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ABSTRACT: Bighorn sheep (*Ovis canadensis*) across North America commonly experience population-limiting epizootics of respiratory disease. Although many cases of bighorn sheep pneumonia are polymicrobial, *Mycoplasma ovipneumoniae* is most frequently associated with all-age mortality events followed by years of low recruitment. Chronic carriage of *M. ovipneumoniae* by adult females serves as a source of exposure of naïve juveniles; relatively few ewes may be responsible for maintenance of infection within a herd. Test-and-remove strategies focused on removal of adult females with evidence of persistent or intermittent shedding (hereafter chronic carriers) may reduce prevalence and mitigate mortality. Postmortem confirmation of pneumonia in chronic carriers has been inadequately reported and the pathology has not been thoroughly characterized, limiting our understanding of important processes shaping the epidemiology of pneumonia in bighorn sheep. Here we document postmortem findings and characterize the lesions of seven ewes removed from a declining bighorn sheep population in Wyoming, USA, following at least two antemortem detections of *M. ovipneumoniae* within a 14-mo period. We confirmed that 6/7 (85.7%) had variable degrees of chronic pneumonia. *Mycoplasma ovipneumoniae* was detected in the lung of 4/7 (57.1%) animals postmortem. Four (57.1%) had paranasal sinus masses, all of which were classified as inflammatory, hyperplastic lesions. *Pasteurella multocida* was detected in all seven (100%) animals, while *Trueperella pyogenes* was detected in 5/7 (71.4%). Our findings indicate that not all chronic carriers have pneumonia, nor do all have detectable *M. ovipneumoniae* in the lung. Further, paranasal sinus masses are a common but inconsistent finding, and whether sinus lesions predispose to persistence or result from chronic carriage remains unclear. Our findings indicate that disease is variable in chronic *M. ovipneumoniae* carriers, underscoring the need for further efforts to characterize pathologic processes and underlying mechanisms in this system to inform management.

Key words: Bighorn sheep, *Mycoplasma ovipneumoniae*, paranasal sinus mass, pneumonia, respiratory disease, test-and-cull, test-and-remove, wildlife-livestock interface.

INTRODUCTION

Bighorn sheep (*Ovis canadensis*) across North America have suffered population-limiting epizootics of respiratory disease since the mid-1800s (Grinnell 1928; Buechner 1960), the etiology of which has been the source of much debate. Outbreaks are often characterized by all-age mass mortality events followed by variable periods of low lamb recruitment that limit recovery and resilience (Besser et al. 2012; Cassirer et al. 2018). The current consensus holds that bighorn sheep pneumonia is multifactorial and polymicrobial, although evidence

for *Mycoplasma ovipneumoniae* as a primary etiology has been demonstrated in multiple instances (Besser et al. 2008, 2013, 2014, 2017; Spaan et al. 2021). The pathogenesis of *M. ovipneumoniae* involves interference with the mucocilliary apparatus (Jones et al. 1985), which can predispose to establishment of other infections (Dassanayake et al. 2010). Bacteria in the Pasteurellaceae family, including *Mannheimia haemolytica*, *Bibersteinia trehalosi*, and *Pasteurella multocida*, are commonly identified along with *M. ovipneumoniae*. The pathogenicity of these agents varies across strains (Besser et al. 2013), resulting in outcomes that range from subclinical infections to high morbidity and mortality epizootics. Subacute to chronic bronchopneumonia within cranioventral consolidation is a classic finding in epizootic pneumonia of bighorn sheep. Lesions may include pleural adhesions, fibrinous pleuritis, pulmonary abscesses, and suppurative to lymphoplasmacytic or mixed inflammation (Foreyt and Jessup 1982; Spraker et al. 1984).

Transmission of respiratory pathogens in bighorn sheep is a frequency-dependent, socially structured process in which pathogens can be maintained in a population through a few infected individuals (Manlove et al. 2017; Plowright et al. 2017, 2019; Almberg et al. 2022). Sometimes referred to as "superspreaders," these individuals, usually adult females, tend toward tolerance as a host defense strategy—that is, deleterious impacts of respiratory pathogens are mitigated by limiting tissue damage rather than by reducing bacterial burden or clearing infection (Plowright et al. 2013). Although this strategy may benefit the individual, it promotes pathogen persistence in populations and may increase prevalence. In the respiratory disease system of bighorn sheep, tolerance in adults is disadvantageous to naïve juveniles (Plowright et al. 2017). Chronically infected females (hereafter chronic carriers) may expose juveniles in nursery groups, directly causing mortality and exacerbating low lamb recruitment, thereby leading to long-term population declines. Removal of chronic carriers has emerged as a potential management tactic to mitigate pathogen prevalence in sheep populations. In western South Dakota (Garwood et al. 2020), removal of chronic carriers defined as adults consistently testing positive for *M. ovipneumoniae* over a 20-mo sampling period resulted in a subsequent absence of detectable *M. ovipneumoniae* in the treatment population along with reduced juvenile and adult hazard mortality in comparison to a control population. A similar observation was noted in Oregon (Spaan et al. 2021), where the loss of a single animal with detectable *M. ovipneumoniae* was followed by improved juvenile survival within the herd. Although such observations have thus far been limited in number, results are supported by models, indicating that test-and-remove programs may aid population recovery (Almberg et al. 2022).

