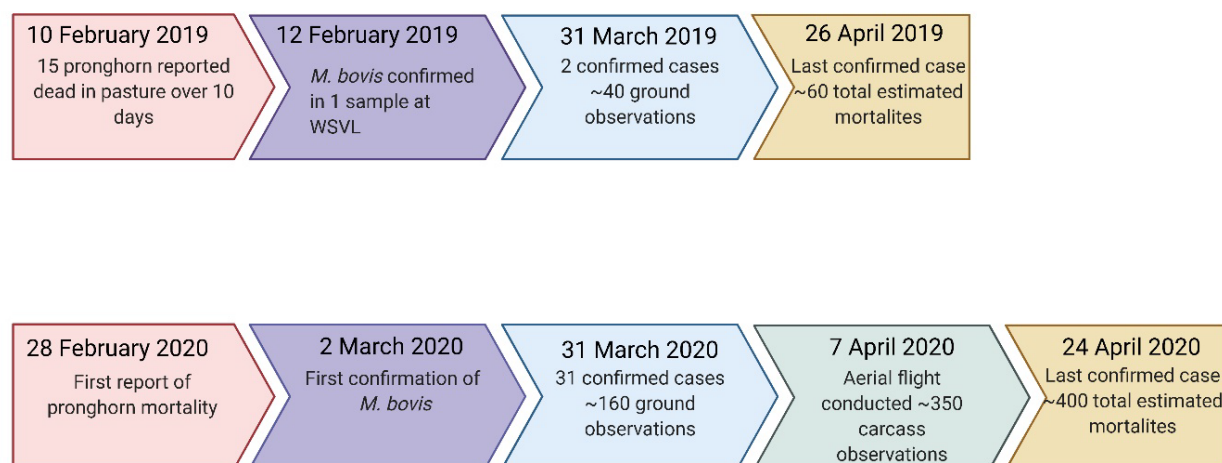
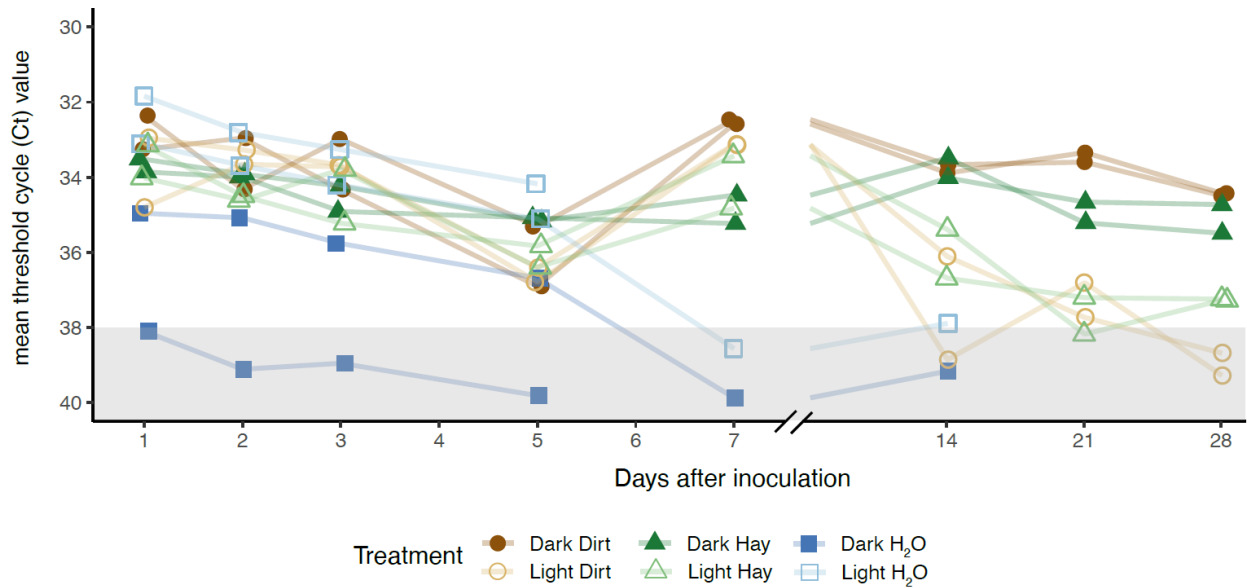


