An observational study of the temporal dynamics of natural Mycoplasma (Mesomycoplasma) ovipneumoniae infection in a range flock of domestic sheep (Ovis aries)

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Objective
To characterize the dynamics of *Mesomycoplasma ovipneumoniae* colonization and serum antibody responses in a rangeland flock of domestic sheep (*Ovis aries*).

Methods
A cohort of 40 female lambs was repeatedly tested for *M ovipneumoniae* from birth to 25 months of age. Nasal swabs were analyzed for *M ovipneumoniae* infection by PCR, and a competitive ELISA was utilized to determine antibody levels. Body weights and body condition scores also were determined.

Results
M ovipneumoniae infection was rapidly acquired, with 80% of the study animals testing PCR positive at 2 months of age and 96.2% testing PCR positive at 4 months of age. The PCR positivity rate then fluctuated between 35% at 10 months and 94% at 25 months. Antibody levels expressed as percentage of inhibition were 57.9% in the dams and 52.7% in their offspring within 24 hours after birth, fell to 18.7% in the lambs at 2 months, and then remained variable, ranging from 36% to 64.2%. No significant association between *M ovipneumoniae* colonization and antibody levels or daily weight gains was found.

Conclusions
In a flock with high *M ovipneumoniae* infection rates, infections were acquired early in life and then fluctuated over time with no clear trends. Importantly, the serum antibody responses that we measured did not appear to enable sustained pathogen clearance.

Clinical Relevance
Our study indicates that, once infected, sheep do not develop effective sterilizing immunity to *M ovipneumoniae*, and serum antibodies cannot be considered a reliable correlate of protection in individual sheep.

Keywords: *Mesomycoplasma ovipneumoniae*, *Mycoplasma ovipneumoniae*, *Ovis aries*, domestic sheep, infection dynamics

Mesomycoplasma ovipneumoniae (previously termed *Mycoplasma ovipneumoniae*) is a facultative respiratory pathogen of domestic sheep (*Ovis aries*), domestic goats (*Capra hircus*), bighorn sheep (*Ovis canadensis*), and other small ruminants.[1–4] *Mycoplasma* spp was first detected in diseased sheep lungs in Scotland in 1963,[5] and its etiological role in ovine interstitial pneumonia was confirmed in an Australian study[6] from 1972. Since then, various strains of *M ovipneumoniae* have been isolated in North America, South America, Europe, Asia, and Africa, with novel strains continuing to be identified.[7–12] *Mesomycoplasma ovipneumoniae* infection is associated with highly variable clinical signs in domestic sheep that range from asymptomatic colonization to moderate or severe acute to chronic atypic pneumonia.[1] Acute *M ovipneumoniae* infection may

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lead to young lambs exhibiting lower weight gains, higher mortality, and decreased feed conversion, resulting in economic losses to sheep producers.[4,13,14] In general, coinfections with other facultative respiratory pathogens, such as *Mannheimia haemolytica*, are required for *M ovipneumoniae* to induce clinical respiratory disease in domestic and wild sheep, leading to a complex disease pathogenesis.[15–17]

Mesomycoplasma ovipneumoniae infection rates in domestic sheep flocks in the US and across the world are high, with 88% of US ewe operations found to be infected.[4] There are currently no effective vaccines or antibiotic therapies that prevent or treat *M ovipneumoniae* infection.[1–3,18–21] A better understanding of natural *M ovipneumoniae* infection and immunity in domestic sheep will be useful for the development of therapeutic regimes or vaccines. Notably, while the epizootics of *M ovipneumoniae* outbreaks in bighorn sheep herds have been well documented,[22] little is known about the spread and maintenance of *M ovipneumoniae* infections within domestic flocks over time. Therefore, the main objective of this observational study was to characterize the dynamics of *M ovipneumoniae* colonization and serum antibody responses in a rangeland flock of domestic sheep (*O aries*). We hypothesized that lambs would acquire *M ovipneumoniae* during the first few months of life and would then clear the infection following the development of adaptive immunity. Therefore, we aimed to determine at what age lambs in a Northwestern US range flock acquire *M ovipneumoniae* infection and how infection is maintained over time. We also analyzed the development of serum antibody responses to *M ovipneumoniae* and interrogated the relationship between antibody responses and infection. Finally, we investigated whether *M ovipneumoniae* infection is associated with reduced daily gains and body condition.

Methods

Study design

This was an observational longitudinal study of sheep in a typical rangeland farm setting. A random subset of lambs in a larger flock, subsequently termed "study lambs," was selected and followed from birth to 25 months of age with repeated collection of nasal swabs and serum samples. Outcome measures were (1) *M ovipneumoniae* detection, (2) antibody responses to *M ovipneumoniae*, and (3) daily gains and body condition as detailed below. Animal Research: Reporting of In Vivo Experiments 2.0 guidelines were followed for reporting.[23]