Pathogen detection is a cornerstone of test-and-remove strategies for managing disease in wildlife populations. More specifically, the ability to distinguish infected from noninfected individuals on the landscape via antemortem sampling and testing is critical to the success of test-and-remove programs. Antemortem diagnosis of *M. ovipneumoniae* in bighorn sheep, however, is complicated by several factors. First, captures are costly and require substantial labor, particularly when serial testing is necessary. Second, suboptimal sensitivity and interassay discrepancies have been documented for *M. ovipneumonaie* (Walsh et al. 2016; Lieske et al. 2022), complicating the interpretation of test results for the relatively few animals that are repeatedly handled and tested. For example, a negative test result occurring between two detections might represent either clearance of infection followed by reinfection, or the occasional false negative, which may mask consistent carriage of the pathogen. These factors constrain our understanding of *M. ovipneumonaie* carriage in bighorn sheep and limit the application of test results to decision making processes by managers.

The range of outcomes of mycoplasma infections, both with and in the absence of coinfections, further muddles our understanding and hinders our ability to predict the

outcomes of management interventions. Although *M. ovipneumoniae* has been implicated as the primary etiology in several epizootics (Besser et al. 2008, 2013, 2014, 2017; Spaan et al. 2021), introductions with minimal morbidity and mortality have also been reported (Johnson et al. 2022). Thus, the carriage of *M. ovipneumoniae* by bighorn sheep, particularly in the upper respiratory tract, does not definitively result in pneumonia, nor does it conclusively lead to juvenile loss within all infected herds. Some animals may in fact, carry *M. ovipneumoniae* in the nasal cavity without colonization of the lung.

Mycoplasma ovipneumoniae has also been identified in other regions of the bighorn sheep respiratory tract, such as the paranasal sinuses. More specifically, *M. ovipneumoniae* and other bacterial pathogens have been documented in association with paranasal sinus masses of bighorn sheep, which comprise a spectrum of proliferative lesions in the frontal and/or maxillary sinuses ranging from inflammatory and hyperplastic to malignant neoplasia (Fox et al. 2011, 2015). A connection between chronic carriage of *M. ovipneumoniae* and the occurrence of paranasal sinus masses is indeed plausible but presents a causality dilemma: Are paranasal sinus masses the result of inflammation induced by chronic carriage of *M. ovipneumoniae*, or do proliferative sinus lesions impair sinonasal clearance of bacteria, thereby promoting chronic carriage? Irrespective of causality, animals with these lesions may contribute disproportionately to transmission within herds. A better understanding of where in the respiratory tract chronic infections are harbored might help elucidate the mechanisms of bacterial persistence and inform sampling and testing approaches to maximize the effectiveness of removal efforts.

Complex interplay between host, pathogen, and environment probably contribute to the variability in outcome of bighorn sheep respiratory infection, both within and among populations (Cassirer et al. 2018; Wagler et al. 2023). Although removal of animals that pose the greatest transmission risk may be inherently beneficial to the herd, many facets of pathogenesis and immunodynamics remain poorly understood. Postmortem evaluation of animals removed from affected herds presents a compelling opportunity to understand the complex interactions that lead to persistent infections, which may inform the definition of chronic carriers and improve our ability to identify such animals. Given the increasing interest in test-and-remove management and the related need to understand persistence of *M. ovipneumonaie* both in individuals and in herds, our objectives were 1) to characterize the pathology in bighorn sheep with chronic carriage of *M. ovipneumoniae*; 2) to assess correspondence between detection of *M. ovipneumoniae* in the nasal cavity and detection in the lung qualitatively; and 3) determine the frequency of co-infecting respiratory pathogens in bighorn sheep persistently infected with *M. ovipneumoniae*.

MATERIALS AND METHODS

Study system

The Whiskey Mountain bighorn sheep herd is located within the northern Wind River Range (43°26′11.2092″, −109°33′4.6764″) near Dubois, Wyoming, USA. Historically considered a thriving population with at least 1,500 individuals, this herd has experienced low lamb recruitment and reduced numbers since a large, all-age respiratory epizootic occurred during the winter of 1990–1991 (Ryder et al. 1992). Declines have been dramatic in the Whiskey Mountain herd, with recent estimates indicating a dwindling population roughly 20% its preepizootic size (Smiley et al. 2022). In 2015, vigorous studies were initiated to understand the population-limiting factors better (Smiley et al. 2022; Wagler et al. 2023). An especially high prevalence of *M. ovipneumoniae* was identified in adult females occupying the Red Creek region of the study area, with 28/94 animal-years testing positive for *M. ovipneumoniae* between March 2015 and December 2021. Recent recruitment rates have been estimated at approximately 20% (Smiley et al. 2022) and pneumonia has been identified as the leading cause of juvenile mortality (Wyoming Game and Fish Department 2021). Test-and-remove efforts therefore specifically targeted adult females occupying the Red Creek region.

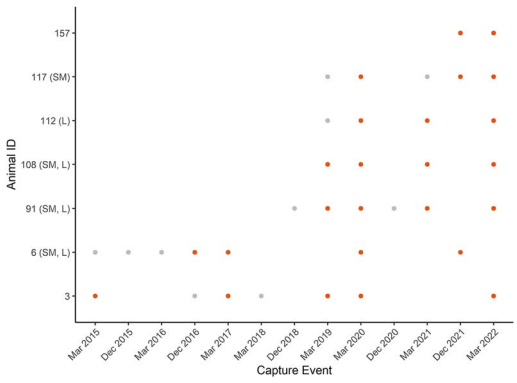

FIGURE 1. Detections of *Mycoplasma ovipneumoniae* in adult female bighorn sheep (*Ovis canadensis*) from the Red Creek subunit of the Whiskey Mountain herd in the Wind River Range, Wyoming, USA. Chronic carriers targeted for removal were defined as individuals testing positive at least twice within a 14-mo period with captures occurring each December and March. Orange dots indicate *M. ovipneumoniae* detections. Gray dots indicate negative test results. L indicates *M. ovipneumoniae* was detected in lung postmortem. SM indicates a paranasal sinus mass was identified postmortem.

