Pathological and Immunological Responses of Calves to *Mycoplasma Bovis*

L. H. Thomas, C. J. Howard *and* **K. R. Parsons**
A.F.R.C. Institute for Research on Animal Diseases
Compton, Newbury, Berkshire, U.K.

Introduction

Mycoplasma bovis was identified in the UK for the first time in association with a severe outbreak of bovine respiratory disease (14). Subsequently it was shown to cause moderate lesions of arthritis and pneumonia in gnotobiotic calves (6).

The following paper describes a hitherto unrecognized necrotic lesions caused by *M. bovis* in the lung parenchyma of gnotobiotic calves; it describes certain features of the immune response to the infection and contrasts the mechanisms of pathogenesis for *M. bovis* with that of other respiratory mycoplasmas. The paper summarizes some of the work described more fully elsewhere (10,16).

Materials and Methods

Friesian or Aberdeen Angus cross Friesian gnotobiotic calves were reared as described (2). Calves were inoculated intratracheally with 10 ml of a culture containing 5 x 10^9 colony forming units (c.f.u.) of *M. bovis* strain Ab/1 and killed 14 or 27 days after inoculation (Table 1). Three gnotobiotic calves previously inoculated endobronchially with 3 x 20 ml of sterile mycoplasma broth served as controls (7).

Calves were sampled, antibody levels measured and mycoplasmas cultured as indicated in Tables (1,2,3 & 4) and as previously described (5,8,9).

Autopsies were carried out on each animal and tissue taken into formol sublimate for fixation, transferred to 80% ethanol after 24 hours and processed for histology. Sections were rountinely stained by haematoxylin/eosin and by a modification of Sternberger's peroxidase antiperoxidase method for immunolabelling of *M. bovis* organisms (16) and specific immunoglobulin (Ig) producing cells (10).

Results

No clinical signs were observed in the control calves and at slaughter one calf only had a small macroscopic lesion scored at 1% pneumonic consolidation. All four calves inoculated with *M. bovis* showed clinical respiratory signs and pneumonic lesions at slaughter (Table 1). In addition calf S160 showed severe lameness associated with swelling of the left stifle joint.

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TABLE 1. Clinical and macroscopic findings from 4 calves inoculated with *M. bovis.**

Calf	Age at 1st inoc. (days)	Slaughter DPI	Clinical signs	% Pneum.
S127	22	14	+	10
S151	39	14	+	6
S157	42	27	+	5
S160	35	27	+	37

* 5 x 10^9 colony forming units in 10 ml, intratracheally.
\+ Transient fever (peak 40-41° C), raised respiratory rate (60-70 respns/min) and inappetance 3-5 days.
++ Protracted fever (peak 40-41° C) raised respiratory rate (70 respons/min), dyspnoea and inappetance 7-10 days plus swelling of the left stifle joint.
DPI Days post inoculation.
% Pneum. Pneumonic consolidation as a percentage of the dorsal lung surface.

TABLE 2. Ig staining cells in lungs from *M. bovis* infected calves.

Calf no.	Week	No. of cells staining with anti-bovine Ig*: M	G1	G2	A
S127	2	1.6±2.30	111.2±15.79	3.4±4.22	1.2±0.84
S151	2	2.6±1.95	56.4±12.48	0	1.4±1.14
S157	4	0.6±0.89	57.2±29.50	65.6±42.49	0
S160	4	0	76.2± 8.41	12.4±8.34	0

***Mean** ± SD of five 0.10 mm² areas of consolidated lung.

TABLE 3. Isolations of *M. bovis* and antibody responses.

Calf	Lung[1]	Lung wash[2]	Blood[3]	Antibody[4]
S127	**8.1**±0.30	**7.4**	**5**	5.5
S151	6.9±0.33	**6.2**	**1.4**	9.6
S157	8.6±0.24	**6.2**	**NI**	35.9
S160	8.8±0.33	**8.2**	**2,3,5**	31.4

1 Mean number (10^n) ± standard deviation per gram at post-mortem M21 single pool titrated.
2 Number (10^n) cfu per ml lung wash.
3 Indicates day on which isolations were made from blood. ND = not done, NI = none isolated.
4 Antibody by SRH, zone of lysis mm² at post-mortem, all zones = 0 at time of inoculation.

