Figure. Direct examination with dark-field microscopy of specimens from a patient with agammaglobulinemia who had *Spiroplasma apis* infection, France. A) Helical and motile bacteria in blood culture. B) Elongated and coccoid bacteria in joint fluid. C) Helical and motile bacteria in culture from joint fluid in modified SP4 broth medium. Scale bar indicates 10 µm.

than honeybees. The insect stings in this patient are a likely gateway of the reported infection.

In summary, clinicians and microbiologists should be aware of fastidious organisms in atypical infections in immunocompromised patients. Our findings indicate a need for prolonged culture on specific agar on all joint fluids in patients with agammaglobulinemia and targeted molecular methods to identify *S. apis* organisms.

About the Author

Dr. Etienne is an infectious disease clinician and a clinical immunologist in Necker-Pasteur Center for Infectious Diseases, Paris. His research interest focuses on infections in immunocompromised patients.

References

1. Schabereiter-Gurtner C, Nehr M, Apfalter P, Makristathis A, Rotter ML, Hirschl AM. Evaluation of a protocol for molecular broad-range diagnosis of culture-negative bacterial infections in clinical routine diagnosis. J Appl Microbiol. 2008;104:1228–37. http://dx.doi.org/10.1111/j.1365-2672.2007.03648.x
2. Devulder G, Perrière G, Baty F, Flandrois JP. BIBI, a bioinformatics bacterial identification tool. J Clin Microbiol. 2003; 41:1785–7. http://dx.doi.org/10.1128/JCM.41.4.1785-1787.2003
3. Meeus I, Vercruysse V, Smagghe G. Molecular detection of *Spiroplasma apis* and *Spiroplasma melliferum* in bees. J Invertebr Pathol. 2012;109:172–4. http://dx.doi.org/10.1016/j.jip.2011.11.006
4. Blanchard A, Bébéar CM. Mycoplasmas of humans. In: Razin S, Herrmann R, editors. Molecular biology and pathogenicity of mycoplasmas. New York: Kluwer Academic/Plenum Publishers; 2002. p. 45–71.</edb>
5. Mouches C, Bové JM, Tully JG, Rose DL, McCoy RE, Carle-Junca P, et al. *Spiroplasma apis*, a new species from the honey-bee *Apis mellifera*. Ann Microbiol (Paris). 1983;134A:383–97.
6. Schwarz RS, Teixeira ÉW, Tauber JP, Birke JM, Martins MF, Fonseca I, et al. Honey bee colonies act as reservoirs for two *Spiroplasma* facultative symbionts and incur complex, multiyear infection dynamics. MicrobiologyOpen. 2014;3:341–55. http://dx.doi.org/10.1002/mbo3.172
7. Mueller NJ, Tini GM, Weber A, Gaspert A, Husmann L, Bloemberg G, et al. Hepatitis from *Spiroplasma* sp. in an immunocompromised patient. Am J Transplant. 2015;15:2511–6. http://dx.doi.org/10.1111/ajt.13254
8. Lorenz B, Schroeder J, Reischl U. First evidence of an endogenous *Spiroplasma* sp. infection in humans manifesting as unilateral cataract associated with anterior uveitis in a premature baby. Graefes Arch Clin Exp Ophthalmol. 2002;240:348–53. http://dx.doi.org/10.1007/s00417-002-0453-3
9. Aquilino A, Masiá M, López P, Galiana AJ, Tovar J, Andrés M, et al. First human systemic infection caused by *Spiroplasma*. J Clin Microbiol. 2015;53:719–21. http://dx.doi.org/10.1128/JCM.02841-14

Address for correspondence: Olivier Lortholary, Necker-Enfants Malades University Hospital, 149 rue de Sevre 75743, Paris CEDEX, France; email: olivier.lortholary@aphp.fr

Mycoplasma ovipneumoniae in Wildlife Species beyond Subfamily Caprinae

Margaret A. Highland, David R. Herndon, Scott C. Bender, Lisa Hansen, Robert F. Gerlach, Kimberlee B. Beckmen