Sampling

Beginning in March of 2015, adult females were captured using helicopter net-gunning as described in (Wagler et al. 2022). Recapture was attempted twice yearly each December and March and new individuals were captured in effort to maintain a target sample size of 25 ewes. All capture and handling procedures were conducted in accordance with Institutional Animal Care and Use Committee guidelines (protocols 20150316KM00148, 20180305KM00296, and 20210303KM00463). Two nasal and two tonsil swabs were collected from each ewe at each capture. Polyester swabs (Puritan Medical Products, Pittsfield, Maine, USA) were stored in tryptic soy broth (Hardy Diagnostics, Santa Maria, California, USA) with 15% glycerol and frozen or stored on dry ice for transport to the Wyoming Game and Fish Department Wildlife Health Laboratory (WHL, Laramie, Wyoming, USA).

Bacterial detection

Pasteurellaceae and *Mycoplasma* spp. culture was performed on tonsil and nasal swabs as described (Wood et al. 2017). Additionally, DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Germantown, Maryland, USA) and PCR assays were performed to detect *M. ovipneumoniae* (Manlove et al. 2019), *P. multocida* (Tocqueville et al. 2017), *Mannheimia* sp. leukotoxin (Shanthalingam et al. 2014), and leukotoxin-positive (lkt+) *Biberstinia trehalosi* using primers and conditions described in Killion et al. (2018). We further assayed DNA with detectable *Mannheimia* sp. leukotoxin using primers specifically targeting *Mannheimia haemolytica* and/or *Mannheimia glucosida* as described (Angen et al. 2009).

Identification and removal of chronic carriers

Chronic carriers were defined as adult ewes that tested PCR positive for *M. ovipneumoniae* at least twice over three sampling events within an approximately 14-mo period (Fig. 1). Chronic carriers targeted for removal were humanely killed by gunshot to the neck in accordance with guidelines for euthanasia of nondomestic animals (American Association of Zoo Veterinarians 2006) and transported to the Wyoming State Veterinary Laboratory (Laramie, Wyoming, USA) for necropsy and diagnostic workup. Nasal and tonsil swabs were collected in the field and stored on ice for transport to the WHL for bacterial diagnostics as previously described.

Postmortem workup

For each animal removed from the herd, a detailed gross necropsy, histopathologic examination was performed by a board-certified pathologist. To assess for common respiratory pathogens of bighorn sheep, we performed bacteriology (aerobic culture and the assays previously described), parasitology (Baermann funnel technique, fecal float), and virology (PCR for bovine herpesvirus-1, parainfluenza virus-3, bovine viral diarrhea virus, and bovine respiratory syncytial virus) as described (Malmberg et al. 2020).

RESULTS

A total of 24 adult female bighorn sheep from the Red Creek subunit of the Whiskey Mountain herd were captured during the 2015–2022 study period. Test-and-remove was initiated in 2021, at which time seven adult females were identified as chronic carriers. The first removal occurred in December of 2021 when

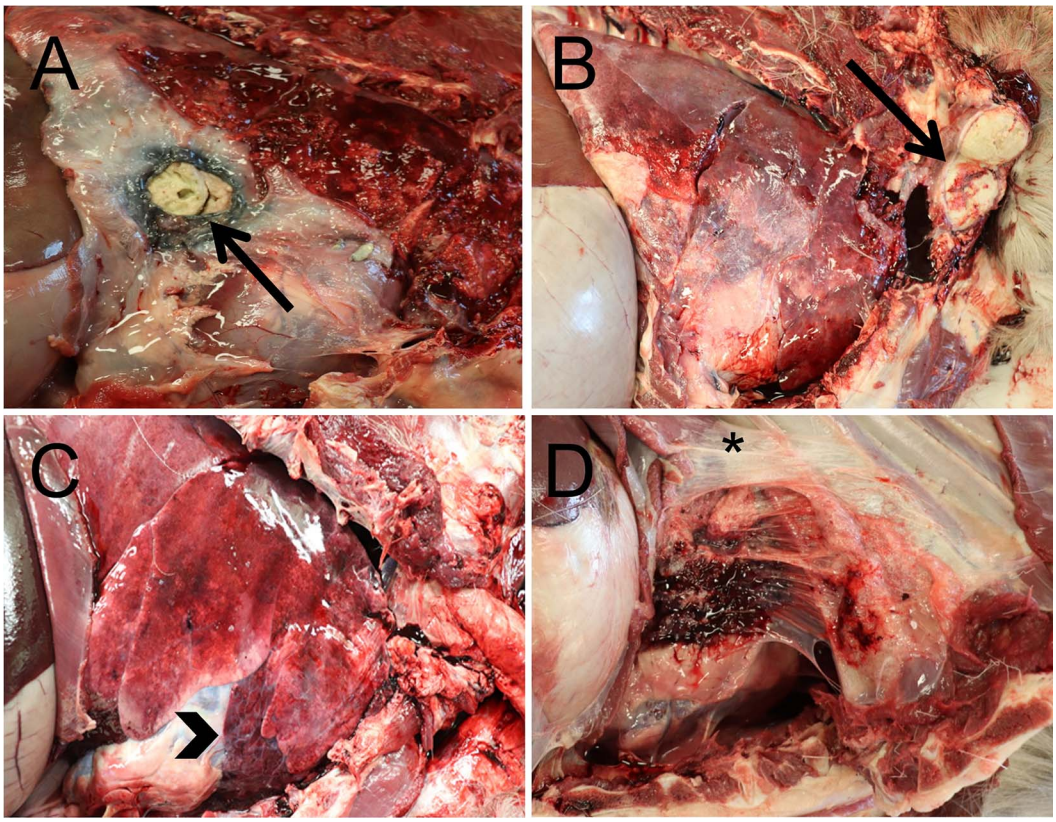

FIGURE 2. Gross features of pneumonia in bighorn sheep (*Ovis canadensis*) ewes chronically infected with *Mycoplasma ovipneumoniae* from the Red Creek subunit of the Whiskey Mountain herd in the Wind River Range, Wyoming, USA; these varied between individuals. Chronic abscesses were occasionally identified (A and B [arrows]). Cranioventral consolidation was the most common finding, best illustrated in C (arrowhead). Thoracic adhesions were a feature in some cases (D [asterisk]).

one ewe was humanely killed due to an atlanto-occipital fracture that occurred during capture. Four adult females were removed in March of 2022. Two additional adult females that tested PCR positive for *M. ovipneumoniae* in March were removed in May of 2022.