Animals, housing, and husbandry

This study was conducted from April 2022 through June 2024 with approval from the Montana State University IACUC (protocol Nos. 2021-AA07 and 2023–296-AA). Domestic sheep from a mixed-breed sheep flock were housed at the Montana State University Red Bluff Research Ranch in Norris, Montana. This flock consists of approximately 450 ewes and was previously found to have a high prevalence of natural *M ovipneumoniae* infection.[3,18] The breeds in the flock included South African Meat Merino crosses, Western Whiteface, Rambouillet, Columbia, and Targhee sheep. The ewes lambed in a drop lot and were then moved into individual indoor bonding pens, approximately 1.2 X 1.2 m. After 1 or 2 days, lambs and their dams were moved to larger mixing pens near the lambing facilities with additional ewes and lambs prior to turnout to summer range at the 2-month time point at approximately 45 to 60 days of age. Ewes were maintained as an extensive commercial rangeland flock, receiving little to no additional supplementation through the months of June through November when on native rangeland. From November through March, ewe diets were supplemented with local mixed-grass hay to provide additional nutrients. Between March and June, ewes were fed a mixed-grass hay and alfalfa mix to increase nutrients needed for late gestation and early lactation. The average weight of an ewe in this flock was 59 kg. The diet of the lambs included ewe colostrum, ewe milk, and native range. The lambs were weaned at the 4-month time point. Once native range was no longer available due to winter weather conditions, the sheep were fed hay or other preserved feedstuffs.

Sample size and inclusion and exclusion criteria

A total of 474 lambs, consisting of 232 females and 242 males, were born in the study flock during the 2022 lambing season. Forty female lambs (subsequently termed "study lambs") born to 30 ewes in 2022 were enrolled in the study within 24 hours after birth from April 17 through April 25 of 2022. The characteristics of the entire original study group, including the general characteristics of their dams, are provided in **Table 1** and **Supplementary Table S1**. Lamb enrollment in the study was random, with any female lamb born on 3 nonconsecutive sample collection days in the middle of the lambing period enrolled until the target number of 40 lambs was reached. Male lambs, which are typically sold within the first year of life, and lambs with obvious failure to thrive were excluded from the study. No additional inclusion and exclusion criteria were applied. With an expected attrition of 25% to 50% over 2 years, a final sample size of 20 to 30 animals would enable the detection of moderate effects (r = 0.5 to 0.6) with a 2-tailed α of .05 at 80% to 90% power.[24] The study animals gave birth to their first set of lambs in April 2024, and these lambs were weaned in August 2024. A timeline of sample collections and other major

Table 1—Lamb and dam characteristics.

Lamb characteristics				Ewe characteristics	
Breed		**Birth type**		**Breed**	
WWF	19	Twin	36	WWF	14
SAMM X	4	Single	2	SAMMX	3
Targhee	11	Triplet	2	Targhee	10
Rambouillet	5			Rambouillet	3
Columbia	1			Columbia	1

SAMM X = South African Meat Merino cross. WWF = Western Whiteface.

events affecting the study group is shown in **Figure 1**. The study time points with corresponding samples that were collected are outlined in **Supplementary Table S2**. Sample collection time points were chosen to coincide with other management events, such as turnout, weaning, or shearing.

Collection of nasal swab samples

Nasal swabs were collected from the caudal nares of the study lambs by inserting flocked 15-cm polystyrene swabs (Puritan Flocked 25–3317-U BT; Harmony Lab and Safety Supplies) into each nostril as far as possible and twisting the swab twice. The swabs were then placed in 15-mL Falcon tubes with 1 mL of nasal swab media (85% Dulbecco's modified Eagle medium and 15% glycerol) on ice for transport. To prepare the nasal swab sample for freezing, the samples were pulse vortexed for 15 seconds prior to removing the nasal swab from the tube. An additional 1 mL of nasal swab media was added to the graduated tube. One milliliter of the nasal swab media was then transferred to a cryovial and frozen at −80 °C. Nasal swabs were collected from each animal in the study group within 24 hours after birth and then at 2 months, 4 months, 7 months, 10 months, 11 months, 15 months, and 25 months (Figure 1; Supplementary Table S2). Nasal swabs were also collected from the dams of each study lamb within 24 hours after birth.

Collection of serum samples

Serum samples were collected from the study lambs within 24 hours after birth after the lambs had received colostrum and then at the same time points as the swabs (Figure 1; Supplementary Table S2). Serum samples were also collected from the dams of each study group animal within 24 hours after birth. Three to 7 mL of blood was collected from the external jugular vein utilizing a Vacutainer system into serum tubes containing a polymer gel clot activator (BD Vacutainer SST 367988; Becton Dickinson) and were stored on ice for transport. The blood samples were centrifuged at 3,210 X *g* for 10 minutes, and 1 mL of serum was aliquoted into labeled tubes and stored for up to 15 months at −20 °C prior to analysis.

Weight and body condition score

Weight was recorded utilizing a built-in scale included in a Racewell Sheep Handler (Te Pari) that restrained the study lambs at each time point. Average daily gain (ADG) was calculated by subtracting the animal's weight at one time point (t_n) from its weight in kilograms at the consecutive time point and then dividing the difference by the number of days (x) between each time point ([weight (t_{n+x}) − weight (t_n)]/x). Body condition score was reported based on the conventional 1-to-5 scale, with 1 indicating an emaciated sheep and 5 indicating an obese sheep.[25] The study lambs were palpated and visually assessed by the same individual, who was blinded to infection status, at each time point to determine the appropriate body condition score. Study lambs were assessed along with all other animals in the flock, and the assessor did not know which animals were included in the study, further limiting potential bias.

