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Risk factors and productivity losses associated with *Mycoplasma ovipneumoniae* infection in United States domestic sheep operations

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ABSTRACT

Association of *Mycoplasma ovipneumoniae* with pneumonia in domestic small ruminants has been described in Europe, Asia, and New Zealand but has received less attention in the United States. In 2011, the US Department of Agriculture's National Animal Health Monitoring System detected *M. ovipneumoniae* shedding in 88% of 453 domestic sheep operations tested in 22 states that accounted for 85.5% of US ewe inventory in 2001. We evaluated factors associated with *M. ovipneumoniae* infection presence and prevalence, and we compared health, lamb production, and ewe losses in infected and uninfected operations. *M. ovipneumoniae* detection was more common in larger operations than in smaller operations. Both likelihood of detection (at the operation level) and within-operation prevalence were higher in operations with more open management practices than in operations with more closed management practices. *M. ovipneumoniae*-positive operations showed significantly lower lambing rates and lower rates of lamb survival to weaning after accounting for differences in operation size and management practice. While its effect on any single rate was not particularly large, in aggregate we estimated that *M. ovipneumoniae* presence was associated with an approximately 4.3% reduction in annual lamb production.

1. Introduction

Mycoplasma ovipneumoniae is a recognized pathogen of Caprinae (Ayling et al., 2004; McAuliffe et al., 2003; Alley et al., 1999), and has been associated with transmissible respiratory disease in domestic sheep in experimental settings (Alley et al., 1999 and references therein). Like other respiratory mycoplasmas, *M. ovipneumoniae* initially colonizes the respiratory tract, where it impedes movement of the ciliary escalator (Niang et al., 1998; Thacker and Minion, 2010). This allows a variety of normally commensal upper respiratory, rumen, and inhaled flora to invade the lungs, where they can cause chronic, polymicrobial pneumonia (Brogden et al., 1998; Niang et al., 1998; Besser et al., 2012).

Veterinary texts describe *M. ovipneumoniae*'s consequences on animal health and operation productivity with characterizations ranging from "mild" (in Smith, 2014 *Large Animal Internal Medicine*) to "proliferative" (in Radostits et al., 2006 *Veterinary Medicine*). The diversity of descriptors leaves ambiguity about how veterinarians and operators should view *M. ovipneumoniae* infection in terms of production loss. Respiratory disease is a serious problem for domestic sheep production in the United States, and is the fifth-highest source of lamb loss, following weather, lambing problems, predation, or unknown causes, and on a par with gastrointestinal problems (USDA-APHIS, 2014, 2015a). Additionally, there are only limited and local data on *M. ovipneumoniae* presence in United States domestic sheep operations (Brogden et al., 1988; USDA-APHIS, 2015b; Heinse et al., 2016). A better understanding of the risk factors associated with *M. ovipneumoniae* infections, and the burden these infections place on domestic sheep production would help producers determine how to prioritize its management.

Here, we investigate whether *M. ovipneumoniae* is associated with

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reduced productivity in United States domestic sheep operations. We postulate that *M. ovipneumoniae* may be an under-recognized pathogen of domestic sheep in the United States for two reasons. First, disease burdens associated with other *Mycoplasma* infections of livestock are well-documented (e.g., Nicholas et al., 2008). This is true, for example, of *Mycoplasma mycoides*, the causal agent of contagious bovine pleuropneumonia, and *Mycoplasma capricolum*, the causal agent of contagious caprine pleuropneumonia, as well as the disease burden associated with *M. ovipneumoniae*'s close relative *Mycoplasma hyopneumoniae*, a pathogen of pigs (e.g., Pieters et al., 2009; Sibila et al., 2009). Second, *M. ovipneumoniae* is thought to cause chronic disease and generate important mortality burdens in domestic sheep in other parts of the world (Alley et al., 1999; McAuliffe et al., 2003); as well as in other caprine hosts, including domestic goats (Rifatbegovic et al., 2011; Rong et al., 2014). However, despite burdens associated with *M. ovipneumoniae*'s close relatives in domestic animals, and with *M. ovipneumoniae* itself in other caprinae hosts, little work has explored the burden of *M. ovipneumoniae* on domestic sheep production within the United States.

We describe the distribution of *M. ovipneumoniae* in domestic sheep operations throughout the United States using data acquired through the U. S. Department of Agriculture National Animal Health Monitoring System. Our inquiry is built around two questions. First, can we identify specific management practices associated with *M. ovipneumoniae* infection that would allow us to predict — and potentially reduce — infection status and prevalence? Second, what are the consequences of *M. ovipneumoniae* infection on specific production and aggregate productivity for United States domestic sheep operations?

2. Materials and methods

2.1. Data collection and laboratory analyses

2.1.1. USDA national animal health monitoring system (NAHMS) data

Data were collected through the USDA-APHIS-VS National Animal Health Monitoring System (NAHMS) on domestic sheep production conducted in 2011 (Fig. 1). Like most NAHMS studies (e.g., Lombard et al., 2013; Stromberg et al., 2015), the survey consisted of two stages. First, premises were selected from a sampling frame of all domestic sheep operations (those with one or more sheep on hand on January 1, 2010; USDA-APHIS, 2012) in 22 states accounting for 85.5% of United States domestic sheep production (USDA-APHIS, 2012). A sample of 4920 operations, stratified by state and operation size, was drawn from this frame. Participants completed a General Survey ("GS"; Phase I) questionnaire between January 1 st and February 11th, 2011 (USDA-APHIS, 2012), which covered a range of issues relevant to domestic sheep production. As in other NAHMS surveys (Lombard et al., 2013; Stromberg et al., 2015), the 3539 Phase I operations with 20 or more ewes (71.9% of all GS operations) were then invited to participate in a second site visit ("SV"; Phase II) phase of the study, which included sample collection. One thousand, two hundred and forty-one (35.1%) of the GS operations with 20 or more animals agreed to participate in the site visit portion of the survey (USDA-APHIS, 2014b pg. 145), of which 761 (61.3%) actually contributed complete information (USDA-APHIS, 2014b pg. 150; Fig. 1). Of those, 453 operations (59.5%) also contributed the biological samples forming the basis for the analysis presented here.