Overall, 6/7 (86%) had chronic bronchopneumonia with cranioventral consolidation (Fig. 2). The proportion of affected lung tissue in cases of bronchopneumonia ranged from 20 to 80% based on gross estimation. Thoracic adhesions were identified in 3/7 (42.9%; Fig. 2D) ewes, the degree of which varied from mild to severe. Two out of six (28.6%) had large abscesses in the thorax, both of which were caseonecrotic (Fig. 2A, B). One abscess was within the lung parenchyma (Fig. 2A); the other was within the mediastinum at the level of the thoracic inlet (Fig. 2B). Histologically, bronchopneumonia was suppurative to lymphoplasmacytic with frequent bronchus-associated lymphoid tissue hyperplasia (Fig. 3A, B). Other common features included variable degrees of bronchiolar epithelial hyperplasia (Fig. 3C) with occasional luminal sloughing. Bronchiole lumens were often filled with degenerate neutrophils and infrequently contained small coccobacilli (Fig. 3D). Features of chronicity included bronchiectasis with peripheral fibrosis as well as foci of mineralization centered on lakes of necrosuppurative debris (Fig. 3E). Lungworms (*Protostrongylus* sp.) were a consistent histologic finding (Fig. 3F) within the caudodorsal lung fields, occasionally forming grossly evident, chronic nodules. *Protostrongylus* sp. eggs

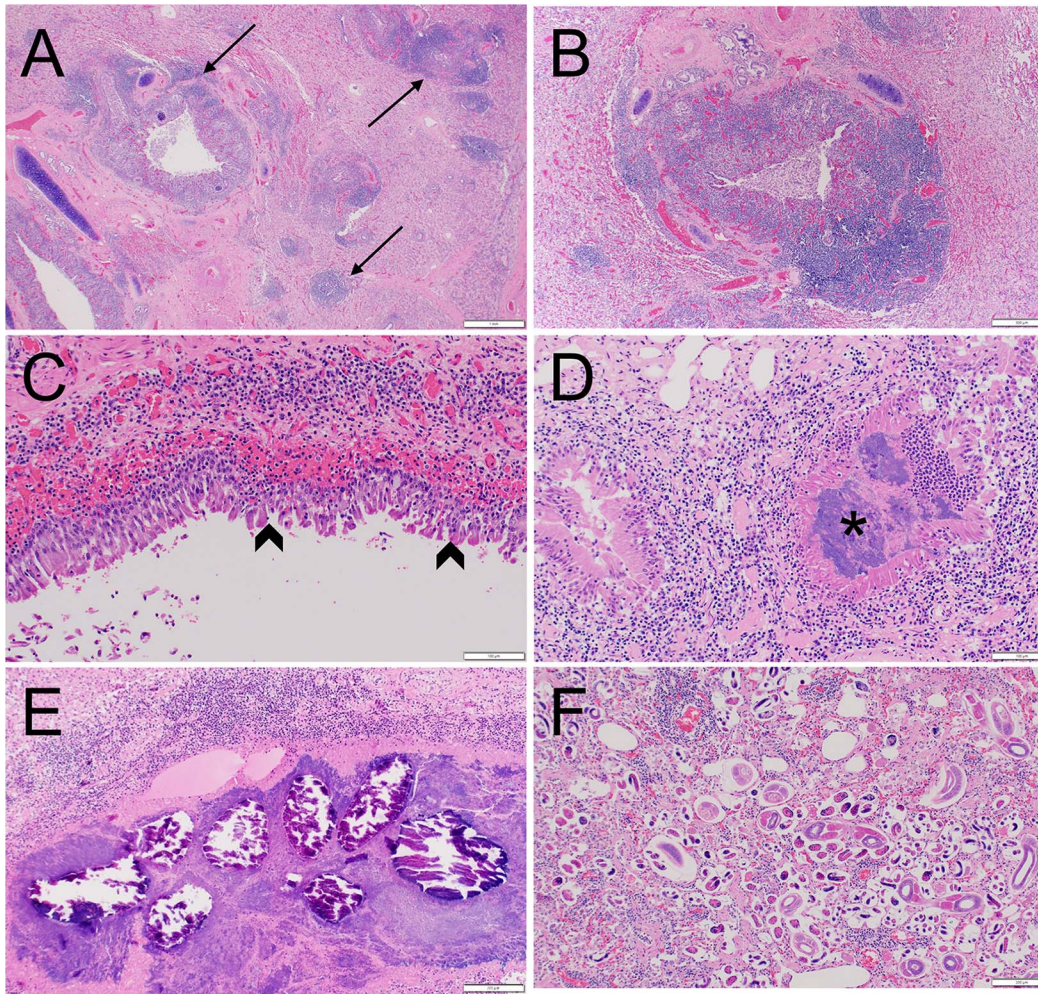

FIGURE 3. Histologic lung lesions in bighorn sheep (*Ovis canadensis*) ewes chronically infected with *Mycoplasma ovipneumoniae* from the Red Creek subunit of the Whiskey Mountain herd in the Wind River Range, Wyoming, USA. These included suppurative to lymphoplasmacytic bronchopneumonia with bronchus-associated lymphoid tissue hyperplasia (A [arrows] and B), bronchiolar epithelial hyperplasia (C [arrowheads]), clusters of bacteria admixed with degenerate neutrophils within bronchiole lumens (D [asterisk]), foci of mineralization (E), and chronic lungworm nodules (F).

were confirmed via the Baermann funnel technique.

