

## **The virulence of T-mycoplasmas, isolated from various animal species, assayed by intramammary inoculation in cattle**

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### SUMMARY

The virulence of T-mycoplasmas for cattle was tested by examining their ability to produce mastitis in cows. It was found that both virulent and avirulent strains of T-mycoplasmas can be isolated from cattle. All of four strains from pneumonic calf lungs and a strain from a case of bovine kerato-conjunctivitis caused mastitis but only two of four strains isolated from the urogenital tract of cows were virulent. None of the human, simian or canine T-mycoplasmas examined were able to cause mastitis in cattle. However, a bovine strain was found to be capable of causing mastitis in goats. Virulent and avirulent strains from the same and different species contain common antigens detected by the metabolic inhibition test. Pathogenicity could not be shown to be characteristic of any particular serotype. The possibility is raised of some species barrier being responsible for the inability of non-bovine strains to infect cattle.

### INTRODUCTION

T-mycoplasmas have been isolated from the urogenital tract of cattle (Taylor-Robinson, Haig & Williams, 1967), pneumonic calf lungs (Gourlay, Mackenzie & Cooper, 1970) and from cases of bovine kerato-conjunctivitis (Gourlay & Thomas, 1969).

The virulence for cattle of two strains of T-mycoplasmas from pneumonic lesions of calves has been demonstrated by the findings that calves inoculated endobronchially developed pneumonia and cows inoculated via the teat canal developed experimental mastitis. However two human T-mycoplasma strains included in these tests did not cause mastitis in cows (Gourlay & Thomas, 1970; Gourlay, Howard & Brownlie, 1972).

An explanation for these findings could be that all human T-mycoplasmas are non-pathogenic. However, since both the bovine strains examined were isolated from the lung whereas the human strains came from the urogenital tract, pathogenicity might be related to the anatomical site of colonization. Furthermore, one of the cows challenged with a human strain (animal L91, Gourlay *et al.* 1972) appeared to be partially resistant to infection with the bovine strain and there may have been some variation in susceptibility among the experimental animals. Another explanation for the failure of human strains to cause mastitis in cows is that there may be an effective species barrier.

Table 1. *Strains used and their sources*

Strain no.	Source	
A417	Pneumonic calf lung	Compton
D32	Pneumonic calf lung	Compton
D20	Pneumonic calf lung	Compton
Vic9	Pneumonic calf lung	Compton
Bu2	Bovine urogenital tract	Compton
B101	Bovine urogenital tract	Compton
M525	Bovine urogenital tract	Compton
U12	Bovine urogenital tract	Compton
O13	Bovine eye	Compton
REOW	Human urogenital tract	Dr D. Taylor-Robinson
CD408	Human urogenital tract	Dr D. Taylor-Robinson
CD573	Human urogenital tract	Dr D. Taylor-Robinson
CD342	Human urogenital tract	Dr D. Taylor-Robinson
M126	Human urogenital tract	Dr B. E. Andrews
CD343	Human oral cavity	Dr D. Taylor-Robinson
Simian	Simian throat	Dr D. Taylor-Robinson
Canine	Canine urogenital tract	Dr D. Taylor-Robinson

The antigenic structure of sixteen of the seventeen strains of T-mycoplasmas used here has been reported previously (Howard & Gourlay, 1972). Strains were examined by a slight modification of the metabolic inhibition (MI) test of Purcell, Taylor-Robinson, Wong & Chanock (1966). The strains were serologically heterogeneous and the possibility existed that virulence might be a characteristic of particular serotypes. The virulence of strains of T-mycoplasmas from various anatomical sites and species was studied by intramammary inoculation of cattle to answer some of the specific questions raised concerning the pathogenicity of T-mycoplasmas.

#### MATERIALS AND METHODS

##### *T-mycoplasma strains*

Strains A417 and D32 were isolated from pneumonic calf lungs and have been described previously (Gourlay *et al.* 1972). Strains Vic9 and D20 were also isolated from the lungs of calves with pneumonia. O13 was isolated from the eye of a cow with kerato-conjunctivitis (Gourlay & Thomas, 1969). Strains Bu2, M525, U12 and B101 were all isolated from the urogenital tract of cows. The bovine strains were purified as previously described (Gourlay *et al.* 1972). All the other strains were obtained from Dr D. Taylor-Robinson except strain M126 (Table 1). The canine and simian strains as well as strains CD343 (Johnson) and REOW have been described by Taylor-Robinson, Martin-Bourgon, Watanabe & Addey (1971). The human strains were isolated from the urogenital tract except strain CD343 which originated from the oral cavity. The strains and their sources are listed in Table 1.