PCR protocol

Real-time PCR was used to detect *M ovipneumoniae* in nasal swab samples and for semiquantitative analyses. Samples collected at 0, 2, and 4 months were analyzed by the Washington Animal Disease Diagnostic Laboratory (WADDL; Pullman, Washington) following a published protocol.[4] Briefly, DNA was extracted from nasal swabs using a MagMAX-96 Isolation Kit (Applied Biosystems) according to the manufacturer's instructions. The following primers and probe were used: forward, GGG GTG CGC AAC ATT AGT TAG TTG GTA; reverse, GAC TTC ATC CTG CAC TCT GT; and probe, 6-FAM TTA GCG GGG CCA AGA GGC TGT-BHQ-1. The nasal swabs from the last 5 time points (months 7, 10, 11, 15, and 25) were analyzed at Montana State University utilizing a previously optimized real-time PCR assay originally described by Yang et al.[3,26] An epMotion 5075 (Eppendorf) robot was utilized with Mag-Bind Viral

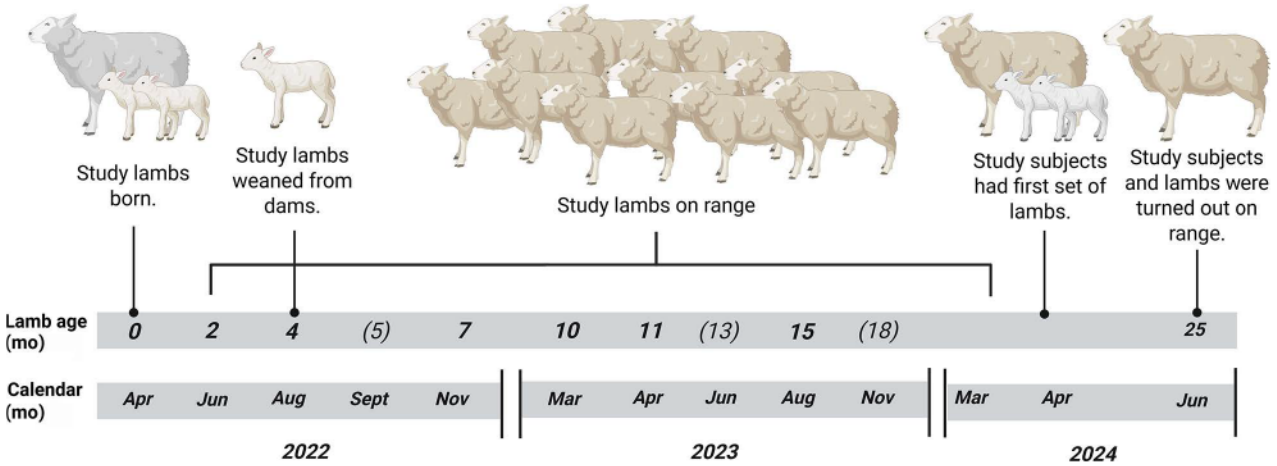

Figure 1—Timeline of major events regarding the study subjects that occurred during the longitudinal study period. Lamb ages in months represent sample collection time points. Numbers in parentheses represent time points where only weights or body condition scores were determined. Created in BioRender (https://BioRender.com/scxnj7t).

DNA/RNA 96 Kit (Omega Bio-tek) reagents to perform DNA extractions on all swab samples. The PCR was performed on a QuantStudio 5 thermocycler (Thermo Fisher) using customized *M ovipneumoniae* premixed forward and reverse primers from BioRad PrimePCR (BioRad Laboratories) targeting the putative adhesin *p113* gene (forward, GGG GTG CGC AAC ATT AGT TA; reverse, CTT ACT GCT GCC TCC CGT AG) and iTaq Universal SYBR Green Mix. For both PCRs, the limit of detection was at cycle threshold (CT) = 35. All PCR CT values were reported as 40-CT, with 40 being the total number of cycles. This conversion allows for a 40-CT of 0 to be considered a negative PCR test, a 40-CT of 0 < 5 being an indeterminate result, and a 40-CT of ≥ 5 being a positive PCR test. Each real-time PCR run included a positive extraction control, consisting of *M ovipneumoniae*–positive culture, and a negative extraction control of buffer or water. Both positive and negative extraction controls underwent the extraction method with the swab samples.