Paranasal sinus lesions were identified in 4/7 (57%) ewes; all were classified as inflammatory, hyperplastic lesions (Figs. 4 and 5). Lesions were most common in the frontal sinus (4/4, 100%; Fig. 4A–C) and less common in the maxillary sinus (n=1/4, 25%) and cornual diverticulum (1/4, 25%; Fig. 4E). Gross appearance ranged from mild expansion of the sinus lining by edematous, hyperemic, soft tissue (Fig. 4A) to complete occlusion by loose fibrous connective tissue and variable amounts of reactive bone (Figs. 4B and 5C). Histologically, all sinus lesions had chronic, suppurative to lymphoplasmacytic, inflammation (Fig. 5) with variable degrees of fibrosis (Fig. 5B), proliferation of immature bone (Fig. 5C), and/or necrosis (Fig. 5D). All sinus lesions were proliferative with elements of hyperplasia (i.e., ductular, glandular or surface epithelial; Fig. 5E, F), though none were identified as neoplastic. In

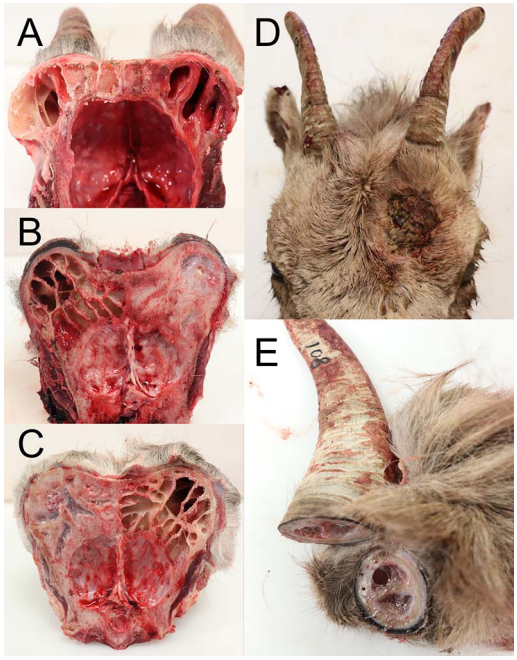

FIGURE 4. Gross lesions in the paranasal sinuses of bighorn sheep (*Ovis canadensis*) ewes chronically infected with *Mycoplasma ovipneumoniae* from the Red Creek subunit of the Whiskey Mountain herd in the Wind River Range, Wyoming, USA. These included thickening of the frontal sinus lining by gelatinous, hyperemic soft tissue (A, left frontal sinus), occlusive proliferation of soft tissue and immature bone (B and C), external skin crusts with frontal bone lysis (D), and extension into the cornual diverticulum (E).

one ewe, the lesion was grossly visible on external examination as a crusty dermal lesion with underlying lysis of the right frontal bone (Fig. 4D). Two of four (50%) cases had copious amounts of mucopurulent nasal discharge, which was also sometimes present in ewes in which sinus lesions were absent.

Mycoplasma ovipneumoniae was detected by PCR in at least one sample type—nasal swab or lung—in all ewes; however, nasal swabs collected postmortem from 2/7 (29%) ewes were negative, and lung tissue from three (43%) ewes tested negative (Table 1). The negative lung samples included those from the ewe without bronchopneumonia. Sinus swabs were collected from ewes (n=4) with proliferative changes in paranasal sinuses; *M. ovipneumoniae* was detected in two (50%; Table 1). Sinus tissue was not tested for *M. ovipneumoniae* in cases that lacked gross lesions.

We detected *P. multocida* by PCR, culture, or both in all seven ewes (100%; Table 1). Detection of *P. multocida* was consistent across sampled sites including nasal cavity, tonsil, and lung. Additionally, *P. multocida* was detected in the sinus tissue of all four ewes with sinus lesions. *Trueperella pyogenes* was frequently isolated on lung culture (n=5, 71%). Leukotoxigenic *Mannheimia haemolytica* was identified in tonsil and nasal swabs from one (14%) ewe, which also had leukotoxigenic *B. trehalosi* in the lung (Supplementary Material Table S1). Respiratory viruses (i.e., bovine herpesvirus-1, parainfluenza virus-3, bovine viral diarrhea virus, bovine respiratory syncytial virus) were not detected in any of the ewes by PCR.

DISCUSSION

Host heterogeneity in infectiousness is a well-described phenomenon in the field of epidemiology (Farrington et al. 2013; Elie et al. 2022). Although disparate individual transmission risk can muddle disease predictions among and between populations, wildlife managers can sometimes capitalize on heterogeneity through selective removal of high-risk individuals, such as chronic carriers (Miguel et al. 2020). Bighorn sheep respiratory disease, which is characterized by epizootic spillover events followed by long-term impacts on juvenile survival, can be mitigated through removal of persistently infected adults (Garwood et al. 2020; Spaan et al. 2021). However, chronic carriers may be difficult to identify (Butler et al. 2017; Plowright et al. 2019) and test-and-removal programs can be costly and laborious (Wolfe et al. 2004; le Roex et al. 2016). Further, postremoval mixing or immigration presents an additional challenge and may hinder recovery efforts (Miguel et al. 2020). Lastly, the long-term impact of test-and-remove actions on naturally occurring host–pathogen coevolutionary processes remains unclear.

