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Pathogen Prevalence in Domestic Sheep in Western Nebraska: Implications for Bighorn Sheep Conservation and Coexistence on a Multi-Use Landscape

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PATHOGEN PREVALENCE IN DOMESTIC SHEEP IN WESTERN NEBRASKA: IMPLICATIONS FOR BIGHORN SHEEP CONSERVATION AND COEXISTENCE ON A MULTI-USE LANDSCAPE

BY

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THESIS ACCEPTANCE PAGE
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This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABSTRACT

PATHOGEN PREVALENCE IN DOMESTIC SHEEP IN WESTERN NEBRASKA: IMPLICATIONS FOR BIGHORN SHEEP CONSERVATION AND COEXISTENCE ON A MULTI-USE LANDSCAPE

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Bighorn sheep (*Ovis canadensis*) and domestic sheep (*Ovis aries*) are members of the same genus and thus share multiple pathogens that can be spread between them. One specific respiratory pathogen of concern is *Mycoplasma ovipneumoniae* (*M. ovipneumoniae*), which has been linked to pneumonia epizootics in bighorn sheep that are often characterized by all age die offs upon initial exposure followed by years of low lamb recruitment. Domestic sheep have been identified as one carrier of *M. ovipneumoniae* with transmission between sheep species occurring when there is close contact on the landscape. To prevent this cross-species transmission, importance has been placed on keeping bighorn sheep and domestic sheep spatially and/or temporally separate. On a landscape of multiple uses that is comprised mainly of privately owned land, this is a difficult goal to achieve. To understand the challenges that arise with having both domestic and wild sheep species in the same landscape, I sent a survey to domestic sheep producers. To understand the perceptions and attitudes of ranchers and hobby farmers about bighorn sheep and their potential to participate in conservation initiatives. Survey results revealed a positive response from domestic sheep producers in support of a wild and domestic sheep advisory committee working toward healthy co-existence of domestic and wild sheep on a shared landscape. Using a Risk of Contact (ROC) tool, we also identified areas on the landscape where domestic and bighorn sheep

are at greatest risk of contact and therefore management focus should be placed on investigating pathogen and disease prevalence while working towards prevention of pathogen spread. Lastly, we examined different strain types of *M. ovipneumoniae* found in domestic and bighorn sheep in western Nebraska to estimate spatial prevalence and possible transmission events of *M. ovipneumoniae*. Through reducing disease prevalence and/or potential for disease transfer from domestic sheep to bighorn sheep and small management changes, we aim to promote coexistence of healthy domestic sheep and bighorn sheep on the western Nebraska landscape.

STUDY SITE

For this research our area of interest was a region of the western high plains. The western high plains are an ecoregion that has a semi-arid to arid climate, annual precipitation ranges from 33-51 cm (Chapman et al. 2001). Natural vegetation is primarily dominated by drought tolerant short-grass prairies paired with large areas of mixed-grass prairies. Large bluffs, escarpments and exposed areas of bedrock make up the landscape (Chapman et al. 2001). Within the western high plains, the two study areas for this project were the Pine Ridge and Wildcat Hills regions, which contain domestic sheep (*Ovis aries*) operations and free-ranging bighorn sheep (*Ovis canadensis*) herds.

The Pine Ridge is in Northwestern Nebraska, near the cities of Chadron and Crawford in the Upper Niobrara-White Nebraska natural resource district. This area spans approximately 161 km though the counties of Dawes, Sioux, and Box Butte. Habitat for the area is comprised of large, steep sandstone and siltstone bluffs, escarpments, and ponderosa pine (*Pinus ponderosa*) woodlands (Chapman et al 2001). Elevation of areas with steep topography range from 1,100-1,500 meters above mean sea level (Wood et al. 2022) with average elevation of 793 meters (Nebraska Department of Agriculture (NDA) 2020, NGPC). As part of the western high plains, ponderosa pine, in association with mixed grass prairie, is found throughout the escarpment areas, on ridge tops, north-facing and east-facing slopes and, in lesser density, on south and west facing slopes (Chapman et al. 2001). Agriculture and livestock play a large role on the multi-use landscape surrounding state parks and national forests (NDA 2022). Livestock on the landscape is primarily comprised of cattle and calve operations. Domestic sheep make up a small number of the livestock found on the landscape. The total number of cattle and

calves in all counties that are included in the study area for the Pine Ridge area was 154,405 (USDA NASS Livestock Inventory [31 December 2017]). In Dawes County, the total number of sheep and lambs was 2,525 (USDA NASS Livestock Inventory [31 December 2017]), in Box Butte County, the total number of sheep and lambs was 1,120 (USDA NASS Livestock Inventory [31 December 2017]), and in Sioux County the total sheep and lambs was 1,896 (USDA NASS Livestock Inventory [31 December 2017]). A grand total of 5,541 sheep and lambs in all counties was included in the Pine Ridge study area in 2017. This area is home to multiple small subherds of bighorn sheep that have been battling disease outbreaks that result in decrease in populations and low lamb recruitment (NGPC, Unpublished data). Many of the domestic sheep operations involved in the study are located near these herds of bighorn sheep.

The second study area for this project was the Wildcat Hills. This area is located approximately 177 km south of the Pine Ridge area. The Wildcat Hills spans approximately 88.5 km starting at the Nebraska-Wyoming border extending East through the counties of Scottsbluff, Morrill, and Banner. This study site is in the North Platte Nebraska natural resource district. Like the Pine Ridge area the habitat for this area includes the high prairie, open grasslands, rangelands, steep bluffs and escarpments. A mixture of sandy and loamy soils supports a combination of mixed-grass prairie and ponderosa pine woodlands on ridge tops and side slopes (Chapman et al. 2001). Similar to the Pine Ridge, elevation of areas with steep topography range from 1,100-1,500 meters above mean sea level (Wood et al. 2022) and an average elevation of 793 meters (NDA 2020, NGPC). Agriculture and livestock play a large role in these counties too, making it a multi-use landscape primarily comprised of privately owned land (NDA

2022). In the Wildcat Hills study area, the total number of cattle and calves was 302,909 (USDA NASS Livestock Inventory [31 December 2017]); in Scotts Bluff County the total number of sheep and lambs was 1,239 (USDA NASS Livestock Inventory [31 December 2017]), in Morill County the total sheep and lambs was 262, and in Banner county the data in the livestock inventory census was withheld to avoid disclosing data for individual operations (USDA NASS Livestock Inventory [31 December 2017]). There was a grand total of 1,501 sheep and lambs in all counties included in the Wildcat Hills study area in 2017. Nebraska is comprised of 97% private land (Nebraska Game and Parks Commission (NGPC Open Fields and Waters [OFW] 2021). This area is home to a thriving herd of bighorn sheep. While disease is present in domestic sheep operations, recent studies (NGPC, Unpublished data) show the herds of bighorns sheep are less impacted by disease in this area.

The annual average temperature for this region in Nebraska is 8.05-10.2 degrees Celsius (NOAA 2022). In both study areas the summer months are hot and dry. The winter months are cold, windy, with an average annual snowfall of 6.15 cm (NOAA 2022).

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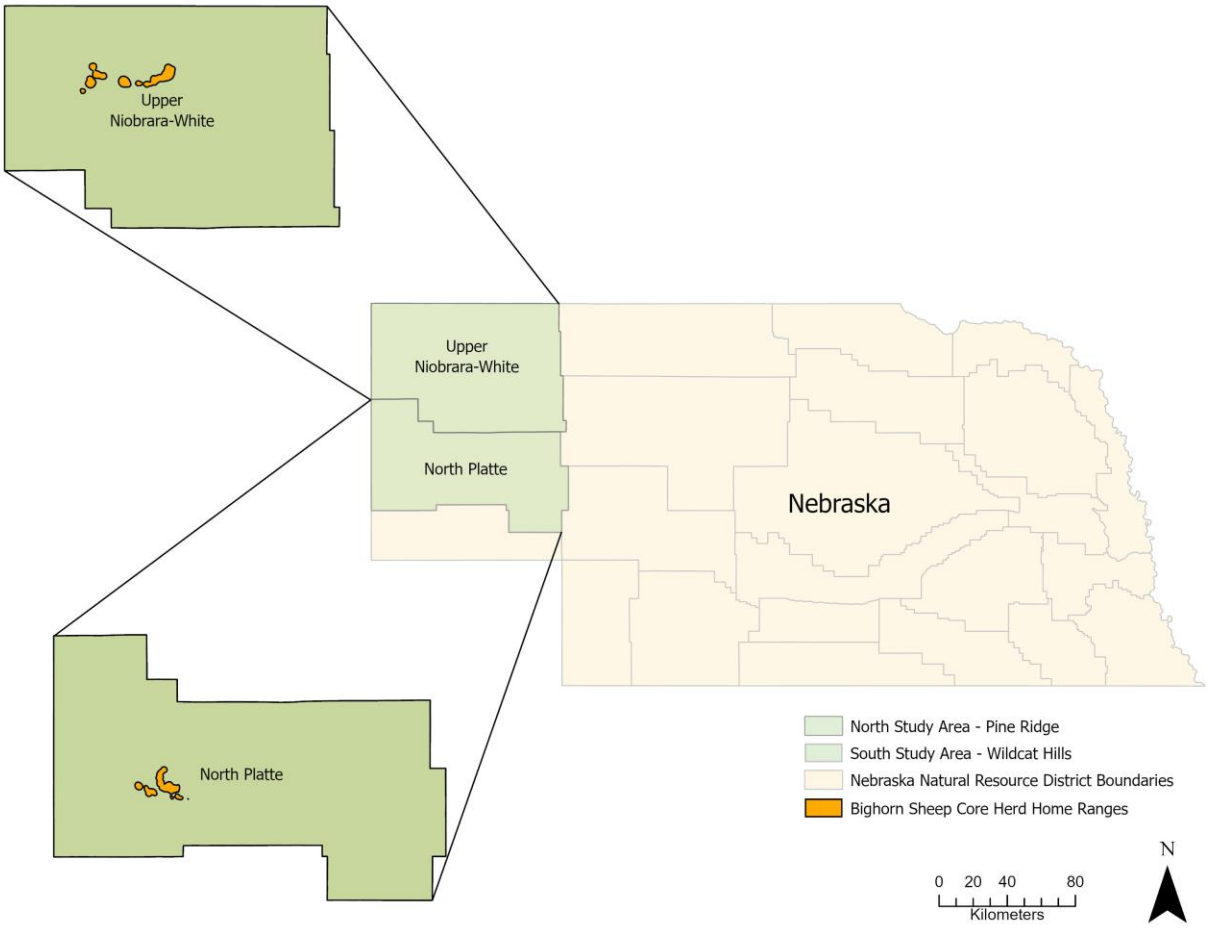

Figure 1: Study sites, Pine Ridge and Wildcat Hills in Western Nebraska.

CHAPTER 1: DOMESTIC SHEEP PRODUCTION AND BIGHORN SHEEP CONSERVATION IN WESTERN NEBRASKA: A SURVEY FOR SHEEP PRODUCERS IN WESTERN NEBRASKA

INTRODUCTION

Bighorn Sheep (*Ovis canadensis*) and domestic sheep (*Ovis aries*) are members of the same genus and share numerous pathogens (Ward et al. 1990, Besser et al. 2013). Along with sharing pathogens, bighorn and domestic sheep co-occur throughout the western United States. Bighorn sheep currently occupy only a small portion of their historical range (Buechner et al. 1960, Heinse et al. 2016). This decline is due to over hunting, habitat fragmentation, and other disruptions, including various disease events (Brewer et al. 2014). One pathogen of particular concern is *Mycoplasma ovipneumoniae*.

M. ovipneumoniae has been linked to epizootic pneumonia disease events in bighorn sheep (Besser et al. 2012, Besser et al. 2012, Cassirer et al. 2018, Kamath et al. 2019, Besser et al. 2021). Upon initial exposure a 10-90% mortality occurs in all age groups of bighorn sheep, followed by high lamb mortality and decreased recruitment persisting for years to follow (Besser et al. 2013, Cassier et al. 2013). Domestic sheep have been identified as carriers of *M. ovipnuemoniae* (McAuliffe et al. 2003). *M. ovipnuemoniae* infections in domestic sheep range from asymptomatic or mild symptoms to significant production loss and disruptions on domestic sheep operations (Manlove et al. 2019).

Domestic sheep most commonly exhibit a mild coughing syndrome affecting lambs (WADDL 2022), while bighorn sheep of all ages are highly sensitive to the pathogen (Cassirer et al. 2017, WADDL 2022). With vaccination and treatment proving

insufficient to prevent widespread population declines of bighorn sheep in North America (Callan et al. 1991, Cassirer et al. 2017), management of pathogens and pneumonia in domestic sheep may be an effective alternative. Focusing on domestic sheep could allow for a more accessible, less costly, and perhaps more accurate assessment of interactions between the two species and provide a means of addressing disease concerns. Transmission occurs due to proximity or contact on a shared landscape (Besser et al. 2014, Cassirer et al. 2018). Much of the scientific discussion has focused on keeping bighorn and domestic sheep spatially separate (Foreyt et al. 1994, Schommer and Woolever 2008, Clifford et al. 2009). For example, efforts attempt to keep domestic and bighorn sheep spatially separate through the management of public grazing allotments throughout the western United States (Schommer and Woolever 2008). Within a landscape of multiple uses comprised mainly of private land, this has proven to be a challenging and difficult goal to achieve. Small scale farming, hobby farms, and recreational farms have become increasingly popular, which has increased the risk of pathogen spillover from smaller domestic sheep operations to bighorn sheep and should be addressed and managed to reduce pathogen prevalence and transmission between species (Zehnder et al. 2006).

Nebraska is 97% privately owned land (NGPC OFW 2021), leading to an overlap between bighorn sheep habitat and domestic sheep operations. Nebraska is home to 2 herds of bighorn sheep located in the western part of the state and this area is also heavily used for agriculture, including cattle and domestic sheep production. Guidelines outlined by the Western Association of Fish and Wildlife Agencies (WAFWA 2012) recommend maintaining a 14.5-km buffer between domestic and wild sheep populations to minimize

the risk of inter-species contact and disease transmission (WSWG 2012). Analysis of existing bighorn sheep movement data in Nebraska indicated rams were nearly always located within the recommended 14.5 km buffer distance of parcels containing domestic sheep/goats (Morrison et al. 2016); and there is evidence that spillover events occurred in both herds in Nebraska.

To help manage and prevent future spillover events we aim to reduce disease prevalence in domestic sheep to promote coexistence of healthy domestic and wild sheep in western Nebraska. Starting by gathering information, we sent surveys to domestic sheep producers that were near bighorn sheep herds. The goal of the survey was to collect perceptions and attitudes of ranchers and hobby farmers with domestic sheep and operations located in western Nebraska regarding their concerns of pneumonia and *M. ovipnuemoniae* in domestic and bighorn sheep. The first objective was to investigate operation type, location, longevity, and general knowledge and concern about respiratory pathogens, specifically *M. ovipnuemoniae,* on their domestic sheep operations. The second objective was to measure the level of concern domestic sheep producers had with pathogen transmission between both species on a shared, multi-use landscape. The final objective was to measure the level of support domestic sheep producers had for management changes and conservation strategies that aid bighorn sheep conservation. With the results of the survey, we aim to work with domestic sheep producers to reduce or eliminate *M. ovipnuemoniae* in domestic flocks and aid in bighorn sheep conservation.

