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The ovine mammary gland as an experimental model to determine the virulence of animal ureaplasmas

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SUMMARY

As an estimate of their virulence, the ability of ovine, bovine, canine, feline and simian ureaplasma strains to cause mastitis in the ovine mammary gland was investigated. Five ovine ureaplasmas produced a clinical mastitis. Broth cultures of seven bovine ureaplasmas were unable to infect the ovine gland, but two of these strains plus one other were able to do so following passage through the bovine udder. One of two canine strains and a feline strain both caused mastitis, but the simian strain persisted at low titre for only 5 days post-inoculation in one of the two ewes tested.

INTRODUCTION

Experimental intramammary inoculation of cows, goats and mice has been used to determine the virulence of bovine, caprine, simian, human and canine ureaplasma strains (Gourlay, Howard & Brownlie, 1972; Gourlay, Brownlie & Howard, 1973; Howard, Gourlay & Brownlie, 1973; Howard *et al.* 1975). In recent studies, the virulence of ovine ureaplasma strains in both the bovine (Mackie & Ball, 1984) and ovine (Ball & Mackie, 1984/5) mammary gland has also been demonstrated.

The present study was initiated as an attempt to extend the previous findings, by examining the use of the ovine mammary gland model as a determinant of the virulence of additional ovine strains and also bovine, canine, feline and simian ureaplasmas.

MATERIALS AND METHODS

Ureaplasma strains

The ureaplasmas used in the experiment were all isolated at this laboratory and consisted of 5 ovine, 8 bovine, 2 canine, 1 feline and 1 simian strains (Table 1). Before experimental inoculation, each was cloned three times following filtration through Millipore filters as recommended by the Subcommittee on the Taxonomy of Mycoplasmatales (1972). This involved 9–12 passages in artificial medium. The bovine ureaplasmas consisted of three each of serogroup A and B strains and two serogroup C strains (Howard & Gourlay, 1981). The canine strain 1702 and 2715 (Ball & Bryson, 1982) and the feline strain MM30c cross-reacted with the Japanese strains D1M-C, D6P-C and FT2-B respectively (Harasawa, Yamamoto & Ogata, 1977; Kotani & Ogata, 1979).

Table 1. *Ureaplasma strains used for intramammary inoculations*

	Ureaplasma strain	Origin
Ovine	WF98	Grossly normal vulva
	MS118	Grossly normal vulva
	53A*	Vulva from an abattoir survey of urogenital tracts
	98A*	Vulva from an abattoir survey of urogenital tracts
	26D*	Uterus from an abattoir survey of urogenital tracts
Bovine A	MC576	Pneumonic calf lungs
	MC534	Pneumonic calf lungs
	MC488	Pneumonic calf lungs
Bovine B	MC566	Pneumonic calf lungs
	MC629	Pneumonic calf lungs
	MC482	Pneumonic calf lungs
Bovine C	73A†	Vulva from an abattoir survey of urogenital tracts
	MC760	Vaginal discharge
Canine	2715‡	Pneumonic dog lungs
	1702‡	Pneumonic dog lungs
Feline	MM30c	Pneumonic cat lung
Simian	MM67a	Chimpanzee vagina

* McCaughey & Ball (1981). † Ball & McCaughey (1979). ‡ Ball & Bryson (1982).

Sheep experimental infection

Fourteen ewes were used in the experiment to test the 17 ureaplasma strains (Table 2). Each was suckling a single lamb of at least 5 weeks of age at the time of the first intramammary inoculation. Ten of the ewes were yearlings (nos. 1–10) with their first lamb, and four were 2 years old (nos. 11–14) and had been used for an ovine experimental mastitis infection experiment during their previous lactation (Ball & Mackie, 1984/5).

Each intramammary inoculation of a ewe consisted of 2 ml of a freshly grown culture in TB broth (Ball & Mackie, 1984/5), into one of the two glands, the other being kept as an uninoculated control. Milk containing bovine ureaplasma strains MC576, MC482 or MC760 from infected quarters of two cows (Ball & Mackie, unpublished results) was also used to inoculate four ewes. The cows' milk was collected on the day of inoculation of the ewes. All inocula were titrated by duplicate 10-fold dilutions (10^1–10^8) in TB broth immediately before inoculation, incubated for 6 days at 37 °C and the titres expressed as colour-changing units (c.c.u.) per 0·2 ml.

