Bighorn sheep show similar in-host responses to the same pathogen strain in two contrasting environments

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Abstract

Ecological context – the particular environment, and how it shapes mixing dynamics and individual susceptibility surrounding infectious disease events – can have major bearing on epidemic outcomes, yet directly comparable disease events with contrasting ecological contexts are relatively rare in wildlife systems due to concurrent differences in host genetics or pathogen strain. Here, we present a case study of one such event: a spillover of a "goat-clade" *Mycoplasma ovipneumoniae* strain into one bighorn sheep population that played out against two very different ecological backdrops. One event occurred on the herd's home range near the Rio Grande Gorge in New Mexico, while the other progressed in a captive facility at Hardware Ranch in Utah. We collected data on antibody and pathogen load patterns through time at the individual level, and examined demographic responses to pathogen invasion to compare the intensity of, and in-host responses to, infection in both settings. While data collection regimens varied between the two sites, general patterns of antibody expansion and gross timing of symptoms were consistent. Symptoms emerged in the captive setting 12.9 days post-exposure, and we estimated an average time to seroconversion among the captive animals of 24.9 days. Clinical signs peaked among the captive animals at approximately 36 days post-infection, consistent with subsequent declines in symptom intensity in the free-ranging herd. At the captive site, older animals exhibited more severe declines in body condition as determined through declines in loin thickness, higher symptom burdens, and a decelerated antibody response to the pathogen. Younger animals were more likely than older animals to clear infection at or before the time of sampling at both sites. This study presents one of the richest datasets on immune responses in bighorn sheep over the course of a newly introduced *M. ovipneumoniae* strain available to-date.

Keywords: ecoimmunology, bighorn sheep, *Mycoplasma ovipneumoniae*, within-host dynamics, wildlife disease

Introduction

Ecological context is thought to have major bearing on epidemiological progressions of infectious diseases, yet direct comparisons of the role of ecological context in wildlife diseases are often hindered by concomitant differences in host genetics or pathogen strain. One pathogen for which context is postulated to be particularly important is *Mycoplasma ovipneumoniae*, a bacte-

rial agent underlying infectious pneumonia in bighorn sheep (Besser et al. 2008, 2012, 2013). *M. ovipneumoniae* is a critical component, and likely the primary causative agent, of the bighorn sheep respiratory disease complex (Dassanayake et al. 2010). It can lead to severe disease across all age classes of bighorn sheep upon introduction, generating immediate-term population declines of 10-90% (Cassirer et al. 2018). In the longer term, infected herds may exhibit years to decades of poor recruitment (Cassirer and Sinclair 2007; Cassirer et al. 2013; Manlove et al. 2016) driven by sustained infection in a subset of chronic carrier adults (Plowright et al. 2017; Garwood et al. 2020). Experts have long speculated the environmental context could have important bearing on epidemic risks and outcomes for bighorn sheep, and these speculations have garnered strength through several comparative studies of bighorn risk of disease over varying environments (Monello, Murray, and Cassirer 2001; Sells et al. 2015).

Despite its hypothesized role, the mechanism by which the environment affects disease dynamics in bighorn sheep is not fully understood. Existing work suggests that herd mixing dynamics can constrain disease burden in lambs (Manlove et al. 2014), and may also play a role in herd recovery (Lula et al. 2020). Environmental context could also affect epidemiological patterns -- for example, varying nutrient availability or stress conditions could lead to differences in susceptibility of hosts from one environment to the next -- but the comparative studies necessary to isolate that pathway do not currently exist.

Here, we capitalize on intensive sampling surrounding a natural experiment to compare epidemiological dynamics of a novel *M. ovipneumoniae* strain invading a single bighorn sheep herd in two different ecological contexts. One group of animals underwent the epidemic in captivity in a holding pen located at Hardware Ranch in northern Utah following capture and translocation from the wild, where crowding, and likely also stress levels, were quite high. The other group experienced the epidemic in a free-ranging setting on the herd's original home range near the Rio Grande Gorge in New Mexico. Though the ecological contexts differed, the events stemmed from the same *M. ovipneumoniae* strain, and hosts in both groups had common genetics and health histories. Here, we compare disease and symptom progressions, antibody expressions, and epidemiological outcomes between the two contexts. We also present novel longitudinal data on *M. ovipneumoniae* progression and antibody response over the course of the epidemic, and provide formal estimates of incubation period, timing of peak of clinical signs, and rates of antibody expansion.

Methods

Study areas

The Taos Pueblo/Rio Grande Gorge in northern New Mexico is home to a reintroduced population of Rocky Mountain bighorn sheep, which is jointly managed by the Taos Pueblo Tribe and New Mexico Department of Game and Fish (NMDGF). Most of the bighorn sheep habitat is within the either Taos Pueblo tribal lands or Rio Grande del Norte National Monument, which is administered by the U.S. Bureau of Land Management. Elevations within the bighorn range extend from approximately 1,800m to 2,150m (roughly 6,000 to 7,500 feet) above sea level. The

population's size was estimated to consist of 375-420 animals in the fall of 2019 (NMDGF unpublished data).

Utah Division of Wildlife Resources (UDWR) aimed to translocate animals from the Taos Pueblo herd to Antelope Island, near Salt Lake City, UT, in February of 2020. The capture was conducted jointly by UDWR and the Taos Pueblo tribe. Twenty-four female bighorn sheep were captured via helicopter (Krausman et al. 1985) and transported to Utah on February 22nd, 2020. To the best of our knowledge, captured animals lived in the same environment as animals that remained on the RGG range and did not represent distinct subunits within the RGG herd. Samples were flown directly from Taos, New Mexico to the Washington Animal Disease Diagnostic Laboratory (WADDL) immediately after capture, and PCR and cELISA tests for *M. ovipneumoniae* were conducted overnight. Observers at the capture saw no clinical signs pneumonia, even among animals running for sustained periods of time. Clinical signs had not been previously observed during Taos Pueblo survey events, and hunter harvested samples submitted for diagnostic testing from 2018-2020 revealed no evidence of *M. ovipneumonia*e. However, the lab results indicated that six of the transported animals were positive for *M. ovipneumoniae*, based on a PCR test (i.e., direct evidence of current infection; (Ziegler et al. 2014), a cELISA test (i.e., antibody response indicating past exposure), or both (laboratory methods in (Ziegler et al. 2014), and further validated in (Manlove et al. 2019).

UDWR then segregated the affected and unaffected animals. Affected animals were euthanized using approved UDWR protocols on February 23rd, and sampled a second time for *M. ovipneumoniae* immediately post-mortem. Gross examinations of lung tissue revealed minimal damage consistent with pneumonia among those animals. Animals that tested negative on both the PCR and the antibody test were moved to a UDWR facility at Hardware Ranch, where they were housed in a solid pen, approximately 3m high and 30m in diameter. Captive animals were fed a diet of grass hay, supplemented with a standard sheep pellet. It took animals several days to adapt to the new food and start eating properly, but they were regularly observed eating after the third day in the pen. Diarrhea was not observed among the captive animals at any point during captivity. Captive animal research was conducted in accordance with Utah State University IACUC protocol #11117.