METHODS

Questionnaire Design

The survey questionnaire was constructed on a 43.2 X 27.9 cm paper folded in half to form a 21.6 X 27.9 cm booklet style questionnaire that was stapled along the spine. The questionnaire was 8 pages in length and contained 20 questions (Appendix 1). Survey questions were created based on the information needed to aid in bighorn sheep conservation and investigate pathogen prevalence. The final draft was approved by all collaborative parties.

The questionnaire contained 4 general sections with an additional 3- 8 questions under each section. The first section "General description of Your Sheep Operation" identified what type and size of domestic sheep operation was owned by the survey recipients. Within this section, the general location of the operation was requested relative to proximity to the Pine Ridge or Wildcat Hills regions of Nebraska. Producers were asked if their operation was located within 16 km (10 miles) or greater than 16 km from Pine Ridge/Wildcat Hills regions. Next, producers were asked how long they anticipated having domestic sheep on their properties. Finally, the survey recipient was asked if they had any prior involvement in the research project; if they allowed their sheep to be tested, were asked but declined, or were not asked to be involved (see Chapter 2).

The next section "Your Concerns about the effects of pneumonia and *M. ovipnuemoniae* on domestic sheep" measured the level of concern and knowledge towards the effects of pneumonia and *M. ovipnuemoniae* in domestic sheep operations. Producers were asked in general if they were familiar with *M. ovipnuemoniae* prior to the

survey being received. We provided information on *M. ovipnuemoniae* to producers on the first page of the survey (Figure 1.1).

Figure 1.1*: M. ovipneumoniae information given to domestic sheep producers in the beginning of the survey.*

> *Mycoplasma ovipneumoniae* (*M. ovi.*) is a respiratory pathogen of domestic sheep, domestic goats, bighorn sheep, and mountain goats. *M. ovi* causes atypical pneumonia and can also predispose sheep and goats to lung infections with many other bacterial species. *M. ovi* infection of domestic sheep and goats is typically associated with mild disease, most often a 'coughing syndrome' of lambs and kids under 6 months of age. Less frequently, *M. ovi* is associated with severe or fatal pneumonia in domestic lambs and adult ewes and rams. *M. ovi* infection is associated with pneumonia in all ages of bighorn sheep upon initial exposure, and often causes recurrent fatal lamb pneumonia outbreaks in subsequent years.

We asked if their operation had been negatively affected by respiratory pathogens such as *M. ovipnuemoniae* that cause pneumonia and to what degree. Next producers were asked their level of concern about a possible negative effect of *M. ovipnuemoniae* on the following aspects of their domestic sheep operation: survival of lambs, survival of adult sheep, lowering lamb rates, birth weight of lambs, rate of weight gain in lambs, or carcass quality.

The third section, "Bighorn Sheep in Western Nebraska" measured the level of importance domestic sheep producers place on having bighorn sheep on the landscape and the level of concern of potential contact and disease/pathogen spread, including pneumonia/*M. ovipnuemoniae* between their domestic sheep and bighorn sheep. Producers were asked how often they observe bighorn sheep on or near their operations, the level of importance producers placed on having bighorn sheep on the landscape in both the Pine Ridge and Wildcat Hills regions, and the level of concern of *M.*

ovipnuemoniae affecting herds of bighorn sheep in both the Pine Ridge and Wildcat Hills regions. Moreover, producers were asked the level of concern they have for bighorn sheep contacting their domestic sheep. The next 4 questions asked about the producer's level of concern regarding general pathogen transmission from bighorn sheep to their sheep and then from their sheep to bighorn sheep. The same question was asked but with regard specifically to *M. ovipnuemoniae* bacteria transmission from bighorn sheep to their sheep and from their sheep to bighorn sheep.

The fourth and final section, "Your Opinion about future Conservation Initiatives and Strategies Regarding Bighorn Sheep in western Nebraska", measured the level of support domestic sheep producers would provide when investigating different potential conservation initiatives and management changes to promote co-existence of domestic sheep and bighorn sheep on the multi-use landscape. Producers were asked what their level of support would be for a domestic sheep/bighorn sheep advisory committee or working group and who would be acceptable representatives. This list included domestic sheep producers, Nebraska Goat and Sheep Producers Association members, Nebraska Game and Parks Commission biologists, Nebraska Extension Educators, 4-H Superintendents, US Department of Agriculture employees, and Nebraska Department of Agriculture employees. The questionnaire provided a line for producers to write in any additional representatives they believed should be included. Producers were asked if they would accept any funding that was available to aid in offsetting the costs associated with any management actions. Potential funding to offset the costs could come from private or non-profit organizations or funding from a government agency. The final question in this section and the survey provided producers with a list of potential management actions

that would reduce or eliminate *M. ovipnuemoniae* and or pneumonia in their sheep. The management actions included the use of a vaccine if available, the use of an antibiotic treatment that targeted *M. ovipnuemoniae* or other respiratory pathogens, the use a medicated feed or supplement that targets *M. ovipnuemoniae* or respiratory pathogens (if legal in the region), the practice of the producer "closing" their flock to exchanging sheep with neighboring sheep or other operations, erecting fences on their property that would provide a second barrier between domestic sheep and bighorn sheep, periodic testing for *M. ovipnuemoniae* in their domestic sheep operation, testing sheep that were sick or have died to check for the presence of *M. ovipnuemoniae,* practice husbandry methods that include quarantine and/or testing new sheep received into the existing flock, and finally culling sheep that have tested positive for *M. ovipnuemoniae*.

A letter located on the inside cover of the questionnaire provided information on the survey contents, research, and the collaborators involved. The letter provided some instructions for the respondents, stressing to producers to not provide their name or address on the survey. This letter also provided additional details on the research project, myself, and the collaborators involved including contact information; and producers were encouraged to contact me with any questions. After review by South Dakota State University IRB committee, the questionnaire did not meet the Federal definition of human subjects research because no personal data was being collected and IRB approval was not required prior to the survey questionnaire being mailed to respondents (4 September 2020).

Survey Implementation

Large manilla envelopes were used to mail the surveys; envelopes measured 22.86 cm X 30.48 cm, with a return address for South Dakota State University Biostress Laboratory and correct postage attached. Recipients of the survey were asked to respond to all questions or send back a blank document if they were not interested in participating in the research. For producers to return the survey, a self-addressed and pre-paid postage envelope that measured 10.48 cm X 24.13cm was enclosed.

Surveys were administered to domestic sheep producers residing in Dawes County, Sioux County, Scottsbluff County, and Morill County, Nebraska. These domestic sheep producers were selected due to previous involvement with the research by having their sheep tested. Other addresses were provided from county 4-H rosters and Nebraska Game and Parks Commission biologists. The goal was to survey as many domestic sheep producers as possible; however, the actual total number of domestic sheep producers in western Nebraska was unknown in these counties. Surveys were delivered by mail (USPS) with a pre-paid and addressed envelope for return. The initial survey was sent on 29 April 2021. This was followed by a post card reminding producers to complete the survey, sent only to producers who had not yet responded. The reminder post cards were sent by mail on 24 May 2021. Finally, a second copy of the survey was sent on 17 June 2021 to producers who had not responded to the survey, with a return deadline of 2 August 2021. Questionnaires were numbered for tracking the returned survey. With this method of managing the returned surveys, multiple mailings of the survey were sent to non-respondents only.

Statistical analysis

Due to the small sample size (n = 23), I only performed descriptive statistics for responses to questions, and rounded percentages to the nearest whole number.

RESULTS

Sample size and Response Rate

The final sample size for domestic sheep producers in western Nebraska was 59 surveys. Five of the surveys were supplied to the Nebraska Game and Parks Commission's Alliance office to have available if a domestic sheep producer did not receive a survey but wanted to participate; however, no questionnaires were distributed from the Nebraska Game and Parks Commission Office. One survey was undeliverable and returned. With those questionnaires removed the total sample size was 53 surveys. A total of 23 surveys was returned from domestic sheep producers, for a final response rate of 43%. The small sample size, unknown true population parameter, and relativity low response rate for this survey, are all possible sources leading to a nonresponse bias. This was taken into consideration when evaluating the results of the generalized attitude and perceptions of domestic sheep producers in western Nebraska.

Demographic and general description of sheep operations: Questions 1-4

Male and female producers were included in the survey sample size, but the gender or age of respondents was not solicited. Questions 1-4 in the survey inquired about the general description of the producer's sheep operations. About 26% of producers indicated they operated a production flock that included more than 50 sheep, whereas 30% indicated they operated a hobby flock with 5-50 sheep. Moreover, 30% of the producers indicated they managed a 4-H flock containing 1-10 sheep and 13% of the

producers selected the "other" option, specifying their operation was seasonal or had sheep for herd dog training purposes.

Within the four different descriptions of domestic sheep operations, producers had the option to check whether they processed their sheep for meat or wool. Of the producers who responded, 21% chose wool and 47% chose meat. Relative to region of Nebraska, 56% of producers were from the Pine Ridge area and 30% were from the Wildcat Hills area; 13% of the producers selected other, indicating their operation was outside of the 2 listed areas. In addition to the general location of the operation, 15% of respondents were located greater than 16 km from the Pine Ridge region, whereas 53% were within 16 km of the Pine Ridge region. Regarding the Wildcat Hills, 57% of respondents were located greater than 16 km from the region, whereas 42% of the operations were located within 16 km of the Wildcat Hills. Eight percent of the domestic sheep producers anticipated having sheep on their property for 1-3 years, whereas 17% of domestic sheep producers, we surveyed, anticipated having sheep on their property for 4-6 years, and 13% of domestic sheep producers anticipated having sheep on their property for 7-10 years. About 60% of domestic sheep producers anticipated having sheep on their property for more than 10 years. The majority of the domestic sheep producers anticipated continuing to raise domestic sheep on their property over the next 10 years.

Approximately 52% of the domestic sheep producers who responded indicated that they were a cooperator in the previous study and allowed their sheep to be tested as part of the research (see Chapter 2), whereas 13% of the domestic sheep producers who responded were asked but declined to be a cooperator in the study and have their sheep

tested. A total of 30% of the domestic sheep producers who responded were not asked to be a cooperator in the study and had no previous involvement in the research.

Producers concerns about the effects of pneumonia and M. ovipnuemoniae on domestic sheep: Questions 5-7

In the next section of the survey for domestic sheep producers, we aimed to measure the level of knowledge of the respiratory pathogen *M. ovipnuemoniae* (Table 1.1). Most of the producers who responded were familiar with the pathogen prior to receiving the survey; 78% of the domestic sheep producers were familiar with the respiratory pathogen *M. ovipnuemoniae* and 21% of the domestic sheep producers were not familiar with the respiratory pathogen *M. ovipnuemoniae*. About 43% of domestic sheep producers had no negative effects from a respiratory pathogen such as *M. ovipnuemoniae* that caused pneumonia, the remaining 57% of producers stated they were unsure if there had been any negative effects on their operation or they had a small number of negative effects from a respiratory pathogen such as *M. ovipnuemoniae* on their sheep operation.

Table 1.1*: Survey results from 23 respondents describing their level of concern about any possible negative effects of Mycoplasma ovipneumoniae on different domestic sheep operations. Results are percentages and are rounded to the nearest whole number.*

Domestic Sheep Operations	None	Small	Moderate	Large	No Opinion
Survival of Lambs	13	30	17	26	0
Survival of adult sheep	13	34	26	17	0
Lowering lamb rates	17	34	8	26	0
Birth weight of lambs	21	34	8	17	4
Rate of gain in lambs	13	30	8	26	4
Carcass Quality	21	34	8	21	4

Majority of domestic sheep producers placed no or a small level of concern on any possible negative effects from *M. ovipneumoniae* in all aspects of domestic sheep operations. However, producers had a large level of concern of *M. ovipneumoniae* negatively affecting the survival of lambs, lowering lamb rates, and rate of weight gain in lambs.

Bighorn Sheep in Western Nebraska: Questions 8-15

The next section of the survey addressed the topic of bighorn sheep in western Nebraska. First domestic sheep producers were asked how often they see bighorn sheep on or near their operation. Sixty-nine percent of producers never saw bighorn sheep near or on their domestic sheep operation whereas 27% of producers rarely or sometimes saw bighorn sheep near or on their domestic sheep operation. Only 4% of producers often saw bighorn sheep near or on their domestic sheep operation. No domestic sheep producers responded they observed bighorn sheep on or near their domestic operation very often. While the domestic sheep producers do not frequently see bighorn sheep on or near their operation 26-30% of producers place a high level of importance on having bighorn sheep on the landscape. While 30-39% placed a moderate importance on having bighorn sheep on the landscape, 4-13% of producers placed no importance, slight importance, or had no opinion on having bighorn sheep on the landscape.

The results measuring the level of concern domestic sheep producers have on *M. ovipnuemoniae* negatively affecting bighorn sheep populations were separated into the two different study areas, the Pine Ridge and Wildcat Hills regions of Nebraska (Table 1.2).

Table 1.2*: Survey results from 23 respondents describing their level of concern domestic sheep producers have about M. ovipnuemoniae negatively affecting bighorn sheep populations. Results are percentages and are rounded to the nearest whole number.*

Study Area Location	Not at all	Slight	Moderate	Very	No Opinion
Pine Ridge	13	21	17	26	17
Wildcat Hills	8	17	21	17	17

In both study areas, the majority of the domestic sheep producers who took the survey placed slight to a very high level of concern on *M. ovipnuemoniae* negatively affecting bighorn sheep populations or had no opinion on *M. ovipnuemoniae* negatively affecting bighorn sheep populations.

Domestic sheep producers were asked their level of concern bighorn sheep would contact their domestic sheep; 47% had no concern at all, while the remaining 53% had slight, moderate, or were very concerned. Next, the question asked the level of concern for transmission of *M. ovipnuemoniae* from bighorn sheep to their domestic sheep (Table 1.3). Over half of producers had no concern at all at the potential of *M. ovipnuemoniae* transmission from domestic sheep to bighorn sheep or transmission of any pathogen, including *M. ovipnuemoniae*, from domestic sheep to bighorn sheep.

Table 1.3*: Survey results from 23 respondents describing their level of concern regarding contact and pathogen transmission between domestic sheep and bighorn sheep. Results are percentages and are rounded to the nearest whole number*

Domestic Sheep and Bighorn Sheep Contact and Transmission	Not at All	Slight	Moderate	Very	No Opinion
Contact will be made between domestic sheep and bighorn sheep.	47	21	21	8	0
Transmission of *M. ovipnuemoniae* from bighorn sheep to domestic sheep.	43	30	13	17	0
Transmission of *M. ovipnuemoniae* from domestic sheep to bighorn sheep.	56	13	8	21	0
Transmission of any disease or pathogen from bighorn sheep to domestic sheep.	35	22	26	17	0
Transmission of any disease or pathogen from domestic sheep to bighorn sheep.	52	13	17	17	0

Overall domestic sheep producers had no to a low level of concern on pathogen transmission amongst the two species.

