Mycoplasmas isolated from the respiratory tract of horses

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SUMMARY

Ten mycoplasmas were isolated from 130 nasopharyngeal swabs from thorough-bred horses with acute respiratory disease and three from 198 apparently normal horses. Two mycoplasmas were isolated from 21 tracheal swabs taken at necropsy. These mycoplasmas, together with six isolated from the equine respiratory tract by other workers, were subjected to biochemical and serological tests. Other properties examined in certain representative strains were appearance under the electron microscope, ability to adsorb or agglutinate the erythrocytes of various animal species and the electrophoretic pattern of the cell proteins.

On the basis of these tests, mycoplasmas from the equine respiratory tract were divided into seven species. Three species belonged to the genus *Acholeplasma*, members of which do not require sterol for growth, and were identified as *A. laidlawii*, *A. oculi* (formerly *A. oculusi*) originally isolated from the eyes of goats, and a recently named species *A. equifoetale*, previously isolated from aborted equine fetuses.

Of the four sterol-dependent *Mycoplasma* species, one was identified as *M. pulmonis*, a common rodent pathogen. Another cross-reacted serologically with *M. felis* and should probably be classified as that species. The other two species probably represent new species peculiar to the horse. One of these, represented by the strains N3 and N11, ferments glucose and is serologically distinct from 19 recognized species of glucose-utilizing mycoplasmas and from two species which do not metabolize either glucose or arginine. The other species, represented by four strains, hydrolyses arginine and, because it is serologically distinct from all the named arginine-hydrolysing *Mycoplasma* species, the name *M. equirhinis* sp.nov. is proposed for it.

Of the seven species, only *M. pulmonis* and the glucose-utilizing species represented by N3 and N11 were found exclusively in horses with acute respiratory disease. *A. oculi* was isolated from an apparently normal horse. The other four species were found in normal horses as well as those with respiratory disease, although three out of the four strains of *M. equirhinis* were from sick horses.

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INTRODUCTION

Mycoplasmas are common inhabitants of the upper respiratory tract of many animals. Some of these micro-organisms are associated with respiratory disease, but others occur as commensals in normal animals. Whereas the mycoplasma flora of the respiratory tract in cattle, sheep, goats and pigs has been extensively investigated (Cottew & Leach, 1969; Switzer, 1969; Leach, 1973; Whittlestone, 1973), there is relatively little information about the incidence and types of mycoplasmas in the equine respiratory tract. Dellinger & Jasper (1972) reported the isolation of two mycoplasmas from the nasal passages of two horses with respiratory disease, but apart from an electrophoretic analysis of their cell proteins, the organisms were not characterized or compared with recognized species of mycoplasmas. Kirchhoff, Deegen, Zeller & Floer (1972) and Kirchhoff (1974) isolated 19 mycoplasmas from the respiratory tract of horses, two of which were identified as *Acholeplasma laidlawii* and 17 as belonging to a new carbohydrate-metabolizing species '*M. equipharyngis*', although the latter was compared with only a few named species of *Mycoplasma*. *Acholeplasma* species, the majority of which were *A. laidlawii*, were also isolated from the oral and nasal cavities and tracheas of slaughtered horses by Ogata, Watabe & Koshimizu (1974).

Since so little is known about equine respiratory mycoplasmas and their association with disease, a search for these micro-organisms was included in a survey sponsored by the Horse Race Betting Levy Board into the problem of equine respiratory disease in Great Britain (Evans, 1971). Some preliminary results on the mycoplasmas isolated have been briefly reported (Allam, Powell, Andrews & Lemcke, 1973) and the serological cross-reactions of some of these equine mycoplasmas with *M. pulmonis* and *M. felis*, which are usually associated with other animal hosts, were discussed by Lemcke & Allam (1974). The present report describes the characterization and identification of the other mycoplasmas isolated and presents some additional information about those which cross-react with *M. felis*.

MATERIALS AND METHODS

Culture media

For the isolation of mycoplasmas from horses, seven different solid or fluid media were used. Horse serum agar (HSA) and horse serum broth (HSB) were of the type described by Hayflick (1965), consisting of Difco PPLO broth or agar supplemented with 20 % (v/v) unheated horse serum (Wellcome No. 3), 10 % (v/v) of a 25 % aqueous extract of dried yeast, penicillin (200 i.u./ml.) and 0·025 % (w/v) thallous acetate, at pH 7·8. For glucose broth (GB) and arginine broth (AB), 0·002 % (w/v) phenol red and 1 % (w/v) of either glucose or arginine was added to HSB. The pH of GB was adjusted to 7·8 and that of AB to 7·0. Solid and fluid lactalbumin hydrolysate (LH) media were similar to those of Whittlestone (1969) except that heat-inactivated pig serum (Flow laboratories, Irving, Scotland) replaced serum from enzootic-pneumonia-free pigs. In addition, DNA agar containing 0·002 % (w/v) sodium deoxyribonucleate from calf thymus and 10 % (v/v)

boiled blood extract (Andrews, 1969) was used. The serum supplement in DNA agar was 10 % (v/v) inactivated pig or human serum.

Isolation procedure

The sources of the nasopharyngeal swabs were described by Allam *et al.* (1973). The exudate from each swab was expressed into 10 ml. of unsupplemented Difco PPLO broth which served as a transport medium. Specimens were transported to the laboratory at ambient temperatures and cultured within 6 hr. of taking the swabs. Samples from the transport media were inoculated into the four fluid media (HSB, GB, AB and LHB) to give dilutions of 10^{-1} and 10^{-2}. Two plates of each of the three solid media (HS, LH and DNA agar) were also inoculated, each with 0·02 ml. of the transport medium. One set of plates was incubated aerobically and one in 5 % CO_2 in nitrogen (N_2/CO_2). Fluid media were subcultured on the corresponding solid medium when there was evidence of growth or after 7 and 14 days at 37° C. Tracheal swabs taken at necropsy from horses slaughtered at the Wellcome Research Laboratories were obtained through the courtesy of Dr G. D. Windsor. These swabs were processed in the same way except that they were expressed into HSB.

Each culture was cloned by picking a single colony from the medium which gave the best growth into the corresponding fluid medium. After incubation, the resulting broth culture was filtered through a 450 nm. Millipore filter and dilutions of the filtrate plated on an appropriate agar to produce well separated colonies. The whole process was repeated twice more.

Other equine mycoplasmas

Five mycoplasmas, BHS5, BHS11, BHS18, BHS19 and BHS20, isolated from the tracheas of horses at necropsy by Dr G. D. Windsor (Wellcome Research Laboratories) were included in this study. BHS18 and BHS19 were from horses with, respectively, a mild upper respiratory tract infection and severe strangles, but the others were from clinically healthy animals (Windsor, 1973). These five cultures were not cloned. '*M. equipharyngis*' isolated from the respiratory tract and *A. equifoetale* from an aborted equine fetus (Kirchhoff, 1974) were also examined.

Filtration characteristics and absence of reversion

Filterability was determined by the method of Leach (1973). To ensure that strains were not bacteria growing in the L-phase, seven serial subcultures were carried out at 2- to 3-day intervals on DNA agar and HSB without penicillin or thallous acetate (Leach, 1973).

Electron microscopy

Cultures grown for 17–24 hr. in HSB containing 2 % (v/v) bovine serum fraction (Difco) instead of horse serum were used. For M432/72 the broth was supplemented with arginine (0·4 %, w/v) and the pH adjusted to 7·0. Before inoculation, media were filtered successively through Millipore membranes of nominal pore diameter 450 and 220 nm. Broth cultures were fixed, the mycoplasmas were

collected on a 220 nm. Millipore filter and post-fixed, embedded and sectioned as described by Lemcke (1972). The basic staining procedure was that of Reynolds (1963) using lead citrate, but some sections were further stained with a mixture of potassium permanganate and uranyl acetate.