2.1.2. Collecting samples from domestic sheep operations

At each participating operation, the Veterinary Medicine Officer conducting the Phase II site visit obtained nasal swab samples from 16 ewes (Fig. 1). This sample size was chosen by NAHMS to balance prespecified precision estimates for detecting *M. ovipneumoniae* presence (not prevalence) given estimated flock and animal-level prevalence against necessary budget constraints. A single swab (BBL CultureSwab EZ #220144) was used to swipe both nares from each sampled animal, and was then placed in mycoplasma transport media. All 16 nasal swabs from each operation were shipped to the Washington Animal Disease Diagnostic Laboratory (WADDL). Blood samples (10 ml) were obtained by jugular venipuncture from the same 16 sampled ewes, collected into red top (clot) tubes, and shipped to the National Veterinary Services Laboratory, where serum was extracted and aliquoted. One set of serum aliquots was then shipped to WADDL for testing.

2.1.3. Detecting Mycoplasma ovipneumoniae by PCR

At WADDL, nasal swabs were placed in mycoplasma broth media (R102, Mycoplasma Broth, Hardy Labs, Santa Maria CA) and incubated at 35C for 72–96 h. Following incubation, aliquots of post-enrichment broths from the 16 ewes were pooled into a single operation-level sample. DNA was extracted from the pooled sample (MagMAX, ThermoFisher Scientific, Waltham MA) and tested using PCR for *M. ovipneumoniae* (Ziegler et al., 2014). Operations with pooled samples producing cycle threshold (CT) values of 34 or less were classified as "infected". If pooled samples produced CT values < 34, individual nasal swab enrichment tube samples were tested. Operations in which all pooled and individual nasal swab enrichment tube samples produced CT values > 34 were classified as "not infected" (Fig. 1).

To determine operation-level prevalence of *M. ovipneumoniae* prevalence, 30 operations were randomly selected for realtime PCR testing of mycoplasma enrichment broths from ten ewes each. After initial analysis of the specimens from this project in 2011, the realtime PCR method for detection of *M. ovipneumoniae* was modified to eliminate false positive reactions associated with the presence of an unidentified *Mycoplasma*-like organism. To ensure consistency in diagnostic methods, this modified realtime PCR method was used to retest all available DNA extracts in which *M. ovipneumoniae* had originally been detected. The modified version of the WADDL realtime PCR included 1) use of a new primer (226Fnew 5′-GGGGTGCGCAACATTAGTTAGTTG GTAG-3′) to replace the previously described forward primer (Ziegler et al., 2014), 2) modified master mix to include bovine serum albumin (final concentration, 400 ng/ml), and 3) modified thermocycling conditions including Stage 1: 50C hold for 2 min followed by 95C hold for 10 min; optics off; Stage 2: 40 repeat cycles of denaturation (95 C, 15 s, optics off) and annealing/extension (66 C, 60 s, optics on). The results of the modified realtime PCR were interpreted as follows: 'detected' if the cycle threshold score (CT) was 36 or lower, 'indeterminate' for CTs between 36 and 40, and 'not detected' for a CT of 40.

2.1.4. Verifying accuracy of M. ovipneumoniae detection

To ensure consistency of *M. ovipneumoniae* detection, the modified WADDL realtime PCR described previously was also used to re-test specimens from all available operations classified as *M. ovipneumoniae*-positive based on the WADDL 2011 test method. Of the 401 operations classified as *M. ovipneumoniae*-positive based on the 2011 test, DNA specimens were available for re-testing from 372 operations.

Both the original and the modified methods were also used for determining sequences of multiple loci for comparison with reference strain data (Cassirer et al., 2017). In addition, for some samples a different partial 16S rDNA target sequence was used, amplified by the primers 226Fnew and 1025Rnew (5′- ATCTCGTTAGCCTCCGCTATA TCT -3′) with the thermocycling conditions Stage 1: 95C hold for 5 min; Stage 2: 45 cycles of denaturation (95 C, 30 s), annealing (66.5C, 30 s) and extension (72 C, 60 s); and State 3: 72C hold for 10 min. Sequence confirmation was sought from DNA extracts from the pooled operation-level enrichment broths from a randomly selected subset of 62 *M. ovipneumoniae*-infected operations. Sequences obtained were used to search GenBank using the Basic Local Alignment Search Tool (BLAST) to detect the most similar database entries. In addition, DNA sequence confirmation was applied to DNA extracts (N = 180), which included all individual ewe samples from the 30 randomly selected operations in which *M. ovipneumoniae* was detected by the modified WADDL realtime PCR method. DNA sequences resulting from these efforts have been

