DOI: 10.7589/JWD-D-22-00094

Journal of Wildlife Diseases, 59(4), 2023, pp. 753–758

Clearance of *Mycoplasma ovipneumoniae* in Captive Bighorn Sheep (*Ovis canadensis*) Following Extended Oral Doxycycline Treatment

Mary E. Wood,[1,2,5] William H. Edwards,[3] Jessica E. Jennings-Gaines,[3] Mariah Gaston,[4] Peach Van Wick,[4] Sierra Amundson,[1] Samantha E. Allen,[2] and Lisa L. Wolfe[1] [1]Colorado Parks and Wildlife, Wildlife Health Program, 4330 Laporte Ave., Fort Collins, Colorado 80521, USA; [2]Wyoming Game and Fish Department, Veterinary Services, 1212 S. Adams St., Laramie, Wyoming 82070, USA; [3]Wyoming Game and Fish Department, Veterinary Services, Wildlife Health Laboratory, 1174 Snowy Range Rd., Laramie, Wyoming 82070, USA; [4]Wyoming Game and Fish Department, Veterinary Services, Thorne-Williams Wildlife Research Center, 2362 WY-34, Wheatland, Wyoming 82201, USA; [5]Corresponding author (e-mail: mary.wood@state.co.us)

ABSTRACT: Respiratory disease is a significant barrier for bighorn sheep (*Ovis canadensis*) conservation, and a need remains for management options in both captive and free-ranging populations. We treated *Mycoplasma ovipneumoniae* infection in six bighorn lambs and five bighorn yearlings at two captive research facilities with twice daily oral doxycycline for 8 wk or longer. Doses of 5 mg/kg twice daily mixed in formula for lambs and 10 mg/kg twice daily mixed in moistened pellets for older lambs and yearlings were tolerated well with minimal side effects. All animals in this case report remain *Mycoplasma ovipneumoniae* free over 2 yr later. Further evaluation is warranted to confirm efficacy of this therapeutic approach.

Key words: Bighorn sheep, doxycycline, *Mycoplasma ovipneumoniae*, *Ovis canadensis*, pasteurella, respiratory disease.

Respiratory disease presents a significant barrier for bighorn sheep (*Ovis canadensis*) conservation. Among bacterial pathogens contributing to the bighorn respiratory disease complex, *Mycoplasma ovipneumoniae* and several *Pasteurellaceae* species appear most commonly detected (Besser et al. 2012). *Mycoplasma ovipneumoniae* can impair mucociliary clearance of bacterial pathogens and may predispose an animal to infection with other bacteria (Dassanayake et al. 2010). Consequently, clearing infections of virulent *M. ovipneumoniae* strains may promote clearance of other respiratory pathogens and lessen severity of disease.

Mycoplasma ovipneumoniae is a fastidious and slow-growing bacteria that is difficult to culture, creating limitations for culture and sensitivity testing to guide antibiotic selection (Weiser et al. 2012). Common therapies for *Mycoplasma* infections include fluoroquinolone, tetracycline, macrolide, cephalosporin, and aminoglycoside antibiotics (Yatoo et al. 2019). Several of these antibiotics aimed at treating respiratory infections have been administered to domestic and wild sheep (Weiser et al. 2009; Sirochman et al. 2012; Politis et al. 2019; Yatoo et al. 2019; Johnson et al. 2022) To date, there are no published reports of successful treatment of *M. ovipneumoniae* in bighorn sheep.

Doxycycline is a second-generation tetracycline antibiotic with broad-spectrum activity against a wide variety of microorganisms, including mycoplasma bacteria (Shaw and Rubin 1986). This antibiotic acts by inhibiting bacterial protein synthesis, causing a bacteriostatic effect, although bactericidal effects may occur at higher doses (Shaw and Rubin 1986). Although doxycycline has broad spectrum activity, it is particularly beneficial in treating infections involving atypical bacteria and bacteria that lack a cell wall, such as *Mycoplasma* and *Rickettsia* species (Holmes and Charles 2009). Both oral and parenteral administration have been described in domestic sheep, goats, and cattle (Meijer et al. 1993; Ole-Mapenay and Mitema 1997; Castro et al. 2009; Castro Robles et al. 2012; Mileva et al. 2020). Tetracycline antibiotics have a wide tissue distribution, but doxycycline is more lipid soluble than other tetracyclines, providing greater tissue penetration and a longer half-life (Shaw and Rubin 1986). Additionally, doxycycline has anti-inflammatory properties that may offset pro-inflammatory responses associated with *M. ovipneumoniae* infection (Shaw and Rubin 1986; Grossman et al. 2021). Here we provide a case report detailing bighorn sheep responses in two captive wildlife facilities following prolonged oral doxycycline treatment for *M. ovipneumoniae* infection.

