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Further studies on caprine and ovine mycoplasmas related to *Mycoplasma mycoides* subsp. *mycoides*

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SUMMARY

Nine caprine and ovine mycoplasma strains, said to be indistinguishable serologically from *Mycoplasma mycoides* subsp. *mycoides* (the causative organism of contagious bovine pleuropneumonia; CBPP) were examined in mice by (1) a mycoplasmaemia test, and (2) a cross-protection test. Of the nine strains, two from goats belonged to a small colony (SC) type; four caprine and three ovine strains belonged to a large colony (LC) type.

The two SC strains – like a single SC strain examined in an earlier study – were indistinguishable from genuine *M. mycoides* subsp. *mycoides* as isolated from CBPP. They produced mycoplasmaemia readily. In a cross-protection test, the two SC strains and a CBPP strain immunized completely against each other.

Of the seven LC strains, six – like six LC strains examined in an earlier study – were easily distinguished from genuine *M. mycoides* subsp. *mycoides*; except for one that was not tested, all were shown to lack the ability to produce mycoplasmaemia readily. In cross-protection tests all six strains immunized partially but not completely against a CBPP strain.

The seventh LC strain (Mankefår 2833) was exceptional: it produced mycoplasmaemia readily, resembling the SC strains in this respect. Like other LC strains, in cross-protection tests it protected only partially against a CBPP strain. Strain Mankefår 2833 was isolated in *ca.* 1965 by Brack from a Barbary sheep (*Ammotragus lervia*) in a German zoo.

The ability of Mankefår 2833 to produce mycoplasmaemia enabled it to be used as a challenge strain in cross-protection tests. For the purpose of such tests the collection of nine mycoplasma strains referred to above was augmented with six LC strains from an earlier study. Partial but not complete protection against Mankefår 2833 was produced by two caprine SC strains, one CBPP strain, and nine LC strains. Three further LC strains gave protection that may have been as strong as that produced by the homologous strain, but confirmatory experiments are needed. A strain of *M. mycoides* subsp. *capri* gave no protection against Mankefår 2833.

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INTRODUCTION

Mycoplasma mycoides subsp. *mycoides* is the causative organism of contagious bovine pleuropneumonia (CBPP) – a disease that is still of exceptional importance in more than 20 African countries, but has long since been eradicated from many other parts of the world.

In recent years numerous mycoplasma strains said to be serologically indistinguishable from *M. mycoides* subsp. *mycoides* have been isolated from goats and occasionally from sheep; the literature has been reviewed by Hooker, Smith & Milligan (1979). Such strains are of two main types – large colony (LC) and small colony (SC); the growth characteristics of the SC type resemble those of CBPP strains of *M. mycoides* subsp. *mycoides* (Cottew & Yeats, 1978; Hooker *et al.* 1979).

Cottew & Yeats (1978) found that eight LC strains – unlike two SC strains – digested casein, liquefied inspissated serum, and survived for considerable periods at 45 °C; the SC strains could not be distinguished from four CBPP strains of *M. mycoides* subsp. *mycoides*. Hooker *et al.* (1979) found that six LC strains – unlike a SC strain – failed to produce mycoplasmaemia readily in mice, and immunized mice partially but not completely against a CBPP strain of *M. mycoides* subsp. *mycoides*; the SC strain could not be distinguished from a virulent CBPP strain of *M. mycoides* subsp. *mycoides*.

The number of SC and LC strains examined in earlier studies is too small to indicate whether further subdivision is likely to be necessary. This paper describes the results of mycoplasmaemia and cross-protection tests on six LC and two SC strains, none of which had been examined by such tests previously. Because the behaviour of one strain proved to be strikingly different from that observed in earlier work (Hooker *et al.* 1979) at this laboratory, it became necessary to include in one experiment a number of the strains examined in the earlier study.

MATERIALS AND METHODS

Mycoplasma strains

The following eight mycoplasma strains have already been described by Hooker *et al.* (1979): Blenheim (*M. mycoides* subsp. *mycoides* from CBPP); Y goat, Ojo I, Ojo II, Cov 2, 74/2488, 143-A66 Conn (LC strains of so-called *M. mycoides* subsp. *mycoides* from goats); Smith 1423 (*M. mycoides* subsp. *capri* from contagious caprine pleuropneumonia). Table 1 gives details of a further nine strains (seven LC, two SC). Of these, two strains from different laboratories were found on receipt to bear the same name ('Vom'), although their colony size showed that they were not identical; these strains were re-named (Vom/Plum Island, Vom/Parkville).

