 43 animal-hours or 19.7% of their time within the 1m of the wire fence (after breaching the EF). On the other hand when the electric fence was not present, animals spent 33 of the observed 42 animal hours, or 78.6% of their time within 1m of the WWF.

4.3.2 Description of Free Ranging Sheep Visitations

Over 88% (237/269) of camera-observed animal hours of visitation involved rams, in contrast to 5.6% (15/269) involving ewes and 6.3% (17/269) for sheep of undetermined sex, based on the photos captured. Of the 43 occurrences of animals seen between the electric fence and the WWF, all involved rams with the exception of one photo of an animal of undetermined sex. While unique identification of free ranging animals was not always possible, based on horn morphology a minimum of 3 different rams were identified crossing the electric fence. The vast majority of visits occurred during the rut between late October and late December (Figure 4-4) and sheep visited the pens most often during daylight hours (Figure 4-5).

4.3.3 Description of Fence Line Breaching Events

All photographed EF breaches involved animals jumping over the fence in front of camera 3 (marked with a green star on Figure 4-1). These breaches were made possible by the breakdown of 3D fence structure at the WWF corners where sheep were able to jump the electric fence and have room to land without crashing into the WWF (Figure 4-3).

Two other electric fence breaches were observed. The first happened soon after the birth of the lambs when a captive lamb (<1 month old) was separated from her mother during handling and went through one of the bottom 15cm² holes of a WWF. The second event involved a breach of the solid walls of the handling area. A yearling ram was able to jump off of a gate and propel itself over the perpendicular adjoining wood wall of the crowding tub to escape back into the larger enclosure. The handling system was not equipped with the metal overhangs and straight wire that the perimeter fence had. Following this occurrence, the entire crowding tub was fully covered over by fencing preventing this from occurring again. None of the mature bighorn sheep crossed the WWF in either direction at any time.

4.4 Discussion

The inability to distinguish the identity of different free ranging bighorn sheep individuals outside of the enclosures made it impossible to discern whether the same several animals were responsible for most of the visitations and electric fence breaches, or whether the trends observed

truly represent characteristic bighorn sheep behaviour in general. Regardless, the abundance of animal-hours captured by camera 3, despite the presence of electric fence at that site, suggests that the electric fence did not dissuade animals from visiting that particular location of the perimeter fence. One explanation is that the area monitored by camera 3 represented the location around the pens with the most elevated terrain and therefore the best visibility. Bighorn sheep use elevated terrains for escape, thus their preference for this area suggests a prioritization of safety and security over the ability to make nose-to-nose contact with the captive sheep. If this is indeed the case, the high terrain areas of an enclosure deserve particular attention to prevent contact or challenge from wild sheep. Similarly, the captive sheep in the pens were most often seen resting in the nearby upper inside corners of the pens, presumably for the same reason. Another contributing factor to this site selection by sheep inside the pens could be that it was the furthest point in the pens from the handling system where sheep experienced substantial stress and therefore sheep may have intentionally chosen to rest as far from the handling area as possible. Free ranging sheep may have selected the areas of the pens adjacent to camera 3 simply to be closest to where the captive sheep chose to rest.

The observation that the presence of the EF correlated with a smaller percentage of time spent within 1 meter of the WWF when compared to areas without it, suggests that the EF reduced, but did not eliminate the opportunities for nose to nose contact. The sex distribution represented in the animal-hours of visitors heavily favoured rams, but this does not distinguish between independent visitors and the frequency of visits made by each individual. This suggests that rams were most driven to contact sheep on the other side of the fence line or that a few interested rams made up the majority of the visitor pool. Regardless, this observation supports the widely held belief that bighorn sheep rams pose the greatest risk to disease transmission between populations due to intermingling with sheep from different herds and greater drive for contact, especially during the rut.

Electric fence breaches were made possible by two notable design flaws that should be mitigated when constructing electric fencing in future. First, the breach location happened at a site where visiting sheep could approach the fence from an uphill direction. This slope makes the functional height of the fence lower and the fence easier to jump as previously described by Paige (2012). However, the fact that the rams were able to jump back out of the fence in the opposite (uphill) direction indicates that even if the angle of terrain were more level, the height selected for the EF in this study is insufficient on its own in preventing bighorn sheep rams from jumping over it.

Secondly, the horizontal component of the 3D electric fence effect no longer applied in corner regions where the fence turns 90 degrees because visiting bighorns had room to jump the electric fence and land without crashing into the second fence (Figure 4-3). This problem with 3D fencing design might be avoided by placing the shorter fence, in this case the EF, on the inside of the corner so that no location is available to jump the EF with any sizable landing area between the fences.

4.5 Conclusions

Fence line breaches signify critical breakdowns in which the fencing structures were insufficient to prevent movement of animals. In general, 2.4m woven wire fencing with 0.9m 45° overhangs were sufficient for housing of captive bighorn sheep. However, in areas of high stress additional security measures such as avoiding 90° corners in fencing, and covered handling areas are necessary. Woven wire fencing with small bottom holes of the dimension 5cm x 15cm is necessary to contain bighorn sheep lambs and prevent escape of young animals.

The addition of 1.4m high electric fencing may reduce the risk of disease transmission through a WWF when used as part of a 3D fence design however using the design implemented in this study was insufficient to prevent all potential nose-to-nose contact events from occurring. Additional attention should be directed towards increasing electric fence security at corners, in areas of high angle terrain where bighorn sheep spend the largest portion of their time, and in areas preferred by the captive sheep population. Future uses of this fence design be improved by putting the shorter fence on the inside of the taller WWF. These weaknesses are especially important to address during the period around the rut when rams are most driven to make contact with ewes.

4.6 Acknowledgements

The authors would like to express their gratitude to Margo Supplies for donating the electric fencing, to the Wild Sheep Society of BC for donating the wildlife game cameras, and to the Penticton Indian Band and the many community volunteers that cared for the captive bighorn sheep from the date of capture until release.

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Pen	Camera	October 3, 2017 to November 8, 2017	November 10, 2017 to January 22, 2018	January 23, 2018 to April 3, 2018
East Pen	1	NA	NA	NA
	2	18	35	NA
	3	62	89	14
West Pen	4	NA	NA	0
	5	23	14	1
	6	NA	NA	4

Table 4-1: Number of animal-hours observed by each motion activated camera during each time period along the perimeter fence of each pen. NA = incomplete camera data during this period of time. WWF = 2.4m woven wire fence, EF = 1.4m electric fence



1 *Figure 4-1: Visual depiction of the two enclosures, associated terrain, camera locations and the location of the electric fence breaching events.*

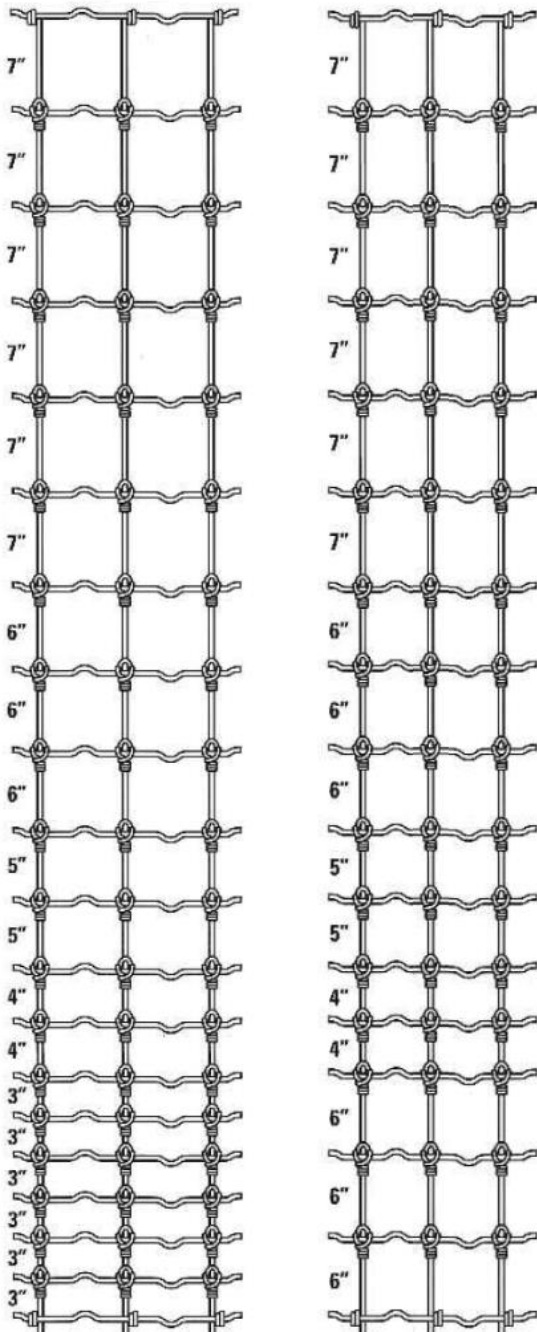


Figure 4-2: Size measurements of the woven wire fencing used in construction of the research pens. left = 20-strand construction, Right = 17-strand construction. Photo credit – modified from: (Tree Island Steel)

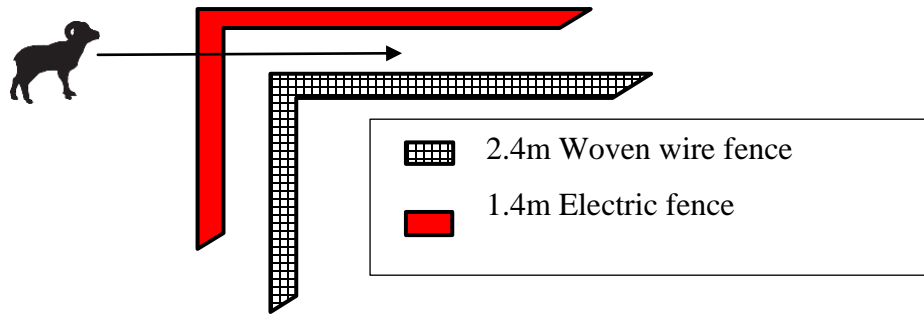


Figure 4-3: Corners create critical breakdowns of the horizontal component of three-dimensional fencing structures.

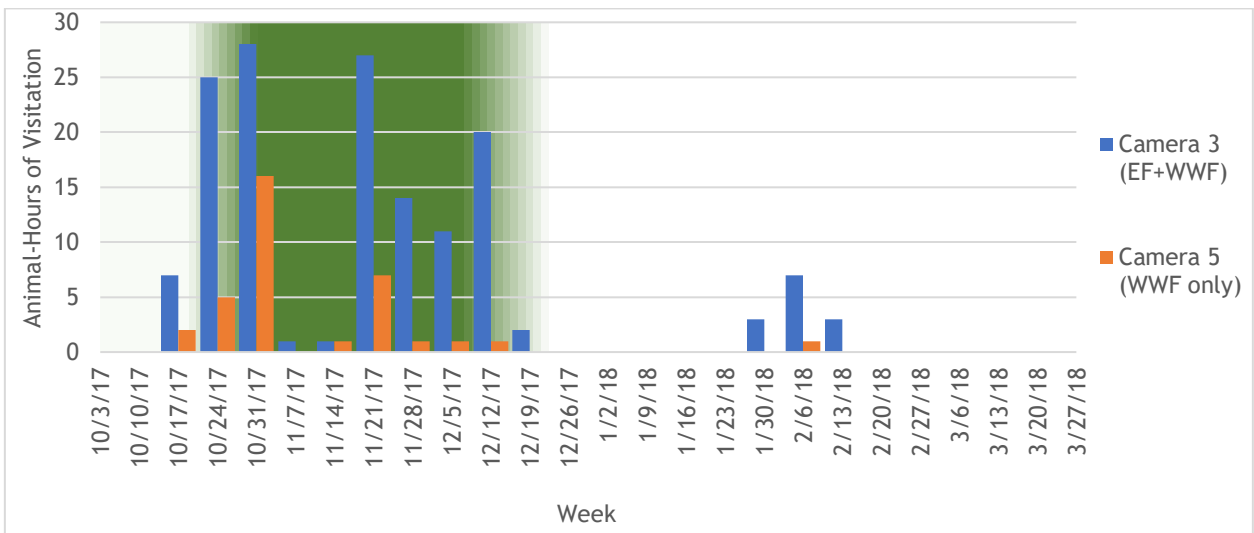


Figure 4-4: Cumulative incidence of free ranging bighorn sheep visitation to captive bighorn sheep pens over six months of observation. Bars represent one week of observation. EF = electric fence, WWF = woven wire fence. Green shading denotes the period of bighorn sheep rut.

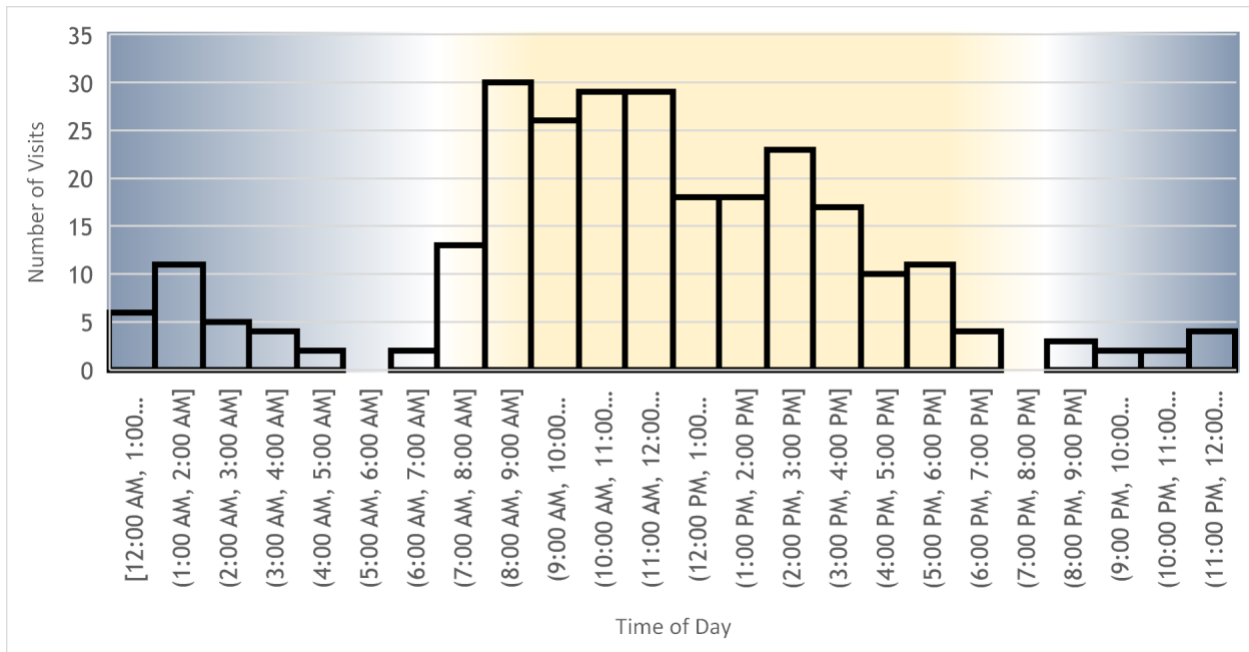


Figure 4-5: Time of day of visits made by free ranging bighorn sheep to captive bighorn sheep pens over six months of observation. Background colours indicate approximate daylight hours (yellow) and nighttime hours (blue)

CHAPTER 5: ASSESSMENT OF NOVEL TREATMENT OPTIONS FOR THE MANAGEMENT OF PSOROPTIC MANGE IN FREE RANGING BIGHORN SHEEP (*OVIS CANADENSIS*)

The research described in Chapter 5 explores new options for the treatment of Psoroptes infestations in bighorn sheep that may facilitate feasible treatment regimens in free-ranging animals. Once disease containment can be achieved by utilizing the findings and tools of chapters 2-4, disease treatment is the necessary next step, however optimal treatment options for free ranging wildlife applications are not yet available. This chapter begins to address this deficit.

The research in this chapter represents the efforts of a partnership between the University of Saskatchewan, the British Columbia Ministry of Forests Lands and Natural Resource Operations and Rural Development (FLNRORD), and the Penticton Indian Band. The concept of this work was suggested by Dr. Helen Schwantje. The suggestion of fluralaner treatment following the failure of eprinomectin came from Dr. Patricia Dowling. Hering coordinated and designed all parts of the study including enclosure design, construction, and animal care practices. Together with research partners and numerous volunteers he performed all field work including sheep capture, handling, treatment, and sample collection. Hering performed all sample processing, microscopy, data analysis and interpretation for this study.

5.1 Introduction

Psoroptic mange is a persistent problem in many wild and domestic animal species around the world. Wild sheep populations in the United States have been afflicted with *Psoroptes* as far back as the 1850s (Lange et al. 1980). Meanwhile, the parasite wasn't identified in Canadian bighorn sheep (*Ovis canadensis canadensis*) populations until 2011 (Scott et al. 2013). Effects of the parasite on bighorn sheep populations range from relatively benign transient infestations (Muschenheim et al. 1990) to severe, population-limiting outbreaks (Lange et al. 1980; Miller et al. 2012). Since its detection in herds of southern British Columbia, severe symptoms, increasing prevalence, and declining populations have been observed, while unaffected nearby populations remain stable (Reid 2013a; Reid 2013b). The severe impacts of disease and currently limited spread in that region makes *Psoroptes* management a time-sensitive concern for local wildlife managers. Parasite eradication requires simultaneous treatment of all individuals in a herd, posing a challenging task for wildlife managers. This further increases the

desire of managers to address the situation before the parasite spreads beyond its current range in Canada.

Management is further complicated by the fact that the parasite has been shown to survive off the host and remain infective in the environment for up to 16 days (O'Brien et al. 1994). Current treatments in domestic sheep provide little residual drug effect and instead rely on repeated application (O'Brien 1999) making them unsuitable for use in free-ranging wild sheep where one missed or reinfested animal results in persistence of the parasite in a herd (O'Brien 1999). Furthermore, widespread reliance on macrocyclic lactones for treatment makes the discovery of *Psoroptes* resistance to this class of drugs a notable concern (Doherty et al. 2018).

All of these factors, in addition to the challenges of wildlife capture and handling, make eradication of this disease in wildlife a complex and expensive proposition. Using the currently available options, treatment of psoroptic mange in bighorn sheep is therefore only appropriate in 3 specific scenarios:

- 1) Small isolated herds, where whole herd treatment may be a possibility,
- 2) Situations where animal welfare or conservation status is impaired to such an extent that treatment is justified even if eradication is unrealistic,
- 3) To ensure freedom from *Psoroptes* infestation among translocated animals.

If a treatment could be administered remotely (Foreyt 1993), had a lasting effect (Bleich et al. 2015), and required only a single usage, its application outside of these situations might be feasible. New treatment options have shown promise in other species and are worthy of exploration in bighorn sheep (D'Alterio et al. 2005; Visser et al. 2013; Prohaczik et al. 2017; Sheinberg et al. 2017). This study evaluated the efficacy of two new drugs, a sustained release eprinomectin, and orally or topically administered fluralaner, in the treatment of psoroptic mange in bighorn sheep.