Opinions about future conservation initiatives and strategies regarding bighorn sheep in Western Nebraska: Questions 16-20

In the final section of the survey questionnaire, domestic sheep producers were asked what their level of support would be for a domestic sheep and bighorn sheep advisory committee/working group. Over half of the producers responded with providing moderate or a strong level of support for the formation of a working group. Should an advisory committee/working group form, domestic sheep producers were asked who they would accept as representatives for the working group. The list included domestic sheep

producers, Nebraska Goat and Sheep Producers Association, Nebraska Game and Parks Commission biologists, Nebraska Extension Educators, 4-H Superintendents, USDA Department of Agriculture, and Nebraska Department of Agriculture. All representatives were acceptable, or producers had no opinion on the representatives. There was an option for the domestic sheep producer to write in any other representatives they thought should be a part of the working group. A representative from the Nebraska Veterinary Association or a local veterinarian was the only additional response regarding membership in the working group.

Producers were asked if they would accept any funding to offset the costs of any management changes done to reduce or eliminate *M. ovipneumoniae* (Table 1.4). Eight percent of the domestic sheep producers responded they would not accept funding from private/nonprofit organizations or government agencies to offset the costs associated with management actions to promote healthy co-existence, 87% responded they would accept or maybe accept funding from a private/nonprofit organization or government funding.

Table 1.4*: Survey results from 23 respondents describing if producers would accept funding to offset any costs associated with management actions on their operation to promote the healthy coexistence of domestic sheep and bighorn sheep. Results are percentages and are rounded to the nearest whole number.*

Type of potential funding	No	Maybe	Yes	No Opinion
Private or non-profit	8	48	39	4
Government	8	48	39	4

The final question listed 10 potential management changes producers would make to reduce or eliminate *M. ovipnuemoniae* and/or pneumonia (Table 1.5).

Table 1.5*: Survey results from 23 respondents describing potential management changes to reduce or eliminate M. ovipneumoniae in domestic sheep operations. Results are percentages and are rounded to the nearest whole number.*

Potential Management Action	No	Maybe	Yes	Undecided
The use of a new **vaccine** that would target *M. ovipnuemoniae* or other respiratory pathogens if available.	0	47	52	0
The use of an **antibiotic treatment** that would target *M. ovipnuemoniae* or other respiratory pathogens.	0	34	65	0
The use of a **medicated feed, supplement or block** that would target *M. ovipnuemoniae* or other respiratory pathogens if available for legal use in the area.	8	26	65	0
"Closing" your flock to the practice of exchanging sheep with neighboring sheep producers/owners.	30	34	17	17
Erecting fences on your property that would prevent contact between wild bighorn sheep and your sheep.	47	30	17	4
Periodic *M. ovipnuemoniae* testing of your sheep.	4	34	56	1
M. ovipnuemoniae **testing of sheep within your operation that are sick or that have died**.	4	21	60	13
Quarantining new sheep prior to turning them in with your existing flock.	17	17	65	0
M. ovipnuemoniae **testing new sheep** prior to turning them in with your existing flock.	8	26	60	4
Culling sheep that have tested positive for *M. ovipnuemoniae*.	14	21	52	8

Of the management actions proposed, producers were willing or potentially willing to participate and adopt the management changes. However, a relatively high percent of producers stated they did not support erecting additional fencing (47%) and closing their flock to the practice of exchanging sheep with neighboring sheep producers/owners (30%).

DISCUSSION

Sample size and domestic sheep in Nebraska

For this research finding the total number of domestic sheep producers residing in the 5 counties was a challenge, and the exact number still is unknown. The 2017 census reported a total of 63,043 domestic sheep in 1,153 farms throughout the entire state of Nebraska. The Nebraska Goat and Sheep Producers association western membership includes only 15 producers; however, this does not include all producers in western Nebraska. To investigate the total population of domestic sheep producers, contact was made with the county extension offices, Nebraska Department of Agriculture, and USDA. Some information was available, such as the total number of sheep and lambs in the State; however, the total number of producers was not available and further research on this topic would aid in livestock and wildlife conflict management.

Paired with the challenge of finding the total number of domestic sheep producers in western Nebraska, the lower response rate (43%) can yield inaccurate results due to missing data leading to a nonresponse bias (Fisher 1996, Gigliotti and Fopma 2019, Vaske 2008). If the non-respondents are significantly different from the survey respondents on the key parameters measured in the survey a non-response bias can play a role when using the results of the survey to estimate population characteristics (Berg 2005, Gigliotti and Fopma 2019, Kreuter 2013). However, a low response rate does not always signify poor quality data (Gigliotti and Fopma 2019, Groves 2006, Groves et al. 2006). Nonresponse is less likely to be an issue in fairly homogeneous survey populations (Becker and Iliff 1983, Gigliotti and Fopma 2019, Kreuter 2013). In many cases, reasons for nonresponse are unrelated to differences between respondents and nonrespondents on

the survey topic, in which case nonresponse may not necessarily lead to significant survey error (Connelly et al. 2003, Czajka and Beyler 2016, Gigliotti and Henderson 2015, Greer et al., 2000, Groves 2006). Gigliotti and Fopma (2019) reported that for surveys used to provide a general description of a population, potential nonresponse bias is less of an issue; however, if important public policy or management decisions are to be made from survey results, they recommend that additional effort should be made to measure and correct for potential nonresponse bias in future surveys (Dillman et al. 2014).

In the Pine Ridge and Wildcat Hills regions the most common livestock species on the landscape is cattle and calves. The most recent livestock inventory census by county was completed by the USDA in 2017. Total number of cattle and calves in all counties that are included in the study area for the Pine Ridge was 154,405 compared to 5,541 sheep and lambs (USDA NASS Livestock Inventory [31 December 2017]). In the Wildcat Hills study area, the total number of cattle and calves was 302,909 (USDA NASS Livestock Inventory [31 December 2017]) in comparison to 1,501 sheep and lambs (USDA NASS Livestock Inventory [31 December 2017]) on the landscape. While there were significantly less sheep and lamb operations in the Pine Ridge and Wildcat Hills compared to cattle and calves, the small number of sheep and lambs can pose a threat to the bighorn sheep that reside in the county through the risk of pathogen transmission. Domestic goats can also pose a risk to bighorn sheep, they are proven to be a carrier of *M. ovipneumoniae* (Besser et al. 2013, Cassirer et al. 2017). The 2017 livestock inventory showed a total number of 2,048 domestic goats in the 5 counties ((USDA NASS Livestock Inventory [31 December 2017])). For this research we did not

include domestic goats and focus was placed on sheep and lamb production in the panhandle of Nebraska. Domestic goats can pose a serious threat for pathogen transmission and for future studies a similar survey sent to domestic goat producers would be helpful in management decisions.

Data gathered from our survey showed over half of the domestic sheep producers represent small operations, housing 1-50 sheep in the flock. A small number of the producers represent larger operations that contain flock sizes of more than 50 sheep. It is estimated most domestic sheep producers in Nebraska have flock sizes of 100 sheep or less. Small flocks still possess the potential for a high pathogen prevalence and high genetic diversity of *M. ovipnuemoniae* strain types making the risk of spillover events difficult to manage (Kamath et al. 2019). With producers anticipating having sheep on the landscape for years to come and as bighorn sheep populations remain on the landscape this highlights the importance and need for a healthy co-existence of domestic sheep and bighorn sheep on the landscape and the need for continued management and pathogen surveillance.

Many of the producers who were recipients of the survey also had participated in previous research by allowing their sheep to be tested. Previous testing performed in 2018-2020 collected 402 nasal swabs from domestic sheep located in the 5 different counties, 199 of the swabs were PCR tested for *M. ovipnuemoniae* and 26 of the positives were further strained typed (see *M. ovipnuemoniae* strain type, Chapter 2, for additional information and results on testing). Just over half (52%) of the producers who allowed testing were included as survey respondents.

Providing brief information on the effects of *M. ovipnuemoniae* on domestic sheep and bighorn sheep was helpful as 22% of respondents were unfamiliar or did not have any prior knowledge of the respiratory pathogen prior to receiving the survey. When attempting to achieve and maintain a healthy co-existence of domestic sheep and bighorn sheep more education on the respiratory pathogen effects and potential transmission would be useful in management decisions. The domestic sheep and bighorn sheep working group could help fill this gap and provide education on *M. ovipnuemoniae* and the effects in domestic sheep and bighorn sheep. In comingling studies between domestic sheep or mouflon and bighorn sheep, bighorn sheep had a near 100% mortality within 90 days of comingling contact with domestics that were *M. ovipnuemoniae* positive (Besser et al. 2014). A similar study was completed with the domestic sheep and bighorn sheep comingled with all sheep being free of *M. ovipnuemoniae*; survival of bighorn sheep in this study was significantly higher, only 1 out of 4 bighorn sheep exhibited respiratory symptoms and died (Besser et al. 2012). The three other bighorn sheep remained symptomless until experiment termination at day 104 (Besser et al. 2012). The risk of bighorn sheep pneumonia following contact with domestic sheep can be reduced significantly if the domestic sheep are free of *M. ovipnuemoniae* (Besser et al. 2012). Promotion of the management actions discussed in the survey to reduce or eliminate *M. ovipnuemoniae* can aid in bighorn sheep conservation, promoting a healthy co-existence on a shared landscape.

Domestic Sheep operations

The consequences of *M. ovipnuemoniae* infections in domestic sheep operations have been described as mild to proliferative (in Smith, 2014 Large Animal Internal

Medicine, in Radostits et al. 2006 *Veterinary Medicine,* Manlove et al., 2019). These consequences not only have effect on animal health but also operation productivity. The results of our survey from domestic sheep producers in western Nebraska provided that 43% of producers stated their operation had no negative effects from a respiratory pathogen such as *M. ovipnuemoniae* that caused pneumonia, the remaining 57% of producers stated they were unsure if respiratory pathogens such as *M. ovipnuemoniae* had a minimal effect on their sheep operation. This could be due to domestic sheep only showing mild to no symptoms when infected (Besser et al. 2019; Manlove et al. 2019). However, respiratory disease is a serious problem for domestic sheep production being the fifth highest source of lamb loss (USDA-APHIS, 2014, 2015a). In the survey, producers were asked about the different aspects that would commonly be part of a domestic sheep operation. The level of concern changed depending on type of operation. Lamb survival, adult sheep survival, lowering lambing rates, lowering lamb birth weight, rate of weight gain in lambs, and carcass quality are all herd performance metrics of domestic sheep operations that can be negatively affected by *M. ovipnuemoniae*. Results varied, with majority of producers having a small level of concern that *M. ovipnuemoniae* would have a negative effect on their operation. *M. ovipnuemoniae* in domestic sheep can cause atypical pneumonia and leave domestic sheep susceptible to other bacterial lung infections (Besser et al. 2008, 2012). One of the producers requested to see more research completed on the effects of *M. ovipnuemoniae* on domestic sheep reproduction. Additional research on this topic would provide domestic sheep producers more information and aid them in managing their ewe/lamb operations.

Bighorn sheep

Domestic Sheep operations surveyed in this study ranged from 1.6-23.3 km from the Pine Ridge bighorn sheep core herd home ranges. Domestic sheep operations that had sheep tested in the previous portion of the study in the Wildcat Hills ranged from 3.6 - 37.6 km from the Wildcat Hills bighorn sheep core herd home ranges. Most of the producers were within the 16 km proximity buffer but stated they never or rarely saw bighorn sheep near their operation; only a small proportion of producers stated they sometimes or often saw bighorn sheep near their operation. Pathogen transmission can occur when wild and domestic species are located within 14.5 km (WSWG 2012). Transmission of the pathogens leading to the disease in bighorn sheep occur when the two species come into contact (Onderka and Wishart 1988, Foreyt 1990, Callan et al. 1991). Transmission of M. *ovipnuemoniae* is thought to be primarily through direct contact, though the pathogens have been shown to spread simply by proximity (Besser et al. 2014, Felts 2020) in an experimental setting. Even though producers do not see bighorn sheep regularly, producers placed a moderate importance level on having bighorn sheep in the Pine Ridge and Wildcat Hills regions of Nebraska.

The levels of concern domestic sheep producers have on *M. ovipnuemoniae* negatively affecting bighorn sheep herds in both the Pine Ridge and Wildcat Hills regions ranged from no concern at all to a slight concern, moderate concern, and very concerned. Equally, some of the domestic sheep producers had no opinion on *M. ovipnuemoniae* affecting bighorn sheep populations in the area. Providing additional education on the lasting consequences of *M. ovipnuemoniae* on bighorn sheep herds may promote a higher level of concern on the effects of respiratory pathogens in bighorn sheep. Information such as *M. ovipnuemoniae* causing recurrent fatal lamb pneumonia outbreaks for

subsequent years to follow initial exposure (Cassirer et al. 2013) may aid in gaining support for bighorn sheep conservation. Questions 11-15 in the survey asked domestic sheep producers the level of concern they place on contact and pathogen transmission occurring between species. Most of the producers (52%) had no concern about *M. ovipnuemoniae* or general disease/pathogen transmission amongst wild and domestic species. The survey indicated producers placed a little more concern on *M. ovipnuemoniae* or general disease/pathogen transmission from bighorn sheep to their domestic sheep than pathogen transmission from domestic sheep to bighorn sheep. Domestic sheep free of *M. ovipnuemoniae* pose minimal risk to bighorn sheep (Besser et al. 2012a; Heinse et al. 2016). Working towards a goal of healthy disease-free domestic sheep on the landscape can help conserve bighorn sheep through reducing or eliminating pathogen transmission.

Conservation

As we work towards a goal of healthy co-existence between bighorn sheep and domestics on a multiple use landscape, the strongest support for this outcome could be paired with the support from producers for a wild sheep/domestic sheep working group/advisory committee. Over half (62%) of the respondents showed moderate to strong support for a working group and accepted the list of representatives to be included in the working group. One additional representative to add would be a member from the Nebraska Veterinary Association or local veterinarian. A veterinarian could provide key insight on the disease process as well as work with biologists if an increase of respiratory disease was observed in domestic sheep flocks in the area and aid in testing if needed. When it comes to making minor management changes within a domestic operation to

reduce or eliminate *M. ovipnuemoniae* producers would be willing to accept some funding to offset this cost if it was available.

In the survey, some management changes were listed to reduce or eliminate *M. ovipnuemoniae* or pneumonia within domestic operations to support a healthy co-existence of both species on the landscape. An overall positive response from domestic sheep producers to participate and make management changes resulted from the survey. All producers who responded to the survey said they would be willing or potentially willing to use a vaccine, antibiotic treatment, or medicated feed if it were available and legal for use. Studies have investigated the safety and immunogenicity of a vaccine, adverse reaction to immunizations were minor and local, and provided evidence that immunization with large antigenic mass combined with an adjuvant can induce active antibody response in ewes and passively immunize lambs (Ziegler et al. 2014). However, at this time there is no vaccine or treatment protocol approved to combat *M. ovipnuemoniae* infections in domestic sheep (Johnson et al. 2022). In addition, no studies have been published on the use of an antibiotic treatment of *M. ovipnuemoniae* in vivo (Johnson et al. 2022). Caution would need to be taken for those operations who process sheep for meat as withdrawal times and vaccine status would need to adhere to standards prior to slaughter. Other bacterial disease vaccine withdrawal times range from 21-60 days and is dependent on the vaccine (Tizard et al. 2021). If using medicated feed for the treatment of respiratory symptoms, there is a risk of antibiotic resistance forming (Love et al. 2011). This concern was brought up by producers, caution should be taken if this management change is put in place. These 3 management options were mostly accepted by producers, proving to be good options when discussing potential management

changes, and also supporting the need for additional research on the use of antibiotics, vaccines, and medicated feed in domestic sheep for the treatment of *M. ovipnuemoniae*.