TABLE 1. Chronology of doxycycline *Mycoplasma ovipneumoniae* treatments, laboratory results, and clinical signs of disease for one bighorn (*Ovis canadensis*) lamb (Ne17) held at the Foothills Wildlife Research Facility, Fort Collins, Colorado, US. Laboratory results reported for the animal's age when sampled; results were not immediately available.

Age (d)	Treatment	*Mycoplasma ovipneumoniae* PCR or culture[a]	Clinical signs
1	None	Not detected (WADDL)	Absent
7	None	Detected	Absent
12–69	Various (see Table S1)	Detected	Coughing, nasal discharge, lethargy, head shaking
73 – 100	Doxycycline syrup[b] (5 mg/kg PO[c] BID[d])	Not tested	Signs resolved
114	None	Not detected	Absent
119–128	Treatments for otitis externa (see Table S1)	Detected	Ear droop, intermittent lateral strabismus
131 – 163	Doxycycline syrup (3.5 mg/kg PO BID)	Not tested	Absent
385, 464, 741, 1,073	None	Not detected	Absent

[a] Swabs were screened for select respiratory pathogens at the Wyoming Game and Fish Department's Wildlife Health Laboratory (Laramie, Wyoming, US) unless noted as being done at the Washington Animal Disease Diagnostic Laboratory (WADDL, Pullman, Washington, US; Walsh et al. 2016; Butler et al. 2017).
[b] Doxycycline calcium syrup (Vibramycin oral syrup, Pfizer, New York, New York, USA).
[c] PO=per os (orally).
[d] BID=twice per day.

Our first attempt involved a bighorn lamb, Ne17, which originated in a herd with a history of respiratory disease and poor lamb recruitment located in northwest Nebraska, US. A field crew captured her within approximately 24 h of birth, on 21 June 2017 (age=1 d), placed a radio collar, and collected nasal and tonsil swabs (Table 1). She was found abandoned 2 d later and brought directly to the Foothills Wildlife Research Facility, Fort Collins, Colorado, US, for hand raising. Because of the source herd's health history, we treated Ne17 prophylactically with 40 mg/kg florfenicol (Nuflor, Merck Animal Health, Madison, New Jersey, USA) and 2.2 mg/kg flunixin meglumine (Banamine, Merck Animal Health) and held her in isolation (Supplementary Table S1). Although apparently healthy early on, at 12 d she was coughing and lethargic and had occasional head shaking. Swabs collected after 7 d were PCR positive for *Mycoplasma ovipneumoniae*. Between 12 and 69 d, we tried a variety of supportive therapies to reduce clinical signs of respiratory disease and antibiotics labeled for bovine respiratory disease to clear *M. ovipneumoniae*, without success (Table S1).

Thereafter, we changed treatment to oral doxycycline calcium (Vibramycin oral syrup, Pfizer, New York, New York, USA) given twice daily in milk and bottle fed for 4 wk (Table 1). The lamb initially rejected the bottle when offered the doxycycline mixture, so we gradually increased the dose per feeding over a week until she accepted a full dose. Respiratory signs gradually resolved. A nasal swab collected on day 114 was PCR negative for *M. ovipneumoniae*. Between 119 and 126 d, we treated Ne17 for necrosuppurative otitis externa with intralesional cocci and plant material identified on ear canal biopsy (Table S1). A nasal swab at 128 d again tested positive for *M. ovipneumoniae* via PCR; we resumed treatment with doxycycline for another 4 wk. Clinical signs resolved, and swabs collected between 385 and 1,073 d remained culture and PCR negative for *M. ovipneumoniae*.