Following detection of *M. ovipneumoniae* among the translocated animals, NMDGF collaborated with the Taos Pueblo tribe to initiate parallel sampling of free-ranging animals remaining at the Rio Grande Gorge (RGG) herd via ground darting in accordance with standard NMDGF protocols. Additional summer field investigation, fall surveys, and hunter harvest samples provided information on disease progression in the free ranging context.

Data collection

Animal handling and sampling

During the initial capture and sampling event, animals were net-gunned from a helicopter by a professional capture crew (Helicopter Wildlife Services, Austin, TX), hobbled and blindfolded, tranquilized with 14 mg haloperidol administered intramuscularly (Haloperidol, 20 mg/ml, Wildlife Pharmaceuticals, Laramie, WY), and transported to a processing location where trained

personnel weighed the animals, collected blood, nasal and tonsillar swabs, applied GPS collars (Advanced Telemetry Systems, Isanti, MN), administered 10 mg midazolam intravenously (Midazolam, 50 mg/ml, Wildlife Pharmaceuticals, Laramie, WY), and assessed body conditions. The captive animals at Hardware Ranch were sampled three times each: on February 21st, 2020; on March 12th, 2020; and at the time of euthanasia on March 26th, 2020 (both ante- and post-mortem). Captive animals were chemically immobilized by herding them into a corner of the pen using a large metal gate, and hand injecting them all with 1.5 ml of butorphanol, azaperone, and medetomidine (BAM, Wildlife Pharmaceuticals, Laramie, WY). After injection, the bighorn sheep were released back into the pen, and allowed to go down. After the bighorn sheep were approachable, they were placed in sternal recumbency, blind folded and sampled. Following sampling, all the bighorn sheep were reversed simultaneously with intramuscular injections of naltrexone and atipamezole (Kreeger and Arnemo 2018). A UDWR veterinarian administered or supervised administration of all drugs. Three captive animals that died prior to euthanasia were necropsied by pathologists at the Utah Veterinary Diagnostic Laboratory in Logan, UT. The 13 remaining animals were euthanized on March 26th and necropsied in the field by a veterinary pathologist-researcher team.

NMDGF personnel ground darted 29 free-ranging animals (19 females and 10 males) in the RGG herd between April 13th and May 13th, 2020. Crews chemically immobilized animals again using BAM. Samples were collected, animal condition and symptoms were recorded, and animals were fitted with ATS GPS collars for on-going tracking. Samples were held at NMDGF facilities and then submitted in bulk to WADDL for diagnostic testing.

Nasal and tonsil swabs and serum were collected during the original capture and all subsequent animal handling events at Hardware Ranch, and all samples were analyzed at the Washington Disease Diagnostic Laboratory (WADDL). Nasal swabs were collected by inserting a single Dacron swab into each nostril and gently swabbing the nasal mucosa by swirling the swab. An additional nasal swab was collected and stored in TSB for whole genome sequencing on the first and second captive sampling events. Blood was collected by jugular venipuncture into serum separator tubes, and the blood was centrifuged within 4 hours of collection. Serum was separated and stored frozen in cryogenic vials until analysis at the laboratory.

Body conditions were estimated using ultrasound, by measuring rump fat and loin thickness of the animals in similar locations as has been described for deer (Cook et al., 2007). Because there is no published formula for translating these measurements into percentage ingesta free body fat as described by Cook et al., 2007, the loin thickness and rump fat measurements were simply compared between each sampling event within and between animals.

Observational scoring of clinical signs and reproductive status

Clinical signs were scored on a daily basis for animals in the captive setting. Observers watched all animals in the pen in tandem for 45 minutes each day and recorded signs including inappetance, nasal discharge, coughing (including number, quality, and pacing of coughs), and lethargy. Photo records of noses (to track nasal discharge) and hips (to track changing body condition) were gathered as frequently as possible (typically ~6 instances per animal over the duration of captivity). We did not attempt to visually score body condition since animals maintained winter coats, though severe declines in body condition were noted on scoring sheets at the observer's discretion.

Nasal discharge at Hardware Ranch was given a numeric score between 0 and 5. Shiny noses were given scores of 1, 2 indicated clear discharge from one nostril, 3 indicated clear discharge from both nostrils, 4 indicated purulent discharge from one nostril, and 5 indicated purulent discharge from both nostrils. Coughs were scored as 1 for isolated coughs, 2 for bouts of five or more consecutive coughs, 2.5-4 for 2 or more bouts of 5 or more consecutive coughs, depending on depth of cough and number of bouts. Nose licking and head shaking were both assigned scores of 1 if present and 2 if consistent throughout the observation period. Total daily scores for each animal were determined by summing the nasal, head shaking, and nose licking scores, and twice the individual's coughing score. Individual daily symptom scores ranged from 0 to 12.

Free-ranging animals at RGG were observed by NMDGF bighorn sheep experts over 31 unique observation days between February 25th and July 18th, 2020. Observers recorded coughing (including quality) at the individual level, and nasal discharge status whenever views were close enough that status could be determined with confidence.

Study termination and follow-up

No suitable location could be found to safely release the captive, infected animals, and the UDWR leadership therefore decided to euthanize them on March 26th, 2020, 34 days after the initial diagnosis. All animals were immobilized with BAM as described above, and sampled live prior to euthanasia via gunshot to the head. All bighorn sheep were then necropsied in full at the Hardware Ranch site by UDWR staff, accompanied by pathologists from the Utah Veterinary Diagnostic Laboratory and researchers from Utah State University. Antemortem sampling included collection of the full suite of samples described previously; postmortem sampling included lung assessments and selective sampling, bronchial junction swabs, extraction and measurements of the fetus, and full tissue collection on all animals. All heads were collected and taken to UVDL for gross sinus tumor assessments (Fox et al. 2011).

Dynamics of *M. ovipneumoniae* in the free-ranging animals at Rio Grande Gorge were allowed to play out in full. Longer-term epidemiological data were garnered through adult survival estimated jointly through survival of the radiocollared adult animals and fall herd population surveys, and summer lamb survival. Field crews measured lamb:ewe ratios in the summer and fall (once in July and once in November), using protocols similar to those used in previous years at RGG. This allowed for a comparison of lamb:ewe ratios in 2020 vs. previous years.

Data analysis

Dynamics of M. ovipneumoniae *infection among the captive animals*

WADDL provided us with cycle threshold values corresponding to all PCR diagnostic tests conducted at both RGG and Hardware Ranch upon request. Though WADDL regards all PCR results with cycle thresholds above 36 as "indeterminate", we include raw values between 36 and 40 in this analysis. Our goal in analyzing the longitudinal captive data was to describe how pathogen load changed over the course of infection. Since we relied on commercially available diagnostic testing, we did not have standard curve values in hand to formally enumerate load at the individual level. However, we were able to track relative load among the samples using the

2^(-Ct') method (Livak and Schmittgen 2001), reliant on the minimum Ct recorded across all samples at both RGG and Hardware Ranch as the calibrator value. Once sample-specific loads were calculated relative to the calibrator, we compared those relative loads among sampling events and age classes.

Immune response to M. ovipneumoniae

Our analysis of the serological data was built around three main objectives: (1) to estimate time to seroconversion among the captive animals; (2) to identify sources of individual-to-individual variation in antibody expansion patterns; and (3) to compare serological dynamics in the captive and wild settings.

