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Abstract

Infectious respiratory diseases are a serious health concern worldwide. However, few models describe the experimental induction of lung infection, or the effect of experimental infection on clinical pathologic parameters in goats. Goats offer benefits compared to cattle because of size and tractability and compared to sheep with regard to specific features of their anatomy. In previous experimental models of infection in goats, coadministration of an immunosuppressive dose of a corticosteroid is common; however, protocols that use corticosteroid often note mortality as an adverse effect. We therefore investigated an infection protocol that did not use immunosuppression but instead relied on 2 intratracheal inoculations of *Pasteurella multocida* in healthy meat goats to induce clinical and hematologic changes associated with respiratory infection. Healthy Boer or Boer-Kiko cross goats ($n = 6$; age, 10 mo) were inoculated with *Pasteurella multocida* and were monitored over a 312-h period for clinical and hematologic parameters of infection. After induction of pneumonia, the goats had a significant 1.2 °C rise in rectal temperature and auscultatable rales for up to 96 h. Lymphocyte counts, serum amyloid A values, and respiratory scores were significantly different before and after induction of disease and were consistent with respiratory infection. No mortality was associated with this experimental infection, and minimal gross pathologic changes were noted at study termination. The clinical and pathologic findings of this study suggest a potentially reproducible method of establishing clinical respiratory infection in goats. The repeated intratracheal inoculation method of inducing caprine respiratory disease can be used to produce experimental respiratory disease in goats when the use of corticosteroid is not desirable. With the feasibility of this method established, additional research evaluating the optimal dose and frequency of *P. multocida* administration is needed.

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Original Research

Evaluation of a *Pasteurella multocida* Respiratory Disease Induction Model for Goats (*Capra aegagrus hircus*)

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Infectious respiratory diseases are a serious health concern worldwide. However, few models describe the experimental induction of lung infection, or the effect of experimental infection on clinical pathologic parameters in goats. Goats offer benefits compared to cattle because of size and tractability and compared to sheep with regard to specific features of their anatomy. In previous experimental models of infection in goats, coadministration of an immunosuppressive dose of a corticosteroid is common; however, protocols that use corticosteroid often note mortality as an adverse effect. We therefore investigated an infection protocol that did not use immunosuppression but instead relied on 2 intratracheal inoculations of *Pasteurella multocida* in healthy meat goats to induce clinical and hematologic changes associated with respiratory infection. Healthy Boer or Boer-Kiko cross goats ($n = 6$; age, 10 mo) were inoculated with *Pasteurella multocida* and were monitored over a 312-h period for clinical and hematologic parameters of infection. After induction of pneumonia, the goats had a significant 1.2 °C rise in rectal temperature and auscultatable rales for up to 96 h. Lymphocyte counts, serum amyloid A values, and respiratory scores were significantly different before and after induction of disease and were consistent with respiratory infection. No mortality was associated with this experimental infection, and minimal gross pathologic changes were noted at study termination. The clinical and pathologic findings of this study suggest a potentially reproducible method of establishing clinical respiratory infection in goats. The repeated intratracheal inoculation method of inducing caprine respiratory disease can be used to produce experimental respiratory disease in goats when the use of corticosteroid is not desirable. With the feasibility of this method established, additional research evaluating the optimal dose and frequency of *P. multocida* administration is needed.

Abbreviations: N:L: Neutrophil: Lymphocyte Ratio; SAA: Serum Amyloid A; TPP: Total Plasma Protein; WBC: White Blood Cell

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Goats, like most ruminant species, have respiratory disease as a major health concern. In the United States, the economics of bovine respiratory disease skew research priorities on cattle as opposed to small ruminants such as sheep or goats. Few models of experimental bacterial respiratory disease in goats have been described in the literature, and those that are described focus on a single intratracheal injection^{17,18} of *Pasteurella* (now *Mannheimia*) *haemolytica*,^{6,8,17,18} and intratracheal or intranasal *Pasteurella multocida*.²² Furthermore, the few models that have been described induce immunosuppression with corticosteroid administration. In addition, the previously described methods of induction of respiratory disease in goats can lead to mortality in the experimental subjects.²² This experimental mortality may be due to multiple factors, including the corticoid-induced immunosuppression.²² Reliable models of inducing respiratory disease in goats without mortality are necessary in order to reduce the number of experimental animals used for respiratory disease studies. Our group

has previously experienced difficulties in inducing pneumonia with single intratracheal inoculation of *P. multocida*. We hypothesized that multiple administrations may induce infection without the need for coadministration of a corticosteroid.

Currently, the majority of models of small ruminant respiratory disease that have been described are specific to sheep. Sheep are useful as potential respiratory disease models, as their lungs contain pulmonary intravascular macrophages (PIM), which increases their susceptibility to bacterial lung injury.¹⁵ Sheep are also easily tractable in laboratory settings, allowing for the collection of samples in unsedated or lightly sedated animals.¹⁶ Likewise, the lungs of sheep are more similar to those of humans than are rodent lungs, based on the tracheobronchial anatomy.^{13,21} These attributes are not distinct to sheep and are shared by goats, which also possess anatomic similarities to human lungs such as airway length:diameter ratio and mixed blunt and sharp airway bifurcations.^{3,19} Goats may prove to be a useful species for respiratory disease models.

In this study, we evaluated the hematologic and clinical signs of pneumonia induced by a modified intratracheal injection model using multiple administrations of *Pasteurella multocida* in meat goats. The tested hypothesis was whether repeated administration of bacteria, without the use of corticosteroid, would induce clinical indicators of infection, as well as the hematologic characteristics of infection in meat goats.

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Materials and Methods

Animals. This study was performed at the Iowa State University (ISU) Livestock Infectious Disease Isolation Facility (LIDIF). Six 8 to 10 mo old female Boer and Boer-Kiko cross goats (*Capra aegagrus hircus*) were enrolled in the study. These goats had no history of prior drug administration or of respiratory disease. They were sourced from a closed herd in State Center, Iowa by Iowa State University Laboratory Animal Resources. The herd's biosecurity protocol tests annually for Caprine Arthritis and Encephalitis virus, and the herd has maintained a negative status for approximately 10 y. Lesions suggestive of ringworm, contagious exanthema, or hoof rot were not observed and were not historically noted in this closed herd. The flock deworming protocol was based on monitoring with FAMACHA and treating anemic animals with ivermectin; however, none of the study goats had been dewormed before enrollment in this study. Goats were group-housed in an individual climate-controlled room at the LIDIF. The ISU Institutional Animal Use and Care Committee approved the research protocol prior to the commencement of trial procedures (protocol number-5-17-8517-F). The animal care program for ISU LIDIF is AAALAC accredited.

Facilities. Goats were group housed on plastisol-coated metal grate flooring with the control and treatment groups in separate rooms. Animals were provided continual access to water, received limited grain (Envigo Teklad Small Ruminant Diet 7060) mixed with beet pulp in the mornings, and were fed brome hay morning and night. Ration parameters met or exceeded those recommended by the NRC guidelines (NRC, 2001). Rooms were maintained between 17.8 and 22.2 °C. Rooms used a 12 h on/off light cycle. Animal housing and husbandry were compliant with the *Guide for the Care and Use of Agricultural Animals in Research and Teaching*. All animals were acclimated for 72 h after arrival.

Respiratory disease model. After acclimation, goats were inoculated intratracheally with 10 mL of 10⁶ CFU/mL of *Pasteurella multocida* strain P1062 (type A3) that was obtained from the National Veterinary Services Laboratory, Ames, IA. The culture was grown from a lyophilized stock culture and prepared as described by others.⁷ The lower third of the trachea was palpated on each goat and the hair was clipped. A local infiltration of lidocaine (Lidocaine 2%, Vet One, Boise, ID) was applied subcutaneously. The skin was then aseptically prepped with alternating scrubs of chlorhexidine and isopropyl alcohol. Next, a 16 gauge 1.5-inch needle was placed into the trachea in the annular ligament between 2 adjacent rings, and the inoculum was released when the animal was mid-inhalation. Approximately 36 h later the injection site was again numbed with lidocaine and aseptically prepared; a second inoculation of 30 mL of 10⁶ CFU/mL was administered into the trachea, together with 5 mL delivered via stylet into each nostril during inhalation.

Monitoring. All study goats were observed daily for physical signs relevant to respiratory disease [pyrexia (rectal temperature), tachypnea (respiratory rate), abnormal respiratory noise (auscultation), coughing, nasal discharge and tachycardia (heart rate)] and were assessed twice daily for general parameters of health such as appetite and responsiveness to stimuli. Blood samples were collected 2 times via jugular venipuncture for CBC and SAA analysis: upon study enrollment prior to inoculation, and 8 h after confirmation of respiratory disease induction. Respiratory disease induction was confirmed by physical and hematological changes as described below. All blood samples were collected by using a jugular venipuncture, with the goat restrained by an assistant who placed one hand behind the poll and one hand beneath the mandible. When working with the

goats, all staff wore latex or nitrile gloves, an N95 respirator, and dedicated barrier clothing that was laundered after each use.

Goats were scored for respiratory disease using a modified clinical respiratory disease scoring system for dairy calves [The California Bovine Respiratory Disease (BRD) scoring system].¹ Goats were assessed for ocular discharge (0 if normal; 2 if any level of severity present), nasal discharge (0 if normal; 4 if any level of severity present), cough (0 if normal; 2 if spontaneous cough present), breathing (0 if normal; 2 if rapid or if abnormal noises auscultated), and temperature (0 if ≤ 39.2 °C; 2 if > 39.2 °C). Scores on confirmation of respiratory disease induction were then compared with control group scores.

Complete blood counts (CBCs) were performed at the Iowa State University Clinical Pathology Laboratory for the control and experimental groups to evaluate overall white blood cell (WBC) count, lymphocytes, monocytes, neutrophils, band neutrophils, neutrophil:lymphocyte (N:L) ratio, total plasma protein (TPP), fibrinogen, and TPP:fibrinogen ratio. A commercial hematology analyzer (Advia 2120i Hematology System, Siemens Healthineers, Erlangen, Germany) was used, with blood films read by a certified medical technologist.²³ Analyzer quality controls were performed at multiple levels daily with a commercial hematology control (CBC-Tech, R and D Systems, Minneapolis, MN). Blood samples for serum amyloid A (SAA) analysis were collected 8 h after confirmation of respiratory disease and analyzed with a commercial ELISA kit (Goat Serum Amyloid A, Life Diagnostics, West Chester, PA). This kit has a typical standard curve that ranges from 1.56 to 100 ng/mL for goat samples and was read on a SpectraMax Plus384 plate reader (Molecular Devices, San Jose, CA). Tests were performed according to manufacturer's recommendations.

At successful induction of respiratory disease (defined as elevation of at least 2 physical exam parameters and at least 2 hematologic parameters), the goats were given a 2.5 mg/kg dose of tulathromycin subcutaneously in the neck as previously described²⁴ and were observed for clinical outcome for the next 13 d. Tulathromycin was administered to determine the feasibility of a model of acute infection that would not necessarily lead to subject mortality. After 13 d, the goats were humanely euthanized,²⁰ and a gross necropsy was performed to evaluate for signs of pneumonia such as consolidation, pleural adhesions, pleural fibrin deposits as well as the distribution of gross lesions, as described by others.²⁵

Statistical Analysis. Descriptive data are expressed as mean and [SD or SEM]. Statistical analysis was performed using R software version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria). Nonparametric tests, Wilcoxon rank-sum test, and signed-rank test were used to compare differences between 2 paired groups (before and after confirmation of disease induction), respectively. If a P-value was less than 0.05, the results were considered statistically significant.

Results

Animals. Study goats weighed 34.7 ± 4.6 kg, and all physical examination parameters were within normal limits prior to inoculation (Table 1). All 6 goats survived to study conclusion. All of the goats demonstrated rales starting at 36 h after the second inoculation and lasting for as long as 72 h (mean duration of rales: 40 ± 20 h). All 6 goats met the criteria for confirmation of disease and received tulathromycin 36 h after the second inoculation with *P. multocida*. Four of the goats were observed coughing, one at 12 h after inoculation and the other 3 at 36 h after inoculation (mean duration of cough: 54 ± 36 h). All goats were

Table 1. Mean and standard deviation values for clinical and hematologic parameters of the study goats ($n = 6$). Values in bold represent a statistically significant difference ($P < 0.05$) between the 2 sample times.

Parameter	Experimental before induction		Experimental after induction		P value
	Mean	SD	Mean	SD	
Heart Rate (beats/minute)	66.3	3.8	70.5	27.9	0.8438
Respiratory Rate (breaths/minute)	22.5	2.8	34.8	21.8	0.2807
Temperature (°C)	38.3	0.5	39.5	0.5	0.0313
Respiratory Disease Score	0.17	0.41	5.33	2.07	0.0022
WBC Count ($\times 10^3/\mu\text{L}$)	12.8	2.0	9.5	4.9	0.0938
Lymphocytes ($\times 10^3/\mu\text{L}$)	6.5	1.7	4.1	2.5	0.0313
Monocytes ($\times 10^3/\mu\text{L}$)	0.23	0.13	0.26	0.14	1
Neutrophils ($\times 10^3/\mu\text{L}$)	5.5	0.4	4.6	3.2	0.5625
Band Neutrophils ($\times 10^3/\mu\text{L}$)	0.0	0.0	0.332	0.384	0.1003
Neutrophil: Lymphocyte Ratio	0.9	0.2	1.2	0.8	0.3125
Total Plasma Protein (gm/dL)	7.4	0.3	7.8	0.2	0.2012
Fibrinogen (mg/dL)	400	219	683	75	0.0731
TPP: Fibrinogen Ratio	22.3	8.9	11.5	1.1	0.0625
Serum Amyloid A (ng/mL)	<1.56	NA	48.8	82.8	0.0284

observed eating at feeding times and all remained ambulatory and alert to stimuli during the experimental period. Tulathromycin was administered subcutaneously in the neck, and all injection volumes were < 2 mL.

Monitoring. Heart rate, respiratory rate, temperature, and CBC values for all groups are shown in Table 1. When comparing values before and after the presence of respiratory disease, no statistically significant differences in heart rate ($P = 0.8438$) or respiratory rate ($P = 0.2807$) were noted; however, a statistically significant ($P = 0.0313$) increase in temperature of 1.2 °C was noted after induction of respiratory disease.

Values from the modified respiratory disease scoring system (mean \pm SD) were 0.17 ± 0.41 before inoculation and 5.33 ± 2.07 after inoculation. These results reflect a statistically significant difference ($P = 0.0022$).

CBC values for all goats are presented in Table 1. When comparing the goats before and after induction of respiratory disease, no statistically significant differences were noted between WBC ($P = 0.0938$), monocytes ($P = 1$), neutrophils ($P = 0.5625$), band neutrophils ($P = 0.1003$), N:L ratio ($P = 0.3125$), TPP ($P = 0.2012$), fibrinogen ($P = 0.0731$), and TPP:fibrinogen ratio ($P = 0.0625$). Statistically significant differences were noted between lymphocyte counts (6.5 vs $4.1 \times 10^3/\mu\text{L}$; $P = 0.0313$). SAA showed a significant difference between time points (<1.6 vs 48.8 ng/mL; $P = 0.0284$). WBC counts, fibrinogen concentration, neutrophil counts, and SAA concentration for all sampling times are displayed in Figure 1.

At necropsy, one of the experimental goats had mild adhesions to the left lung lobe. In another, a region of the right lung lobe (approximately 30% of the affected lobe) was pale and friable.

Discussion

The goal of this study was to investigate whether a protocol involving multiple intratracheal inoculations of *P. multocida* in meat goats could successfully induce respiratory disease as defined by altered clinical parameters, signs, and hematologic indices. Clinical parameters such as heart rate and respiratory rate not altered to a statistically significant degree. Rectal temperature was significantly higher after disease induction. All goats survived until the conclusion of the study, indicating that this method may be useful for inducing respiratory disease while avoiding animal mortality.

The only significant difference noted in CBCs was a decrease in lymphocyte counts after infection. In ruminants, lymphopenia can occur with stress as well as acute bacterial and viral infections.¹² Other parameters showed no statistically significant changes.

The respiratory disease scoring system was initially modified to exclude head tilting, which is used for calves.¹ Goats typically do not present with head tilts from respiratory disease at the same frequency as calves, and as *Mycoplasma* spp. organisms were not used in this study, the risk of otitis was deemed to be low. Moreover, none of the goats displayed a head tilt.

SAA concentrations can be elevated in the presence of inflammation in goats.^{9,10} Specifically, in a caprine inflammation model using turpentine, SAA levels were significantly increased one day after injection.⁹ Furthermore, in a study of 55 healthy goats, SAA levels demonstrated minimal variation, suggesting it may be used as a reliable biomarker of inflammation in goats.¹⁰ The results from these studies suggest that SAA measurement could have diagnostic utility in experimental models of inflammation and infection in goats. In our study, the infected goats had a statistically significant elevation in SAA levels as compared with the control group. The subcutaneous tulathromycin injections were not a likely a source of inflammation due to route of administration, however intramuscular injection of tulathromycin has been associated with increased muscle damage in cattle.^{5,14}

While not a specific aim of this study, the administration of tulathromycin most likely had a therapeutic effect on the goats, as other studies reported 40% and 20% mortality from intratracheal and intranasal inoculation respectively with *P. multocida* B:2 in goats.²² B:2 is a hemorrhagic septicemia strain and is much more likely to produce clinical disease and mortality than is the strain we used.²² Tulathromycin is efficacious against 100% of *P. multocida* isolates recovered from the lungs of pneumonic goats in one study.⁴ The lack of severe lesions at gross necropsy in our study could also suggest clinical efficacy with tulathromycin treatment in our model of induction of respiratory disease. The presence of respiratory disease reportedly alters the pharmacokinetics of tulathromycin in goats.²⁴ However the recovery of animals after challenge, as occurred in our study, suggests these changes have minimal effect on the short-term health of the goats in this model. The administration of tulathromycin could potentially confound the model, as the presumptive protective

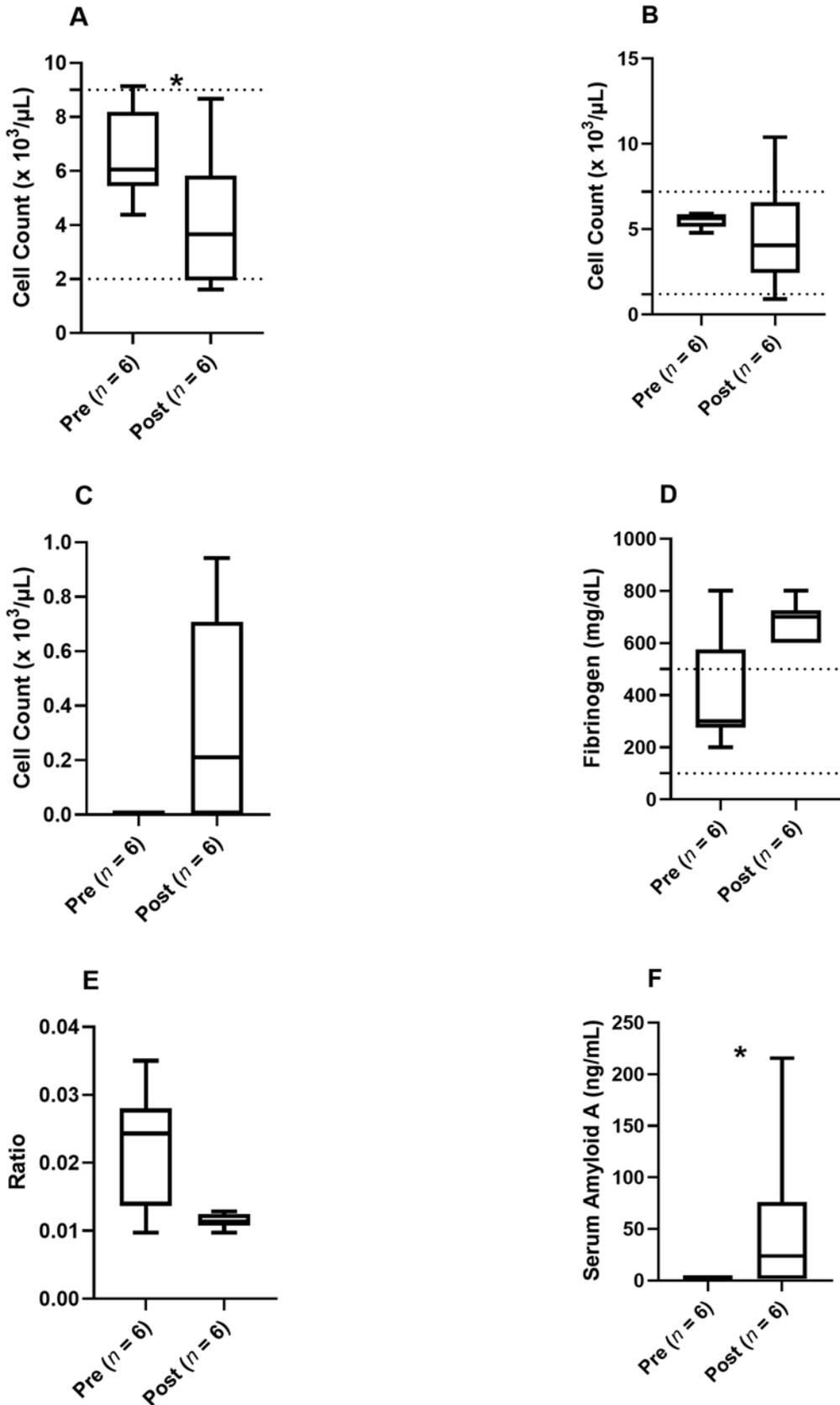


Figure 1. Distribution of White blood cell counts, fibrinogen concentration, neutrophil counts, and Serum Amyloid A (SAA) concentration for the experimental group pre and postinduction of disease ($n = 6$). (A) lymphocyte, (B) neutrophils, (C) band neutrophils, (D) fibrinogen, (E) TPP: fibrinogen ratio, (F) SAA. Note: dashed lines indicate normal ranges established by the Iowa State University College of Veterinary Medicine's Clinical Pathology lab. An "*" indicates a statistically significant difference ($P < 0.05$).

effect of antimicrobial therapy could preclude a complete evaluation of pathogenicity. The presumptive protective effect could be purely antimicrobial, or if tulathromycin possesses other properties (for example, anti-inflammatory or epigenetic), those properties could influence disease as is the case with several other antimicrobials.^{2,11} Without the administration of an antimicrobial, significantly more morbidity and possibly mortality could be observed. While more research is needed, specifically in terms of histopathologic evaluation, the lack of significant gross changes on necropsy may suggest that multiple intratracheal administrations of *P. multocida* is a reproducible method of inducing mild respiratory disease in goats.

Limitations of this study include the small number of animals. However, other caprine respiratory disease models have also used groups as small as 5.²² Additional information could have been gathered with advanced imaging, such as thoracic ultrasound and radiography; however, the study's location in a biosecure isolation facility made the importation of such equipment extremely limited due to institutional policy. The modified respiratory disease scoring system identified statistically significant score increases between the experimental and control groups despite the small number of animals. The administration of tulathromycin may be a limitation as this antimicrobial therapy would be effective for treatment; however, other studies using the corticosteroid induction model saw significant mortality despite the administration of antimicrobials.^{17,18} The modified scoring system will need to be further refined to determine the sensitivity and specificity when applied to caprine respiratory disease, as it was designed for bovine respiratory disease. Future directions for this research include evaluating the model with larger sample sizes to ensure appropriate power for the variables of interest, comparing the hematologic and clinical parameters in goats that have had respiratory disease induced with traditional glucocorticoid methods, and investigating the effects of the intratracheal injection on disease parameters. Additional avenues of investigation include refining the *P. multocida* dosing schedule (dose, frequency). Similarly, the effect of a sham injection of the infusion broth alone, without *P. multocida*, would provide a more appropriate control for these experiments.

In conclusion, multiple intratracheal inoculations of *P. multocida* appear to successfully induce respiratory disease in goats. Clinical signs of respiratory disease, such as altered respiratory sounds and increased temperature, were observed. Similarly, differences in lymphocyte counts, SAA levels, and temperatures were consistent with infection. Because no mortality occurred and minimal changes were noted on gross necropsy, researchers should consider multiple intratracheal administrations for inducing experimental respiratory disease in goats.

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