Competitive ELISA for M ovipneumoniae *antibodies*

Serum antibodies to *M ovipneumoniae* were detected using a competitive ELISA (cELISA) performed at the WADDL.[3,18,20] The cELISA is designed to detect total *M ovipneumoniae*–specific antibodies based on competition with a monoclonal antibody directed against a 71-kDa immunodominant antigen epitope of the organism that has not been further characterized. The *M ovipneumoniae* antigen used in this assay was produced in house at the WADDL. Briefly, *M ovipneumoniae* (strain 06SM90) was propagated in SP4 broth supplemented with glucose under CO_2 incubation conditions until the culture reached the appropriate growth phase as indicated by medium color change. Organisms were harvested by centrifugation (15,000 X *g* for 30 minutes), washed 3 times in sterile PBS, and resuspended in PBS. Antigen was diluted in coating buffer to a standardized working concentration based on internal quality-control procedure and then coated onto 96-well plates. Plates were equilibrated to room temperature and washed once with 200 µL/well of wash buffer consisting of PBS with 0.05% Tween-20. Undiluted serum samples and controls were added at 50 µL/well, with milk-based blocking buffer added to conjugate control wells. Plates were sealed and incubated for 1 hour at room temperature. Plates were then washed 3 times with 200 µL/well of wash buffer. An in-house anti–*M ovipneumoniae* monoclonal antibody (clone F141/224.2.1), produced internally, was diluted 1:500 in antibody diluent to a final working concentration of 1 µg/mL and added at 50 µL/well. Plates were sealed and incubated for 1 hour at room temperature, followed by 3 additional wash steps. Thereafter, a horseradish peroxidase–conjugated anti-mouse immunoglobulin G secondary antibody (LGC Clinical Diagnostics), diluted 1:250 in milk-based diluent, was added at 50 µL/well and incubated for 1 hour at room temperature. Plates were washed 4 times, and 60 µL/well of tetramethylbenzidine substrate was added. Plates were protected from light and incubated at room temperature for 15 to 30 minutes depending on optical density (OD) development. The enzymatic reaction was stopped with 60 µL/well of 0.5 M HCl, and absorbance was measured at 450 nm. Each assay run included positive and negative reference controls. Acceptance criteria required that the mean percentage of inhibition of the positive control fall within established internal trend limits. The mean OD of triplicate negative control wells was required to be ≥ 0.6 and < 2.0, and the OD of the monoclonal antibody (conjugate) control well was required to be > 0.6 and < 2.5; runs failing these criteria were repeated. Based on internal validation, samples producing > 50% inhibition were interpreted as antibody detected, < 40% inhibition as not detected, and 40% to 50% inhibition as indeterminate. Interpretation was based on relative inhibition compared with assay controls rather than fixed absorbance cutoffs or SD-based thresholds.

Statistical analysis

Statistical analyses and graphing were completed in Prism (version 10; GraphPad Software Inc) or with RStudio (version 4.3.1; R Foundation for Statistical Computing, Posit Software PBC). Data are shown as individual values with mean ± SD where appropriate. Competitive ELISA and PCR results collected at different time points were analyzed by using a mixed-effects model with multiple-comparisons testing **(Figure 2)**. Correlations between antibody levels in dams and in their lambs **(Figure 3)** were analyzed with a simple linear regression model that evaluated if the slope was significantly nonzero (with a reported *P* value), with 95% CIs. To determine whether serum antibody levels predict *M ovipneumoniae* infection status determined by PCR **(Table 2)**, data for the PCR outcomes were analyzed using a cumulative link mixed model with a logit link for the ordered categorical outcome reflecting the PCR result (0, negative; 1, intermediate; 2, positive). Of note, the 0-month time point was excluded from this analysis as there was no time for the lambs to become infected. The model was implemented in R using the ordinal package. Fixed effects for the model included month and cELISA values, whereas nested random intercepts for lamb identification (ID) and ewe ID were used for maternal clustering and repeated measurement. Odds ratios and 95% CIs were reported for the fixed effects. The data for predicting the ADG were analyzed using a linear mixed-effects model **(Table 3)**. Fixed effects for the model included PCR, ELISA, and month, whereas nested random intercepts for lamb ID and ewe ID were used for maternal clustering and repeated measurements. The model was implemented in R using the *lme4* package. Odds ratios and 95% CIs were reported for the fixed effects.

Results

To analyze the temporal dynamics of *M ovipneumoniae*, we enrolled a cohort of 40 lambs at birth and followed them for 25 months. Detailed information about the breeds, litter sizes, sample collection time points, and reasons for attrition, where applicable, is presented in Supplementary Table S1. Due to attrition,