All strains were grown in U-broth without Hepes (Gourlay *et al.* 1972).

##### *Inoculation of animals*

Cows and goats in milk were inoculated via the teat canal with 10 ml of actively growing mycoplasma cultures. The number of T-mycoplasmas and the number of

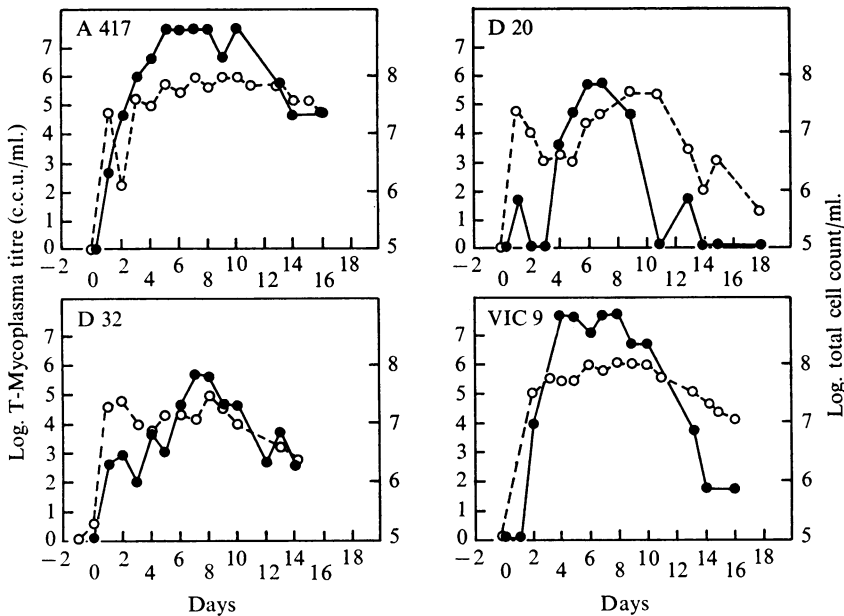


Fig. 1. Number of *T-mycoplasmas* and cells in the milk of cows inoculated with four strains from pneumonic calf lungs. Inocula: A417,  $10^{5.7}$ ; D20,  $10^{5.7}$ ; D32,  $10^{5.7}$  and Vic9,  $10^{6.7}$  c.c.u./ml. ●—● *T-mycoplasmas*; ○--○ cells.

cells present in milk were measured as previously described (Gourlay *et al.* 1972) except that the *T-mycoplasma* titre was recorded as the 50% endpoint (Gourlay & Domermuth, 1967). The criteria used to determine whether strains produced mastitis following intramammary inoculation were the continued increase in the number of cells in milk associated with the consistent reisolation of *T-mycoplasmas* from milk. Strains which did not cause infection were tested in at least two animals. Each animal was inoculated at the same time in another quarter with strain A417 as a control. The possibility of a concurrent bacterial mastitis occurring was excluded by spreading blood agar plates with milk and examining them for bacterial colonies.

## RESULTS

### *Strains from calf lungs*

Of 20 cows inoculated with strain A417, 19 developed mastitis. In one case the animal appeared to be partially immune to infection (animal L91, Gourlay *et al.* 1972). Another cow was found to be refractory to infection with strain A417, although it was susceptible to infection with the bovine urogenital strain U12. A typical response to strain A417 is shown in Fig. 1. The maximum *T-mycoplasma* titre and number of cells in the milk occurred about 5–10 days after injection. *Mycoplasmas* have been found to be excreted for as long as 6 months after inoculation, the longest time studied. The lowest dose of actively growing *T-mycoplasmas* that has been inoculated was  $10^4$ /ml. strain A417, and this caused mastitis.

