Experimental infection of calves with *Mycoplasma mycoides* subspecies *mycoides* Large Colony

Inoculation of calves with the goat pathogen *Mycoplasma mycoides* subspecies *mycoides* Large Colony (MmmLC) showed that calves were refractory to infection and that horizontal transmission occurred but did not lead to clinical disease. Neither clinical signs nor lesions developed in orally inoculated or in-contact calves despite the organism being present. Intravenously inoculated calves became pyrexic and recumbent with septic arthritis.

An outbreak of disease caused by *Mycoplasma mycoides* subsp *mycoides* Large Colony (MmmLC) occurred recently in goat kids and young calves that had been fed unpasteurised bulk goat milk and colostrum[1].

The disease in calves manifested as fibrinosuppurative polyarthritis and multifocal pneumonia[2]. An experiment was designed to investigate the potential role of this organism as a significant pathogen of calves and to determine whether infected calves would transmit infection horizontally to other calves.

Experimental design

Twelve five- to seven-day-old calves from a commercial dairy herd were used in the trial. The herd had no contact with goats. The calves had been fed pooled bovine colostrum that tested negative for MmmLC. They were then transported to the Silverstream Isolation Unit where they were housed indoors in two pens.

Six of the calves were dosed orally with MmmLC ($5.4x10^{11}$ colony forming units or cfu) and the following day four control calves were placed with them in the same pen.

Six days later the two remaining calves were inoculated intravenously (IV) with MmmLC ($7x10^{10}$ cfu) and placed with the other calves. The calves were monitored for clinical signs and their temperatures were measured daily for the first 14 days.

Nasal swabs and blood samples were collected from the day of oral inoculation (day 0) until the day each calf was euthanased (the last ones on day 43). Nasal swabs were collected on days 0, 2, 4, 6, 8 and at necropsy; blood samples weekly and at necropsy. Necropsies were carried out at regular intervals during the trial (see table) and samples taken from tonsil, retropharyngeal and mesenteric lymph nodes, trachea, lung, spleen, pericardial fluid and joint fluid (stifle, carpal and hip). Both fresh and fixed samples were collected.

The nasal swabs and tissues were cultured for MmmLC. The same samples plus bloods were tested in the CAP-21 polymerase chain reaction (PCR)[3] for *Mycoplasma mycoides* cluster. The nasal swabs were also tested in a generic PCR for mycoplasma[4] on days 0 and 2. Serum samples were tested in the *M mycoides* complement fixation test (CFT) using whole cell antigen[6]. Histopathology was carried out on the formalin fixed tissues.

Trial results

Most of the laboratory results are summarised in the table but some, especially sequential data, are captured only in text.

Clinical signs

There was no significant difference between the temperatures recorded in the orally inoculated group and the in-contact group. Individual temperatures ranged between 38.3°C and 39.5°C depending on the day. No significant clinical signs were seen in these two groups apart from coughing in one in-contact calf (calf 11).

In contrast, the two calves inoculated intravenously (numbers 7 and 8) developed elevated temperatures (>39.5°C) from day 7 post inoculation and the temperatures remained high until euthanasia, reaching 40.4°C in one case. Both became anorexic and reluctant to move, spending an increasing amount of time in sternal recumbency. However, their joints were not obviously enlarged.

Bacteriology

Nasal swabs: Non-MmmLC mycoplasmas were isolated from many of the nasal swabs throughout the experiment, from pre-inoculation (day 0) to the last necropsy on day 43. MmmLC was isolated from two orally inoculated calves on days 4 and 7. It was also isolated from both intravenously inoculated calves at necropsy (days 10 and 15).

Tissues: In the orally inoculated animals MmmLC was isolated from the tonsil (4/6), retropharyngeal lymph node (1/6), trachea (3/6), lung (1/6) and spleen (1/6). Most isolates were from tissues collected up to 14 days after inoculation, although in calf 6 MmmLC could still be isolated from the tonsil and trachea on day 43. MmmLC was isolated from all tissues, including joint fluids, from the intravenously inoculated calves. No MmmLC was isolated from the tissues of the four in-contact calves.

PCR testing

Nasal swabs: Four mycoplasmas were detected in the generic mycoplasma PCR on day 0 but none was MmmLC positive. On day 2, one of 12 swabs was positive for MmmLC. By day 4, six of 12 were positive. Positives continued to be detected until day 14, after which only the intravenously inoculated animals were positive.

