Reproduction of respiratory mycoplasmosis in calves by exposure to an aerosolised culture of *Mycoplasma bovis*

Anna Kanci[a], Nadeeka K. Wawegama[a], Marc S. Marenda[b], Peter D. Mansell[c], Glenn F. Browning[a], Philip F. Markham[a].

[a] Asia-Pacific Centre for Animal Health, Melbourne Veterinary School, Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Parkville, Victoria 3010, Australia; [b] Asia-Pacific Centre for Animal Health, Melbourne Veterinary School, Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Werribee, Victoria 3030, Australia; [c] Melbourne Veterinary School, Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Werribee, Victoria 3030, Australia.

Corresponding author

Anna Kanci

Address:

Asia-Pacific Centre for Animal Health, Melbourne Veterinary School, Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Parkville, Victoria 3010, Australia

Phone: +61 3 8344 7368, Fax: +61 3 83447374,

Email: akanci@unimelb.edu.au

Running title: Reproducing mycoplasmal pneumonia in calves

Key words: experimental infection, aerosol, mycoplasma, bovis

Abstract

Mycoplasma bovis is an important pathogen of cattle, causing pneumonia, arthritis and otitis media in young calves, and mastitis in lactating cows, resulting in increased morbidity and, in some instances, mortality. The objective of this study was to evaluate the survival of a *M. bovis* isolate following nebulisation and to establish whether respiratory disease similar to that seen in the field could be induced in calves by exposing them to an aerosolised culture of *M. bovis*. A group of eight *M. bovis*-free calves 14-28 days old were exposed to an aerosolised culture of a field isolate of *M. bovis* that had originally been recovered from a joint lesion in a calf. Three weeks after aerosol exposure necropsies were conducted on all calves. Lung lesions were seen in 7 of 8 calves exposed to the aerosol of *M. bovis*, whilst calves exposed to the culture medium alone did not develop lesions. Two calves in the infected group had detectable concentrations of serum antibody against *M. bovis* on day 7 post infection and 4 calves had detectable concentrations of serum antibody against *M. bovis* on day 21 post infection when tested by MilA IgG ELISA. *M. bovis* was reisolated from the upper trachea of 6 of the 8 infected calves. The infection method described here appeared to induce lung lesions typical of naturally occurring disease associated with infection with *M. bovis* and should be applicable to testing the safety and efficacy of attenuated vaccine candidates to control disease caused by this pathogen.

1. Introduction

Mycoplasma bovis is an important pathogen of cattle worldwide, causing pneumonia (Caswell and Archambault, 2007), polyarthritis and contagious mastitis (Wilson et al., 2007). It has also been associated with outbreaks of meningitis (Stipkovits et al., 1993), keratoconjunctivitis (Alberti et al., 2006), otitis media (Lamm et al., 2004) and abortion (LaFaunce and McEntee, 1982). Most disease associated with *M. bovis tends to be chronic and as a result causes substantial economic losses in both the beef and dairy industries,* reducing weight gain, carcass value and milk production, as well as increased morbidity and, in some instances, mortality (Aebi et al., 2012; Gourlay et al., 1989). The costs of treatment are typically high and antimicrobial therapy is often ineffective (Maunsell et al., 2011).

On dairy farms, *M. bovis* can be readily transmitted to the udder via the teat canal and young calves can be exposed to organisms in uterine fluids during parturition or in raw milk (Gonzalez and Wilson, 2003). *M. bovis* can be transmitted by asymptomatically infected, clinically healthy animals (Maunsell et al., 2011), which may start shedding and develop disease after exposure to stresses such as comingling, transport and environmental changes (Assié et al., 2009; Pfützner and Sachse, 1996). The primary mode of transmission of *M. bovis* in the field is via the respiratory route as a result of inhalation of infectious aerosols, ingestion of milk from cows with mastitis, or via nose-to-nose contact (Maunsell et al., 2011; Nicholas et al., 2002).

