Serological Comparison of Bovine T-mycoplasmas

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SUMMARY

Eight bovine T-mycoplasmas examined serologically by the metabolism inhibition test, growth inhibition on agar and by immunofluorescence showed considerable serological heterogeneity. None of the eight strains was serologically identical with any other, but three formed a group of similar organisms. No evidence was obtained for one particular serotype being confined to a specific anatomical site or being isolated typically from any particular pathological condition. Antisera to the bovine T-mycoplasmas were tested against eight serologically distinct human strains and a caprine, a simian and a canine T-mycoplasma. None of the non-bovine strains was identical to these bovine T-mycoplasmas.

INTRODUCTION

Most studies on the serology of human T-mycoplasmas have been made with the metabolism inhibition test (Purcell, Taylor-Robinson, Wong & Chanock, 1966) and these have demonstrated the serological diversity of this group of micro-organisms (Purcell *et al.* 1967). No correlation between serotype and anatomical site of colonization, or serotype and isolation from pathological conditions or normal subjects, has been found (Ford, 1967; Purcell, Chanock & Taylor-Robinson, 1969). Lin, Kendrick & Kass (1972) examined human T-mycoplasma strains using a complement-dependent mycoplasmacidal test and came to the same conclusions.

Black (1971) examined human T-mycoplasmas by three additional serological tests – growth inhibition on agar, immunofluorescence, and indirect haemagglutination. On the basis of his results he postulated that at least seven distinct serotypes of human T-mycoplasmas exist.

Preliminary examinations of the serology of bovine T-mycoplasmas have been made with only the metabolism inhibition test. These studies have indicated that bovine strains are also serologically heterogeneous (Taylor-Robinson, Thomas & Dawson, 1969; Howard & Gourlay, 1972).

We have now examined the antigenic structure of bovine T-mycoplasmas using three serological methods, to provide a basis from which further studies on the pathogenicity of the group can be made.

METHODS

T-mycoplasma strains. Strains isolated from cattle and against which antisera were raised are listed in Table 1. Strains were purified by filtration of broth cultures through 450 nm Millipore filters and propagation of single colonies, on three successive occasions, as recommended by the Sub-committee on the Taxonomy of *Mycoplasmatales* (1972). Strains BU2 and F801 were isolated from the urogenital tract of cattle and strain 013 originated from

the eye of a cow with kerato-conjunctivitis. The other strains (A417, Gra383, D48, vic9 and mmb143) were isolated from pneumonic calf lungs.

The human T-mycoplasma strains 7, 23, 27, 58, 354, Pirillo, Cook and t960 were provided by Dr F. T. Black (Aarhus, Denmark). All except t960 have been examined by him and proposed as seven distinct serotypes of human T-mycoplasmas (Black, 1971).

Canine strain sp1701 and simian strain sp1625A were obtained from Dr D. Taylor-Robinson (M.R.C. Clinical Research Centre, Northwick Park, London) and have been described by him (Taylor-Robinson, Martin-Bourgon, Watanabe & Addey, 1971). The caprine T-mycoplasma strain G1 was isolated from the urogenital tract of a goat (Gourlay, Brownlie & Howard, 1973).

Media. The liquid medium used was U2 broth (Howard & Gourlay, 1973). Solid medium was made according to Gourlay *et al.* (1973).

Serological tests. The metabolism inhibition test of Purcell *et al.* (1966) was performed according to Howard & Gourlay (1973). Guinea-pig serum was not added to the system.

The ability of antisera to inhibit the growth of strains on solid media was examined in a similar manner to that of Black (1971). Solid medium was inoculated with a 20 μ l drop of broth culture; a well, 1.5 mm in diameter, was then cut in the agar in the centre of the drop and filled with antiserum. Plates were incubated for three days at 37 °C under 5 % (v/v) CO₂ in N₂. Zones of inhibition of growth were measured under a microscope with $\times 40$ magnification.

Strains were examined by the indirect immunofluorescent technique for unfixed mycoplasma colonies according to Rosendal & Black (1972) with antisera raised in rabbits and conjugated goat or pig anti-rabbit sera (Nordic Diagnostics, Fraburg Ltd, Maidenhead, Berkshire).

The schedule used for the preparation of antisera in New Zealand white rabbits to T-mycoplasma strains grown in U2 broth was as previously stated (Howard & Gourlay, 1972). All antisera were stored at -20 °C and heated at 56 °C for 30 min before use.