Blood samples (EDTA): All calves were negative on day 0. By day 7, eight of nine were positive in the CAP-21 PCR. MmmLC continued to be detected in the blood leucocyte fraction from most calves

throughout the trial and until day 43 in the last remaining calves.

Tissue samples: The tonsils, retropharyngeal lymph nodes, tracheas, spleens and mesenteric lymph nodes were almost all positive in the calves necropsied up to day 14. This included one in-contact calf. Thereafter, apart from the two intravenously inoculated calves, most tissues were negative. All tissues in the two intravenously inoculated animals were positive for MmmLC.

Serology

Pre-inoculation, ten of the 12 calves had variable titres in the CFT ranging from 3:10 to 3:80. A positive titre is regarded as = or > 2:20. The two calves (8 and 12) with no detectable antibody (<1:5) may have had an inadequate colostrum intake. Most of the calves became seronegative by day 21. No animals seroconverted to MmmLC.

Necropsy

Gross lesions were largely confined to the two calves inoculated intravenously. These animals had several focal areas of red consolidation in the cardiac and anterior caudal lobes of the lungs. There was excess cloudy fluid in the joints, which was under pressure when incised. Two in-contact calves (calves 11 and 12) also had lung lesions. The one that had been coughing (calf 11) had several small areas of red consolidation in the right middle and caudal lobes. Calf 12, which was necropsied on day 43, had the most obvious lung lesions with multifocal areas of red consolidation (2-3 cm in diameter) in midzonal areas of the posterior cranial, middle and anterior caudal lobes of both lungs.

Histopathology

Six calves showed respiratory tract lesions, which included subacute to chronic mucopurulent bronchitis and bronchiolitis (three calves), mild suppurative bronchopneumonia (two calves) and mild subacute non-suppurative tracheitis (four calves).

Because of the failure to demonstrate MmmLC, further investigations were carried out on the lung tissues of the in-contact calf (calf 12) with the most severe lesions. The lesions were consistent with the inhalation route of infection. Gram stains showed the presence of sparse Gram-negative coccobacilli in the exudate. A PCR for *Pasteurella multocida* was negative as was virus isolation. Joint lesions were present in the two calves inoculated intravenously. There was ulceration of the synovial membrane and a subacute fibrinopurulent synovitis.

Discussion

Only the two intravenously inoculated animals showed significant clinical signs. Both animals developed protracted pyrexia and signs related to polyarthritis, which was confirmed by histopathology.

Calf 11 was coughing and had bronchiolitis on necropsy. The calf with the most severe lung lesions (calf 12) had evidence of a bacterial bronchopneumonia. Both were in-contact animals. The responsible organism was probably environmental as the animals were housed indoors in pens with straw over a concrete floor. Calf 12 may also have had an inadequate colostral intake as indicated by the negative initial MmmLC CFT.

Although several animals had lung lesions, MmmLC was only isolated from the lungs of the two animals inoculated intravenously. All the calves except two had received variable levels of colostral antibodies that reacted in the MmmLC CFT. The two that did not have antibodies may not have received adequate colostrum. These antibodies may not have been specific but may have been caused by exposure of their dams to other mycoides cluster mycoplasmas or other mycoplasmas that share antigens with MmmLC[6]. By three weeks these antibodies had virtually disappeared or were at low levels.

None of the calves developed detectable antibody to MmmLC during the course of the trial in spite of the fact that MmmLC was detected in blood and other tissue. This implies specific humoral responses to this organism are being delayed. MmmLC may have evolved methods to elude the humoral immune response. It has been shown that mycoplasmas have mechanisms that produce