The cELISA test for *M. ovipneumoniae* should not produce negative 'percent inhibition' results, and the fact that negative results are sometimes reported is an artifact of a formula applied to the lab-generated data. Therefore, we first reset all negative values to 0, and then added 1 to every reported value to force the data into a space that is biologically appropriate for the test, and statistically appropriate for a log transformation.

cELISA models were fit using the glm function in R's stats package, with family set to "Gamma" with an identity link function. We compared models with four covariate structures: days post-detection, age, days post-detection plus age, and days post-detection plus both linear and quadratic effects for age; assessed model assumptions through standard diagnostic plots; and critiqued model validity by examining predictions arising from each model. Models were compared to one another in terms of AIC, and coefficients were interpreted only for the most parsimonious model within the competitive model set (i.e., within 2 AIC points of the best-performing model). Dispersion and shape parameter estimates were refined separately following model fitting using the gamma.shape function in R's MASS package, and those estimates were used in the Wald's tests to assess coefficient significance.

We estimated the number of days until cELISA percent inhibition exceeded 40 (the cut-off to be classified as non-negative at WADDL) by fitting a Poisson regression model that treated days since February 22nd as a function of logged cELISA percent inhibition and individual age. We then used that model to predict the day upon which cELISA percent inhibition exceeded 40 across a spectrum of ages.

Disease progression and severity of clinical signs

Our exploration of symptom dynamics was focused around three objectives: (1) to estimate the lag between exposure and emergence of clinical signs among the captive animals; (2) to describe the rise and fall of clinical signs following their emergence in the captive setting; and (3) to qualitatively compare the timing of clinical signs between the two field sites.

We estimated the time to emergence of clinical signs through a changepoint analysis that examined symptom score as a piecewise-linear function of days post-exposure using the piecewise.linear function in R's SiZeR package (Sonderegger 2012). This analysis identified the most likely breakpoint in the pattern of clinical signs across all captive animals. We built bootstrapped confidence intervals to quantify uncertainty in the changepoint location and pre- and post-changepoint slopes. We elected to use this approach, as opposed to time until first recorded

symptoms for each animal to account for occasional sneezes or nasal discharge that might be expected among translocated and captive animals independent of *M. ovipneumoniae* infection.

We described the rise and fall of symptoms post-onset by first subsetting the symptom data down to just those data arising after symptom onset (with onset identified using the change-point identified above), and then fitting a linear model of symptom score as a function of linear and quadratic effects of days post-exposure, along with a fixed effect for individual age and a random intercept for each individual.

We compared six different models of body condition (as measured by loin thickness) among the captive animals. All models assumed residual normality and included a random intercept for individual to account for the repeated measure structure of the data. Fixed effect combinations included models with age; days post-February 22nd; cELISA percent inhibition value; age, days post-February 22nd and an age-by-days interaction term; cELISA, days-post-February 22nd and a cELISA-by-days interaction term; and a model with both cELISA-by-days and age-by-days interaction terms. Models were fit using R's lme4 package (Bates et al. 2007).

Epidemiological outcomes

We tracked survival of the captive animals at Hardware Ranch. Though none of those animals had delivered lambs by the time of euthanasia, we assessed pregnancy status throughout the disease event and weighed and examined all fetuses post-mortem. At RGG, three epidemiological outcome measurements exist: survival of the instrumented animals captured by NMDGF in April and May; survival of lambs associated with instrumented ewes; and aggregate lamb:ewe ratios collected during survey events throughout the summer.

Results

Dynamics of *M. ovipneumoniae* infection in the captive and free-ranging herds

Longitudinal data from the captive animals are presented in Table 1. All captive animals showed evidence of *M. ovipneumoniae* infection at the sampling event 19 days post-exposure. We tracked temporal dynamics of pathogen load within animals by comparing measured load to the maximum load (minimum Ct) detected across the entire study. The minimum Ct was 23.1, obtained from a bronchial junction swab at Hardware Ranch; the minimum Ct from a nasal swab was 23.96, also from Hardware Ranch. All samples were calibrated against the bronchial junction value.

In the destructive sampling at 34 days post-exposure, most animals exhibited reduced *M. ovipneumoniae* load (corresponding to higher *M. ovipenumoniae* Cycle threshold ("Ct") values; Figure 1A); though load increased incrementally in three individuals (Eartags 36, 38, and 39) between the second and third sampling events. Older animals achieved slightly higher pathogen burdens in their noses over the course of infection than younger animals (bluer lines rise to higher values on the y-axis in Figure 1B than do greener lines), though the correlation between age and maximum load did not differ substantially from 0 (Pearson's r = 0.22; 95% CI = [-0.38, 0.69]). No evidence of sinus tumors was detected upon necropsy, though *Pasteurella multocida*

was cultured from both oropharyngeal swabs (detected in 10 of 10 sampled individuals) and lung tissue (detected in 10 of 13 sampled individuals) at necropsy.

Table 1. Longitudinal data from 16 captive female bighorn sheep. "Tag" is eartag number. "Age" is an age estimate based on tooth eruption and ware patterns. "Ct" is the WADDL-derived cycle threshold from a real-time PCR for *M. ovipneumoniae* (40 corresponds to no detection). "%I" is percent inhibition from the WADDL cELISA serological test (values >40 are regarded as indeterminate, and >50 are regarded as seropositive). "Rump fat" is an ultrasound-based rump fat measurement. "Loin" is an ultrasound-derived measure of loin thickness. "Weight" is weight in kilograms.

		Feb 22nd						March 12th				March 27th				
Tag	Age	Ct	%I	BCS	Rump fat	Loin	Weight	Ct	%I	BCS	Loin	Ct	%I	BCS	Loin	Weight
26	6.5	40	-0.73	2.5	1	35	70	24.42	0.04	2	31	27.7	76.1	1.75	29	66
27	6	40	-6.31	2	0	38	65	28.18	-4.55	2	28	28.7	40.5	1.5	27	51
28	7.5	40	4.86	2.25	0	37	56	25.76	6.90	1.75	26	Mortality — pneumonia, 03/25/20				
30	4+	40	-35.85	NA	2	38	73	Mortality — jumped fence during second capture, then euthanized								
31	2.5	40	3.56	2.5	2	36	58	29.47	23.02	2	31	30.5	18.2	1.75	32	57
33	8	40	0.94	2	0	38	65	Mortality — second night in pen, myopathy								
35	5.5	40	9.58	2.5	2	42	78	33.07	46.06	2.25	31	33.3	60.8	2.5	32	72
36	6	40	19.45	2	1	38	70	32.48	-4.68	1.75	29	29.1	21.6	1.5	27	62
38	7.5	40	13.75	2	1	37	65	33.1	6.90	2.25	32	28.4	39.8	2	30	62
39	4	40	22.42	2	2	38	60	35.11	-7.48	2	32	28.3	-1.4	2.25	31	57
40	0.9	40	-7.70	2.5	0	34	48	30.65	15.79	2	31	40	13.1	2	30	40
41	6	40	-8.59	2.25	1	38	68	23.96	15.96	1.75	26	31.9	67.4	1	24	60
42	3.5	40	14.42	2	1	35	55	28.6	2.91	2.5	34	30.8	11	1.75	28	50
43	1.5	40	-10.16	2.75	1	35	55	26.69	39.40	2.5	35	28.1	54.2	2.25	32	51
45	4+	40	-3.82	3.25	2	42	75	35.69	25.95	2.5	35	40	84.8	2	34	69
46	2.5	40	-5.31	2.5	1	36	55	26.17	29.68	2.25	33	34.1	60.8	1.75	28	47

Upper and lower respiratory tracts (measured through Ct values from nasal and bronchial junction swabs shown in Figure 1C) generally agreed in their infection status, though this agreement was not absolute. Two animals had discrepant outcomes. One (Eartag 45) exhibited very

low levels of *M. ovipneumoniae* at the bronchial junction, but was positive on the nasal swab; another (Eartag 39) continued to harbor *M. ovipneumoniae* in the bronchial junction, but had no detectable nasal infection. A third animal (Eartag 40, the youngest animal in the captive group) appeared to have completely cleared infection from both the nose and the bronchial junction by the final day of the study. It is notable that that animal also never mounted an antibody response that began to approach a conventionally "detectable" magnitude (her cELISA percent inhibition on the final sampling event 34 days post-exposure was 13.1; in order to be classified as anything other than negative on the WADDL serological test, percent inhibition should exceed 40%).