Of all the management options provided, domestic sheep producers showed the least support for fencing and closing flocks to exchange (30-47% said NO to erecting extra fences and "closing" their flock to exchanging sheep with neighboring operations or producers). This was due to the cost and labor it would entail. Studies in other areas have had high success if help and funding was given to producers to aid in offsetting the cost of materials and labor for erecting the additional fences. Producers closing their flock would limit potential breeding opportunities with neighboring sheep operations and/or getting new sheep through exchanging sheep with neighboring operations. Testing sick or deceased sheep can provide information for the flock owner and biologists. If sheep are ill and showing respiratory symptoms, there are several different respiratory diseases that can account for the illness and having potential diagnostic answers can help the producers manage the flock and prevent illness from spreading to other sheep in the flock and potentially to bighorn sheep. Another time period when testing is helpful for producers is when they acquire or purchase new sheep. Often when sheep are purchased and travel is involved, sheep are more susceptible to illness from the stress of travel (Sevi et al. 2001). Quarantining and testing new sheep prior to turning them out with the existing flock can prevent disease transmission and aid in documenting illness prior to the illness becoming a more serious problem. Periodic testing of healthy sheep can provide necessary data for pathogen prevalence and surveillance found in domestic sheep operations, as many of the sheep positive for *M. ovipnuemoniae* will show little to no symptoms.

Testing for *M. ovipnuemoniae* is generally a simple process; when working with producers the optimal time to perform testing is when sheep will be run through a chute or caught for shearing, confined for lambing, or in hand for other reasons. Using a dry swab to swab both nostrils is quick, generally the sheep tolerate this procedure well. Further research could be done on pathogen prevalence in domestic herds depending on the time of the year and if change in prevalence occurs in response to reproduction. The final management change is to cull sheep that test positive for *M. ovipnuemoniae*; domestic sheep producers showed a strong support (52%) for this option. If the management change was to cull positive sheep, a trade out program or compensation may help producers and provide more support for this management option. Having a *M. ovipnuemoniae* free zone is a lengthy goal but has been accomplished in other species. In the swine industry *M. hyopneumoniae* is one of the most prevalent and economically significant respiratory pathogens (Holst et al. 2015). Efforts have been made to eradicate *M. hyopneumoniea* in farms creating certified *M. hyopneumoniae* free operations. This was completed with the use of treatments and/or vaccines (Holst et al. 2015). Similar options could be investigated for large scale domestic sheep operations with the goal of having *M. ovipnuemoniae* free operations near areas of bighorn sheep habitat. When considering management changes, producers and other members of the domestic sheep/bighorn sheep advisory committee could discuss and develop optimal solutions for producers, working towards having a healthy co-existence between free-ranging bighorn sheep and domestics on the landscape. Management actions range from making small changes to more invasive changes to help reduce or eliminate *M. ovipnuemoniae* in a domestic sheep flock. These management actions are obtainable by working individually

with each producer to tailor the management change to their specific flock needs. Management actions taken provide support in reducing disease prevalence in domestic sheep herds and obtaining a healthy co-existence between domestics and bighorn sheep.

This research provides the groundwork for a domestic sheep and wild sheep working group/advisory committee. Further education on the consequences of respiratory pathogen *M. ovipnuemoniae* for domestic sheep producers could be done at 4-H events, providing informative brochures, working with local veterinarians, and the Nebraska Goat and Sheep Producers Association. On a landscape of multiple uses, working together is key to achieve the goal of healthy domestic sheep and bighorn sheep coexisting on the landscape.

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CHAPTER 2: DOMESTIC SHEEP SAMPLING AND STRAIN TYPING OF *MYCOPLASMA OVIPNEUMONIAE* IN DOMESTIC SHEEP AND BIGHORN SHEEP IN WESTERN NEBRASKA

ABSTRACT

Archived domestic sheep samples were used to investigate the pathogen prevalence in domestic sheep operations in western Nebraska near the Pine Ridge and Wildcat Hills areas of Nebraska that are inhabited by bighorn sheep. We collected samples throughout February and March 2018-2020 at private operations. In May and August of 2019, we collected samples at 3 different 4-H weigh-in events/fairs. These samples represented 14 different operations in the Pine Ridge region near Chadron, Nebraska and 24 operations in the Wildcat hills region near Scottsbluff, Nebraska. We collected 402 samples of those samples, 199 were PCR tested for *M. ovipneumoniae*, and 26 of the PCR *M. ovipneumoniae* detected samples were further strain typed using a multi loci sequence typing (MLST) approach. We evaluated the effect of pathogen status (*M. ovipneumoniae* detected vs non detected) on rate of weight gain in domestic sheep at the three 4-H events. Multi-Locus Sequence Typing (MLST) a portion of the PCR Positive *M. ovipneumoniae* samples allowed an evaluation of the pathogen diversity in both domestic and bighorn sheep. We hypothesized domestic sheep flocks in Nebraska would have a high genetic diversity of strain types within operations.

INTRODUCTION

Infectious disease has influenced bighorn sheep (*Ovis canadensis*) population dynamics (Cassirer et al. 2018); pathogen transmission between domestic sheep and bighorn sheep is thought to have occurred as European settlers expanded westward (Grinenell 1928, Kamath et al. 2019). Bighorn sheep are susceptible to infectious agents carried by domestic sheep, this is not unexpected given the genetic similarity between the two species (Pedersen et al. 2007, Cassirer et al. 2018). Domestic hosts are key risk factors for pathogen spillover and associated disease-induced population declines in wildlife (Pedersen et al. 2007, Cassirer et al. 2018, Kamath et al. 2019). *Mycoplasma ovipneumoniea* (*M. ovipneumoniae*) is strongly linked to pneumonia outbreaks in bighorn sheep and is thought to facilitate the respiratory disease (Dassanayake et al. 2010, Besser et al. 2013, 2014). Domestic sheep are a known carrier of *M. ovipneumoniae* and source of pathogen infection to naïve bighorn sheep populations (Kamath et al. 2019).

Pneumonia outbreaks in bighorn sheep often have profound effects on herd population dynamics with initial mortality across all age groups ranging from 10-90% (Besser et al. 2013, Cassirer et al. 2013). Following these initial mortality events, high lamb mortality and decreased recruitment may persist for years, further depressing population growth and increasing risk of local extinction (Cassirer and Sinclair 2007, Sells et al. 2015). Disease outbreaks have occurred in free-ranging wild sheep populations post contact with domestic sheep, which paired with 12 domestic-wild sheep commingling experiments conducted have resulted in similar deadly results for bighorn sheep (Wehausen et al. 2011, Besser et al. 2012a, Cassirer et al. 2018). Results of previous studies show, that management of bighorn sheep also involves management of

pathogen transmission from domestic sheep (Council for Agricultural Science and Technology 2008, Western Association of Fish and Wildife Agencies Wild Sheep Working Group 2012, The Wildlife Society 2015, Cassirer et al. 2018). Management of pathogens and pneumonia in domestic sheep may be an effective alternative to combat pathogen transmission between species. Focusing on domestic sheep could allow for a more accessible, less costly, and perhaps more accurate assessment of interactions between the two species and provide a means of addressing the disease concerns. Genetic data from pathogens have recently proven valuable for gaining insights into pathogen spillover and transmission between livestock and wildlife (Kamath et al. 2019). Our study seeks to provide insight into reducing disease prevalence in domestic sheep to promote coexistence of healthy domestic and wild sheep in western Nebraska. We investigated the prevalence and spread of pathogens linked to pneumonia within flocks of domestic sheep on ranches and hobby farms near wild, free-ranging bighorn sheep herds in the Pine Ridge and Wildcat Hills regions of the State.

METHODS

Samples collected from private operations

The participating operations included private production, hobby, and 4-H flocks located in Pine Ridge and Wildcat Hills study sites in western Nebraska. Samples were collected onsite, and the sampling protocol for sample collection at the operations was tailored to the producer's requests and flock style. Each producer and sheep were administered a unique ID number to keep the owner's information confidential relative to disease status and location of their operation. At each private operation additional information was collected on the body condition of the sheep and if there was visible

nasal discharge or coughing observed when collecting samples. Adult sheep were tested when it suited the producer best, usually prior to lambing when producers were "working" their sheep or running the sheep through a chute. Adult sheep were run single file down a chute system or confined in a smaller pen where they could easily be hand grabbed and restrained. Producers secured the head of the animal while the samples were collected. With the animal safely restrained, a single dry cotton swab was carefully inserted into the nasal cavity of the sheep with precautions taken to not touch the outer portion of the nose or sides of the nasal cavity. Once the swab was fully inserted into the nare of the sheep, the swab was rotated 360 degrees 2-3 times in the nasopharyngeal area to collect the sample. The swab was removed and then inserted into the other nare of the sheep and the same procedure was repeated. The swab was carefully placed back into the paper sheath and placed in a plastic Ziplock bag labeled with the producers ID and sheep ID and placed in a cooler. At least 1 swab was collected, but, if the flock owner allowed and sheep tolerated sampling, we repeated multiple nasal swabs for archival purposes. If we collected more than 50 nasal swabs at the two larger operations, a random selection of 7-30 swabs were selected for *M. ovipneumoniae* PCR testing. Domestic sheep samples collected were representative of lambs, ewes, and rams. Single swabs were selected for *M. ovipneumoniae* PCR testing and were shipped overnight to Washington Animal Diagnostic Disease Lab (WADDL) on ice packs. All handling and sample collection from the animals followed IACUC guidelines and were approved by the South Dakota State University Institutional Animal Care and Use Committee (Approval No. 18-035 A).

Samples collected at 4-H weigh in events

In 2019, sheep were weighed, and samples were collected from domestic sheep participating in the 4-H county sheep shows using the same sampling protocol that was used at private operations; this was completed at 3 county 4-H sheep shows. Dawes county is in the northern study area near the Pine Ridge region. Weights and nasal swabs were collected on 32 sheep. Sheep of all ages and sex were included when we collected nasal swabs and weights. The 32 sheep were representative of 9 different flocks located in the county. Initial weights were obtained on all the sheep on 1 June 2019, followed up with a second weight obtained at the Dawes County Fair, 4-H Sheep Show on 1 August 2019. A minimum of 1 sheep representing each operation was sampled, if an operation had multiple sheep represented at the weigh in, up to 4 sheep were sampled and tested for *M. ovipneumoniae* via PCR.

Scottsbluff and Morill counties are located in the southern study site near the Wildcat Hills. Weights and nasal swabs were collected on 63 sheep, representative of 21 different flocks located in these counties. Sheep of all ages and sexes were included in sampling. Initial weights were obtained on all the domestic sheep participating in the Morrill County Fair on 4 May 2019 and domestic sheep participating in the Scottsbluff County Fair on 5 May 2019, followed up with a second weight obtained at the County Fair 4-H sheep shows held on 24 July 2019 in Morrill County and 31 July 2019 in Scottsbluff County. A minimum of 1 sheep representing each operation was sampled, if an operation had multiple sheep at the weigh in, up to 8 sheep were sampled and tested for *M. ovipneumoniae* via PCR.

Across all counties, nasal swabs for *M. ovipneumoniae* PCR testing were collected at the initial weigh-in, and 60-84 days later the second weight of the sheep was collected at the 4-H fair. Following the same protocol used when sampling the private operations, sheep were given an individual identification number, and then samples were labeled with their ID and stored in a cooler. Subsequently, they were refrigerated overnight prior to being sent to WADDL for PCR testing. Samples were sent to the lab on ice packs the day following sampling was completed. Permission for sheep to be tested was acquired by the guardians and 4-H participants prior to samples being collected. All the samples collected at 4-H weigh in events were sent for PCR testing. These methods also were approved by the South Dakota State Institutional Animal Care and Use Committee (Approval No. 18-035 A).

Domestic Sheep Mortality

To further monitor pathogens in domestic sheep flocks, we asked the producer to contact a technician if they had observed sick sheep or any mortalities in their operation. If notified of sick or deceased sheep, we collected information, pictures, and samples on these cases. In cases involving mortalities, carcasses were obtained to collect a sample of lung tissue to send with nasal swabs to WADDL. All samples collected were sent for *M. ovipneumoniae* PCR testing with additional histopathology completed on a small number of samples when lung tissue was also submitted.

Bighorn Sheep Samples

Samples were collected during 2018-2020 from bighorn sheep at winter captures, mortalities, and opportunistically using a protocol of swabbing the nasopharyngeal area

in both nares. Samples were sent to WADDL for *M. ovipneumoniae* PCR testing with a subset of the positive samples further strain typed using the same MLST approach.

PCR detection of M. ovipneumoniae

Samples were tested at WADDL using real-time PCR for detection of *M. ovipneumoniae*. This method was developed by WADDL, and the real-time assay can detect 6 cfu/ml of *M. ovipneumoniae* with high sensitivity and specificity (WADDL 2022, Kamath et al. 2019, Manlove et al. 2019). Results of the real time PCR were interpreted as follows: 'detected' if the cycle threshold score (CT value) was 36 or lower, 'indeterminate' for CT values between 36 and 40, and 'not detected' for a CT of 40 (Kamath et al. 2019). Indeterminate results may be caused by sampling or transport issues, low level of shedding at time of collection, PCR inhibitors such as dirt, or in rare cases, cross-reacting *Mycoplasma* species (WADDL 2022).

Multi-Locus Sequence Typing (MLST) Strain Typing

For samples from which *M. ovipneumoniae* was detected, we conducted further diagnostics at WADDL to characterize *M. ovipneumoniae* strains using partial DNA sequences with a suite of PCR primers that targeted the 16S- 23S intergenic spacer region (IGS), the small ribosomal subunit (16S), and the genes encoding RNA polymerase B (rpoB) and gyrase B (gyrB). These 4 loci exhibit sequence polymorphism and could be used independently or together as a highly discriminatory test to determine different strain types of *M. ovipneumoniae* (Cassirer et al. 2017).

Data Analysis

To investigate the pathogen prevalence on a domestic sheep operation level, we used the mean percent of infected individuals for the domestic sheep operations in the

Pine Ridge and Wildcat Hills regions. We tested for correlation between the weight gain of the sheep at weigh-ins and PCR pathogen status on the sheep tested at 4H weigh in events. Due to a non-normal distribution and not all assumptions being met by the data for common parametric approaches, we used a non-parametric Kruskal Wallis test to analyze this data for differences in weight gain based on *M. ovipneumoniae* infection status. When assessing pathogen diversity, the strain types were determined to be the same strain if there were less than 4 differences when comparing base pairs using a pair-wise evaluation of detected strains (Kamath et al. 2019). A phylogenetic tree was completed using a multiple sequence alignment using program software, Clustal Omega (Sievers et al. 2011). The DNA sequences of the strain types were imported into the multiple sequence alignment software that uses seeded guide trees and Hidden Markov Model (HMM) profile-profile techniques to generate alignments. Specifically, phylogeny trees used for data visualization of the strain types were completed using Tree View software (Page 1996). This information was used and compared with bighorn sheep strain types identified using the same MLST approach.