The response of cows to inoculation with three other *T-mycoplasma* strains isolated from cases of calf pneumonia is shown in Fig. 1. All of these strains caused

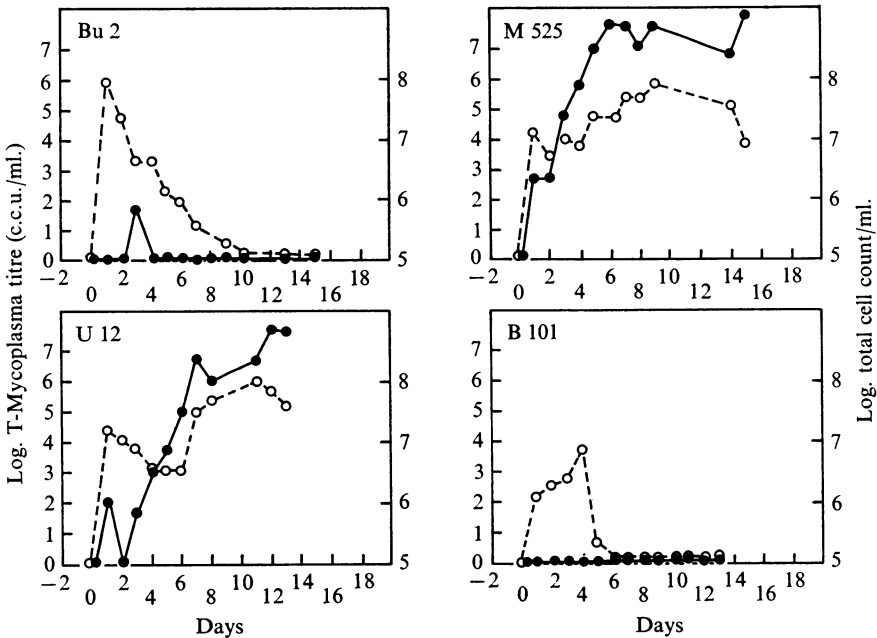


Fig. 2. Number of T-mycoplasmas and cells in the milk of cows inoculated with four strains from the urogenital tract of cows. Inocula: Bu2,  $10^{6.7}$ ; M525,  $10^{6.7}$ ; U12,  $10^7$  and B101,  $10^{6.7}$  c.c.u./ml. ●—● T-mycoplasmas; ○--○ cells.

mastitis, but they varied in their ability to persist and multiply in the udder. Infection with strain D20 was resolved rapidly compared to infection with A417. Infection with all of these strains, except D20, caused the milk to become yellow and produced clots and could thus be considered to have caused clinical mastitis. Strain D20 caused only subclinical mastitis.

#### *Strains from the urogenital tract of cows*

Four strains isolated from the urogenital tract of cattle were tested for virulence. Two of these four strains, U12 and M525, proved virulent on intramammary inoculation and caused the milk to become yellow and produced clots. The number of milk cells and T-mycoplasmas found in the milk after inoculation is shown in Fig. 2. The infection was as severe as that caused by strains isolated from the lung. Two of the urogenital strains examined, B101 and Bu2, were avirulent, the response to their injection was essentially similar to that produced by non-viable cells of strain A417 (Gourlay *et al.* 1972). Inoculation of B101 and Bu2 caused a transient cell response in the milk (Fig. 2).

#### *A T-mycoplasma strain from the eye of a cow*

The results of inoculation with strain O13 are shown in Fig. 3. This strain caused clinical mastitis as evidenced by the yellow milk produced subsequent to injection.

#### *T-mycoplasmas isolated from man*

Six human T-mycoplasmas listed in table 1 have been tested for their ability to cause mastitis in cows. Five of the strains were from the urogenital tract and one