The experimental inoculation, the collection and testing of milk samples and the electronic measure of the cell count total was carried out as described before (Ball & Mackie, 1984/5). When a ewe was used to test more than one isolate, the second test was carried out in the control gland of the first test. Any strains subsequently inoculated into the same ewe were inoculated at least 3 weeks after the final re-isolation from the previous infection of the particular gland.

Table 2. *Ureaplasma re-isolation from experimental intramammary inoculation of ewes*

Ewe no.*	Infected with ureaplasma strain	Titre of inoculum (c.c.u./0·2 ml)	Delay between ureaplasma inoculation and re-isolation	Duration of infection (from first re-isolation)
	Ovine			
1	WF98	10^4	None	Until after weaning (>12 weeks)
2	MS118	$10^{5\cdot6}$	24 days†	12 weeks
3 (a)	53A	10^4	15 days†	9 weeks
4	98A	10^3	—	No infection
5	98A	10^4	None	Until after weaning (>12 weeks)
6	26D	$10^{3\cdot4}$	None	Until after weaning (>12 weeks)
	Bovine A			
11 (a)	MC576	$10^{2\cdot3}$	—	No infection
11 (b)	MC576‡	10^5	—	1 day
7 (b)	MC576‡	10^2	6 days	16 days
3 (b)	MC534	$10^{4\cdot5}$	None	2 days
12 (a)	MC488	10^5	2 days	1 day
	Bovine B			
8 (a)	MC566	10^3	—	No infection
8 (b)	MC629	$10^{3\cdot4}$	—	No infection
9	MC482‡	10^2	None	15 days
	Bovine C			
11 (c)	73A	$10^{4\cdot5}$	—	No infection
13 (a)	MC760	$10^{4\cdot5}$	—	No infection
13 (b)	MC760‡	10^1	None	4 weeks
	Canine			
3 (c)	2715	$10^{4\cdot5}$	—	No infection
10 (a)	2715	$10^{5\cdot6}$	—	No infection
7 (a)	1702	10^6	None	10 days
	Feline			
12 (b)	MM30c	$10^{4\cdot5}$	None	17 days
	Simian			
14	MM67a	$10^{2\cdot3}$	1 day	5 days
10 (b)	MM67a	10^2	—	No infection

* (a, b and c) Order of intramammary inoculation of strains.
† Delay before sustained isolation.
‡ Infected milk from experimental bovine mastitis.

RESULTS

Clinically the mastitis produced by all ureaplasma strains was similar, infection being limited to the inoculated gland, giving rise to an increase in the cell count from 10^5–$10^{5\cdot5}$ cells/ml in normal glands to 10^7–$10^{7\cdot5}$. The result of this was atrophy of the gland and reduction in the volume of milk produced. A rise in the cell count corresponded to the re-isolation and rise in titres of ureaplasmas in the milk, up to a maximum of 10^8 c.c.u./0·2 ml.

The results of the inoculations are summarized in Table 2.

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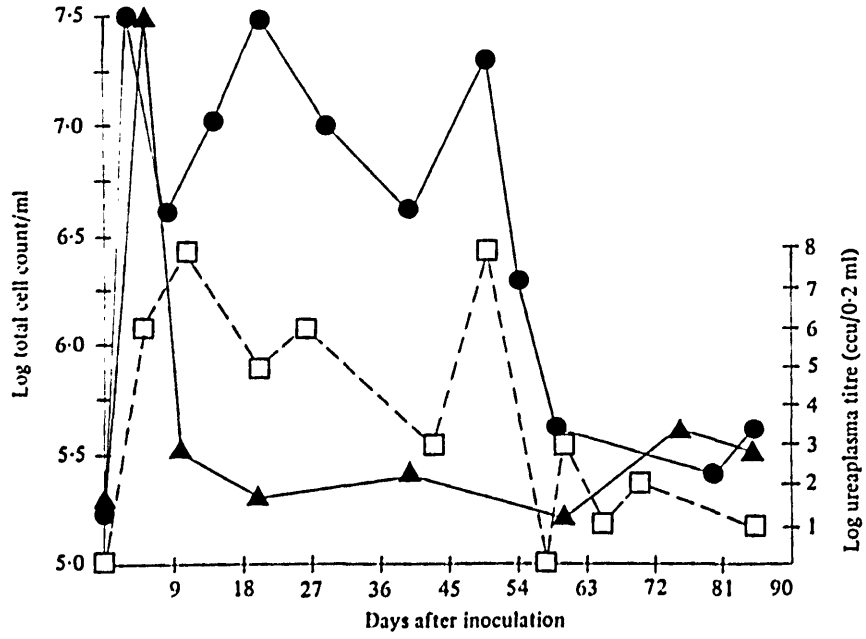

Fig. 1. Number of cells and ureaplasmas in milk of ewe no. 1 following intramammary inoculation with ovine strain WF98. ●–●, Cells in infected gland; ▲–▲, cells in control gland; □–□, ureaplasma.