RESULTS

Pathogen prevalence

We collected 402 nasal swabs from domestic sheep, representing flocks in both study areas. Of these, 199 of those nasal swabs were submitted for *M. ovipneumoniae* PCR testing. Forty percent (n = 80) of the samples were positive for *M. ovipneumoniae* detected, 41% (n = 83) were negative for *M. ovipneumoniae*, and 19% (n = 36) were considered indeterminate. These results do not include domestic sheep mortality data (n = 4) because there was low compliance by producers reporting sick sheep or mortalities for

M. ovipneumoniae sample testing. Nasal swabs were collected from domestic sheep represented 14 flocks throughout the Pine Ridge and 24 flocks in the Wildcat Hills area. In the Pine Ridge area, 76 samples were PCR tested for *M. ovipneumoniae*, of which 30% (*n* = 23) were *M. ovipneumoniae* positive, 50% (*n* = 38) were negative, and 20% (*n* = 15) were indeterminate. For the Wildcat Hills region, 126 nasal swab samples were PCR tested for *M. ovipneumoniae*; 45% (*n* = 57) of these were *M. ovipneumoniae* positive, 37% (*n* = 46) were negative, and 18% (*n* = 23) were indeterminate. For indeterminate samples, it is recommended to have the sheep retested using the *M. ovipneumoniae* PCR. For this study, none of the domestic sheep were retested based on a result of indeterminate. By grouping each 4-H event as its own "operation", there were 11 operations (8 private operations + 3, 4-H events) in the study, and only 1 operation did not have *M. ovipneumoniae* detected by PCR, whereas 10 out of the 11 (90%) operations had at least 1 PCR *M. ovipneumoniae* detected sample within their domestic sheep flock.

4H pathogen prevalence and weight gain

We collected 92 samples along with obtaining weights of domestic sheep at 3 different 4-H weigh in/show events. The Dawes County 4-H weigh in event was in the Pine Ridge study area. Nasal swab samples and weights were obtained from 29 (*n* = 29) domestic sheep at this event. Of these, 24% (*n* = 7) tested positive for *M. ovipneumoniae*, 76% (*n* = 22) were negative, and no samples were found to be indeterminate. Scottsbluff County and Morrill County 4-H events were located in the Wildcat Hills study area. Nasal swabs and weights were obtained on 35 (*n* = 35) sheep in Scottsbluff County and 28 (*n* = 28) in Morrill County. Nasal swabs were submitted to WADDL for *M. ovipneumoniae* testing via PCR. At the Scottsbluff County 4-H event, 77% (*n* = 27) of

the samples had *M. ovipneumoniae* detected, 14% (*n* = 5) were negative, and 8% (*n* = 3) of the samples were found to be indeterminate. At the Morrill County 4-H event, 53% (*n* = 15) of the samples had *M. ovipneumoniae* detected, 35% (*n* = 10) were negative, and 11% (*n* = 3) were indeterminate. Results of the Kruskal Wallis test were non-significant (*n* = 58, p-value = 0.2571). Results of the correlation test were non-significant (*n* = 58, p-value = 0.3769, cor=0.12). Sheep with a non-detected *M. ovipneumoniae* pathogen status had a similar rate of weight gain compared to sheep that had a detected *M. ovipneumoniae* status. The mean rate of weight gain for *M. ovipneumoniae* detected domestic sheep was 0.28 kg compared to an average weight gain 0.29 kg for domestic sheep that tested negative for *M. ovipneumoniae*.

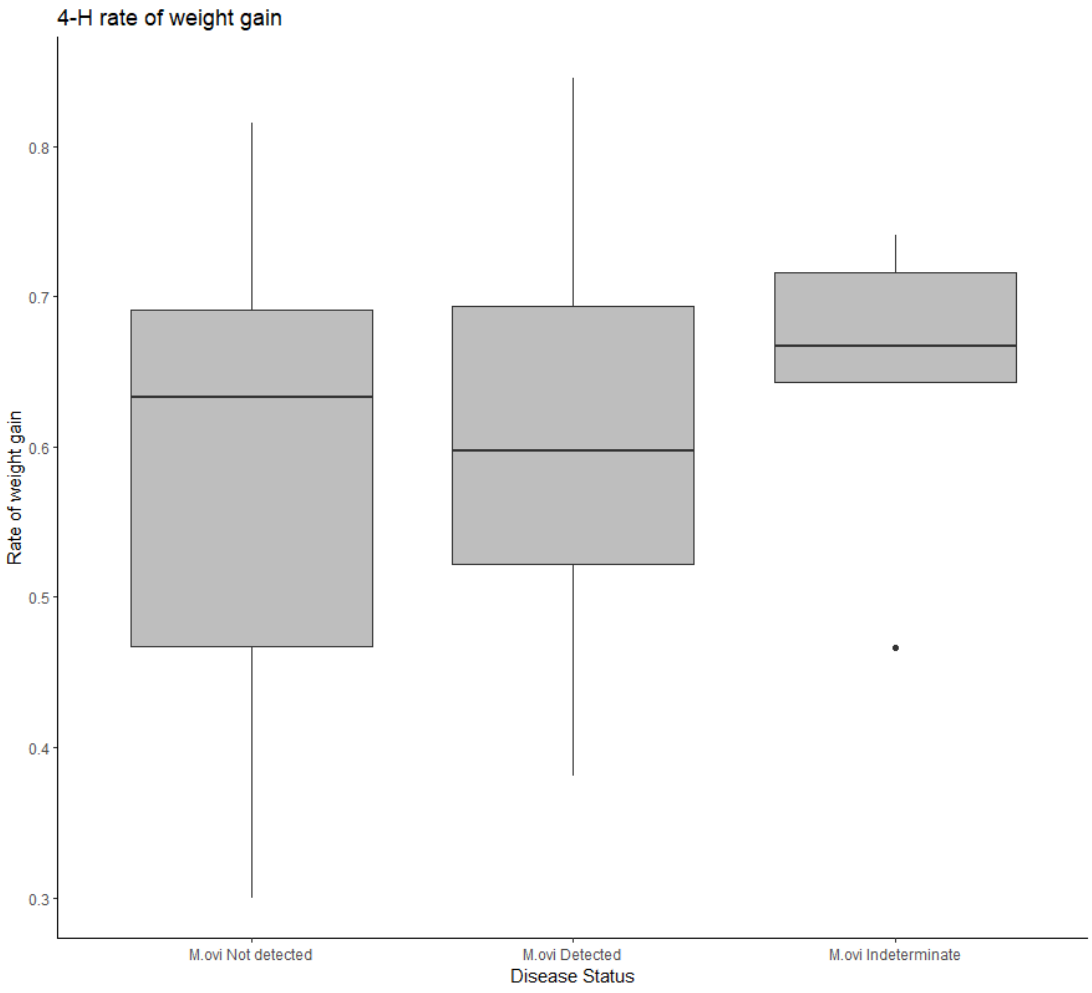

Figure 2.1: Box plot showing the results of the rate of weight gain in lambs at 4-H county sheep shows in 2019 based on *Mycoplasma ovipneumoniae* PCR pathogen status. The black horizontal line represents the median value for the rate of weight gain, vertical lines represent the minimum and maximum rate of weight gain.

Strain Types of Domestic Sheep

To further investigate *M. ovipneumoniae* in domestic sheep located on operations in western Nebraska, 26 *M. ovipneumoniae* PCR detected samples were further strain typed using the MLST approach at WADDL. The 26 samples represented both study areas and 15 different domestic sheep operations. We found high genetic variation amongst the samples we strain typed. Of the 26 PCR positive samples tested, 12 unique strains were identified (12 of 26, 46%). One strain that was identified twice was in two

different domestic sheep that were part of the same flock, housed within the same operation. This contrasted with the data on the bighorn sheep located in study areas. Studies have shown bighorn sheep in Nebraska have 5-6 *M. ovipneumoniae* strain types that have been circulating amongst herds since 2010 (Nebraska Game and Parks Commission (NGPC)). Although the sample size of *M. ovipneumoniae* strain typed samples from domestic sheep in western Nebraska was small, results support the high genetic diversity of *M. ovipneumoniae* found within domestic sheep flocks. Multiple strains can be found within a flock and individual sheep (Kamath et al. 2019, Manlove et al. 2019).

DISCUSSION

M. ovipneumoniae is present in domestic sheep flocks across western Nebraska and throughout other states and regions (NGPC, WAFWA 2012). The presence and prevalence of the pathogen, strain types found, and effects on operations can range from mild to proliferative (Manlove et al. 2019). The pathogen prevalence found in each flock and operation may constantly change based on number of sheep, operation type, and sale/processing of sheep. Respiratory disease is a problem for domestic sheep operations; however, there is limited data on the burden of *M. ovipneumoniae* detected in a flock of domestic sheep (Manlove et al. 2019). Evidence of *M. ovipneumoniae* in domestic sheep in Nebraska was detected in 40% of the samples collected from 2018-2020. By selecting operations near bighorn sheep herds, the number of domestic sheep tested was limited. In a larger comprehensive survey investigating *M. ovipneumoniae* in U.S. domestic sheep operations, evidence of *M. ovipneumoniae* infections was detected in over 85% of domestic sheep operations (Heinse et al. 2016, Manlove et al. 2019). There are few

studies specifically evaluating presence and prevalence of *M. ovipneumoniae* in domestic sheep operations (Manlove et al. 2019, McAuliffe et al. 2003); our results of 10 out of 11 (90%) operations with *M. ovipneumoniae* detected in flocks aligns with results of other studies showing a prevalence rate of 72-85% (Manlove et al. 2019, McAuliffe et al. 2003). In a 2011 USDA National Animal Health Monitoring System (NAHMS) study, *M. ovipneumoniae* was detected by PCR in one or more domestic sheep on 88.5% (401/453) of sheep premises and 29.4% (1,199/4,073) of individual sheep tested (NAHMS USDA 2011, Manlove et al. 2019). This study found the prevalence of *M. ovipneumoniae* was correlated with flock size (NAHMS USDA 2011, Heinse et al. 2016). Medium and larger sized flocks had a higher percentage of *M. ovipneumoniae* PCR detected samples compared to smaller flocks, which had an average size of flock of less than 100 sheep (NAHMS USDA 2011, Heinse et al. 2016). Our result of 80 PCR *M. ovipneumoniae* detected samples out of 199 samples (40%) aligns with smaller flocks that tend to have a smaller percentage of *M. ovipneumoniae* detected samples (NAHMS USDA 2011, Heinse et al. 2016, Manlove et al. 2019).

The high percentage of *M. ovipneumoniae* PCR detected flocks indicate *M. ovipneumoniae* is a ubiquitous pathogen in United States domestic sheep flocks; *M. ovipneumoniae* has been found in the respiratory tracts of "healthy" animals and in animals showing clinical symptoms respiratory disease (NAHMS USDA 2011). Both 4-H sheep shows and events at county fairs bring many domestic sheep from different flocks together causing a risk of pathogen transmission. Transmission occurs via respiratory droplets or secretions when animals are in close contact (USDA 2015). Domestic sheep can be asymptomatic and have a *M. ovipneumoniae* PCR detected result.

Clinical signs vary from mild respiratory disease to severe pneumonia and sudden death (USDA 2015). Variation in clinical symptoms is believed to be related to differences in strain virulence, host immune response, and secondary pathogens (USDA 2015). Further research is warranted to investigate *M. ovipneumoniae* strain type virulence in domestic sheep and bighorn sheep.

The weights of domestic sheep that participated in the 4-H events that we sampled for *M. ovipneumoniae* PCR were collected as part of the 4-H event. We found there was only minimal to no difference in the rate of weight gain in domestic sheep that had a negative PCR result compared to the rate of weight gain in domestic sheep that tested PCR positive for the pathogen. Despite this finding, for producers who have a meat production operation, the burden of *M. ovipneumoniae* could affect rate of weight gain causing economic concern for the producer (Besser et al. 2019). While there are many incentives to have disease free flocks including increased operation productivity, a decreased lamb loss, and general financial benefit, many of the producers who participated in the research had little concern on *M. ovipneumoniae* negatively affecting their operation. A recent study found *M. ovipneumoniae* may impair lamb growth and productivity even in the absence of overt respiratory disease (Besser et al. 2019). The absence of overt respiratory symptoms poses a challenge when expressing the need for general surveillance of *M. ovipneumoniae* on domestic sheep operations. However, with continued pathogen surveillance, flocks free of *M. ovipneumoniae* could be maintained, particularly for operations that are in proximity of bighorn sheep. This would benefit the producers as well as aid in bighorn sheep conservation. Additional research on the presence and prevalence of *M. ovipneumoniae* and its impact on domestic sheep

operation productivity and flock health should be explored as pathogen status is frequently changing in flocks with evolving *M. ovipneumoniae* strain types.

The strain types of *M. ovipneumoniae* found in the domestic sheep herds we sampled support the findings of domestic sheep having a high genetic diversity in *M. ovipneumoniae* strain types. This suggests the pathogen *M. ovipneumoniae* in domestic sheep is likely endemic and domestic sheep are an important reservoir host and source of infection (Kamath et al. 2019, Manlove et al. 2019). Domestic sheep and bighorn sheep are closely related sharing a common ancestor and a high degree of genome synteny, these similarities pose a risk for pathogen spillover (Poissant et al. 2010, Kamath et al. 2019). The high genetic diversity of *M. ovipneumoniae* found in domestic sheep can make it challenging to detect spillover events that occur while increasing the risk of a spillover event (Kamath et al. 2019, Manlove et al. 2019). Phylogenetic analysis in other studies revealed the majority of bighorn *M. ovipneumoniae* strains were most closely related to those from domestic sheep. The samples we collected from domestic sheep did not match *M. ovipneumoniae* strain types found in the bighorn sheep herds in Nebraska. To investigate a spillover event, it is crucial to collect samples from domestic sheep in real time. For this research, samples were collected starting in 2018, bighorn sheep epizootics related to *M. ovipneumoniae* began in 2010, with outbreaks occurring in 2014, 2016, 2018, and present (NGPC, unpublished data). Because bighorn sheep lack cross-strain immunity (Cassirer et al. 2017, Felts 2020), the high genetic diversity in domestic sheep of *M. ovipneumoniae* strains poses a risk of sequential introductions of different *M. ovipneumoniae* strains into bighorn sheep populations with resulting repeated severe disease outbreaks should contact occur with domestic sheep.

The use of the MLST approach to investigate the strain types of *M. ovipneumoniae* found in both domestic sheep and bighorn sheep provides the opportunity to identify the potential strain types involved in a potential spillover event or when an outbreak occurs, particularly when assessing bighorn sheep to bighorn sheep transmission. Genetic data identify domestic sheep as an infection reservoir with multiple and ongoing spillovers to bighorn sheep (Kamath et al. 2019, Manlove et al. 2019). The results from this research support the need for continuing surveillance of *M. ovipneumoniae* in both domestic sheep and bighorn sheep. The knowledge of pathogen presence and prevalence paired with the *M. ovipneumoniae* strain types can aid producers by increasing operation productivity and help bighorn sheep conservation by limiting pneumonia outbreaks from spillovers. As strain types of *M. ovipneumoniae* are ever evolving in both domestic and bighorn sheep, future work should be continued to monitor pathogen prevalence and *M. ovipneumoniae* strain types while working towards eliminating *M. ovipneumoniae* in domestic sheep and bighorn sheep.