A total of 21 of the RGG animals were PCR-positive for M. ovipneumoniae at capture, and another three were PCR-negative but showed serological signals consistent with exposure (cELISA percent inhibitions of 69.2, 64.2, and 64.1). All animals that had cleared and seroconverted were under four years of age (1, 3, and 3.5), though a formal statistical relationship between age and probability of clearance could not be detected at this sample size. One male and two females were among the individuals that showed evidence of clearance.

Across the entire study, the four animals that cleared infection (i.e., tested both seropositive at the 40% inhibition level, and also PCR negative) were significantly younger than those with evidence of exposure that had not cleared infection ($p = 0.048$ in a randomization test of animal ages).

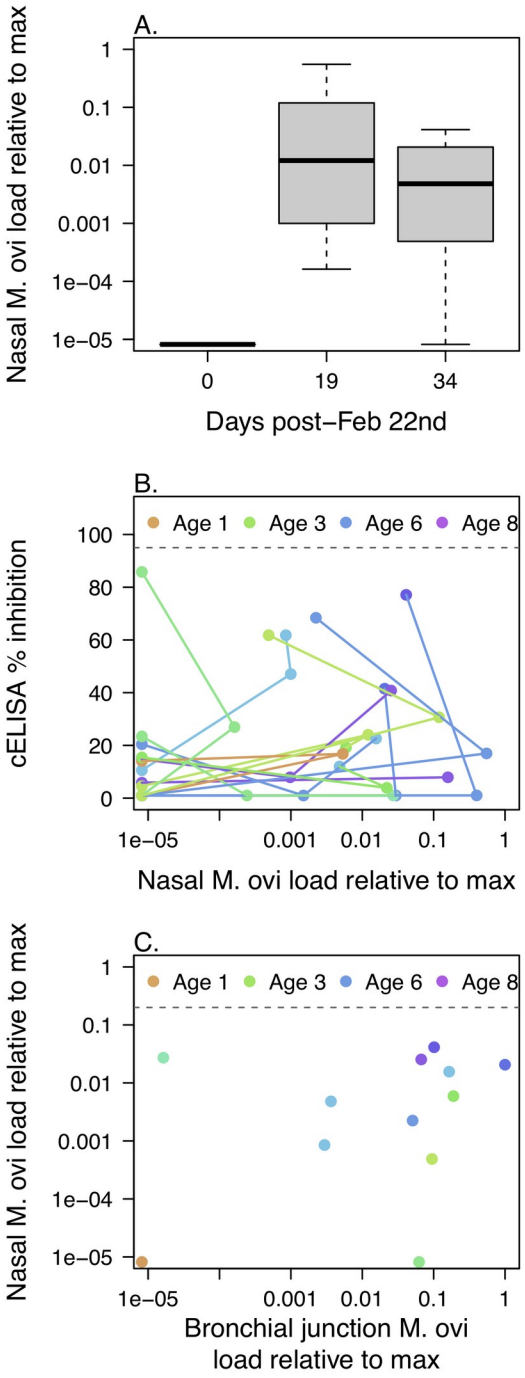

Figure 1. Dynamics of *M. ovipneumoniae* ("M. ovi") infection in the captive bighorns. A. *M. ovipneumoniae* load relative to maximum observed in the study obtained from nasal swabs associated with captive animals over each sampling event. In this case, a value of relative load equal to 1e-05 approximately corresponds to a PCR-negative animal with a Ct of 40. All animals retained for captivity were PCR-negative on the initial sampling event. B. Relative *M. ovipneumoniae* loads and serological values for each individual at each sampling event at Hardware Ranch. Points within an individual are connected. All individuals started uninfected (left-hand side of the x-axis), with relatively low cELISA values (points low on the y--axis) in the first sampling event. As infections progressed, first relative loads and then antibody expression levels increased, shifting points to the right on the x-axis, and then up on the y-axis. Animals that completely cleared infection returned to a relative load of approximately 1e-

05, but with higher cELISA values during the third sampling event. C. Relative *M. ovipneumoniae* loads in the nasal passage and bronchial junction in the final sampling event.

Immune response, disease progression, and severity of clinical signs

Serological dynamics of M. ovipneumoniae

Serological expansion rate varied weakly with age among the captive females at Hardware Ranch (Figures 1B, 2A). AIC-based comparisons indicated that no better support for models that included age than for a model reliant exclusively on days post-detection (Table 2). Predictions from the Poisson regression model of days since February 22nd as a function of cELISA percent inhibition and age suggested that the typical female would cross a value 40% inhibition on the cELISA test at 24.9 days post-exposure (95% CI for the average days to crossing = [22.6, 27.2]; Table S1).

Table 2. Comparison of plausible models for cELISA expansion rates.

Model	**AIC**	**Δ AIC**	**AIC weight**
Age	309.62	12.36	0.00
Days post-February 22nd	297.26	0.00	0.47
Days post-February 22nd + Age	298.16	0.90	0.30
Days post-February 22nd + Age + Age^2	298.73	1.47	0.23

Antibody expression appeared to follow relatively similar time courses in the captive and free-ranging settings. Although we do not have explicitly concurrent sampling across the two sites, general trends in antibody expression among animals with active *M. ovipneumoniae* infections aligned well (e.g., Figure 2B). In the free-ranging RGG herd, 11 of 15 animals sampled prior to April 20th had already seroconverted to a cELISA percent-inhibition value above 40 prior to sampling (Figure 2C). The disease event was still underway, however, as evidenced by the five PCR-positive animals that had yet to seroconvert (Figure 2C; Figure 2D). Over the course of the RGG sampling effort, three animals were detected with antibodies to *M. ovipneumoniae* but no evidence of current infection, suggesting they may have already cleared infection (Figure 2D).