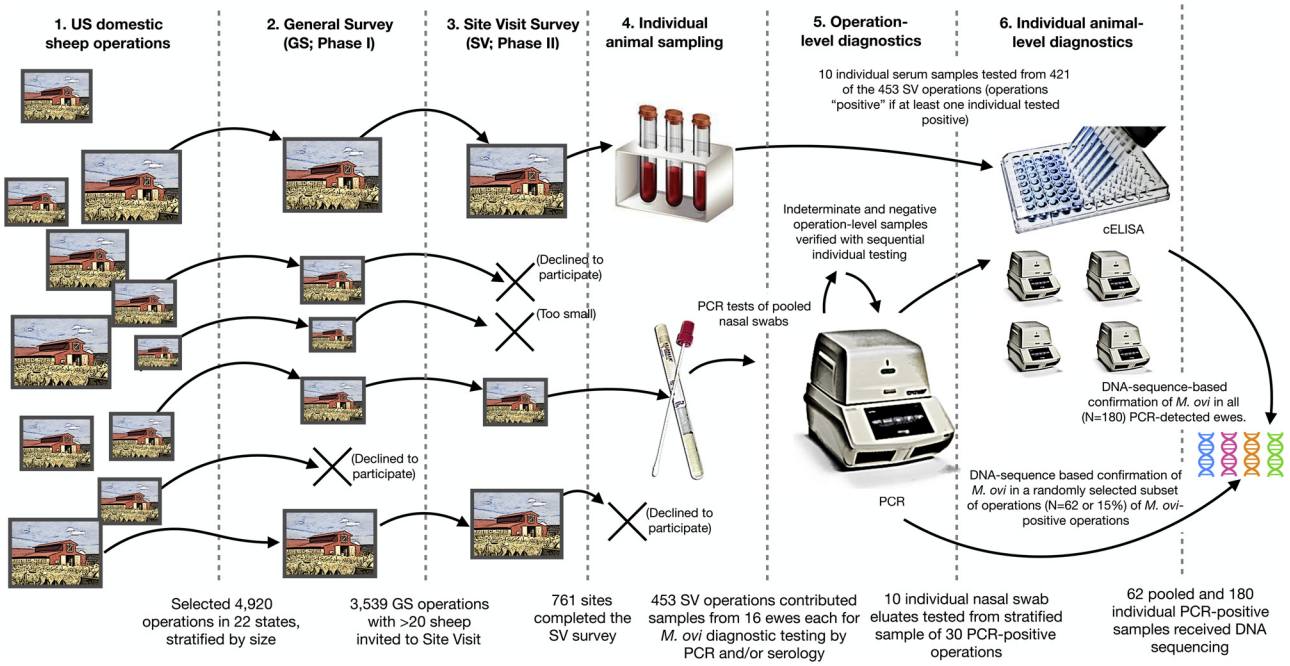

Fig. 1. Sampling and data development. Operations were identified prior to Phase I of the NAHMS Sheep-2011 survey, and a size-stratified sample of 4920 operations were invited to participate in the General Survey Phase I. A subset of General Survey Phase I operations elected to participate in the Site Visit (Phase II) portion. Of that subset, a smaller group (761) completed the survey. Of these, 453 participated in the *Mycoplasma ovipneumoniae* portion of the study. Sixteen animals at each of those 453 operations contributed nasal swabs and venous blood samples. Those samples were developed individually, and then pooled and tested by PCR and cELISA. When an operation's pooled PCR result was indeterminate or negative, individual samples from that operation were tested sequentially until an animal tested PCR-positive, or all tests were queried. Ten animals each from a structured subsample of 30 operations that tested PCR-positive on the pooled test were then tested at the individual level to establish operation-level prevalence estimates. A random subset of 62 pooled operation samples, and all 180 individual animal samples that tested PCR-positive in the prevalence estimation were then sequence-confirmed as being *M. ovipneumoniae*-positive.

deposited in GenBank (MH042304-MH042516; MH045511-MH045514; MH087248-MH087420; MH107389-MH107763).

2.2. Data analysis

2.2.1. Response variables

The analysis was built around two separate blocks of response variables. In the first block, *M. ovipneumoniae* was treated as a response, and we analyzed *M. ovipneumoniae* presence (0 or 1, based on the pooled operation-level samples) and, given a positive operation, prevalence (proportion of 10 ewe samples that tested PCR-positive, for the subset of operations receiving follow-up testing) as a function of a set of potential risk factors. In the second block, *M. ovipneumoniae* was treated as a predictor, and we explored how ewe survival and productivity – measured by number of pregnant ewes lambing at full term, number of lambs born per ewes bred, number of birthed lambs surviving to weaning, lamb weight at weaning, and number of sheep dying from respiratory disease — varied according to *M. ovipneumoniae* presence and prevalence.

2.2.2. Potential predictors of M. ovipneumoniae infection

We considered five risk factors that we hypothesized could influence a flock's likelihood of being infected with *M. ovipneumoniae*, and might affect *M. ovipneumoniae* prevalence within infected flocks. The risk factors were 1) operation size; 2) primary management type; 3) an index for operation biosecurity (ranging -1 to 10); 4) an index for overall disease burden (ranging 0 to 11); and 5) an index for operation-level antibiotic use practices (ranging 0 to 5). Primary management types were "fenced" operations where animals were contained in any fenced area not specifically cultivated to raise forage or browse; "herded" operations where animals were maintained on *any* unfenced acreage; or "pastured" operations where animals grazed fenced areas specifically cultivated to raise forage or browse (USDA-APHIS, 2012). Data from 35 dry lot and feedlot operations that contributed biological samples through the Site Visit portion of the study but that did not allow grazing were excluded due to limited occurrence in our dataset. The three index variables were created by aggregating each operation's responses across a series of General Survey and Site Visit questions. Contributing survey questions are listed in Table S1, and univariate distributions of each index are shown along the main diagonal of Figure S1.

Regions varied substantially in management type and operation size (Fig. 2), which generated confounding between management type and region. In particular, operations that pastured their animals were disproportionately located in the East region, whereas operations that used fenced or herded management practices were disproportionately located in the Central region (Fig. 2). Since there is no clear mechanism for regional difference in *M. ovipneumoniae* persistence outside the host (*M. ovipneumoniae*'s environmental persistence is thought to be uniformly poor due to its lack of a cell wall, Razin and Herrmann, 2002), and in an effort to focus our analyses and protect participant privacy, we chose to omit Region throughout the remainder of this investigation.

We first evaluated univariate relationships between each risk factor and *M. ovipneumoniae* presence across all fenced, pastured, and herded operations contributing biological samples to NAHMS Sheep 2011 Phase II using Wilcoxon rank-sum tests, and then we fit a logistic regression model in which operations were assigned a one if they were *M. ovipneumoniae*-positive, and a zero otherwise. All covariates were included as additive effects, since a subset of models examining covariate interactions showed limited, or no improvement in model performance on the basis of AIC (all models with interactions were > 2 AIC points above the model with only additive terms). Covariates were not substantially collinear with one another (absolute value of all correlations < 0.4 throughout; see Figure S1), so we included them all in subsequent models. An identical model with a binomial residual structure with n = 10 (number of animals individually tested by PCR in