Author affiliations: Washington Animal Disease Diagnostic Laboratory, Pullman, Washington, USA (M.A. Highland); US Department of Agriculture, Pullman (M.A. Highland, D.R. Herndon); Navajo Technical University, Crownpoint, New Mexico, USA (S.C. Bender); Barron Veterinary Clinic, Barron, Wisconsin, USA (L. Hansen); Alaska Department of Environmental Conservation, Anchorage, Alaska, USA (R.F. Gerlach); Alaska Department of Fish and Game, Fairbanks, Alaska (K.B. Beckmen)

Elucidating the emergence of *Mycoplasma ovipneumoniae*–associated respiratory disease in ruminants requires identification of the pathogen host range. This bacterium was thought to be host restricted to subfamily *Caprinae*, but we describe its identification in healthy moose, caribou, and mule deer and diseased mule and white-tailed deer, all species in subfamily *Capreolinae*.

Mycoplasma ovipneumoniae was identified in Queensland, Australia, in 1972 as an infectious agent associated with pneumonia in domestic sheep (*Ovis aries*) (*1*). Since then, it has most frequently been identified in healthy and diseased domestic sheep, domestic goats (*Capra aegagrus hircus*), and bighorn sheep (*Ovis canadensis*). Although *M. ovipneumoniae* was identified in respiratory disease outbreaks in bighorn sheep as early as 1980 (*2*), the past decade has brought it under scrutiny because of evidence supporting its association with bighorn sheep pneumonia in western North America (*3*). Because most reports have described this bacterium in sheep and goats, and fewer in muskoxen (*Ovibos moschatus*) (*4*), some have concluded that *M. ovipneumoniae* is specific to the subfamily *Caprinae* (*5*) or has a host range limited to *Caprinae* (*6*), despite publications describing *M. ovipneumoniae* in non-*Caprinae* species, including Beira antelope (*Dorcatragus megalotis*) with respiratory disease in Qatar (*7*) and in 9 cattle (*Bos taurus*) in Colorado, USA (*8*). Unfortunately, description of the method(s) used to identify *M. ovipneumoniae* in those reports was limited to stating the use of PCR with no supporting sequence data.

In general, definitive claims of host range restrictions are absent from mycoplasma literature, because "assumptions about restricted host range of mycoplasmas, based on the host from which they were first or frequently isolated, are usually made in the context of nearly complete absence of representative sampling of the vast majority of potential vertebrate hosts" (*9*). In addition to insufficient sampling of potential hosts, the fastidious and variably culturable nature of *M. ovipneumoniae* often requires molecular techniques for identification. We used molecular techniques to analyze multiple species from the subfamily *Capreolinae* for the presence of *M. ovipneumoniae*.

During July 2017–January 2018, the US Department of Agriculture Agricultural Research Service in Pullman, WA, USA, received nasal swab samples from 230 moose (*Alces alces*) and 243 caribou (*Rangifer tarandus*) from Alaska and 5 mule deer (*Odocoileus hemionus*) from Arizona (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/24/12/18-0632-Techapp1.pdf). Also received in February 2018 was an isolate of *M. ovipneumoniae* that had been cultured by Newport Laboratories (Worthington, MN, USA) from lung tissue from a white-tailed deer (*Odocoileus virginianus*) that died during a pneumonia outbreak at a captive facility in the upper Midwest region of the United States in 2016. We extracted DNA from swab samples and from the white-tailed deer isolate, performed PCR using a modified published PCR method (*10*) to amplify part of the 16S rRNA gene, and sequenced amplicons of the correct size (online Technical Appendix). Forward and reverse sequences were merged, manually inspected for errors, and trimmed to 290 bp using Sequencher 5.2.2 (Gene Codes, Ann Arbor, MI, USA) corresponding to a 103–392-bp region of the 16S rRNA gene of type strain Y98 obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). Sequences were blastn queried (https://www.ncbi.nlm.nih.gov/BLAST/) and identified as *M. ovipneumoniae* at 100% coverage and ≥97% identity to Y98 (GenBank accession no. NR_025989.1). The analyzed region represents the most divergent region of the 16S rRNA gene among strains of *M. ovipneumoniae* and between *Mycoplasma* spp. of the highest percentage identity to *M. ovipneumoniae* (online Technical Appendix).