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CHAPTER 3: ESTIMATING THE RISK OF CONTACT BETWEEN DOMESTIC SHEEP AND BIGHORN SHEEP IN WESTERN NEBRASKA USING A RISK OF CONTACT TOOL

INTRODUCTION

In the last 35 years, extensive research has been done on the effects of pathogen transmission when domestic sheep (*Ovis aries*) and bighorn sheep (*Ovis canadensis*) come in contact on the landscape (Onderka and Wishart 1988, Foreyt 1990, Callan et al. 1991). This research identified pathogens that are commonly carried by domestic sheep and are serious threats to bighorn sheep populations (Cassirer et al 2017). In particular, *Mycoplasma ovipneumoniae* (*M. ovipneumoniae*) has been linked to pneumonia outbreaks in bighorn sheep with an initial all age die-off, followed by years of low lamb recruitment (Dassanayake et al. 2010, Besser et al. 2013, 2014, Cassirer et al 2017). For managers and biologists keeping domestic sheep and wild sheep spatially separate is a high priority but is challenging across a shared multi-use landscape where 97% of the land is privately owned. Once a die-off has occurred and *M. ovipneumoniae* is found within a herd it is challenging to manage the disease and prevent recurrent infection and spread (Cassirer et al. 2017, 2018). With these challenges, prevention of contact is a preferred method to reduce the risk of pathogen spread (WAFWA 2012). The behavior of the bighorn sheep must be taken into consideration when investigating the risk of contact between wild and domestic sheep species (O'brien et al. 2014). Bighorn sheep and domestic sheep are gregarious and can be attracted to one another, which is especially common when females are in estrus and during the rut (Young and Manville 1960, O'Brien et al. 2014).

The term "foray" is used to describe the movement of an individual bighorn sheep when they leave their core herd home range (O'Brein et al. 2014). These movements are usually exploratory and increase during different seasons such as rut (Festa-Bianchet 1986, DeCesare and Pletscher 2006, O'Brein et al. 2014). These movements can be short or long in distance and the risk of bighorn sheep encountering a domestic sheep operation during the foray excursion is increased. With the potential of becoming infected during a foray, bighorn sheep could act a vector for transmission of the respiratory pathogen when returning to the core herd home range, spreading the pathogen to members of the herd or adjacent herds (O'Brien et al. 2014).

The risk of contact (ROC) tool was created following a remand by USDA Forest Service in 2005, later this tool was used by the Payette National Forest who developed a ROC tool for calculating probability rates of contact between bighorn sheep and active domestic sheep allotments (O'Brien et al. 2014). This tool contains 6 data components: (1) a core herd home range; (2) a habitat model; (3) foray distance and rate; (4) relative habitat preference based on the proportion of location points on each class of the source habitat model; (5) bighorn sheep herd size and sex dynamics, and (6) domestic sheep allotments. Combining the data components, the ROC model will produce maps of the relative probability an individual ewe or ram will reach each domestic sheep operation on the landscape surrounding the core herd home range (O'Brien et al. 2014). These maps are then combined with the herd population estimates to evaluate the annual probability that at least one bighorn sheep in a herd will reach a given area on the landscape (O'Brien et al. 2014). In this study, we used the ROC tool for bighorn sheep herds located in western Nebraska to provide biologists with an assessment of the risk of contact between

bighorn sheep and the domestic sheep found on public grazing allotments, or in the case of western Nebraska, private domestic sheep operations. Using this tool, we aimed to estimate the risk and probability of contact between the bighorn sheep herds found in the Wildcat Hills and Pine Ridge regions and private domestic sheep operations in the surrounding areas.

METHODS

GPS/Telemetry data and core herd home range formation

Satellite telemetry provided GPS data used in the ROC tool. These data comprised of radio collar locations taken from 2018-2021 from 30 GPS satellite collars affixed to bighorn sheep. In the Pine Ridge study area, the GPS data consisted of 138,132 telemetry points. In total, 12 Vectronic (Vectronic Aerospace GmbH, Berlin Germany) GPS satellite collars were deployed on bighorn sheep ewes collecting up to 5 points daily in the Pine Ridge region. In the Wildcat Hills study area, a total of 222,853 telemetry points was used. The telemetry data were collected from 18 Vectronic GPS satellite collars that were deployed on bighorn sheep ewes collecting up to 5 points per day. The GPS data were downloaded from the Vectronics Aerospace Inventa wildlife monitoring website (Vectronic Aerospace GmbH, Berlin Germany). Prior to entering the GPS data into the ROC tool the points were cleaned by removing any GPS points outside of the study area that were collected in transportation prior to deployment on bighorn sheep. The GPS data were viewed and a shapefile was created for the ROC tool in ArcGIS Pro (ESRI, ArcGIS Pro, Version 2.8.0). To include the space use of bighorn sheep in the model, a standard core herd home range estimation technique was used (O'Brien et al. 2014). The cleaned GPS telemetry points were imported into the ROC Tool to create the core herd home

range. Due to the distance between the two study sites the model was run separately for each study area. The core herd home ranges were estimated using a fixed kernel density estimator to calculate utilization distributions for each individual bighorn sheep. Specifically, a bivariate-normal kernel was used with fixed bandwidths (Worton 1995, O'Brien et al. 2014). In the ROC model, maps of the individual utilization distributions were superimposed, summing the values in each pixel (O'Brien et al. 2014). Last, 95% isopleths of the estimated kernels were calculated for core herd home ranges (Hawth's Analysis Tools for ArcGIS Beyer 2004, O'Brien et al. 2014).

Habitat Classification and Relative Preference of Habitat:

The habitat raster layer included three classes; habitat, connectivity, and non-habitat. The habitat layer is based on the preference of bighorn sheep to use areas on the landscape where steep sloped escape terrain and ruggedness is available (Bleich et al. 1997, Valdez and Krausman 1999, O'Brien et al. 2014). Suitable access to escape terrain was modeled using a 2-step process, by first delineating and then buffering around areas of steep and rough topography. Areas greater than or equal to 1.6 ha, with a slope and ruggedness values that meet the criteria for escape terrain were deemed suitable as escape terrain (Smith et al. 1991, Gudorf et al. 1996, O'Brien et al. 2014). The connectivity layer borders the habitat layer and is located within 350 m of habitat (or within 525 m if located between 2 patches of habitat) (O'Brien et al. 2014). The remainder of the mapped habitat is classified as non-habitat. The habitat raster layer created for the ROC tool has a boundary of 35 km around the core herd home range. All areas within the 35 km boundary are assigned to one of the 3 habitat classes. After the habitat raster layer was created for the study site, the relative preference was calculated for bighorn sheep

with a use–availability-based resource selection function (Manly et al. 1993, Boyce et al. 2002, O'Brien et al. 2014):

$$\mathrm{Pref}_h = \frac{(\mathrm{Use}_h/\mathrm{Area}_h)}{(\mathrm{Use}_{\mathrm{habitat}}/\mathrm{Area}_{\mathrm{habitat}})},$$

where *h* indexes habitat class, Use_h is the number of telemetry points found in the habitat class *h*, Area_h is the area of the habitat class *h* (O'Brien et al. 2014).

Allotments and Private domestic sheep operations

The allotment layer is then added to the model. The original model used public domestic sheep grazing allotments on public land. The study sites in Nebraska for this research were comprised of mainly private land; thus, we were interested in the risk of contact between private domestic sheep operations and bighorn sheep herds. The locations of domestic sheep operations that had participated in testing (see Chapter 2) were used as the allotments in this component of the ROC model. The location of these domestic sheep operations were buffered with a 0.5 km circular buffer, and these buffered locations comprised our allotment layer.

Foray rate and distances and herd dynamics

A foray movement was defined as any sequence of observations of an animal outside the core herd home range, followed by its return to the core herd home range (Singer et al. 2001, O'Brien et al. 2014). Other foray components of the ROC model include ram and ewe foray frequency rates and foray distances. Herd dynamics including sex ratio and population size also contribute to the data used in the ROC tool. For this study, we used the default values of 0.141 ram foray frequency and 0.015 ewe foray frequency rates as well as, a default 35 km foray distance for both rams and ewes. The

default foray distance distributions were derived from 12 years of Hell Canyon telemetry data, authors of the ROC tool found these values were consistent with published observations of bighorn sheep movement in other areas of western North America (Bighorn Sheep Risk of Contact Tool V2 User Guide 2015). GPS satellite collar data from the herds of bighorn sheep in Nebraska were primarily from ewes only. Due to the lack of GPS satellite collared ram data, we used default values in the model when running models. The herd dynamics for the bighorn sheep in Nebraska have changed each year. Herd dynamics and total population numbers were provided by Nebraska Game and Parks Commission. The ROC tool was run with a total population of 115 sheep (65 rams, 50 ewes) in the Pine Ridge study area. In the Wildcat Hills, the ROC tool was run based on a total population of 195 sheep (65 rams, 130 ewes).

Estimating rate of contact

Estimated rate of contact is calculated by the model. Once all of the data were entered into the ROC tool, contact rates were estimated and paired with a map showing the different 1km bands extending out from the core herd home range of bighorn sheep inhabiting the region. To estimate the rate of contact, first the annual probability of a single bighorn sheep intersecting the allotment or in our case the domestic sheep operation in each of the 35, 1 km wide bands surrounding the core herd home range is calculated (O'Brien et al. 2014). Next, to calculate the overall probability of contact with an allotment (domestic sheep operation) forays were treated as linear movements and the probability that a bighorn sheep will reach an allotment is equivalent to the probability that it will reach the 1 km band within the allotment that has the greatest probability of being contacted (O'Brien et al. 2014). Lastly the herd level contact rates were calculated

by summing the contact probabilities of individual rams and ewes within each herd. (O'Brien et al. 2014).

RESULTS

Habitat Creation and Relative Preference of habitat use by bighorn sheep

Habitat creation for the habitat raster layer used in the ROC tool was created for both study areas. The Pine Ridge study area criteria that made up the habitat area was defined as a minimum slope of 16 degrees and a minimum ruggedness measure of 78. With these criteria defined as habitat, 96% of satellite telemetry points were located in the area classified as habitat. The total area used in the ROC tool was comprised 19% of habitat. In the Wildcat hills, habitat criteria were defined with a minimum slope of 20 degrees and a minimum ruggedness measure of 85. The proportion of satellite telemetry GPS located in the area classified as habitat was 98% of data points. The area proportioned as habitat in the three-class habitat model was 24.5% of the total area.

We evaluated relative preference for each habitat class. In the Pine Ridge Study area, 132,291 (95.8% of all telemetry points) satellite GPS telemetry points were in the habitat class, the total area of the habitat class was 188.9 km^2. $Pref_h$ for the habitat class was 700.24. In the connectivity class, there were 5,310 (3.84% of all telemetry points) satellite GPS telemetry points, the total area of the connectivity class was 122.9 km^2. $Pref_h$ for the connectivity class was 41.8. In the non-habitat class there were 531 (0.4% of all telemetry points) satellite GPS telemetry points, the total area of the non-habitat class was 9350 km^2. $Pref_h$ for the non-habitat class was 0.6. Relative to a preference of 1.00 for

habitat, bighorn sheep showed a preference of 0.95 for habitat, 0.06 for connectivity, and 0.0001 for non-habitat.

In the Wildcat Hills study area, 214,458 (98.4% of all telemetry points) satellite GPS telemetry points were in the habitat class, the total area of the habitat class was 378.2 km^2. $Pref_h$ for the habitat class was 567.0. In the connectivity class, there were 2,512 (0.01% of all telemetry points) satellite GPS telemetry points, the total area of the connectivity class was 268.3 km^2. $Pref_h$ for the connectivity class was 9.4. In the non-habitat class there were 1,003 (0.005% of all telemetry points) satellite GPS telemetry points, the total area of the non-habitat class was 723.6 km^2, and $Pref_h$ for the non-habitat class was 1.39. Relative to a preference of 1.00 for habitat, bighorn sheep showed a preference of 0.98 for habitat, 0.02 for connectivity, and 0.002 for non-habitat.

Pine Ridge study area ROC:

The results of the ROC tool calculated annual contact rates including ram contact probability, ewe contact probability, all rams contact rate, all ewes contact rate, and the herd contact rate. The operation P1 was located within the core herd home range with a probability and contact rate of infinite or 100% risk of contact between domestic sheep and bighorn sheep. Private domestic sheep operation P2 that was located outside of the core herd home range had the second highest risk. The ram contact probability was 0.2%, the ewe contact probability was 0.02%, the all ram contact rate was 0.166, the all ewe contact rate was 0.011, and the herd contact rate was 0.177. This operation was located just outside the core herd home range in an area that was classified as ideal habitat in the three-class habitat model. Domestic sheep operation PRDW-19_7 had the lowest herd contact rate of 0.0000234. On average for all contact categories and private domestic

sheep operations in the Pine Ridge area there was a 0.07-0.08 contact rate per year (Table 3.1). The map of the study area produced in the ROC tool (Figure 3.3) shows the core herd home range created in the ROC tool for the bighorn sheep, the domestic sheep operation imported in the allotment layer, the habitat layer on the landscape, and the 1 km bands used in the tool.

Table 3.1. Risk of Contact Tool results for Pine Ridge, Nebraska. Ewe, ram, and herd contact rates calculated within the ROC model. Allotments are the domestic sheep operations on the landscape, Ring corresponds to the 1 km band the allotment falls in around the core herd home range.