We introduced two bighorn lambs into her pen in October 2019; both lambs and Ne17 remain free of clinical respiratory disease as of April 2023. Nasal swabs from those pen mates collected 233 d later were PCR negative for *M. ovipneumoniae*. No other bighorns at the Fort Collins facility became infected. Notably, no lambs survived in the Nebraska source herd in 2017. Of the 22 lambs monitored from this herd in 2017, five were abandoned, 13 mortalities were attributed to respiratory disease, three were attributed to predation, and one was unknown (Nebraska Game and Parks Commission 2017).

Our subsequent attempts to treat mycoplasmosis involved two cohorts of captive bighorns held at the Thorne-Williams Wildlife Research Center, Wheatland, Wyoming, US. In February 2014, we moved a group of 15 free-ranging bighorn ewes into captivity from a population in northwest Wyoming known to harbor multiple respiratory pathogens. We sampled them every 3–6 mo for the presence of respiratory pathogens throughout their time in captivity. Most members of the group shed *M. ovipneumoniae*, leukotoxigenic *Mannheimia* spp., leukotoxigenic *Bibersteinia trehalosi*, and *Pasteurella multocida* between February 2014 and July 2019, with intermittent coughing and nasal discharge noted in most animals. Additionally, we documented paranasal sinus masses (Fox et al. 2011) in eight of 13 ewes euthanized from the group. Out of nine lambs born to these ewes in 2015, seven died or were euthanized due to severe respiratory disease, with clinical signs and pathology similar to previous captive lamb work at the facility (Wood et al. 2017); one died of complications from hydrocephalus; and one was euthanized due to nutritional insufficiency. We did not breed these ewes in the fall of 2015 or 2016.

Five bighorn lambs were born to these captive ewes in the spring of 2018 and another five in 2019. We removed these lambs 24–72 h after birth for hand raising and administered 8 mg/kg tildipirosin (Zuprevo, Merck Animal Health) prophylactically upon removal from the ewes and housed them together. The lamb enclosure was approximately 400 m from the ewe enclosure, and facility staff typically drove between the enclosures.

Among the 2019 cohort, one lamb began coughing at 21 d old. Nasal swabs were PCR positive for *M. ovipneumoniae* in three of four lambs swabbed (Table 2). It is unclear whether *M. ovipneumoniae* transmission occurred from the ewes prior to removal of the lambs or if it was due to a failure in biosecurity associated with facility staff or fomites moving between the two groups. We treated all five lambs with doxycycline twice daily for 4 wk (Table 2). Clinical signs resolved within 10 d, at which point one of five lambs was still PCR positive for *M. ovipneumoniae*. No additional diagnostics were conducted to confirm clearance after the 4-wk treatment. Coughing and nasal discharge recurred at 87 d, and all five lambs tested PCR positive for *M. ovipneumoniae* (Table 2). We reinitiated twice-daily treatment for 4 wk. Clinical signs were still present, so we increased the dose and treated for another 4 wk (Table 2). Most clinical signs resolved, but nasal swabs from three of five lambs were still PCR positive for *M. ovipneumoniae*. Lambs were too old for bottle feeding, so we continued treatment by opening 100 mg capsules of doxycycline hyclate (Amneal Pharmaceuticals, Bridgewater, New Jersey, USA) and sprinkling the powder over moistened pelleted feed. Animals remained together during feeding, and we supplied doxycycline-coated feed in five separate feed bins at 10 mg/kg, based on estimated animal weight, twice per day for an additional 30 d (Table 2). Clinical signs fully resolved and did not recur. Nasal swabs collected between 221 and 1,058 d were PCR negative for *M. ovipneumoniae* (Table 2); however, we did detect leukotoxigenic *Bibersteinia trehalosi* in all five lambs on days 507, 912, and 1,058.

The five bighorn sheep born in 2018 remained apparently healthy for more than a year with no detection of respiratory pathogens. Routine sampling at 479 d revealed all five yearlings were PCR positive for *M. ovipneumoniae* and leukotoxigenic *B. trehalosi* (Table 2). All developed clinical signs of coughing and nasal discharge shortly thereafter. This cohort was in a fenced enclosure adjacent to the 2019 cohort. While nose-to-nose contact was not

TABLE 2. Chronology of doxycycline *Mycoplasma ovipneumoniae* treatments, laboratory results, and clinical signs of disease for bighorn lambs held at the Thorne-Williams Wildlife Research Center, Wheatland, Wyoming, US. Laboratory results reported for the approximate animal's age when sampled; results were not immediately available.