Summary of laboratory results

		Necropsy days post inoc	Culture results		PCR results			Pathology results
			Nasal swab	Tissues	Nasal swab	Blood	Tissues	
Group	**Calf**							
Oral	1	4	+ (1)	+ (1)	+ (1)	-	+ (2)	
Oral	2	7	+ (1)	+ (5)	+W (1)	+ (1)	+ (4)	
Oral	3	14	-	+ (2)	+ (4)	+ (2)	+ (5)	
Oral	4	21	-	-	+W (1)	+ (2)	+W (3)	Tracheitis
Oral	5	28	-	-	+W (1)	+ (4)	-	Tracheitis
Oral	6	43	-	+ (2)	+ (2)	+ (4)	+ (2)	Tracheitis/bronchopneumonia
IV	7	10	+ (1)	+ (10)	+ (2)	+ (1)	+ (10)	Synovitis
IV	8	15	+ (1)	+ (10)	+ (2)	+ (2)	+ (10)	Synovitis/bronchitis
In-contact	9	14	-	-	+ (3)	+ (2)	+ (5)	
In-contact	10	21	-	-	+ (1)	+ (2)	-	
In-contact	11	28	-	-	+ (2)	+ (2)	-	Bronchitis
In-contact	12	43	-	-	+ (1)	+ (5)	+(2)	Tracheitis/bronchopneumonia

Key

IV = Intravenous
\+ = MmmLC positive
( ) = Number of positives
\- = Negative
+W = Weak positive

variations in their surface antigens, which may allow subpopulations of mycoplasmas to avoid the immune response[5]. It is also possible that some mycoplasmas may avoid the immune response by becoming intracellular rather than surface pathogens. Additionally, the CFT is recognised as having a low sensitivity[6].

MmmLC could be detected from nasal swabs of most calves by PCR from about day 4. However, it could be isolated from only two calves on one occasion, if the calves that were inoculated intravenously were excluded. The isolation of non-MmmLC mycoplasmas from many swabs illustrated the fact that mycoplasmas can reside in certain sites without causing harm. Because of the mixed cultures present, nasal swabs are not good diagnostic samples. Culture and PCR results from tissues obtained at necropsy were similar. If the intravenously inoculated animals were excluded then most positives were obtained from the tonsil, retropharnygeal lymph node and trachea. In the IV inoculated animals, every tissue sampled was positive.

The PCR tests on blood leucocytes were negative on day 0 but by day 7 all except one of the 12 calves were positive. This animal was positive by day 14 and the animals remained persistently positive until the end of the project. No cultures were attempted from blood. The results suggest a persistent bacteraemia without progression to clinical disease over the six weeks of the project.

Conclusion

The trial showed that under normal husbandry conditions calves appear to be relatively refractory to infection with the goat pathogen MmmLC. Infection of calves held in close contact with inoculated calves did occur but numbers of the organism were low as it could not be detected by culture alone.

Disease can be produced with artificially high doses of the organism given intravenously and factors such as inadequate intake of colostrum may contribute. It is not known if the cross-reacting colostral antibodies detected in these calves are found commonly in New Zealand dairy herds or were a feature of this herd. If commonly present they may confer some level of passive protection to calves in the national herd. However, it is probable that the feeding of contaminated goats' milk is a more important factor.

It was not possible to duplicate exactly the conditions of the field outbreak. In that case calves were fed bulk goats' milk containing an unknown titre of MmmLC over a number of days. It is known that individual goats can excrete high titres of MmmLC in their milk (up to 10^9/ml)[7]. Another variable is the colostral intake as there was evidence that many of the affected calves in the field outbreak had received inadequate colostrum.

In this trial, calves were given one large dose of MmmLC orally. A confounding factor was that the calves might have received some protection from cross-reacting antibodies present in the pooled colostrum. The colostrally derived antibody decayed at the expected half-life and had almost gone by day 21. This did not prevent the inoculated and in-contact calves from becoming infected as evidenced by the presence of MmmLC in blood leucocytes and the isolations from tonsil, retropharyngeal lymph node and trachea. However, there were no clinical signs or lesions in the orally inoculated or in-contact calves. Neither did they seroconvert during the 43-day duration of the experiment. In contrast, when 51 goat kids were given a single oral dose containing fewer organisms ($1x\ 10^6$ cfu) 72% died[8].

The lung lesions seen in several of the in-contact calves appeared to be caused by environmental bacteria rather than MmmLC.

Disease was produced in the two calves given a large dose of MmmLC intravenously. This manifest as persistent pyrexia and polyarthritis, but the organism could be recovered from all tissues. The calves did not seroconvert before euthanasia. The calf with a low titre (1:40) of colostral antibody succumbed to the infection before the second calf, which was seronegative, suggesting these antibodies were not protective.

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