

Studies on *Mycoplasma mycoides* subsp. *mycoides* (LC) in Lambs and Calves

R.B. Truscott and G.G. Finley*

ABSTRACT

Six cesarean-derived lambs were inoculated either with 4.5×10^4 , 4.5×10^6 or 4.5×10^8 *Mycoplasma mycoides* subsp. *mycoides* intratracheally. One animal receiving the intermediate dose died four days post-inoculation, the two receiving the high dose died six days postinoculation, while one receiving the low dose died eight days postinoculation. The two surviving lambs were challenged on day 20 postinoculation with 1×10^8 organisms subcutaneously and 2×10^9 organisms intravenously. One animal died eight days following this challenge while the other survived and was killed. Six conventionally reared lambs challenged with 90 to 8500 organisms by intranasal and intraocular instillation failed to become infected. Three conventionally reared calves were each inoculated with 1×10^8 organisms by each of intratracheal, subcutaneous and intravenous routes. They were killed 20 days postinoculation without having shown any clinical signs.

Key words: *Mycoplasma mycoides*, infection, lambs, calves.

RÉSUMÉ

Cette expérience consistait à introduire dans la trachée de six agneaux obtenus par césarienne, les doses suivantes de *Mycoplasma mycoides* subsp. *mycoides*: $4,5 \times 10^4$; $4,5 \times 10^6$ ou $4,5 \times 10^8$. Un des deux qui avaient reçu la dose intermédiaire mourut au bout de quatre jours; les deux auxquels on avait donné la plus forte dose moururent au bout de six jours, tandis que l'un des deux qui avaient reçu la dose la plus faible mourut au bout de

huit jours. Au bout de 20 jours, les deux survivants subirent une infection de défi qui consistait en une inoculation sous-cutanée de 1×10^8 mycoplasmes et en l'injection intraveineuse de 2×10^9 des mêmes mycoplasmes. Un de ces deux agneaux mourut au bout de huit jours, tandis que l'autre survécut et fut éventuellement sacrifié. Six agneaux élevés de façon conventionnelle et soumis à une infection de défi par l'instillation intranasale et intratrachéale de 90 à 8500 mycoplasmes, ne développèrent pas d'infection. Trois veaux élevés de façon conventionnelle reçurent 1×10^8 mycoplasmes, par chacune des voies suivantes: intraveineuse, sous-cutanée et intratrachéale. On les sacrifia au bout de 20 jours, sans qu'ils aient manifesté de signes cliniques.

Mots clés: *Mycoplasma mycoides*, infection, agneaux, veaux.

The presence of *Mycoplasma mycoides* subsp. *mycoides* in goats in Ontario (1) prompted an investigation to determine the possible hazard this organism might present to Canadian sheep and cattle.

The test strain D44, had been isolated from an affected goat herd. It was from an animal showing polyarthritis and young kids in this herd were affected with polyarthritis and septicemia. It had been cloned three times and was in low passage when it was kindly provided by Dr. Rosendal (University of Guelph). The organism was grown for 24 h in Hayflick type broth (2), centrifuged at $35000 \times g$ for 30 min and the pellet suspended in PBS. Dilutions were made to provide doses of 4.4×10^4 , 4.4×10^6 and 4.4×10^8 colony forming units (CFU) or as further stated. Plate counts were

performed to determine CFU.

Six cesarean-derived lambs were held in a newly constructed isolated barn and transferred to isolation cubicles at five months of age. Each of two lambs were inoculated with one of the above doses intratracheally (IT). Deaths occurred following a brief period of fever and inappetance from four to seven days postinoculation (p.i.). Lambs in extremis were euthanized and considered to have died. Two surviving lambs were clinically normal 19 days p.i. and were challenged on day 20 p.i. with 1×10^8 organisms subcutaneously (SC) and 2×10^9 organisms intravenously (IV). One animal died eight days following challenge while the other survived and was killed. Animals were necropsied shortly after death. Tissue samples were taken from all major organs as well as synovial membranes of hip and carpal joints and were immediately fixed in 10% buffered formalin or placed in sterile containers for culture. Tissues were trimmed, embedded in paraffin, sectioned at $5 \mu\text{m}$ and stained with hematoxylin and eosin (H&E). Gross examination revealed a two to fourfold increase in synovial fluid in carpal joints of three lambs, one from each of the three treatment groups. One animal receiving the intermediate dose had extensive polyarthritis with fibrin in and around all joints examined. Five of six lambs had 1 to 5 cm diameter yellow areas in the subcutaneous tissue adjacent to the site of IT inoculation. Histologically the subcutaneous lesions were areas of acute cellulitis consisting of necrosis, fibrin and large numbers of neutrophils. The lamb with polyarthritis had abundant fibrin and neutrophils both in the joints and adjacent to synovial membranes. The surviving lamb had no gross lesions. Sites of recovery of

*Agriculture Canada, Animal Pathology Laboratory, P.O. Box 1410, Sackville, New Brunswick E0A 3C0.