Allotment	Ring	ramContactProb	eweContactProb	allRamsContactRate	allEwesContactRate	herdContactRate
HobbyOp	5	0.000001	0.000001	0.0000648	0.0000028	0.0000676
P2	2	0.0025594	0.0002231	0.166296	0.0111609	0.1774569
PRDW-19_1	15	0.0001611	0.0000048	0.0104678	0.0002426	0.0107104
PRDW-19_2	24	0.0000275	0.0000007	0.0017836	0.0000368	0.0018205
PRDW-19_3	15	0.0001886	0.0000057	0.0122553	0.000284	0.0125393
PRDW-19_4	10	0.0003197	0.0000066	0.0207739	0.0003319	0.0211057
PRDW-19_5	21	0.0000927	0.0000023	0.0060231	0.0001157	0.0061388
PRDW-19_6	5	0.000001	0.000001	0.000063	0.0000027	0.0000657
PRDW-19_7	3	0.0000003	0.00	0.0000222	0.0000012	0.0000234
PRDW-19_8	24	0.0000413	0.0000011	0.0026819	0.0000554	0.0027372
PRP18001	3	0.0000004	0.00	0.0000229	0.0000013	0.0000242
PRP19002	9	0.0000148	0.0000003	0.0009638	0.0000167	0.0009805
PRP20001	2	0.0000004	0.00	0.0000258	0.0000017	0.0000275
PRP20005	9	0.000004	0.0000001	0.0002619	0.0000045	0.0002665
P1	CHHR	INF	INF	INF	INF	INF

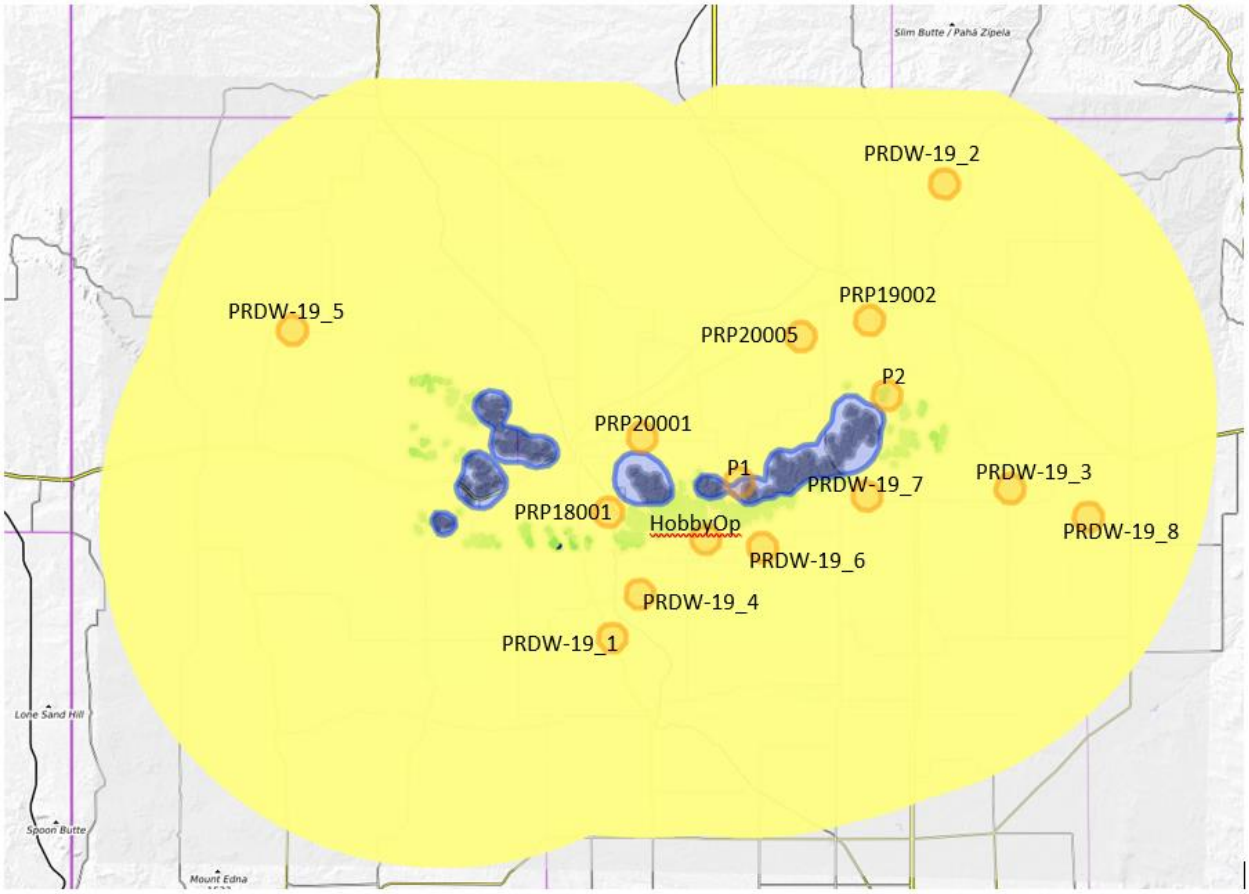

Figure 3.3. Risk of Contact Tool results for Pine Ridge, Nebraska. Map showing habitat is shown in green, domestic operations are represented by the light orange circles, and bighorn sheep core herd home range in blue. The yellow shows the 35 km boundary in the model and area classified as non-habitat.

Wildcat Hills ROC Study area:

The results of the ROC tool calculated annual contact rates including ram contact probability, ewe contact probability, all rams contact rate, all ewes contact rate, and the herd contact rate. Private domestic sheep operation WCW20004 was located outside of the core herd home range but had the highest risk of contact. The ram contact probability was 0.006%, the ewe contact probability was 0.0002%, the all ram contact rate was 0.004, the all ewe contact rate was 0.0002, and the herd contact rate was 0.0.004. This

operation was located just outside the core herd home range in an area that was classified as ideal habitat in the three-class habitat model. There were 3 domestics sheep operations that were located outside the 35-km boundary, the ROC was 0.00 due to the location. Of the remaining domestic operations, WCSW-19_5 had the lowest herd contact rate of 0.000016. On average for all contact categories and private domestic sheep operations in the Wildcat Hills there was a 0.000222 annual contact rate (Table 3.2). The map of the Wildcat Hills study area produced in the ROC tool (Figure 3.4) shows the core herd home range created in the ROC tool for the bighorn sheep, the domestic sheep operations imported in the allotment layer, the habitat layer on the landscape, and the 1 km bands used in the tool.

Table 3.2. Risk of Contact Tool results for Wildcat Hills, Nebraska. Ewe, ram, and herd contact rates calculated within the ROC model. Allotments are the domestic sheep operations on the landscape, Ring corresponds to the 1 km band the allotment falls in around the core herd home range.

Allotment	Ring	ramContactProb	eweContactProb	allRamsContactRate	allEwesContactRate	herdContactRate
WCW20003	13	0.0000089	0.0000002	0.0005775	0.0000296	0.0006071
WCW20002	25	0.0000058	0.0000002	0.0003789	0.0000244	0.0004033
WCW20004	8	0.0000564	0.0000017	0.0036603	0.0002174	0.0038777
WCSW-19_1	18	0.0000043	0.0000002	0.0002796	0.0000206	0.0003002
WCSW-19_2	3	0.0000111	0.0000008	0.0007193	0.0001025	0.0008217
WCSW-19_3		0.0	0.0	0.0	0.0	0.0
WCSW-19_4	9	0.0000081	0.0000002	0.0005232	0.0000236	0.0005469
WCSW-19_5	35	0.0000002	0.0	0.0000155	0.0000005	0.000016
WCSW-19_6	35	0.0	0.0	0.0000018	0.0000001	0.0000019
WCSW-19_7	16	0.0000093	0.0000003	0.0006024	0.0000393	0.0006417
WCSW-19_8		0.0	0.0	0.0	0.0	0.0
WCSW-19_9	24	0.0000033	0.0000001	0.0002167	0.0000116	0.0002284
WCSW-19_10	20	0.0000043	0.0000001	0.0002821	0.0000175	0.0002996
WCSW-19_11	17	0.0000056	0.0000002	0.0003652	0.0000256	0.0003908
WCSW-19_12	12	0.0000051	0.0000001	0.0003322	0.0000157	0.0003479

Allotment	Ring	ramContactProb	eweContactProb	allRamsContactRate	allEwesContactRate	herdContactRate
WCMW-19_13	22	0.0000061	0.0000001	0.0003942	0.0000174	0.0004116
WCMW-19_14	20	0.0000046	0.0000001	0.0003014	0.0000187	0.0003201
WCMW-19_15	21	0.0000043	0.0000001	0.0002762	0.0000138	0.00029
WCMW-19_16	20	0.0000049	0.0000002	0.0003199	0.0000198	0.0003398
WCMW-19_17	27	0.000007	0.0000003	0.000457	0.0000447	0.0005017
WCMW-19_18	7	0.0000173	0.0000007	0.0011232	0.0000919	0.0012152

Table 3.2. Continued (Above). Risk of Contact Tool results for Wildcat Hills, Nebraska. Ewe, ram, and herd contact rates calculated within the ROC model. Allotments are the domestic sheep operations on the landscape, Ring corresponds to the 1 km band the allotment falls in around the core herd home range.

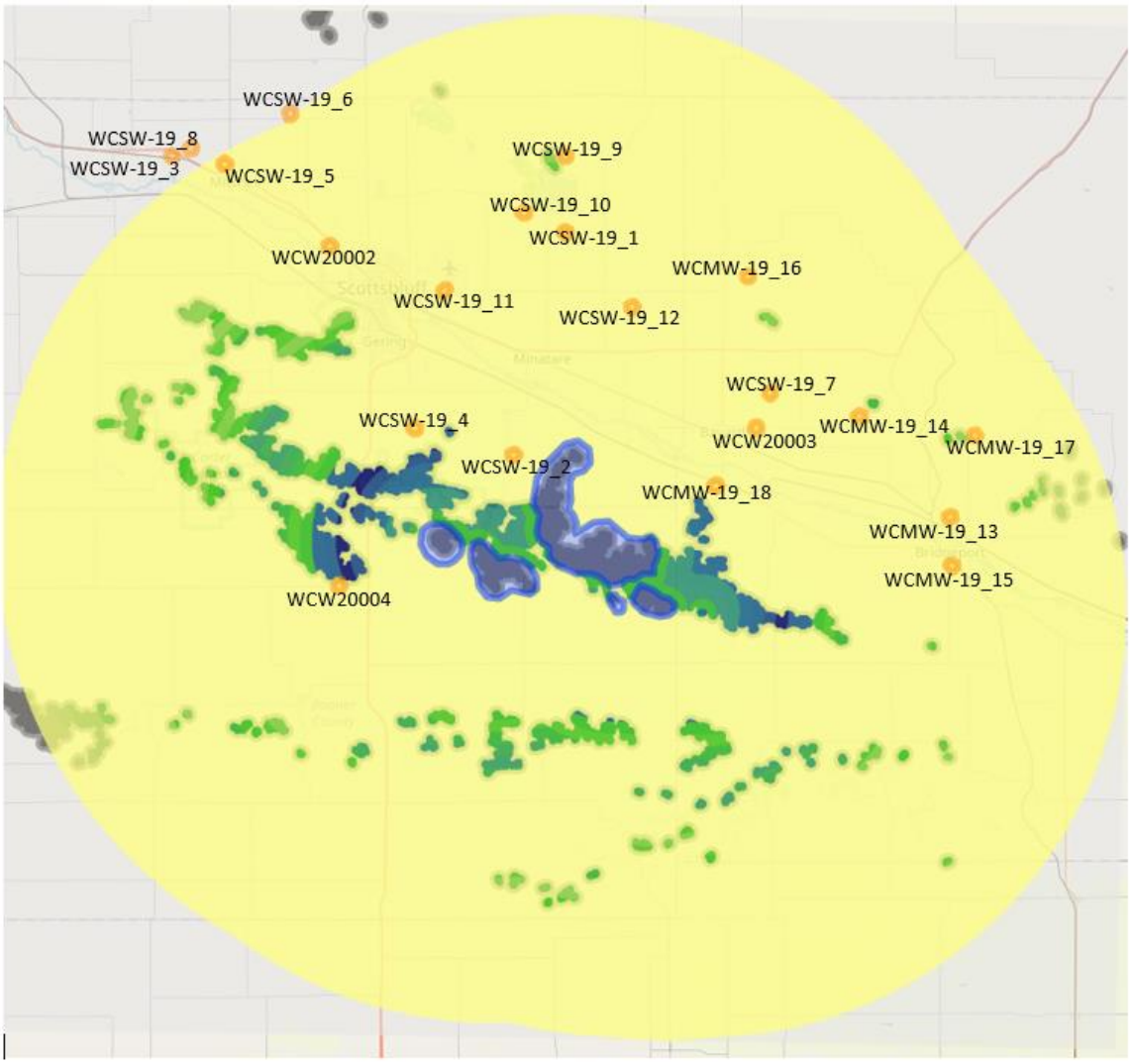

Figure 3.4. Risk of Contact Tool results for Wildcat Hills, Nebraska. Map showing habitat is shown in green, domestic operations are represented by the light orange circles, and bighorn sheep core herd home range in blue. The yellow shows the 35 km boundary in the model and area classified as non-habitat.

DISCUSSION AND MANAGEMENT IMPLICATIONS

Domestic sheep and bighorn sheep are members of the same genus, sharing multiple pathogens including those widely believed to cause pneumonia (Ward et al.

1990, Besser et al. 2013). *M. ovipneumoniae* is a recognized pathogen of Caprinae (Ayling et al., 2004; McAuliffe et al., 2003; Alley et al., 1999, Manlove et al. 2019), and domestic sheep have been identified as carriers of *M. ovipneumoniae* with strong evidence that transmission of the pathogen occurs when the two species come in contact (Onderka and Wishart 1988, Foreyt 1990, Callan et al. 1991). There have been numerous reports of pneumonia outbreaks occurring in bighorn sheep following contact with domestic sheep, this is validated with the results of 13 different commingling experiments (Cassirer et al. 2018). Through investigation of the pathogen prevalence found in domestic sheep flocks and the strain types of *M. ovipneumoniae* found within each species, as discussed in the previous chapters, there is evidence of potential spillover of *M. ovipneumoniae having* occurred in western Nebraska with potential for additional spillover events. The Risk of Contact Model found a range of ROC values (0.02%-infinite [100%]). Using the ROC tool can aid in management by provided potential for contact and allowing for management efforts to decreasing the risk of pathogen transmission.

The habitat raster layer created for this model was based off of the use of escape terrain; slope, and the ruggedness of the landscape. An archived habitat raster was available (United States Forest Service [USFS] 2009), however, only a small proportion of the satellite telemetry points were located in what was classified as habitat. Thus, for better accuracy in the ROC tool, we created a new habitat layer for our study area with the goal to have maximum telemetry points with minimum area on the landscape classified as habitat for resident bighorn sheep. When creating a habitat model for the ROC tool for bighorn sheep habitat selection, the following factors were considered:

proximity to steep sloped escape terrain, forage availability, horizontal visibility, proximity to a water source (Bleich et al. 1997, Valdez and Krausman 1999, O'Brien et al. 2014). In other states, the role of horizontal visibility and proximity to a water source are an important factor in the source habitat models (O'Brien et al. 2014). In environments where desert bighorn species are found, the distribution of sheep appears to be correlated with the proximity to free water (Dolan 2006). For this research, the concern of an available water source or proximity to an available water source was not of concern when creating the source habitat used by bighorn sheep. On a shared multi use landscape there are a substantial amount of livestock water facilities used by ranchers for their grazing cattle that are also used by the bighorn sheep.

Vegetation cover or horizontal visibility will frequently play a role when investigating habitat selection for bighorn sheep. Bighorn sheep prefer sufficient vegetation cover to have coverage from predators but not too much as it would impair their ability to see predators and communicate with other members of the herd (Valdez and Krausman 1999, O'Brien et al. 2014). When investigating and creating the habitat coverage for the model, the digital LANDFIRE map was used to look at the vegetation cover for the area (Keane et al. 2002, O'Brien et al. 2014, LANDFIRE 2021). The majority of the study area's vegetation cover was >10% but <30% cover, providing sufficient horizontal visibility for bighorn sheep habitat (Valdez and Krausman 1999, O'Brien et al. 2014) and therefore, that component was not considered in the creation of the three-class source habitat layer for the ROC model. The final components used for the source habitat model created and used in the ROC tool were the slope of the landscape and ruggedness of the landscape (Sappington et al. 2007). This ruggedness value was

developed from a vector ruggedness measure (VRM) of terrain based on a geomorphological method for measuring vector dispersion that is less correlated with slope (Sappington et al. 2007).