Biochemical tests

The breakdown of glucose, arginine and urea was tested in GB, AB or UB by the method of Aluotto, Wittler, Williams & Faber (1970). Ability to reduce 2,3,5-triphenyl tetrazolium chloride aerobically and anaerobically was determined on solid medium as described by Leach (1973). The production of 'film and spots' (Edward, 1954) was tested on HSA and on DNA agar supplemented with pig serum (10%, v/v) and containing 10% (v/v) egg-yolk emulsion (Oxoid) instead of boiled blood extract. Plates were incubated for up to 3 weeks. Aesculin hydrolysis of *Acholeplasma* species was determined by the method of Williams & Wittler (1971).

Differenitation of Acholeplasma from Mycoplasma

The ability to grow in medium without serum or at 22° C. was tested as described by Leach (1973). Inhibition of growth by sodium polyanethol sulphonate (SPS; 'Liquoid', Roche Products Ltd., Welwyn Garden City, Hertfordshire) and by digitonin was tested on HS and DNA agar by the disk method of Freundt *et al.* (1973). The cholesterol-dependence test of Edward (1971) was used to confirm that two strains, 377 and N93, were able to grow without sterol.

Haemadsorption and haemagglutination

Haemadsorption of erythrocytes to colonies was determined as described by Del Giudice & Pavia (1964), using 40 hr. cultures with about 100 colonies per plate. Haemagglutination was tested by the method of Manchee & Taylor-Robinson (1968) using *M. gallisepticum* Prem C1/69 (Goel, 1973) and an unstained *M. gallisepticum* HA antigen (Wellcome Research Laboratories, Beckenham, Kent) as controls.

Preparation of specific antisera

Rabbit antiserum was prepared against strains representative of the different species isolated, 377, N93, N14, N29A, N29B, N20, N3 and M432/72. The mycoplasmas were grown on HSA and in HSB modified by the substitution of 15% (v/v) unheated rabbit serum (Flow Laboratories) for horse serum, and were passaged twice on rabbit serum agar and at least six times in rabbit serum broth to eliminate traces of foreign serum proteins from the growth medium. Washed organisms from 125 ml. of rabbit serum broth were resuspended in 0·5 ml. of phosphate buffered saline (PBS), pH 7·3, and emulsified in 1 ml. of adjuvant (Esso Markol 52:Arlacel, 9:1, v/v). Half of the emulsion was given subcutaneously inside the right hind leg and half intramuscularly into the left flank. After 13–14 days a similar emulsion was inoculated by the same routes in collateral positions.

Thirteen days later a series of intravenous (I.V.) inoculations was begun; organisms from 250 ml. of culture resuspended in 3 ml. of PBS were administered in six I.V. doses (0·25, 0·25, 0·5, 0·5, 0·5 and 1 ml.) on alternate days. Serum was obtained 5–7 days after the last I.V. inoculation.

The sources of antisera against recognized species of *Acholeplasma* and *Mycoplasma* are given in Table 1.

Serological tests

Strains were examined by the paper disk growth-inhibition (GI) technique (Clyde, 1964) on DNA agar, and by the metabolic inhibition (MI) test described by Purcell, Taylor-Robinson, Wong & Chanock (1966). Metabolic inhibition tests were carried out in duplicate in the presence of guinea-pig serum (2·25 %, v/v, final concentration) and without guinea-pig serum. Complement fixation (CF) tests were performed as described by Hollingdale & Lemcke (1970) and immunodiffusion tests by the method of Hollingdale & Lemcke (1972).

Electrophoresis of cell proteins

The method of polyacrylamide disk electrophoresis was that of Razin (1968).

RESULTS

Isolation of mycoplasmas from swabs

Of 130 nasopharyngeal swabs from thoroughbred horses with acute respiratory disease, ten yielded mycoplasmas – an isolation rate of approximately 8 %. Three out of 198 similar swabs from apparently normal horses were positive – an isolation rate of 1·5 %. Two of the cultures, N29 and N60, which belonged to different species, were obtained from the same horse from swabs taken at an interval of 3 weeks. Two mycoplasmas were isolated from 21 tracheal swabs, an isolation rate of 10 %.

Twenty-two of the nasopharyngeal specimens could not be examined for mycoplasmas because of heavy bacterial contamination on all the primary plates and in the broths. With 14 other specimens, some of the primary plates and broths were contaminated so that the specimens were not fully examined. The rate of contamination was therefore about 11 %. None of the media inoculated with tracheal swabs became contaminated.

Nine of the 15 positive swabs produced growth on only one or two of the four fluid and three solid media inoculated from the transport medium (Table 2), but there was no consistency as to the type of medium on which growth was obtained. In five of these nine cases only one or two colonies grew on the positive plates. The highest isolation rate, 8 cultures from 15 positive swabs, was obtained in HSB or on LH or DNA agar incubated in N_2/CO_2.

Except for 1617 and 1618, only one of the cultures from each positive swab was cloned and characterized (Table 2). The swab N29 yielded two colony types on HS agar. Since these bred true, two clones, N29A and N29B, were established. Colonies of N29B were brown in colour and had larger centres than those of N29A. Thus, 16

Table 1. *Antisera used in serological tests*

	Antisera to species (and strain)	Antiserum provided by
A. Used to test *Acholeplasma* species	*A. laidlawii* (PG8 and PG9) ⎫ *A. granularum* (BTS39) ⎭	N.I.H.*
	A. axanthum (S743) ⎫ *A. modicum* (PG49) ⎬ *A. oculi* (19L) ⎭	R. M. Lemcke
B. Used to test glucose-utilizing *Mycoplasma* species	*M. lipophilum* (Maby)	M. F. Barile
	M. pneumoniae (FH) ⎫ *M. fermentans* (PG18) ⎪ *M. mycoides* subsp. mycoides (Gladysdale) ⎬ *M. bovigenitalium* (PG11) ⎭	R. M. Lemcke
	Mycoplasma sp. (bovine group 7) (N29) ⎫ *M. bovoculi* (M165/69) ⎭	R. H. Leach
	M. dispar (462/2)	R. H. Leach, E. A. Freundt
	M. bovirhinis (PG43)	N.I.H.*
	M. conjunctivae (HRC/581)	M. F. Barile
	M. hyorhinis (7)	N.I.H.*
	M. neurolyticum (KSA)	R. M. Lemcke
	M. pulmonis (Ash) ⎫ *M. canis* (PG14) ⎭	N.I.H.*
	M. edwardii (PG24)	E. A. Freundt
	M. felis (CO)	D. G. ff. Edward, E. A. Freundt, R. M. Lemcke
	M. feliminutum (Ben)	E. A. Freundt
	M. gallisepticum (X95) ⎫ *Mycoplasma* sp. (Kleckner's avian group D) (A36) ⎬	R. M. Lemcke
	M. anatis (1340)	N.I.H.*
	M. synoviae (Lasswade)	R. H. Leach
C. Used to test arginine-hydrolysing *Mycoplasma* species	*M. hominis* (PG21) ⎫ *M. orale* (Hilverda) ⎬ *M. buccale* (CH20247) ⎪ *M. salivarium* (PG20) ⎭	R. H. Leach
	M. primatum (Navel) ⎫ *M. fermentans* (PG18) ⎭	R. M. Lemcke
	M. faucium (DC333)	N.I.H.*
	M. alkalescens (D12) ⎫ *M. arginini* (M265/68) ⎪ *M. arginini* (G230) ⎬ *M. hyosynoviae* (A40) ⎪ *M. arthritidis* (Preston) ⎪ *M. meleagridis* (17529) ⎭	R. H. Leach
	M. iners (PG30)	N.I.H.*
	M. gallinarum (Fowl)	R. M. Lemcke
	M. maculosum (PG15) ⎫ *M. spumans* (PG13) ⎬ *M. gateae* (CS) ⎭	R. H. Leach

* National Institutes of Health, Bethesda, Maryland, U.S.A.; Research Reference Reagent antisera prepared in donkeys.

cloned cultures were established, 14 from 13 nasopharyngeal swabs and 2 from 2 tracheal swabs. None of the cultures showed signs of reversion to a bacterial form. The colony form was maintained with no evidence of bacterial growth after seven transfers in media without inhibitors, and microscopy of the final cultures on inhibitor-free medium also failed to reveal bacteria. Films stained with Giemsa showed minute coccal or coccobacilliform particles, and sometimes ring-shaped and other pleomorphic forms. Broth cultures of all strains passed through a 450 nm. Millipore membrane filter with a reduction in the number of colonies not exceeding 10^2.