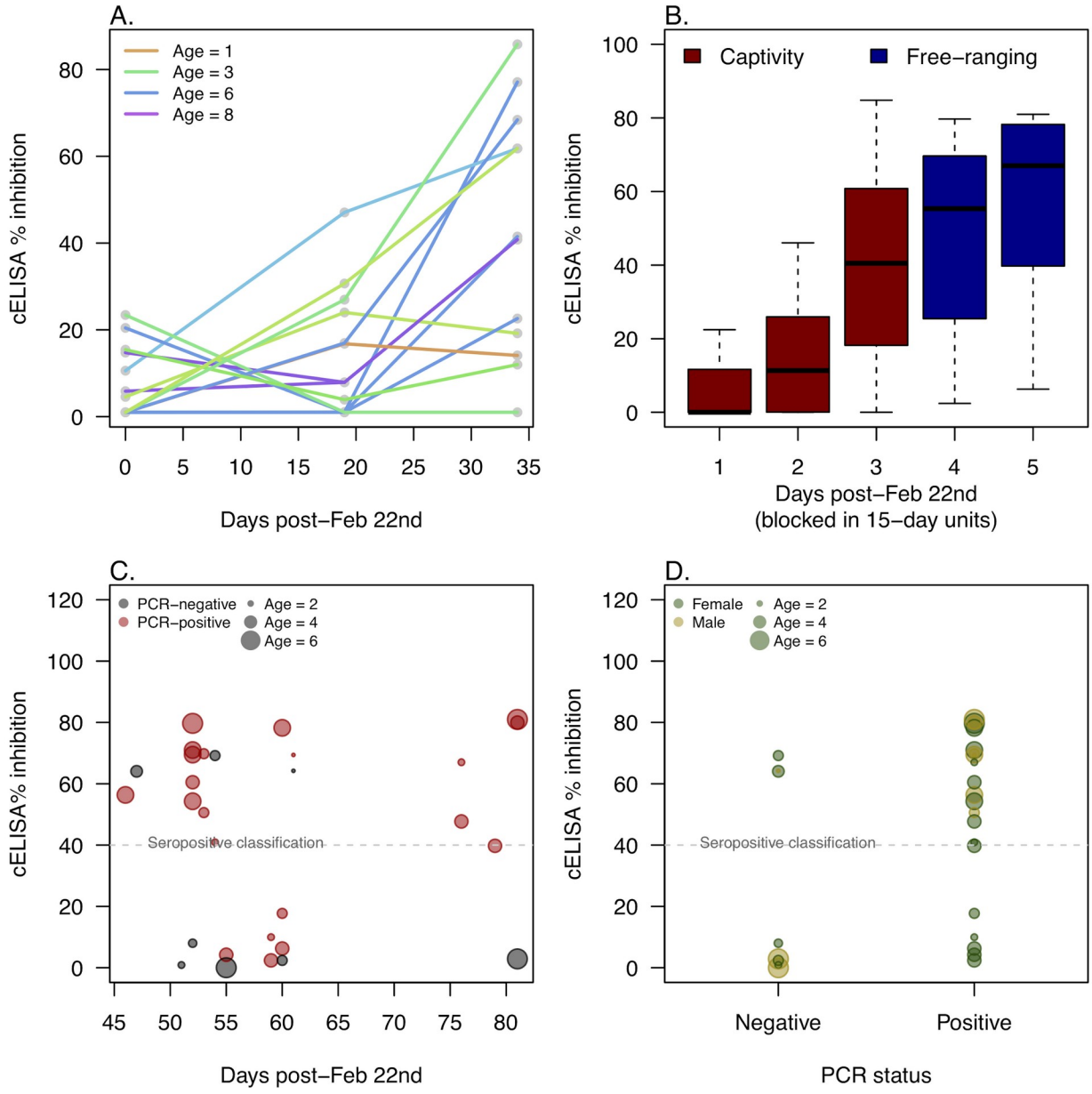

Figure 2. Serological dynamics of *M. ovipneumoniae* infection. A. *M. ovipneumoniae* cELISA percent inhibition through time by animal among the captive individuals at Hardware Ranch. B. Aggregate *M. ovipneumoniae* cELISA percent inhibition values through time among PCR-positive animals across both the captive and free-ranging settings. C. *M. ovipneumoniae* cELISA percent inhibition as a function of date of sampling for the RGG animals. Point colors indicate whether the animal was also PCR-positive for *M. ovipneumoniae* (red), or PCR-negative (grey). D. *M. ovipneumoniae* PCR status by cELISA percent inhibition among the animals captured at RGG. The dashed lines in panels C and D indicate the 40% inhibition level that WADDL uses as a cut-off value for classifying animals as seropositive.

The specific structure of the samples from RGG left us limited ability to determine whether immune responses varied between the two sexes. PCR-positive males generally exhibited higher antibody levels at capture than did PCR-positive females (median percent inhibition among PCR-positive males = 69.5, IQR = [62.9, 74.9]; among PCR-positive females = 44.3, IQR = [11.9, 65.4]), but this difference could be attributable to when individual sampling events oc-

curred relative to the individual's exposure, which may not be well-approximated for the free-ranging animals.

Clinical signs

All animals within the pen developed symptoms following exposure, though timing of symptom onset and patterns of severity varied (Figure 3A). Signs were initially mild, with coughing first observed 8 days post-exposure. Once symptoms emerged, however, they rapidly intensified, to the point that at epidemic peak, we observed over 19 coughing bouts (including one of 85 deep coughs) in a single affected individual -- Eartag 35 -- over one 45-minute observation period. In particular, younger animals tended to exhibit lower clinical sign scores, and clinical signs emerged more gradually in that group (contrast between purple and gold lines in Figure 3A).

The changepoint analysis indicated a significant shift in symptoms among the captive animals at 12.9 days post-exposure (95% bootstrapped CI [11.50, 18.00]). Symptom scores did not increase significantly with time prior to that day ($\beta_{\text{days post-exposure-pre}} = 0.04$; 95% CI [-0.01, 0.25]), but did increase in its aftermath ($\beta_{\text{days post-exposure-post}} = 0.34$; 95% CI [0.33, 0.50]).

Symptom scores post-onset (i.e., 12.9 days post-exposure and beyond) were best described by a model with linear and quadratic effects of time, and an additive effect of individual age. The quadratic effect was significantly negative, suggesting that symptoms would likely have peaked at about 36 days post-exposure ($\beta_{\text{days\^2}} = -0.02$, SE = 0.01). Symptom scores were generally higher among older animals ($\beta_{\text{additional year of age}} = 0.22$, SE = 0.19), though this effect was only marginally significant, and the timing and rate of symptom increase through time did not vary significantly according to age.

Among the RGG animals, clinical signs declined steadily over the course of the sampling period from April 13th through May 13th. However, clinical signs were observed sporadically (in 7 of 73 groups observed from May 1st through July 18th) over the course of the summer of 2020 in the RGG herd in both ewes and lambs (Figure 3B).