Control and management of *M. bovis* has been hampered by the lack of sensitive diagnostic assays to detect carrier animals and the limited efficacy of antimicrobial therapy. Recent studies have developed an improved serological assay (Wawegama et al., 2016), but effective vaccination will be essential for future control (Ayling et al.,

2000). Evaluation of the safety and efficacy of vaccine candidates requires a reliable and reproducible infection model that emulates the route of infection seen in the field. Different methods have been used previously to reproduce disease caused by *M. bovis* under experimental conditions. Oral (Maunsell et al., 2012), endobronchial (Bartram et al., 2016) and intraarticular (Ryan et al., 1983) routes have been used in some studies, but intranasal (Zhang et al., 2014) or intratracheal inoculation of cultured organisms have been most widely used (Hermeyer et al., 2012; Prysliak et al., 2011). However, these methods require high doses to induce clinical disease (Prysliak et al., 2011) and may not induce lesions typical of natural infection. Aerosol exposure to *M. bovis* using a mask has also been used to induce pneumonia in calves (Nicholas et al., 2002), but this method requires individual handling of each animal, and is therefore laborious and could induce stress. Aerosol exposure is the preferred method for reproduction of other respiratory mycoplasmoses as it simulates the natural route of infection, and thus the pathogenesis of the disease that is induced is more likely to closely resemble that of naturally occurring disease (Czaja et al., 2002; Whithear et al., 1996). The aim of this study was to develop an approach that could deliver infectious aerosols of *M. bovis* to calves and assess its capacity to reproduce respiratory mycoplasmosis.

2. Materials and methods

2.1. Experimental animals

Thirteen Friesian-cross calves aged between 2 and 3 weeks were obtained from a dairy herd with no previous history of *Mycoplasma bovis* and bovine herpesvirus type 1 in Gruyere, Victoria, and transported to the Asia-Pacific Centre for Animal Health

(APCAH) at the Veterinary Clinical Centre, Werribee, Victoria, Australia. The animals were allowed to acclimatise for a week before infection. A nasal swab was collected from each calf on arrival and used to inoculate *M. bovis* agar, which was then incubated at 37°C. No growth was observed on the agar plates. We did not investigate the BHV-1 status of the calves upon arrival at the facility as the source herd was selected because there was no previous history of BRD on this particular farm. All calves appeared clinically healthy at the time of arrival and after the acclimatising phase and no clinical signs of respiratory disease were observed. Three days prior to experimental infection, a serum sample was collected from all calves to detect passive transfer of immunoglobulins, using the qualitative zinc sulphate turbidity test (McEwan et al., 1970). Calves were given unique ear-tags, randomly allocated into groups, weighed and placed into one of two rooms, one for the uninfected calves and the other for infected animals, in which they were kept throughout the study. The calves were fed milk replacer twice per day and provided water *ad libitum*. Clinical signs were monitored in both groups throughout the study. The experiment was conducted with the approval of the University of Melbourne Animal Ethics Committee (approval number: 0911327.1).

2.2. Mycoplasma bovis culture

M. bovis wild-type strain 3683 (isolated from a joint lesion in a calf in Millmerran, Queensland, Australia) was obtained as frozen culture from the Department of Primary Industries (DPI), Queensland (a kind gift of J. Forbes-Faulkner), and cultured in mycoplasma culture medium (21 g PPLO without crystal violet, 37 ml of 15% yeast extract, 100 ml inactivated swine serum, 4 ml of 1.6% phenol red solution, 859 ml distilled water, pH adjusted to 7.8). The cultures were grown in a final volume of

100 ml at 37°C without agitation for 17 h, until the mid to late log phase of growth was reached, as determined by the colour change of the medium. Titres were determined using a limiting dilution method and the final titre was expressed in colour changing units (CCU) (Meynell and Meynell, 1970).