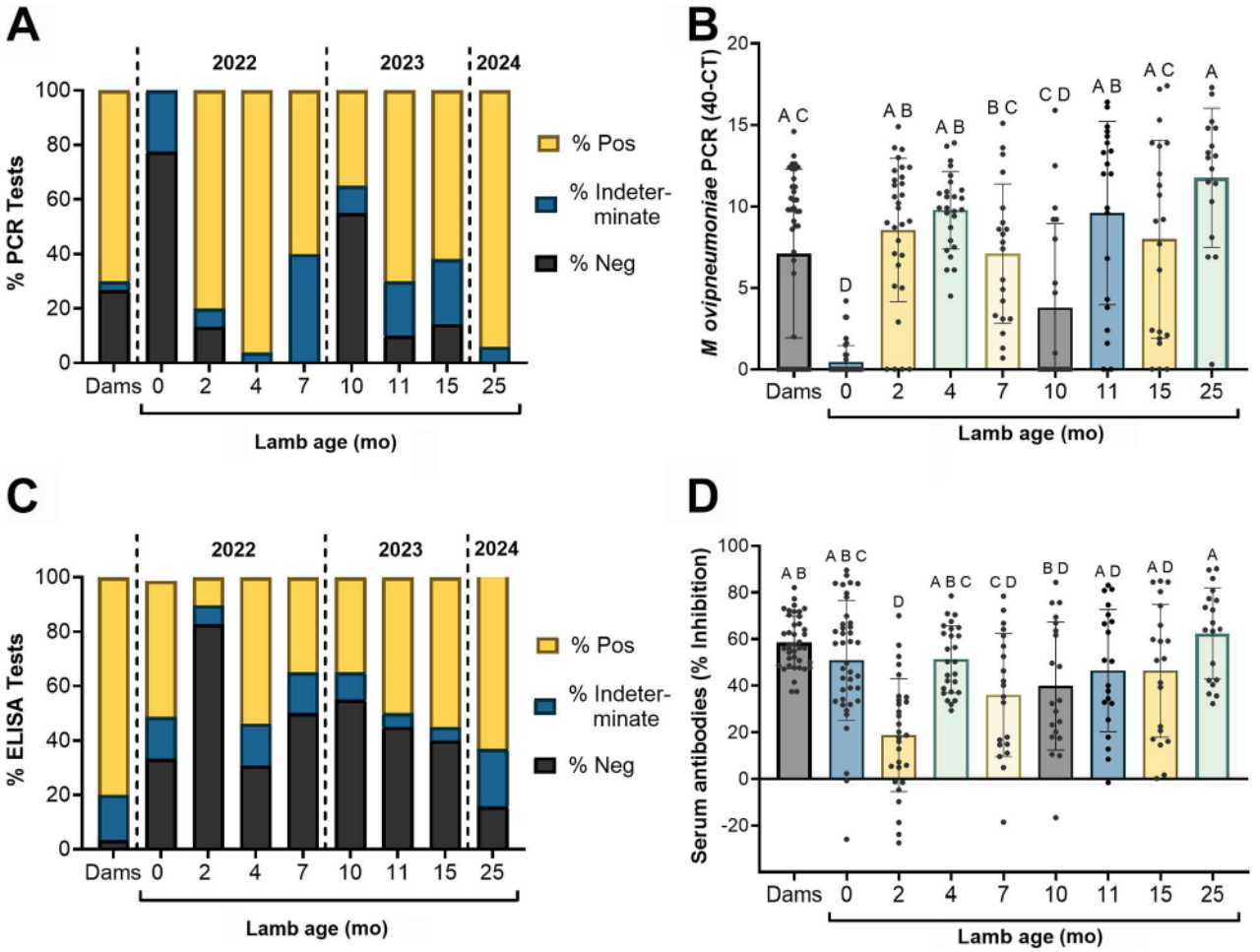

Figure 2—A—Percentage of study group animals at each time point that tested positive (Pos), negative (Neg), or indeterminate for *Mesomycoplasma ovipneumoniae* based on real-time PCR analysis of nasal swabs. 40-CT values of ≥ 5 were considered a Pos result, 0 to 5 an indeterminate result, and 0 a Neg result. B—Semiquantitative analysis of *M ovipneumoniae* infection over time determined by real-time PCR. Data are shown as 40-CT and were analyzed using a mixed-effects analysis with a Tukey multiple comparisons test. Different letters indicate statistically significant differences at $P \leq .05$. The PCR assays for the dams and the 0-, 2-, and 4-month time points were performed at the Washington Animal Disease Diagnostic Laboratory, whereas PCR assays for the 7- to 25-month time points were performed at Montana State University. C—Percentage of study group animals at each time point that tested Pos, Neg, or indeterminate for *M ovipneumoniae* antibodies in a competitive ELISA (cELISA). Greater than 50% inhibition was considered Pos, 40% to 50% inhibition was considered indeterminate, and < 40% was considered Neg. D—Mean percentage of inhibition from a cELISA assay is represented by bars, with an SD represented by error bars. Values of > 50% indicate that the antibody was detected. Data were analyzed using a mixed-effects analysis with a Tukey multiple comparisons test. Different letters indicate statistically significant differences at $P \leq .05$. The initial study group included 40 animals, but attrition occurred over time, and the study group decreased to 17 animals (see Supplementary Tables S1 and S2). 40-CT = 40 minus cycle threshold.

only 17 out of 40 animals remained at the final time point, and complete datasets were obtained for 14 animals. Five of the study lambs (12.5%) died within the first 2 months of life, and an additional 4 lambs (10%) died of unknown causes at later stages. Summarized data about the age of the lambs at each time point, the number of samples, and the types of samples collected are provided in Supplementary Table S2.

Longitudinal analysis of M ovipneumoniae *infection using PCR analysis of nasal swabs*

Nasal swab samples collected within 24 hours after birth were PCR positive for *M ovipneumoniae* in 21 of 30 dams (70%) but in none of the study lambs (0 of 40 [0%]), although 9 of 40 study lambs (22.5%) had indeterminate results (Figure 2). In sharp contrast, 24 of 30 study lambs (80%) tested PCR positive at 2 months of age. The prevalence of *M ovipneumoniae* peaked at 4 months of age, when 25 of 26 lambs (96.2%) tested PCR positive, with only 1 indeterminate test result. At 7 months, 12 of 20 study lambs (60%) had positive and 8 of 20 (40%) had indeterminate PCR results. The incidence of positive PCR tests for *M ovipneumoniae* was lowest at the 10-month sampling time point, with only 7 of 20 positive tests (35%). However, 14 of 20 (70%) and 13 of 21 study lambs (61.9%) tested PCR positive at 11 and 15 months, respectively. The prevalence of