RESULTS

Comparison of bovine T-mycoplasmas by the metabolism inhibition test. The titres of antisera to eight bovine T-mycoplasmas were measured against the same eight strains by the metabolism inhibition test (Table 1). High titres were obtained with homologous antisera. None of the strains was identical with any other, but strains A417, F801 and Gra383 appeared to be similar. The other five strains were distinct from each other and from the group A417, F801 and Gra383. Because a normal rabbit serum titre of 20 in the metabolism inhibition test with bovine T-mycoplasmas has been reported (Howard & Gourlay, 1973), titres of 20 were ignored. Antisera did have low titres to some heterologous strains suggesting some degree of relatedness between distinguishable strains (Table 1).

Comparison of bovine T-mycoplasmas by growth inhibition on agar. When strains were compared by this method essentially the same result was found as with the metabolism inhibition test (Table 2). The three strains A417, F801 and Gra383 formed a group of similar but not identical isolates. The most obvious difference between these strains was the inability of antiserum to strain Gra383 to inhibit the growth on agar of strain A417. The other five strains were distinct from this group and from each other.

Comparison of bovine T-mycoplasmas by immunofluorescence. This method (Table 3) gave a similar result to the metabolic and growth inhibition tests, the only difference being the detection of antibody to strain vic9 in antisera to D48.

Table 1. *Relation between bovine T-mycoplasma strains measured by the metabolism inhibition test*

Strain	Titre* with antisera to							
	A417	F801	Gra383	D48	vic9	O13	BU2	Mmb143
A417	≥ 20480	10240	160	20	< 20	< 20	20	< 20
F801	320	≥ 20480	5120	160	< 20	< 20	160	40
Gra383	640	≥ 20480	10240	80	< 20	40	160	160
D48	40	20	< 20	≥ 20480	20	20	< 20	< 20
vic9	40	80	20	160	≥ 20480	40	20	< 20
O13	40	20	< 20	20	20	≥ 20480	20	40
BU2	160	80	80	40	20	80	≥ 20480	40
Mmb143	40	20	20	40	40	40	20	≥ 20480

* Reciprocal of the highest dilution of antiserum that inhibited breakdown of urea during growth.

Table 2. *Relation between bovine T-mycoplasma strains measured by growth inhibition by antisera*

Strain	Zone* of inhibition of growth by antisera to							
	A417	F801	Gra383	D48	vic9	O13	BU2	Mmb143
A417	7	6	0	0	0	0	0	0
F801	4	6	4	0	0	0	0	0
Gra383	4	6	4	0	0	0	0	0
D48	0	0	0	8	0	0	0	0
vic9	0	0	0	0	7	0	0	0
O13	0	0	0	0	0	8	0	0
BU2	0	0	0	0	0	0	7	0
Mmb143	0	0	0	0	0	0	0	4

* Figures indicate diameter, in mm, of zones of inhibition of growth of organisms by antiserum on solid medium. Incubation period three days.

Table 3. *Relation between bovine T-mycoplasma strains measured by indirect immunofluorescence*

Strain	Titre* with antisera to							
	A417	F801	Gra383	D48	vic9	O13	BU2	Mmb143
A417	160	160	80	< 5	< 5	< 5	< 5	< 5
F801	160	160	40	< 5	< 5	< 5	< 5	5
Gra383	80	80	80	< 5	< 5	< 5	< 5	< 5
D48	5	< 5	< 5	160	5	5	5	< 5
vic9	< 5	< 5	< 5	80	80	10	< 5	< 5
O13	< 5	< 5	< 5	5	5	80	< 5	< 5
BU2	< 5	< 5	< 5	< 5	< 5	< 5	320	< 5
Mmb143	< 5	< 5	5	< 5	< 5	< 5	< 5	320

* Reciprocal of the highest dilution of rabbit antiserum resulting in fluorescence of colonies on agar after addition of fluorescein-conjugated anti-rabbit serum.

Relation between T-mycoplasma strains isolated from different animal species measured by the metabolism and growth inhibition tests. None of the antisera raised against bovine T-mycoplasmas had high titres against human strains by the metabolism inhibition test (Table 4). Some of the antisera had low titres to the human strains, the highest being a titre of 320 of antiserum to bovine strain BU2 against human strain 27.