2.3. Infection Model

The calves were randomly allocated into two treatment groups containing 5 or 8 calves each. Individual weights were recorded at day 0 and 21. Five calves were exposed on days 0 and 3 to an aerosol of medium containing 0.1% (v/v) antifoam A (Sigma Aldrich) (negative control, uninfected group). Eight calves were exposed on days 0 and 3 to an aerosol of an undiluted culture of *M. bovis* wild-type strain 3683 containing 0.1% (v/v) anti-foam A (infected group) in a specially constructed 500 litre infection chamber (Figure 1). The chamber contained two or three calves each time they were exposed. Industrial grade compressed air was passed through two Collison 6-jet CN25 nebulisers (BGI Instruments, USA), positioned on either side of the chamber, each containing 30 ml of medium or culture at a flow rate of 15 litres/minute through each nebuliser for 15 minutes. The nebulised medium or culture was blown into the chamber containing the calves with additional compressed air at a rate of 25 litres/minute for each nebuliser, resulting in a total flow rate of 80 litres/minute through the chamber. Exhaust air from the chamber was passed through a high efficiency particulate arrestance (HEPA) filter (MTM Technologies, Australia). Twenty litres of air were withdrawn from the chamber during the period of exposure and passed through mycoplasma culture medium using an air-sampler pump (A.P. BUCK Inc, USA) and the number of organisms per litre of air was determined by titration. Temperature and humidity within the chamber were also determined using

145 an external temperature and relative humidity data logger (HOBO®, Australia). After
146 each exposure, the calves were returned to their respective rooms. The calves were
147 monitored for numerous clinical signs associated with infection with *M. bovis*
148 throughout the length of the study. These included attitude, nasal discharge,
149 respiratory effort, lameness, coughing, ataxia, head tilt and ear drooping.
150 Development of signs of respiratory disease, arthritis and otitis media (individually or
151 in combination) were also recorded. At 21 days post challenge, blood was collected
152 from the calves, and they were weighed, euthanised and necropsied.
153

154 *2.4. Pathological examination*

155 The lungs were removed at *post mortem* and scored based on the weighted percentage
156 of the lungs with acute lesions in the apical, middle, diaphragmatic and accessory
157 lobes. The gross lesion scoring method used was based on the method of Goodwin *et*
158 *al*. (1969), with minor modifications (the lesions in the right cranial lobe were scored
159 out of 5 for each of the two sections of the lobe). The proportion of each lung lobe
160 with lesions was weighted using the following corrections to adjust for their
161 contributions to the total lung mass: left apical, 5%; left cardiac, 6%; left
162 diaphragmatic, 32%; right apical, 6%; right accessory, 5%; right cardiac, 7%; right
163 diaphragmatic, 35%; and intermediate, 4%. The weighted lung lobe proportions were
164 then summed to yield the proportion of the lung with lesions for each animal (lung
165 lesion score). Tissue sections from areas with gross lesions were collected, fixed in
166 10% formal saline, processed, embedded in paraffin wax, sectioned and stained with
167 hematoxylin and eosin for histopathological examination. Lesions were only
168 examined histologically if they were found by culture to be associated with infection
169 with *M. bovis.*

2.5. Serological examination

Sera collected at days -3, 7 and 21 were tested using the MilA IgG ELISA described previously (Wawegama et al., 2014) and *M. bovis*-specific antibody concentrations were obtained.

2.6. Re-isolation and identification

Swabs were collected from all calves at *post mortem* from a number of sites in the respiratory tract, including the trachea (upper and lower), apical lung lobes (left and right), left carpal joint, pleural fluid and the right ear canal and cultured for re-isolation of *M. bovis*. Microbial re-isolation was performed in *M. bovis* culture medium. A swab was also collected from areas with lesions (if present). If no lesions were present, the sample for bacteriological analysis was collected from the right cranial lobe. Where lesions were present, a sample was also inoculated onto sheep blood agar (SBA) for identification of bacteria, including, but not limited to, *Pasteurella multocida* and *Mannheimia haemolytica*. The identity of re-isolates was confirmed using a PCR assay targeting a species-specific sequence within the *M. bovis polC* gene (Marenda et al., 2005). The PCR was conducted on all cultures, including those that showed no observable growth.