5.2 Materials and Methods

5.2.1 Study Animals

The study population began with twelve adult ewes, and six young of the year (five ram lambs and one ewe lamb), totaling 18 naturally *Psoroptes* infested Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) from two free-ranging herds in southern British Columbia. They were captured between January and March of 2017 from the *Psoroptes* infested region of

British Columbia, Canada. Sixteen of the individuals originated from the Penticton Indian Band reserve, one from the Macintyre bluff area and one from the Keremeos area (all within 50km of the study enclosure). Every captured animal was heavily symptomatic for *Psoroptes* infestation with severe, hyperkeratotic, exudative lesions in both ears and abundant live *Psoroptes* mites present on microscopic examination of the ear exudates. Eleven additional lambs, seven males and four females, were born in the research pens between April 15 and May 30, 2017 and were included in phases 2 and 3 of the trial.

Captures involved a combination of ground based chemical immobilization, helicopter net gunning and helicopter based chemical immobilization. Chemical immobilization was achieved using an intramuscular injection of 1 ml of a premixed combination of butorphanol (27.3 mg/ml), azaperone (9.1 mg/ml) and medetomidine (10.9 mg/ml) (ZooPharm 2015) delivered by a remote delivery device (dart). When chemical immobilization was used sedation was reversed using 2 ml of atipamezole (25 mg/ml) and 0.5 ml of naltrexone (50 mg/ml) injected intramuscularly prior to transportation. Following capture, animals were administered 5 mg/kg of haloperidol lactate intramuscularly for long term tranquilization and then transported to the research pens in a stock trailer with covered windows (University of Saskatchewan Animal Use Permit 20160068, British Columbia Wildlife Permit PE16-240045).

5.2.2 Sampling and Treatments

The study was conducted in three phases: (1) Eprinomectin treatment trial, (2) Fluralaner pilot study, (3) Fluralaner substantiation (figure 5-1). Animals were handled and sampled monthly throughout the three phases and the following samples and data were collected at each handling:

1. Pictures of each ear
2. Blood (serum extracted and frozen for ELISA testing)
3. Fecal Samples
4. Ear exudate samples from each ear
5. Body weight measurement using a hanging spring scale
6. Subjective body condition score

Phase 1 used an extended-release formulation of eprinomectin, a macrocyclic lactone sold under the trade name Longrangetm. Eprinomectin was administered at a dosage (1 ml/25kg) in a single subcutaneous injection on the side of the neck. Saline treated control animals received the

same handling and sampling and were administered an equivalent volume of saline as a subcutaneous injection in the same site as the treatment group animals. In phase 1, study animals were divided into 3 treatment groups:

- a. Eprinomectin single treatment (EST) (n=6)
- b. Eprinomectin double treatment (EDT) (n=6)
- c. Saline control animals (SC) (n=6)

All animals were treated at the onset of phase 1 and EDT animals were treated a second time with Longrangetm three months after the initial treatment (Figure 5-1). Monthly examination and sampling took place for five months following the first treatment.

Phase 2 took place after the conclusion of phase 1 and investigated the efficacy of fluralaner, an isoxazoline drug sold under the trade name Bravecto™. Both oral and topical applications were tested. The six saline-treated control animals and their three lambs from phase 1 were randomly subdivided into four treatment groups composed of a high dose and a low dose group for each route of drug administration (Figure 5-1).

- a. Fluralaner high-dose oral (FHO) 25 mg/kg (n=3)
- b. Fluralaner low-dose oral (FLO) 5 mg/kg (n=2)
- c. Fluralaner high-dose topical (FHT) 10 mg/kg (n=2)
- d. Fluralaner low-dose topical (FLT) 5 mg/kg (n=2)

Topical treatments used a feline topical liquid formulation of Bravecto™ with the administered volume rounded up to the nearest 28mg (0.1ml) and deposited on the skin of the back of the neck (Merck Animal Health 2016). Oral treatments used canine chewable Bravecto™ tablets rounded up to the nearest 175mg (¼ tab) and were force fed using a balling gun to physically restrained sheep. Monthly examination and sampling took place for 3 months following treatment with fluralaner.

Phase 3 took place after phase 2 and involved the treatment of all bighorn sheep with 25mg/kg of oral fluralaner (FHO-all) prior to release from the pens at the conclusion of the trial. Because five of the animals had been recently cleared of disease using the oral fluralaner treatment during phase 2 (FLO and FHO animals), only those animals never before treated with oral fluralaner (n=24) were included in the analysis of this phase of the trial (Figure 5-1). Examination, sampling, and release from the pens occurred one month after phase 3 was initiated.

5.2.3 Randomization and Allocation of Treatment Group

In phase 1 animals were assigned to treatment groups using an alternating pattern such that every third animal to enter the chute was placed into one of the EST, EDT or the SC group with the first group selected by coin toss. Following the first three animals, this order was reversed to reduce stress by allowing animals to be released into pens in pairs². In phase 2 the six control animals from phase 1 and their 3 lambs were randomly allocated into one of four fluralaner treatment groups using a Microsoft Excel (2017) random number generator.

While blinding of observers to the treatment status was not possible due to separation of treatment groups into different pens, observers were blinded to the treatment group when multiple treatment groups were present in the same pen. This was the case for the comparison of EST and EDT animals as well as for the comparison of all fluralaner treatment groups in phase 2. Microscopic detection of live mites was performed with the microscope user blinded to the sample identity for phases 1 and 2 however phase 3 did not involve a control group so no blinding could be done.

5.2.4 Husbandry

Two purpose-built 5-acre enclosures were constructed on Penticton Indian Band (PIB) land, within the natural range of the PIB bighorn sheep herd. Each pen included a separate handling system and pens were separated by at least 2 metres to prevent unintentional transmission of mites between pens. Following the allocation of animals into treatment groups at the beginning of phase 1, no trial animals were moved between pens.

Sheep had continuous access to alfalfa/grass mix hay, fresh water, salt and mineral blocks, and a wind/sun shelter in each pen. Animals were inspected from a distance and a grain mix formulated for domestic sheep was offered by pen checkers daily to desensitize animals to human activity and as supplemental nutrition. All pen checkers entered the treatment pen prior to the control pen. Monthly handling and sampling was performed on treatment groups first and control groups second. Pen-specific materials including buckets and supply-transport sleds were used for each pen. Personal protective equipment including boot covers and gloves were disposed of between pens and clean blindfolds were used for each animal and machine washed

² Because of a capture related mortality that occurred prior to the beginning of the treatment trial, the 6th control animal was caught on the day of the first eprinomectin and automatically assigned to the control group to fill out the target study population size.

and dried between handlings to reduce likelihood of disease transmission between pens as a result of handling.

5.2.5 Microscopic Parasite Evaluation

Samples were collected from the ear canal or the lower third of the auricle, and from the deepest portion of the hyperkeratosis adjacent to the skin where live mites are most likely to be found. Inspection for live mites was performed using fresh, unprocessed exudate within 2-12 hours of sample collection and examined under a stereoscopic microscope (10-40X magnification). Quantification of mites in samples was highly variable when performed on multiple subsamples from a single animal and therefore only the presence or absence of live mites in a sample was recorded. Samples were inspected until a live mite was found or the entire sample was visualized. Animals were categorized as having live mites found in samples from both ears (2), from just one of their ears (1) or no live mites in the samples collected from either ear (0).

5.2.6 Subjective Clinical Lesion Severity

Ear lesion severity was estimated using a modified scoring rubric from Pan et al. (2006) for *Psoroptes* infestations in rabbits which presents similarly to bighorn sheep (Figure 5-2). This scoring rubric was as follows:

0 - Ears are normal in appearance, ear canals patent, and no active hyperkeratotic lesions are apparent.

1 – Ear canals are patent. Hyperkeratotic lesions are present but confined to the ear canal (visible on otoscopic exam only).

2 - Hyperkeratosis extends outside of the ear canal onto the lower 1/3 of the auricle.

3 –Hyperkeratosis is present on the lower 2/3 of the auricle and the ear canal is occluded.

4 – Hyperkeratosis is present over the entire auricle and the ear canal is occluded.

When chronically damaged ears showed clear evidence of healing and healthy epithelial regeneration, scoring was based on the extent of the distal-most apparently-active hyperkeratotic lesions present on the auricle.

5.2.7 Statistical Analysis

Statistical analysis was considered necessary for phase 1 results because of a lack of clinical relevance given the observed results. Statistically analysis was not performed on the phase 2 results because of the extremely small sample size in this pilot study. Statistical analysis

was performed on phase 3 results through comparison of ear lesion severity scores before and after treatment using Wilcoxon signed rank test performed in R-studio (Version 1.1.423).

5.3 Results

5.3.1 Live Mite Presence

Microscopic evaluation revealed live mites in both ears of all animals at the time of initial treatment (Table 5-1). Live mites were found in one or both ears of all animals throughout the eprinomectin trial. Live mites could also be found in both ears of all animals treated with topical fluralaner at all handling times, but no live mites were found in the ears of any animals treated with oral fluralaner (at either dosage) one month after treatment. One individual in the high-dose group (FHO) was free of live mites in both ears for the entire 3-month follow-up period. Live mites were subjectively more easily located in samples from saline control (SC) animals than eprinomectin and fluralaner treated animals. Saline control animals had live mites throughout the lesions whereas, when found on treated animals, they were often seen only in exudate collected from the lower third of the auricle or ear canal. No live mites were found in the ears of any of the animals following treatment with high-dose oral fluralaner in phase 3 (FHO-All).

5.3.2 Clinical Ear Lesion Scores

Ear lesions were moderate to severe (Grade ≥ 2) in all animals at the time of initial treatment in each of the 3 phases. Clinical signs of eprinomectin-treated animals in phase 1 and topical fluralaner-treated animals in phase 2 often appeared to be improving but the portion of auricle affected generally remained unchanged so these improvements were not reflected in the lesion severity scores (Figure 5-3). Ear canals did not become patent in any eprinomectin or topical fluralaner treated animals at any time following treatment. Oral fluralaner treatment resulted in marked improvement of ear lesions (figure 5-4) and patent ear canals (grade <2) in all animals. Ear lesions improved dramatically and canals remained patent in these animals over the course of the study follow-up period (Figure 5-5). When lesions were present on other parts of the body prior to treatment they appeared to be healing as well but were not specifically graded or quantified.

In phase 3, all ear lesion severity scores decreased over the course of the month. All but one animal started with a score of 3 or 4 in both ears at the time of treatment and all were significantly reduced to 0 or 1 with patent ear canals by the time of release one month later (Wilcoxon signed rank test $p < 0.001$, R-studio Version 1.1.423).

5.3.3 Mortalities

Two animals died during phase 1 of the trial; the ewe that was captured at McIntyre Bluff, and the animal believed to be her lamb. Both ewe and lamb were among the most chronically stressed and most difficult animals to bring into the handling enclosure. They were also regularly assessed to be in poor body condition during handling and sampling prior to their death. The ewe died 2.5 months after initial treatment and the lamb died approximately 3 months later. No evidence was found to suggest that the mortalities were related to drug administration, however liver toxicity panels were not performed due to degradation of the carcass prior to collection and no definitive cause of death was determined in either animal.

5.4 Discussion

It is clear from these trials that the extended-release formulation of eprinomectin and topically applied fluralaner were not effective in treating *Psoroptes* infestations in heavily infested bighorn sheep at the dosages used. However, oral fluralaner was effective in treatment of *Psoroptes* infestations and prevention of re-infestation for at least 4 weeks when administered at a dosage of either 5 mg/kg or 25 mg/kg. While all treatment groups likely benefited from improvements in symptom severity, the persistence of live mites on eprinomectin and topical fluralaner treated animals makes these treatment approaches of little clinical value. It is believed that the two eprinomectin treated animals found to have one ear without live mites one-month after treatment (table 5-1) was the result of poor sample collection technique rather than true freedom from live mites in those ears. It was at that one-month assessment that the necessity of careful sampling involving collection from the deep aspects of the skin lesions adjacent to the live skin was appreciated and incorporated into future sampling procedure. All sampling after that date followed this new, more rigorous procedure.

To the author's knowledge, this is the first report of the use of fluralaner in any ruminant species. Drug safety studies have not been published for fluralaner in ruminant species making it difficult to assess optimal dosages or other potential drug effects. Starting dosages of 5mg/kg were chosen based on discussion with pharmacologists and parasitologists regarding anticipated dosages for effective use of this drug in ruminant species. Mortalities occurred in animals treated with eprinomectin only and both died more than 2 months after treatment, suggesting that cause of mortality was not related to the eprinomectin effects. Neither animal was treated with fluralaner. No localized or systemic effects were observed in any of the treated animals in any

treatment groups from distance exam or at the time of hands-on exam one month following treatment.

The duration of drug activity for fluralaner-treated animals could not be assessed through this study design. At least four weeks of drug persistence following oral treatment is likely based on the lack of live mite observations on FHO and FLO animals one month after treatment, despite cohabitation with persistently infested FHT and FLT animals. The observation of live mites on the FHO and FLO animals two and three months post-treatment was thought to be the result of reinfestation from FHT and FLT animals, however the possibility of low-level undetected continuous infestation of those animals cannot be ruled out. It was also not possible to determine whether the mites found two and three months after oral treatment represented viable infestations or transferred mites from (unsuccessfully) topically-treated animals that would eventually succumb to persistent effects of the oral fluralaner treatment. There may have been increased effect or persistence of activity of fluralaner when used at 25 mg/kg compared to 5 mg/kg, but there was insufficient study power to confirm this suspicion based on the phase 2 sample size.

The reason for the small phase 2 sample size was that this treatment trial was initially developed for the phase 1 trial and only after the eprinomectin trial produced disappointing results was the phase 2 trial developed. To prevent confounding between the residual effects of the extended release eprinomectin and the fluralaner, the pilot study initially only involved the untreated control animals from phase 1. By the conclusion of phase 2, however, the risk of residual effects of eprinomectin was considered to be low, so in an effort to add power to the phase 2 findings, and consider the welfare of the animals prior to release, the most efficacious treatment (FHO) was applied to all animals.

It would have been valuable to continue the phase 3 portion of the treatment trial for longer in order to ensure that infestations did not return two or three months after treatment since no new exogenous source of infestation would have been present. The extension of phase 3 was not pursued because it was important to respect the wishes of the many stakeholders that contributed to and supported this research project and follow-through on the promise that these wild sheep would only remain in captivity for one year. These local stakeholders and research partners are excited for future research possibilities to answer follow-up questions on the use of fluralaner in bighorn sheep presented in this thesis.

The separation of treatment and control animals made blinding of observers to treatment status impossible during the phase 1 trial. Because ear lesion severity scoring was, by necessity, performed animal-side, the potential for observer bias could not be eliminated; however, this bias would be expected to exaggerate treatment effect. Given the lack of treatment success in eprinomectin treated animals, the potential for observer bias may account for some or all of the reported improvement of clinical signs, but likely did not change the outcome given that negative trial results were ultimately reported in Phase 1. Observer bias was eliminated during live mite evaluation for phases 1 and 2 by blinding the microscope user to the treatment used on the sample being evaluated. During phase 2, observers were also blinded as to treatment group for fluralaner treated animals because topical and oral fluralaner treated animals were co-housed, and these treatments resulted in profoundly different results. The difference in clinical lesion score improvement reported between phase 2 FHO animals and phase 3 animals who received the same treatment (Figure 5-3) may have been the result of the elimination of co-housing with other persistently infested animals in phase 3, the result of observer bias, or of some combination of both. Clinical lesion scores were susceptible to some subjectivity because ears that were heavily impacted by *Psoroptes* could not fully heal within just 1 month, regardless of treatment. Observers attempted to grade lesions based on where the distal-most active portion of the lesion appeared to be on the ear so that a grade of 0 or 1 could be awarded to an ear with a patent ear canal and healing wounds despite the ongoing presence of skin crusts still adherent to distal aspects of the pinna (Figure 5.4).

The efficacy of oral fluralaner following a single use for the treatment of *Psoroptes* provides an easily accessible promising new option for the potential treatment and management of this parasite in wild sheep. Remote applications of this drug using medicated feeds or salt licks may enable low-cost effective treatment in remote areas with severely affected herds however assuring consistent dosages and herd-wide treatment may present a challenge. Further studies are necessary to better assess the duration of drug effect through experimental re-infection trials. Pharmacokinetics and safety studies are advised to evaluate drug safety and withdrawal times in this hunted species, and the palatability of free-choice oral formulation could be assessed to explore hands-off application options. Investigation of the environmental impact of fluralaner must also be considered prior to field administration (Wall 2007).

5.5 Conclusion

Extended release injectable eprinomectin (Longrangetm) was not effective in eradicating *Psoroptes* infestations in bighorn sheep following single or multiple administrations when used at a dosage of 2mg/kg. Topical fluralaner (Bravecto™) was similarly ineffective in treatment of *Psoroptes* following a single administration at 5 or 10 mg/kg but was effective for a period of at least four weeks when administered orally at either 5mg/kg or 25mg/kg. It is possible that 25mg/kg of fluralaner, administered orally to bighorn sheep, may result in protection from reinfection for 2-3 months following treatment; however, additional replication and modification of this study design to prevent continuous exposure over this period of time is needed to confirm this observation.

5.6 Acknowledgements

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Time (months)	0			1			2			3			4			5		
# of animals with live mites in 0, 1, or both ears.	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2
EST (n=6)			6		1	5			5 ^a			5 ^b			5 ^b			5 ^b
EDT (n=6)			6		1	5			6			6			5 ^a			6
FLO (n=3)			3	3					3		1	2						
FHO (n=2)			2	2			1	1		1		1						
FLT (n=2)			2			2			2			2						
FHT (n=2)			2			2			2			2						
FHO-All (n=24)			24	24														
SC (n=6)			6			6			6			5 ^a			6			5 ^a

Table 5-1: Number of bighorn sheep in each treatment group with live mites found in their otic exudate.

Columns indicate handling month (0 through 5) subdivided by the number of animals per treatment group with live mites found in 0, 1 or both (2) ears.

Rows indicate treatment group: EST = Eprinomectin single treatment, EDT = Eprinomectin Double treatment, FLO = Fluralaner low oral (5mg/kg), FHO= Flurlaner high oral (25mg/kg), FLT = Fluralaner low topical (5mg/kg), FHT = Fluralaner high topical (10mg/kg) FHO-All = Fluralaner high dose oral (25mg/kg) phase 3 , SC = Saline control.