Submitted June 8, 1984.

TABLE I. Recovery of *Mycoplasma mycoides* subsp. *mycoides* from Intratracheally Infected Cesarean-derived Lambs

# Animal	Dose (CFU)	Clinical Symptoms	Days to Death	Organisms Recovered									
				Nasal Secretions	Blood	Liver	Heart	Spleen	Lungs	Kidneys	Bladder	Brain	Joints
1	4.4 x 10 ⁴	Fever	Sur ^a	+ ^b	+ ^b	—	—	—	—	—	—	—	—
2	4.4 x 10 ⁴	Fever; Inappetance	7	+	+	+	+	+	+	+	+	+	+
3	4.4 x 10 ⁶	Fever	8 ^c	+	+	+	+	+	+	+	+	+	+
4	4.4 x 10 ⁶	Fever; Inappetance	4	NA ^d	NA	+	+	+	+	+	NT ^e	NT	+
5	4.4 x 10 ⁸	Fever; Inappetance	6	—	+	+	+	+	+	+	—	+	+
6	4.4 x 10 ⁸	Fever; Inappetance	6	+	+	+	+	+	+	+	+	+	+

^aSurvived — killed 14 days following second challenge

^bPositive at five and seven days following initial challenge only

^cSurvived and was rechallenged, died eight days later

^dNot applicable — animal died before first samples collected

^eNot tested

the organism are presented in Table I.

Six conventionally reared lambs five months of age were inoculated with the organism by instillation of 0.2 mL into each nostril and 0.1 mL into the right eye only. The total dosages given to each of two lambs was 90, 900 and 8500 CFU. These lambs remained clinically normal and were killed at 17 and 18 days p.i. Isolation attempts were made from the nostrils, eyes and lungs. There were no significant gross lesions in any of the lambs. Microscopic examination of tissues of each lamb including trachea, turbinates, liver, eyes, heart, synovial membranes (2), spleen, kidneys or lungs did not reveal significant lesions. Organisms were not isolated from the tissues cultured.

Three, three month old conventionally reared calves were placed in isolation and inoculated with organisms which had been isolated from one of the dead lambs. Each calf was inoculated with 1 x 10⁸ organisms IV, 1 x 10⁸ IT and 1 x 10⁸ SC. These animals remained clinically normal and were killed 20 days p.i. No gross pathology was evident in two calves

while the third had increased synovial fluid in joints of the right side including carpal, fetlock, hip and stifle. Slight subcutaneous adhesions were evident at the neck injection site of this animal as well. No microscopic lesions were evident in the first two calves. The latter calf had a very slight peri-arthritis (carpal joint) and cellulitis at the site of subcutaneous injection. *Mycoplasma mycoides* subsp. *mycoides* was not isolated from any of the tissues and organs cultured.

Sera were collected from all animals prior to inoculation and euthanasia and were tested by passive hemagglutination test using an antigen (3) prepared from the test strain. Only the cesarean-derived lamb which survived showed a response with a titer of 1/40, eight days after initial inoculation and 1/160 just prior to euthanasia.

The results indicate that *Mycoplasma mycoides* subsp. *mycoides* is capable of producing illness in experimentally inoculated lambs when 4.5 x 10⁴ organisms were given intratracheally whereas 3 x 10³ organisms instilled into each nostril along with 1.5 x 10³ into the eye did not. It is

interesting that detectable antibodies were present in only one of the cesarean-derived lambs. This individual survived both the initial and secondary inoculations which suggests that vaccination might be of very limited assistance should this organism become a problem in sheep flocks. On the other hand the resistance to infection by the calves tends to suggest that the large colony type as presently found within the goat population does not present a serious hazard for the cattle population in Canada.

REFERENCES

1. RUHNKE HL, ROSENDAL S, GOLTZ J, BLACKWELL TE. Isolation of *Mycoplasma mycoides* subspecies *mycoides* from polyarthritis and mastitis of goats in Canada. Can Vet J 1983; 24: 54-56.
2. HAYFLICK L. Tissue cultures and mycoplasmas. Texas Report Biol Med 1965; 23 (Suppl. 1): 285-303.
3. CHIMA JC, ONOVIRAN O. A passive haemagglutination test for detection of antibodies against *Mycoplasma mycoides* subsp. *mycoides* using glutaraldehyde-fixed sheep erythrocytes. Vet Microbiol 1982; 7: 343-349.