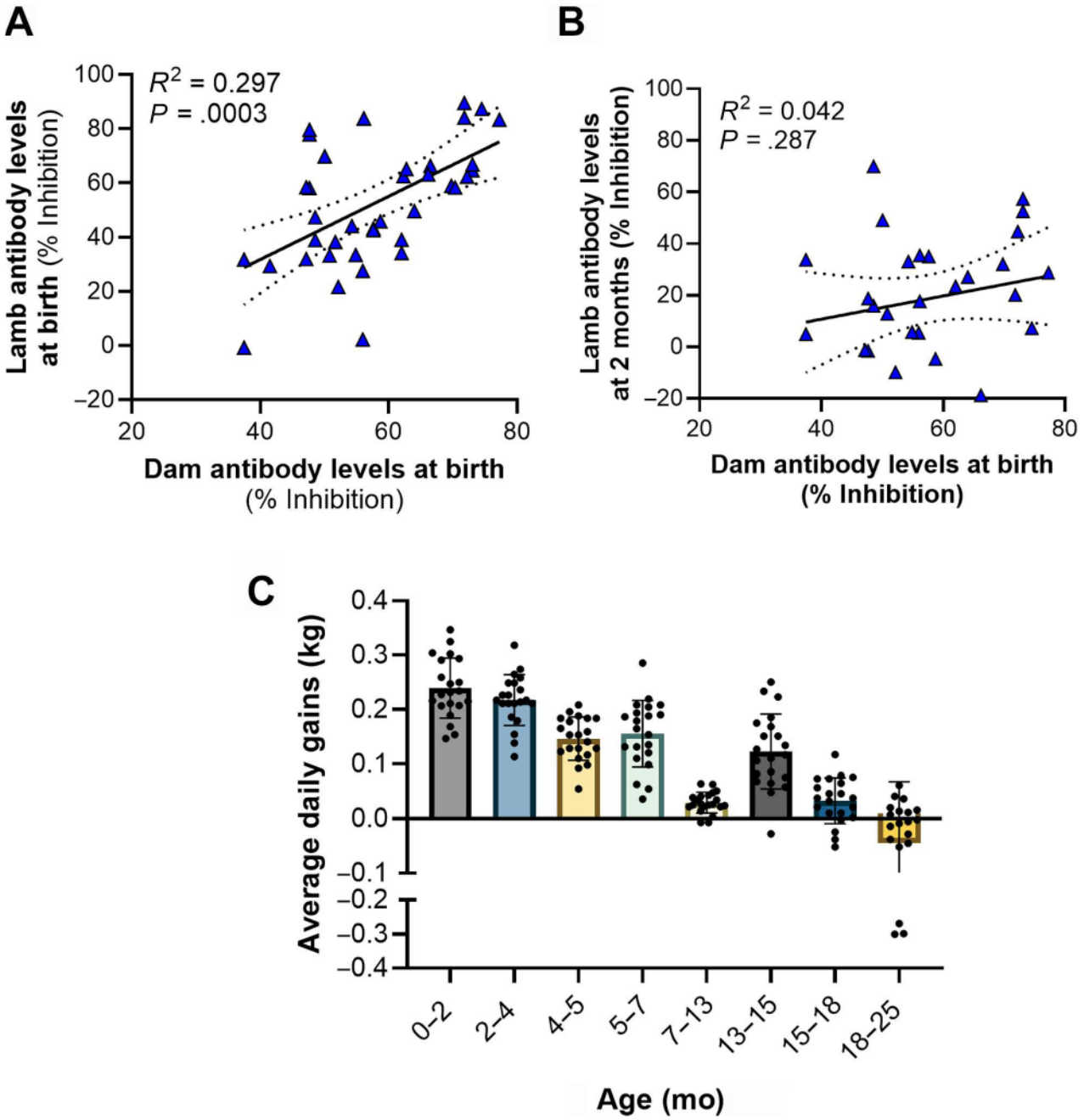

Figure 3—A and B—Correlation between *M ovipneumoniae* antibody levels in study lambs and their dams. Antibody levels in lamb sera collected within (A) 24 hours after birth or (B) 2 months after birth were compared to serum antibody levels in the respective dams determined within 24 hours after birth. *Mesomycoplasma ovipneumoniae* antibody levels are shown as percentage of inhibition determined by cELISA. A significant correlation between dam and lamb antibody levels at birth was found (R^2 = 0.297; P = .0003) using a simple linear regression model. C—Weight development shown as average daily gain for the study lambs over time. Average daily gain was calculated between time points by subtracting the animal's weight at one time point from the consecutive time point and then dividing the difference by the number of days between the 2 time points. The figure shows individual data points, mean, and SD.

M ovipneumoniae infection then increased again at 25 months, with 16 of 17 (94.1%) testing PCR positive. Analysis of 40-CT values as a semiquantitative measure of pathogen load showed the same trend, with *M ovipneumoniae* levels at all time points except 10 months significantly higher than at the 0-month time point ($P \leq .05$). Overall, all study lambs that were tested multiple times after the 0-month time point had positive PCR test results at some of the time points, with no animal remaining *M ovipneumoniae*–negative throughout the study **(Supplementary Figure S1)**.

Table 2—Mixed model for association between measured serum antibody responses and *Mesomycoplasma ovipneumoniae* detection by PCR.

	***M ovipneumoniae* PCR**		
Predictors	**OR**	**CI**	***P* value**
0|1	0.22	0.09–0.56	.001*
1|2	0.64	0.27–1.52	.311
Mo	1.00	0.94–1.06	.913
cELISA (% inhibition)	1.01	0.99–1.02	.371
Random effects			
σ^2	3.29		
$\tau_{00\ lamb:ewe}$	0.00		
$\tau_{00\ ewe}$	0.00		
N_{lamb}	14		
N_{ewe}	11		
Observations	98		
Marginal R^2/conditional R^2	0.011 / NA		

Coefficients for PCR category predicted based on serum antibody concentration are expressed as percentage of inhibition; a cumulative link mixed model with a logit link for the ordered categorical outcome representing diagnostic PCR results (0, negative; 1, intermediate; 2, positive) was used.