^a Sample collection failures in one or both ears resulted in loss of data points

^b Mortality of one study animal resulted in loss of data for the remainder of study

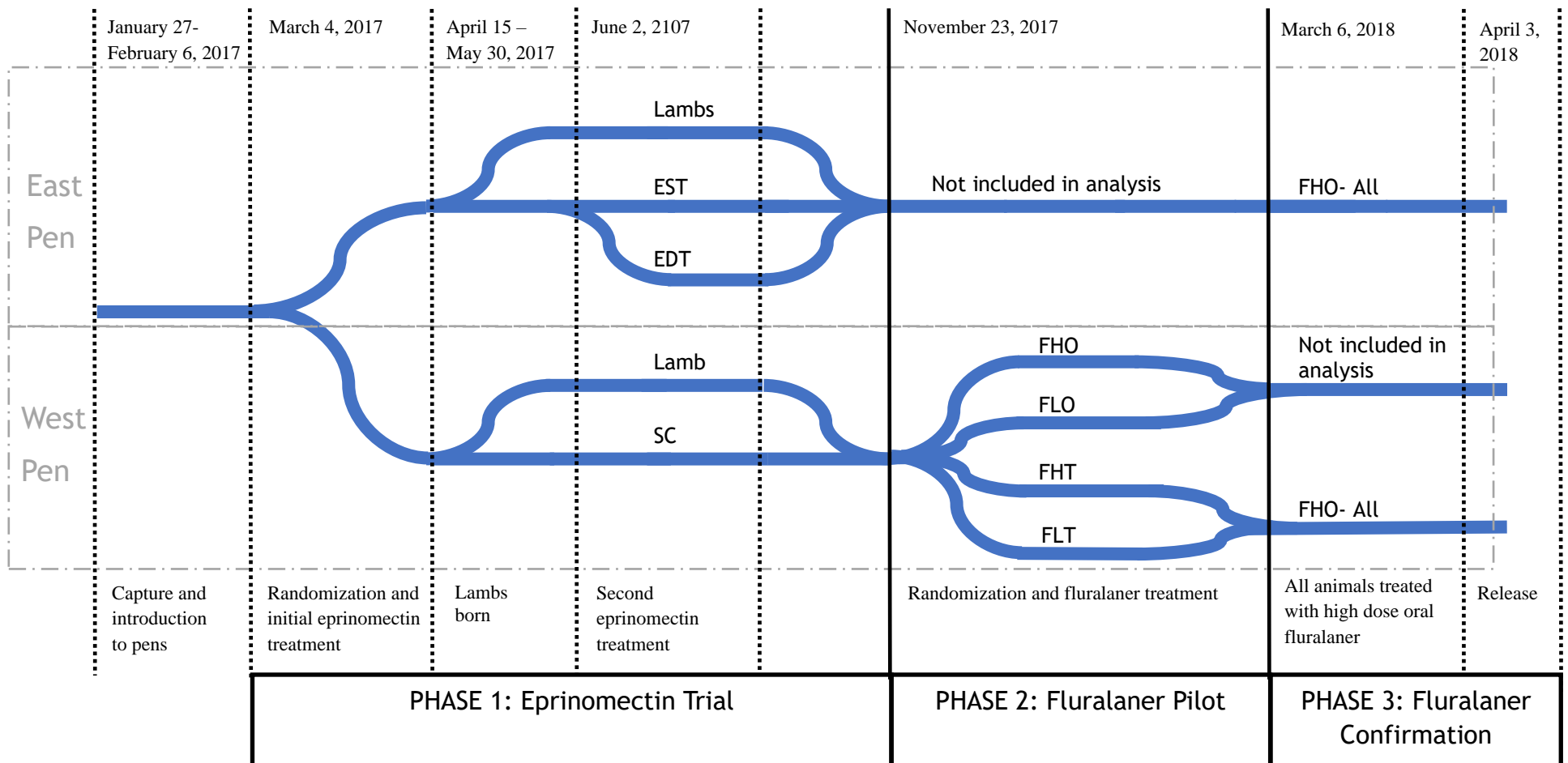


Figure 5-1: Bighorn sheep *Psoroptes* treatment trial study timeline depicting treatment timing and location/co-housing of study animals. EST = Eprinomectin single treatment, EDT = Eprinomectin double treatment, SC = saline control, FHO = Fluralaner high-dose oral, FLO = fluralaner low-dose oral, FHT = Fluralaner high-dose topical, FLT = Fluralaner low-dose topical

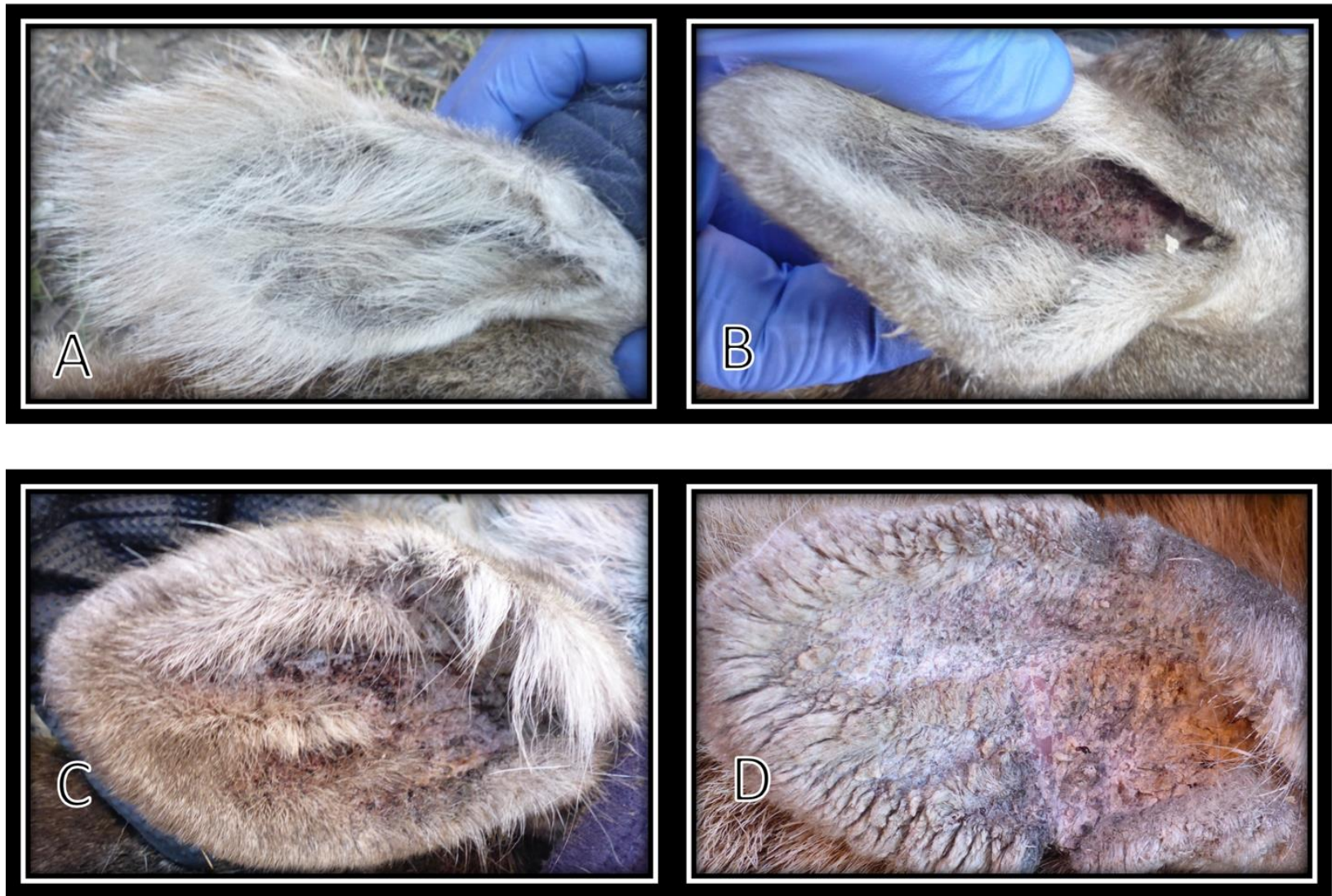


Figure 5-2: Examples of ear lesion severity scores. (A) Grade 0 (non-infested) or 1 (lesions restricted to ear canal) depending upon otoscopic findings, (B) Grade 2- lower 1/3rd of auricle affected, (C) Grade 3- lower 2/3rd of auricle affected, (D) Grade 4- entire auricle affected

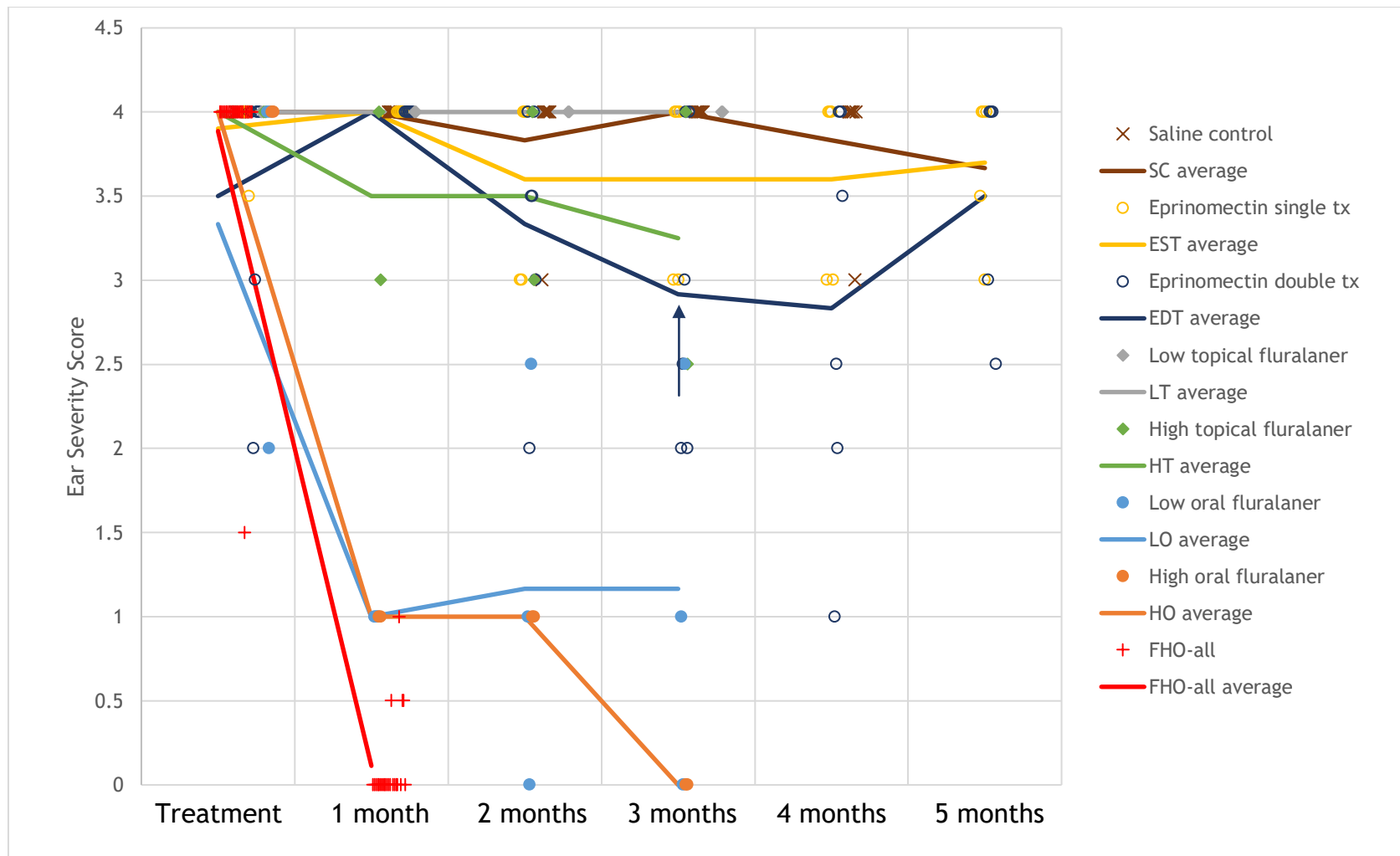
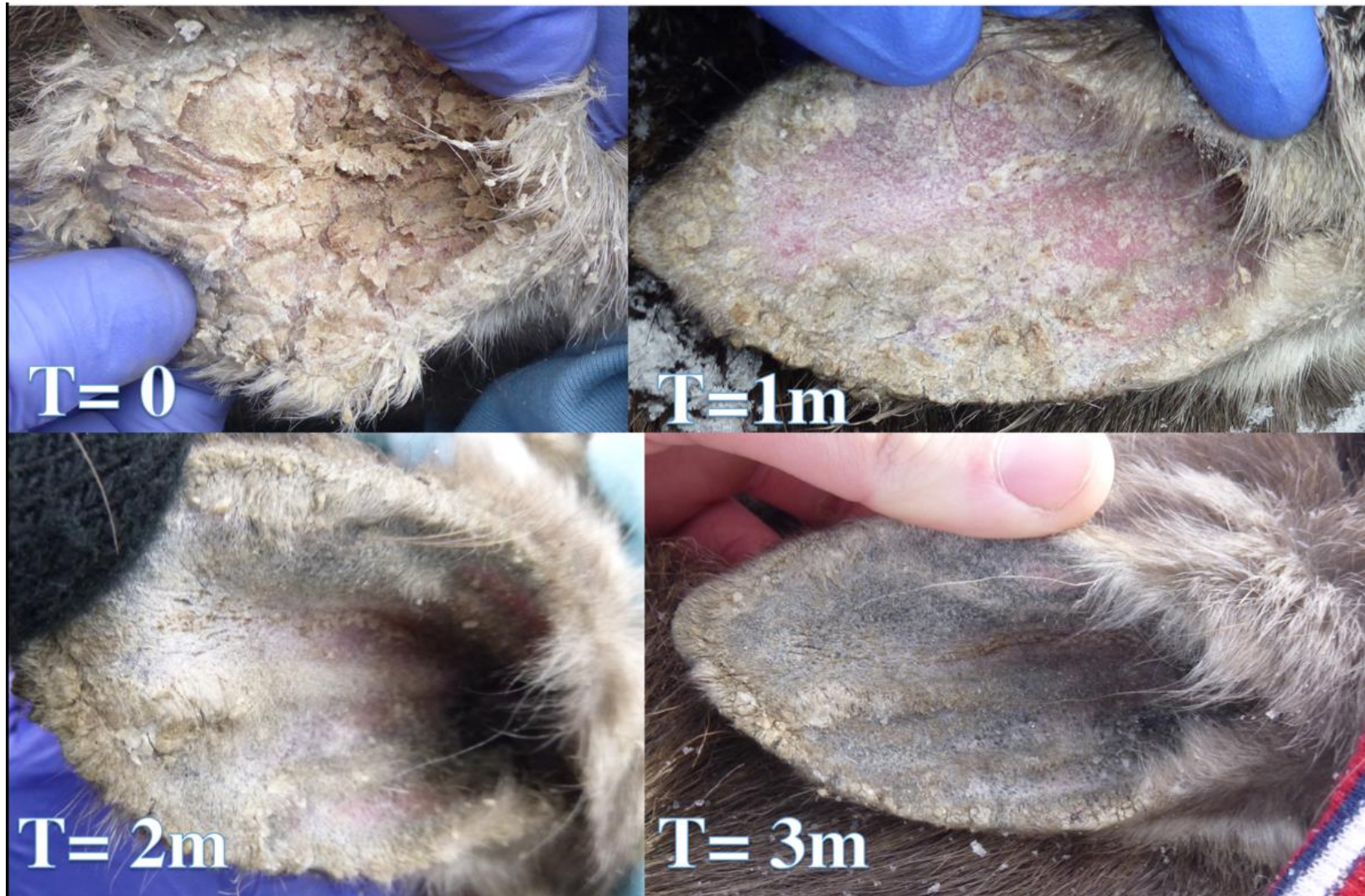


Figure 5-3: Individual and average ear lesion severity scores of bighorn sheep following different treatments. Treatment groups include: Injectable extended-release eprinomectin delivered once (EST) or twice (EDT), saline control (SC), topical fluralaner administered at 5mg/kg (LT) and 10mg/kg (HT), oral fluralaner administered at 5mg/kg (LO) and 25mg/kg (HO). Results of HO treatment of animals previously treated with eprinomectin or topical fluralaner are marked in red (FHO-all). The arrow indicates the date of second eprinomectin treatment for the EDT group.



Figure 5-4: Characteristic resolution of clinical signs following treatment with high dose (25mg/kg) oral fluralaner (FHO) (A1) The right ear of animal #19 at the time of treatment (A2) the same ear one month after treatment.



*Figure 5-5: Improvement of the ear lesion of one characteristic *Psoroptes*-infested bighorn sheep over the course of three months following a single treatment with oral fluralaner at a dosage of 25 mg/kg (FHO).*

T0=treatment day, T1= 1 month after treatment, T2= 2 months after treatment, T3= 3 months after treatment.

CHAPTER 6: THE USE OF ADAPTIVE MANAGEMENT IN WILD SHEEP RESPIRATORY DISEASE AND PSOROPTIC MANGE MANAGEMENT IN NORTH AMERICA

The research described in Chapter 6 completes the management process by addressing the challenge posed in converting theoretical knowledge into management action and proceeding with management despite uncertainty. It aims to expedite the efficiency of this process through identification of challenges and mistakes in a comparable wildlife disease management scenario, respiratory disease, and then adapt those lessons learned to the management of Psoroptes in bighorn sheep. Findings of this chapter are important considerations to be accounted for in the development of a Psoroptes management plan in Canadian bighorn sheep.

Hering completed all interviews, interpretation, thematic analysis and data synthesis for this manuscript. Suggestions for interview subjects were provided by Drs. Mike Cox and Helen Schwantje of the Adaptive Wild Sheep Disease Management Venture. Guidance in performance of thematic analysis was provided by Dr. Craig Stephen.

6.1 Disclaimer

Interview subjects who participated in this study work in a variety of regions and settings. The information discussed here is a reflection of their experiences, perspectives, and opinions as well as those of the author but are not necessarily representative of the official perspective or opinion of the agency for which they work. Further, these interview findings should not be considered a comprehensive summary of all of the actions or approaches being pursued in each of the jurisdictions represented in this study.

6.2 Introduction

Practitioners of wildlife management are regularly faced with the challenge of applying meaningful management action in the face of ongoing uncertainty (Possingham 2000; Armitage et al. 2008; Williams and Brown 2016). The application of wildlife disease knowledge in the form of management action is a complex process that necessarily involves an understanding of a wide variety of biological and social considerations. Biological systems include agent, host and ecosystem-level factors, while social considerations, commonly referred to as the “human dimensions of wildlife management” include stakeholder desires, economic limitations and political dynamics (Wobeser 2002; J. Riley et al. 2003). This complicated and sometimes delicate

reality contributes to a phenomenon called “analysis paralysis”; a desire to constantly learn more before taking action (Urquhart 2012). The need to refine and optimize the process of knowledge application in the form of wildlife disease management interventions is therefore apparent.

Adaptive management (AM) is described as a method for addressing complex problems laden with a high degree of uncertainty concerning which management actions are best suited to achieve desired outcomes (Williams et al. 2009). The concept of AM has been the topic of much academic consideration, in part because of how appealing it is in theory and simultaneously how challenging it is to execute (Walters 2007; Allen and Gunderson 2011). The theory of AM describes the process as “learning by doing” (Armitage et al. 2008; Cundill and Fabricius 2009; Fontaine 2011). Putting theory into practice has been thoroughly discussed in the literature, with a wide variety of AM examples (National Research Council 2004). On one end of the spectrum is “passive” AM where managers unceremoniously choose what they believe to be the best course of action, monitor the outcomes of their actions and then adapt their management actions to the situation at hand (National Research Council 2004). Others describe this “trial and error process” as “maladaptive management”, instead, suggesting one of several far more prescriptive processes (Williams et al. 2009; McFadden et al. 2011; Conroy and Peterson 2013; Chadès et al. 2017). What remains consistent between approaches is the recommendation to apply scientific methods, addressing uncertainty, to select management strategies that are most effective. To do so there is a clear need to: 1) define the goal of management, 2) implement multiple strategies to achieve that goal (based on particular hypotheses), 3) monitor and compare the results of the selected interventions and 4) make changes or “adapt” the management strategy(ies) accordingly.