2.7. Statistical analyses

The lung lesion scores were compared using a Mann-Whitney U test, and the antibody concentrations at each time point and mean percentage weight gains for each

193 experimental group were compared using Student's t test with GraphPad Prism 6
194 (GraphPad Software, La Jolla, CA, USA).
195

196 **3. Results**

197 *3.1. Challenge dose of M. bovis*

198 The predicted dose of the challenge culture received by individual animals was
199 calculated by determining the titre of *M. bovis* in the samples of the chamber air, and
200 the estimated respiratory volume and rate for a calf of 50 kg (the average weight of
201 the calves was 49.1 kg). The inoculum dose, the concentration of *M. bovis* in the
202 chamber air samples and the estimated number of *M. bovis* inhaled by each calf are
203 summarised in Table 1. Each calf in the infected group was estimated to have
204 inhaled 2.02 x 10^4 CCU of *M. bovis* over a 15 min period on both days. The
205 temperature and humidity within the chamber did not vary significantly from run to
206 run.
207

208 *3.2. Clinical observations*

209 All the calves in the uninfected group appeared clinically healthy throughout the
210 experiment. Some calves in the infected group had mild pyrexia (3 calves had
211 temperatures above 39.4°C at 6 days after first exposure), which persisted for between
212 1 and 5 days. Five calves were also observed coughing during daily monitoring
213 periods, for periods lasting from 2 to 10 days. One animal also showed signs of
214 unilateral facial paralysis. There was no significant difference in mean weights or
215 mean percentage weight gains of calves in the uninfected and infected groups (Table
216 2).

3.3. Pathological examination

Post mortem examination of lungs detected gross lesions in 7 of the 8 infected calves (Table 3, Figure 5). The lesions were similar in appearance and distribution to those described previously in calves infected *M. bovis* - dark purple lobular consolidation and most commonly located in the apical and cardiac lobes (Figure 2A). Purulent material was seen in the bronchi of several of the infected calves (Figure 2B). The lung lesion scores differed significantly between the uninfected [0 (0 - 0.22)] and infected groups [9.4 (1.6 – 21.5)] ($P < 0.01$). Histopathological evaluation of the lung lesions revealed peri-bronchiolar, peri-bronchial and peri-vascular accumulation of lymphoid cells, lymphoid follicle formation, loss of alveolar architecture and the presence of purulent exudates and leukocytes in the bronchial lumen (Figure 3B and C). In severe lesions total destruction of lung architecture was seen, with formation of abscesses at the sites of bronchioles/bronchi (Figure 3D), while the lungs of the uninfected calves had a normal architecture (Figure 3A). The very small gross lesions observed in two uninfected calves were not associated with *M. bovis*, so these lesions were not examined histologically.

3.4. Serological analysis

All calves were confirmed to have serum immunoglobulin concentrations greater than 8 g/l using the zinc sulfate turbidity test prior to infection, and thus to have had adequate levels of passively transferred IgG. In the MilA IgG ELISA, four calves had detectable concentrations of antibody against *M. bovis* prior to infection, which were attributed to passive transfer of maternal antibody (1 in the uninfected group and 3 in the infected group), but the uninfected calves remained negative for antibody against

M. bovis throughout the remainder of the experiment (Table 3). In the infected group, 2 calves had concentrations of serum antibody against MilA above the cut off on day 7 post infection and 4 calves had concentrations of antibody against MilA above the cut off on day 21 post infection (Figure 4). The mean serum antibody concentrations against MilA did not differ significantly between the two groups on any of the 3 days samples were collected.

3.5. Recovery of M. bovis

The *M. bovis* specific *polC* PCR assay detected *M. bovis* in broth cultures from 8 of the 8 calves (100%) exposed to strain 3683 (Table 3). The greatest rates of re-isolation of *M. bovis* were from the trachea, followed by the apical lung lobes. *M. bovis* was not detected in the uninfected calves at any of the sites that were sampled. There was no evidence of co-infections with other secondary bacterial respiratory pathogens, such as *Pasteurella multocida* or *Mannheimia haemolytica.*

4. Discussion

An aerosol infection model was developed for *M. bovis* that closely emulated the natural route of infection and appeared to induce lung lesions typical of naturally occurring disease associated with *M. bovis*. Unlike other aerosol methods (Nicholas et al., 2002), this model was able to deliver the infectious aerosol to more than one calf at a time with minimal handling of the animals. An infection model with these characteristics is essential for testing the safety and efficacy of new vaccine candidates.