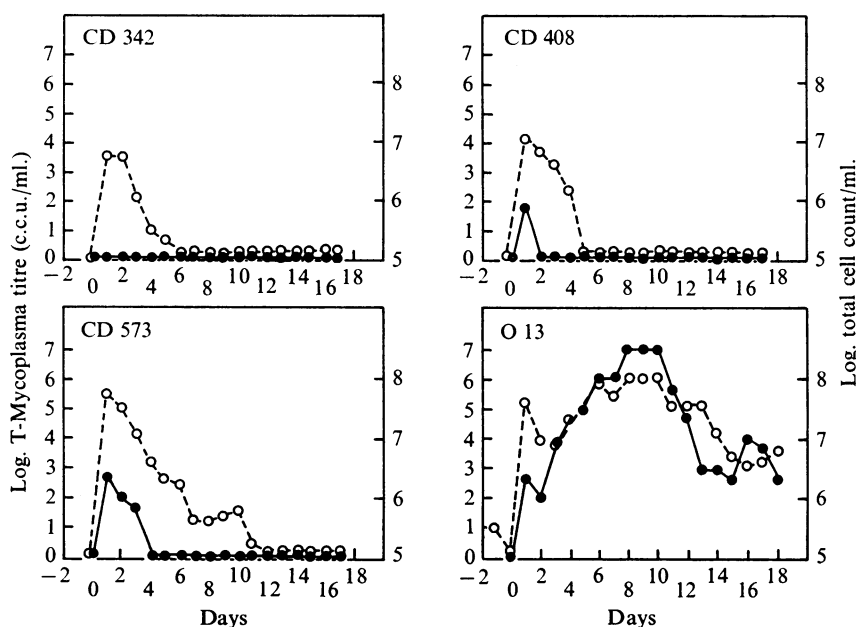


Fig. 3. Number of T-mycoplasmas and cells in the milk of cows inoculated with three human strains and a strain (O13) from the eye of a cow. Inocula: CD342,  $10^{6.7}$ ; CD408,  $10^6$ ; CD573,  $10^{6.7}$  and O13,  $10^{6.7}$  c.c.u./ml. ●—● T-mycoplasmas; ○—○ cells.

was from the oral cavity (CD343). All the strains were inoculated into at least two different cows. None of the human strains was virulent for cattle. The response induced by three of the human T-mycoplasmas is shown in Fig. 3. In all cases on the day after injection a cell response was found in the milk but the high level of cells did not persist. T-mycoplasmas were sometimes isolated on the first or even second and third day after inoculation but this was considered to be due to the persistence of the inoculum. No gross milk changes or udder abnormalities were observed following inoculation with human T-mycoplasmas. The response of cows to human strains was essentially the same as that produced by inactivated bovine T-mycoplasma strain A417 (Gourlay *et al.* 1972) and by the avirulent bovine strains.

#### *Simian and canine T-mycoplasmas*

Neither of these strains caused mastitis in cows. The type of response produced by their inoculation was identical with that of human T-mycoplasmas and the avirulent bovine strains. Neither of the strains multiplied in the udder and they were not re-isolated from the milk. The cell response induced was maximal on day one after injection and thereafter the number of leucocytes in the milk gradually declined. No gross milk or udder abnormalities were observed.

#### *Infection of goats with bovine T-mycoplasma*

Goats were challenged in the same way as cows with strain A417. An infection with clinical signs of mastitis was produced in four out of four animals. An increase

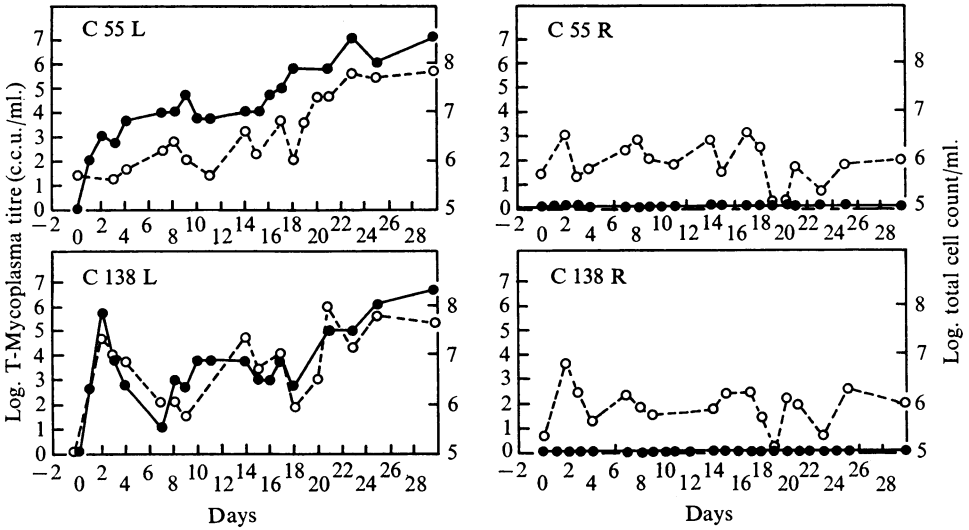


Fig. 4. Number of T-mycoplasmas and cells found in the milk of two goats, nos. C55 and C138, inoculated with bovine strain A417. Inocula: left (L) mammary gland A417,  $10^{4.7}$  c.c.u./ml.; right (R) mammary gland U-broth. ●—● T-mycoplasmas; ○—○ cells.

in the number of leucocytes in the milk was observed and T-mycoplasmas were reisolated from the milk samples (Fig. 4). The number of cells found in control glands injected with U-broth was higher than in cows. The infections caused an increase in the number of cells present and gross milk changes were apparent.