σ^2 = Within-group variance. τ_{00} = Intercept variance. cELISA = Competitive ELISA. N = Number of animals. NA = Not available. *Significant difference at $P \leq .05$.

Table 3—Linear mixed-effects model for association between measured serum antibody responses and *M ovipneumoniae* detection by PCR and average daily gains (ADGs).

	ADGs		
Predictors	**Estimates**	**CI**	***P* value**
(Intercept)	0.30	0.25 to 0.35	< .001*
PCR	0.00	−0.01 to 0.02	.618
ELISA	0.00	−0.00 to 0.00	.178
Mo	−0.03	−0.03 to −0.02	< .001*
Random effects			
σ^2	0.00		
$\tau_{00\ lamb}$	0.00		
$\tau_{00\ ewe}$	0.00		
N_{ewe}	11		
N_{lamb}	14		
Observations	56		
Marginal R^2 / conditional R^2	0.764 / NA		

Coefficients for ADGs based on PCR category and serum antibody concentration are expressed as percentage of inhibition linear mixed-effects model. Fixed effects for the model included PCR, ELISA, and month, whereas nested random intercepts for lamb identification and ewe identification were used for maternal clustering and repeated measurements.

*Significant difference at $P \leq .05$.

Longitudinal analysis of serum antibody responses to M ovipneumoniae *infection*

At birth, 80% of the dams had positive cELISA test results for *M ovipneumoniae*, with antibody concentrations at 57.9 ± 10.6% inhibition (Figure 2). For the study lambs, antibody concentrations of 52.7 ± 22.7% inhibition were detected in serum samples collected within 24 hours after birth (0 months), with 50% of lambs testing positive. Between the 0-month and 2-month time points, antibody concentrations decreased significantly to 18.7 ± 24.2% inhibition ($P < .0001$), and only 10.3% of lambs tested positive at that time point. At 4 months of age following weaning, antibody levels increased to a mean of 51.2 ± 14.4%, with 53.8% of study lambs testing positive, and then decreased again at 7 months. Between months 7 and 25, average antibody levels increased over time, with mean antibody levels of 36.0 ± 26.42% inhibition at 7 months, 39.8 ± 27.5% inhibition at 10 months, 46.4 ± 26.2% inhibition at 11 months, and 46.4 ± 28.5% inhibition at 15 months. Antibody levels peaked at 25 months of age, with a mean percentage of inhibition of 64.2 ± 19.5%. The percentages of lambs with positive serological tests at these time points were 35.0% at 7 and 10 months, 50.0% at 11 months, 55.0% at 15 months, and 63.2% at 25 months. Individual cELISA test results for each lamb and time point by diagnostic category are presented in Supplementary Figure S1.

Comparison between M ovipneumoniae *antibody levels in lambs and their dams and analysis of association between antibody levels and PCR detection of* M ovipneumoniae

When comparing *M ovipneumoniae*–specific antibody levels in serum samples collected from the study lambs and their dams within 24 hours after birth (0-month time point), we detected a significant positive correlation ($P = .0003$; $R^2 = 0.297$; Figure 3). By 2 months of age, the antibody levels of the study lambs were no longer correlated with their dams ($P = .287$; $R^2 = 0.042$). Further statistical analysis of the study period between 2 and 25 months indicated that the fixed effects of month and cELISA were not significantly associated with PCR status (marginal $R^2 = 0.011$; Table 2). Month had no detectable effect on the odds of a lamb testing PCR positive (OR, 1.00; 95% CI, 0.94 to 1.06; $P = .913$). Similarly, antibody levels measured by cELISA did not predict concurrent PCR detection status (OR, 1.01; 95% CI, 0.99 to 1.02; $P = .371$).

Analysis of potential associations between M ovipneumoniae *infection, antibody levels, and weight gains*

Analysis of ADGs in the study lambs found consistent weight gains up to 7 months of age, followed by variable weight development (Figure 3). Linear mixed modeling, performed for the period between 2 and 10 months when significant growth occurred, confirmed that ADG declined significantly over time (Wald test; $P < .001$). However, neither PCR status defined by diagnostic category nor cELISA percentage of inhibition values were significantly associated with ADGs (Table 3). Throughout the study, body condition scores remained within a narrow range of 2 to 3 (2.5 ± 0.43), with no apparent trends for differences associated with *M ovipneumoniae* detection or cELISA positivity (data not shown).

Discussion

Our study describes the natural dynamics of *M ovipneumoniae* infection and serum antibody responses in a rangeland flock of domestic sheep. Our PCR analysis of nasal swabs confirmed a high prevalence of *M ovipneumoniae* in the study lambs and their dams, with the percentage of positive PCR tests fluctuating between 45% and 95% throughout the 25-month study period. This prevalence was similar to previous results from a large-scale study[4] performed by the USDA in US sheep operations in 2011, where the median prevalence of *M ovipneumoniae* in individual ewes based on positive PCR tests was 60%. Conversely, prevalence rates for *M ovipneumoniae* were somewhat lower in recent studies[27] from India, where 34% of sheep with respiratory signs were PCR positive , and China, where 2 studies[11,28] reported PCR positivity rates of 10% to 41%.