The clinical signs observed during the study were not pronounced, but pyrexia,

coughing and/or facial paralysis were observed in some animals. The calves exposed to *M. bovis* showed no signs of reduced appetite or weight loss, and their body weight gains were not significantly reduced. The mild clinical signs may be attributable to the virulence of the *M. bovis* strain, the relatively low dose to which the calves were exposed and the absence of secondary pathogens, such as *Trueperella pyogenes* or *Pasteurella multocida*. Our findings are similar to those of others who have described experimental infection with *M. bovis* (Rodriguez et al., 1996; Tegtmeier and Arnbjerg, 2000). In these previous studies the clinical signs associated with infection varied and in some cases were minimal or absent, particularly in calves with minor and/or chronic lung lesions (Tegtmeier and Arnbjerg, 2000).

We were able to re-isolate *M. bovis* from the respiratory tracts of all the infected calves at day 21 post infection. The highest rate of recovery of *M. bovis* was from swabs of the upper trachea (6/8 calves). Previous studies of naturally occurring *M. bovis* infections in calves suggest that the upper respiratory tract is the initial site of colonisation (Nicholas et al., 2002; Pfützner and Sachse, 1996) and the organism is able to persist in the host for an extended time (Nicholas et al., 2002). The relative ease of re-isolation of *M. bovis* from upper trachea in our study suggests that the aerosol produced by the nebuliser was deposited at the site of colonisation during natural infection, the upper respiratory tract. The administration of the aerosolised culture of *M. bovis* was straightforward, and, unlike intratracheal and intransal inoculation, did not require sedation or excessive handling of the calves prior to inoculation (Hermeyer et al., 2012). There was no evidence of co-infection of the lungs with secondary pathogens, such as *Pasteurella multocida*, which has been seen in studies using intratracheal inoculation (Poumarat et al., 2001). The aerosol method minimized the possibility of contamination with other agents, and avoided the

291 introduction of a bolus of exogenous material, thus reducing the likelihood of other
292 factors confounding the interpretation of the role of *M. bovis* in the respiratory disease
293 that was induced in the infected calves. The clinical signs approximated those seen in
294 the field and the disease progression was similar to that seen during natural outbreaks
295 in the field in calves of this age.
296 Two exposures to an aerosolised culture of *M. bovis* were able to reproduce
297 respiratory disease in calves. The calves received the same dose of *M. bovis* on both
298 inoculation days (day 0 and 3), indicating the reproducibility of the delivery method.
299 While 7 of the 8 infected animals had detectable macroscopic lung lesions, *M. bovis*
300 was re-isolated from a range of tissues in the infected calves. Mild microscopic
301 lesions may have been overlooked in the one calf without gross lesions, as only gross
302 lesions were assessed. The lung lesions predominantly involved the apical and
303 cardiac lobes, as is the case in younger calves naturally infected with *M. bovis*
304 (Adegboye et al., 1995; Nicholas et al., 2002; Rodriguez et al., 1996).
305 The bronchial lumina in the infected lungs were occluded with mucus, cell debris,
306 neutrophils and macrophages, surrounded by necrotic eosinophilic material, the pre-
307 cursor of the pyogranulomatous inflammatory lesions of classical *M. bovis*
308 bronchopneumonia (Khodakaram-Tafti and Lopez, 2004). It has been suggested that
309 the classical *M. bovis* lesion of coagulative necrosis is superimposed on an exudative
310 bronchopneumonia (Gourlay et al., 1989). Infiltration of neutrophils and
311 mononuclear cells between the epithelial cells has been seen previously in
312 experimental *M. bovis* infections (Howard et al., 1986; Rodriguez et al., 1996).
313 Extension of lymphocytes into the lamina propria and obliteration of the smooth
314 muscle is a hallmark of cuffing pneumonia (Dungworth, 1993), which progresses to
315 formation of a lymphoepithelium, which becomes flattened, changes also seen in the

lung lesions in this study. These pathological changes may have occurred due to the presence of *M. bovis* in the bronchial lumen and within luminal exudates (Howard et al., 1987a, b; Rodriguez et al., 1996). We also observed possible early stages of abscess formation in areas where the lesions were severe (Figure 3D), as is seen in chronic natural *M. bovis* lesions (Adegboye et al., 1995).