#### DISCUSSION

All four T-mycoplasmas isolated from pneumonic calf lungs and the strain from a case of bovine kerato-conjunctivitis were found to be virulent for cattle. However, both virulent and avirulent T-mycoplasmas have been isolated from the urogenital tract of cows. Pathogenicity is not therefore a specific feature of bovine strains isolated from a particular site.

It is possible that the urogenital tract of cows acts as a reservoir of T-mycoplasmas. The upper respiratory tract could become infected as calves pass down the birth canal. Klein, Buckland & Finland (1969) considered that the oral cavity of babies can become infected with T-mycoplasmas during parturition.

Although T-mycoplasmas have been incriminated in urogenital tract infections of man, there is still doubt regarding their role in these conditions (Shepard, 1969; Taylor-Robinson, 1971; Ford, 1970) and it has been suggested that T-mycoplasmas may usually be commensals in man (Klein *et al.* 1969; Biberfeld, 1971) and in the urogenital tract of bulls (Taylor-Robinson, Thomas & Dawson, 1969).

The possibility that T-mycoplasmas may be of aetiological significance in calf pneumonia has been suggested by their isolation from 58% of pneumonic calf lungs (Gourlay *et al.* 1970) but not from non-pneumonic lungs (Thomas & Smith, 1972). Furthermore, they cause pneumonia in calves inoculated endobronchially

(Gourlay & Thomas, 1970). The finding that all four strains isolated from calf pneumonia were virulent, unlike the bovine urogenital strains, is consistent with the possibility that *T-mycoplasmas* are of aetiological significance in calf pneumonia. This finding may be the result of a selective pressure being present in the respiratory tract which is not present in the urogenital tract.

Taylor-Robinson (1971) reported that the inoculation of a bovine *T-mycoplasma* into the urethra of a Caesarian-derived pathogen-free bull-calf caused infection but failed to produce disease. However, bovine *T-mycoplasmas* are capable of causing clinical mastitis in cows infected experimentally. This group of microorganisms should not be regarded as merely commensals in cattle.

None of the human, canine or simian strains tested caused infection in cows. An explanation for this finding could be that all the strains examined were avirulent. Since both virulent and avirulent *T-mycoplasmas* have been isolated from cattle there is by analogy no reason for assuming that all human, simian and canine strains are avirulent *per se* and an alternative explanation is that some host specific factors are involved which prevent the non-bovine strains from infecting cows. However, since the bovine A417 strain was capable of causing experimental mastitis in goats, host specificity is not absolute, although specific strains may only be able to infect a limited range of animals. The findings reported by Taylor-Robinson *et al.* (1971) that only human *T-mycoplasmas* adsorbed to HeLa cells, not bovine, simian or canine strains, and only simian strains adsorbed to chicken erythrocytes indicates that some specificity exists in cell adsorption by *T-mycoplasmas*.

Human and bovine *T-mycoplasmas* have been found to be serologically heterogeneous. Strains which contain common antigens can be isolated from various anatomical sites and from normal or diseased conditions (Ford, 1967; Purcell, Chanock & Taylor-Robinson, 1969; Taylor-Robinson *et al.* 1969; Howard & Gourlay, 1972). Furthermore, strains from different animal species have been found to cross react in the MI test (Howard & Gourlay, 1972).

Since strains that had been reisolated from milk reacted with the antisera in the same way as they did before injection, the antigenic structure of the organisms is apparently a stable characteristic.

Sixteen of the strains tested for virulence have been examined by the MI test for cross-reacting antigens using antisera raised against six of the strains (Howard & Gourlay, 1972).

Both virulent and avirulent bovine *T-mycoplasmas* possess common antigens. Moreover, strains from other species which contain antigens present in virulent bovine *T-mycoplasmas* are avirulent for cows. Judging from the results presented here, no particular serotype, as indicated by the MI test, appears to be characteristically pathogenic.

The results reported by Taylor-Robinson *et al.* (1971), noted above, indicated differences in the cell adsorptive properties of strains from different species. Our results indicate that important differences between strains from the same and different animal species exist which affect the virulence of strains for a particular species of animal.

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