We detected *M. ovipneumoniae* in 6 moose (2.6%), including 3 from 2 captive facilities and 3 free-ranging; 5 free-ranging caribou (2.1%); and 2 of 5 mule deer, 1 of which was coughing and had nasal discharge at the time of sample collection. The identity of the lung isolate, cultured from the white-tailed deer that had died from pneumonia, was confirmed as *M. ovipneumoniae*. For sequence comparison, we generated a percent identity matrix with the *M. ovipneumoniae* sequences from the *Capreolinae* species, nasal swabs collected from 2 healthy *M. ovipneumoniae*–positive wild sheep (online Technical Appendix), Y98, and bacteria of the closest identity to Y98 and sequences generated in this study based on blastn queries (*M. dispar*, *M. hyopneumoniae*, and *M. flocculare*) (online Technical Appendix). The percent identity matrix revealed 2 groupings of *M. ovipneumoniae* and illustrates the divergence from the other *Mycoplasma* spp. of closest identity to *M. ovipneumoniae*. Sample sequences have been submitted to GenBank (online Technical Appendix).

This report describes *M. ovipneumoniae* carriage in multiple members of the subfamily *Capreolinae* (moose, caribou, and mule deer), and emergence of *M. ovipneumoniae*–associated respiratory disease in deer. These findings are of importance to epidemiologic investigations because current dogma regarding host specificity may dissuade laboratories from pursuing identification of this fastidious bacterium in hosts beyond the subfamily *Caprinae*. Improved diagnostic methods to increase detection sensitivity are warranted based on information provided in this report (online Technical Appendix). Full-length genome sequencing and phylogenetic analysis of *M. ovipneumoniae* isolates are necessary next steps in inferring evolutionary relationships and origin of this bacterium in identified host species.

Acknowledgments

About the Author

Dr. Highland is a veterinary medical officer at the US Department of Agriculture Agricultural Research Service Animal Disease Research Unit in Pullman, WA, USA, and is an adjunct faculty member in the department of veterinary microbiology and pathology and the School for Global Animal Health at Washington State University. Her research interests include infectious diseases of small ruminants, with special focus on respiratory disease agents of domestic and wildlife species.

References

1. Carmichael LE, St George TD, Sullivan ND, Horsfall N. Isolation, propagation, and characterization studies of an ovine *Mycoplasma* responsible for proliferative interstitial pneumonia. Cornell Vet. 1972;62:654–79.
2. Bunch TD. Chronic sinusitis in desert bighorn sheep in northwestern Arizona. In: Douglas CL, Krausman PR, Leslie DM, editors. Desert Bighorn Council meeting 1985. Las Vegas (NV, USA): the Council; 1985. p. 1–3.
3. Besser TE, Highland MA, Baker K, Cassirer EF, Anderson NJ, Ramsey JM, et al. Causes of pneumonia epizootics among bighorn sheep, Western United States, 2008–2010. Emerg Infect Dis. 2012;18:406–14. http://dx.doi.org/10.3201/eid1803.111554
4. Handeland K, Tengs T, Kokotovic B, Vikøren T, Ayling RD, Bergsjø B, et al. *Mycoplasma ovipneumoniae*—a primary cause of severe pneumonia epizootics in the Norwegian muskox (*Ovibos moschatus*) population. PLoS One. 2014;9:e106116. http://dx.doi.org/10.1371/journal.pone.0106116
5. Cassirer EF, Manlove KR, Plowright RK, Besser TE. Evidence for strain-specific immunity to pneumonia in bighorn sheep. Journal Wildl Manag. 2017;81:133–43. http://dx.doi.org/10.1002/jwmg.21172
6. Besser TE, Cassirer EF, Potter KA, Foreyt WJ. Exposure of bighorn sheep to domestic goats colonized with *Mycoplasma ovipneumoniae* induces sub-lethal pneumonia. PLoS One. 2017;12:e0178707. http://dx.doi.org/10.1371/journal.pone.0178707
7. Gull JM, Hebel C, Deb A, Arif A, Clauss M, Hatt JM, et al. Blood values of captive beira antelope (*Dorcatragus megalotis*) prior to and during an outbreak of fibrinous pleuropneumonia