None of the calves in the uninfected group seroconverted, whereas four calves in the infected group were seropositive by day 21 post infection. This is not unexpected as the diagnostic sensitivity of the MilA ELISA is only 14% at day 17 post-infection, even though it rises to over 90% at 24 days post infection (Wawegama et al., 2014).

In summary, we developed an experimental method for aerosol infection of calves with *M. bovis* that results in disease that closely emulates that seen in naturally occurring infections. The route of infection was similar to the exposure to infectious aerosols via inhalation that is believed to be the major route of infection in the field, suggesting that this is a useful model for assessing the efficacy of vaccines against not only *M. bovis,* but also other bovine respiratory diseases.

Conflict of Interest

The authors have no potential conflicts of interest (financial, professional or personal) related to the research reported here.

Acknowledgements

The authors gratefully acknowledge the assistance of Judy Forbes-Faulkner at The Queensland Department of Primary Industries for the *M. bovis isolate*, Cheryl Colson for care of the animals, and Faye Doherty for assistance with histopathology. This

work was supported by Australian Research Council Linkage Project grant LP0989620.

Ethics

The animal experiment was conducted with the approval of The University of Melbourne Animal Ethics Committee, project number 1111970.

References

Adegboye, D.S., Hallbur, P.G., Cavanaugh, D.L., Werdin, R.E., Chase, C.C., Miskimins, D.W., Rosenbusch, R.F., 1995. Immunohistochemical and pathological study of *Mycoplasma bovis*-associated lung abscesses in calves. J. Vet. Diagn. Invest. 7, 333-337.

Aebi, M., Bodmer, M., Frey, J., Pilo, P., 2012. Herd-specific strains of *Mycoplasma bovis* in outbreaks of mycoplasmal mastitis and pneumonia. Vet. Microbiol. 157, 363-368.

Alberti, A., Addis, M.F., Chessa, B., Cubeddu, T., Profiti, M., Rosati, S., Ruiu, A., Pittau, M., 2006. Molecular and antigenic characterization of a *Mycoplasma bovis* strain causing an outbreak of infectious keratoconjunctivitis. J. Vet. Diagn. Invest. 18, 41-51.

Assié, S., Seegers, H., Makoschey, B., Désiré-Bousquié, L., Bareille, N., 2009. Exposure to pathogens and incidence of respiratory disease in young bulls on their arrival at fattening operations in France. Vet. Rec. 165, 195-199.

Ayling, R.D., Baker, S.E., Peek, M.L., Simon, A.J., Nicholas, R.A., 2000. Comparison of in vitro activity of danofloxacin, florfenicol, oxytetracycline,

365 spectinomycin and tilmicosin against recent field isolates of *Mycoplasma*
366 *bovis*. Vet. Rec. 146, 745-747.
367 Bartram, D.J., Moyaert, H., Vanimisetti, B.H., Ramage, C.P., Reddick, D.,
368 Stegemann, M.R., 2016. Comparative efficacy of tulathromycin and
369 tildipirosin for the treatment of experimental *Mycoplasma bovis* infection in
370 calves. Vet. Med. Sci. 2, 170-178.
371 Caswell, J.L., Archambault, M., 2007. *Mycoplasma bovis* pneumonia in cattle. Anim.
372 Health. Res. Rev. 8, 161-186.
373 Czaja, T., Kanci, A., Lloyd, L.C., Markham, P.F., Whithear, K.G., Browning, G.F.,
374 2002. Induction of enzootic pneumonia in pigs by the administration of an
375 aerosol of in vitro-cultured *Mycoplasma hyopneumoniae*. Vet. Rec. 150, 9-11.
376 Dungworth, D.S., 1993. Pathology of Domestic Animals., Vol II. Academic Press:
377 New York.
378 Gonzalez, R.N., Wilson, D.J., 2003. Mycoplasmal mastitis in dairy herds. Vet. Clin.
379 North. Am. Food. Anim. Pract. 19, 199-221.
380 Goodwin, R.F., Hodgson, R.G., Whittlestone, P., Woodhams, R.L., 1969. Immunity
381 in experimentally induced enzootic pneumonia in pigs. J. Hyg. (Lond) 67,
382 193-208.
383 Gourlay, R.N., Thomas, L.H., Wyld, S.G., 1989. Increased severity of calf pneumonia
384 associated with the appearance of *Mycoplasma bovis* in a rearing herd. Vet.
385 Rec. 124, 420-422.
386 Hermeyer, K., Buchenau, I., Thomasmeyer, A., Baum, B., Spergser, J., Rosengarten,
387 R., Hewicker-Trautwein, M., 2012. Chronic pneumonia in calves after
388 experimental infection with *Mycoplasma bovis* strain 1067: characterization of