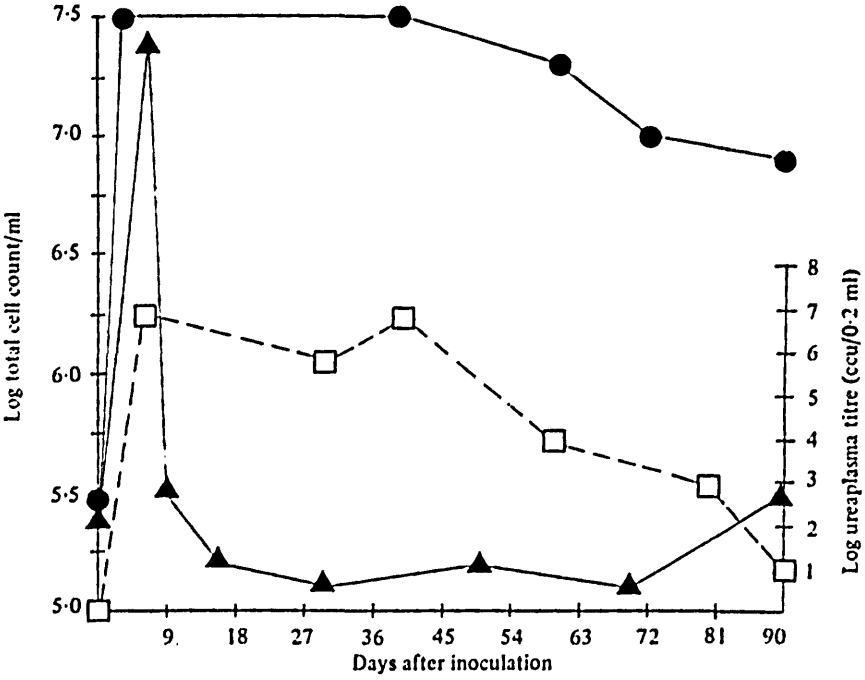

Fig. 2. Number of cells and ureaplasmas in milk of ewe no. 6 following intramammary inoculation with ovine strain 26D. ●–●, Cells in infected gland; ▲–▲, cells in control gland; □–□, ureaplasma.

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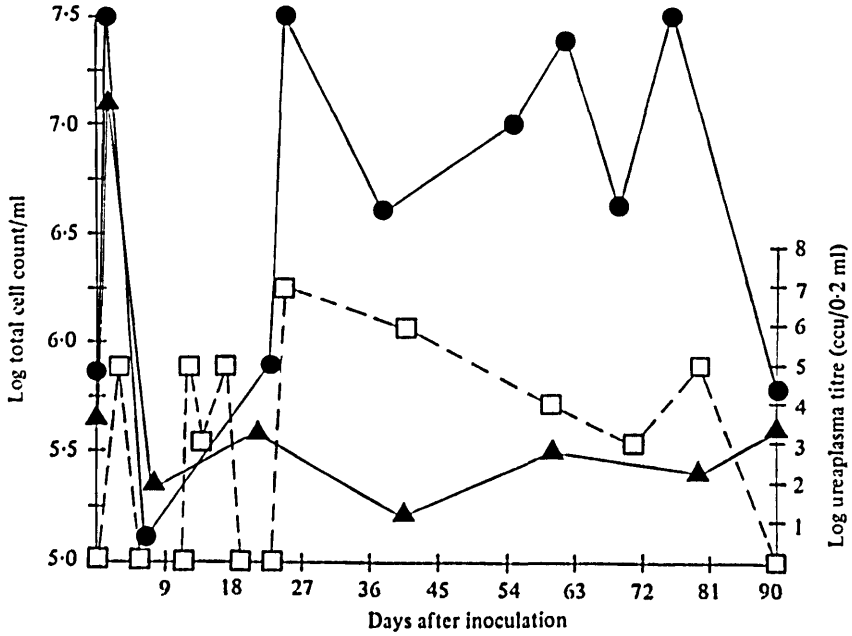

Fig. 3. Number of cells and ureaplasmas in milk of ewe no. 2 following intramammary inoculation with ovine strain MS118. ●–●, Cells in infected gland; ▲–▲, cells in control gland; □–□, ureaplasma.