Age (d)	Day of year	Treatment	*Mycoplasma ovipneumoniae* PCR or culture[a,b]	Clinical signs
		Five Wyoming lambs born 2019		
21	165	None	Detected (3/4)	Coughing
21–30	165–174	Doxycycline syrup[c] (2.2 mg/kg PO[d] BID[e])	Detected (1/5)	Intermittent soft stool
30–49	174–193	Doxycycline syrup (2.2 mg/kg PO BID)	Not tested	Signs resolved
87–117	252–282	Doxycycline syrup (2.2 mg/kg PO BID)	Detected (5/5)	Coughing, nasal discharge, intermittent soft stool
118–147	283–312	Doxycycline syrup[f] (5 mg/kg PO BID)	Not tested	Coughing, nasal discharge
158	323	None	Detected (3/5)	Rare coughing
166–196	331–361	Doxycycline powder[g] (10 mg/kg PO BID)	Not tested	Signs resolved
221, 312, 409, 507, 912, 1,058	NA[h]	None	Not detected (5/5)	Absent
		Five Wyoming lambs born 2018		
39, 109, 263, 381	NA	None	Not detected (5/5)	Absent
479	266	None	Detected (5/5)	Absent
489–531	276–318	Doxycycline powder (10 mg/kg PO BID)	Not tested	Coughing, nasal discharge
537–574	324–361	Doxycycline powder (10 mg/kg PO BID)	Not tested	Signs resolved
599, 690, 787, 885, 1,272, 1,418	NA	None	Not detected (5/5)	Absent

[a] Swabs were screened for select respiratory pathogens at the Wyoming Game and Fish Department's Wildlife Health Laboratory (Laramie, Wyoming, US; Butler et al. 2017).
[b] Numbers in parentheses depict the number of animals where *M. ovipneumoniae* was detected out of the total number of animals sampled.
[c] Doxycycline calcium syrup (Vibramycin oral syrup, Pfizer, New York, New York, USA).
[d] PO=per os (orally).
[e] BID=twice per day.
[f] Compounded doxycycline calcium syrup (Good Day Pharmacy, Fort Collins, Colorado, USA).
[g] Doxycycline hyclate capsules (Amneal Pharmaceuticals, Bridgewater, New Jersey, USA).
[h] NA=not applicable.

possible, only a 1-m fenced alleyway separated the two enclosures, and animal care staff moved between enclosures after stepping in a footbath. We initiated treatment with doxycycline powder sprinkled over moistened pelleted feed (Table 2). Animals remained together during feeding, and we supplied antibiotic-coated feed twice per day in five separate feed bins at 10 mg/kg based on estimated animal weight. We treated for approximately 12 w with a 1-wk gap after 6 wk of treatment because of a delay in medication refills. Clinical signs fully resolved, and swabs collected between 599 and 1,418 d were PCR negative for *M. ovipneumoniae*; however, leukotoxigenic *B. trehalosi* was still detected in all five sheep.

Our work demonstrates potential efficacy of prolonged oral doxycycline application to treat *M. ovipneumoniae* in bighorn sheep. Doses of 5 mg/kg twice daily mixed in formula for young lambs and 10 mg/kg twice daily powder mixed in pellets for older lambs and yearlings were

tolerated well with some soft stool noted as dosing was increased (Table 2). In the absence of untreated controls, we cannot rule out the possibility of natural clearance. We have never observed natural clearance of *M. ovipneumoniae* in lambs at either research facility; however, numerous factors may contribute to differing disease outcomes, including bacterial strain variation, changes in animal management, and timing of infection. Additionally, hand rearing lambs eliminates repeated ewe-to-lamb transmission events, potentially leading to less complicated infections that are more readily cleared by natural immune responses.