lung pathology, persistence of variable surface protein antigens and local immune response. Acta. Vet. Scand. 54, 9.

Howard, C.J., Parsons, K.R., Thomas, L.H., 1986. Systemic and local immune responses of gnotobiotic calves to respiratory infection with *Mycoplasma bovis*. Vet. Immunol. Immunopathol. 11, 291-300.

Howard, C.J., Thomas, L.H., Parsons, K.R., 1987a. Comparative pathogenicity of *Mycoplasma bovis* and *Mycoplasma dispar* for the respiratory tract of calves. Isr. J. Med. Sci. 23, 621-624.

Howard, C.J., Thomas, L.H., Parsons, K.R., 1987b. Immune response of cattle to respiratory mycoplasmas. Vet. Immunol. Immunopathol. 17, 401-412.

Khodakaram-Tafti, A., Lopez, A., 2004. Immunohistopathological findings in the lungs of calves naturally infected with *Mycoplasma bovis*. J. Vet. Med. A. Physiol. Pathol. Clin. Med. 51, 10-14.

LaFaunce, N.A., McEntee, K., 1982. Experimental *Mycoplasma bovis* seminal vesiculitis in the bull. Cornell. Vet. 72, 150-167.

Lamm, C.G., Munson, L., Thurmond, M.C., Barr, B.C., George, L.W., 2004. *Mycoplasma otitis* in California calves. J. Vet. Diagn. Invest. 16, 397-402.

Marenda, M.S., Sagné, E., Poumarat, F., Citti, C., 2005. Suppression subtractive hybridization as a basis to assess *Mycoplasma agalactiae* and *Mycoplasma bovis* genomic diversity and species-specific sequences. Microbiology 151, 475-489.

Maunsell, F., Brown, M.B., Powe, J., Ivey, J., Woolard, M., Love, W., Simecka, J.W., 2012. Oral inoculation of young dairy calves with *Mycoplasma bovis* results in colonization of tonsils, development of otitis media and local immunity. PLoS One 7, e44523.