8. syndrome (FPPS). J Zoo Wildl Med. 2014;45:735–43. http://dx.doi.org/10.1638/2013-0073.1
8. Wolfe LL, Diamond B, Spraker TR, Sirochman MA, Walsh DP, Machin CM, et al. A bighorn sheep die-off in southern Colorado involving a *Pasteurellaceae* strain that may have originated from syntopic cattle. J Wildl Dis. 2010;46:1262–8. http://dx.doi.org/10.7589/0090-3558-46.4.1262
9. Brown DR, Zacher LA, Wendland LD, Brown MB. Emerging mycoplasmoses in wildlife. In: Blanchard A, Browning GF, editors. Mycoplasmas: molecular biology pathogenicity and strategies for control. Norfolk (UK): Horizon Bioscience; 2005. p. 382–414.
10. McAuliffe L, Hatchell FM, Ayling RD, King AI, Nicholas RA. Detection of Mycoplasma ovipneumoniae in Pasteurella-vaccinated sheep flocks with respiratory disease in England. Vet Rec. 2003;153:687–8. http://dx.doi.org/10.1136/vr.153.22.687

Address for correspondence: Margaret A. Highland, US Department of Agriculture, Animal Disease Research Unit, Agricultural Research Service, ADBF 3003, Washington State University, Pullman, WA 99164, USA; email: maggie.highland@ars.usda.gov

Locally Acquired Leptospirosis in Expedition Racer, Manitoba, Canada

Sameer S. Kassim, Antonia Dibernardo, L. Robbin Lindsay, Terence C. Wuerz

Author affiliations: University of Manitoba, Winnipeg, Manitoba, Canada (S.S. Kassim, T.C. Wuerz); Public Health Agency of Canada, Winnipeg (A. Dibernardo, L.R. Lindsay); St. Boniface General Hospital, Winnipeg (T.C. Wuerz)

Leptospirosis is found worldwide, except in northern regions. We report a case associated with a backcountry adventure race in Manitoba, Canada. Initially, nonspecific symptomatology and diagnostic pitfalls contributed to a delay in identification. Careful attention needs to be paid to exposure to and risk for leptospirosis in northern and temperate climates.

Leptospirosis is a zoonotic disease caused by *Leptospira interrogans* (motile bacterial spirochetes). Human transmission occurs by direct contact with contaminated urine or animals (*1*). The organism has a worldwide distribution outside of polar regions and is common during the rainy season in tropical and temperate climates (*2*).

Mycoplasma ovipneumoniae in Wildlife Species beyond Subfamily *Caprinae*

Technical Appendix

Materials and Methods

Animals (Study Samples and PCR Controls)

We collected nasal swab samples from moose and caribou during October 2013–December 2017, and from mule deer in January 2018, during routine health monitoring captures. Nasal swabs were placed into universal viral transport media (UVT; Becton Dickinson, Franklin Lakes, NJ, USA), sterile phosphate buffered saline, or a dry transport cylinder. Samples collected during 2017–2018 were kept cool and shipped on ice packs within 48 hours of collection, whereas samples collected before 2017 were maintained at −70°C following collection and shipped on dry ice. Nasal swab samples from *M. ovipneumoniae*-positive healthy wild sheep, including a Dall's sheep in Alaska, USA and a bighorn sheep in Colorado, USA, were collected during health monitoring captures, placed in UVT, and sent on ice packs following collection in November 2017 and March 2017, respectively. Sequence data from these *M. ovipneumoniae*-positive wild sheep are used for sequence identity comparison, representing species that have previously been reported as *M. ovipneumoniae* hosts.

PCR-positive control samples included DNA extracted from a nasal swab collected from a *M. ovipneumoniae*-positive domestic sheep in February 2016 in Idaho, USA, and from American Type Culture Collection type strain Y98 culture (domestic sheep origin). Negative control samples included DNA extractions, performed alongside test samples, on PCR-grade water, and/or *M. ovipneumoniae*-negative domestic sheep. Negative control sheep were raised and maintained under specific pathogen-free conditions (*1*) and confirmed *M. ovipneumoniae*-free by serology (Washington Animal Disease Diagnostic Laboratory, Pullman, WA) and repeat nasal swab sample analysis, as described in this study.