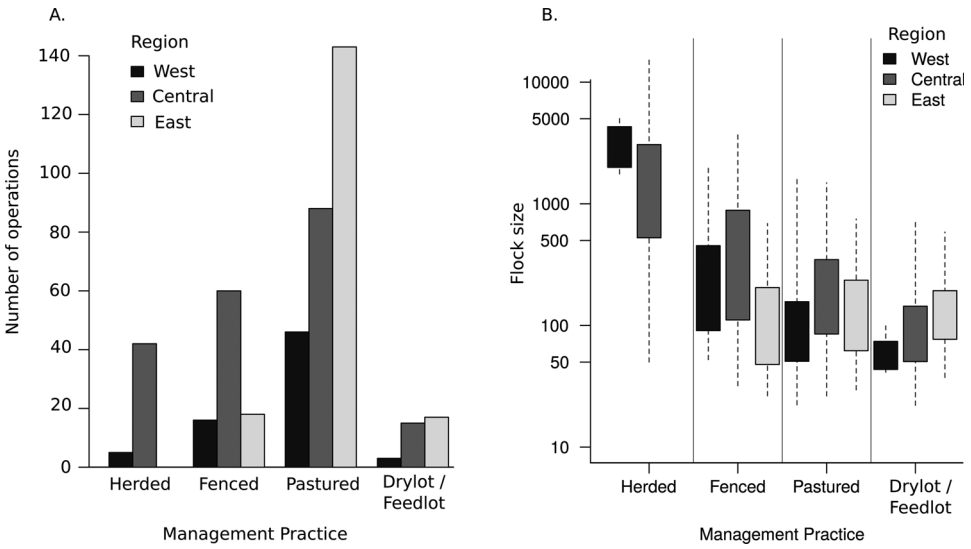

Fig. 2. Sheep management styles. (a) Distribution of region-level flock management practices across 453 operations that contributed biological samples during the Site Visit portion of the NAHMS Sheep-2011 survey. (b) Logged herd size across four different herd management practices, and split out by region. Boxes capture the middle 50% of operations in each group; lines extend to the 2.5th and 97.5th quantile in each group.

each operation) was used to characterize associations between the same set of risk factors and *M. ovipneumoniae* prevalence on the subset of 62 size-stratified operations subject to individual-level testing (see Detecting *Mycoplasma ovipneumoniae* by PCR).

2.2.3. Associations between vital rates and M. ovipneumoniae infection status

We examined the association of operation-level *M. ovipneumoniae* presence (zero for no *M. ovipneumoniae* detection) and prevalence in positive operations (number of individuals testing positive out of 10) with seven response variables linked to productivity and animal health. Response variables were full-term births among pregnant ewes, number of lambs born per bred ewe, lambs born that survived to weaning, average lamb weight at weaning, number of ewes culled, and number of ewes and lambs that died from respiratory disease. We built one set of models in which *M. ovipneumoniae* presence or absence was a predictor (fit to all operations with complete biological samples), and a second set in which *M. ovipneumoniae* prevalence was a predictor (fit to prevalence data from the stratified set of 62 operations where individual-level prevalences were established). All models also contained management type and operation size covariates to account for vital rate differences among production styles. We checked for correlations among transformed response variables, and they were negligible following transformation (Figure S4).

The proportion of pregnant ewes that deliver lambs at full term was treated as a binomial variable. The number of lambs born per bred ewe was treated as Poisson, with total number of lambs born. Lamb survival to weaning was treated as a binomial response variable. Lamb weight at weaning was modeled as Gaussian with an identity link; that model also incorporated average age at weaning, and an indicator variable of whether lambs were fed high-energy diets. We also examined the number of ewe, pre-weaning lamb, and weaned lamb deaths due to respiratory disease. These were treated as negative binomial variables to account for over-dispersion in the raw counts (many operations had no respiratory deaths in any age group). The negative binomial models also included total number of sheep on the operation as offset terms.

Additional descriptions of response variables, formal statements of all models, and specific model fits, are included in the Supplementary Materials.

2.2.4. Statistical analyses

All statistical analyses were conducted in R (R Core Development Team, 2017). Linear and most of the generalized linear models were fit using the bayesglm function in the arm package (Gelman and Su, 2016) to circumvent any issues associated with complete separation – the situation in which a covariate completely splits a response, such that all positive (or negative) responses occur under a single level of the covariate. This might occur, for instance, if all operations of a particular management type tested PCR-positive for *M. ovipneumoniae*. bayesglm is a simple augmentation of the usual iteratively reweighted least squares that is classically used to fit generalized linear models. It works by first incorporating a small data augmentation to adjust for complete separation, and then running a step of iteratively weighted least squares and a step of expectation-maximization algorithm to estimate coefficient variances (Gelman et al., 2008). While inference under bayesglmis fundamentally Bayesian, it is structured to analogize cleanly with iteratively reweighted least squares; thus our inferences are presented as a hybrid of Bayesian and frequentist norms. Student's T-distributions with 2.5 degrees of freedom (or 10 degrees of freedom in the case of the intercept) was used as a prior for all model coefficients with expectation-maximization updates.

To model over-dispersed count variables (e.g., count variables for which there are more zeroes than expected), negative binomial regression models were fit using the bayes_glm function in the rstanarm package (Stan Development Team, 2016). Negative binomial models are typically chosen as alternative models for over-dispersed data because they naturally account for the extra-Poisson variability via an additional variance parameter (Lawless, 1987; Ridout et al., 1998). The bayes_glm function fits Bayesian generalized linear regression models in the Stan programming language (Carpenter et al., 2017), which uses adaptive Hamiltonian Monte Carlo sampling to simulate draws from the posterior distribution. These are fully Bayesian models, unlike those fit by the bayesglm function and thus do not generate p-values. Instead, posterior credible intervals are used to make inference about predictor variables of interest. Again, Student's T-distributions with 2.5 degrees of freedom (or 10 degrees of freedom in the case of the intercept) was used as the prior distributions for all model coefficients.

During a preliminary investigation, we relied on AIC-based model selection to identify best-performing models, and considered models with all possible combinations of covariates in the model suites. This exploration indicated that models including additive effects of operation size and management nearly always performed best, and we used that covariate structure as the basis for all models presented here. We report McFadden's pseudo R-squared values as a coarse metric of performance on logistic regression model fits. Though widely used, pseudo R-squared values are not identical to conventional R-squared values available through ordinary least squares. Pseudo R-squared values are often lower than conventional R-squared values, and in general, values