Differentiation of Acholeplasma *from* Mycoplasma

Of the 16 cultures established by cloning, four strains, 358, N99, 377 and N93, grew on serum-free media through five successive subcultures and at 22° C. through three subcultures and were not inhibited by 5 and 10 % SPS or by 1·5 % digitonin (Table 3). Both the strains which were tested for sterol-dependence, 377 and N93, grew in the absence of cholesterol. On this basis 358, N99, 377 and N93 were identified as members of the genus *Acholeplasma*. All four *Acholeplasma* strains fermented glucose. The other 12 cultures did not grow without serum or at 22° C. and were inhibited by 5 and 10 % SPS or 1·5 % digitonin. They were there-fore identified as belonging to the genus *Mycoplasma*. Nine of these fermented glucose and three hydrolysed arginine. None hydrolysed urea (Table 3).

The strain BHS11 isolated from an equine trachea at necropsy by Windsor (1973) was also assigned to the genus *Acholeplasma* because it grew on serum-free medium and at 22° C. and was not inhibited by SPS or digitonin.

Identification of Acholeplasma *strains*

In GI tests using antisera against five recognized species of *Acholeplasma* (Table 1A), 358 and N99 were inhibited only by antisera to *A. laidlawii* (PG8 and PG9), and 377 only by *A. oculi* (formerly *A. oculusi*) antiserum. The relationship of 377 to *A. oculi* was confirmed by MI tests (Table 4). The electrophoretic patterns of 377 and *A. oculi* were also very similar but distinct from those of *A. laidlawii* and *A. modicum* (Plate 1); 377 also resembled *A. oculi* in hydrolysing aesculin strongly and reducing tetrazolium both aerobically and anaerobically.

N93 and BHS11 were inhibited in GI tests by N93 antiserum but not by antisera against any of the five recognized species of *Acholeplasma*. Moreover, N93 antiserum did not inhibit *A. laidlawii*, *A. granularum*, *A. axanthum*, *A. modicum*, *A. oculi* or three unclassified *Acholeplasma* strains, D-1, TD-3 and S2, which were recognized as distinct serological types by Tully (1973). However, a mycoplasma isolated from an aborted equine fetus and recently named *A. equifoetale* (Kirchhoff, 1974) was inhibited by N93 antiserum. N93, BHS11 and *A. equifoetale* also shared the properties of reducing triphenyl tetrazolium chloride strongly anaerobically and weakly aerobically, of weakly hydrolysing aesculin and producing 'film and spots' after 14–21 days on egg yolk or HS agar.

Table 2. *Isolation of equine mycoplasmas on various media*

Swab no.	Site swabbed*	Presence of respiratory disease	HS agar		LH agar		DNA agar		HS broth	G broth	A broth	LH broth	No. of positive cultures
			Air	N_2/CO_2	Air	N_2/CO_2	Air	N_2/CO_2					
358	N	−	−	−	−	−	−	−	+†	−	−	−	1
N99	N	+	−	−	−	−	+†	−	−	−	−	−	1
377	N	−	−	−	−	−	−	−	+†	−	−	−	1
N93	N	+	−	−	−	+	−	−	+	−	−	−	2
N14	N	+	+	−	+	−	−	+†	+	−	−	−	4
1617	N	+	−	+	−	+	−	+	+	−	−	+†	5
1618	N	+	+	+†	+	+	+	+	−	+	−	+†	8
N29	T	+	−	−	−	+	−	−	−	−	+	−	2
N20	T	−	−	+†	+	+†	+	+	−	−	−	−	5
N53	N	−	+	−	+	+†	+	+†	+	+	−	−	7
N3	N	+	−	−	−	+	−	+	−	−	−	−	2
N11	N	+	−	−	−	−	−	−	+	−	−	−	1
M432/72	N	+	−	+†	−	−	−	−	+	−	−	−	2
N60	N	+	−	−	−	+	−	+†	−	+	−	−	3
506	N	−	−	−	−	−	−	+†	−	−	−	−	1
Total of positive cultures from 15 swabs			3	4	4	8	4	8	8	3	1	2	

* N, Nasopharynx; T, trachea.

† Culture subsequently cloned and characterized.

Table 3. *Biochemical characters of mycoplasmas from the equine respiratory tract*

Strain no.	Growth without serum	Growth at 22° C.	Inhibition by:		Growth without cholesterol	Utilization of:			Tetrazolium reduction		'Film and spots'	Aesculin hydrolysis
			SPS	Digitonin		Glucose	Arginine	Urea	Air	N_2/CO_2		
358	+	+	–	–	NT	+	–	–	–	+	–	±
N99	+	+	–	–	NT	+	–	–	–	+	–	±
377	+	+	–	–	+	+	–	–	+	+	–	+
N93	–	+	–	–	+	+	–	–	±	+	+ (s)	±
N14	NT	–	+	+	NT	+	–	–	+	+	–*	NT
1617	NT	NT	+	+	NT	+	–	–	NT	NT	NT	NT
1618	NT	NT	+	+	NT	+	–	–	NT	NT	NT	NT
N29A	–	–	+	+	NT	+	–	–	–	± (t)	+ (s)	NT
N29B	–	–	+	+	NT	+	–	–	–	± (t)	+ (s)	NT
N20	–	–	+	+	NT	+	–	–	–	± (t)	+ (s)	NT
N53	–	–	+	+	NT	+	–	–	–	–	+ (s)	NT
N3	–	–	+	+	NT	+	–	–	+	+	–	NT
N11	–	–	+	+	NT	+	–	–	+	+	–	NT
N432/72	–	–	+	+	NT	–	+	–	–	–	+	NT
N60	–	–	+	+	NT	–	+	–	–	–	+	NT
506	–	–	+	+	NT	–	+	–	–	–	+	NT

(s) Produced slowly (14–21 days). (t) Very faint, transient reaction.

+, ±, –, Positive, weak positive, negative reactions. NT, Not tested.

* Grew poorly on egg yolk agar, but 'film and spots' developed on LH agar incubated 10–15 days.

Table 4. *Identification of equine mycoplasma 377 by the metabolic inhibition (MI) test*

(Results expressed as reciprocal of antiserum titre.)

Species of mycoplasma against which anti-serum produced	Titre of antiserum in MI tests with:		
	Homologous mycoplasma	A. oculi (19L)	Equine 377
A. laidlawii (PG8)	**5120**	< 10	< 10
A. laidlawii (PG9)	NT	< 10	< 10
A. granularum (BTS39)	**640**	< 10	< 10
A. axanthum (S743)	**640**	< 10	< 10
A. modicum (PG49)	**20,480**	< 10	< 10
A. oculi (19L)	(160)	**160**	640
Equine 377	(320)	80	**320**

NT, Not tested.

Table 5. *Comparison by growth inhibition (GI) tests of glucose-utilizing mycoplasmas from horses*

Mycoplasma	Zone of inhibition (mm.) with antiserum to:			
	N14	N29A	N29B	N3
N14	**4**	0	0	0
1617	3	0	0	0
1618	3	0	0	0
N29A	0	**2–3**	2 p.i.	0
N29B	0	2	**2–3**	0
N20	0	2–3	2–3	0
N53	0	2–3	2–3	0
N3	0	0	0	**3**
N11	0	0	0	3

p.i., Partial inhibition; some colonies within zone of inhibition.

Characterization of glucose-utilizing mycoplasmas

On the basis of growth and biochemical characteristics (Table 3), the nine glucose-fermenting mycoplasmas isolated from swabs fell into three groups. The first comprised N14, 1617 and 1618, the second N29A, N29B, N20 and N53, and the third N3 and N11. This classification was confirmed by GI and MI tests using antisera to representative strains (Tables 5 and 6). Despite the differences in colony morphology, N29A and N29B belonged to the same species.