DNA Isolation, PCR, Sequencing, Sequence Analysis

We extracted DNA from swab samples and from the white-tailed deer isolate, and performed PCR using commercial kits (QIAamp DNA Mini Kit and QIAGEN Multiplex PCR Kit; QIAGEN, Germantown, MD, USA). We performed DNA extractions using the manufacturer's protocol titled "DNA Purification from Buccal Swabs (Spin Protocol)" (QIAamp DNA Mini and Blood Mini Handbook, 05/2016) with the following modifications: 1 mL of digest was applied to the spin column by placing 500 μL of sample digest onto the column, centrifuged, then repeated; samples were eluted with 100 μL of elution buffer provided in the kit. For samples arriving in 3 mL UVT media, 400 μL of media, in place of the initial PBS step, was used to perform the same extraction procedure. DNA eluent from dry swab samples or swabs arriving in small volumes of PBS were diluted 10-fold before performing PCR, to dilute potential inhibitor carry-through. Published primers (*2*) that amplify part of the 16S rRNA gene, base range 66–426 of type strain NCTC 10151 [Y98] (ATCC 29419; Manassas, VA, USA), were used at a 1 μM combined concentration in 20 μL reactions containing 2 μL of sample DNA and 10 μL of Multiplex PCR Master Mix (QIAGEN). PCR cycler conditions were as follows: 15 min denaturation, followed by 40 cycles, each 30 s, of 95°C denaturation, 58°C annealing, and 72°C extension, then a final 10 min extension, and 4°C hold. We performed gel electrophoresis on an aliquot of each PCR reaction, including negative and positive controls. Each reaction having a visualized amplicon of correct size was Sanger sequenced (Eurofins Genomics, Louisville, KY, USA). Sequences generated in this study were GenBank accessioned (Technical Appendix Table).

PCR Method Detection Limit

The method detection limit (MDL) for DNA extracted from Y98 culture was determined to be 10^1 templates per 2 μL. MDL for biologic samples collected and submitted as dry nasal swabs was determined to be 10^4 (10^4 per 1.2 mL digestion solution, which equates to 14 copies per 2 μL of the final 10-fold diluted eluent used in PCR reaction). This was determined by spiking *M. ovipneumoniae* negative domestic goat nasal swab samples with log dilutions of extracted DNA from Y98 culture before performing DNA extraction on the swab sample. The MDL for 10-fold diluted extracted DNA from negative swab samples spiked with Y98 extracted DNA was determined to be 10^2 per 2 μL. This supports high DNA extraction efficiency and low

PCR inhibitors from the biologic sample with ≤1 log fold decrease in MDL between pure culture and biologic samples.

Divergence Analysis

To illustrate the 290 bp region of the 16S rRNA gene analyzed in this study, in positional relation to variable regions of the 16S rRNA gene of *M. ovipneumoniae* and bacteria of the closest identity, a conservation plot was generated by graphing conservation of a multiple sequence alignment using the default settings in CLC Genomics Workbench (Redwood City, CA, USA) (Technical Appendix Figure 1). Bacteria of closest identity were *Mycoplasma* spp. and are referenced in the Technical Appendix Table.

Percent Identity Matrix

To compare sequences described in this study to the Y98 *M. ovipneumoniae* type strain and bacteria of the closest identity to Y98, a multiple sequence alignment was performed followed by a pairwise sequence comparison using default setting in CLC Genomics Workbench (QIAGEN, Redwood City, CA, USA); we then applied conditional formatting in Microsoft Excel (Redmond, WA, USA) (Technical Appendix Figure 2).

References

1. Highland MA, Berglund AK, Knowles DP. Total IgG in ewe sera and colostrum and serum IgG kinetics in lambs following colostrum ingestion are similar in domestic sheep and bighorn sheep (*Ovis aries* and *Ovis canadensis*). Sheep Goat Res J. 2017;32:36–42.