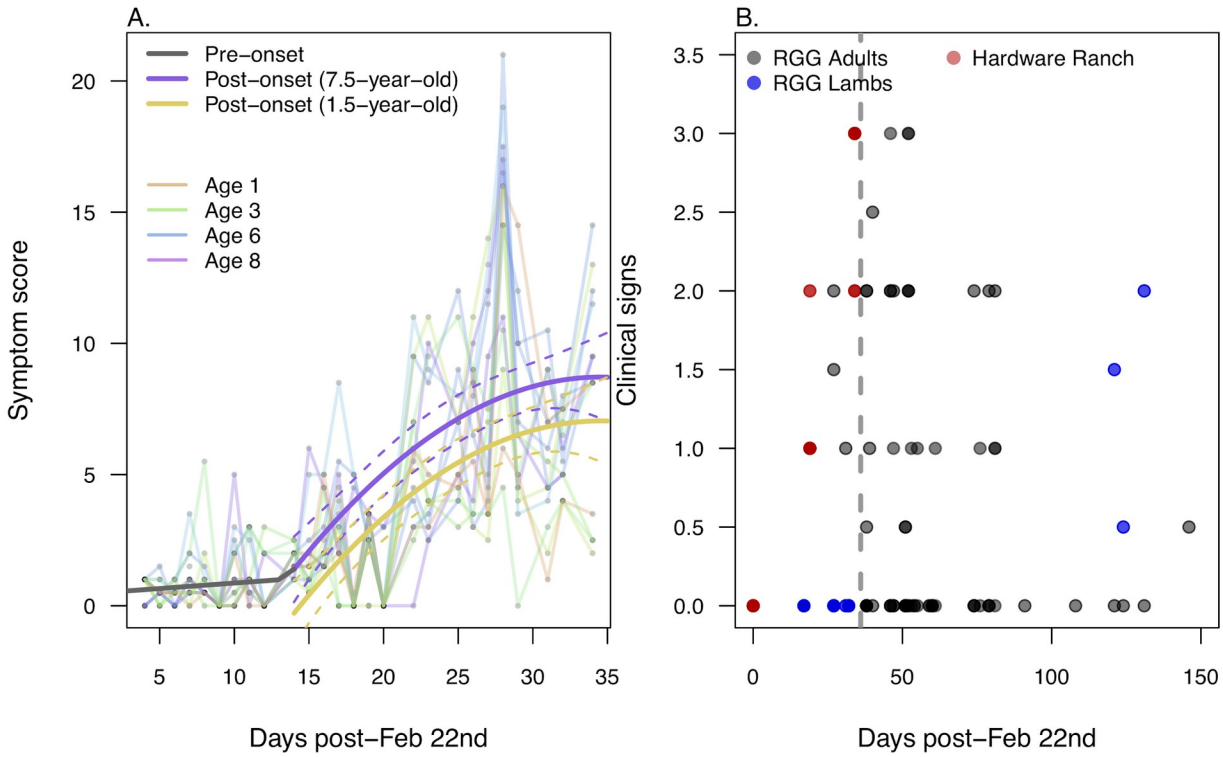

Figure 3. Clinical signs progression. A. Clinical signs among the captive animals at Hardware Ranch. Partially transparent lines show observed scores for each animal. The bold grey line indicates pre-symptom-onset patterns. Bold purple and gold lines indicate expected symptom scores for 7.5-year-old and 1.5-year-old animals, respectively, with dashed lines indicating 95% confidence intervals associated with each fit. B. Clinical signs across both sites (scores are recalibrated to allow for comparability between the RGG and Hardware Ranch data; 3 indicates animals that were coughing severely at capture, 0 indicates no clinical signs). The increase in clinical signs over summer in lamb groups is consistent with endemic-phase *M. ovipneumoniae* dynamics reported elsewhere (Cassirer et al. 2017). The grey vertical line in B is at 36 days post-February 22nd, the estimated timing of peak symptoms based on the captive data.

Decline in body condition, and use of loin thickness for quantification

The six captured animals that were PCR- or cELISA-positive for *M. ovipneumoniae* exposure on the February 22nd capture event showed similar, and usually more, fat reserves than captured animals that were PCR- and cELISA-negative for *M. ovipneumoniae* on the initial capture (median loin thickness among positives = 37.0 mm; median loin thickness among negatives = 37.5 mm; Figure S1). Rump fat, which is the conventional target for ultrasound measurement of ungulate body condition (Stephenson et al. 1998; Cook et al. 2007), was very low in most animals at the first sampling event (median among positives = 1.25 mm; median among negatives = 1.00 mm). This led us to track loin thickness as a relative measure of condition changes within an animal in subsequent sampling events. Loin thickness was correlated with both rump fat and aggregate body weight (r = 0.52, 95% CI = [-0.04, 0.83] between loin thickness and rump fat; r = 0.53, 95% = CI [0.18, 0.77] between loin thickness and body weight; Figure S2), and tracked consistently within animals through time (Figure 4A).

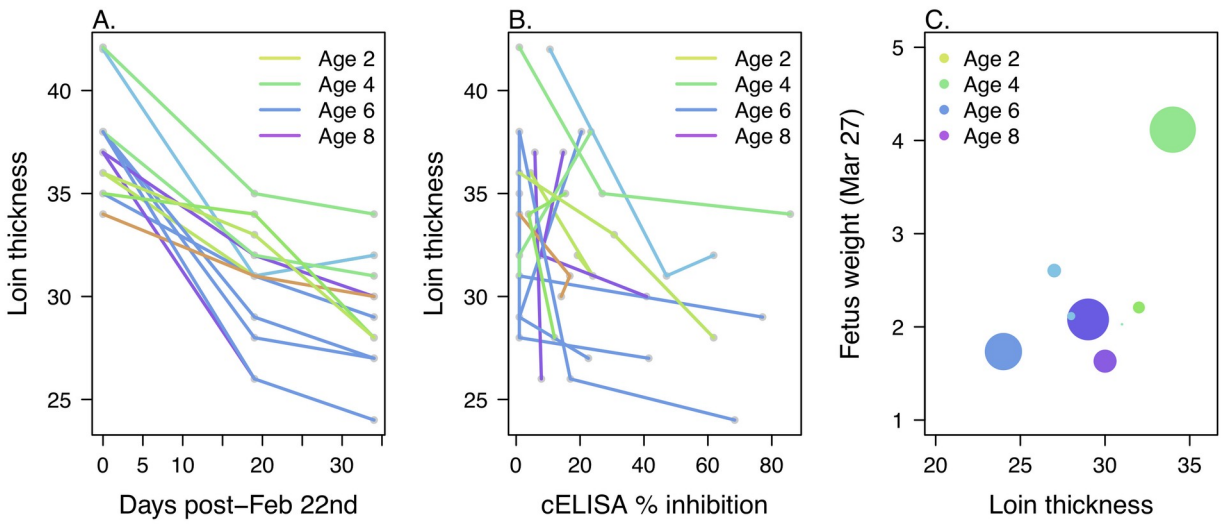

Figure 4. Patterns of loin thickness and *M. ovipneumoniae* serology among the captive animals. A. Loin thickness through time by animal. B. Loin thickness by *M. ovipneumoniae* cELISA percent inhibition by animal. C. Fetus weight at necropsy as a function of maternal loin thickness at the final sampling event (point sizes correspond to cELISA percent inhibition at that same event; larger circles indicate % inhibition of ~80; smallest circles indicate % inhibition of ~20).

Upon entering captivity, loin thickness declined significantly with days in captivity, though this effect diminished in magnitude through time. Declines were more severe among older animals than younger animals (Figure 4A), and patterns in loin thickness were better explained by age than serology (Figure 4A vs. 4B). The best model of loin thickness included age, days post-February 22nd, and an age x days-post-February-22nd interaction (AIC = 123.40). The next-best model included days post-February 22nd only (AIC = 127.13). Loin thickness in the final sampling event was positively correlated with fetus weight at necropsy (Figure 4C), though this relationship was estimated with substantial uncertainty (r = 0.58; 95% CI = [-0.21, 0.91]). No parallel decline in body condition was documented at RGG.