Polymerase chain reaction testing of nasal swabs showed that the study lambs rapidly acquired *M ovipneumoniae*, likely from infected adult ewes that they were housed with, with 80% of lambs testing PCR positive at 2 months of age and 96% positive at 4 months. Interestingly, the prevalence of positive *M ovipneumoniae* PCR tests in lambs at 2 months of age exceeded the prevalence in their dams at birth. Clinical disease associated with *M ovipneumoniae* is generally thought to affect mostly lambs. However, the prevalence of infection in our experimental cohort reached similar levels at 4 months and at 25 months, when the study lambs had matured. Still, pathogen clearance may occur at a more advanced age, or, alternatively, age could impact susceptibility to clinical disease rather than *M ovipneumoniae* colonization of the upper respiratory tract.

Despite the success of passive transfer of *M ovipneumoniae* antibodies to the study lambs, as evidenced by positive cELISA tests in > 50% of lambs immediately after birth, colostral antibodies did not appear to protect lambs from acquiring *M ovipneumoniae* infection given that most lambs had become infected by 2 months of age. No relationship between pathogen detection and antibody levels was found in this study. However, sampling during early life was limited, and it is unclear whether a drop in maternal antibodies preceded nasal colonization with *M ovipneumoniae* or whether infection occurred despite high antibody levels. Alternatively, the quality of the antibody response, which the cELISA did not measure, may be the key determining factor for protection.

One interesting finding from our study was the significant fluctuations in *M ovipneumoniae* PCR positivity and *M ovipneumoniae*–reactive antibody responses. At 10 months of age, more than half of the study lambs tested negative; however, by the age of 25 months almost all animals tested positive again. These fluctuations could be due to the development of protective immunity that decreased colonization followed by reinfection with a different *M ovipneumoniae* strain after initial clearance or reemergence of infection from an internal reservoir. Interestingly, the fluctuations in *M ovipneumoniae* colonization appeared to follow seasonal trends, with higher rates of infection observed during the summer months. Similarly, seasonal trends for *M ovipneumoniae* infection were described for lung colonization in market lambs in Spain[9] and in sheep with respiratory disease Italy.[29] We speculate that such seasonal trends could be due to environmental impacts on pathogen stability, seasonal differences in sheep husbandry or diet, hormonal changes, or seasonal stressors and could be a subject of further studies.

Notably, the cELISA used to detect serological responses is not recommended as a diagnostic tool to determine *M ovipneumoniae* infection in individual sheep.[30] However, a positive serological test result after colostral antibodies have waned (4 months and later) does show that a particular animal has been exposed to *M ovipneumoniae* and has developed an adaptive immune response. In 2 previous studies,[3,18] results from the cELISA showed a typical induction of a serum antibody response following experimental *M ovipneumoniae* infection of domestic lambs. In this study, there was no significant association between antibody levels and the presence of *M ovipneumoniae* at any given time point. This observation indicates that a serum antibody response does not protect sheep from *M ovipneumoniae* infection. Notably, as the cELISA assay used in this study can only detect antibodies to 1 immunodominant 71-kDa *M ovipneumoniae* epitope, a different type of serological assay may have yielded more informative results. Also, given the large intervals between sampling time points, seroreversion with loss of protection and subsequent seroconversion upon reinfection may have occurred without being detected. Nevertheless, our study confirms that serum antibodies to *M ovipneumoniae* are not sufficient for achieving sterile immunity. Other immune mechanisms, such as T cells or innate mechanisms, may be required to successfully combat *M ovipneumoniae* infection.[31]

Previous studies[3,4,13] had shown a significant impact of *M ovipneumoniae* on weight gains in lambs, which we did not observe. However, the effects of *M ovipneumoniae* infection on weight development may have been masked by the overall high prevalence of infection in our study cohort or by the presence of 5 genetically different breeds with different growth characteristics in our flock. There were some additional limitations to this study. First, there was > 50% attrition over time, which decreased the statistical power of the analyses, limiting our ability to detect smaller effects on weight gains or body condition. Second, the *M ovipneumoniae* PCR results were not truly quantitative, and 2 different PCR protocols were used, which precluded statistical analysis of pathogen loads. Third, due to the extensive management practices within the rangeland flock, the presence of respiratory disease or causes of death were not consistently analyzed.

Our study has potential implications for clinical practice and diagnostics. First, the fluctuations in both *M ovipneumoniae* itself and *M ovipneumoniae* antibodies suggest that testing may need to be performed at multiple time points to obtain accurate herd-level infection data. Moreover, given

the absence of effective sterilizing natural immunity in sheep up to 2 years of age, the development of a vaccine may prove to be challenging and may require optimized adjuvants. The observed reinfection or reemergence of *M ovipneumoniae* following negative test results also indicates that treatment of *M ovipneumoniae* with antimicrobials may not yield sustained pathogen clearance. These issues should be taken into consideration in the future development of treatment or preventive strategies.

In summary, our study shows a high rate of early acquisition of *M ovipneumoniae* in the upper respiratory tract when lambs are exposed to infected animals in the flock. Despite evidence of significant antibody responses to *M ovipneumoniae*, there was no evidence that the sheep developed sterilizing immune responses that could achieve clearance of *M ovipneumoniae* within the first 25 months of life.

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The authors have nothing to disclose. No AI-assisted technologies were used in the composition of this manuscript.

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Supplementary Materials

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