Viable counts

The method was that used by Hooker *et al.* (1979).

Table 1. *Mycoplasma strains*

Strain	Reference	Colony	Number of subcultures since isolation	Further information
Mankefår 2833*§	Brack (1966), Al-Aubaidi *et al.* (1972), Ernø *et al.* (1972)	Large	?	From fatal infection of Barbary sheep in a German zoo
2605-Razi*§	Cottew *et al.* (1969), Al-Aubaidi *et al.* (1972)	Large	?	From disease resembling contagious agalactia in a goat, Iran
Vom/Plum Island*§	Al-Aubaidi *et al.* (1972)	Large	?	From a goat, Nigeria
222-69 N.Y.*§	Al-Aubaidi *et al.* (1972)	Large	?	From a goat, presumably U.S.A.
Ghaleh Morghi-16†§	Al-Aubaidi *et al.* (1972)	Large	< 20	From milk of a sheep, Teheran, Iran
S-5-64†§	Al-Aubaidi *et al.* (1972)	Large	Few	From milk of a sheep, Shiraz, Iran
F 30‡	MacOwan (1976)	Large	*ca.* 20	From caprine pleuro-pneumonia, Kenya
P goat§||	Cottew & Yeats (1978)	Small	?	From a goat, Sudan
Vom/Parkville||	Cottew & Yeats (1978)	Small	?	From a goat, Nigeria

* Supplied by Dr A. H. Dardiri, U.S.A.
† Supplied by Dr H. Ramyar, Iran.
‡ Identified as *M. mycoides* subsp. *mycoides* by MacOwan (1976); supplied by Dr R. H. Leach, Norwich.
§ Identified by Al-Aubaidi *et al.* (1972) as *M. mycoides* subsp. *mycoides* ('group 8' strain).
|| Identified and supplied as *M. mycoides* subsp. *mycoides* by Dr G. S. Cottew, Australia.

Table 2. *Mycoplasmaemia test with four LC strains (one ovine, three caprine)*

Mycoplasma strain	Dose of mycoplasmas per mouse (c.f.u., 10^6)	Positive blood cultures in groups of 8 mice at the stated times (days) after inoculation		
		1	2	3
Blenheim (for comparison)	10	8	8	6
	1	8	8	7
	0·1	7	6	6
Mankefår 2833*	53	8	8	4
	5·3	7	5	2
	0·53	5	1	0
2605-Razi	81	6	3	0
	8·1	3	1	1
	0·81	0	0	0
Vom/Plum Island	32	3	3	1
	3·2	2	0	0
	0·32	0	0	0
222-69 N.Y.	2	0	1	0
	0·2	0	0	0

c.f.u., colony forming units. * Ovine strain.

Table 3. *Mycoplasmaemia test with two ovine strains from Iran*

Mycoplasma strain	Dose of mycoplasmas per mouse (c.f.u., 10^6)	Mycoplasmaemia in groups of mice at the stated times (days) after inoculation		
		1	2	3
Blenheim (for comparison)	2·58	8/10	7/10	5/10
Mankefår 2833 (for comparison)	3·27	9/10	6/10	2/10
Ghaleh Morghi-16	34·5	2/10	2/10	2/10
	3·45	1/10	0/10	1/10
S-5-64	31·3	2/11*	0/20	0/20
	3·13	1/20	0/20	0/20

* Nine cultures contaminated.

Mycoplasmaemia and cross-protection tests in mice

The methods were essentially those used by Hooker *et al.* (1979) and described earlier by Smith (1969*a*, 1971). The selective blood-culture medium was invariably ONB-OS containing penicillin and thallium acetate. Mycoplasmaemia tests were made by culturing the tail-blood of mice at three daily intervals after intraperitoneal inoculation. In the cross-protection tests mice were immunized intravenously with heat-killed cultures. Three weeks later they were challenged intraperitoneally with a strain capable of producing mycoplasmaemia; protection was assessed by means of blood cultures made after a further 24 h.

RESULTS

Mycoplasmaemia tests on four LC strains

The results are given in Table 2. Of the four strains, three (2605-Razi, Vom/Plum Island, 222-69 N.Y.) differed from the CBPP strain Blenheim in being unable to produce mycoplasmaemia readily. They thus resembled the six LC strains described by Hooker *et al.* (1979). The fourth strain (Mankefår 2833) was strikingly different; its ability to produce mycoplasmaemia closely approached that of Blenheim. This finding was confirmed in numerous subsequent experiments.

Mycoplasmaemia tests on two LC strains from sheep in Iran

Of the 10 strains examined thus far, either in the present study or by Hooker *et al.* (1979), all except the ovine strain Mankefår 2833 originated from goats. Because it seemed possible that the ovine origin of this strain was related to its ability to produce mycoplasmaemia, two further ovine strains (Ghaleh Morghi-16 and S-5-64) were obtained and examined. Table 3 shows that, despite their ovine origin, they were far less capable than Blenheim or Mankefår 2833 of producing mycoplasmaemia; in this they resembled the majority of LC strains.

Table 4. *Mycoplasmaemia test with two SC caprine strains (P goat and Vom/Parkville)*

Mice inoculated with culture diluted 1 in	Positive blood cultures in groups of 8 mice at the stated intervals (days) after inoculation with strain											
	Blenheim (for comparison)			P goat			Vom/Parkville			Cov 2 (for comparison)		
	1	2	3	1	2	3	1	2	3	1	2	3
1*	8	8	8	8	8	8	8	8	6	5	4	2
10	8	8	8	8	8	5	8	7	0	4	3	0
10^2	8	8	7	8	8	6	8	5	1	1	0	0
10^3	8	7	7	8	8	5	6	3	1	0	0	0
10^4	8	8	8	7	5	4	3	1	1	0	0	0

* Undiluted cultures supplied the following numbers of c.f.u. (10^6) per mouse: Blenheim, 1420; P goat, 1325; Vom/Parkville, 786; Cov 2, 1980.

Mycoplasmaemia tests on two SC strains from goats

During the course of this study Cottew & Yeats (1978) reported that the caprine strains P goat and Vom/Parkville were SC types of *M. mycoides* subsp. *mycoides*. As only one SC caprine strain – strain O goat – had previously been subjected to mycoplasmaemia tests (see Hooker *et al.* 1979), P goat and Vom/Parkville were obtained for examination. They were tested in parallel with the CBPP strain Blenheim and the LC caprine strain Cov 2. The results are shown in Table 4. The behaviour of both strains was different from that of Cov 2 but similar to that of Blenheim; in this they resembled strain O goat (see Hooker *et al.* 1979). Although high, the ability of Vom/Parkville to produce mycoplasmaemia was slightly less than that of P goat and Blenheim.

Cross-protection against challenge with the LC strain Mankefår 2833

The unusual properties of strain Mankefår 2833 made possible, for the first time, the use of an LC strain for challenge purposes.

In each of three experiments made on different occasions, heat-killed cells of the 12 strains listed in Table 5 were used to immunize groups of mice. Three weeks later the mice were challenged with Mankefår 2833. Table 5 shows the results of blood cultures made 24 h after challenge in each of the three experiments; it also gives the aggregated results.

The aggregated results show that strain Smith 1423 – the only representative of the species *M. mycoides* subsp. *capri* – gave no protection. All other heterologous strains gave protection ($P < 0{\cdot}02$ for strain 143-A66 Conn and $P < 0{\cdot}001$ for the remainder); this protection was significantly less ($P < 0{\cdot}016$) than that produced by vaccination with the homologous strain and is therefore described as 'partial'.

Table 5 shows that the results of a single cross-protection test may be grossly misleading and emphasizes the need for repetition of such tests on different occasions, with different challenge doses.

Table 5. *Challenge (Mankefår 2833) of mice vaccinated with (1) the homologous strain, (2) the CBPP strain Blenheim, (3) caprine strains of so-called* M. mycoides *subsp.* mycoides, (4) M. mycoides *subsp.* capri

Mice vaccinated with strain	Mycoplasmaemia in vaccinated mice 24 h after challenge with the stated dose (c.f.u.) of Mankefår 2833 in experiment no.			
	1 (173×10^6)	2 (79×10^6)	3 (286×10^6)	1–3 (aggregated results)
Mankefår 2833	0/12	0/12	3/12	3/36
Blenheim	1/12	1/12	11/11	13/35
2605-Razi	4/12	2/12	11/12	17/36
Vom/Plum Island	4/12	3/12	11/12	18/36
222-69 N.Y.	4/12	4/12	12/12	20/36
Y goat	4/12	1/11	9/12	14/35
Ojo I	1/12	0/11	10/12	11/35
Ojo II	0/12	0/12	12/12	12/36
Cov 2	6/12	1/12	11/12	18/36
74/2488	1/12	1/11	12/12	14/35
143-A66 Conn	10/12	3/11	12/12	25/35
Smith (1423)	11/12	10/12	12/12	33/36
Controls	30/30	19/29	30/30	79/89

Cross-protection given by four LC strains against the CBPP strain Blenheim

The four strains, which included the unusual strain Mankefår 2833, are shown in Table 6, together with the results of three experiments made on different occasions. The aggregated results show that the four strains each produced cross-protection against Blenheim ($P < 0{\cdot}001$); the protection was partial ($P < 0{\cdot}001$). In this respect they resembled the six LC strains already examined by Hooker *et al.* (1979).

Cross-protection against challenge with Blenheim, P goat, Vom/Parkville, and Mankefår 2833

The receipt of several strains during the course of the study necessitated a final cross-protection experiment to complete the examination of the entire collection. Table 7 shows the design and results of three similar experiments made on different occasions. Groups of mice immunized with Blenheim, P goat and Vom/Parkville vaccines were subdivided to allow for challenge with Blenheim, P goat, Vom/Parkville and Mankefår 2833. Further groups immunized with Mankefår 2833, Ghaleh Morghi-16, S-5-64 and F 30 vaccines were subdivided to allow for challenge with Blenheim and Mankefår 2833.

The aggregated results (Table 7) show that strains Blenheim, P goat and Vom/Parkville immunized virtually completely against themselves and against each other. Strains P goat and Vom/Parkville thus resembled the SC strain O goat (Hooker *et al.* 1979) in being indistinguishable from a genuine CBPP strain of *M. mycoides* subsp. *mycoides*. Strains P goat and Vom/Parkville – like Blenheim – protected against Mankefår 2833 ($P < 0{\cdot}001$); this protection was partial ($P <$

Table 6. *Partial cross-protection given by vaccines prepared from one ovine and three caprine strains of so-called* M. mycoides *subsp.* mycoides *against challenge with the CBPP strain Blenheim*

Mice vaccinated with strain	Mycoplasmaemia in vaccinated mice 24 h after challenge* with strain Blenheim, in experiment no.			
	1	2	3	1–3 (aggregated results)
Blenheim	0/8	0/8	1/8	1/24
Mankefår 2833	7/8	2/8	3/8	12/24
2605-Razi	5/8	1/8	5/8	11/24
Vom/Plum Island	2/8	2/8	11/20	15/36
222-69 N.Y.	4/8	2/8	6/8	12/24
Controls	20/20	17/20	20/20	57/60

* The challenge doses in experiments 1, 2 and 3 were (c.f.u., 10^6): 480, 32 and 30 respectively.

0·025), i.e. less than that given by homologous vaccine. Strains Ghaleh Morghi-16, S-5-64 and F 30 resembled Mankefår 2833 in protecting against Blenheim ($P <$ 0·001); this protection was partial ($P < 0·001$). Strains Ghaleh Morghi-16, S-5-64 and F 30 protected against Mankefår 2833 ($P < 0·001$). The protection appeared to be as strong as that afforded by homologous vaccine, but this requires confirmation in view of the partial protection given by nine LC strains against Mankefår 2833 in an earlier experiment.

DISCUSSION

The strains used were reported by various workers (see Table 1) to be indistinguishable serologically from *M. mycoides* subsp. *mycoides*; as far as is known the serological examinations did not include the use of absorption tests.

The in-vivo methods (mycoplasmaemia and cross-protection tests) used in the present study showed that, with one exception, nine strains could be divided into the two categories described by Hooker *et al.* (1979). In assessing the mycoplasmaemia tests, it should be borne in mind that the number of subcultures to which the strains had been subjected since isolation was usually unknown (see Table 1).

The exceptional strain, Mankefår 2833, was isolated by Brack (1966) from one of 15 Barbary sheep (*Ammotragus lervia*) that died from fatal mycoplasmosis at a zoo in Frankfurt; at necropsy the main abnormalities were myocarditis and polyarthritis. The strain was identified as *M. mycoides* subsp. *mycoides* by Ernø *et al.* (1972) and Al-Aubaidi, Dardiri & Fabricant (1972). The present study showed that Mankefår 2833 – unlike genuine *M. mycoides* subsp. *mycoides* – produced colonies resembling those of the LC type described by Cottew & Yeats (1978) and Hooker *et al.* (1979). The present study also showed that Mankefår 2833 – unlike all other LC strains examined so far – readily produced mycoplasmaemia in mice. In its ability to cross-protect only partially against challenge with a CBPP strain it resembled typical LC strains. Further cross-protection tests, in which Mankefår

Table 7. *Further cross-protection tests between the CBPP strain Blenheim, and related strains (LC and SC types) from sheep and goats*

Mice vaccinated with strain	Mycoplasmaemia in vaccinated mice 24 h after challenge* with Blenheim (B), P goat (P), Vom/Parkville (V), and Mankefår 2833 (M), in experiment no.															
	1				2				3				1–3 (aggregated results)			
	B	P	V	M	B	P	V	M	B	P	V	M	B	P	V	M
Blenheim	0/8	0/8	0/8	5/7	0/8	0/8	0/8	6/8	1/8	0/8	0/8	5/8	1/24	0/24	0/24	16/24
P goat	2/8	0/8	1/8	8/8	0/8	0/8	0/8	4/8	0/8	0/8	0/8	4/8	2/24	0/24	1/24	16/24
Vom/Parkville	1/8	1/8	1/8	6/7	0/8	0/8	0/8	1/8	1/8	1/8	0/8	4/8	2/24	2/24	1/24	11/23
Mankefår 2833	8/8	—	—	3/8	5/8	—	—	0/8	5/8	—	—	1/8	18/24	—	—	4/24
Ghaleh Morghi-16	7/8	—	—	6/8	2/8	—	—	2/8	3/8	—	—	1/8	12/24	—	—	9/24
S-5-64	4/8	—	—	5/8	0/8	—	—	0/8	2/8	—	—	1/8	6/24	—	—	6/24
F 30	6/8	—	—	6/7	1/8	—	—	0/8	4/8	—	—	0/8	11/24	—	—	6/24
Controls	19/19	19/20	20/20	20/20	19/19	18/20	15/20	18/20	20/20	19/20	18/20	20/20	58/58	56/60	53/60	58/60

Numerator, mice with mycoplasmaemia; denominator, mice challenged.

* Challenge doses (c.f.u., 10^6) in experiments 1, 2 and 3 were as follows: (Blenheim) 560, 27·6, 44, respectively; (P goat) 696, 32·5, 62, respectively; (Vom/Parkville) 533, 28·9, 44, respectively; (Mankefår 2833) 625, 64·1, 179, respectively.

2833 was used as the challenge strain, indicated that the majority of LC strains gave only partial protection, i.e. protection significantly less than that produced by vaccine prepared from the homologous strain.

These observations indicate that it would be unwise to regard the LC strains as a homogeneous group. Further support for this view stems from the rather low degree of cross-protection produced by the LC strain 143-A66 Conn against Mankefår 2833 (Table 5) and against the SC strain O goat and the CBPP strain Blenheim (Hooker *et al.* 1979).

Strain Mankefår 2833 deserves further study in respect of (1) the reason for its ability to produce mycoplasmaemia, and (2) its pathogenicity and immunogenicity for cattle, sheep and goats.

Bovine, caprine and ovine mycoplasma strains, some isolated many years ago, are constantly exchanged between laboratories in various parts of the world. The practice, though unavoidable, is not without danger. This point is illustrated by the receipt from America and Australia of two African mycoplasma strains, one of the SC type and one of the LC type, both bearing the name 'Vom'. The availability of caprine SC strains appears to be less than that of LC strains. A new isolate of a caprine SC strain, with a full and accurate history, would be of particular value.

The growth characteristics of strain Smith 1423 (*M. mycoides* subsp. *capri*) resemble those of LC strains. Two-dimensional gel electrophoresis of cell proteins (Rodwell & Rodwell, 1978) gave results which suggested that the LC strains are more closely related to *M. mycoides* subsp. *capri* than to the SC strains. The results obtained in the present study and in the study by Hooker *et al.* (1979) suggest the opposite. The LC strains gave partial cross-protection against SC strains (including a CBPP strain), whereas *M. mycoides* subsp. *capri* gave none; moreover, the SC strains and the typical LC strains gave cross-protection – usually partial – against the atypical LC strain Mankefår 2833, whereas *M. mycoides* subsp. *capri* gave none. However, judgement should be reserved until a comparison has been made of the ability of SC and LC strains to cross-protect against challenge with *M. mycoides* subsp. *capri* in mucin (Smith, 1967); Smith (1969*b*) showed that a CBPP strain of *M. mycoides* subsp. *mycoides* gave partial cross-protection against the Smith 1423 strain of *M. mycoides* subsp. *capri*.

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