Mycoplasmas identified as M. pulmonis

Strain N14, as representative of the group comprising N14, 1617 and 1618, was identified by GI, MI, CF tests and electrophoresis of cell proteins as belonging to the species *M. pulmonis*. Strains 1617 and 1618 were also inhibited in GI tests by *M. pulmonis* antisera (Lemcke & Allam, 1974).

Table 6. *Comparison by metabolic inhibition (MI) tests of glucose-utilizing mycoplasmas from horses*

	Reciprocal of titre of antiserum			
Mycoplasma	N14	N29A	N20	N3
N14	**2560**	< 10	< 10	< 10
N29A	< 10	**20**	≤ 10	< 10
N20	< 10	640	**160–320**	< 10
N3	< 10	< 10	< 10	**5120**

Titres of corresponding preimmunization sera < 10.

Mycoplasmas cross-reacting with M. felis

The cross-reactions of N29A, N29B, N20 and N53 with the type strain (CO) of *M. felis* in GI and MI tests and a comparison of the electrophoretic patterns of these strains has been reported (Lemcke & Allam, 1974). To summarize, in GI and MI tests, *M. felis* was inhibited by antisera to N29A and N29B, but these strains were not inhibited by *M. felis* antisera from three different sources (Table 1B). Strains N20 and N53 were inhibited by *M. felis* antiserum in MI, but not in GI tests. In polyacrylamide gels, only part of the electrophoretic pattern of *M. felis* was identical with that of the equine strains. To elucidate the relation of these equine mycoplasmas to *M. felis*, therefore, further tests were carried out.

Besides N29A, N29B, N20 and N53, three other strains, BHS5, BHS19 and BHS20, which were isolated from the tracheas of horses at necropsy (Windsor, 1973) and a strain representative of a newly proposed species '*M. equipharyngis*' (Kirchhoff, 1974) were inhibited in GI tests by antisera against N29A and N29B. These eight strains thus constitute a single serological group. Although *M. felis* was inhibited in GI tests by N29A and N29B antisera, '*M. equipharyngis*' was the only equine strain which was inhibited by *M. felis* antisera. The same results were obtained with all three *M. felis* antisera.

The reactions in immunodiffusion tests of *M. felis*, N29A, N29B, N20, N53 and '*M. equipharyngis*' with antisera to *M. felis*, N29B and N20 are shown in Plate 2. With *M. felis* and N20 antisera, the reaction of all six strains is virtually identical except for an extra line given by *M. felis* with its homologous antiserum (Plate 2A) and by '*M. equipharyngis*' with N20 antiserum (Plate 2B). The reactions of the antigens with N29B antiserum, however, suggest that in *M. felis* and N20 two of the precipitating components are present in lower concentration than in the other four strains. This is indicated by the faintness of the two precipitin lines (arrowed in Plate 2C) and their greater proximity to the antigen well. This may correlate with the observation that in the electrophoretic patterns produced by *M. felis* and N20, only a faintly staining band was present in the position occupied by a band which stained very intensely in strains N29A, N29B, N53 and BHS19 (Lemcke & Allam, 1974).

Of the three strains N29B, N20 and *M. felis* which were tested for haemadsorption, only N29B adsorbed sheep and chicken erythrocytes, although adsorption occurred at the periphery of the colonies and not all over the surface as with *M.*

Table 7. *Summary of the characters of* M. felis *and related equine mycoplasmas*

Test	M.felis (CO)	Equine strains				
		N29A	N29B	N20	N53	'M. equi-pharyngis'
Glucose breakdown	+	+	+	+	+	+
Tetrazolium reduction						
Aerobic	–	–	–	–	–	–
Anaerobic	+	±	±	±	–	–
'Film and spots' production	+	+ (s)	+ (s)	+ (s)	+ (s)	+ (s)
Haemadsorption of erythrocytes:						
Sheep	–	NT	+	–	NT	NT
Horse	–	NT	–	–	NT	NT
Chicken	–	NT	+	–	NT	NT
Haemagglutination* of erythrocytes:						
Sheep	2	NT	4	2	NT	NT
Horse	2–4	NT	4	2	NT	NT
Chicken	2	NT	4	2	NT	NT
Growth inhibition	Inhibited by N29A and N29B antisera	Only 'M. equipharyngis' inhibited by M. felis antisera				
Metabolic inhibition	Inhibited by N29A and N29B antisera but not by N20 antiserum	N20 and N53 inhibited, but N29A and N29B not inhibited by M. felis antiserum				
Immunodiffusion	Pattern almost identical to equine strains with M. felis and N20 antisera. Two components reacting only faintly with N29A and N29B antisera	Pattern almost identical to M. felis and N20 antisera. Two components of N20 reacting only faintly with N29A and N29B antisera				

(s) Slow, developing in 14–21 days.
NT, Not tested.
±, Very faint, transient reaction.
* Expressed as reciprocal of titre.

Table 8. *Metabolic inhibition (MI) tests with M432/72 and antisera to recognized species of arginine-metabolizing mycoplasmas*

(Results are expressed as reciprocal of titre and represent mean of tests carried out in presence and absence of guinea-pig serum).

| | Titre of antiserum in MI tests with: | |
Species of *Mycoplasma* against which antiserum was produced	Homologous mycoplasma	Mycoplasma M432/72
Mycoplasma M432/72	(1280–2560)	1280–2560
M. hominis (PG21)	5120	< 10
M. orale (Hilverda)	1280	< 10
M. buccale (CH20247)	≥ 5120	< 10
M. salivarium (PG20)	640	< 10
M. primatum (Navel)	640	< 10
M. alkalescens (D12)	81920	< 10
M. arginini (M265/68)	NT	< 10
M. arginini (G230)	≥ 10,240	< 10
M. hyosynoviae (A40)	2560	80
M. arthritidis (Preston)	≥ 5120	< 10
M. meleagridis (17529)	640	< 10
M. iners (PG30)	2560	< 10
M. gallinarum (Fowl)	≥ 5120	< 10
M. maculosum (PG15)	5120	< 10
M. spumans (PG13)	1280	< 10
M. gateae (CS)	5120	< 10

gallisepticum. Horse erythrocytes were not adsorbed. Washed suspensions of N29B, N20 and *M. felis* representing a 50-fold concentration of 40 hr. broth cultures agglutinated sheep, chicken and horse erythrocytes at titres of 2–4.

Electron microscopy of thin-section preparations of N20 and N29B showed that both strains had a fringe of loose amorphous material on the outer surface of the membrane (Plate 3A). This layer may be associated with the haemadsorbing or haemagglutinating activities of the organisms.

M. felis strongly reduced tetrazolium anaerobically whereas the equine strains gave, at most, a weak transient reaction. The equine strains and *M. felis* all produced 'film and spots' on HS and egg-yolk agar, but the reaction appeared only after incubating the equine strains for 15–21 days, compared with 7–14 days for *M. felis*. The equine strains were previously reported as not producing 'film and spots' (Lemcke & Allam, 1974) because plates were incubated for only 14 days. The properties of *M. felis* and the equine mycoplasmas are summarized in Table 7.

Characterization of strains N3 and N11

Both N3 and N11 grew very rapidly in all four types of fluid medium. On solid media, typical 'fried-egg' colonies which often exceeded 1 mm. in diameter were produced in 24 hr., whether incubated aerobically or in N_2/CO_2. A rapid fall occurred in the pH of glucose-containing media and tetrazolium was reduced aerobically and anaerobically.

Electron microscopy of thin-section preparations of N3 and N11 revealed mainly

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round but sometimes rod-shaped forms containing DNA and ribosomes, and limited by a triple-layered membrane (Plate 3B, a). An undifferentiated, electron-dense layer was seen on the external surface of the membrane (Plate 3B, b). This layer was more compact than that seen in preparations of N20 similarly stained (Plate 3A).

Colonies of N3 and N11 did not adsorb sheep, chicken or horse erythrocytes. Washed suspensions concentrated 50-fold from 24 hr. broth cultures agglutinated sheep, chicken and horse erythrocytes at titres of 2–4. In GI tests, N3 and N11 were not inhibited by antisera to the 18 glucose-fermenting *Mycoplasma* species, *M. pneumoniae, M. fermentans, M. mycoides* subsp. *mycoides, Mycoplasma* sp (bovine group 7), *M. bovoculi, M. dispar, M. bovirhinis, M. conjunctivae, M. hyorhinis, M. neurolyticum, M. pulmonis, M. canis, M. edwardii, M. felis, M. feliminutum, M. gallisepticum, Mycoplasma* sp (Kleckner's avian, group D), and *M. anatis*, nor by antisera to two species which metabolize neither glucose nor arginine, *M. lipophilum* and *M. bovigenitalium*. Zones of growth inhibition exceeding 2 mm. were given by all these antisera in GI tests against their homologous strains. In reciprocal GI tests, N3 antiserum did not inhibit any of the eight glucose-fermenting *Mycoplasma* species against which it was tested.

In MI tests, N3 was tested against all the antisera used in GI tests except *M. bovigenitalium*. *M. synoviae* antiserum, which was available only as a dilution, was also included in these tests. All 20 antisera had homologous MI titres exceeding 80 and only 3 had titres below 640. However, MI titres of these antisera with N3 never exceeded 10. Thus N3 and N11 appeared to be serologically distinct, by GI or MI or by both tests, from 21 recognized species of *Mycoplasma*.

Characterization of arginine-hydrolysing mycoplasmas

Cultural characteristics

Strains M432/72, N60 and 506 produced colonies of typical 'fried-egg' morphology on solid media. The strains grew both aerobically and in N_2/CO_2, but more luxuriant growth was obtained in N_2/CO_2 than in air, and cultures often grew only in N_2/CO_2 when recovered from the lyophilized or frozen state.

Morphology

Electron microscopy of thin sections of M432/72 showed mainly round or oval forms together with some elongated cells which probably represented organisms in the process of division. Organisms were bounded by a triple-layered limiting membrane (Plate 4a). There was no evidence of a cell wall, but there was a thin amorphous layer on the outside of the membrane (Plate 4b). The internal structure comprised fibrillar DNA, which was variable in configuration, and more electron-dense ribosomes.

Metabolic characteristics

All three strains hydrolysed arginine, with a resulting increase in the pH of arginine broth, but did not break down glucose or urea. Tetrazolium was not

Table 9. *Results of serological tests to compare* M432/72 *with* M. hyosynoviae

(Results of GI tests expressed as zones of inhibition (mm.) and results of MI and CF tests as reciprocal of serum titre.)

		Reaction with serum		
Mycoplasma	Test	Pre-immuniz-ation*	Against M432/72	Against *M. hyosynoviae* (A40)
Mycoplasma M432/72	Growth inhibition (GI)	0	**3**	0
	Metabolic inhibition (MI)	< 10	**1280–2560**	20–40
	Complement fixation (CF)	< 10	**10,240**	NT
M. hyosynoviae (S16)	Growth inhibition	0	0	**3–4**
	Metabolic inhibition	< 10	40	**1280**
	Complement fixation	< 10	80	NT

NT, Not tested.

* Corresponding to M432/72 antiserum (no preimmunization serum available for *M. hyosynoviae*).

reduced aerobically, or anaerobically (Table 3). The most characteristic feature was the rapid formation of 'film and spots' on HS, LH, DNA and egg-yolk agar. BHS18 from an equine trachea (Windsor, 1973) also hydrolysed arginine and produced 'film and spots'. Only M432/72 was tested for ability to adsorb guinea-pig, chicken and sheep erythrocytes to its colonies; no haemadsorption was observed.

Serological properties

In GI tests, M432/72, N60 and 506 were inhibited only by M432/72 antiserum and not by the corresponding preimmunization serum or antisera against the 17 species of arginine-metabolizing or arginine and glucose-metabolizing *Mycoplasma* listed in Table 1C. These antisera gave satisfactory inhibition (zones > 2 mm. in diameter) of the homologous mycoplasma. In reciprocal GI tests, M432/72 antiserum did not inhibit the growth of any of the 13 arginine-hydrolysing mycoplasmas against which it was tested. The antiserum thus seemed to be specific. BHS18 was inhibited by M432/72 antiserum, but not by the corresponding preimmunization serum.

Strain M432/72, selected as representative of this group of mycoplasmas, was also subjected to MI tests with 16 antisera to 15 recognized species of arginine-hydrolysing mycoplasmas (Table 8). M432/72 was not inhibited by any of the antisera except that against *M. hyosynoviae* strain A40. However, inhibition occurred only to a titre representing 1/32 of the homologous titre. Titres obtained with and without guinea-pig serum did not differ by more than two dilutions, but the presence of guinea-pig serum stabilized the titre for a longer period. No inhibition was observed with any of the other 15 antisera at the lowest dilution of 1 in 10, either in the presence or absence of guinea-pig serum.

The cross-reaction between M432/72 and *M. hyosynoviae* was further explored by GI, MI and CF tests (Table 9). In these tests the type strain *M. hyosynoviae* S16 was used since A40, the strain against which the antiserum was made, could not

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Table 10. *Identification of mycoplasmas from the equine respiratory tract in relation to presence of respiratory infection*

Species identified	Strain no.	Isolation site*	Acute respiratory disease	Normal†
Acholeplasma laidlawii	358	N	.	+
	N99	N	+	.
A. oculi	377	N	.	+
A. equifoetale	N93	N	+	.
	BHS11‡	T	.	+
Mycoplasma pulmonis	N14	N	+	.
	1617	N	+	.
	1618	N	+	.
M. felis	N29A and B	N	+	.
	N20	T	.	+
	N53	T	.	+
	BHS5‡	T	.	+
	BHS19‡	T	+	.
	BHS20‡	T	.	+
	'M. equi-pharyngis'§	U	U	U
Mycoplasma sp.	N3	N	+	.
	N11	N	+	.
Mycoplasma sp. (name proposed: *M. equirhinis* sp. nov.)	M432/72	N	+	.
	N60	N	+	.
	506	N	.	+
	BHS18‡	T	+	.

* N, Nasopharynx; T, trachea; U, unknown.
† Without overt signs of respiratory infection.
‡ Isolated by Dr G. D. Windsor (Wellcome Research Laboratories).
§ Isolated by Dr H. Kirchhoff (Hanover).

be obtained. No cross-reaction was observed between the two organisms by GI, but both M432/72 and *M. hyosynoviae* reacted to low titre with the heterologous antiserum in MI tests. *M. hyosynoviae* also reacted to low titre with M432/72 antiserum in the CF test. It was not possible to titrate M432/72 against *M. hyosynoviae* antiserum in the CF test since the rabbit antiserum was produced for GI and MI tests by the inoculation of organisms which were not grown in a rabbit serum medium.

Electrophoresis of cell proteins

Strain M432/72 and *M. hyosynoviae* were also compared by polyacrylamide disk electrophoresis. The electrophoretic patterns were different for each organism, the bands differing both in number and arrangement (Plate 5), although both organisms were grown under identical conditions for the same length of time (48 hr.). The figure shows gels from the same electrophoretic run.

Association of equine mycoplasmas with acute respiratory disease

The 16 mycoplasmas isolated in this laboratory together with the six mycoplasmas isolated by other workers from the equine respiratory tract were thus divided into seven species (Table 10). Of these, only *M. pulmonis* and the N3/N11 group were found exclusively in horses with acute respiratory disease. *A. oculi* was isolated only once from an apparently normal horse. The other four species were found in normal horses as well as those with respiratory disease, although three out of the four strains belonging to the M432/72 group were from sick horses.

Eight of the ten sick horses which yielded mycoplasmas from nasopharyngeal swabs were simultaneously swabbed for the isolation of viruses. The only virus isolated was a slow-growing herpes virus from the horse from which N3 was obtained (D. G. Powell, personal communication). None of the apparently normal horses or those swabbed at necropsy were examined for viruses.

DISCUSSION

The isolation rate from nasopharyngeal swabs, even from horses with acute respiratory infection (8 %), was quite low, and probably does not reflect the actual incidence of mycoplasmas in the upper respiratory tract. A much higher isolation rate (33 %) was obtained by Windsor (1973) from tracheal swabs taken at necropsy from 21 horses, most of which were clinically healthy. Kirchhoff (1974) isolated 19 mycoplasmas from the respiratory tract of 127 horses, an isolation rate of 15 %, although four sites – nose, trachea, pharynx and guttural pouch – were swabbed. A similar isolation rate for *Acholeplasma* species was obtained by Ogata *et al.* (1974) from the nasal cavities and tracheas of slaughtered horses. In our investigations, sites other than the nasopharynx were not explored because the survey was initiated among valuable thoroughbred horses with respiratory disease and it was essential that the disturbance to the animals during swabbing should be minimal. Windsor (1973) suggested several possible reasons for our low isolation rate, e.g. the type of horse population, the sensitivity of the media, the type of swab or the time elapsing before the swabs were cultured. The effects of the type of population are impossible to assess, because so little is known about the incidence of mycoplasmas in horses. It seems unlikely that the media were inadequate, since several different media with three types of serum supplement were used and incubation was in two different atmospheres.

However, the use of nasopharyngeal swabs was associated with a relatively high incidence of bacterial contamination (11 %), probably due to the introduction of swabs through the external nares. This probably accounted for some reduction of the isolation rate. The most important factor, however, was probably the method of expressing material from the swab into 10 ml. of transport medium, thereby diluting the concentration of any mycoplasmas picked up on the swab. If mycoplasmas were present in only small numbers on the swab and were then subjected to further dilution, it was probably a matter of chance whether the small volumes of transport medium used to inoculate the various media contained mycoplasmas.

This theory is borne out by the observation that 9 out of 15 positive swabs yielded growth on only 1 or 2 media and that in 5 cases only 1 or 2 colonies grew on the primary isolation plates. It seems probable that the number of mycoplasmas picked up on swabs from the equine respiratory tract is often small. Windsor (personal communication) found that four of the seven strains he obtained from tracheal swabs (Windsor, 1973) grew only from subcultures of the liquid medium in which the swabs were incubated and not on plates incubated directly from the swabs. Ogata et al. (1974) also obtained a higher isolation rate from subcultures of broths inoculated from nasal and tracheal swabs than from direct agar cultures.

It is also possible that the use of unsupplemented PPLO broth as the transport medium for nasopharyngeal swabs did not afford sufficient protection for small numbers of mycoplasmas, in spite of the presence of mucus from the swab, and that there was some decline in numbers. The use of a small volume of a more protective transport medium containing serum proteins and incubation of the swab in one of the liquid media before subculture might well improve the isolation rate.

From most of the positive swabs only one culture was cloned and characterized. However, where growth was obtained on more than one medium the growth characteristics of the other positive cultures usually suggested that the same type of mycoplasma was present in all cultures. The only exception was N20, where a glucose-fermenting mycoplasma was cloned from the culture on LH agar, but the presence of an arginine-hydrolysing organism was suggested by an increase in the pH of the primary arginine broth. However, no arginine-hydrolysing mycoplasma was subsequently isolated, and it is possible that a mycoplasma of this type was lost owing to the more rapid growth of the glucose-metabolizing strain. That a horse may carry more than one species of mycoplasma in the nasopharynx is also suggested by the recovery of the arginine-hydrolysing N60 and the glucose-fermenting N29A and N29B from the same animal, although at different times.

The morphological, cultural and filtration characteristics of the 16 cloned cultures from horses were consistent with those of members of the Order Mycoplasmatales (Subcommittee, 1972). Representatives of three species were shown by electron microscopy to have a structure typical of mycoplasmas. All three showed the presence of an amorphous layer or fringe on the outside of the limiting membrane, although the appearance of this differed between the three species. The presence of this fringe in N20 and N29B, the organisms related to *M. felis*, is noteworthy since nothing similar was reported in *M. felis*, strain CO, during an investigation by electron microscopy (Boatman & Kenny, 1970).

Three different *Acholeplasma* species were found in the equine respiratory tract. *A. laidlawii* obviously occurs fairly frequently (Ogata et al. 1974; Kirchhoff, 1974), and its presence in the equine respiratory tract is yet another example of the ubiquity of this species. *A. oculi* has previously been isolated only from the eyes of goats with keratoconjunctivitis (Al-Aubaidi, Dardiri, Muscoplatt & McCauley, 1973) and tissue cultures (Tully, 1973), although its occurrence in the latter suggested that it might be present in other animals, since tissue cultures are so frequently contaminated from the serum used to supplement the medium. Two other species, *A. granularum* and *A. axanthum*, which are normally associated with other

hosts were isolated from the conjunctiva and oral cavity, respectively, of horses by Ogata *et al.* (1974). Other acholeplasmas besides *A. laidlawii* may therefore have a variety of habitats. The third *Acholeplasma* species comprises N93, BHS11 and *A. equifoetale*. This name was proposed by Kirchhoff (1974), although her strains were compared with only three of the named species: *A. laidlawii*, *A. granularum* and *A. axanthum*. However, we have shown that N93 is also serologically distinct from the other two named species *A. oculi* and *A. modicum*. Since N93 is identical with *A. equifoetale* this name would seem to be valid, and we can identify the two respiratory strains N93 and BHS11 as belonging to this species. N93 was also serologically distinct from three unnamed *Acholeplasma* serotypes recognized by Tully (1973). Although certain *Acholeplasma* species are believed to be pathogenic (Switzer, 1969; Stipkovits *et al.* 1974) it seems unlikely that any of the *Acholeplasma* species from horses were pathogenic, since three of the five strains were from apparently healthy animals.

Since none of the sterol-dependent mycoplasmas isolated from swabs hydrolysed urea, they were excluded from the genus *Ureaplasma* (Shepard *et al.* 1974). The identification of the three glucose-utilizing mycoplasmas N14, 1617 and 1618 with *M. pulmonis* was unequivocal (Lemcke & Allam, 1974). However, it was less clear from GI and MI tests and an electrophoretic comparison of cell proteins whether N29A, N29B, N20 and N53 should be included in the feline mycoplasma species *M. felis* (Lemcke & Allam, 1974). Antigenic heterogeneity is known to be revealed in other *Mycoplasma* species by MI tests, and strains resistant to inhibition by homologous antisera in GI tests occur in species such as *M. hyorhinis*. However, the results of immunodiffusion tests and the observation that one of the equine strains belonging to this group, '*M. equipharyngis*', is inhibited in GI tests by *M. felis* antisera suggest that this group is sufficiently closely related to *M. felis* to be included in that species, despite its origin in a different host species. There were some differences in biological characters, notably in the ability to reduce tetrazolium, but such properties are known to vary within a species. In view of the relation of '*M. equipharyngis*' to *M. felis*, the former name can no longer be regarded as valid.

The eight cultures related to *M. felis* were isolated in three different laboratories from at least two different sites in the equine respiratory tract. Moreover, Kirchhoff (1974) reported that she obtained 17 strains belonging to '*M. equipharyngis*' from the respiratory tract of horses. This type of mycoplasma therefore seems to occur quite frequently in horses, and since it has been isolated from apparently healthy horses as well as from those with acute respiratory disease, it may well be a commensal. In cats, *M. felis* may have a pathogenic role in conjunctivitis and respiratory disease (Tan & Miles, 1974).

The discovery in horses of organisms formerly isolated only from rodents or cats further erodes the concept of the host-specificity of mycoplasmas. As a wider variety of animal hosts is examined, more examples of this sort are being recorded (Rosendal, 1974; Langford, 1974). It now appears that some mycoplasmas may be able to colonize more than one host species, although their pathogenicity for hosts different from those in which they are usually found is not known.

The rapid growth and large colony size of the third glucose-utilizing *Mycoplasma* species, comprising strains N3 and N11, suggested that it might be the L-phase of a bacterium, but no reversion was observed in transfers made immediately after isolation on inhibitor-free medium. It seems probable that this represents a new species, since it was serologically distinct from 21 recognized species of *Mycoplasma*. However, there are at least seven named species of glucose-metabolizing mycoplasmas (e.g. *M. mycoides* subsp. *capri*, *M. capricolum*, *M. putrefaciens*, *M. suipneumoniae*, *M. caviae*, *M. molare* and *M. cynos*) with which no comparisons were made. We therefore propose, before suggesting a name, to submit N3 and N11 to the FAO/WHO International Centre for Animal Mycoplasma, Aarhus, Denmark, for further examination. Strains N3 and N11 were both isolated from horses with respiratory disease, although the growth characteristics of this species are not those associated with other respiratory pathogens such as *M. pneumoniae* and *M. suipneumoniae*.

The four arginine-hydrolysing mycoplasmas examined all belonged to a single biochemical and serological group. The cross-reaction observed between the representative of this group, M432/72, and *M. hyosynoviae* in MI and CF tests was of a low order, and since there was no cross-reaction in GI tests and the electrophoretic patterns of the two organisms were quite distinct, it seems justifiable to regard M432/72 as belonging to a distinct species. Antigenic similarities have been reported between other arginine-hydrolysing species. Lemcke (1965) and Kenny (1973) found a higher incidence of cross-reactions in immunodiffusion tests among these mycoplasmas than among glycolytic species.

The cross-reaction of M432/72 with a porcine mycoplasma raised the question whether M432/72 and 506, the first members of the group to be isolated, could have originated in the pig serum used to supplement the DNA agar on which they were isolated, despite inactivation of that serum. However, similar cultures from the swab which yielded M432/72 were also obtained in broth supplemented with horse serum. Moreover, N60 was isolated on DNA agar supplemented with human serum, and BHS18 in a fluid medium containing horse serum.

In fact the species represented by M432/72 may occur fairly frequently in the respiratory tract of horses. Not only was it recovered three times from nasopharyngeal swabs but also once from a tracheal swab in another laboratory using an entirely different procedure (Windsor, 1973). Moreover, arginine-hydrolysing mycoplasmas which are inhibited in GI tests by our antiserum against M432/72 have been isolated by Dr J. Poland (personal communication) from the respiratory tract of slaughtered horses. However, three of the four strains which we examined were isolated from horses with respiratory disease, so a pathogenic role for this species cannot be excluded.

This group of arginine-hydrolysing mycoplasmas from horses is thought to represent a new species because it is serologically different from 17 other named arginine-hydrolysing species. The proposals for minimal standards for descriptions of new species of Mycoplasmatales (Subcommittee, 1972) suggest that, as a minimum, any new mycoplasma should be shown to be antigenically different from all species having the same habitat and/or showing the same general biological

properties. Since arginine was hydrolysed, the type strain, M432/72 was compared serologically with as many other arginine-hydrolysing *Mycoplasma* species as possible by GI and MI tests. All the recognized species with which it was compared were from other hosts, since no arginine-hydrolysing mycoplasmas from horses have been characterized or named. As far as can be as ascertained at present, the only other recognized arginine-hydrolysing mycoplasma with which no comparison was made in our tests is the unnamed avian serotype L (Fabricant, 1969). We therefore propose the name of *Mycoplasma equirhinis* for the new species, and designate M432/72 as the type strain. The specific epithet indicates the host and one of the isolation sites, the equine nose or nasopharynx. Examples of such nomenclature already exist in the names *M. bovirhinis* (Leach, 1967) and *M. hyorhinis* (Switzer, 1955). A culture of this strain has been deposited in the National Collection of Type Cultures, Colindale, under the number NCTC 10148.

The classification of the mycoplasmas examined during this investigation into seven different species has provided a basis, previously lacking, for the identification of mycoplasmas from the equine respiratory tract. The pathogenicity of these species for horses remains to be determined.

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EXPLANATION OF PLATES

PLATE 1

Comparison of cell proteins of equine mycoplasma 377 with recognized *Acholeplasma* species by polyacrylamide disk electrophoresis. (A) 377, (B) *A. oculi* (19L), (C) *A. laidlawii* (PG9), (D) *A. modicum* (PG49).

PLATE 2

Immunodiffusion reactions of equine mycoplasmas N29A, N29B, N20, N53, '*M. equipharyngis*' and *M. felis* (CO); (A) with antiserum to *M. felis*; (B) with antiserum to N20; (C) with antiserum to N29B. Antiserum in central wells. Peripheral wells contain antigens at 5 mg. protein per ml., lysed with Triton X-100 at 10 mg. per ml.; 1, *M. felis* (CO); 2, N20; 3, N53; 4, N29A; 5, N29B; 6, '*M. equipharyngis*'. Arrows in (C) indicate faintly reacting components in *M. felis* and N20.

PLATE 3

Electronmicrographs of thin-section preparations of equine mycoplasmas stained with Reynold's lead citrate and a mixture of potassium permanganate and uranyl acetate. (A) Mycoplasma N20; (*a*) × 72,000; (*b*) × 260,000. (B) Mycoplasma N3; (*a*) × 72,000; (*b*) × 266,000.

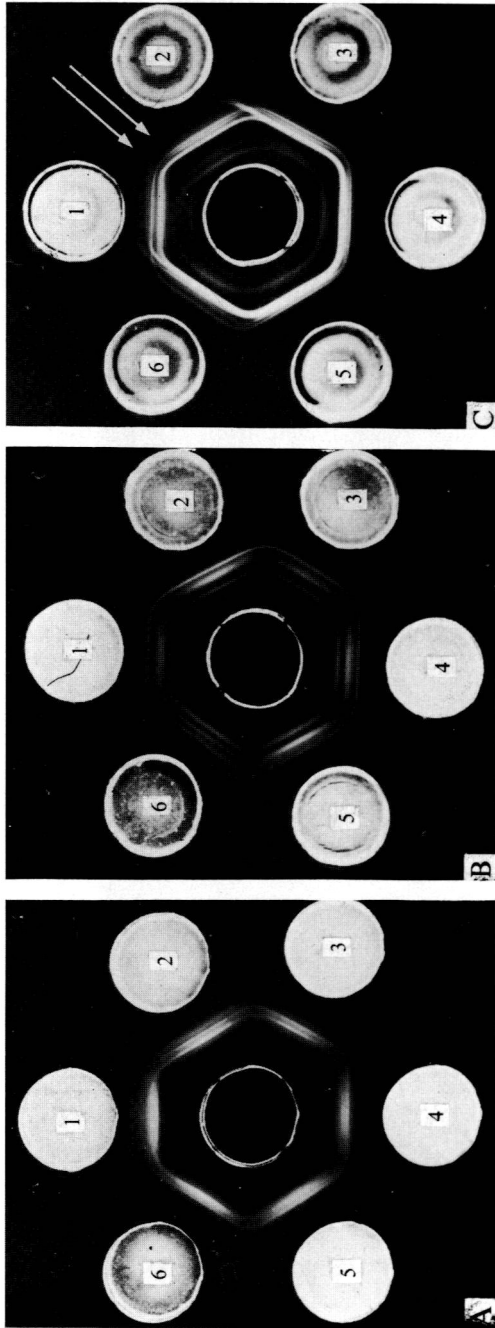### PLATE 4

Electronmicrographs of thin-section preparations of equine mycoplasma M432/72 (*M. equirhinis*). (*a*) × 64,000; (*b*) × 241,000. Stained with Reynold's lead citrate.
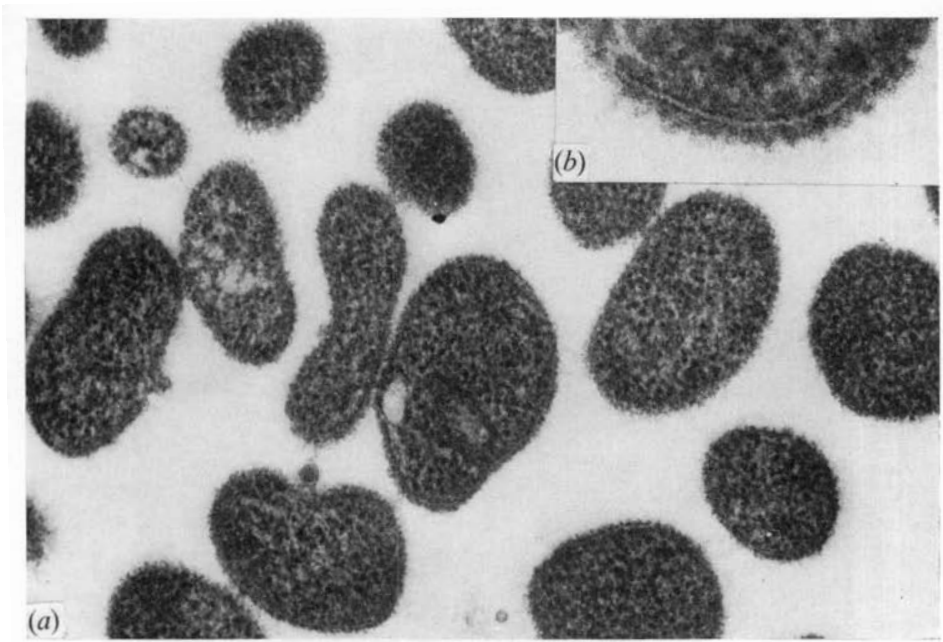
PLATE 5

Comparison of cell proteins of M432/72 and *M. hyosynoviae* by polyacrylamide disk electrophoresis. (A) M432/72 (*M. equirhinis*); (B) *M. hyosynoviae* (S16).
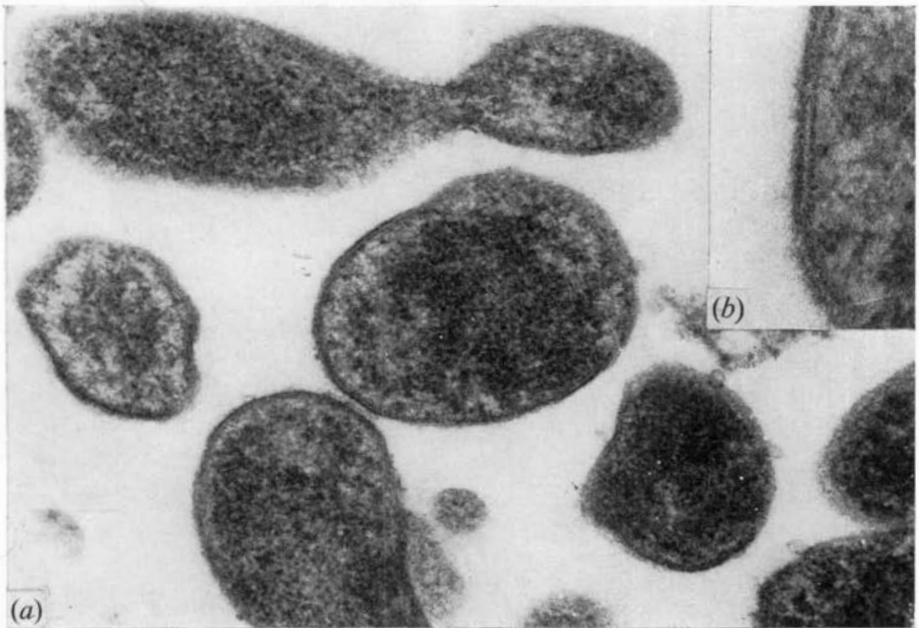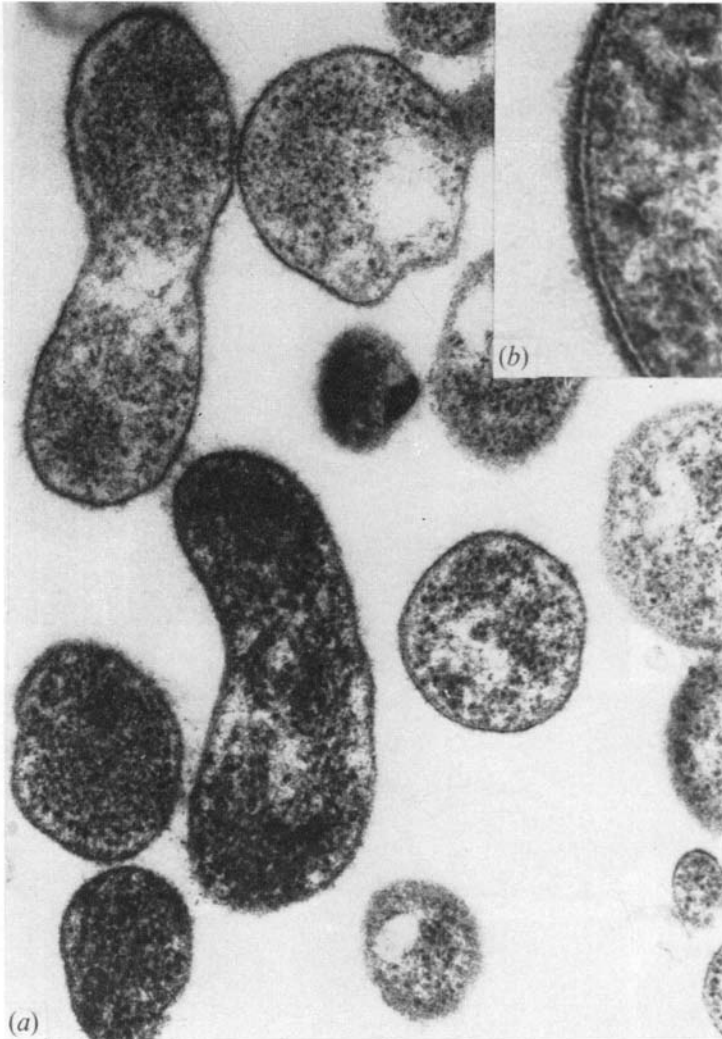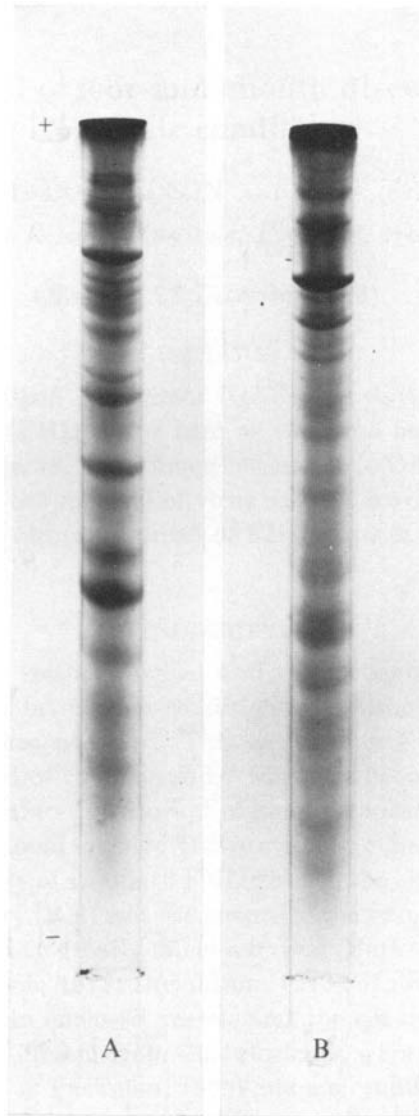
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Plate **3**

A

B

N. M. ALLAM AND R. M. LEMCKE

N. M. ALLAM AND R. M. LEMCKE

A   B

N. M. ALLAM AND R. M. LEMCKE