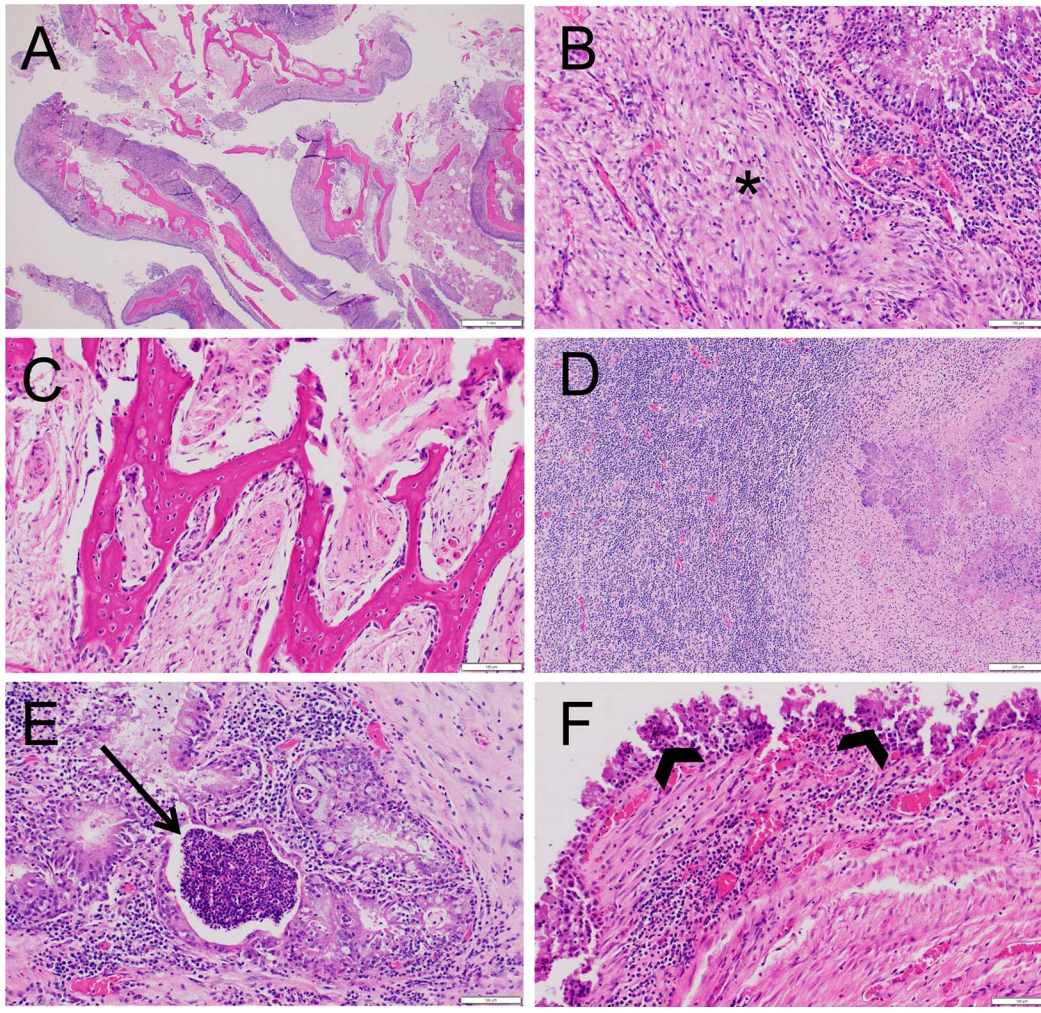

FIGURE 5. Histologic lesions in the paranasal sinuses of bighorn sheep (*Ovis canadensis*) ewes chronically infected with *Mycoplasma ovipneumoniae* from the Red Creek subunit of the Whiskey Mountain herd in the Wind River Range, Wyoming, USA. Findings included sinus expansion by chronic-suppurative to lymphoplasmacytic inflammation (A), fibrosis (B [asterisk]), bone proliferation (C), necrosis (D, right), glandular ectasia and hyperplasia with suppurative inflammation (E [arrow]), and surface epithelial hyperplasia (F [arrowheads]).

To understand the pathophysiology of chronic carriers of *M. ovipneumoniae* better, we characterized the degree and distribution of lesions in carrier ewes in conjunction with postmortem testing for *M. ovipneumoniae*. Most ewes had moderate to severe lung lesions, while some also had lesions in the paranasal sinuses, ranging from mild to severe. The degree and extent of tissue damage varied across individuals and anatomic sites (i.e., lung versus paranasal sinuses). Lung lesions had features of chronicity including dispersed bronchus-associated lymphoid tissue hyperplasia and epithelial to glandular hyperplasia of the airways. All lesions were regionally extensive and centered on the cranioventral lung fields, as is typical of bacterial pneumonia, with variable amounts of unaffected or lesser affected lung in the caudodorsal region. Absence of bronchopneumonia was found in only one ewe; this individual did not have detectable *M. ovipneumoniae* in the lung at the time of death. These findings indicate that the immune

TABLE 1. Postmortem findings in adult female bighorn sheep (*Ovis canadensis*) removed from the Red Creek subunit of the Whiskey Mountain herd in the Wind River Range, Wyoming, USA.