Habitat of bighorn sheep in western Nebraska is part of the historic home range of bighorn sheep (Buechner et al. 1960, WAFWA WSWG 2012), however, it is located in the eastern extent of the historic distribution of bighorn sheep. The topography of the landscape is uniquely different than the majority of what is considered bighorn sheep habitat. Bighorn sheep in Nebraska still inhabit a steep rocky terrain, however it is much less steep than other areas like the Payette National Forest. Escape terrain was classified in Nebraska as a minimum ruggedness index of 78-85 and a minimum slope of 16-20 degrees. The maximum slope found in Nebraska was 53. In contrast, escape terrain in the Payette National Forest was determined as a ruggedness index of >310 and a slope between 31˚-85˚ (Smith et al. 1991, Gudorf et al. 1996, O'Brien et al. 2014). With this difference in habitat landscape, further research should be done moving forward with rams collared in western Nebraska to determine if the unique reduced ruggedness plays a role in foray distance. O'Brien et al. (2014) found the average foray distance was dependent on the herd location and season. Foray probabilities in the winter and summer differed significantly for ewes but not rams (O'Brien et al. 2014). As further research is completed with the herds of bighorn sheep in the Great Plains region of western Nebraska, region-specific foray rates and distances can be used in the ROC tool to improve estimates of ROC.

Domestic sheep allotment data used in the ROC tool was point data with a 0.5 km buffer to represent the area where the domestic sheep are contained at private domestic

sheep operations. Unlike large public grazing allotments, smaller operations house sheep in smaller pens within operations with grazing opportunities that are fenced that provide less area than large public grazing allotments. For this research, the exact size of areas within which producers kept their sheep was unknown; thus, an average 0.5-km buffer was used in the model. The results of the ROC tool showed varying probability of contact based on whether the private domestic operation was in proximity to the core herd home range and its location relative to bighorn sheep habitat in the area. The ROC table paired with the results map provided a good visual of the risk and location of private domestic sheep operations in relation to the bighorn sheep core herd home range. As more information on private domestic sheep operation locations become available, the ROC tool can continually be updated to show the probability of contact with the new operations.

The results from the ROC Tool indicated there was great risk of contact between domestic sheep and bighorn sheep. Many of the private domestic sheep operations were in close proximity to the core herd home ranges of bighorn sheep. The ROC Tool estimated 100% probability of interspecies contact when any of the private operations overlapped with the bighorn sheep home ranges. In our study for the Pine Ridge study area this included 1 small hobby flock. In the Wildcat Hills there were no domestic operations that were located within the core herd home range. In the Pine Ridge study area, an increased risk occurred compared to the Wildcat Hills study area. Historically, during 2018-2021, the 3 subherds that make up the bighorn sheep population in the Pine Ridge study area have been battling the long-term effects of a pneumonia epizootic (NGPC, unpublished). Through monitoring pathogen prevalence in the bighorn sheep, we

found multiple common strain types circulating amongst the subherds. This indicates that there was movement and contact amongst the subherds and the consequence of contact between a single bighorn sheep and domestic sheep can have a snowball effect leading to pathogen transmission within bighorn sheep subherds. While *M. ovipneumoniae* has been found in both bighorn sheep populations in both study areas there has been a decreased number of *M. ovipneumoniae* strains found in the Wildcat Hills populations and less severe effects of pneumonia within the herd. These results align with our finding of a decreased ROC between bighorn sheep and domestic sheep in that region.

As the population and herd dynamics of the bighorn sheep fluctuate the probability of contact also changes. In both study areas private domestic sheep operations varying in operation size fall within the management recommendation of a 14.5 km spatial buffer to the bighorn sheep core herd home ranges (WAFWA WSWG 2012). Maintaining this wide spatial separation poses a great challenge and is often an impossible task especially on a multi-use landscape. As mentioned in the previous chapters we aimed to use this ROC tool paired with promoting management changes on domestic operations to have a healthy co-existence on the shared landscape. Future goals within the state agency include the formation of a wild and domestic sheep advisory committee (see Chapter 1). The ROC Tool can estimate where the risk of contact is greatest and where efforts should be placed to work with private domestic sheep operations in preventing contact and promoting healthy flocks.

The ROC Tool can also provide biologists with other information. When there is the need to augment a population, the tool can be useful when looking at potential release sites and the risk of contact with private domestic sheep operations. With this tool,

managers can consider if release sites fall within the historic core herd home range of bighorn sheep that previously inhabited the area. Identifying the area on the landscape that was determined as habitat, connectivity, and non-habitat from the three-class source habitat layer, can aid in decisions on where to release translocated bighorn sheep on the landscape. On the multi-use landscape of western Nebraska that is primarily privately owned land, the management of bighorn sheep can be a challenge, the risk of pathogen transmission from domestic sheep to bighorn sheep primarily comes from smaller domestic sheep operations and/or small hobby flocks found on the landscape; this tool can aid in the management and prevention of pathogen spread through knowledge of probability of contact. Despite the availability and use of this tool, further conservation of bighorn sheep should continue, and further research should be done to decrease pathogen transmission between the wild and domestic species.

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APPENDIX

1.1 Copy of survey sent to domestic sheep producers in western Nebraska.

Domestic Sheep Production and Bighorn Sheep Conservation in Western Nebraska

A SURVEY FOR SHEEP PRODUCERS IN WESTERN NEBRASKA

Dear Nebraska Sheep Producer,

My name is Kaytlin Bohr, I am a graduate research assistant with South Dakota State University. I am conducting this survey in partnership with Nebraska Game and Parks Commission as part of a project called “Pathogen Prevalence in Domestic Sheep in Western Nebraska: Implications for Bighorn Sheep Conservation, Domestic Sheep Fitness, and Coexistence”.

The goal of this survey is to provide insight on the domestic sheep flocks of Western Nebraska in close proximity to bighorn sheep herds. We are investigating if there has been any impact within your flock due to *Mycoplasma ovipneumoniae* (*M. ovi*.) a common respiratory pathogen of both domestic sheep and bighorn sheep. The last part of this survey involves bighorn sheep and your opinion on the potential future conservation initiatives and strategies regarding bighorn sheep in Western Nebraska.

Mycoplasma ovipneumoniae (*M. ovi*.) is not only a pathogen that affects the respiratory tract in sheep causing coughing and nasal discharge. Studies have shown *M. ovi* can have a negative effect on lambing rates, lamb survival, weight gain and the general health of flocks. By working together, our goal is to generate solutions to promote domestic sheep flocks that are free of *M. ovi* to benefit the health of your flock and the health of bighorn sheep herds.

Your response and input are important to our project and the future of bighorn sheep in Nebraska. With this information you will be helping us develop strategies to promote co-existence of domestic sheep and bighorn sheep on the Western Nebraska landscape.

I estimate this survey will take about 20 minutes to complete. Your participation is completely voluntary (returning a blank survey will let us know that you do not wish to participate). You may leave any question in the questionnaire blank that you do not want to answer. Your name and contact information will never, in any way, be released or associated with the reported data. Each questionnaire has an identification number that I will use to check off your name when I receive your questionnaire, to try to ensure that I do not bother you with more mailings related to this study effort. In order to protect your anonymity, please **DO NOT** provide your name or address on the survey. In addition, there are no known risks or direct personal benefits associated with your participation in this study.

Please use the pre-paid, addressed envelope to return your completed survey. Feel free to contact me at 605-688-6121 or by email kaytlin.bohr@sdstate.edu with any questions or if you would like more information on this project.

Thank-you for your time and cooperation.

Sincerely,

Kaytlin Bohr
McFadden Biostress Laboratory 138
Natural Resource Management-Box 2140B
South Dakota State University
Brookings, SD 57007

South Dakota State University
Department of Natural Resource Management

General Description of Your Sheep Operations

1. Which answer below best describes your sheep operation? (select all that apply)

❑ Production flock (more than 50 sheep)

- ❑ Wool
- ❑ Meat

❑ Hobby flock (5-50 sheep)

❑ 4-H sheep (1-10 sheep)

❑ Other: Please specify

_____

2. Where is your sheep operation located?

❑ Pine Ridge :

❑ Within 10 miles ❑ Greater than 10 miles away

❑ Wildcat Hills :

❑ Within 10 miles ❑ Greater than 10 miles away

❑ Other: Please specify

_____

3. How many years do you anticipate continuing to run domestic sheep on your property?

❑ 1-3 years

❑ 4-6 years

❑ 7-10 years

❑ More than 10 years

4. Which answer best describes your involvement with the recent research regarding respiratory bacteria presence and pneumonia prevalence in domestic sheep in western Nebraska and the effects of those bacteria on domestic sheep.

❑ I was a cooperator in the study and allowed my sheep to be tested.

❑ I was asked to be a cooperator in the study but declined involvement/testing.

❑ I was not asked to be a cooperator in the study

***Mycoplasma ovipneumoniae* (*M. ovi.*) information**

Mycoplasma ovipneumoniae (*M. ovi.*) is a respiratory pathogen of domestic sheep, domestic goats, bighorn sheep, and mountain goats. *M. ovi* causes atypical pneumonia and can also predispose sheep and goats to lung infections with many other bacterial species. *M. ovi* infection of domestic sheep and goats is typically associated with mild disease, most often a 'coughing syndrome' of lambs and kids under 6 months of age. Less frequently, *M. ovi* is associated with severe or fatal pneumonia in domestic lambs and adult ewes and rams. *M. ovi* infection is associated with pneumonia in all ages of bighorn sheep upon initial exposure, and often causes recurrent fatal lamb pneumonia outbreaks in subsequent years.

Your concerns about the effects of pneumonia & *Mycoplasma ovipneumoniae* (*M. ovi*) on domestic sheep.

5. Before receiving this survey, have you heard about the respiratory pathogen *Mycoplasma ovipneumoniae* (*M. ovi*.)?

❑ Yes ❑ No

6. Has your sheep operation been negatively affected by a respiratory pathogen such as *M. ovi* that caused pneumonia?

Degree of Negative Effect

None	Very Small	Small	Moderate	Large	Very Large	Not Sure
❑	❑	❑	❑	❑	❑	❑

7. How concerned are you about any possible negative effects of *M. ovi* on each of the following aspects of your sheep operation? (please circle one response for each operation)

Sheep Operations	Level of Concern				
	None	Small	Moderate	Large	No Opinion
a) Survival of lambs	1	2	3	4	5
b) Survival of adult sheep	1	2	3	4	5
c) Lower lambing rates	1	2	3	4	5
d) Birth weight of lambs	1	2	3	4	5
e) Rate of gain in lambs	1	2	3	4	5
f) Carcass quality	1	2	3	4	5

Bighorn Sheep in Western Nebraska

8. How often do you see bighorn sheep on or near your sheep operation?

Never	Rarely	Sometimes	Often	Very Often
❑	❑	❑	❑	❑

9. How important is it to you to have bighorn sheep in the…

	Importance				
	Not at All	Slightly	Moderately	Very	No Opinion
a) Pine Ridge of western Nebraska	❑	❑	❑	❑	❑
b) Wildcat Hills of western Nebraska	❑	❑	❑	❑	❑

10. How concerned are you regarding *M. ovi* negatively affecting bighorn sheep populations in the…

	Level of Concern				
	Not at All	Slightly	Moderately	Very	No Opinion
a) Pine Ridge of western Nebraska	❑	❑	❑	❑	❑
b) Wildcat Hills of western Nebraska	❑	❑	❑	❑	❑

11. How concerned are you that wild bighorn sheep will make contact with your sheep?

Not at All Concerned	Slightly Concerned	Moderately Concerned	Very Concerned	No Opinion
❑	❑	❑	❑	❑

12. How concerned are you about the transmission of the *M. ovi* bacteria from wild bighorn sheep to your sheep?

Not at All Concerned	Slightly Concerned	Moderately Concerned	Very Concerned	No Opinion
❑	❑	❑	❑	❑

13. How concerned are you about the transmission of the *M. ovi* bacteria from your sheep to wild bighorn sheep?

Not at All Concerned	Slightly Concerned	Moderately Concerned	Very Concerned	No Opinion
❑	❑	❑	❑	❑

14. How concerned are you about the transmission of ANY diseases/pathogens from wild bighorn sheep to your sheep?

Not at All Concerned	Slightly Concerned	Moderately Concerned	Very Concerned	No Opinion
❑	❑	❑	❑	❑

15. How concerned are you about the transmission of ANY diseases/pathogens from your sheep to wild bighorn sheep?

Not at All Concerned	Slightly Concerned	Moderately Concerned	Very Concerned	No Opinion
❑	❑	❑	❑	❑

Your Opinions about Future Conservation Initiatives & Strategies Regarding Bighorn Sheep in Western Nebraska

16. What would your level of support be for a domestic sheep/bighorn sheep advisory committee or working group to develop and promote strategies that work toward the coexistence of healthy domestic and wild bighorn sheep herds in western Nebraska?

No Support	Slight Support	Moderate Support	Strong Support	No Opinion
❑	❑	❑	❑	❑

17. If a domestic sheep/wild sheep advisory committee or working group existed, here is a list of potential members that could be invited to participate. Please rate whether representative(s) from each group would be acceptable or not to you to participate on the advisory committee/working group.

Committee/Group Representative(s) from…	Acceptable	NOT Acceptable	No Opinion
a) Domestic sheep producers	❑	❑	❑
b) Nebraska Sheep & Goat Producers Association	❑	❑	❑
c) Nebraska Game & Parks Commission biologist(s)	❑	❑	❑
d) Nebraska Extension Educator(s)	❑	❑	❑
e) 4-H Sheep Superintendent(s)	❑	❑	❑
f) US Dept. of Agriculture	❑	❑	❑
g) Nebraska Dept. of Agriculture	❑	❑	❑

h) Can you suggest any other group(s) that should be represented on a Nebraska domestic sheep/wild sheep advisory committee or working group?

_____

18. If private or non-profit funding was available to help you offset costs associated with management actions on your operation to promote the healthy coexistence of domestic and bighorn sheep, would you accept funding?

No	Maybe	Yes	No Opinion
❑	❑	❑	❑

19. If government funding was available to help you offset costs associated with management actions on your operation to promote the healthy coexistence of domestic and bighorn sheep, would you accept funding?

No	Maybe	Yes	No Opinion
❑	❑	❑	❑

20. Below is a list of possible management actions to reduce or eliminate *M. ovi* and/or pneumonia in your sheep. For each action, please tell us if you would consider taking that action for you sheep operation.

Management Action	No	Maybe	Yes	Undecided
The use of a new **vaccine** that would target *M. ovi* or other respiratory pathogens if available.	❑	❑	❑	❑
The use of an **antibiotic treatment** that would target *M. ovi* or other respiratory pathogens.	❑	❑	❑	❑
The use of a medicated feed, supplement or block that would target *M. ovi* or other respiratory pathogens if available for legal use in the area.	❑	❑	❑	❑
"Closing" your flock to the practice of exchanging sheep with neighboring sheep producers/owners.	❑	❑	❑	❑
Erecting fences on your property that would prevent contact between wild bighorn sheep and your sheep.	❑	❑	❑	❑
Periodic *M. ovi* testing of your sheep.	❑	❑	❑	❑
M. ovi testing of sheep within your operation that are sick or that have died.	❑	❑	❑	❑
Quarantining new sheep prior to turning them in with your existing flock.	❑	❑	❑	❑
M. ovi testing new sheep prior to turning them in with your existing flock.	❑	❑	❑	❑
Culling sheep that have tested positive for *M. ovi*.	❑	❑	❑	❑

Other, please specify:

Additional Comments: