Development of a Challenge Model for *Mycoplasma bovis*–induced Pneumonia in Cattle

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ABSTRACT

Mycoplasma bovis is an important pathogen of young dairy and veal calves and is a causative agent of mastitis in adult cows and respiratory disease and arthritis in feedlot and stocker calves. Although most cases of bovine respiratory disease (BRD) involve more than one pathogen, there are reports of *M. bovis* being the predominant bacteria isolated from the lungs of calves with BRD. This study was conducted to develop a method (challenge model) for inducing pneumonia with *M. bovis* in young cattle. Thirty healthy colostrum-deprived crossbred calves, 3 to 6 months of age were randomly allocated to 10 replicates of three animals each. Within each replicate, one calf was randomly allocated to each challenge group: 60 mL of sterile saline endobronchially (control) on day 0; 60 mL suspension of *M. bovis* endobronchially on Day 0; 60 mL suspension of *M. bovis* endobronchially on Days 0, 1 and 2, respectively. Depression scores, respiratory scores, and rectal temperatures were measured daily from Day 0 through Day 14. Calves were humanely euthanized Day 14, and lungs were harvested and scored for percent consolidation. Depression scores, respiratory scores, and rectal temperatures increased dramatically on Day 1 for all animals challenged with *M. bovis* and generally remained elevated for 5 or 6 days. These three endpoints did not increase for controls throughout the study. At necropsy, mean percentage lung consolidation for the *M bovis* challenged calves was considerably greater than that of saline control calves. Results of this study support the use of a one-day challenge of *M. bovis* as well as the use of clinical criteria depression and respiratory character scores ≥ 1 and rectal temperature ≥103.5°F as indicative of induced pneumonia in future studies.

INTRODUCTION

Mycoplasma bovis is a member of the class Mollicutes, bacteria that are distinguished by the lack of cell walls, having instead a complex plasma membrane.[1,2] *Mycoplasma bovis* is an important pathogen of young dairy and veal calves and is a causative agent of mas-

titis in adult cows and respiratory disease and arthritis in feedlot and stocker calves. Although most cases of bovine respiratory disease (BRD) involve more than one pathogen, including *Mannheimia haemolytica, Pasteurella multocida*, and *Histophilus somni*, there are reports of *M. bovis* being the predominant bacteria isolated from the lungs of calves with BRD.[1,2] Experimental infections of *M. bovis* and *M. haemolytica* were more severe when calves were inoculated with *M. bovis* before *M. haemolytica*.[3,4] The authors hypothesized that *M. bovis* might play a major role in the infectious process and lead to more severe pneumonia when present with other BRD pathogens. *Mycoplasma bovis* is resistant to antibiotics of the β lactam family, which express their activity by inhibiting synthesis of the bacterial cell wall. Although treatments have been effective against *M. bovis* in vitro, it is not uncommon to have treatments for this bacteria fail to resolve respiratory disease, arthritis, and mastitis in cattle.[2] This study was conducted to develop a method (challenge model) for inducing pneumonia with *M. bovis* in young cattle.

MATERIALS AND METHODS

Animals

Thirty healthy crossbred calves, 3 to 6 months of age, weighing approximately 72 to 139 kg (160–306 lb), including both males and females, were acquired for the study. The calves had been reared colostrum deprived at the source facility. General health and suitability for inclusion in the study were determined. They had no history of bacterial pneumonia, vaccination against M. bovis, or therapeutic antibiotic administration in the 7 days prior to challenge.

Management

Animals were managed similarly and with due regard for their well-being. Animals were handled in compliance with Merial Institutional Animal Care and Use Committee (IACUC) approvals, all applicable local regulations, and requirements of local IACUC. The Investigator and Monitor determined that these procedures were in compliance with the protocol.

Calves were individually identified by uniquely numbered duplicate ear tags, and were housed indoors at the Central States Research Centre, Oakland, Nebraska beginning on Day -11 in an environmentally modified confinement building. The room was ventilated using HEPA filtered air (in and out) and heated as needed using gas-fired propane heaters located outside the room. The pens measured 80 × 25 ft and consisted of concrete floors, concrete block walls, and glass board ceilings.

Calves were fed a suitable pelleted calf ration containing sodium monensin ad libitum. Water was supplied ad libitum by automatic water tanks within each pen.

Allocation and Challenge

Animals (n=30) were ranked by ascending weight and randomly allocated to 10 replicates of three animals each. Animals within each replicate received challenge on Day 0. Within each replicate, one calf was randomly allocated to each challenge group using the PROC PLAN procedure of SAS® version 8.2, as follows:

Group 1: 60 mL of sterile saline endobronchially (control).

Group 2: 60 mL (approximately $1.26×10^{10}$ colony-forming units [CFU]) suspension of M. bovis endobronchially on Day 0

Group 3: 60 mL (approximately $1.26×10^{10}$, $1.32×10^{10}$, and $3.54×10^{10}$ CFU) suspension of M. bovis endobronchially on Days 0, 1, and 2, respectively.

An additional 60 mL of saline and 60 mL of air were used to flush the endoscope port following challenge administration for each calf.

The sterile saline and *M. bovis* isolates (Strain KL-100) for challenge were provided by Midwest Veterinary Services. Challenge suspension preparation and enumeration documentation were provided by laboratory personnel at the test facility. Prior to infection, the *M. bovis* isolate was

cultured to determine the strain and purity. Minimum inhibitory concentration testing was performed to obtain an indication of the susceptibility of the challenge strain to antimicrobials in vitro.

Primary Endpoints

Each animal was assessed daily starting on Day 0. Clinical evaluations were conducted on Day 0 (prior to challenge) and from Days 1 through 14 at approximately the same time of day (in the morning). The following scales were used for scoring depression and respiratory character:

Depression

   0 = normal (no depression observed)

   1 = mild depression (off-feed, moved when person entered pen)

   2 = moderate depression (off-feed, moved when physically prompted)

   3 = severe depression (pronounced; very reluctant to move when physically prompted)

   4 = moribund (recumbent, near death)

Respiratory character

   0 = normal (nothing unusual in respiratory character)

   1 = mild respiratory distress (clinical signs included mild cough, sneezing; mild increase in rate or shallow breathing, mild dyspnea)

   2 = moderate respiratory distress (clinical signs included increased cough, sneezing; moderate increase in rate or shallow breathing, moderate dyspnea)

   3 = severe respiratory distress (clinical signs included: open-mouth breathing, or marked dyspnea or "thumping")

Rectal body temperatures

Rectal temperatures were taken daily from Day 0 (before challenge) through Day 14.

Necropsy

Calves were humanely euthanized on Day 14. Necropsy procedures included evaluations to confirm a clinical diagnosis of BRD and to describe pneumonic lesions, percent consolidation of each lung lobe and other findings. Pneumonic lesions (if applicable) were examined grossly. Individual swabs of the pneumonic lesions and the trachea (near the bifurcation) were taken for microbiologic culture. If no pneumonic lesion(s) were observed at necropsy, swabs from the right middle lung lobe tissue and the trachea were collected for microbiologic culture.

Percentage lung consolidation

During necropsy, lungs were harvested and scored for percent consolidation. Each lung lobe was palpated, and the percent consolidation on the dorsal and ventral surface was estimated and recorded. The percent consolidation for the total lung was calculated by multiplying the percentage of each lobe by the percent weight for that specific lobe. The percentage of each lobe is as follows: left cranial (apical) 5%; left posterior cranial (cardiac) 6%; left caudal (diaphragmatic) 32%; intermediate 4%; right cranial (apical and accessory) 11%; right middle (cardiac) 7%; and right caudal (diaphragmatic) 35%.

Secondary Endpoints

Lung/pneumonic lesion and tracheal swab samples were cultured for BRD pathogens.

Blinding

All individuals assigned to collect data were blinded to challenge assignments. The blinding codes were revealed upon completion of the last necropsy within the study.

Adverse experiences

Calves were observed daily during the study for adverse experiences other than those specified as primary endpoints.
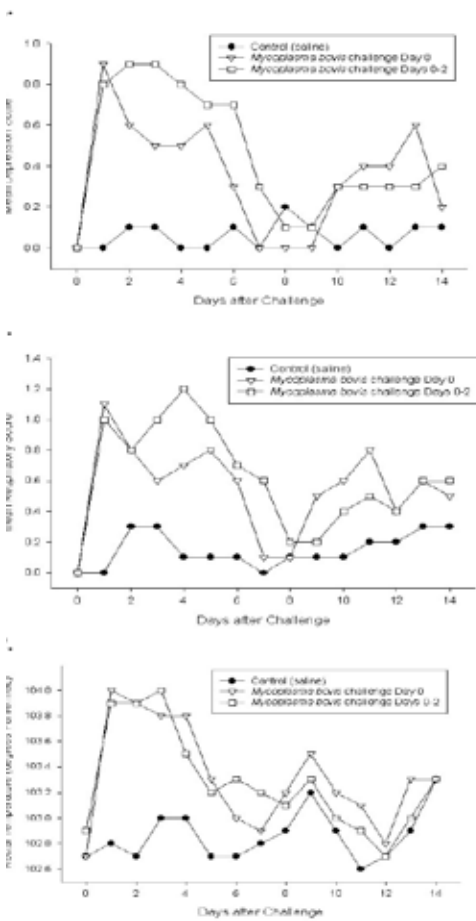
Statistical analysis

Analyses included descriptive statistics for quantitative physical examination data and lung lesion scores for all animals. Comparisons between groups were not planned.

RESULTS

Depression scores, respiratory scores, and rectal temperatures all increased dramatically on Day 1 for animals challenged with *M. bovis* on Day 0 and Days 0, 1 and 2 (Figure 1A-1C). These endpoints remained

Figure 1. Results of daily depression scores, respiratory character scores, and rectal temperatures before challenge and throughout the study period

relatively stable for control animals challenged only with saline on Day 0. Depression and respiratory scores generally remained elevated for 5 or 6 days for the calves challenged with M. bovis (Figure 1A-1B). Rectal temperatures began to return to normal by Day 5 (Figure 1C).

Using depression and respiratory scores greater than or equal to one and a rectal temperature greater than or equal to 103.5°F (39.7°C) as criteria to indicate the presence of pneumonia induced by *M. bovis*, seven calves in each of the two groups challenged with *M. bovis* (a total of 14 calves) satisfied these criteria on Day 1, whereas none of the calves challenged with saline met these

criteria (data not shown). Over the entire 14-day study period, two calves challenged with saline, 10 calves challenged with *M. bovis* on Day 0, and nine calves challenged with M. bovis on Days 0, 1 and 2 satisfied the above-mentioned criteria.

The percentages of total lung consolidation at necropsy on Day 14 were somewhat variable within each of the groups; however, the mean percentages of lung consolidation for the two groups challenged with *M. bovis* (10.42 ± 7.25 and 7.12 ± 5.38) were clearly and considerably greater than the percentage for the control group (1.74 ± 1.99) (Table 1).

No adverse experiences were reported and no calves died before the scheduled day of humane euthanasia and necropsy (Day 14).

DISCUSSION

Intratracheal challenge of gnotobiotic calves with *M. bovis* to induce pneumonia has been previously described.[2,5] Authors have reported that challenge with *M. bovis* as the sole pathogen resulted in wide variance in the severity of clinical signs and lung lesions observed.[2,3,5]

In the present evaluation of a challenge model for *M. bovis* in young calves, clinical signs of depression and respiratory character as well as rectal body temperatures were relatively similar among challenged calves; however, the degree of lung consolidation was not as similar within the challenged groups. Nevertheless, it was clear that the percentage of lung consolidation was relatively extensive compared with that observed for control calves, which received saline. There was no appreciable difference in the severity of clinical signs or percentage of lung consolidation between calves challenged once on Day 0 and those challenged daily for three days (Days 0, 1 and 2).

Results of this study support the use of a one-day challenge of M. bovis as well as

Table 1. *Individual and mean lung consolidation scores*

Treatment Group	Animal ID	Replicate	Total Lung Score (% Consolidation)
Control (saline)			
	83	1	0.65
	107	2	7.11
	116	3	0.68
	122	4	0.17
	119	5	2.37
	96	6	1.32
	55	7	1.43
	44	8	1.57
	31	9	1.58
	38	10	0.54
Mean ± SD			1.74 ± 1.99
Challenge Day 0			
	93	1	12.35
	68	2	5.69
	47	3	6.66
	32	4	18.35
	89	5	4.00[a]
	48	6	10.12
	57	7	22.50
	46	8	5.75
	33	9	0.49
	25	10	18.30
Mean ± SD			10.42 ± 7.25
Challenge Days 0-2			
	52	1	5.92
	71	2	4.35
	90	3	15.59
	95	4	14.48
	140	5	6.39
	87	6	13.50
	53	7	2.01
	37	8	1.42
	29	9	5.09
	26	10	2.44
Mean ± SD			7.12 ± 5.38

[a]Contained large abscesses not typical of *M. bovis* (*M. hemolytica* determined by culture)

the use of clinical criteria depression and respiratory character scores ≥ 1 and rectal temperature ≥103.5°F, as indicative of induced pneumonia in future studies.

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