Despite awareness of *Psoroptes* presence in wild sheep populations of North America for over a century, it has received relatively little attention from wildlife managers for several reasons, one being a lack of feasible practical management options (Scott et al. 2013). The one exception where *Psoroptes* was actively eradicated from a wild sheep population occurred in the San Andres Mountains of New Mexico once the population had declined to only one ewe that was caught, brought into captivity, treated with ivermectin, and then re-released with other animals to rebuild the population (Boyce and Weisenberger 2005). Ideally management interventions should take place before the population dwindles to just one animal. Attention to other wild sheep disease management challenges might provide valuable insight into how to develop effective management plans for *Psoroptes*. Psoroptic mange has also received less

attention because most managers agree that there are other significant threats to bighorn sheep conservation more deserving of their time and attention, specifically respiratory disease (Brewer et al. 2014; Hurley et al. 2015).

An abundance of effort has been directed towards better understanding the risk factors, disease dynamics, and population impacts of epizootic polymicrobial respiratory disease complex in bighorn sheep (Cassirer and Sinclair 2007; Besser, Highland, et al. 2012; Besser et al. 2013; Plowright et al. 2013; Besser et al. 2014; Sells et al. 2015; Sells et al. 2016; Borg et al. 2017) and yet the application of knowledge into management action presents its own challenges. In dissecting the struggle to implement management action for respiratory disease in bighorn sheep, conclusions can be drawn about common challenges and opportunities in wild sheep management. Considering the Wild Sheep Working Group of the Western Association of Fish and Wildlife Agencies' (WAFWA) Adaptive Wild Sheep Disease Management Venture (DMV) as a case study is a useful exercise to highlight some of the practical barriers to the implementation of AM to wild sheep health challenges.

The DMV's mission is to act as a source of expertise and guidance for jurisdictional wildlife management agencies and to accelerate collective learning by facilitating the implementation and evaluation of the AM of respiratory disease in wild sheep (Western Association of Fish & Wildlife Agencies 2017). Since the DMV was established in 2015, several examples of management interventions that could be described as adaptive management of respiratory disease can be found (Bernatowicz et al. 2017; Garwood 2018), however applications have only been attempted in select limited situations (Montana Department of Fish Wildlife and Parks 2010; Bernatowicz et al. 2017; Garwood 2018). This chapter aims to review the current state of respiratory disease management in bighorn sheep across North America and seeks to understand the factors preventing the translation of acquired knowledge into respiratory disease management action by conducting semi-structured interviews with wildlife health professionals. Lessons learned through this process are discussed in the context of improving wildlife management for respiratory disease as well as their potential application to development of *Psoroptes* management plans in bighorn sheep.

6.3 Materials and Methods

6.3.1 Respiratory Disease and the DMV

Respiratory disease has caused at least 175 major die-off events, involving almost all jurisdictions in North America containing bighorn sheep populations, resulting in significant population declines throughout bighorn range over the past 50 years (WAFWA - Wild Sheep Working Group 2017; Cassirer et al. 2018). Respiratory disease mortality events are the result of polymicrobial infections but new research within the last decade confirms that *Mycoplasma ovipneumoniae* is the primary causative agent, resulting in mortality rates of 10-90% in infected populations (Besser et al. 2014; Butler et al. 2017). There is agreement that a major source of respiratory disease-causing pathogens in wild sheep is contact with domestic sheep which are asymptomatic carriers of *M. ovipneumoniae*. Therefore, minimizing contact between wild and domestic sheep is a necessary component of wild sheep respiratory disease management (Besser, Highland, et al. 2012; Sells et al. 2015; Cassirer et al. 2018).

The key question defined by the DMV at its inception was: “What contributes to this variation in herd response to respiratory disease and how can management actions improve herd performance?” (WAFWA - Wild Sheep Working Group 2017). In 2017 the DMV created guidelines for improved herd monitoring, as well as proposed AM actions for jurisdictions to implement (WAFWA - Wild Sheep Working Group 2017). Six AM strategies were suggested, three of which were described in detail with recommended actions and criteria for evaluation of success, while the remaining three were listed without additional guidance. Briefly, those described in detail were: 1) selective test and cull to eliminate *M. ovipneumoniae* shedders or other pathogens of interest from a herd, 2) depopulation of a respiratory disease affected herd and repopulation with unaffected animals, and 3) translocation of animals based on matching pathogen profiles between source and recipient herds. The remaining three included in the DMV strategy without additional direction for managers were: 4) “Nonselective culls – ewe hunts”, 5) “Breakup herds- actions to disperse/distribute/hazing” and 6) “Fertility control to reduce herd densities” (WAFWA - Wild Sheep Working Group 2017). Specific guidance in the selection of a particular AM school of thought was not provided in the DMV strategy, leaving managers to select their own approach to implementing AM interventions.

6.3.2 Study Population

Thirteen wildlife professionals with personal experience working on respiratory disease management in bighorn sheep were selected from seven different states and provinces. Interview

participants were non-randomly chosen using suggestions from the chair of the DMV, and the recommendations made by the interviewees. Care was taken to include a wide variety of roles, perspectives, and related experience from these different regions, and included both DMV committee members and non-members. Interview subjects worked in and/or represented British Columbia, Washington, Idaho, Montana, South Dakota, Oregon, and Utah. Seven interview subjects were government biologists, managers, or supervisors, three were government-employed wildlife veterinarians, and three were researchers or biologists who work closely with government officials but have consulting roles and are not directly tasked with management decision making. Enrollment of interview participants continued until information saturation was achieved (no additional unique viewpoints were provided).

6.3.3 Study Design

Semi-structured interviews were conducted by phone at pre-arranged times between August and October 2018 with one or two wildlife managers at a time. Each lasted between 45-90 minutes in a single session. The goal of each interview was to learn about the current state of respiratory disease management in their jurisdiction, the interventions being performed, and the perceived barriers preventing action. Interviewees were promised anonymization of results to ensure honesty when expressing their concerns or disagreements with the DMV approach or other potentially controversial topics around respiratory disease management. Subjects were encouraged to discuss both the aspects of respiratory disease management that they felt were currently going well and key challenges being faced in their respective regions. Semi-structured interview questions were used with additional prompts when necessary to help clarify unclear key points, specific context, and important details as needed. The core questions were provided to interviewees ahead of time and were kept intentionally general to limit the biasing of answers by the interviewer.

Semi-structured interview questions were as follows:

1. Tell me about your role with regard to wildlife management in your area.
2. Does your region state specific goals regarding the management of respiratory disease in your bighorn sheep?
3. What are some of the main actions you're implementing or trying to implement to manage respiratory disease in your region?
4. What are the main challenges you face in selecting or implementing these actions?

5. What are some things your region does well?
6. Is there anything else you would like to add or anyone else you would suggest that I talk with?

Where available, bighorn sheep management plans for each region as well as other supplemental texts were examined ahead of time by the interviewer and were discussed during the interviews to add additional context and information about the unique and common challenges faced in each region.

6.3.4 Interview Evaluation

All interviews were audio-recorded for future review and a thematic analysis was performed on the recordings to ascertain key commonalities and points of disagreement between interview subjects. Shared management goals, actions, and barriers preventing action were grouped into unifying themes. Management actions discussed by interview subjects were divided into the four basic wildlife disease management categories: prevention, control, eradication, and doing nothing, as defined by Wobeser (2002). In this case, control is distinguished from eradication in that control refers to actions aimed at reducing the frequency of disease or its effects in a population to an acceptable level without aiming to remove the disease or pathogen from the population entirely. The expectation with control actions is therefore that the disease will persist in the population at some level and therefore control actions are generally intended to be carried out in perpetuity (Wobeser 2002). Meanwhile, eradication measures aim to eliminate the pathogen from the population entirely. Where desired management actions were not being actively implemented, and a do-nothing approach was the de-facto strategy employed, the key barriers preventing action presented by the interview subjects were discussed and grouped into unifying themes.

The management actions carried out by agencies and how they were implemented were reviewed and evaluated for adherence to an AM approach. There is no widely accepted definition of what constitutes AM; however, it is generally understood to refer to situations in which management actions themselves are viewed as experiments to address uncertainty around how best to accomplish some management objective (Walters 1986). Since the DMV did not provide additional guidance on what they meant by AM and managers were free to employ their own

preference of AM styles, a broadminded perspective on what constitutes AM was taken when evaluating management actions.

Interview subjects were questioned about their management goals, challenges they faced, actions they carried out, how outcomes were monitored, how success was evaluated, how outcomes were compared, and how the integration of acquired knowledge was achieved. The management processes described by interviewees, and any supplemental documents on their interventions, were evaluated for whether they were implemented in a way that would allow experimentation and comparison of multiple strategies, and therefore could be considered AM. For added clarity, a set of 9 key adaptive management principles for success identified by McFadden et al (2011) through a meta-analysis of different approaches to AM was considered. The 9 key principles are 1) stakeholder involvement, 2) defined objectives, 3) multiple actions, 4) prediction of consequences, 5) specification of constraints, 6) acknowledgement of uncertainty, 7) explicit experimentation, 8) monitoring, and 9) emphasis on active learning. While these do not define what qualifies as AM, they provide insight into how to perform AM effectively or where to focus efforts when it is not being implemented.

Interviews were used to assess whether the jurisdictions were using AM and to assess how the DMV could best direct future efforts to improve respiratory disease management in wild sheep. The thematic analysis of the interview information was used to assess how the DMV and an AM approach could help address the barriers identified.

6.4 Results

6.4.1 Overview

All four wildlife disease management strategies (prevention, control, eradication, and do-nothing) (Wobeser 2002) were represented in the challenges, goals, and actions described by the interview subjects. Some aspects, namely prevention and eradication, received considerably more time and effort from most managers than others. Where no action was being taken (Do-nothing), despite a desire by managers to do something, four unifying themes of barriers to action emerged (Figure 6-1).

6.4.2 Prevention Actions

The largest portions of managers' time and effort have been directed towards the prevention of disease, and all jurisdictions included in this study were working towards preventing disease entry into unaffected herds in multiple ways. Three primary routes of disease

exposure were identified by interview subjects for which action is being taken: 1) exposure from contact with domestic sheep and goats, 2) exposure from human-assisted translocation of bighorn sheep, and 3) exposure from the natural movement of bighorns between herds of differing pathogen profiles.

To prevent introduction of disease, a key foundation identified by all interview subjects was knowledge of the current pathogen distribution. All regions invest substantial amounts of time and resources in monitoring the infection statuses of the wild sheep populations under their care. Meaningful comparison of disease profiles between jurisdictions is made possible by the development of standardized disease testing recommendations. Recommendations for respiratory disease testing were published by the Wildlife Health Committee of the WAFWA in 2015 (Wildlife Health Committee, 2015) and enhanced monitoring guidelines were published by the DMV in 2017. Higher priority herds such as those that provide better wildlife viewing or hunting opportunities are subject to more frequent retesting, but most regions reported to have baseline information on the majority of herds in their territory even if some baseline information was several years old. When asked what aspect of respiratory disease management they did well, at least one representative of each of the 7 jurisdictions considered baseline surveillance to be among their strengths.

After establishing an understanding of the location and disease status of herds, managers directed significant effort to identifying and addressing sources of disease exposure in at-risk populations. The first potential route of disease entry was through contact with domestic sheep and/or goats. All interview subjects agreed that the science was clear that domestic sheep and goats represent a significant threat to wild sheep and that isolation from these domestic species must be a priority. All regions have put significant effort into identifying domestic sheep and goat producers and landowners with these species on their properties or making use of public lands close to bighorn sheep habitat. Locating new owners of domestic sheep and goats was a common challenge identified by managers from four of the seven regions. Several strategies were presented for locating and identifying properties that represent risk of disease introduction. One region has an independent program aimed specifically at locating and addressing the risk presented by domestic sheep and goats and in all other cases this work is done primarily by government-employed wildlife managers.

Several interview subjects also discussed having an interest in testing domestic sheep for *M. ovipneumoniae* in the hopes that this might help identify the degree to which those tested domestic herds represent a disease spillover risk to wild sheep. Research is underway to investigate options for treatment or eradication of *M. ovipneumoniae* from domestic species (Besser et al. 2018) however, none of the interviewees discussed this strategy being applied as a management action at the time when interviews were performed. The barriers preventing exploration of this approach are discussed later in the “do nothing” section.

The second potential route of pathogen entry identified by wild sheep managers was through bighorn sheep translocations. Translocation involves the intentional movement of wildlife from one or more “source” herd(s) to another “recipient” herd or region. Translocations are used to control source herd size or density, or to establish, reintroduce, or expand/augment recipient herds. Interviewees from all included jurisdictions reported having carried out translocations in their region. All describe performing pathogen testing on both the source and recipient herds before translocations as per DMV recommendations, however all expressed hesitation around the DMV’s suggested AM approach of performing translocations between herds based on matching of *M. ovipneumoniae* strain types. There was a general reluctance to translocate *M. ovipneumoniae* positive animals unless absolutely necessary, and when done, managers expressed a strong preference for doing it between herds with a historical connection through previous translocations in addition to matching of pathogen profiles. The main reason presented for this hesitation is a lack of confidence in the current ability to identify all relevant pathogens or strain types in donor and recipient populations and a reluctance to do anything that potentially puts bighorn sheep at risk. None of the interviewees described any plans for direct comparison of their translocation outcomes, regardless of pathogen profiles. However, all regions are performing these translocations after thorough testing of the source and recipient herd pathogen profiles, and all are carrying out post-translocation monitoring that should detect respiratory disease outbreaks if they occur.

The third potential route of disease introduction presented by the interviewees is through the natural movement of bighorn sheep between herds of differing pathogen profiles thereby exposing new herds to pathogens not previously familiar to them. Six of the 13 interview subjects, each from a different region, discussed a lack of ability to prevent bighorn movement

between herds that are part of a larger metapopulation as a key challenge in preventing disease introduction into naïve herds.

"We can do [a test and cull] with a sheep herd like the [location removed] that's isolated in a relatively small piece of sheep habitat, but things are going to get much more challenging if we're talking about the [location removed] for example, where you have 1000 sheep scattered across 150 or 200km of river." – Wildlife Biologist/manager

Few options have been proposed to prevent the movement of wild sheep between herds; however, population reduction was identified by interviewees as a potential method to address this challenge. This theory is based on evidence that respiratory disease outbreaks often occur when herds are close to their maximum population size (Monello et al. 2001). It is believed that the mechanism for these disease outbreaks was through increased likelihood of contact between *M. ovipneumoniae* free bighorns and nearby domestic sheep, goats, or infected bighorn herds. When population sizes were large and herd densities were high, animals would have been more likely to venture further away from core habitats into surrounding areas (Sells et al. 2015), thereby increasing the risk of exposure to *M. ovipneumoniae* carriers. In light of this, some managers and management plans described specific population targets for herds based on an estimated minimum-viable population and a population size that could be sustainably hunted. Interview subjects described carrying out translocations rather than ewe hunting for population reduction in 3 of the regions interviewed. When action was targeted at population size or density reduction in hopes of impacting the likelihood of disease exposure, none of the regions described any way of evaluating whether this strategy was successful in changing animal movement patterns in the source herd.

6.4.3 Control versus Eradication Actions

Effective control actions aimed at reducing the impact of disease in affected herds have not yet been identified. While research is underway to find actions that may be taken to either reduce the impact of disease on affected animals or the degree of spread within affected herds, no

demonstrably effective control strategies were discussed by interview subjects or in wildlife management plans.

Because of the lack of successful options for disease control once disease is present within a population, disease eradication is the goal of much of the management effort as well as a main focus of the DMV initiative. The two main eradication interventions proposed by the DMV are 1) test and cull, and 2) depopulate and repopulate. At the time of interviewing, test and cull efforts focusing on *M. ovipneumoniae* were carried out in two of the seven regions with a third effort in the planning stages. Depopulation and repopulation efforts were also described in two of the regions, one of which had reintroduced animals back into that landscape while the other was still confirming the success of the depopulation attempt before reintroducing disease-free sheep.

All managers agreed that depopulation and repopulation can be an effective strategy for eradicating respiratory disease; however, most managers expressed reluctance to consider this option because of the many social, political and logistical challenges that come with this approach. Some examples of challenges discussed include lack of public support for killing wildlife, lack of public support for re-introduction of bighorn sheep if they were removed, the logistical challenge of locating and removing all individuals in challenging terrains, and the expense of such a proposition. Some managers also see this option as undesirable because of the loss of local genetic diversity and local landscape knowledge that cannot be quickly replaced when completely naïve animals are introduced into a foreign habitat (Jesmer et al. 2018). Of the two regions implementing this strategy, one case selected this option due to a severe pneumonia die-off leaving few remaining animals, and the other chose it because of multiple failed population augmentation efforts and chronic poor recruitment. The interview subjects from the region that had reintroduced sheep into the previously depopulated area indicated that while follow-up monitoring had not seen any evidence of respiratory disease in the newly reintroduced animals yet, continued observation was still necessary before the reintroduction effort could be considered a success.

6.4.4 Do-Nothing

The “do nothing” approach is sometimes considered the best management option when the effects or probability of disease are mild enough that intervention is not justified, but in many cases it is the default that is selected when no practical or viable options are thought to offer a high chance of success. All interviewees described numerous situations where action was

desirable but was not being taken. Significant amounts of time were spent discussing these examples and the numerous barriers that prevented the implementation of desired active interventions. Of the numerous barriers described during interviews, the thematic analysis revealed 4 general categories of barriers: 1) social/ political, 2) physical, 3) resource, and 4) information/knowledge barriers (figure 6-2).

Social and political barriers were those where aspects of human dimensions were the limiting factor identified. In general, these existed because the managers did not have the political leverage to achieve the desired action through regulatory means nor the social support among at least some of the target audience to achieve that same action voluntarily. Physical barriers were those where some aspect of the physical landscape or the herd dynamics on that landscape made the desired management intervention physically impossible. Resource barriers referred to shortages in personnel time or money that interviewees felt were needed to carry out a particular management action in the appropriate way. Finally, knowledge and information barriers were present when interviewees felt that the necessary understanding needed to carry out a particular intervention in a responsible way was not yet available.

Social and Political Barriers

A lack of legal power over domestic sheep and goat owners on either private or public lands was identified by 10 of the 13 interviewees as a barrier that limited their ability to reduce the risk of contact between wild sheep and domestic hosts. Interviewees explained that while relationships with most domestic sheep producers, especially larger producers, were on good terms, 11 of the 13 interview subjects described situations in their region where a lack of trust or rapport between a landowner or producer and the interview subject/agency they worked for resulted in the landowner being unwilling to work cooperatively with wild sheep managers to reduce the risk presented by their livestock. Almost half (6 of the 13) reported that they faced situations in which a stakeholder was unwilling to accept the scientific consensus regarding the risk of pathogen spillover from domestic species. Interview subjects had variable opinions about whether they felt the most effective strategy was likely to be something involving voluntary incentive-based or regulation-enforced compliance with the necessary actions.

A recurrent theme throughout interviews was that the involvement of livestock producers in consultation and planning was regularly low despite the key role that their domestic species posed to wild sheep. Even when stakeholder engagement was a part of management planning,

often the primary stakeholders represented were hunters, leaving domestic sheep owners and producers without a substantive contribution to the process. For example, in one management plan involving a public scoping procedure at the time of its creation, thirty-one responses were received during the process and seven organizations were represented in the commenting period, 5 of which were wildlife or hunting related organizations and only one of which was a domestic sheep related organization. Of the 18 comments made specifically on the topic of bighorn and domestic sheep and goat interactions, 13 recommended reducing, limiting or preventing domestic sheep and goats from being raised or grazed in bighorn-adjacent areas. Given this reality, it is not surprising that so many of the domestic sheep and goat affiliated stakeholders felt threatened and expressed a lack of trust.