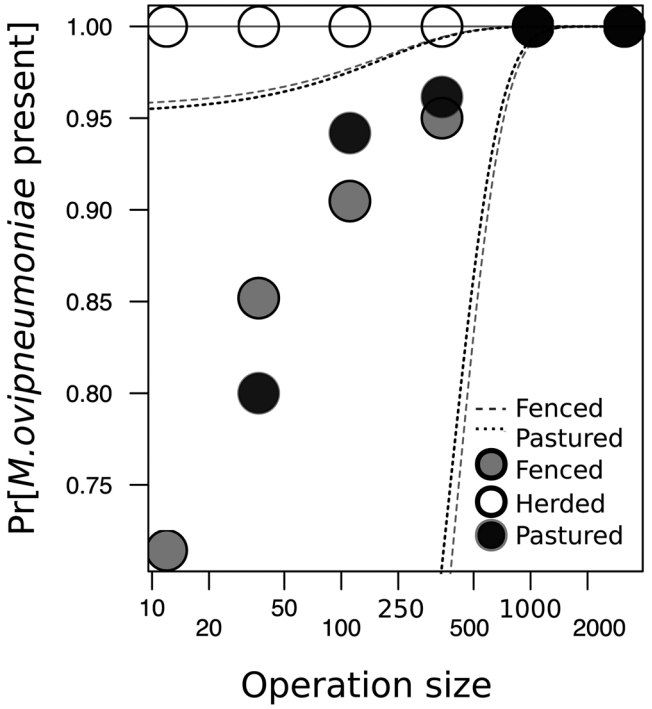

Fig. 3. *Mycoplasma ovipneumoniae* risk factors. Points show empirical proportions of operations containing PCR-detectable *M. ovipneumoniae* infection, grouped by operation size and management type. Shaded regions show 95% credible intervals for the probability of *M. ovipneumoniae* detection in an operation, in a model that also accounted for operation size and management type. The dotted interval is for Pastured operations; the dashed interval is for Fenced (intervals overlap extensively). No interval could be calculated for operations with herded management, since all herded operations in this study showed active *M. ovipneumoniae* infections.

of 0.2 - 0.4 are considered "excellent fits" (McFadden, 1973).

3. Results

3.1. Data overview and structure of sampled operations

A total of 453 operations voluntarily contributed biological samples for *M. ovipneumoniae* testing as part of the Sheep 2011 study. Ninety-two percent (418) of those operations were under fenced, pastured, or herded management practices, and of those 418, 10 contributed swabs but not serum samples. A cross-tabulation of operations where *M. ovipneumoniae* was detected via PCR, and where antibodies to *M. ovipneumoniae* were detected via cELISA, is shown in Table S2, along with a full description of cELISA methods.

Operation size and production type varied by region. Herding occurred in the Central and Western regions of United States, but not in the East. Operations using herded management systems were on average much larger (median = 1750 animals; 95% of operations between 50 and 15,200 animals) than operations using pastured (median = 128 animals; 95% of operations between 26 and 1444 animals) or fenced (median = 195 animals; 95% of operations between 31 and 2867 animals) management. Although only 3.1% of operations used herded or open-range management, those operations accounted for 26.1% of ewes on operations with 20 or more animals (USDA-APHIS, 2012). A comparison of the NAHMS Site Visit sites with data from all operations surveyed during the Sheep 2011 NAHMS General Survey suggested that Site Visit sites provided a reasonable cross-section of production sizes and management strategies used across all surveyed sites.

3.2. Reliability of diagnostic tests

M. ovipneumoniae was detected by the modified realtime PCR method in 369 of the 372 available specimens. Of the three discrepant samples, one produced 'indeterminate' results by the modified realtime PCR, while *M. ovipneumoniae* was not detected in the other two specimens by either the original or the modified realtime PCRs, suggesting that the DNA template had degraded during storage. Overall, the modified realtime PCR method detected *M. ovipneumoniae* in 369 of 370 (99.7%) of the available, template-intact specimens. We also used DNA sequencing to evaluate the accuracy of the realtime PCR methods using DNA extracts obtained from 1) 62 (15%) randomly selected operations classified as *M. ovipneumoniae*-infected, and 2) all enrichment broth specimens (N = 180) in which *M. ovipneumoniae* was detected while estimating individual animal prevalence. All 62 randomly selected, pooled (operation-level) enrichment broths and all 180 individual ewe enrichment broths (composed of all samples in which *M. ovipneumoniae* was detected after 10 samples each were tested from 30 randomly selected operations) produced DNA sequences highly similar to *M. ovipneumoniae* GenBank entries. Together, these findings provide strong support for the accuracy of identification of *M. ovipneumoniae*-infected operations and individuals, and therefore, all results found to be positive by the original WADDL PCR test are defined as positive for these analyses. False negative misclassification of operations is another potential problem, and the limited numbers of ewes sampled per operation (N = 16) predicts that there is a 5% or larger risk of false-negative misclassification of operations with an individual animal prevalence of 0.17 or lower (binary distribution). This risk of false negative classification may be the explanation for some or all of the 36 operations classified as *M. ovipneumoniae*-negative by PCR, but in which *M. ovipneumoniae* cELISA-reactive ewes were detected (Table S2).

3.3. M. ovipneumoniae presence and prevalence in surveyed operations

Of the operations contributing biological samples, 88.5% (401/453; 95% binomial confidence interval [85.2%, 91.3%]) tested PCR-positive for *M. ovipneumoniae*, and 85.3% (348/408; 95% confidence interval [81.5%, 88.6%]) showed signs of exposure to *M. ovipneumoniae* under the cELISA antibody assessment. *M. ovipneumoniae* prevalence varied substantially among the 62 size-stratified operations selected for individual-level testing (Figure S2). The median prevalence among those operations was 60%, though that figure should not be regarded as widely representative, given its basis on a stratified sample intended to cover a range of covariate values.

3.4. M. ovipneumoniae-associated risk factors

All 47 herded operations tested PCR-positive for *M. ovipneumoniae,* as did 237 of 277 (86%) pastured operations, and 83 of 94 (88%) fenced operations. The median operation size of *M. ovipneumoniae*-positive operations was significantly larger (median size = 171 ewes) than negative operations (median size = 70 ewes, Wilcoxon p-value < 0.0001; Fig. 3).

Biosecurity risk scores were marginally significantly higher in operations testing PCR-positive for *M. ovipneumoniae* than in operations testing negative (W = 1503.5, p-value = 0.069). There was evidence that PCR-positive operations were also subject to higher overall disease burden than negative operations (Wilcoxon rank-sum test W = 7708.5, p-value = 0.013). We found no significant association between antibiotic use and *M. ovipneumoniae* presence (Wilcoxon rank-sum test W = 307.5, p-value = 0.599).

In a logistic regression of *M. ovipneumoniae* presence as a function of the five postulated risk factors, only operation size and contact score retained their significance, and contact score only marginally so (Table 1).