After initiation of doxycycline treatment, we observed rapid reduction of clinical signs leading to full clinical resolution in all treated animals. Clinical signs did not resolve after other treatment approaches. The combination of both bacteriostatic and anti-inflammatory properties of doxycycline may limit clinical disease, facilitate more appropriate immune responses, and promote pathogen clearance in combination with natural immune responses. The spectrum of activity of doxycycline also includes *Pasteurellaceae* bacteria, including *P. multocida* and *Mannheimia spp.* (Politis et al. 2019). Therefore, doxycycline treatment may limit concurrent *Pasteurellaceae* infections and reduce severity of disease to enable *M. ovipneumoniae* clearance. It is worth noting that doxycycline treatment, despite its broad spectrum of activity, failed to clear leukotoxigenic *B. trehalosi* in the 2018 cohort.

Clinical signs of respiratory disease resolved following 4 wk of doxycycline treatment in both Ne17 and the 2019 cohort; however, signs recurred after treatment stopped in both cases. Additionally, the lower dose of 2.2 mg/kg in the 2019 cohort did not resolve clinical signs when the 2019 cohort was older; clinical resolution occurred only after dosing was increased. Learning from these experiences, we treated the 2018 cohort with a higher initial dose of 10 mg/kg and a longer duration, to maximize the likelihood for successful treatment in animals that were difficult to handle regularly.

All sheep described here harbored recent infections that had not reached a chronic state. It is unclear whether similar treatment would be beneficial in animals with chronic infections or advanced paranasal sinus masses. Our work reflects early trial and error to determine antibiotic selection, dosing, and methods of administration. Further evaluation with experimental controls and consistent initial dosing or treatment with higher doses administered once daily (Turk et al. 2020; Castro et al. 2009) warrants evaluation.

Our work was supported by Colorado Parks and Wildlife and the Wyoming Game and Fish Department. We thank the dedicated animal care staff at both facilities who provided daily care and treatments for the animals, including Matt Huizenga, Cole Hansen, Ashlynn Harris, Mark Fisher, and Shari Singleton. Christine Ellis consulted on mycoplasma therapies and Michael Miller provided helpful comments on an early draft of the manuscript. Todd Nordeen, Erin Wood, and Dane Horowski with Nebraska Game and Parks Commission kindly provided Ne17 and results from the Nebraska source population. We also thank the laboratory staff at the Wyoming Game and Fish Department Wildlife Health Laboratory for diagnostic support, including Katie Luukkonen and Tara Stitzlein.

SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/JWD-D-22-00094.

LITERATURE CITED

Besser TE, Highland MA, Baker K, Cassirer EF, Anderson NJ, Ramsey JM, Mansfield K, Bruning DL, Wolff P, et al. 2012. Causes of pneumonia epizootics among bighorn sheep, western United States, 2008–2010. *Emerg Infect Dis* 18:406–414.

Butler CJ, Edwards WH, Jennings-Gaines JE, Killion HJ, Wood ME, McWhirter DE, Paterson JT, Proffitt KM, Almberg ES, et al. 2017. Assessing respiratory pathogen communities in bighorn sheep populations: Sampling realities, challenges, and improvements. *PloS One* 12:e0180689.

Castro LJ, Sahagún AM, Diez MJ, Fernández N, Sierra M, García JJ. 2009. Pharmacokinetics of doxycycline in sheep after intravenous and oral administration. *Vet J* 180:389–395.

Castro Robles LJ, Sahagún Prieto AM, Diez Liébana MJ, Fernández Martínez N, Sierra Vega M, García Vieitez JJ. 2012. Pharmacokinetic behavior of doxycycline

after intramuscular injection in sheep. *Am J Vet Res* 73:714–718.

Dassanayake RP, Shanthalingam S, Herndon CN, Subramaniam R, Lawrence PK, Bavananthasivam J, Cassirer EF, Haldorson GJ, Foreyt WJ, et al. 2010. *Mycoplasma ovipneumoniae* can predispose bighorn sheep to fatal *Mannheimia haemolytica* pneumonia. *Vet Microbiol* 145:354–359.

Fox KA, Wootton SK, Quackenbush SL, Wolfe LL, LeVan IK, Miller MW, Spraker TR. 2011. Paranasal sinus masses of Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*). *Vet Pathol* 48:706–712.