TABLE 4. Antibody to *M. bovis* in sera and lung washings from inoculated calves.[Θ]

Sample*	Week	Titre[x] with anti-isotope (10^n)			
		M	G1	G2	A
S (4)	0	<1	<1	<1	<1
S (4)	1	2.4±0.34	1.3±0.17	<1	<1
S (4)	2	3.4±0.67	2.9±0.32	1.4±0.36	<1
S (2)	3	2.0 (2.0,3.3)	3.7 (3.3,4.0)	2.8 (3.0,2.5)	1.3 (<1,1.9)
S (2)	4	1.9 (1.6,2.3)	4.0 (4.0,4.0)	3.3 (3.1,3.4)	1.5 (<1,2.3)
LW (2)	2	0.6 (0.4,0.7)	0.6 (0.5,0.7)	<1	0.8 (0.1,1.4)
LW (2)	4	<0	2.0 (2.0,2.))	0.9 (0.04,1.7)	1.5 (1.2,1.7)

* S = serum, LW = lung-washing; number of animals sampled in parenthesis.

[x] Geometric mean titre and standard deviation, except lung washings and sera from *M. bovis* infected calves on weeks 3 and 4 where mean is given with duplicate values in parenthesis.

[Θ] No antibody was detected in equivalent samples from 3 uninoculated control calves.

Consolidated areas of lung were red, hepatised and frequently interspersed with raised white indurated nodules. The interlobular septa were prominent and the respiratory lymph nodes enlarged and oedematous. Turbid synovial fluid was present in the left stifle joint of calf S160 and the synovial membrane was covered by a cream coloured caseous layer, 1-2 mm thick. Microscopic lesions in the lungs of all four calves comprised variable sized areas of coagulative necrosis in the lung parenchyma and a suppurative bronchiolitis, with varying degrees of peribronchiolar lymphoreticular hyperplasia. The necrotic lesions was located primarily in the peribronchiolar and perivascular areas of the lung parenchyma and within the larger areas of necrosis vestigial, or "ghost" lung structures could be clearly discerned with cell nuclei in varying stages of necrosis. *M. bovis* organisms were located by immunoperoxidase labelling in these lesions especially at the periphery. Similar lesions of necrosis were identified in the connective tissue of the joint capsule of calf S160 in which *M. bovis* organisms could also be identified. Accumulated around the necrotic lesions in both lung and joint capsule were large numbers of mononuclear cells: macrophages, lymphocytes and plasma cells, varying numbers of which could be shown to be Ig producing cells. In the two calves killed 14 days post inoculation, significant numbers of IgM, IgG1 and IgA producing cells could be identified with IgG1 producing cells predominating. In the two calves killed at 27 days post inoculation, IgM and IgA producing cells were insignificant in number of absent and IgG2 producing cells were present in similar numbers to IgG1 producing cells (Table 2).

Substantial thickening of the interlobular septa was due to oedema and macrophage infiltration of the connective tissue and extensive dilatation of the lymphatic vessels. The suppurative bronchiolitis was often advanced to the stage of abscessation and bronchiectasis with organization of peribronchiolar fibrous tissue. Large numbers of *M. bovis* organisms could be identified in the bronchiolar exudate.

M. bovis was recovered from the lungs of all 4 calves and from the blood of 3 inoculatd with *M. bovis* (Table 3). Specific IgG1, IgG2, IgA and IgM antibody responses to *M. bovis* in the sera and lung washes of the 4 calves and 3 uninoculated control calves at varying times post inoculation are shown in Table 4. Levels of IgG1, IgG2, IgA but not IgM were higher at 27 days compared with 14 days post inoculation.

Appearance of specific antibody reflected the numbers of immune globulin producing cells identified in the lung parenchyma.