Sheep identity	AID[a] 3	AID 91	AID 117	AID 6	AID 108	AID 112	AID 157
Number of times tested	7	6	5	7	4	4	2
History of (+, −−, +)	Yes	Yes	Yes	No	No	No	No
Bronchopneumonia	Severe	Severe	Marked	Marked	Severe	Severe	ND
Sinus mass[b]	ND[c] (0)	Yes (2)	ND (0)	Yes (1)	Yes (2)	ND (0)	No (0)
Mycoplasma ovi pneumonia PCR (nasal swab)	ND	Detected	Detected	Detected	ND	ND	PCR
Mycoplasma ovipneumonia PCR (lung)	ND	Detected	ND	Detected	Detected	Detected	ND
Trueperella pyogenes culture (lung)	Detected	Detected	Detected	Detected	Detected	ND	ND
Pasteurella multocida culture (lung)	Detected	Detected	Detected	Detected	Detected	Detected	Detected

[a] AID=animal ID.
[b] Paranasal sinus score according to Fox et al. (2015).
[c] ND=not detected.

response of chronic carriers does not prevent disease in most cases, but rather permits host survival in the face of persistent infection, albeit with unknown fitness consequences.

Paranasal sinus masses in chronic carriers of *M. ovipneumoniae* were an inconsistent finding in this study. The absence of sinus lesions in some chronic carriers indicates that occlusion of the sinuses is not a requirement for chronic carriage of *M. ovipneumoniae*. We did not perform histopathology or bacterial testing on grossly normal sinus tissue, which is a limitation of this study. However, our findings lend support to a putative association between paranasal sinus masses and common pneumonia -associated bacteria (Fox et al. 2015). In our study, sinus lesions were not neoplastic. These findings emphasize the spectrum of lesions reported in (Fox et al. 2011) and suggest that space-occupying masses in the paranasal sinuses of bighorn sheep often comprise inflammation and reactive change. We highlight the need for histopathology and bacterial assays on grossly unremarkable sinus tissue from bighorn sheep, including those with and without pneumonia, to understand the relationship between bacterial carriage, chronic antigenic stimulation, and reactivity to neoplastic change.

In our study, commonly detected co-infecting bacteria included *P. multocida* and *T. pyogenes* (Table 1), both of which are considered common commensals that can act as opportunistic pathogens. Given the commonality of these bacteria in bighorn sheep, the significance of detection in this study is unclear. Although it is plausible that these bacteria have the potential to exacerbate pneumonia, it is difficult to assess the association with chronic carriage of *M. ovipneumoniae* specifically. We did not identify any co-infecting viruses in this study, nor did we consistently identify any co-infecting bacteria with known virulence factors. Overall, these findings indicate that virulent co-infections are not required for the persistence of *M. ovipneumoniae* in the respiratory tract of bighorn sheep. Our study involved capture and testing of adult females twice yearly in March and December. We defined chronic carriers as those testing

positive at least twice in a 14-mo period, which encompassed three testing events while allowing for a flexible window around each scheduled capture. This definition allowed for a single negative result in a series of the three most recent tests, which accounted for the possibility of false negatives and facilitated a more aggressive removal strategy that targeted both intermittent and persistent carriers. We did not differentiate between intermittent versus persistent shedding, but instead assumed that both posed a putative herd health risk.

The number of times an animal was handled and tested varied in our study. Some animals had been tested numerous times prior to initiation of the targeted removal efforts, and others were represented only by more recent captures (Fig. 1). Some animals (n=3/7, 43%) tested positive intermittently, and others (n=4/7, 57%) remained consistently positive following the first detection. Though not a requirement for inclusion, the two most recent tests were positive for all animals in this study.

Our definition of chronic carriers was more inclusive than that used by Garwood et al. (2020). A comprehensive definition of chronic carriage has yet to be established; however, classification should consider testing uncertainty as well as variability in colonization of anatomic site (i.e., nasal cavity versus lung). For instance, our study revealed that two ewes defined as chronic carriers did not have detectable *M. ovipneumoniae* in nasal swabs collected postmortem. This may be because of clearance from the upper respiratory tract before removal or imperfect sensitivity of detection assays (Butler et al. 2017). Findings such as this indicate that managers investing in test-and-removal efforts will be faced with trade-offs, such as whether it is prudent to remove some animals that may eventually clear the bacteria versus, leaving even a single possible superspreader on the landscape. Postremoval assessment of more animals that intermittently test positive will help inform the role of these individuals in the maintenance of *M. ovipneumoniae* within herds and aid the refinement of criteria for removal. Even if standard criteria are developed, however, the optimal strategy for test-and-remove programs may vary depending on the focal system at hand. Managers should consider the ecology and epidemiology of herds to determine the optimal design of test-and-remove programs, which may range from extensive to selective and proactive to reactive, depending on the unique demography, social structure, and connectivity of the populations in question. Although this study only considered the infection status of adult females, future consideration of other demographics (i.e., adult males, yearlings) will be important to understand the socioecological determinants of *M. ovipneumoniae* persistence within a herd better.

Previous work has shown that strain variability of *M. ovipneumoniae* limits the effectiveness of the adaptive immune response (Walsh et al. 2023). Our study did not evaluate antibodies or *M. ovipneumoniae* strain types. Future studies that aim to understand the relative importance of strain-associated virulence and immune-driven tissue damage—that is, immunopathology—in bighorn sheep respiratory disease might aid the development of strategies for maintaining healthy wild sheep populations. Future test-and-remove efforts should incorporate thorough postmortem examinations and diagnostic testing, as well as strain typing and serology, to understand host–pathogen interactions in this system and better inform identification of animals for targeted removal.