414 Maunsell, F.P., Woolums, A.R., Francoz, D., Rosenbusch, R.F., Step, D.L., Wilson,
415 D.J., Janzen, E.D., 2011. *Mycoplasma bovis* infections in cattle. J. Vet. Intern.
416 Med. 25, 772-783.
417 McEwan, A.D., Fisher, E.W., Selman, I.E., Penhale, W.J., 1970. A turbidity test for
418 the estimation of immune globulin levels in neonatal calf serum. Clin. Chim.
419 Acta. 27, 155-163.
420 Meynell, G.G., Meynell, E. 1970. Theory and practice in experimental bacteriology.,
421 In: Cambridge University Press, Cambridge, 232-233.
422 Nicholas, R.A.J., Ayling, R.D., Stipkovits, L.P., 2002. An experimental vaccine for
423 calf pneumonia caused by *Mycoplasma bovis*: clinical, cultural, serological
424 and pathological findings. Vaccine 20, 3569-3575.
425 Pfützner, H., Sachse, K., 1996. *Mycoplasma bovis* as an agent of mastitis, pneumonia,
426 arthritis and genital disorders in cattle. Rev. Sci. Tech 15, 1477-1494.
427 Poumarat, F., Le Grand, D., Philippe, S., Calavas, D., Schelcher, F., Cabanie, P.,
428 Tessier, P., Navetat, H., 2001. Efficacy of spectinomycin against *Mycoplasma*
429 *bovis* induced pneumonia in conventionally reared calves. Vet. Microbiol. 80,
430 23-35.
431 Prysliak, T., van der Merwe, J., Lawman, Z., Wilson, D., Townsend, H., Van Drunen
432 Littel-van den Hurk, S., Perez-Casal, J., 2011. Respiratory disease caused by
433 *Mycoplasma bovis* is enhanced by exposure to bovine herpes virus 1 (BHV-1)
434 but not to bovine viral diarrhea virus (BVDV) type 2. Can. Vet. J. 52, 1195-
435 1202.
436 Rodriguez, F., Bryson, D.G., Ball, H.J., Forster, F., 1996. Pathological and
437 immunohistochemical studies of natural and experimental *Mycoplasma bovis*
438 pneumonia in calves. J. Comp. Pathol. 115, 151-162.

Ryan, M.J., Wyand, D.S., Hill, D.L., Tourtellotte, M.E., Yang, T.J., 1983. Morphologic changes following intraarticular inoculation of *Mycoplasma bovis* in calves. Vet. Pathol. 20, 472-487.

Stipkovits, L., Rady, M., Glavits, R., 1993. Mycoplasmal arthritis and meningitis in calves. Acta. Vet. Hung. 41, 73-88.

Tegtmeier, C., Arnbjerg, J., 2000. Evaluation of radiology as a tool to diagnose pulmonic lesions in calves, for example prior to experimental infection studies. J. Vet. Med. B. 47, 229-234.

Wawegama, N.K., Browning, G.F., Kanci, A., Marenda, M.S., Markham, P.F., 2014. Development of a recombinant protein-based enzyme-linked immunosorbent assay for diagnosis of *Mycoplasma bovis* infection in cattle. Clin. Vaccine. Immunol. 21, 196-202.

Wawegama, N.K., Markham, P.F., Kanci, A., Schibrowski, M., Oswin, S., Barnes, T.S., Firestone, S.M., Mahony, T.J., Browning, G.F., 2016. Evaluation of an IgG enzyme-linked immunosorbent assay as a serological assay for detection of *Mycoplasma bovis* infection in feedlot cattle. J. Clin. Microbiol. 54, 1269-1275.

Whithear, K.G., Harrigan, K.E., Kleven, S.H., 1996. Standardized method of aerosol challenge for testing the efficacy of *Mycoplasma gallisepticum* vaccines. Avian Dis. 40, 654-660.

Wilson, D.J., Skirpstunas, R.T., Trujillo, J.D., Cavender, K.B., Bagley, C.V., Harding, R.L., 2007. Unusual history and initial clinical signs of *Mycoplasma bovis* mastitis and arthritis in first-lactation cows in a closed commercial dairy herd. J. Am. Vet. Med. Assoc. 230, 1519-1523.

463 Zhang, R., Han, X., Chen, Y., Mustafa, R., Qi, J., Chen, X., Hu, C., Chen, H., Guo,
464 A., 2014. Attenuated *Mycoplasma bovis* strains provide protection against
465 virulent infection in calves. Vaccine 32, 3107-3114.
466
467

Author/s:
Kanci, A; Wawegama, NK; Marenda, MS; Mansell, PD; Browning, GF; Markham, PF

Title:
Reproduction of respiratory mycoplasmosis in calves by exposure to an aerosolised culture of Mycoplasma bovis

Date:
2017-10-01

Citation:
Kanci, A., Wawegama, N. K., Marenda, M. S., Mansell, P. D., Browning, G. F. & Markham, P. F. (2017). Reproduction of respiratory mycoplasmosis in calves by exposure to an aerosolised culture of Mycoplasma bovis. Veterinary Microbiology, 210, pp.167-173. https://doi.org/10.1016/j.vetmic.2017.09.013.