Another social/political barrier identified was a lack of public support for lethal tools needed to limit bighorn population size. This was presented by five interview subjects representing four different jurisdictions and referred to a lack of precedent for ewe hunts in two regions, lack of support for increasing hunting tags sufficiently in one region, and lack of access to lethal population reduction in parks in one region. In all cases this created a situation where translocation was the only remaining option for population reduction; however; it was not always a suitable solution due to an inability to meet appropriate translocation pre-requisites such as those suggested by the DMV.

Physical Barriers

Challenging terrain that made capture work or even depopulation next to impossible was discussed by 7 of the 13 interviewees from 5 of the 7 regions. In certain habitats, neither of the strategies aimed at disease eradication (test and cull or depopulation) were considered feasible because of an inability to find, capture, or even kill all sheep regardless of the tools and money available.

High levels of inter-herd connectivity preventing action on one herd without working on all subpopulations of a metapopulation simultaneously was also cited as a significant physical barrier by six of the interview subjects representing all of the regions.

“The problem is we don’t have any good candidate herds... there’s so much connectivity to other locations.” -Wildlife veterinarian

Resource Barriers

Limited access to the financial and personnel resources necessary to complete an AM style intervention was discussed by 11 of the 13 interviewees representing all jurisdictions. It was noted that both the cost of the action itself and the obligatory follow-up, monitoring, and analysis were aspects of this limitation. Most did not feel that simply increasing access to funding would be sufficient to address this challenge because the time to coordinate, complete, and analyze the desired work was not available to many interviewees, especially those in government-funded positions. One similarity among all three of the test and cull interventions discussed was a high level of cooperation between regional wildlife departments and academic institutions, independent researchers, or both. Access to these additional resources was discussed as a valuable component in helping those highly involved test and cull interventions occur and enabled the post-treatment evaluation that was necessary to determine the success of those actions.

Knowledge and Information Barriers

Many areas of curiosity and uncertainty were discussed; however, in many cases these should almost be seen as reasons why AM should be carried out rather than reasons why it could not. Numerous uncertainties were presented as barriers that prevented action. These included which pathogens and which strains of *M. ovipneumoniae* were most important (6/13 interviewees), the nature of intermittent shedding of *M. ovipneumoniae* (3/13 interviewees), whether *M. ovipneumoniae* could be cleared from a live animal or a herd without depopulation (8/13 interviewees), what could be done to reduce movement between herds in a metapopulation (5/13 interviewees), why some herds experienced more severe outcomes to *M. ovipneumoniae* introduction than others (8/13 interviewees) and whether any actions could be taken to reduce the impact of disease in these *M. ovipneumoniae* positive herds (3/13 interviewees). Three interview subjects were not convinced that *M. ovipneumoniae* should be receiving as much priority as it has been; however, all agreed that isolation from domestic sheep and goats was still a necessary priority.

6.4.5 Intentional Use of Adaptive Management

The use of an AM approach to wildlife management was not a stated goal in any of the regions' management plans, nor was it referenced in additional supplemental literature describing the management interventions discussed (Oregon Department of Fish and Wildlife 2003; Hells

Canyon Bighorn Sheep Restoration Committee 2010; Idaho Department of Fish and Game 2010; Montana Department of Fish Wildlife and Parks 2010; Utah Division of Wildlife Resources 2012; Washington Department of Fish and Wildlife 2015; Bernatowicz et al. 2017; South Dakota Game Fish and Parks 2018; Utah Division of Wildlife Resources 2018). While many of the management plans were written and published before the establishment of the DMV none of the interview subjects identified AM as an intended framework in which management interventions were being implemented. When asked about it directly, several managers expressed a need for clarity around what is meant by AM, while those that felt they had a clear understanding of AM did not think they were truly implementing it.

One interview subject discussed a lack of control group availability as a key challenge preventing the utilization of an AM approach. The difficulty in finding a population that was a reasonable candidate for the implementation of any management action is extremely difficult, but to find two that are both good candidates for action and are similar enough to each other to allow one to act as a comparable control group to the other was next to impossible. This interviewee expressed that one of their hopes for the DMV was to increase the geographic pool from which candidate herds could be identified and consequently more eligible control herds might be available, but this has proven more challenging than anticipated. This interview subject suggested that this barrier could be overcome by increased uptake of a “before-after-control impact” (BACI) design for assessing the effect of the intervention.

When asked whether translocations were being done through any AM framework, one interview subject responded by asking “what is an adaptive management experiment?” and another said, “you could say that almost any time we move sheep it’s adaptive management”. When no framework or formal plan describing how a management intervention was selected, designed, carried out, and interpreted was available, objectively evaluating whether management action qualified as AM was difficult. In these cases, both the fulfillment of the nine AM principles proposed by McFadden et al. (2011) and the more general intent to carry out the intervention in a way that allows comparison and analysis of multiple strategies were considered.

One example of this ambiguity is the fact that all regions had some stakeholder involvement and public consultation process in their management planning, but whether stakeholders were informed or had a meaningful opportunity to be involved in management planning was often unclear. Despite the fact that AM was not a stated goal, the application of

management in a way that enabled the evaluation and comparison of different actions through the outcomes they achieved, therefore making them examples of AM, could still be found in some cases.

With regard to prevention, none of the DMV proposed AM actions address the uncertainty expressed around how best to prevent disease introduction from domestic sheep and goats. None of the regions identified a formal set of objective separation goals, or a mechanism of evaluating whether their separation efforts were successful.

"There's no adaptive management in terms of evaluating the intensity of prevention strategies for example, and how that's affecting risk. We're not really doing that in any systematic way. It's hard without a clear program. We have a clear program in terms of general health monitoring, but we don't have any [region] wide program for sheep respiratory disease management" – Disease Ecologist

Interview subjects discussed actions directed at reducing the risk posed by domestic sheep and goats but none of these were applied in a way that would qualify as AM. For example, it is common practice for stray animals found in high-risk areas (whether domestic or wild) to be removed and tested for respiratory pathogens. There is little uncertainty about the fact that these animals constitute a significant disease spillover risk; the most effective way of detecting these animals, however, is less obvious. None of the regions discussed any specific strategy for detecting these animals let alone attempting to compare which strategies are most effective in a systematic way that might be described as AM.

In terms of mitigating the risk of disease introductions via translocations, it is difficult to determine if all nine AM principles received consideration without having a formal description of the decision processes and actions of each individual translocation event. The lack of multiple actions and explicit experimentation between translocation events precludes most of these translocations from qualifying as true AM. However, the high degree of testing and monitoring that goes into each of these translocations as well as the following of standard recommendations from the DMV about pre-and post-translocation data collection makes them well suited to post-hoc comparison and recovery of that otherwise missed opportunity. Because AM is an iterative process, the comparison of management outcomes and integration of lessons learned are

essential; if these are completed for this data set, by the DMV or another body, and lessons are incorporated into future management planning, this would be an example of AM.

The only mechanism discussed to prevent disease introduction through reduction of movement between herds of wild sheep in a metapopulation was population reduction. There were three management strategies suggested by the DMV adaptive management recommendations that pertain to population size or density: 1) Non-selective culls – ewe hunts, 2) “Break-up” herds – actions to disperse/distribute/hazing, and 3) Fertility Control to reduce herd densities. Neither fertility control nor actions aimed at breaking up herds were mentioned by any regional management plans or interview subjects as a strategy they were considering or had carried out. Four of the bighorn management plans described non-selective ewe hunts as an acceptable method of population reduction but none of the interview subjects reported actively using this strategy for population control (excluding depopulation efforts) in their region. Population reduction via translocation of excess animals was discussed but no methods of reviewing the impact of these removals on source herd movement patterns or herd dynamics were reported. Without this necessary follow-up, the evaluation of the success of this action on reducing likelihood of disease or animal movement between herds could not be evaluated and therefore this action too does not qualify as AM in its current form. Another theory regarding the benefit of population reduction may be to reduce the potential effect of density-dependent factors on disease (Monello et al. 2001), but again, no examples of comparison of pneumonia-related mortality rates between reduced and unreduced herds were reported.

When searching for interventions that could be used for disease control, interview subjects discussed ongoing investigation into why some herds are more severely impacted by respiratory disease than others. The research described was generally aimed at comparison of herd or ecosystem characteristics and was therefore observational in nature rather than incorporating active management interventions that could be considered AM. In four of the seven regions, actions aimed at habitat improvement were discussed including controlled burning and weed control. While some interview subjects discussed the idea that habitat improvement might lead to improved health and therefore reduced susceptibility to pneumonia, none described an experimental comparison or AM methodology that would enable the evaluation of that hypothesis.

A large portion of DMV attention was directed towards recommended AM interventions aimed at disease eradication. Two regions completed a test and cull attempt and one had plans to begin one when the interviews were completed. All 3 were directed at *M. ovipneumoniae* eradication from the herd. The two completed interventions involved thorough monitoring including *M. ovipneumoniae* testing and observation of both lamb recruitment and mortality rates, before and after application of the proposed strategy. Both involved comparison of outcomes between herds subjected to different management approaches. This enabled an objective comparison of the different approaches to further support any cause and effect associations of implemented interventions and observed outcomes. The third proposed plan did not explicitly identify a control group. Instead, pre-treatment data describing relevant herd characteristics including recruitment and mortality rates has been collected for several years since the outbreak of disease occurred in the affected herd. That data should be sufficient to allow a before and after comparison to be performed that will provide similar confidence in the validity of the conclusions. While the application of these management approaches did not explicitly state the use of an AM framework, publications on the two completed test and cull efforts combined with interviews of involved parties clearly demonstrated consideration of the nine AM principles. The third test and cull intervention similarly did not include an AM plan but based on an interview with involved personnel they had plans to address all of the AM principles except for the presence of a control group. In all cases, the experimental approach to evaluating the effect of test and cull management interventions clearly qualify as AM.

With regard to the use of AM in depopulation and repopulation cases, both regions that used this approach had stakeholder involvement in their management planning and one even used stakeholders (hunters) to help with the depopulation effort. Meanwhile, other AM principles were less self-evident. A defined objective of having a sustainable disease-free herd of bighorn sheep in the target area was the objective of each intervention but the specifics of what that meant was not defined. Neither situation discussed a specific direct comparison of the herd outcome to a control herd to fulfill the multiple actions for comparison principle as proposed by McFadden et al. (2011). One of the two depopulation events had numerous years of pre-treatment data for comparison while the other carried out the depopulation effort immediately following the outbreak and therefore did not. Both had predicted consequences even if they didn't directly discuss them. Constraints and uncertainty were included in discussions around difficulty in

performing the management actions. Explicit experimentation was not described as an aim of either of these efforts; however, monitoring and emphasis on active learning was clearly a priority and so one could consider these principles met as well. Ultimately, the comparison of pre- and post- depopulation herd dynamics could make this an example of AM in one case if lessons learned helped shape future management, while the lack of pre-depopulation data in the other case makes the identification of a suitable control herd a necessary requirement to qualify as an example of AM.

6.4.6 The Challenges Not Addressed by the DMV

This research observed that the recommendations provided by the DMV were helping to facilitate the implementation of AM style interventions for certain management challenges while not addressing other necessary priorities. Many of the interviewees spent large portions of time discussing challenges and uncertainties faced in managing respiratory disease not identified by the DMV that need to be addressed before jurisdictions can consider implementing DMV recommended interventions. Seven major management challenges were identified where the optimal strategy remains unclear, and of those only three were addressed by the DMV strategy (Table 6-1).

6.5 Discussion

This research demonstrates that there are some strong opinions and theoretical strategies about how we might best manage wild sheep respiratory disease through efforts directed at elimination of *M. ovipneumoniae* from affected herds, but that the opportunities to use this information are still limited because isolation from sources of pathogen reintroduction is rare. There is ongoing uncertainty about how best to achieve pathogen eradication; however, AM is already being used to examine how eradication might be best achieved, and two feasible alternatives (depopulation/repopulation and test and cull) might each be well suited to particular disease scenarios. In cases where the entire AM cycle is not already being completed, the standardized recommendations for follow-up and testing provided by the DMV should allow comparisons to be done by the DMV at a later date. Managers are already focused on prevention, the necessary pre-requisite of eradication, though prevention efforts are lacking the systematic organization currently applied to eradication. Additional progress towards improved pathogen isolation is needed otherwise the opportunities to make use of the meaningful work the DMV is

currently doing on optimizing pathogen eradication strategies will always be limited to a small subset of the respiratory disease affected herds.

These primary lessons are valuable when adapting them to considering how psoroptic mange management should be approached. *Psoroptes* management benefits from a less complicated management process than respiratory disease for several key reasons. First, while *Psoroptes* is infectious to domestic sheep, it is currently absent from North American domestic sheep making isolation from domestic animals less important for *Psoroptes* management (van den Broek and Huntley 2003). Second, chronic smoldering infestations of *Psoroptes* are often more easily identified by the presence of external symptoms in at least some animals in a herd (van den Broek and Huntley 2003). Third, because there is only a single pathogen involved attention can be focused on the specific cause without requiring the decades of research effort and debate that has been needed to identify *M. ovipneumoniae* as the primary causal pathogen in the respiratory disease complex (Besser et al. 2013). Finally, experience in domestic species suggests that treatment of *Psoroptes* at the herd level is only possible by treating each and every animal (O'Brien 1999) unlike respiratory disease which requires removal of the “chronic shedders” of *M. ovipneumoniae* who may only be shedding *M. ovipneumoniae* intermittently therefore potentially requiring multiple captures of each animal.

On the other hand, numerous similarities between respiratory disease and *Psoroptes* are apparent in this study. For example, isolation of naïve bighorn herds from infected herds or isolation of subpopulations from a larger metapopulation poses the same challenge for *Psoroptes* as it does for respiratory disease. Action aimed at disease prevention (isolation) to subdivide larger metapopulations into manageable subpopulations must be undertaken in many situations before extensive disease eradication efforts should be considered otherwise pathogen reintroduction is likely. Additionally, current treatments used in domestic animals require multiple applications for herd level pathogen eradication (O'Brien 1999) so until a *Psoroptes* treatment can be shown to have residual effects long enough to allow treatment of an entire herd before the first treated animals become susceptible again, multiple captures would also be necessary.

The selection criteria suggested by the DMV for choosing respiratory disease situations suited to AM intervention and the recommendations made to standardize follow-up procedures were valuable guidance for wild sheep managers. Creation of similar guidelines for AM of

Psoroptes would be useful for managers wishing to manage *Psoroptes* in their regions. The challenges of improving detection of stray wild sheep and reducing emigration from core habitats are equally applicable to improving isolation for *Psoroptes* management as they are to respiratory disease management. Translocation procedures can be easily adapted to include *Psoroptes* either through improved detection or through treatment of translocated individuals with anthelmintics. Detection can be performed at both the herd level and the individual level; however, because of the possibility of sub-clinical carriers, reliance on detection should involve herd level pathogen testing to determine the suitability of a population to act as a source herd for translocations (van den Broek and Huntley 2003). Routine treatment of animals used in translocations would provide an extra level of protection to prevent transmission of *Psoroptes* to a recipient herd, however an interim housing facility would be required for 10-12 days depending on the treatment selected to ensure all of the mites are killed prior to introduction to the new herd (O'Brien 1999). Significant differences in herd-level disease dynamics exist between respiratory disease and psoroptic mange. Current evidence suggests that only select animals act as maintenance hosts for *M. ovipneumoniae* and pathogen eradication can be accomplished if those animals are identified and removed. Meanwhile, in domestic species, all animals appear to be potential maintenance hosts for *Psoroptes* (O'Brien 1999); however, it is possible that in a wildlife setting, lower density would allow some animals with a higher level of natural resistance to clear the infestation without treatment and so the same might be true for *Psoroptes* management. Actions aimed at control or eradication of psoroptic mange therefore need their own AM experiments.

6.5.1 Barriers

The barriers presented by interviewees provide direction on where management of respiratory disease can generally be improved in North America. The six information barriers described (Figure 6-2) are evidence that significant uncertainties exist regarding fundamental aspects of respiratory disease and how best to accomplish management goals, indicating that AM continues to have a role in respiratory disease management. Some barriers may be insurmountable such as the physical barrier of inaccessible terrain for capture or animal removal, while others could be greatly aided by an AM approach to test a particular hypothesis. The AM approach is especially necessary in situations where a reductionist approach is not valid. For example, the question of “Can we remove *M. ovipneumoniae* from domestic sheep flocks using antibiotics?” can likely be answered by first asking the question of “can we remove M.

ovipneumoniae from an individual sheep using antibiotics?” and then the findings of that reduced question can be applied to an entire flock. Meanwhile, to ask “Can we prevent intermingling of wild sheep between multiple herds of a larger metapopulation?” necessarily involves consideration of interactions between sheep in a herd and so a reductionist approach is simply not possible. The incentive for sheep to disperse between sub-herds may be driven by factors that cannot be studied in isolation, such as drive to find mating opportunities or the search for improved forage conditions when densities are high and habitat is heavily grazed. As a result, this important question must be investigated at the landscape scale through AM.

When considering these same barriers through a *Psoroptes* lens, some remain relatively unchanged, some become less relevant, and some provide new possible directions for management exploration. For example, the lack of importance of domestic sheep in the management challenge of *Psoroptes* alleviates many of the social and political barriers discussed. By contrast, high levels of connectivity in larger metapopulations represent similarly important challenges that require attention before *Psoroptes* eradication efforts can be entertained. Adaptive management approaches aimed at metapopulation fragmentation and reduction of connectivity through strategies such as population reductions to reduce emigration rates are equally applicable to *Psoroptes* management as they are respiratory disease management. Unlike respiratory disease, however, chapter 5 of this thesis added to the literature on effective treatment options for psoroptic mange (Boyce et al. 1990; Boyce et al. 1992; Foreyt 1993). Because we found that fluralaner could be administered orally, the use of a medicated feed or salt lick may be an option for treatment in even highly inaccessible terrains and may help alleviate some of the physical barriers with regard to their impedance of *Psoroptes* management. Significant additional research to further evaluate the safety, and efficacy of fluralaner would be needed before a free choice application of fluralaner could be considered. Some of these next steps will be discussed further below.

Regardless of the disease being managed, a unified vision of the process of AM would be beneficial in order to ensure that wild sheep managers are proceeding in a manner that allows the results of individual AM experiments to be compared and compiled in a meaningful way. It is important to be clear that the goal of any management should not be to fulfill the “criteria” of AM, and as Walters (1986) states, the goal shouldn’t even be to learn unless it helps fulfill the ultimate objective which is to optimize management (Conroy and Peterson 2013). So, while the

principles described by McFadden et al. (2011) may not be relevant to every management scenario, many of the barriers preventing implementation of respiratory disease management action as presented by interview subjects, could be addressed through the implementation of a more structured and unified vision of AM and therefore additional guidance for managers on what constitutes AM would be beneficial.