The best explanatory model of *M. ovipneumoniae* prevalence within

Table 1
USDA NAHMS Sheep 2011 survey coefficient estimates from a model of *M. ovipneumoniae* presence as a function of operation size, management type (baseline = Fenced), contact score, disease burden score, and antibiotic use score. This model was fit using the 408 operations that contributed biological samples, and where management was herded, pastured, or fenced.

Variable	Coefficient estimate	Standard error	Test statistic (P-value)
(Intercept)	0.754	0.406	1.855 (0.064)
management = Herded	1.768	1.469	1.203 (0.229)
management = Pastured	0.008	0.549	−0.515 (0.607)
operation size	0.005	0.002	2.819 (0.005)
contact score	0.072	0.062	1.722 (0.085)
disease burden score	−0.004	0.120	−0.029 (0.977)
antibiotic use score	0.379	0.295	1.281 (0.200)

an operation for the 62 PCR-positive, individually sampled operations included only a single predictor, management type (AIC = 27.5). Models that also included antibiotic use score (AIC = 28.4), operation size (AIC = 28.6), and disease burden score (AIC = 28.9) in addition to management type were competitive with management type alone, but these multivariate models were not selected because the individual effects were not significant ($p > 0.05$ delta-AIC > 2, Table S3).

3.5. Associations between animal health measures and M. ovipneumoniae presence

M. ovipneumoniae-infected operations often exhibited significantly less-productive vital rates than comparable operations without *M. ovipneumoniae*, after accounting for differences in management type and operation size (Fig. 4). Birth rates among pregnant ewes were lower in *M. ovipneumoniae*-positive operations than in *M. ovipneumoniae*-negative operations (posterior mode for $e^{\beta} = 0.744$, 95% credible interval= [0.646, 0.870]; pseudo R-squared = 0.142, Table S4). Number of lambs born per pregnant ewe was higher in *M. ovipneumoniae*-positive operations than in *M. ovipneumoniae*-negative operations (posterior mode for $e^{\beta} = 1.055$, 95% credible interval = [1.024, 1.088]; pseudo R-squared = 0.082, Table S5), but *M. ovipneumoniae*-positive operations also had a higher proportion of lambs born dead than *M. ovipneumoniae*-negative operations (median proportion of full-term lambs born dead = 0.052 in *M. ovipneumoniae*-positive operations vs. 0.037 in *M. ovipneumoniae*-negative operations). Lamb survival to weaning was also lower, though not significantly so, in the presence of *M. ovipneumoniae* (posterior mode for $e^{\beta} = 0.922$, 95% credible interval = [0.833, 1.020]; pseudo R-squared = 0.171, Table S6). Overall ewe culling rates were higher in the presence of *M. ovipneumoniae* than in *M. ovipneumoniae*'s absence (posterior mode for $e^{\beta} = 1.178$, 95% credible interval = [1.036, 1.339]; pseudo R-squared = 0.147, Table S7). *M. ovipneumoniae* was associated with marginally higher lamb weaning weights (posterior mode for $\beta = 4.890$, 95% credible interval = [-0.953, 10. 733], $p = 0.102$, Table S8, though this effect was not statistically significant at a 0.05 significance level, and may be due to unmodeled differences in twinning rates between *M. ovipneumoniae*-positive and -negative operations.

The data analyzed here did not provide conclusive evidence that culling rates increased for ewes, pre-weaning lambs, or weaned lambs at *M. ovipneumoniae*-positive operations over rates at *M. ovipneumoniae*-negative operations, after accounting for management type and operation size (posterior mode for $e^{\beta} = 4.903$, 95% credible interval = [0.917, 30.204], Table S9; pre-weaning lambs' posterior mode for $e^{\beta} = 4.333$, 95% credible interval = [0.720, 23.313], Table S10; weaned lambs' posterior mode for $e^{\beta} = 1.907$, 95% credible interval = [0.474, 6.615], Table S11).

3.6. Associations between vital rates and M. ovipneumoniae prevalence

WADDL and TEB's laboratory conducted additional testing on a subsample of 62 *M. ovipneumoniae*-positive operations to determine individual ewe prevalence. Higher prevalence (number of individuals testing PCR-positive for *M. ovipneumoniae* out of ten) was associated with lower full-term birth rates among pregnant ewes (posterior mode for $e^{\beta} = 0.709$, 95% credible interval = [0.572, 0.878], $p = 0.002$, pseudo R-squared = 0.877, Table S12) in models that also accounted for operation size and management type. Increased prevalence was associated with a decreased number of lambs born per ewe (posterior mode for $e^{\beta} = 0.758$, 95% credible interval = [0.726, 0.792], $p < 0.0001$, Table S13). Increased *M. ovipneumoniae* prevalence was associated with lower lamb survival to weaning among lambs born alive (posterior mode for $e^{\beta} = 0.455$, 95% credible interval = [0.391, 0.530], $p < 0.0001$, pseudo R-squared = 0.159, Table S14). Ewe culling rates increased significantly with increasing *M. ovipneumoniae* prevalence (posterior mode for $e^{\beta} = 1.722$, 95% credible interval = [1.484, 1.998], $p < 0.0001$, pseudo R-squared = 0.092, Table S15). There was no relationship between lamb weight at weaning and *M. ovipneumoniae* prevalence (posterior mode for $\beta = 0.784$; 95% credible interval = [-0.532, 2.101], $p = 0.908$, Table S16). Predicted changes in vital rates in response to the changes in *M. ovipneumoniae* prevalence reported in Table 2 are based on these models.

3.6.1. Consequences of M. ovipneumoniae infection on operation productivity

To evaluate the association between infection and production, we compared two median-sized operations of 155 ewes each, one infected with *M. ovipneumoniae* and one uninfected. Of those ewes, we would expect 10.2% (15.8) to be culled in the *M. ovipneumoniae*-positive operation, and 9.0% (14.0) to be culled in the *M. ovipneumoniae*-negative