Supplementary materials for Journal of Wildlife Diseases DOI: 10.7589/JWD-D-21-00117: Marguerite Johnson, Christopher MacGlover, Erika Peckham, Halcyon Killion, Samantha E. Allen, Terry Creekmore, William H. Edwards, Madison Blaeser, McKenzi Davison, Erin Schwalbe, Amy K. Wray, Thomas K. Bragg, Kerry S. Sondgeroth, and Jennifer L. Malmberg. SOURCE AND SEASONALITY OF EPIZOOTIC MYCOPLASMOSIS IN FREE-RANGING PRONGHORN (*ANTILOCAPRA AMERICANA*).



Supplemental Figure 1. Timeline of documented *Mycoplasma bovis* infections in pronghorn. In 2019, mortalities were first identified on February 10th and the last confirmed cases were on April 26th. In 2020, the first reports of pronghorn mortalities were received on February 28th. *M. bovis* was first confirmed on March 2nd and last confirmed on April 24th. No *M. bovis* infections were detected in pronghorn in 2021. Created with BioRender.com.



Supplemental Figure 2. Environmental persistence of *Mycoplasma bovis* DNA in dirt, water, and grass hay environmental substrates. Each treatment (light vs. dark) was performed in duplicate, where cq value determined by real-time PCR is on the y-axis (axis inverted) and days post-inoculation is on the x-axis. Gray shaded area represents the threshold for determining whether samples were positive or negative.

Supplemental Table 1. Summary of diagnostic evaluations conducted during the 2020 mortality event near Gillette, Wyoming, USA

Case #	Age	Sex	Histopathology	<i>M.bovis</i> PCR Lung	<i>M. bovis</i> PCR Nasal	Aerobic Culture	Bovine Respiratory Panel & Virus Panel	Lungworm <i>Protostrongylus</i> sp.
1	3	M	Pleuropneumonia, suppurative with caseous necrosis	Detected	Detected	<i>Enterococcus</i> sp, <i>Bacillus</i> sp,	Undetected	Present
2	3	F	Suppurative to fibrinopurulent pleuropneumonia	Detected	Detected	<i>Streptococcus dysgalactiae</i> , <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , Mixed Contaminants	Undetected	Present
3	4+	F	NE	NE	Detected	Mixed contaminants	Undetected	NE
4	4+	F	NE	NE	Detected	Mixed contaminants	Undetected	NE
5	3	F	NE	NE	Detected	Mixed contaminants	Undetected	NE
6	4+	F	NE	NE	Detected	NE	Undetected	NE
7	2	F	NE	Detected	Undetected	<i>Mycoplasma bovis</i> , <i>Escherichia coli</i> , <i>Mannheimia</i> sp. <i>Streptococcus lutetiensis</i>	Undetected	Present
8	4+	F	NE	Detected	Undetected	<i>Mycoplasma bovis</i> , <i>Escherichia coli</i> , Mixed Contaminants	Undetected	Present
9	3	F	NE	Detected	Detected	<i>Mycoplasma bovis</i> , <i>Escherichia coli</i>	Undetected	NE
10	1	NE	NE	Detected	Detected	<i>Escherichia coli</i> , mixed contaminants	Undetected	NE
11	NE	NE	NE	Detected	Detected	<i>Escherichia coli</i> , <i>Enterobacter</i> sp.	Undetected	NE
12	3	M		Detected	Detected	Mixed contaminants, <i>E. coli</i> , <i>T. pyogenes</i>	Undetected	NE
13	NE	NE	NE	NE	Detected	NE	NE	NE
14	4+	F	NE	NE	Detected	NE	NE	NE
15	4+	F	NE	NE	Detected	NE	NE	NE
16	4+	M	Necrosuppurative bronchointerstitial pneumonia, with severe fibrinous pleuritis	Detected	Detected	Mixed Contaminants	Undetected	Present
17	4+	F	Necrosuppurative bronchointerstitial pneumonia, severe, diffuse, subacute, with severe fibrinous pleuritis and multifocal dystrophic mineralization	Detected	Detected	<i>Mycoplasma bovis</i>	Undetected	Present
18	3	M	Necrosuppurative bronchointerstitial pneumonia, severe, diffuse, acute, fibrinous pleuritis	Detected	Detected	<i>Mycoplasma bovis</i>	Undetected	Present
19	1	F	NE	Detected	Detected	<i>Mycoplasma bovis</i> , <i>Escherichia coli</i> , Mixed Contaminants	Undetected	NE