2. McAuliffe L, Hatchell FM, Ayling RD, King AI, Nicholas RA. Detection of *Mycoplasma ovipneumoniae* in *Pasteurella*-vaccinated sheep flocks with respiratory disease in England. Vet Rec. 2003;153:687–8. PubMed http://dx.doi.org/10.1136/vr.153.22.687

Technical Appendix Table. GenBank accessioned partial 16S rRNA *Mycoplasma ovipneumoniae* sequences and reference strains

Species	Animal identification	GenBank accession no.
Alaskan moose (*Alces alces*)	13_12–20–2017	MH133232
	30_01–24–2018	MH133235
	25_01–25–2018	MH133233
	41_01–25–2018	MH133236
	28_02–09–2018	MH133234
	86_01–24–2018	MH133237
Caribou (*Rangifer tarandus*)	76_02–14–2018	MH133229
	94_02–14–2018	MH133230
	51_12–20–2017	MH133228
	24_12–29–2017	MH133227
	23_01–09–2018	MH133226
Mule deer (*Odocoileus hemionus*)	93_12–04–2017	MH133239
	90_12–04–2018	MH133238
White-tailed deer (*Odocoileus virginianus*)	01_03–16–2018	MH133240
Bighorn sheep (*Ovis canadensis*)	62_04–07–2016	MH133224
Dall's sheep (*Ovis dalli*)	77_12–04–2017	MH133231
Reference strains	Strain designation	
Mycoplasma ovipneumoniae	NCTC 10151 [Y98]	NZ_JOTL01000000
Mycoplasma dispar	NCTC 10125	NZ_CP007229
Mycoplasma flocculare	[Ms42, NCTC 10143]	CP007585
Mycoplasma hyopneumoniae	NCTC 10110; J	NC_007295
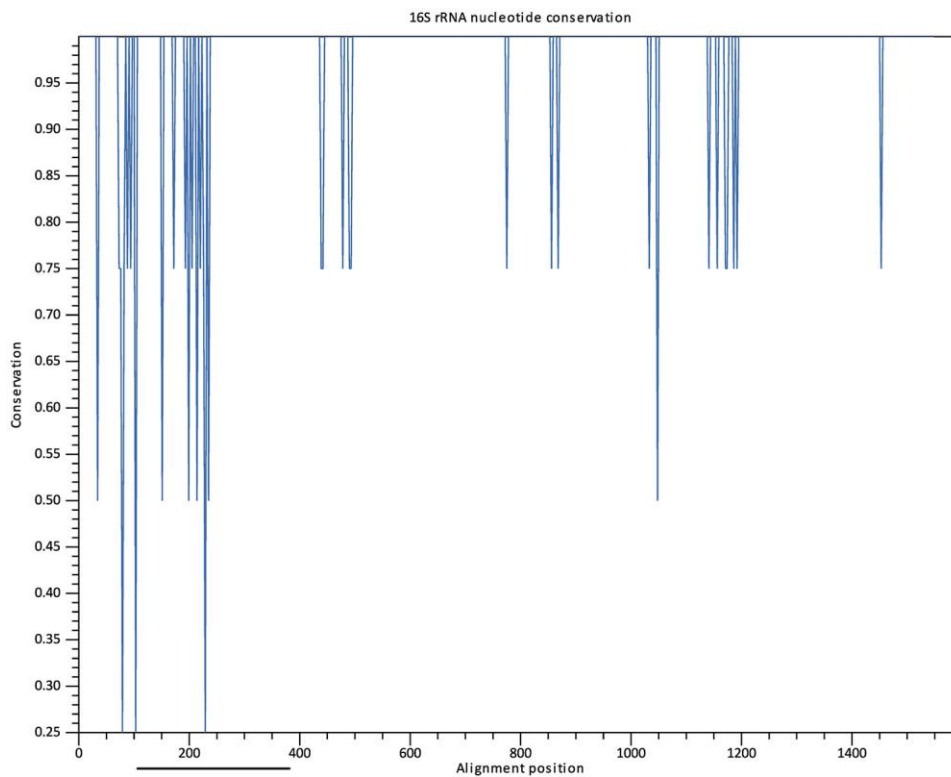
Technical Appendix Figure 1. Full-length 16S rRNA gene multiple sequence alignment conservation graph of *M. ovipneumoniae* type strain Y98 and the three mycoplasmas of the highest percent identity to Y98: *M. dispar*, *M. flocculare*, and *M. hyopneumoniae*. Black line illustrates the 290 bp region used in this study to identify *M. ovipneumoniae* in samples.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Mycoplasma ovipneumoniae	1		0	1	1	1	1	1	2	7	7	7	7	7	7	7	7	9	15	17	20
Moose 25 01-25-18	2	100		1	1	1	1	1	2	7	7	7	7	7	7	7	7	9	15	17	20
Moose 30 01-24-18	3	100	100		2	0	0	2	1	7	7	7	7	7	7	7	7	9	15	17	19
Moose 41 01-25-18	4	100	100	99.31		2	2	2	3	8	8	8	8	8	8	8	8	10	16	18	21
Mule deer 90 02-04-17	5	100	100	100	99.31		0	2	1	7	7	7	7	7	7	7	7	9	14	16	19
Mule deer 93 12-04-17	6	100	100	100	99.31	100		2	1	7	7	7	7	7	7	7	7	9	14	16	19