Grossman PC, Schneider DA, Herndon DR, Knowles DP, Highland MA. 2021. Differential pulmonary immunopathology of domestic sheep (*Ovis aries*) and bighorn sheep (*Ovis canadensis*) with *Mycoplasma ovipneumoniae* infection: A retrospective study. *Comp Immunol Microbiol Infect Dis* 76:101641.

Holmes NE, Charles PGP. 2009. Safety and efficacy review of doxycycline. *Clin Med Ther* 1:CMT-S2035.

Johnson T, Jones K, Jacobson BT, Schearer J, Adams N, Thornton I, Mosdal C, Jones S, Jutila M, et al. 2022. Experimental infection of specific-pathogen-free domestic lambs with *Mycoplasma ovipneumoniae* causes asymptomatic colonization of the upper airways that is resistant to antibiotic treatment. *Vet Microbiol* 265:109334.

Meijer LA, Ceyssens KGF, de Grève BIJAC, de Bruijn W. 1993. Pharmacokinetics and bioavailability of doxycycline hyclate after oral administration in calves. *Vet Q* 15:1–5.

Mileva R, Subev S, Gehring R, Milanova A. 2020. Oral doxycycline pharmacokinetics: Lambs in comparison with sheep. *J Vet Pharmacol Ther* 43:268–275.

Nebraska Game and Parks Commission. 2017. *Barrel Butte Herd quarterly reports May–September 2017: Lamb information*. Unpublished internal agency document, Nebraska Game and Parks Commission, Lincoln.

Ole-Mapenay IM, Mitema ES. 1997. Efficacy of doxycycline in a goat model of *Pasteurella pneumonia*: Research communication. *J S Afr Vet Assoc* 68:55–58.

Politis AP, Vasileiou NGC, Loannidi KS, Mavrogianni VS. 2019. Treatment of bacterial respiratory infections in lambs. *Small Rumin Res* 176:70–75.

Shaw DH, Rubin SI. 1986. Pharmacologic activity of doxycycline. *J Am Vet Med Assoc* 189:808–810.

Sirochman MA, Woodruff KJ, Grigg JL, Walsh DP, Huyvaert KP, Miller MW, Wolfe LL. 2012. Evaluation of management treatments intended to increase lamb recruitment in a bighorn sheep herd. *J Wildl Dis* 48:781–784.

Turk E, Corum O, Tekeli IO, Sakin F, Uney K. 2020. Effects of single and repeated doses on disposition and kinetics of doxycycline hyclate in goats. *Animals* 10:1088.

Walsh DP, Cassirer EF, Bonds MD, Brown DR, Edwards WH, Weiser GC, Drew ML, Briggs RE, Fox KA, et al. 2016. Concordance in diagnostic testing for respiratory pathogens of bighorn sheep. *Wildl Soc Bull* 40:634–642.

Weiser GC, Drew ML, Cassirer EF, Ward ACS. 2012. Detection of *Mycoplasma ovipneumoniae* and *M. arginini* in bighorn sheep using enrichment culture coupled with genus- and species-specific polymerase chain reaction. *J Wildl Dis* 48:449–453.

Weiser GC, Miller DS, Drew ML, Rhyan JC, Ward ACS. 2009. Variation in *Pasteurella* (*Bibersteinia*) and *Mannheimia* spp. following transport and antibiotic treatment in free-ranging and captive Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*). *J Zoo Wildl Med* 40:117–125.

Wood ME, Fox KA, Jennings-Gaines J, Killion HJ, Amundson S, Miller MW, Edwards WH. 2017. How respiratory pathogens contribute to lamb mortality in a poorly performing bighorn sheep (*Ovis canadensis*) herd. *J Wildl Dis* 53:126–130.

Yatoo MI, Parray OR, Bhat RA, Gopalakrishnan MA, Saxena A, Chakraborty S, Tiwari, R, Khurana SK, Singh SV, Dhama K. 2019. Emerging antibiotic resistance in *Mycoplasma* microorganisms, designing effective and novel drugs/therapeutic targets: Current knowledge and futuristic prospects. *J Pure Appl Microbiol* 13:27–44.

Submitted for publication 21 July 2022.
Accepted 18 May 2023.