6.5.2 Adaptive Management and Human Dimensions

A key challenge that all wild sheep managers discussed facing is minimizing the potential for disease spillover from domestic sheep. This challenge differs from any of the situations where the DMV is currently proposing AM in that it involves not only biological uncertainty, but also uncertainty about how the social system will respond to a selected action. For example, in some regions livestock producers may be amenable to having their domestic sheep tested for *M. ovipneumoniae* and working with government employees to eliminate it from their flock, while in other regions interviewees described some local producers as being generally suspicious and/or uncooperative and so efforts to test and remove *M. ovipneumoniae* from domestic flocks would be unlikely to succeed. There is an essential human dimensions component here. The question of how to minimize spillover from domestic sheep is currently being addressed through the haphazard application of numerous different strategies in different regions rather than in a way that would enable controlled learning about which strategies are most likely to be successful. According to Enck et al. (2006) “management actions should be considered experiments, and this should apply to both the human dimensions of management (i.e., beliefs, attitudes, and behaviors) and the biological and ecological dimensions”. Numerous preferences and opinions were presented for how best to address this challenge which is exactly why an AM style comparison of these different approaches is worthwhile.

6.5.3 Stakeholder Involvement

It is no coincidence that the very first AM principle is stakeholder involvement (McFadden et al. 2011). Divergent stakeholder priorities, interests, and concerns are common reasons for the failure of management (Gregory et al. 2006; Allen and Gunderson 2011). Especially where strategies require compliance of those stakeholders, early involvement in the management decision-making process can identify conflicts of interest or opinion that must be addressed to maximize the chances of creating a sustainable management plan (S.J. Riley et al. 2003). Increased involvement of stakeholders leads to increased trust and a greater likelihood of

selecting management strategies that have the support of divergent stakeholders (Lauber and Decker 2012).

Many interview subjects expressed skepticism that any policy or regulation that might impinge on livestock agriculture, including access to public grazing lands or people's rights and freedoms on their private property, is likely to gain traction in their region. In these regions, the promotion of wild-domestic sheep separation through regulatory means is unlikely, leaving cooperation and compliance as the necessary route to create disease isolation where regulatory enforcement is not an option. At the same time, regulated domestic sheep-free areas may be the most effective option for long term wild sheep protection and so in areas where managers believe it is feasible, especially those areas without a long history of domestic sheep farming, managers should prioritize regulatory options. Because of the role of domestic sheep in pathogen spill-over, some of the most important stakeholders are the domestic sheep and goat owners. Because of the diversity of social and political contexts, one particular strategy is unlikely to work in all regions faced with this challenge, but additional attention to human dimensions challenges and involvement of stakeholders is the first step to addressing these key common barriers (S.J. Riley et al. 2003).

The likelihood of facing drastically different stakeholder priorities and opinions is far lower when discussing management of *Psoroptes*, but beyond building trust and a shared set of goals, higher levels of stakeholder engagement could also allow for improved public monitoring as a means of detecting sheep in undesirable locations. All regions have a policy of removing wild sheep thought to pose a high risk for pathogen exposure. For respiratory disease management these are the animals that may potentially make contact with domestic sheep, but this tool could also be used in psoroptic mange management through the use of wild sheep-free buffer areas between *Psoroptes* infested and uninfested herds. These animals represent a significant potential source of disease transmission between infected and naïve populations, and yet the best way to detect these high-risk sheep is also uncertain. Low-frequency chance occurrences such as movement of wild sheep outside of their core habitat can be extremely difficult to detect, but in other scenarios public awareness and reporting as a form of passive surveillance was both a more sensitive and more cost-effective way of detecting stray animals than active surveillance (Hering 2015). Not only could the engagement of private citizens help achieve the goal of surveillance more effectively, but evidence suggests that this engagement of

stakeholders, a strategy known as collaborative monitoring, further builds sorely needed social capital (Cundill and Fabricius 2009). Similarly, the involvement of private citizens such as forestry professionals or guide outfitters could be valuable in helping maintain disease isolation between two herds of a larger metapopulation as part of a disease eradication effort for either *Psoroptes* or respiratory disease.

6.5.4 Defined Objectives, Multiple Actions, and Explicit Experimentation

Defined objectives, and multiple actions are the next two key AM principles identified by McFadden et al (2011). An explicit experimental approach to achieve these defined objectives through comparison of multiple actions is the basis of the scientific method. Concrete measurable objectives were not always identified during interviews. While their apparent deficit may have been in part a result of the informal interview style, the intentional selection and statement of objectives commonly receives less attention than it deserves (S.J. Riley et al. 2003). The process of defining objectives together with stakeholders is valuable in developing a shared vision, an important step in building trust with stakeholders (Conroy and Peterson 2013; Videira et al. 2016). The lack of clear objectives and involvement of stakeholders in their formulation is a likely contributor to the frequency with which the issues relating to a mistrust and non-compliance were cited by interview subjects.

Objectives take two forms, both of which require consideration. Fundamental objectives can be thought of as the ultimate goal of the management action whereas enabling objectives describe smaller actions generally related to the hypothesis being tested, and are used to achieve the fundamental objective (S.J. Riley et al. 2003; Enck et al. 2006). Numerous enabling objectives are often identified that might achieve a single fundamental objective. For example, preventing disease transmission between domestic sheep and wild sheep is a fundamental objective that might be met by the enabling objectives of eliminating domestic sheep farming from an area, or by increasing detection and reporting of stray wild sheep, or by creating test and remove programs in domestic sheep flocks to remove concerning pathogens from those flocks. Establishing fundamental objectives together with stakeholder groups highlights common ground to build on, after which a variety of enabling objectives can be proposed and considered. As one interview subject said, “a die-off is bad for everyone”.

Similarly, for *Psoroptes* management, co-development of management objectives would be a valuable process to get managers and stakeholders to have a shared set of goals. An example

of a fundamental objective may be to prevent spread of the *Psoroptes* parasite into nearby naïve herds. Several enabling objectives such as removal of bighorn sheep from high risk corridors where disease transfer is likely, attempts to capture and treat high risk animals such as young rams or herds in high risk locations, or placement of medicated feeding stations at the extent of the current range of the parasite might be worthy of consideration.

A process of structured decision making (SDM) can be used to systematically select between the multiple actions or “enabling objectives” most likely to achieve the fundamental objective (Robinson and Fuller 2016). An excellent example of this SDM process for selection between bighorn respiratory disease enabling objectives was published by Sells et al. (2016). While SDM can be used to select management options worthy of implementation, it should be seen as a component of AM not a replacement for it because the complexity of challenges suited to AM approaches are beyond what can be accurately predicted by SDM and modeling alone. The assessment of which strategy best achieves the desired result when applied in a management setting requires the comparison of multiple strategies through explicit experimentation; this is the foundation of AM (Possingham 2000; McFadden et al. 2011; Sells et al. 2016).

6.5.5 Prediction of Consequences, Specification of Constraints, and Acknowledgment of Uncertainty

Throughout the exploration of management challenges, managers should aim to clearly understand where the uncertainty lies, what constraints exist, and how selected actions might result in differing outcomes; however, the role and necessity of these AM principles is often less clear. Without explicitly considering these, managers risk overlooking key considerations when developing and selecting management options together with stakeholders. One example of this is the fact that many managers cited metapopulation connectivity as a barrier to herd eligibility for respiratory disease eradication; however, in at least some cases the frequency of animal movement between subpopulations is uncertain. Global positioning system (GPS) collar data may show that animals have on occasion moved between herds and this represents the potential for connectivity from a disease standpoint, but now with new evidence suggesting that many animals in a herd may not be chronic shedders, those animals may not pose a high transmission risk. For example, of the 24 animals involved in the South Dakota test and remove intervention on the Custer State Park herd (composed of 17 females and 7 males), only 2 animals were classified as “chronic shedders” after serial testing for *M. ovipneumoniae*, both of which were female. While

the proportion of animals acting as chronic shedders are likely variable, it is possible that these infrequent animal dispersals, often involving rams (O'Brien et al. 2014), may not represent as much of a respiratory pathogen transmission risk as was once thought.

Hells Canyon is one area where numerous subpopulations are considered to be part of one larger metapopulation. The Hells Canyon bighorn sheep metapopulation's range includes some 22,500km² alongside the Snake River's drainage of Washington, Idaho and Oregon (Hells Canyon Bighorn Sheep Restoration Committee 2010). The Hells Canyon metapopulation is affected by both respiratory disease and *Psoroptes*; however, in Chapter 3 of this thesis, the samples collected from the Wenaha and Mountain View herds of Hells Canyon were found to be almost entirely (14/15) seronegative for *Psoroptes* exposure while active mite infestations were found in the nearby Asotin herd of Hells Canyon. Presumably, the *Psoroptes* infestation spread through the larger metapopulation through animal movement at one time and so this connectivity does exist, but at the same time the different serological status of these two neighboring herds may indicate a break in this connectivity or that it is far less frequent or relevant than might have otherwise been thought. The Asotin herd of Hells Canyon which continued to be infested with *Psoroptes* was also the site of a successful test and remove management intervention directed at *M. ovipneumoniae* based on nasal PCRs (Bernatowicz et al. 2017). This strategy could take advantage of temporary breaks in connectivity of larger metapopulations to attempt disease eradication throughout a metapopulation in a stepwise format by working on one sub-herd at a time. There is often uncertainty around the actual degree of connectivity, and the likelihood of disease transmission with the connectivity that does still exist. This uncertainty needed to be acknowledged in order for feasible management options to be inappropriately considered.

6.5.6 Monitoring

The development of standardized monitoring recommendations has already been established by the Wild Sheep Working Group and the DMV for many of the key respiratory disease herd and animal-level parameters (WAFWA - Wild Sheep Working Group 2017) and interviews confirmed that herd surveillance and monitoring are already a strength in most regions. The infrastructure that is currently in place for respiratory disease management can also help address the challenge of *Psoroptes* management. Thanks to the externally visible symptoms of psoroptic mange, monitoring and herd health assessments can and do collect samples and observations for *Psoroptes* at the same time as respiratory disease.

The high degree of disease monitoring and pathogen surveillance is commendable and a significant area of success of the DMV and regional wildlife management efforts. One area where additional monitoring could be of value is in evaluation of objectives not directly related to wild sheep populations. For example, additional emphasis on monitoring how new domestic sheep owners in high-risk areas are detected would enable managers to become more strategic in selecting and adapting management strategies aimed at these enabling objectives.

6.5.7 Active Learning Emphasis

The significant role that learning already plays in the work and attitudes of wild sheep managers is evidenced by the willingness of interview subjects to participate in this study, the development of the DMV with its emphasis on active learning, and the numerous information barriers discussed by interview subjects. Managers need to continue being strategic about how best to address ongoing areas of uncertainty. Adaptive management can help address problems that are not suited to a reductionist approach such as whether actions aimed at disrupting connectivity of larger metapopulations are valuable in preventing transmission of respiratory disease causing pathogens between subpopulations, or whether actions taken by managers can reduce the impact of respiratory disease in affected populations. Meanwhile, primary research efforts directed at simpler problems like treating domestic sheep to clear *M. ovipneumoniae*, or preparing and testing an oral free-choice preparation of fluralaner to treat bighorns with psoroptic mange should be pursued.

6.6 Recommendations for the DMV

1. Utilize non-government personnel to work alongside government employees in developing and implementing management actions.
 - Most interviewees identified scarcities of finances or time as major challenges preventing more proactive implementation of disease management strategies. The creation of dedicated sheep separation coordinators and fostering of partnerships with academic institutions are valuable ways to address these deficits.
2. Invite human dimensions experts to aid in understanding the social dynamics of respiratory disease management and the development of positive working relationships with key stakeholder groups.

- Human dimensions of wildlife management are a significant challenge that must be considered in minimizing the risk of respiratory disease spillover from domestic sheep and goats to wild sheep. Differing social and political climates in different states and provinces are key considerations that can be used to guide the selection of strategies. Managers are often well positioned to work with local stakeholders in selecting which strategies are most likely to be effective in their region; however, the explicit consideration of stakeholder engagement as a part of an AM approach can both increase the likelihood of success in each region and increase the learning that can be done during this process.
3. Strategies involving regulatory based restrictions on domestic sheep and goat farming should be approached with caution and in some cases entirely discontinued.
- In regions with long-standing histories of domestic sheep farming the continued exploration of these options severely undermines the necessary relationship foundation with these important stakeholder groups. Increased stakeholder involvement, public education, and the development of trusting relationships with different stakeholder groups and individuals are essential steps in the reduction of disease risk to wild sheep, especially with regard to respiratory disease.
 - In wild sheep adjacent areas where domestic sheep and goat farming is not already present, the pursuit of regulatory restrictions on the presence of these domestic species is strongly encouraged.
4. Animal-level treatments should continue to be explored in the primary research arena before implementation in a management context.
- New options for treating individual animals harbouring select respiratory disease pathogens could offer promising management alternatives. For example, these options may enable pathogen elimination from wild herds without requiring depopulation or may be more palatable alternatives for removal of those pathogens to domestic flocks in high risk areas.
5. Respiratory disease should continue focusing on harm and risk reduction.
- The complex polymicrobial nature of respiratory disease is such that true eradication will likely never be achieved. Adaptive management interventions

should focus first and foremost on the herd level response to management interventions rather than on a single pathogen. This insures that AM continues to work towards the fundamental goal of improving the health of wild sheep.

6. Continue providing key guidance and direction to wild sheep managers that acts to standardize aspects of wild sheep management procedure and expand recommendations to include other disease of concern to wild sheep health.

- The wild sheep surveillance and testing guidelines, AM recommendations, synthesis of knowledge, and availability of expertise provided by the DMV are invaluable contributions to the management of wild sheep in North America. Surveillance and health monitoring recommendations allow comparison of actions carried out in different jurisdictions and should be expanded to include other diseases of concern. These actions and DMV services should be continued and expanded to help provide additional guidance on how managers can use the principles of AM to better accomplish their management goals.

6.7 Recommendations for Adaptive Management of Psoroptic Mange

Specific management recommendations are context specific. While numerous management options have been discussed throughout this thesis and will be referenced in these recommendations, the selection of specific actions for implementation should be made together with relevant stakeholder groups and to suggest otherwise would be to forget one of the key lessons of these interviews.

1. Reach out to all potential stakeholder groups that may be interested in being involved in the management of psoroptic mange.
 - This should include other neighbouring jurisdictions, local first nations and aboriginal governments, relevant parks and other governance bodies, domestic producers harbouring *psoroptes* susceptible species, wildlife and bighorn sheep specific conservation groups, sportsmen associations and hunters groups, guide outfitters, and any other locally relevant individuals and organizations.
2. Together with any interested stakeholder groups identify shared fundamental and enabling objectives.

- Depending on the situation, these should begin with initial research to better understand the local context, distribution, and potential sources/reservoirs of infestation.
 - Next, actions aimed at prevention of spillover to other species or populations, mitigations of the effects of infestation, reduction of prevalence, or even eradication of the mite from a population when possible should be considered.
3. Identify multiple actions that can realistically be used to achieve the desired outcome and how eligible herds should be selected for application of these actions.
- These would be dependent on the objectives selected together with the stakeholders. In most situations, once the initial understanding of the parasite distribution and prevalence is understood, prevention of disease spread to unaffected herds is an appropriate next step. Options to achieve this objective might include: population reduction of herds at the periphery of the known mite distribution to reduce the likelihood of emigration, complete depopulation of small herds thought to pose a high risk of transmission of the mite to a naïve metapopulation, development of oral treatment bait stations on suspected high likelihood animal movement corridors, fencing solutions to prevent animal movement, annual capture and treatment of high risk herds, wild sheep-free buffer areas where citizens are asked to report any wild sheep sightings, etc.
4. Anticipate consequences and potential constraints of the management actions and thoroughly explore them with the body of stakeholders involved in selection of management options.
- While this step can be tedious, it is an essential component of due diligence. For example, if the proposed management action involves building of physical barriers such as fences, the impacts of that habitat fragmentation on other species should be considered, and where possible mitigated. Furthermore, it may be understood that fencing would be the most effective way of preventing disease movement, but that option may be cost prohibitive or the effects on other species too great. The shared understanding of these constraints can help build a shared vision among AM collaborators.
5. Explicitly discuss uncertainty of management outcomes with AM partners.

- Improved understanding of the range of potential outcomes will help alleviate frustration that may otherwise be felt if management actions do not achieve the desired results or follow desired timelines. This uncertainty also helps explore possible reasons why management efforts may not be successful and identify areas where further investigation may be necessary before a management strategy is ready for implementation in an AM situation.
 - For example, there are many areas of uncertainty that should be considered before a free-choice form of oral fluralaner could be used. Some of these require additional research before this management option is ready to be implemented including: establishing safe and effective dose ranges, palatability, duration of protection, effects in pregnant animals, impacts on non-target species, drug residues and human safety in hunted species, impacts on invertebrates performing ecosystem functions, etc.
6. Develop experimental plans to observe and monitor the outcomes of management interventions.
- Development of a research plan prior to implementation of the AM action will ensure that the hypothesis that drove the management action can be adequately evaluated in order to improve the application of that management strategy into the future.
7. Identify a specific timeline sufficient to complete the initial AM intervention and observe the results. These can then be reviewed and discussed with management partners in order to adapt and refine the management strategy being implemented.

6.8 Conclusions

The DMV is well situated to help regions implement AM approaches to improving disease management in many bighorn herds; however, a broader perspective of the significant challenges faced by wild sheep managers and the potential management options is essential to maximize the utility of the DMV's outputs. While it may be outside of its current scope, if the DMV wants to act as a valuable resource to managers in addressing their key barriers, it needs to not only address the knowledge gaps but also consider and strategize around the diverse social and political barriers that prevent action from being taken and ensure these barriers are considered in AM planning. Prevention of respiratory disease pathogen transmission is often

impaired by social and political challenges rather than by uncertainty about the biological systems involved. These human dimensions can not only be better understood, but also be directly addressed by incorporating the principles of AM. While the DMV is not in a position to directly implement any particular actions, it can help managers and facilitate learning through an AM approach. It should continue to suggest management actions to be implemented and outcomes to be monitored and can then take an active role in completing the comparison of outcomes observed from management actions performed in different regions. This will enable shared learning across jurisdictional lines about which strategies have the greatest likelihood of success.

Despite a lack of consistent positive results in the management of respiratory disease in wild bighorn sheep populations, the research findings of members of the DMV and WAFWA's Wild Sheep Working Group present very promising new options for the management of respiratory disease in wild sheep that may alter how this problem is managed in the coming years. Additionally, new information provided by this thesis on the detection and treatment of *Psoroptes* could be seamlessly added into some management actions including disease surveillance and test and remove programs already being carried out by wild sheep managers. Lessons learned in this study about common challenges of bighorn sheep disease management can help avoid common challenges while directing the development of new AM plans aimed at the management of psoroptic mange. The addition of expertise on human dimensions would aid in addressing and guiding wild sheep managers through the many social and political barriers they face. Development of adaptive management recommendations and inclusion criteria aimed at addressing the currently underexplored management challenges identified in this study such as how best to prevent emigration of bighorns between sub-herds of a metapopulation, or how to maximize detection of stray sheep would be valuable additions to the current topics of interest for the DMV and be useful in improving the management of numerous infectious diseases including respiratory disease and *Psoroptes*.