The long-term impact of selective removal on evolving host defense mechanisms also merits consideration. The ability of mycoplasmas to establish chronic, subclinical infections has been demonstrated in other systems. In some cases, these infections are well tolerated by the host. Domestic sheep infected with *M. ovipneumoniae*, for example, exhibit minimal morbidity and mortality (US Department of Agriculture Animal and Plant Health Inspection Service 2015; Heinse et al. 2016). This can be attributed to a long history of host and pathogen co-evolution, resulting in an

equilibrium that minimizes damage to the host while permitting pathogen persistence (Carval and Ferriere 2010; Soares et al. 2017). Therefore, although it may be ideal to select for animals with an ability to clear *M. ovipneumonaie* (i.e., resistance), tolerant animals, especially those that avoid severe disease, may also be important to the long-term evolutionary trajectory of the system. Our study identified one animal that met the definition for removal but had no detectable disease at necropsy. If similar animals can be identified through postmortem assessment of other populations, these individuals may be of particular interest, especially given that tolerance of *M. ovipneumoniae* is influenced by genetics (Råberg et al. 2009; Plowright et al. 2017; Martin et al. 2021; Mousel et al. 2021).

Our study indicates that postmortem findings from test-and-remove studies can advance our understanding of bighorn sheep respiratory pathophysiology and thereby inform management actions that account for individual heterogeneity in efforts to control pneumonia. Further, our findings indicate that although most carriers of *M. ovipneumoniae* have chronic bronchopneumonia, it is possible for an animal to carry *M. ovipneumoniae* in the nasal cavity without pneumonia and without colonization of the lung. We recommend that future studies assess the degree and extent of pathology in conjunction with adaptive immune response and strain-specific exposure status to understand the defense mechanisms of chronic carriers in this disease system better. Efforts to identify and characterize host factors that contribute to chronic carriage are needed to optimize testing, to minimize sampling effort, and to help understand the utility of test-and-remove actions in wild sheep conservation. More broadly, knowledge gleaned from detailed bighorn sheep test-and-remove studies may inform the general use of wildlife culling as a disease-management tool. Long-term, detailed data collection is necessary to understand the benefits and consequences of culling, progressively refine criteria and approaches, and inform adaptive wildlife management.

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SUPPLEMENTARY MATERIAL

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Table S1. Postmortem laboratory assay results in adult female bighorn sheep (*Ovis canadensis*) removed from the Red Creek subunit of the Whiskey Mountain herd in the Wind River Range, Wyoming, USA. *B. trehalosi= Bibersteinia trehalosi;* lkt+= leukotoxin-positive; *M. ovi=Mycoplasma ovipneumoniae*; ND= not detected; NT=not tested; *P. multocida=Pasteurella multocida*; T. pyogenes=*Trueperella pyogenes.*

Animal ID	**Sample**	**Aerobic culture**	***M. ovi* culture**	***B. trehalosi* lkt+ qPCR**	***Pasteurella* culture**	***P. multocida* PCR**	***Mannhemia* sp. lkt+ PCR**	***M. haemolytica /glucosida* PCR**	***M. ovi* PCR**
AID91	Lung	*T. pyogenes, P. multocida*	ND	Detected	*P. multocida*	Detected	ND	NT	Detected
	Nasal swab	NT	ND	ND	*P. multocida*	Detected	Detected	Detected	Detected
	Tonsil swab	NT	NT	ND	Non-hemolytic *B. trehalosi*, *Mannheimia* sp.	Detected	Detected	Detected	NT

	Sinus swab	NT	ND	ND	*P. multocida*	Detected	Detected	NT	Detected
AID3	Lung	*T. pyogenes, P. multocida*	ND	ND	*P. multocida*	Detected	ND	NT	ND
	Nasal swab	NT	ND	ND	*P. multocida*	Detected	NT	NT	ND
	Tonsil swab	NT	NT	ND	Non-hemolytic *B. trehalosi*	Detected	Detected	ND	NT
	Sinus swab	NT	NT	NT		NT	NT	NT	NT
AID112	Lung	*P. multocida, S. lutetiensis*	ND	ND	No signifcant isolates	Detected	ND	NT	Detected
	Nasal swab	NT	ND	ND	*P. multocida*	Detected	ND	NT	ND
	Tonsil swab	NT	NT	ND	*P. multocida*, Non-hemolytic *B. trehalosi*	Detected	ND	NT	NT

	Sinus swab	NT	NT	NT	NT	NT	NT	NT	NT
AID108	Lung	*T. pyogenes, P. multocida*	ND	ND	*P. multocida*	Detected	ND	NT	Detected
	Nasal swab	NT	ND	ND	*P. multocida*	Detected	ND	NT	ND
	Tonsil swab	NT		ND	Non-hemolytic *B. trehalosi*	Detected	ND	NT	NT
	Sinus swab	NT	ND	ND	*P. multocida*	Detected	ND	NT	ND
AID157	Lung	Mixed contaminants	ND	ND	*P. multocida*	Detected	ND	NT	ND
	Nasal swab	NT	ND	ND	*P. multocida*	Detected	ND	NT	Detected
	Tonsil swab	NT	NT	ND	Non-hemolytic *B. trehalosi*	Detected	ND	NT	NT

	Sinus swab	NT	NT	NT	NT	NT	NT	NT	NT
AID117	Lung	*T. pyogenes, P. multocida*	ND	ND	*P. multocida,* Non-hemolytic *B. trehalosi*	Detected	ND	NT	ND
	Nasal swab	NT	ND	ND	*P. multocida*	Detected	ND	NT	Detected
	Tonsil swab	NT	NT	ND	*P. multocida*	Detected	ND	NT	NT
	Sinus swab	NT	ND	ND	*P. multocida*	Detected	ND	NT	ND
AID6	Lung	*P. multocida, T. pyogenes,* Non-hemolytic *B. trehalosi*	ND	ND	No significant isolates	Detected	ND	NT	Detected
	Nasal swab	*P. multocida, Moraxella bovoculi*	*Myco-plasma* spp.	ND	*P. multocida*	Detected	ND	NT	Detected
	Tonsil swab	NT	NT	ND	No significant isolates	Detected	ND	NT	NT

Sinus swab	NT	ND	ND	*P. multocida*	Detected	ND	NT	Detected