Epidemiological outcomes

Thirteen of the 16 animals that entered captivity at Hardware Ranch were censored prior to clearing infection. None of the captive females lambed while in captivity, so we have very few ultimate epidemiological outcomes for that setting. One of the three animals that died in captivity died from pneumonia (Eartag 28 on March 25, 2020). A second died overnight following arrival at the pen due to complications from capture, and the third was euthanized after jumping out of the pen during the capture event on March 13th (Table S1). Removing the two non-pneumonia mortalities yields a per-animal mortality probability of 0.071 in captivity (95% binomial confidence interval = [0.01, 0.34]). All remaining animals at Hardware Ranch were euthanized on March 26, 2020. In the free-ranging population, 23 of the 29 radiocollared animals remained alive as of March 2nd, 2021, and five of the six mortalities were due to hunter harvest. The RGG lamb:ewe ratio on November 12th, 2020 was 22:100 (as compared to 48:100 in a comparable count in 2019, and an average of approximately 53:100 from 2007-2019). Thus in aggregate, this event appears to have imposed very little mortality burden on free-ranging adults (95% binomial confidence interval for disease-induced adult mortality = [0.00, 0.21], which overlaps extensively with the confidence interval from the captive animals). However, there was a more sub-

stantial effect on lamb survival, in the range of post-die-off summer lamb survival rates reported elsewhere (Cassirer et al. 2018).

Discussion

We provide a detailed description of comparative in-host dynamics associated with a novel *Mycoplasma ovipneumoniae* strain introduction to a Rocky Mountain bighorn sheep herd, and compare outcomes in two different ecological contexts. We estimated an approximately 12.9-day incubation period of very low clinical signs, followed by a period of symptom expansion which we projected would likely peak around 36 days post-exposure. Seroconversion was estimated to occur at an average of 24.9 days post-exposure among the females held in captivity. Symptom, pathogen load, and serological dynamics varied among individuals, and in one animal, the infection was insufficient to generate an immune response classically indicative of exposure. Across both the free-ranging and captive groups, younger animals were more likely to clear the pathogen prior to sampling than older animals. Gross patterns of antibody expansion and symptom emergence were consistent across the two very different environments, suggesting that pathogen and host-specific factors may be more critical determinants of *M. ovipneumoniae* epidemiology, at least in this case.

Comparing disease outcomes in captive and wild settings

The epidemic progressed in very different environments at RGG and Hardware Ranch. While the RGG animals were free-ranging, the captive animals experienced unnaturally high contact rates, along with stress associated with translocation, living in captivity, dietary shifts, etc. Moreover, the RGG and Hardware Ranch data collection efforts were not perfectly aligned in time, and may reflect different phases of the disease event. Despite those contextual differences, a gross comparison indicated at least a few similarities in disease dynamics between the two sites. First, serological expansion at Hardware Ranch and RGG followed relatively similar patterns (Figure 2B). The captive data are our only line of information surrounding the antibody expansion phase of the disease event (Figure 2A); the RGG data, which arose slightly later in the epidemic, generally showed relatively high levels of expansion, especially in the later samples. When temporally aligned with one another, however, the patterns at the two sites are relatively seamless (Figure 2B). The timing at which clinical signs peaked was also grossly consistent between the two sites: the apparent plateau in clinical signs in the latter part of the captive study (Figure 3A) is consistent with subsequent declining signs in the RGG animals after approximately April 1st (Figure 3B). The estimated date of maximum symptom burden based on the Hardware data is consistent with the observed maximum symptom burden date at RGG (Figure 3B). However, direct comparison of the intensity of clinical signs is not possible using these data, since observation opportunities differed substantially between the captive and wild settings, and overall epidemic intensity may have varied between locations.

Epidemiology of a "goat clade" M. ovipneumoniae strain in wild sheep

These data provide a unique view of a "goat clade" *M. ovipneumoniae* strain invading a bighorn herd. Strains derived from domestic goats (as opposed to domestic sheep) tend to cluster genetically (Kamath et al. 2020; Maksimović et al. 2017), and have been shown to produce less-severe

infections in experimental settings (Besser et al. 2017). In the free-ranging RGG animals, the strain studied here posed a low mortality burden on adult animals relative to what has been reported elsewhere for strains from outside the "goat clade" (Cassirer et al. 2018), though disease burden on lambs was near the range of values associated with more severe *M. ovipneumoniae* strains (Manlove et al. 2016; Cassirer et al. 2018). Thus, this report provides another line of evidence suggesting a potentially lower -- but not entirely negligible -- burden of goat strains on bighorn sheep.

Importantly, several animals studied here failed to seroconvert even following known — and PCR-confirmed — infection with *M. ovipneumoniae*. This underscores the variation in individual immune responses to this pathogen. The processes leading to this variation may merit more investigation in the future.

In captivity, the bighorns exhibited substantial declines in weight and loin thickness over the course of their infections. We cannot separate environmental from disease-associated drivers in these data, but it is worth noting that loin thickness in the animals that were already infected during the first capture event did not differ significantly (and were typically in incrementally better condition, Figure S2) from those of uninfected animals during that same sampling event, perhaps providing a weak indication that *M. ovipneumoniae* alone was not responsible for the body condition declines.

Individual heterogeneity in response to infection

While all individuals in the captive setting developed infection and exhibited symptoms, symptom severity, infection duration, and antibody responses all varied (even within a single host sex, from a single host source population). The proximal driver of this variation appeared to be animal age: younger animals experienced less-severe declines in body condition, mounted slightly more rapid antibody responses, and experienced slightly lower peak loads. However, the particular factors generating these differences among ages remains unclear.

Other studies of bighorn sheep pneumonia have reported associations between age and chronic carriage rates (Plowright et al. 2017), and age and transmission potential (Manlove et al. 2017), though the latter of these could not separate behavioral drivers (i.e., young animals had lower contact rates) from in-host factors (i.e., young animals bore lower pathogen burdens or exerted lower volumes of pathogen into the environment). The data presented here suggest that immunodynamics do vary with age in the bighorn sheep-*Mycoplasma ovipneumoniae* system, consistent with patterns of immune function observed in other free-ranging sheep populations (Nussey et al. 2012; Watson et al. 2016).

In the wild setting, we saw differences among sexes in pathogen load and antibody response (both were higher in males). However, the data available here are insufficient to determine whether those effects are attributable to individual differences in date of initial infection or to actual differences in immune function between the sexes.

Study limitations

The data analyzed here arose through a natural experiment, and a number of factors limit our ability to draw direct comparisons between sites or animals. First, while we speculate that most captive animals were exposed on or very shortly after the initial capture event on February 22nd,

we do not know this definitively. It is reasonable to assume that some animals may have avoided exposure for a brief period of time. However, this avoidance would likely have occurred at random (young and old animals were well-mixed in the trailer), and should not substantially confound the general patterns reported here.

Timing of exposure in the free-ranging RGG herd is much less certain than among the captive animals, thus the symptom dynamics in Figure 3B probably represent individuals at varying stages of infection. Nevertheless, the clear decline in symptoms at the RGG herd suggests that most animals resolved acute symptoms of infection within no more than 100 days of exposure. This is fairly consistent with the symptom peak ~36 days post-exposure in the captive animals.