Table 4. *Metabolism inhibition titre of antisera to bovine T-mycoplasmas tested against human T-mycoplasmas*

Human T-mycoplasma strain	Titre* with antiserum to bovine strain							
	A417	F801	Gra383	D48	vic9	013	Bu2	Mmb143
7	< 20	80	< 20	20	< 20	< 20	< 20	< 20
23	40	40	< 20	20	< 20	< 20	20	40
27	< 20	160	< 20	< 20	< 20	40	320	< 20
58	< 20	< 20	< 20	< 20	< 20	< 20	< 20	< 20
354	< 20	< 20	< 20	< 20	< 20	40	< 20	< 20
Pirillo	< 20	20	< 20	< 20	< 20	20	< 20	< 20
Cook	< 20	< 20	< 20	< 20	< 20	< 20	20	< 20
T960	< 20	< 20	< 20	< 20	< 20	< 20	< 20	< 20

* Reciprocal of the highest dilution of antiserum that inhibited breakdown of urea during growth.

When the same anti-bovine T-mycoplasma sera were examined for their ability to inhibit the growth on agar of the same eight human strains and also of simian, canine and caprine T-mycoplasmas, no zones of inhibition were observed. Thus none of the non-bovine strains examined was found to be serologically the same as the bovine strains.

Ability to identify other bovine strains. A further 27 bovine strains were examined by the metabolism inhibition test with the eight available antisera. Eleven of these strains could be serotyped, i.e. high titres were obtained when the eight antisera were tested against them. Four strains isolated from lungs were serologically similar to strain 013 which was isolated from the eye of a cow. Serologically similar strains were also isolated from the lungs and urogenital tract of cattle.

DISCUSSION

The three serological tests gave essentially the same, but not identical, results. Since these tests vary in their sensitivity, the metabolism inhibition test being the most sensitive and growth inhibition on agar the least sensitive (Taylor-Robinson, 1968), some divergence of the results obtained with the different methods is to be expected. However, the finding that shared antigens were not detected by all the tests cannot be explained in terms of the sensitivity of the tests alone. Antibody to strain A417 was demonstrated in antiserum to strain Gra383 by immunofluorescence and at a low titre by metabolism inhibition, but it was not demonstrated by growth inhibition. Similarly, antibody to vic9 was demonstrated in antiserum to strain D48 by immunofluorescence and to a lesser extent by metabolism inhibition, but not by growth inhibition on agar. The three strains A417, Gra383 and F801 are similar and can perhaps be grouped together. Strains vic9 and D48 also appear to share a common antigen but only a one-way cross-reaction was demonstrated. The other three strains are distinct from each other and from the five already mentioned. The low metabolism inhibition titres of antisera, when tested against heterologous bovine strains, indicate the existence of common shared antigens besides those antigens by which the strains are distinguished. The finding that the three strains A417, F801 and Gra383 form a similar but not identical group indicates that amongst bovine T-mycoplasmas, groups of similar but not identical organisms exist. This is analogous to the serologically similar but not identical strains amongst the species of *Mycoplasma pulmonis* (Forshaw & Fallon, 1972). These results may indicate a system by which the bovine T-mycoplasmas could be classified, perhaps with groups of similar organisms being given the status of species or subspecies.

As a group, bovine T-mycoplasmas are serologically heterogeneous. Strains which possess common antigens have been isolated from different anatomical sites of cattle, and we have no evidence for any serotype being confined to a particular organ or being associated with any particular pathological condition. The same situation occurs in man in that human T-mycoplasma strains are serologically heterogeneous and no serotype is characteristically isolated from any one anatomical site or disease (Ford, 1967; Purcell *et al.* 1969; Lin *et al.* 1972). Studies on the virulence of bovine T-mycoplasma strains have not been extensive enough to say whether or not any particular serotype is characteristically virulent or avirulent. However, serologically distinct strains have been shown to be virulent for the bovine udder (Howard, Gourlay & Brownlie, 1973).

Of the further 27 bovine T-mycoplasmas examined by the metabolism inhibition test, 11 (40 %) could be serotyped. Clearly many other serotypes of bovine T-mycoplasmas must exist besides those encountered here.

None of the human, canine, simian or caprine strains examined was serologically the same as any of the bovine strains. However, the low metabolism inhibition titres observed when antisera to bovine strains were tested against human strains presumably indicates some shared antigens; these must be distinct from the major antigens which distinguish the bovine serotypes. Low metabolism inhibition titres of antisera to strains isolated from one animal species have been observed when tested against strains isolated from other animal species (Taylor-Robinson *et al.* 1971; Howard & Gourlay, 1973).

The T-mycoplasmas isolated from different animal species appear no more different serologically from each other than are some strains from the same animal species. Serologically identical strains from different animal species have so far not been isolated.

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