Ovine ureaplasmas

Ovine strains WF98, 98A and 26D inoculated into ewes nos. 1, 5 and 6 respectively caused an immediate infection following inoculation, persisting until the lambs were weaned (>12 weeks). The infection in ewe 1 was two-phase, the cell count dropping to control gland levels from 55 days post-inoculation, corresponding to a drop in the ureaplasma titres to 10^1–10^2 c.c.u./0·2 ml (Fig. 1). Both cell count and ureaplasma titre remained high until the end of the experiment in the infection in ewes 5 and 6 (Fig. 2).

Strain 98A, which infected ewe 5, failed to cause an infection in ewe 4.

Delays between inoculation and re-isolation were seen with the other two ovine strains, MS118 and 53A respectively. Initially, both strains were re-isolated for a short period for 4 and 3 days respectively, immediately following inoculation into ewes 2 and 3. Strain MS118 was again isolated from 8 to 20 days post-inoculation, during a period when the cell count had dropped to normal levels after the peak following inoculation (Fig. 3). The re-isolation period corresponding to a rise and maintenance of the high cell count was from 24 days to 12 weeks post inoculation for ewe 2 and from 15 days to 9 weeks post inoculation for ewe 3.

Bovine ureaplasmas

Inoculations of broth cultures of bovine strains MC576, MC534, MC488, MC566, MC629, 73A and MC760 failed to cause an infection in six ewes (three yearlings and three 2-year-olds). Strains MC534 and MC488 were the only strains re-isolated, for 2 days and on the third day after inoculation respectively.

Ureaplasmas were re-isolated from all four ewes (two yearlings and two

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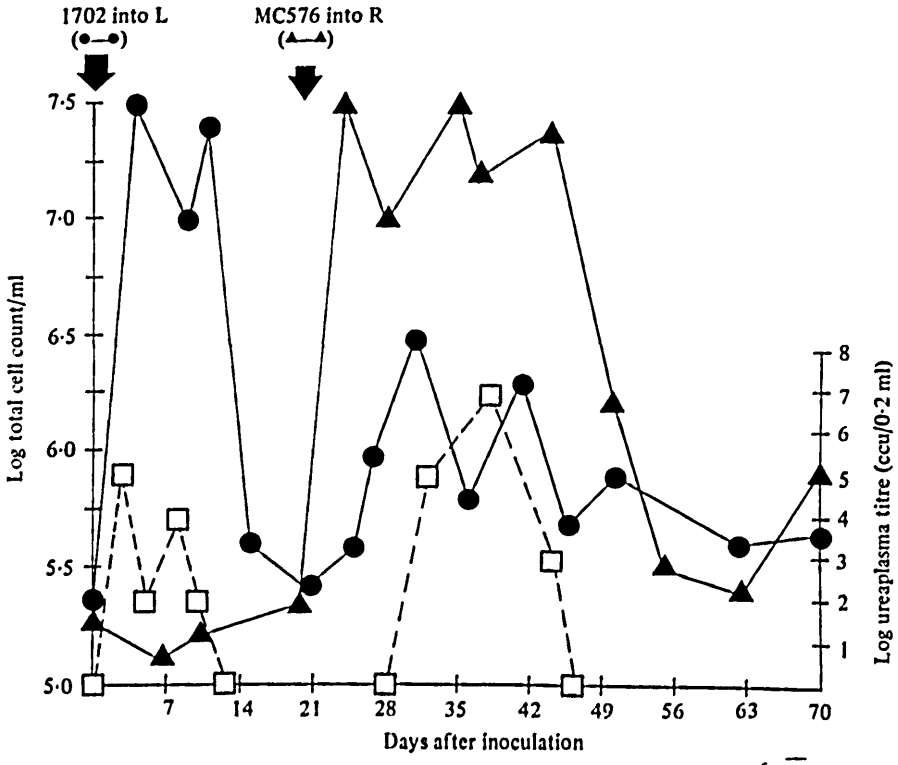

Fig. 4. Number of cells and ureaplasmas in milk of ewe no. 7 following initial intramammary inoculation of canine strain 1702 into the left gland (●–●) followed by intramammary inoculation of bovine strain MC576 in infected bovine milk into the right gland (▲–▲). □–□, Ureaplasmas.