20	2.5	M	NE	Detected	Detected	<i>Mycoplasma bovis</i>	Undetected	NE
21	4+	F	Bronchopneumonia, fibrinosuppurative and necrotizing, severe, acute, with fibrinous pleuritis	Detected	Detected	<i>Mycoplasma bovis</i>	Undetected	Present
22	4+	M	Pleuritis, focally extensive, fibrinosuppurative to necrotizing	Detected	Detected	<i>Clostridium perfringens</i> , <i>Mixed contaminants</i>	Undetected	Present
23	4+	F	Fibrinous pleuritis, mild, focal acute	Detected	Detected	<i>Pantoea agglomerans</i>	Undetected	Present
24	4+	M	Fibrinous pleuritis, mild, focal acute	Detected	Undetected	<i>Staphylococcus chromogenes</i>	Undetected	Present
25	NE	NE	Fibrinonecrotizing pleuritis, marked, regionally extensive, acute to subacute	Detected	NE	<i>Mycoplasma bovis</i> , <i>Mixed contaminants</i>	Undetected	Present
26	NE	NE	Pleuritis, mild, regionally extensive, fibrinonecrotizing, acute	Detected	NE	<i>mixed contaminants</i>	Undetected	Present
27	NE	NE	Pleuropneumonia, severe, fibrinosuppurative and necrotizing, subacute to chronic, with multifocal dystrophic mineralization	Detected	NE	<i>Mannheimia sp.</i>	Undetected	Absent
28	NE	NE	Pleuropneumonia, severe, fibrinosuppurative and necrotizing, acute to subacute	Detected	Detected	<i>Neisseria sp.</i>	Undetected	Present
29	4+	F	Pleuropneumonia, fibrinosuppurative and necrotizing, diffuse	Detected	NE	<i>No Growth</i>	Undetected	Absent
30	4+	M	Fibrinonecrotizing pleuritis, marked, acute, regionally extensive, with multifocal to coalescing, necrosuppurative subpleural pneumonia.	Detected	NE	<i>Escherichia coli</i> , <i>Pantoea Sp.</i>	Undetected	Absent
31	4+	F	Subpleural pneumonia, fibrinosuppurative to necrotizing, acute.	Detected	NE	<i>Pseudomonas sp.</i> , <i>Pantoea agglomerans</i>	Undetected	Absent
32	4+	F	Pleuropneumonia, severe, fibrinosuppurative and necrotizing, acute	Detected	Detected	<i>Pseudomonas sp.</i>	Undetected	Absent
33	4+	F	Bronchointerstitial pneumonia, severe, suppurative and fibrinonecrotizing, diffuse, acute	Detected	Detected	<i>Escherichia coli</i>	Undetected	Present
34	4+	M	NE	Detected	NE	<i>Mixed Contaminants</i>	Undetected	NE
35	4+	M	NE	Detected	Detected	<i>Pseudarthobacter sp</i>	NE	NE
36	NE	NE	NE	Detected	Detected	<i>No Growth</i>	Undetected	NE
37	NE	F	NE	Detected	NE	<i>Truperella pyogenes</i> , <i>mixed contaminants</i>	NE	NE

M. bovis = *Mycoplasma bovis*

NE = Not examined

Supplemental Table 2. Summary of temperature and relative humidity during culture assessment between time of inoculation and hour eight.

	Light Treatment		Dark Treatment	
	Temperature °C	% Relative Humidity	Temperature °C	% Relative Humidity
Hour 0	12.11	60	9.28	70
Hour 1	13.00	56	10.89	66
Hour 2	15.61	52	12.50	60
Hour 3	18.50	34	16.00	48
Hour 4	18.39	35	17.11	44
Hour 5	18.61	37	15.72	45
Hour 6	22.11	34	16.89	45
Hour 7	26.28	26	19.11	40
Hour 8	27.22	24	19.11	39