The creation of centralized working groups for compiling and analyzing information to tackle common goals represents a significant advancement in North American wildlife disease management. The benefits gained through this centralization can help refine the process from knowledge acquisition to application if used strategically. Added support in the form of additional funding, expertise, and personnel would help the DMV initiative provide guidance,

coordination, and synthesis of knowledge for wildlife management agencies in tackling the negative impacts of infectious diseases on wild sheep conservation and recovery in North America.

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Category	Management challenge	Addressed by DMV?
Prevention	Detection of domestic sheep operations	No
Prevention	Detection of high-risk stray sheep	No
Prevention	Strategies to reduce stray bighorn emigration from core habitat	No
Prevention	Optimal translocation strategies to mitigate respiratory disease risk	Yes
Control	Habitat modification and resilience to respiratory disease pathogens	No
Eradication	Test and Cull	Yes
Eradication	Depopulation/repopulation	Yes

Table 6-1: Primary management challenges identified during interviews and their consideration in the DMV strategy.

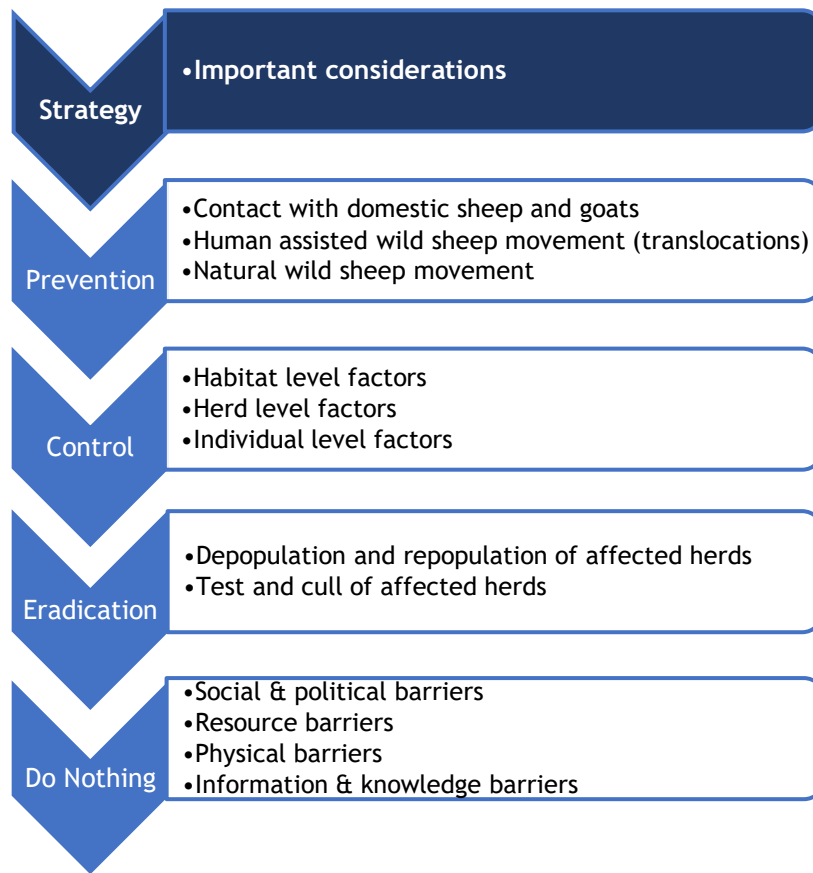


Figure 6-1: Respiratory disease management considerations identified by thematic analysis of interviews divided into the four wildlife disease management strategies. Where no action is being taken, the major groups of barriers to action presented are listed.

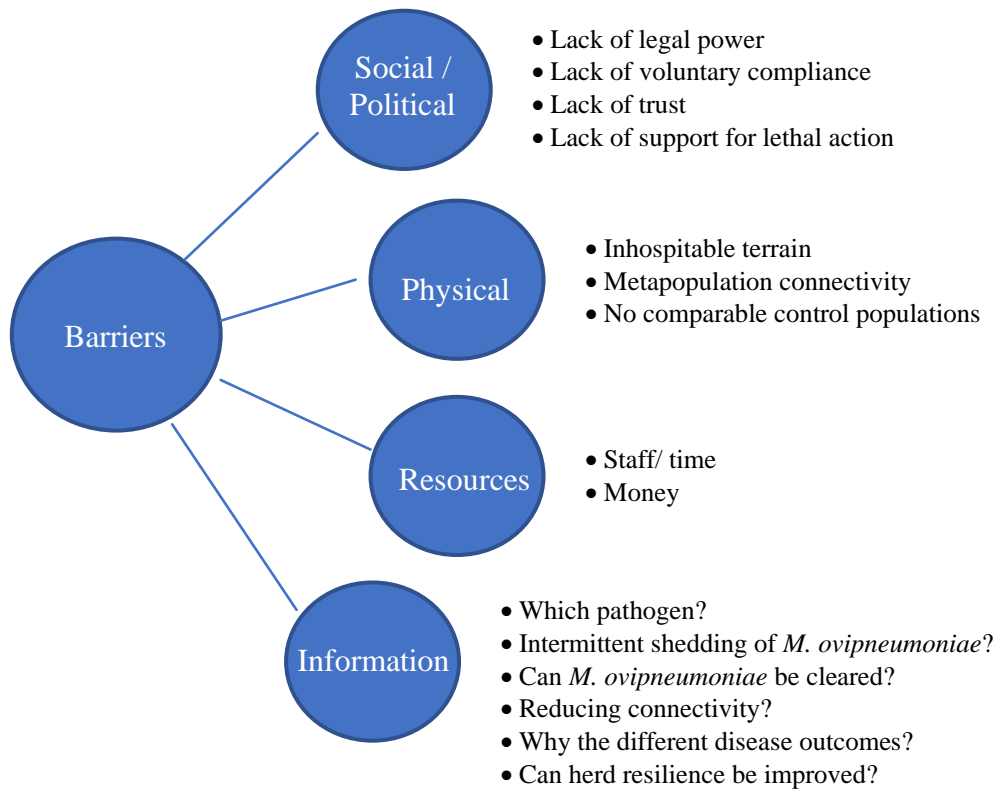


Figure 6-2: Major barriers to action identified through thematic analysis revealed four common groups, each suited to different methods of approach.

CHAPTER 7: DEVELOPMENT OF TOOLS AND INFORMATION FOR THE ADAPTIVE MANAGEMENT OF *PSOROPTES* IN CANADIAN BIGHORN SHEEP (*OVIS CANADENSIS*)

7.1 Introduction

Bighorn sheep (*Ovis canadensis*) are an iconic wildlife species that have come to represent the enduring wilderness of North America. Following European contact with North America, bighorn sheep populations declined from one to two million animals to the low tens of thousands in the early 1900s (Buechner 1960). Since that time, in part thanks to conservation effort throughout their range, bighorn populations have rebounded to over eighty five thousand animals (Wild Sheep Foundation 2017). Numerous anthropogenic factors were responsible for their decline, one of which was the introduction of exotic diseases (Buechner 1960). Psoroptic mange is one such disease thought to have been brought to North America with domestic livestock from Europe in the 1800s. After its introduction, it crossed over to numerous North American wildlife species including all three subspecies of bighorn sheep (*O. canadensis*), as well as elk (*Cervus canadensis*), white-tailed deer (*Odocoileus virginianus*), and mule deer (*Odocoileus hemionus*) (Lange et al. 1980; Sandoval 1980).

When observed in bighorn populations, *Psoroptes* infestations have often been managed by benign neglect, while in select cases population declines following outbreaks have necessitated that action be taken (Sandoval 1980). This appears to be the case currently in British Columbia, Canada, where the outbreak of psoroptic mange in several southern bighorn sheep herds was associated with a >50% population decline (Reid 2013a). It has spurred local wildlife biologists and the provincial wildlife veterinarian to direct efforts towards improving understanding of the parasite and the outbreak with the intention of developing management options to address the situation.

The non-burrowing obligate ectoparasitic mite *Psoroptes* parasitizes ungulates and lagomorphs around the world (Zahler et al. 2000). The taxonomy and host specificity of *Psoroptes* has been the subject of much academic debate and reclassification. Increasing evidence supports the assertion that all *Psoroptes*, with the possible exception of *P. natalensis* in Egyptian

water buffalo, are conspecific based on the fact that distinguishing morphologic traits, genetic data, host species, and site predilection on the host are not mutually exclusive as was once thought (Sweatman 1958; Zahler et al. 1998; Amer et al. 2015). Psoroptic mange continues to plague domestic sheep in the United Kingdom despite decades of management effort (Smith et al. 2001; Losson 2012b), and any disease management gets more challenging in a wildlife context, where free ranging animals are involved (Lange et al. 1980; Sandoval 1980). Despite the added complexity of a wildlife context, the principles of infectious disease management are conserved. In order to manage a disease outbreak, a process of disease anticipation, early detection, containment, mitigation, and finally elimination/eradication should take place (World Health Organization 2018).

This thesis addresses several gaps in knowledge that will aid in the development of an adaptive management plan for psoroptic mange in Canadian bighorn sheep. Included is identification of the source of the outbreak, new tools for detection of infested animals, observations of fencing structures and their utility in controlling bighorn sheep movement, and new *Psoroptes* treatment options that may be suited to remote application in free-ranging animals. Because of the lack of active *Psoroptes* management in bighorn sheep, a review focusing on respiratory disease management provided valuable insights into the challenges of turning knowledge into management action. Lessons learned through this review will help in the development of a psoroptic mange management plan moving forward.

7.2 Results and Discussion

The first step of developing a psoroptic mange management plan for Canadian bighorn sheep was identifying the source of the outbreak. There were two dominant theories about the source *Psoroptes* in Canada. First, that mites travelled north through natural bighorn sheep movement or on contaminated wildlife handling equipment from infested bighorn populations in the USA, and second, that infestation originated from another locally endemic species, rabbits.

Psoroptes mites recovered from Canadian bighorn sheep were compared to those found on pet rabbits in western Canada and to mites collected from infested wild bighorn sheep populations in the USA. *Psoroptes* mites acquired from Canadian bighorn sheep were more morphologically and genetically similar to those collected from rabbits than those of American bighorn origin (Chapter 2). Outer opisthosomal setae (OOS) lengths of mature male mites, the previously accepted method of differentiating species of *Psoroptes*, measured an average length

of 81.7 μm (+/-7.7 μm) in Canadian bighorn mites and 88.9 μm (+/-12.0 μm) in domestic rabbit mites; meanwhile, the OOS of *Psoroptes* mites recovered from American bighorn populations averaged 151.2 μm (+/-16.6 μm). Observation of opisthosomal lobe morphology also differed between the groups with Canada bighorn mites and rabbit mites appearing morphologically similar to each other but distinct from USA bighorn mites. Findings in USA mites matched previously observed descriptions of bighorn *Psoroptes* mites (previously classified as *P. ovis*) which have significantly longer OOS lengths than those collected from rabbits (previously classified as *P. cuniculi*; Boyce et al. 1990). This is the first report of mites matching the rabbit ecotypes infesting free-ranging bighorn sheep.

Comparison of mitochondrial genes Cytochrome B (Cyt B) and Cytochrome C oxidase subunit I (COI) corroborated these findings. The Canadian bighorn mites were more similar to mites collected from rabbits than mites collected from American bighorns at the Cyt B locus. None of the rabbit origin mites were successful in producing amplicons at the COI locus however, when compared to a Genbank sequence of *P. cuniculi*, the Canadian bighorn mites were more similar to the Genbank sequence than they were to the American bighorn mite sequence at the COI locus. This information provides a high degree of confidence that the Canadian bighorn *Psoroptes* mites are the rabbit variant (previously called *P. cuniculi*) suggesting that the source of *Psoroptes* introduction into Canadian bighorn populations was through a disease spillover event from rabbits rather than from spread of the parasite through bighorn sheep movements.

It seems likely that this host-species jump occurred prior to 1999 when a wildlife park called the Okanagan Game Farm was operating in the area now affected by psoroptic mange. The park housed a herd of bighorn sheep in close proximity to a breeding colony of *Psoroptes*-infested rabbits, used for feeding carnivores (Schwantje 2019). This captive situation would have put the two species in unnaturally close quarters and increased the chances of contaminated feed or other fomites spreading the infestation between species. The bighorn sheep were also known to escape their enclosure periodically, creating a conduit for the infestation to spread to the surrounding free-ranging bighorn sheep populations. If true, the source of disease introduction has been removed by the closure of the game farm and wildlife managers can turn their attention to better understanding the current spread of disease, application of control measures, and pursuit of eradication efforts.

Subclinical *Psoroptes* infestations are also reported, making detection, control and determination of the current distribution of the mite a challenge. The availability of a serologic diagnostic test optimized for bighorn sheep will help address this challenge by increasing sample throughput, allowing for detection of previous exposure, and enabling retrospective evaluation of archived samples. The *LilliTest* Sheep Scab ELISA is a commercially available indirect ELISA aimed at detection of IgG antibodies to *Psoroptes* antigen developed for use in domestic sheep. This ELISA was applied to bighorn sheep serum from *Psoroptes*-infested Canadian and American animals as well as unexposed Canadian bighorn sheep. Serum from treatment-trial animals (Chapter 5) before and after treatment, and serum from their newborn lambs handled monthly as they developed infestations was also tested (Chapter 3). After optimizing the ELISA test conditions and creating pooled positive and negative control serum of bighorn sheep with known disease status, the *LilliTest* Sheep Scab ELISA achieved a test sensitivity of 98.7% and specificity of 94% using a cutoff of 1.5 times the negative control optical density (Chapter 3). The positive-test cutoff could be adjusted up or down to maximize sensitivity or specificity depending on the ecological context and the consequences of false positives or negatives, thereby increasing the ELISA's utility in a variety of scenarios.

Antibody detection was unreliable in lambs under 3 months of age and clinical symptoms of infestation appeared in young animals before antibody titres exceeded the cut-off threshold. As previous studies observed, these antibody titres take time to decline following successful treatment and therefore, this ELISA cannot be used as a measure of treatment success. In the process of validating this ELISA, we found two of the herds in the Hells Canyon metapopulation presumed to be infested with *Psoroptes* that may in fact be free of the disease, demonstrating the utility of this test when applied at the herd level. This test can be a valuable addition to *Psoroptes* management in Canada and is well suited to being applied retroactively to archived samples or to any suspect herds to confirm the true distribution of the parasite. For example, samples collected from the Okanagan Mountain Park herd which is outside of the current known distribution of *Psoroptes* in Canada, but was augmented with bighorn sheep taken in 2007 from the area now infested with *Psoroptes*, should be tested. At that time *Psoroptes* had not been detected in Canadian bighorn sheep; however, based on the findings of Chapter 2 it had likely already been introduced into the wild bighorn sheep population used as a source herd at the time of that translocation and augmentation.

Once the extent of the *Psoroptes* spread is understood, preventing further movement should be the next priority, while mitigation and eradication efforts are prepared. A key tool in the prevention of animal movement, and with it disease movement, is wildlife fencing. For example, the current distribution of psoroptic mange in British Columbia is prevented from spreading further east by a wildlife game fence that runs alongside highway 97 dividing the *Psoroptes*-free bighorn populations on the east side of the Okanagan valley from the infested western populations, separated in some cases by only a few hundred meters (Reid 2013a). Because of the varying athletic abilities and social drives of different animals, species-specific knowledge of fencing efficacy is essential. The circumstances leading to the escape of bighorn sheep from the Okanagan Game Farm are not known, but it is possible that the use of species-appropriate fencing in the design and construction of the bighorn sheep enclosures may have prevented them from escaping the facility, and the *Psoroptes* outbreak in Canada may never have happened (Chapter 2). Chapter four of this thesis addresses this gap in the literature on bighorn sheep wildlife fencing.

Two adjacent wildlife enclosures were constructed to house bighorn sheep for a *Psoroptes* treatment trial (Chapter 5). One enclosure was surrounded by 2.4m tall, fixed knot, woven wire fencing (WWF) with 0.9m overhangs protruding inward at a 45° angle from horizontal from the top of the WWF. The second enclosure had the same WWF plus a secondary electric fence (EF) installed 1 m outside of the WWF designed to prevent nose to nose contact between sheep on either side of the fence line. The EF was composed of three positive and one negative strand with the top strand at a height of 1.4 m. A group of 18 bighorn sheep ewes and lambs were introduced into the pens and housed there for 14 months. Eleven lambs were born in the pens midway through the year and two rams were temporarily introduced into the pens for the purpose of breeding but were released after the rut. Perimeter fences were monitored using three motion activated game cameras along each enclosure's perimeter. The 2.4 m WWF with the 0.9 m overhangs was successful in preventing any bighorn sheep crossing events, with the exception of one handling day when a baby lamb that was less than one month old ran through the large holes at the bottom of the perimeter WWF during a high stress handling event. Because its dam remained in the pens, the lamb was easily recovered and reunited with the ewes. The EF on the other hand, was not successful in preventing animals from crossing it. Wild rams frequently jumped over the 1.4m EF to gain closer access to the ewes, though they never managed to get

inside of the WWF. Utilization of a 2.4m WWF with 0.9m overhang structures is a good option for any circumstance where bighorn movement needs to be fully restricted.

Once isolation from sources of disease reintroduction is established, managers' attention can shift towards disease eradication efforts. Elimination of *Psoroptes* in domestic flocks generally involves multiple sequential treatments of every animal in the herd using a macrocyclic lactone anthelmintic (O'Brien 1999). This approach would necessitate bringing wildlife into a captive setting in order to treat them multiple times. This approach was taken in a New Mexico *Psoroptes*-affected herd, but not before the bighorn sheep herd had dwindled to just one remaining animal (Boyce and Weisenberger 2005). This strategy comes at great financial cost and wildlife capture is inherently risky to the animals (as demonstrated in this study); it is also dependent on being able to catch every single animal and is therefore not well suited to application in many free ranging wildlife scenarios. For treatment of psoroptic mange in free ranging bighorns to be an option, effective single use treatments need to be available. Ideally those treatments should be administered remotely, and preferably they should have a duration of action long enough to allow treatment of all the animals in a herd before the first treated animals become susceptible to reinfection. This thesis discussed the results of a treatment trial conducted on captive, naturally-infested bighorn sheep in Penticton, British Columbia.

We tested two different drugs administered in three different ways for their efficacy in treating psoroptic mange. Longrangetm, a macrocyclic lactone that uses an extended release formulation of eprinomectin to achieve 150 days of residual effect against internal parasites in cattle, was tested at twice the label dosage in our bighorn sheep but was not effective in eliminating the infestation. The other, of a relatively new class of drugs called isooxazolines, has been highly effective in treating ectoparasites in other species but its use had not been reported in ovids (Prohaczik et al. 2017; Taenzler et al. 2017; Sheinberg et al. 2017). One of the drugs in this class, Fluralaner®, advertises an efficacy window of 12 weeks for preventing ectoparasites in dogs and cats. Two formulations, a topical and an oral form, were tested on the *Psoroptes* infested bighorn sheep, each at two different dosages. The oral form was effective in clearing the infestation at both the 5mg/kg and 25mg/kg dosages, but the topical form was not effective at either the 5mg/kg or the 10mg/kg dosage. The duration of efficacy of the oral fluralaner could not be fairly evaluated in this trial because of cohabitation of all fluralaner treated animals. As a result, it was not possible to differentiate new developing infestations on orally treated animals

from mites acquired from topically treated animals that might still succumb to the persistent effects of the oral drug and therefore would not result in another infestation. Follow up studies are needed to clarify the duration of protection provided by the oral fluralaner. Ideally, these studies should involve artificially controlled *Psoroptes* exposures at timed intervals rather than continuous exposure from cohousing with persistently infested animals as was the case in this study. That would allow researchers to differentiate between viable re-infestations and the transfer of mites that are likely to succumb to the persistent effects of the drug in the orally treated animal.