2-year-olds) inoculated with bovine ureaplasma infected bovine milk. In the 2-year-old and the yearling inoculated with MC576 the re-isolation was limited to 1 day and from 7 to 23 days post-inoculation respectively (Fig. 4). In the yearling and 2-year-old inoculated with MC482 and MC760, the infection persisted for 15 days and 4 weeks after inoculation respectively.

Canine, feline and simian ureaplasmas

Canine ureaplasma strain 2715 failed to cause an infection in two yearlings, one of which had been previously infected with an ovine strain. Strain 1702 was re-isolated from another yearling for 10 days after inoculation (Fig. 4).

Feline strain MM30c caused an infection which persisted for 17 days in a 2-year-old ewe.

The simian ureaplasma strain was not re-isolated from the yearling ewe but was re-isolated in low titres for 5 days after inoculation into the 2-year-old ewe.

DISCUSSION

The ovine mammary gland was more susceptible to ovine ureaplasma than to bovine ureaplasma infection, and the duration of infection was also more prolonged in the homologous than in the heterologous infections. This suggests a role for ovine

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ureaplasmas in naturally occurring ovine mastitis. A similar role was suggested for bovine ureaplasmas in bovine mastitis (Gourlay, Howard & Brownlie, 1972) and a limited number of naturally occurring bovine ureaplasma mastitis cases have been reported (Gourlay & Howard, 1979). It is probable that ureaplasma mastitis has not been extensively looked for in either species.

The failure of broth cultures of the bovine ureaplasmas to cause an infection in ewes contrasts with the ability of broth cultures of ovine strains to cause mastitis in cows (Mackie & Ball, 1984). Bovine strains MC566, MC629 and 73A also failed to cause an infection in the bovine gland (Ball & Mackie, unpublished results), but two of the three strains that caused mastitis in ewes after passage through the cows, were unable to do this as broth cultures. In a previous investigation, ureaplasma-infected ovine milk was found to infect the udder of a ewe that had resisted infection by a broth culture of the same strain (Ball & Mackie, 1984/5). It is possible that the previous passage of the strains is a selection of virulence in strains cultured in artificial medium.

Insufficient numbers of ewes became infected with bovine ureaplasmas to determine whether the previous exposure to ovine ureaplasmas of the 2-year-olds afforded any protection against subsequent bovine ureaplasma infection. Infection of the two ewes with bovine strain MC576 persisted for longer in the yearling than in the 2-year-old but the longest bovine ureaplasma infection of 4 weeks was in a 2-year-old. Similarly a previous ovine ureaplasma infection did not prevent infection with feline strain MM30c in ewe 12, and a previous infection with canine strain 1702 did not prevent infection with bovine strain MC576 in ewe 7. These results indicate that the ureaplasma strains from different hosts are sufficiently different serotypically to be unable to confer cross-protection from infection.

The patterns of infection caused by the canine strain 1702 and feline strain MM30c were similar to those caused by the bovine strains, being shorter but clinically similar to the ovine infections. The two canine strains could be separated into virulent and non-virulent strains in the ovine gland. Although this contrasts with the inability of a single canine strain to infect the bovine mammary gland (Howard, Gourlay & Brownlie, 1973), a greater number of both canine and feline strains need to be tested to evaluate the ovine model. Similarly the inability of the simian strain to persist for longer than 5 days in one of two ewes is similar to the findings of Howard, Gourlay & Brownlie (1973) with a single strain tested in the bovine gland, but again it is only the second strain to have been tested.

This study has confirmed the pathological role of ovine ureaplasmas in the mammary gland of the ewe and has demonstrated the potential use of the model for testing the virulence of other animal ureaplasmas. The ewe as an experimental model has the advantage of being less costly than the cow, and is probably a more natural model for the ovine and bovine ureaplasmas than the mouse. The development of a simple method of infecting the ovine gland with the bovine strains other than passaging through the cow needs investigation.

In conclusion, it is difficult to assess the usefulness of the ovine mastitis model for comparing the virulence of the ovine strains isolated from the urogenital tract. Although a wide variation in the duration of infection has been observed, in the present and previous study (Ball & Mackie, 1984/5) only one of the 14 strains tested, several of which were isolated from clinically normal vulvas, has failed to

infect the udder. This contrasts with the proportionately greater number of bovine strains that have been found to be nonpathogenic in the bovine mammary gland by other workers and ourselves (Howard, Gourlay & Brownlie, 1973; Ball & Mackie, unpublished results). It is possible that cross-infections, as demonstrated by the bovine, canine and feline strains in the present study using the ovine udder, might be more meaningful than homologous infections in the detection of virulence in ureaplasmas.

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