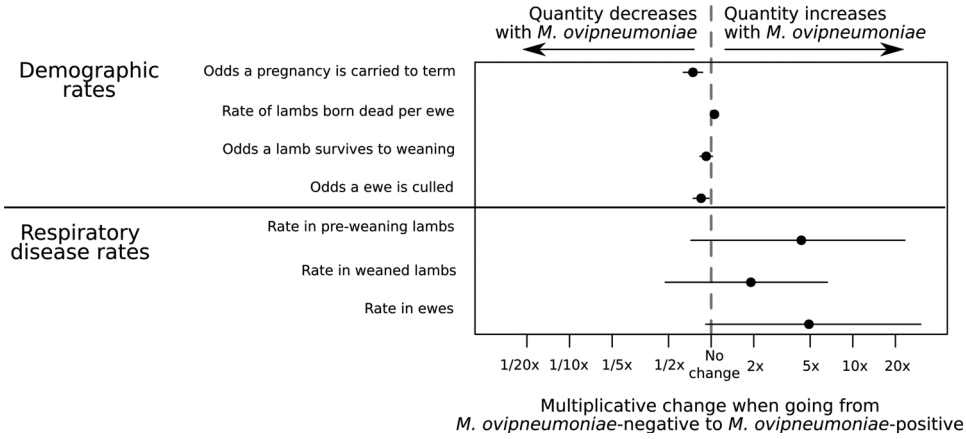

Fig. 4. Associations between *Mycoplasma ovipneumoniae* presence and production rates. Estimated changes in vital rates and rates of reported respiratory disease incidence in operations with PCR-detectable *M. ovipneumoniae*, relative to uninfected operations. The x-axis shows the multiplicative change in each rate in the presence of *M. ovipneumoniae*, relative to the rate in the absence of *M. ovipneumoniae*. For instance, an interval extending from 1.2 to 2.6 would indicate that the corresponding rate is expected to be between 1.2 and 2.6 times higher in *M. ovipneumoniae*-positive flocks than in *M. ovipneumoniae*-negative flocks.

Table 2
USDA NAHMS Sheep 2011 survey vital rate estimates in the presence and absence of *M. ovipneumoniae*, and estimated effects of increasing prevalence. Median vital rate estimates are based on the entire dataset. Estimated effects of increasing prevalence from 25% to 50% are derived from prevalence models that also accounted for management type and operation size. Effect sizes are calculated for the median operation size across the entire dataset (155 animals), but are reported separately for each management type. Complete coefficient tables are reported in Tables S4-S11.

Vital rate	Median in presence of *M. ovipneumoniae*	Median in absence of *M. ovipneumoniae*	Estimated effect of increasing *M. ovipneumoniae* prevalence from 25% to 50% on vital rates under each management type			
			Quantity	Pastured	Herded	Fenced
Pregnant ewes who deliver full-term lambs	0.954	1	Reduction in probability that a pregnant ewe carries to term.	0.0045	0.0058	0.0032
Number of lambs born per bred ewe	1.60 lambs/bred ewe	1.47 lambs/bred ewe	Change in expected number of lambs born per bred ewe	0.0940	0.1143	0.0784
Lamb survival to weaning	0.933	0.957	Reduction in probability that a lamb survives to weaning	0.0090	0.0301	0.0262
Ewes culled	0.102	0.090	Increase in probability that a ewe is culled.	0.0107	0.0114	0.0145

operation, leaving 139.2 and 141.0 ewes, respectively. If all surviving ewes are bred at both operations, then the NAHMS data suggests that 141.0 (100%) of the ewes from *M. ovipneumoniae*-negative operation would deliver full-term lambs, but only 133.0 (95.4%) of the ewes from the *M. ovipneumoniae*-positive operation would do so. In the *M. ovipneumoniae*-positive operation, we would expect each bred ewe to produce 1.53 live lambs, for a total of 203.5 lambs born alive. In the *M. ovipneumoniae*-negative operation, that rate drops to 1.47 lives lambs per ewe, for an expected total of 207.3 lambs born alive. Of those lambs, we would then expect 198.4 (95.7%) to survive to weaning at the *M. ovipneumoniae*-negative operation, but only 189.9 (93.3%) to survive to weaning at the *M. ovipneumoniae*-positive operation. Therefore, in aggregate, we would anticipate a reduction of roughly 8.5 lambs (4.3%) for an *M. ovipneumoniae*-positive operation, in comparison with an *M. ovipneumoniae*-negative but otherwise identical operation. If we finally assume that lambs can be sold for $75-$150 per head, this translates to an annual *M. ovipneumoniae*-imposed cost of $637–$1275 for a median-sized flock.

4. Discussion

4.1. Conclusions

Evidence of *M. ovipneumoniae* infection was in detected over 85% of the herded, pastured, and fenced domestic sheep operations that contributed biological samples to the first comprehensive survey of *Mycoplasma ovipneumoniae* in U.S. domestic sheep operations. Prevalences varied widely among the operations that received individual-level follow-up testing, from a single infected animal out of 10 sampled to universal infection in 10 sampled individuals. *M. ovipneumoniae*'s near ubiquity across domestic sheep operations means that even if infection has very small economic consequences at the individual level, those consequences likely accumulate over large numbers of animals and operations. This potentially imposes a heavy but unrecognized disease cost on domestic sheep production in the United States that contrasts with rare-but-devastating diseases like Scrapie which have the potential to accumulate high and visible economic burdens over a relatively small number of animals and operations (e.g., Boden et al., 2012). While there are relatively few survey studies of *M. ovipneumoniae* presence and prevalence in domestic sheep, our findings are on the same order as those of McAuliffe et al. (2003). Those authors reported *M. ovipneumoniae* presence (as determined by PCR) in 8 of 11 tested flocks in the United Kingdom. In their case, however, seven of those flocks had a reported history of respiratory disease, and it is not entirely clear if flocks were chosen for testing on the basis of that history. Akwuobu et al. (2014) reported 25 *M. ovipneumoniae* detections among 172 sample sheep from small ruminant operations in Nigeria, and Amin et al. (2013) found a prevalence of 16.5% across 1047 samples from 139 flocks in Baluchistan, Pakistan. These individual-level prevalences are lower than those from our stratified sample, but production conditions are likely to be quite different.