Caribou 76 02-14-18	7	100	100	99.31	99.31	99.31	99.31		3	8	8	8	8	8	8	8	8	10	15	17	20
Moose 13 12-20-17	8	99.31	99.31	100	98.97	100	100	98.97		8	8	8	8	8	8	8	8	10	15	17	20
Bighorn sheep 62 04-07-17	9	97.59	97.59	97.59	97.24	97.59	97.59	97.24	97.24		0	0	0	0	0	0	0	2	15	19	18
Caribou 23 01-09-18	10	97.59	97.59	97.59	97.24	97.59	97.59	97.24	97.24	100		0	0	0	0	0	0	2	15	19	18
Caribou 24 12-29-17	11	97.59	97.59	97.59	97.24	97.59	97.59	97.24	97.24	100	100		0	0	0	0	0	2	15	19	18
Caribou 51 12-20-17	12	97.59	97.59	97.59	97.24	97.59	97.59	97.24	97.24	100	100	100		0	0	0	0	2	15	19	18
Caribou 94 02-14-18	13	97.59	97.59	97.59	97.24	97.59	97.59	97.24	97.24	100	100	100	100		0	0	0	2	15	19	18
Dall's sheep 77 12-04-17	14	97.59	97.59	97.59	97.24	97.59	97.59	97.24	97.24	100	100	100	100	100		0	0	2	15	19	18
Moose 86 01-24-18	15	97.59	97.59	97.59	97.24	97.59	97.59	97.24	97.24	100	100	100	100	100	100		0	2	15	19	18
White-tailed deer NL 06-22-16	16	97.59	97.59	97.59	97.24	97.59	97.59	97.24	97.24	100	100	100	100	100	100	100		2	15	19	18
Moose 28 02-09-18	17	96.90	96.90	96.90	96.55	96.90	96.90	96.55	96.55	99.31	99.31	99.31	99.31	99.31	99.31	99.31	99.31		17	19	18
Mycoplasma dispar	18	94.83	94.83	95.17	94.48	95.17	95.17	94.83	94.83	94.83	94.83	94.83	94.83	94.83	94.83	94.83	94.83	94.14		15	13
Mycoplasma hyopneumoniae	19	94.14	94.14	94.48	93.79	94.48	94.48	94.14	94.14	93.45	93.45	93.45	93.45	93.45	93.45	93.45	93.45	93.45	94.83		15
Mycoplasma flocculare	20	93.13	93.13	93.47	92.78	93.47	93.47	93.13	93.13	93.81	93.81	93.81	93.81	93.81	93.81	93.81	93.81	93.81	95.53	94.85	

Technical Appendix Figure 2. Percent identity matrix illustrating pairwise sequence comparison of percent identity (lower left) and number of nucleotide changes (upper right) for the analyzed 290 bp of the 16S rRNA gene (range 103–392 of type strain Y98 *Mycoplasma ovipneumoniae*) from samples, type strain Y98 *M. ovipneumoniae*, and the next 3 closest blastn (https://www.ncbi.nlm.nih.gov/BLAST/) sequence query matches: *M. dispar*, *M, hyopneumoniae*, and *M. flocculare*. Percent identity and nucleotide change values grouped as follows: 98%–100% (<3 changes), 96%–98% (4–12 changes) and 90%–96% (≥12 changes).