Finally, the vast majority of data assessed here are from females of reproductive age, and in particular, none of the longitudinal patterns we describe should be extended to males without additional validation. A few lines of evidence suggest that symptom dynamics in males may correspond relatively closely to those of females (for instance, the 10 males captured at RGG were not extreme outliers in terms of symptom expression), but we lack sufficient replication to fully document those differences here.

Implications for Management

Longitudinal data on in-host measurements hold huge value for this system -- and wildlife disease management more generally -- since they can provide context on how repeatable serological or PCR values might be, and at what timescale. Our data suggest that researchers and managers wishing to clearly document an emerging disease event in bighorn sheep should concentrate sampling efforts within the first month of the event if they wish to capture early infections and immune expansion; if the intent is to measure epidemic extent, it might be better to wait several months until most animals have had an opportunity to seroconvert and potentially clear infection. In this study, very few animals reached a point of pathogen clearance by the time of sampling. It follows that managers planning test-and-remove efforts surrounding new disease events should proceed with caution within the epidemic's first few months in order to avoid removing animals who might eventually recover naturally (especially if the disease-induced mortality burden of the event among adults is low, as was the case here). Lastly, though they have more limited immediate-term management utility, clear data on quantities like incubation period, timing of seroconversion, and timing of clinical signs through naturally acquired infections have direct utility for epidemiological models to characterize management efficacy (e.g., Almberg et al. in review). We hope these data can prove useful for both modeling efforts and research design in addition to management going forward.

Funding

Funding for this project was provided by Utah Division of Wildlife Resources, New Mexico Department of Game and Fish, and Utah State University faculty start-up support for KRM.

Data Accessibility

All data contributing to analyses presented here will be uploaded to Dryad during manuscript revision. We have also provided the original data in four files for use during the review process. HardwareCaptureData_Final and RGGCaptureData_Final contain the diagnostic testing results and other metrics obtained during each capture event for the captive and free-ranging animals, respectively. These data serve as the basis for Figures 1, 2, and 4. HardwareSymptomData_Final and RGGSymptomData_Final contain the symptom observations used to build Figure 3. All four datasets are provided as .csv files for reviewers.

Acknowledgements

We thank Drs. Tom Besser and Frances Cassirer for their on-going consultation on the captive project; and Drs. Logan Weyand, Arnaud Van Wettere, and Jacqueline LaRose for providing necropsy expertise at Hardware Ranch. We also thank Talisa Ortega, Mike Martinez, and the Natural Resources staff of the Taos Pueblo Tribe for their assistance in animal capture and monitoring over the course of this project.

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Supporting information

Model of days to seroconversion

The model was of the following form:

$\log(\text{Days post-exposure}_i) = \beta_0 + \beta_1 * \ln(\text{cELISA \% inhibition}_i) + \varepsilon_i$. Model coefficient estimates are shown in Table S1. The model was fit exclusively to data from the captive females at Hardware Ranch. Age was not predictive in this context, and we generate predictions for four-year-old animals only in the main text.

Table S1. Model coefficient estimates for the days-to-seroconversion model.

	Estimate	Standard error	Z-value	p-value from Wald's test
Intercept	1.89	0.346	5.52	<0.0001
ln(cELISA % inhibition)	0.36	0.11	3.26	0.002

Starting body condition

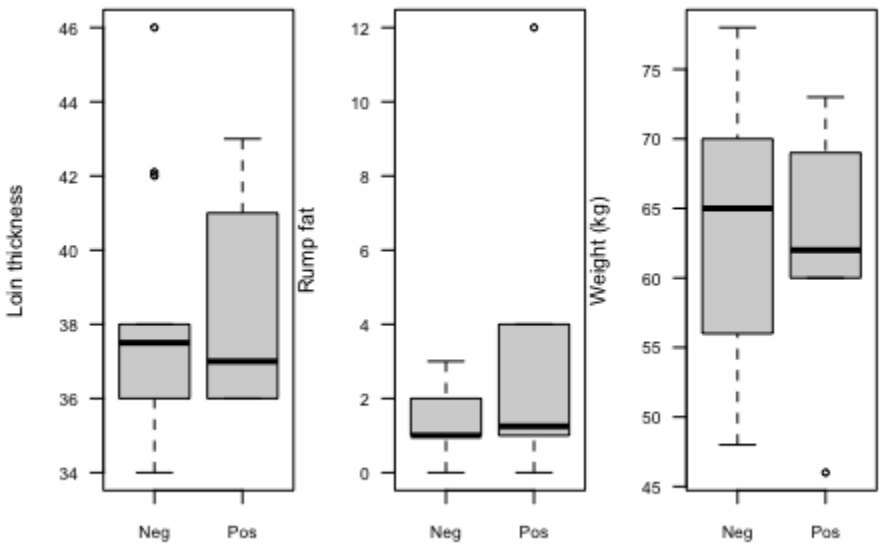

Figure S1. Comparison of loin thickness, rump fat, and weight at original capture for animals that tested PCR- or Seropositive for *M. ovipneumoniae* on the February capture event (group "Pos") to animals who did not test positive on that event ("Neg").

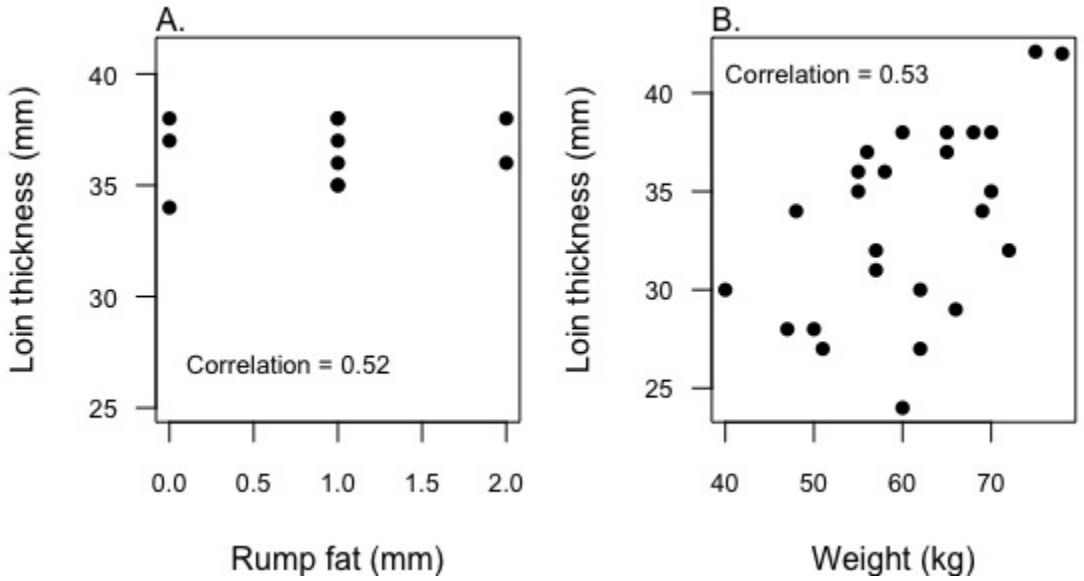

Figure S2. Relationship between rump fat and loin thickness (A) and body weight and loin thickness (B). Rump fat was only measured during the first sampling event, and was excluded in subsequent samplings due to its lack of underlying variation. Weight was only measured on the first and last sampling, due to timing constraints in the middle sampling event.