Our estimate of *M. ovipneumoniae*'s burden on domestic sheep operations is subject to a number of sources of variation. In particular, we used vital rate estimates at the population level to estimate disease effects that actually operate at the individual level. Therefore, our individual-level burden estimates may be biased low, and are subject to additional variation associated with flock-level prevalences. *M. ovipneumoniae*'s burden was distributed across a variety of vital rates, as is true for other chronic ovine diseases like Johne's disease (Morris et al., 2006). While the social costs of *M. ovipneumoniae* are likely much lower than those of diseases with zoonotic potential, *M. ovipneumoniae* is nevertheless associated with substantial costs for production. Our estimate of *M. ovipneumoniae* burden may also be somewhat confounded by the overall higher disease burden exhibited by *M. ovipneumoniae*-positive operations. Further work is needed to separate *M. ovipneumoniae* effects from those of other pathogens. Nevertheless, losses of this

magnitude may merit additional inquiry into management practices that could mitigate *M. ovipneumoniae* presence and prevalence *per se*. The ubiquity of *M. ovipneumoniae* infection in domestic sheep poses a major challenge for control. Exploration into novel approaches that might be more widely applicable and cost-effective is a worthy avenue for future work.

We were surprised by the positive effect of *M. ovipneumoniae* presence on the number of lambs born per ewe. Our analysis showed apparently divergent effects: while *M. ovipneumoniae* presence (binary) was associated with more lambs born per bred ewe, increased *M. ovipneumoniae* prevalence (continuous, and only among *M. ovipneumoniae*-positive operations) was associated with fewer lambs born per bred ewe. It is possible that some unmeasured factor associated with more lambs born per ewe was more common among *M. ovipneumoniae*-positive operations (potentially due to divergent management practices in positive and negative operations). When prevalence (only measured among infected operations) was considered, however, the coarse factor separating positive and negative operations may have been removed, allowing the weaker, yet still present *M. ovipneumoniae*-related vital rate shift to emerge. One plausible explanatory factor is mode of breeding. Smaller operations, which were also more likely to be *M. ovipneumoniae*-negative, may have used less reliable methods to determine how many of their ewes were bred than those used by larger operations. If small operators tended to over-estimate their numbers of “bred" ewes relative to large operators, that would inflate their denominator values in estimated number of lambs born per bred ewes, producing the apparently lower multiple birth rate reported here. Regardless of whether this is in fact the case, all vital rates presented here, and especially the multiple birth rate estimate, should be the topic of future investigation.

We saw significantly higher weaning weights in flocks that had any *M. ovipneumoniae* presence than in flocks where no *M. ovipneumoniae* was detected (i.e., a categorical variable where 1 = *M. ovipneumoniae*-positive and 0 = *M. ovipneumoniae*-negative), though this effect might be partially explained by differences in twinning rates between *M. ovipneumoniae*-positive and *M. ovipneumoniae*-negative operations. When we limited our inquiry to just *M. ovipneumoniae*-positive flocks, we saw no effect of prevalence on weaning weights. The direction of the effect was still positive (which is to say, higher prevalence was associated with higher weaning weight), but the standard error on that estimate was high enough that the estimated effect could have easily been due to sampling variation.

Operation size and management type were strongly related in the NAHMS dataset. We suspect that this relationship did not have a strong deleterious effect on our model results, however, since standard error estimates associated with size and operation effects did not change dramatically when models included only one or both variables as predictors. We present results from models containing both risk factors here, with the understanding that in practice, some operation sizes are only feasible under certain management types.

4.2. Project strengths and limitations

4.2.1. Scope of inference

The NAHMS data presented here represent health patterns in operations with 20 or more ewes as of 2011, included in one of the 22 sampled states (USDA-APHIS, 2014b pg. 145). These are the strongest data available for studying prevalence of under-reported pathogens in domestic sheep throughout the United States, since invitation to participate in the NAHMS survey is determined through a rigorously designed, state-of-the-art sampling protocol. However, any sampling protocol that incorporates some element of participant choice (in this case, the invitees' ability to decline the invitation to participate) can potentially depart from its intended stratification. As a consequence, and to avoid compounding even small biases due to participant choice, the sample data were not weighted back to the population totals in the present analysis.

Although our results are correlative in nature, our findings nonetheless suggest that *M. ovipneumoniae* may pose substantial yet underrecognized costs for domestic sheep production in the United States. Additional efforts to limit *M. ovipneumoniae* infection in domestic sheep operations could be of financial benefit to operators, and cost-effective methods to reduce infection burden should be explored.

4.2.2. Confounding with other respiratory diseases, and with region

The relationship between *M. ovipneumoniae* presence and vital rates and disease burden may be somewhat confounded by the generally higher overall disease burden exhibited by *M. ovipneumoniae*-positive operations. Further work is needed to separate *M. ovipneumoniae* effects from those of other pathogens, in particular ovine progressive pneumonia. Nevertheless, losses of this magnitude merit some additional inquiry about management practices that could mitigate *M. ovipneumoniae* presence and prevalence, along with those of other respiratory pathogens.

M. ovipneumoniae diagnostic testing continues to be an area of active refinement. The method initially used to test these samples is now known to cross-react on occasion with another *Mycoplasma*-like organism, but the re-testing we conducted using a modified test that eliminates that cross-reaction, together with the sequence confirmation of 180 test-positive samples from 30 randomly selected operations, confirms the lack of significant cross-reaction in the original testing for this study. Nevertheless, there is some possibility that additional refinement or recategorization of *Mycoplasma* spp. might produce different testing results in the future.

We omitted region information from our analysis, and this may have some consequences on our inferences. Region was confounded with management type and operation size, which were important predictors of *M. ovipneumoniae* prevalence and presence. We could not separate region effects from management type effects within this dataset, so here we attribute the *M. ovipneumoniae* risk to management type. However, geographic effects should be addressed explicitly in follow-up work, if possible.

4.2.3. Within-operation sampling

We had limited information on how individual animals were selected for sampling within a flock. If *M. ovipneumoniae* transmission occurs predominantly within groups, and all sampled individuals were from the same group, then there might be a lack of independence among animals sampled within a particular operation. This correlation is unlikely to have introduced substantial bias into inferences based on *M. ovipneumoniae* presence, but could have produced biased results related to prevalence. In an effort to limit the role of that potential bias here, we chose not to emphasize overall prevalence rates, and focused instead on relationships between prevalence and various vital rates. Nevertheless, clustering should be explored in future investigations.

An additional issue associated with sampling is sample size within operations. These small sample sizes limited the precision of estimated relationships between *M. ovipneumoniae* prevalence and vital rates. For this reason, we primarily focused on the direction of estimated effects, and based our cost projections on an aggregate across all estimated vital rate relationships. Larger sample sizes from a carefully chosen set of focal operations would add valuable clarity to both the prevalence-vital rate relationships, and the cost projections.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prevetmed.2019.04.006.

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