Despite increased knowledge acquired about this disease, translating knowledge into wildlife management action is often more challenging than it would appear. While psoroptic mange has rarely been actively managed in wild sheep, review of other wild sheep disease management programs may help provide valuable insight to the process and application of wild sheep infectious disease management. This thesis explored the transition of knowledge into action in the management of respiratory disease throughout the USA and Canada. Particular attention was placed on illuminating challenges to be avoided and lessons to be learned in the development of management plans for psoroptic mange.

Through interviews with wildlife professionals across North America we learned that developing disease eradication options is currently a primary focus but that prevention of disease introduction is a key pre-requisite that needs additional attention. Many uncertainties continue to exist regarding how best to create and maintain isolation from sources of disease introduction. The three sources of respiratory pathogen introduction were identified as 1) natural movement of wild sheep between herds, 2) human-assisted translocation of animals between herds, and 3) contact with domestic sheep harbouring respiratory disease pathogens. Of these three, only the first two are relevant to *Psoroptes* prevention. Preventing contact with domestic sheep adds significant human dimensions considerations to respiratory disease isolation that is not relevant to managing *Psoroptes*. This aspect highlights both the importance of considering sources of disease introduction from other reservoirs including domestic species, as well as the importance of addressing the human dimensions challenges of wildlife management in a strategic way.

Other components of respiratory disease management are more directly applicable to *Psoroptes* management. For example, considerable effort is expended on population surveillance and herd pathogen profiling, and substantial progress has been made on improving and

standardizing disease testing of translocated animals to reduce the likelihood that human-assisted animal movements contribute to the spread of disease. Managers also explained that natural animal movements that maintain connectivity of metapopulations also prevent disease isolation. Further exploration into whether this connectivity can be temporarily interrupted through actions such as herd reductions is needed. The results of these adaptive management efforts is equally relevant to both *Psoroptes* and respiratory disease management.

Disease eradication efforts for respiratory disease and psoroptic mange are also different in the strategy that must be applied because of the dynamics of each disease. Research on domestic flocks shows that *Psoroptes* may infest every single animal necessitating treatment of each individual, while respiratory disease appears to be maintained by a few “super shedders” of *Mycoplasma ovipneumoniae*. However, effective treatment of respiratory disease has not been demonstrated and therefore those carrier animals must be found and removed through a test and cull type strategy (Bernatowicz et al. 2017). Both of these scenarios involve the capture and handling of each animal. The discovery of an effective oral treatment for *Psoroptes* creates the possibility of treating a herd remotely using a free-choice medicated salt lick or grain that could be strategically placed in the field to treat affected herds.

Interviews with wildlife professionals revealed several key barriers faced during implementation of management action against respiratory disease. These barriers fit into four basic categories: political and social, physical, resource shortages, and informational. The first of these involve a lack of political power or social will to execute a desired management action. These barriers impact respiratory disease management more so than *Psoroptes* management because of the key role that domestic sheep play in the epidemiology of respiratory disease. Despite this difference, interviews highlighted that stakeholder engagement can be a major challenge and that the human dimensions component of wildlife management needs to be addressed at the beginning of the management process in order to identify shared goals and objectives that will allow managers and stakeholders to work cooperatively towards management success.

The second category, physical barriers were those that prevent action outside of the human or social dynamics of the situation. For example, many bighorn sheep live in terrain that is inaccessible where wildlife capture is next to impossible. Capture of each animal in a herd is unrealistic in those areas. The development of effective anthelmintic treatments that can be

delivered remotely may help overcome this barrier for *Psoroptes* treatment. Resource shortages, the third category, refers to the high financial and time cost of intensive wildlife management interventions. This barrier has been addressed in some circumstances through collaboration between provincial and state management agencies and other groups such as academic institutions as is the case for this research program.

Finally, the many gaps in knowledge that still exist around respiratory disease and how best to manage it are represented by the informational barriers category. In Chapter 6 we demonstrated how an adaptive management approach, utilizing the 9 principles of adaptive management proposed by McFadden et al. (2011), can address these uncertainties and other barriers that deter management progress. Adaptive management should begin with a significant investment of time and energy into meaningful stakeholder engagement. Multiple management actions should then be implemented in a way that allows objective comparison and assessment of their ability to achieve predefined management goals. The development of a centralized body of wildlife management knowledge such as the one created by the Western Association of Fish and Wildlife Agencies (WAFWA) for respiratory disease in the form of the Adaptive West-wide Disease Management Venture (DMV) is a worthwhile investment to help achieve desired outcomes across bighorn range. Because *Psoroptes* represents a significantly smaller threat to bighorn sheep conservation than respiratory disease, and the fact that many of the disease isolation research priorities will be useful for both *Psoroptes* and respiratory disease, directing dedicated management effort to *Psoroptes* management in areas where both diseases are present will only act to further divide these already limited resources. Release of *Psoroptes* monitoring and translocation prevention recommendations through the WAFWA's DMV may be sufficient to meet most of the needs of this disease management scenario for all but the most heavily impacted herds.

7.3 Conclusion

The development of an adaptive management program for psoroptic mange in British Columbia is worthwhile. Numerous important governments and stakeholder groups should be consulted and invited to be involved in the creation of an adaptive management plan including BC fish and wildlife, Washington fish and wildlife, the Penticton Indian Band, the Okanagan Nation Alliance, local conservation groups such as the Wild Sheep Society of BC, willing biologists, academics, invested members of the public, and others. Together these groups can

establish shared goals and values that will guide a strategic roadmap to managing this disease. It is recommended that any future adaptive management program use the tools developed and information collected through this thesis to ascertain the actual geographic and herd specific extent of the parasite, limit its spread, further investigate treatment options and delivery methods, and evaluate suitability of herds to the application of a disease eradication effort.

Similar to the situation for respiratory disease management discussed in chapter 6, it would be important for this adaptive management effort to consider the entire disease triad: the agent, the host, and the ecosystem, and to include the larger human dimensions and social aspects when considering the ecosystem. Future studies are recommended to determine if wild sheep herds can be isolated from sources of disease introduction which would be valuable for both respiratory disease and *Psoroptes* management. Next, further evaluation of the *Psoroptes* ELISA specifically regarding its utility in detection of subclinical *Psoroptes* carriers and the use of this test in surveillance should be pursued. Additional evaluation of the true impacts of *Psoroptes* on the affected Canadian herds will help clarify the population level impacts of this parasite and the necessity of direct management action. Further research should also be directed at the use of fluralaner for this application to determine the duration of protection provided by the drug. A form of the drug suited to free choice consumption would be valuable, and research on whether there are ecosystem level consequences of herd-wide use of this drug or on non-target species. Opportunities for application of this information through disease eradication efforts on smaller isolated populations such as the Penticton Indian Band herd should be considered.

There is no doubt that any disease management effort aimed at tackling disease in free ranging wildlife situations is an extremely difficult challenge. Yet, I have also been humbled and amazed at the amount of support and enthusiasm displayed by people from all walks of life who are willing to donate their time and energy to wildlife projects. The work described in this thesis could not have happened without the time, effort, and money donated by a wide variety of people and companies who simply wanted to help wildlife in whatever ways they could. That support inspires me to persevere despite the numerous setbacks experienced throughout this PhD program and to encourage other passionate conservationists to work with me in tackling these tricky wildlife challenges into the future.

7.4 Literature Cited

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Appendix A: Cytochrome B Sequences

. = no sequence readable here

Group USA= (18RB15, 19081, 18LH20, 18RB33, 19901, 11RB08, 5158)
 Group Canada BHS = (F20, 2F, 4F, 10F)
 Group Rabbit = (PSC003, PSC001)
 GenBank accession # KJ957822- *Psoroptes Cuniculli* - Cyt B gene

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RABBIT1 .....
RABBIT2 .....GGTTAATAAGATAACGACTCTCACAAA
CAN BHS .....GGTTAATAAGATAACGACTCTCACAAA
GRP USA .....AGGTTAGGAGGATAACGACTCTCACAAA
GB-P. cun. TGTATGGGGGCTCTACAGGGTTTGCCTCCAATTTCATGTTAGCAGGATAACGACTCTCACAAA
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RABBIT1 .....
RABBIT2 ACATCAGAACGATAAATTTTTTTGTTGGGTTAAATTTTTTATTAAATTTTCTTTTTATTAAA
CAN BHS ACATCAGAACGATAAATTTTTTTGTTGGGTTAAATTTTTTATTAAATTTTCTTTTTATTAAA
GRP USA ACATCAGAACGATAAATTTTTTTGTTGGGTTAAATTTTTTATTAAATTTTCTTTTTATTAAA
GB-P. cun. ACATCAGAACGATAAATTTTTTTGTTGGGTTAAATTTTTTATTAAATTTTCTTTTTATTAAA
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RABBIT1 .....AAACCCCCCTAGTTTTGAGG
RABBIT2 CATAGTACTATTAAAGAAAGGATCGAAAGTCCTATAGCGCAAACCCCCCTAGTTTTGAGG
CAN BHS CATAGTACTATTAAAGAAAGGATCGAAAGTCCTATAGCGCAAACCCCCCTAGTTTTGAGG
GRP USA CATAGTACTATTAAAGAAAGGATCGAAAGTCCTATAGCGCAAACCCCCCTAGTTTTGAGG
GB-P. cun. CATAGCACTATTAAAGAAAGGATCGAAAGTCCTATAGCGCAAACCCCCCAGTTTTGAGG
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RABBIT1 GAATTGCCCGTAAAATGGCGTAGGCCAATAAGAAATATCACTCAGGTTGAATATGGGTTGG
RABBIT2 GAATTGCCCGTAAAATGGCGTAGGCCAATAAGAAATATCACTCAGGTTGAATATGGGTTGG
CAN BHS GAATTGCCCGTAAAATGGCGTAGGCCAATAAGAAATATCACTCAGGTTGAATATGGGTTGG
GRP USA GAATTGCCCGTAAAATGGCGTAGGCCAATAAGAAATATCACTCAGGCTGAATATGGGTTGG
GB-P. cun. GAATTGCCCGTAAAATGGCGTAGGCCAATAAGAAATATCACTCAGGTTGAATATGGGTTGG
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RABBIT1 GGTTGATGTTGGTATAGCTGGTGTGAAGTTTTCAACATCTCCTAGAAGGTTGGGGTTCAGA
RABBIT2 GGTTGATGTTGGTATAGCTGGTGTGAAGTTTTCAACATCTCCTAGAAGGTTGGGGTTCAGA
CAN BHS GGTTGATGTTGGTATAGCTGGTGTGAAGTTTTCAACATCTCCTAGAAGGTTGGGGTTCAGA
GRP USA GGTTGATGTTGGTCTAGCTGGTGTGAAGTTTTCAACATCTCCTAGAAGGTTGGGGTTCAGA
GB-P. cun. GGTGATGTTGGTATAGCTGGTGTGAAGTTTTCAACATCTCCTAGAAGGTTGGGGTTAAA
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RABBIT1 CACACCAAGTAGATAATTATTATTATTGATAACAAA
RABBIT2 CACACCAAGTAGATAATTATTATTATTGATAACAAA
CAN BHS CACACCAAGTAGATAATTATTATTATTGATAACAAA
GRP USA CACACCAAGTAGATAATTATTATTATTGATAACAAA
GB-P. cun. CAGATCAAGTAGATAATTATTATTATTGATAACAAA
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Appendix B: Cytochrome Oxidase 1 Sequences

. = no sequence readable here

Group USA= (18RB15, 19081, 18LH20, 18RB33, 19901, 11RB08, 5158)

Group CAN BHS = (F20, 2F, 4F, 10F)

GenBank accession # KJ957822- *Psoroptes Cuniculli* - COI gene

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BHS_CAN4 .....TTTAGTAGTTT
BHS_CAN1 AACATTATATTTTATCTTTGGTGTGTGAAGTGGTATATTGGGGACCAGGTTTAGTAGTTT
BHS_USA3 AACATTATATTTTATCTTTGGTGTGTGAAGTGGTATATTGGGGACCAGGTTTAGTAGTTT
BHS_USA4 .....GGTGTGTGAAGTGGTATATTGGGGACCAGGTTTAGTAGTTT
GB-P.cun. AACATTATATTTTATCTTTGGTGTGTGAAGTGGTATATTGGGGACCAGATTAGTAGTTT
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BHS_CAN4 TATTCGGTTAGAGTTGTCTCAACCTGGTGACCTCTTAATAGATTATGATTACTATAATTC
BHS_CAN1 TATTCGGTTAGAGTTGTCTCAACCTGGTGACCTCTTAATAGATTATGATTACTATAATTC
BHS_USA3 TATTCGGTTAGAGTTGTCTCAACCTGGTGACCTCTTAATAGATTATGATTACTATAATTC
BHS_USA4 TATTCGGTTAGAGTTGTCTCAACCTGGTGACCTCTTAATAGATTATGATTACTATAATTC
GB-P.cun. TATTCGGTTAGAGTTGTCTCAACCTGGTGACCTTTAATAGATTACGATTACTATAATTC
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BHS_CAN4 TGTGTACTGCTCATGCTTTTATTATAAATTTTTTTTATAGTTATACCTATTATAATAGG
BHS_CAN1 TGTGTACTGCTCATGCTTTTATTATAAATTTTTTTTATAGTTATACCTATTATAATAGG
BHS_USA3 TGTGTACTGCTCATGCTTTTATTATAAATTTTTTTTATAGTTATACCTATTATAATAGG
BHS_USA4 TGTGTACTGCTCATGCTTTTATTATAAATTTTTTTTATAGTTATACCTATTATAATAGG
GB-P.cun. TGTGTACTGCTCATGCTTTTATTATAAATTTTTTTTATAGTTATACCTATTATAATAGG
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BHS_CAN4 GGGTTTTGGTAATTTATTTGGTACCACCTAATGATTGGTGCAACTGATATGGCTTATCCTCG
BHS_CAN1 GGGTTTTGGTAATTTATTTGGTACCACCTAATGATTGGTGCAACTGATATGGCTTATCCTCG
BHS_USA3 GGGTTTTGGTAATTTACTGGTACCACCTAATGATTGGTGCAACTGATATGGCTTATCCTCG
BHS_USA4 GGGTTTTGGTAATTTACTGGTACCACCTAATGATTGGTGCAACTGATATGGCTTATCCTCG
GB-P.cun. GGGTTTTGGTAATTTACTAAATACCACCTAATGATTGGTGCAACTGATATGGCTTATCCTCG
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BHS_CAN4 TTTAAATAATAAAGTTTTTGGCTTCTTCCCCCATCTTTGTCATTACTTATTAGATCTGC
BHS_CAN1 TTTAAATAATAAAGTTTTTGGCTTCTTCCCCCATCTTTGTCATTACTTATTAGATCTGC
BHS_USA3 TTTAAATAATAAAGTTTTTGGCTTCTTCCCCCATCTTTGTCATTACTTATTAGATCTGC
BHS_USA4 TTTAAATAATAAAGTTTTTGGCTTCTTCCCCCATCTTTGTCATTACTTATTAGATCTGC
GB-P.cun. TTTAAATAATAAAGTTTTTGGCTTCTTCCCCCATCTTTGTCATTACTTATTAGATCTGC
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BHS_CAN4 TGTGGTAGGTTGTGGAGTGGGGACCGGTTGAAGTGTCTATCCCCCTTATCTAATG....
BHS_CAN1 TGTGGTAGGTTGTGGAGTGGGGACCGGTTGAAGTGTCTATCCCCCTTATCTAATGGTAT
BHS_USA3 TGTGGTAGGTTCTGGGGTGGGGACTGGTTGAAGTGTCTATCCCCCTTATCTAATGGTAT
BHS_USA4 TGTGGTAGGTTCTGGGGTGGGGACTGGTTGAAGTGTCTATCCCCCTTATCTAATGGTAT
GB-P.cun. TGTGGTAGGTTGTGGAGTGGGGACCGGTTGAAGTGTCTATCCCCCTTATCTAATGGTAT
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BHS_CAN4 .....
BHS_CAN1 TTTTCACCATGGCCCAGCGGTTGATTTTGGTATTTTAAGCCTTCATATCGCTGGTATTTTC
BHS_USA3 TTTTCACCATGGCCCAGCGGTTGATTTTGGTATTTTAAGCCTTCATATCGCTGGTATTTTC
BHS_USA4 TTTTCACCATGGCCCAGCGGTTGATTTTGGTATTTTAAGCCTTCATATCGCTGGTATTTTC
GB-P.cun. TTTTCACCATGGCCCAGCGGTTGATTTTGGTATTTTAAGCCTTCATATCGCTGGTATTTTC
  
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BHS_CAN4 .....
BHS_CAN1 TTCAATTTTAGGGGCTATTAAATTTTATTGTTACTATTTTTAATATGAAAGTTGAAGGGAT
BHS_USA3 TTCAATTTTAGGGGCTATTAAATTTTATTGTTACTATTTTTAATATGAAAGTTGAAGGGAT
BHS_USA4 TTCAATTTTAGGGGCTATTAAATTTTATTGTTACTATTTTTAATATGAAAGTTGAAGGGAT
GB-P.cun.TTCAATTTTAGGGGCTATTAAATTTTATTGTTACTATTTTTAATATGAAAGTTGAAGGGAT
*****

BHS_CAN4 .....
BHS_CAN1 AAAATGATCTAATGTTCCTTTGTTTGTTTGATCTGTATTTATTACTTCTTTTCTTTTACG
BHS_USA3 AAAATGATCTAATGTTCCTTTGTTTGTTTGATCTGTATTTATTACTTCTTTTCTTTTACG
BHS_USA4 AAAATGATCTAATGTTCCTTTGTTTGTTTGATCTGTATTTATTACTTCTTTTCTTTTACG
BHS_USA1 AAAATGATCTAATGTTCCTTTGTTTGTTTGATCTGTATTTATTACTTCTTTTCTTTTACG
GB-P.cun.AAAATGATCTAATGTTCCTTTGTTTGTTTGATCTGTATTTATTACTTCTTTTCTTTTACG
*****

BHS_CAN4 .....
BHS_CAN1 TTTTTCCTTTACCTGTTTTAGCAGCAGCTTTAACTATGTTATTAACAGACCGAAATTTTAA
BHS_USA3 TTTCTCTTTACCTGTTTTAGCAGCAGCTTTAACTATGTTATTAACAGACCGAAATTTTAA
BHS_USA4 TTTCTCTTTACC.....
GB-P.cun.TTTTTCCTTTACCTGTTTTAGCAGCAGCTTTAACTATGTTATTAACAGACCGAAATTTTAA
*** *****

BHS_CAN4 .....
BHS_CAN1 TTCTACATTCTTTGACCCATTGGGGTGGTGACCCATTCTTTATCAACATCTTTTC
BHS_USA3 TTCTACATTCTTTGACCCATTGGGGTGGTGACCCATTCTTTATCAACATCTTTTC
BHS_USA4 .....
GB-P.cun.TTCTACATTCTTTGACCCATTGGGGTGGTGACCCATTCTTTATCAACATCTTTTC
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