Discussion

The lesion caused by *M. bovis* in the lung parenchymas is both striking and characteristic. It may be regarded as a combination of the acute coagulative necrosis induced by the invading mycoplasma and the surrounding immune response of the host. Similar lesions have been recognized and *M. bovis* organisms identified by immunoperoxidase labelling in formalin fixed tissue from field cases from which *M. bovis* organisms had been isolated (Thomas, et al., unpublished observations). Examination therefore of field material by this method may be a useful adjunct to diagnosis.

No less striking than the lesion in the lung parenchyma is the suppurtive bronchiolitis and the marked thickening of the interlobular septa. Both lesions may be readily recognized macroscopically and the former is responsible largely for the multiple, focal, indurated, lesions that may be palpated in the consolidated tissue.

The proliferative element of the lesion in the lung parenchyma is similar in character albeit located differently to the peribronchiolar and perivascular lymphoid hyperplasia seen in other mycoplasma infections (3) and this similarity is reinforced by the demonstration of specific immune globulin producing cells amongst the mononuclear cell accumulations. The sequential appearance of the antibodies (IgM, IgG1 and IgG2) is consistent with the ontogeny of the bovine immune response (12). IgG1 and IgG2 clearly represent the major isotypes available in the sera or lung secretions which may act directly on the mycoplasmas or promote their killing by macrophages and neutrophils. Although IgA antibody was present in sera and tracheobroncial washings, few IgA producing cells were located in the lung. It seems probably therefore that this antibody was secreted locally in the tracheobronchial or nasal mucosa.

The difference in location of the lesion compared with

other mycoplasma infections may be related to the invasive nature of *M. bovis*. Whereas other respiratory mycoplasmas, e.g. *M. dispar* and *M. pneumoniae* locate on the respiratory epithelium of tracheal organ cultures (1, 13) from where they exert their pathogenic effect, *M. bovis* has the capacity to penetrate between the cells of the respiratory epithelium into the lamina proprias below (15). This invasiveness would also appear to relate to the appearance of *M. bovis* organisms in the blood stream and the occasional resulting arthritis.

The pathogenic mechanism of the lesion in the lung parenchyma has not yet been elucidated, a toxin has been described for *M. bovis* (4) but no evidence of such a toxin has yet been demonstrated in organ cultrue (L. H. Thomas and C. J. Howard, unpublished observations). Furthermore, *M. bovis* is non-motile (W. Bredt and L. H. Thomas, unpublished observations) and does not have the flask shaped morphology possessed by other pathogenic mycoplasmas such as *M. pneumoniae*.

In conclusion *M. bovis* has been shown previously to be capable of causing acute mastitis in cattle (11) and in the present work of causing severe progressive pulmonary lesions. These findings coupled with its ability to invade the bloodstream must place it with *M. mycoides* subsp. *mycoides* as one of the most pathogenic mycoplasmas for cattle. The present description of the macroscopic and microscopic lesions and the immunolabelling of the organism in formalin fixed tissue could be a useful aid to diagnosis.

Summary

Mycoplasma bovis has been shown to produce pneumonia in gnotobiotic calves following intratracheal inoculation. At post mortem 14 or 27 days later macroscopic lesions involved 5 to 37% of the lung. Microscopic examination revealed hitherto unrecognized but characteristic focal areas of coagulative necrosis in the lung parenchyma surrounded by infiltrating mononuclear cells, comprising macrophages, lymphocytes and plasma cells. *M. bovis* antigen was demonstrated in the necrotic areas by immunoperoxidase staining, particularly at the interface with the infiltrating leucocytes. Immunoperoxidase staining of lung sections demonstrated IgG1 and IgG2 producing cells amongst the leucocyte accumulations. A marked suppurative bronchiolitis, with remarkably little damage to the bronchial epithelium, was also a prominent feature of the histopathology. The lesion in the lung appeared to be due partly to the necrotising effect of *M. bovis* and partly to the host's immune response. The pathogenesis of this lesion may be related to the ability of *M. bovis* to penetrate the respiratory epithelium as demonstrated in organ cultures and contrasted with that induced by less invasive mycoplasmas, such as *M. dispar* and *M. pneumoniae*, which remain localized in the ciliated epithelium of the airways.

References

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