BRIEF NOTE

Isolation of *Mycoplasma hyosynoviae* and Other Mycoplasmas from the Respiratory Tracts of Pigs by Aerobic and Anaerobic Cultivation

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Although there have been several reports on mycoplasmal flora of the respiratory tracts of pigs, especially those with enzootic pneumonia [1, 4-6, 8, 13], the incidence and distribution of *Mycoplasma hyosynoviae* in pigs have been poorly investigated. Our previous report [11] stated that anaerobic cultivation is effective in selective isolation of *M. hyosynoviae*. The present report describes isolation and identification of mycoplasmas other than *M. hyopneumoniae* and *M. flocculare* by anaerobic cultivation from the respiratory tracts of pigs from various herds.

The media used for isolation of mycoplasmas were MAG-Broth and M-Agar as described previously [11]. The pigs examined for mycoplasmas are characterized in Table 1. All pigs except A5 and A6 were derived from herds with enzootic pneumonia. Isolation of mycoplasmas was performed in the same manner as described in the previous report [11]. In addition, pneumonic lesion of about 5 mm³ was homogenized with 4 ml of MAG-Broth and the supernatant was used as an inoculum.

Identification of isolates was first con-

ducted with antisera against *M. hyorhinis* BTS7 and *M. hyosynoviae* S16 by epi-immunofluorescence and in some cases the growth inhibition test and the metabolism inhibition test were also performed. Isolates not identified with these antisera were further tested with those against the following strains: *M. arginini* G230, *M. gallinarum* PG16, *M. iners* PG30, *M. salivarium* PG20, *Acholeplasma laidlawii* PG8 and *A. granularum* BTS39.

Distribution of mycoplasmas in the respiratory tracts of seven pigs in group A is given in Table 2. Both *M. hyorhinis* and *M. hyosynoviae* were isolated frequently from the nasal cavities. However, their incidence was lower in the lower respiratory tracts.

The incidence of mycoplasmas in the nasal cavities and pneumonic lungs of pigs from group A, B, C and D is summarized in Table 3. The overall isolation rates of *M. hyorhinis* and *M. hyosynoviae* from the nasal cavities were very close each other (70%), and the rates are almost the same in all the groups examined. Moreover, the coexistence of these two species in the nasal

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Table 1. Pig groups examined

Group	Case No.	Number of cases	Gross lesion	Sample ^a	Remark
A	A5-A6	2	None	N, T, P	Experimental animal
	A7-A11	5	Pneumonia	N, T, PL	Farm animal
B	BN1-BN17	17	NI ^b	N	Slaughtered animal
	BL1-BL6 BL10-BL21	18	Pneumonia	PL	Slaughtered animal
C	CN1-CN20	20	NI	N	Slaughtered animal
	CL1-CL18	18	Pneumonia	PL	Slaughtered animal
D	D1-D60	60	NI	N	Slaughtered animal

^a, N: Nasal cavities. T: Trachea. P: Pharynx. PL: Pneumonic lungs.

^b, NI: Not inspected.

Table 2. Distribution of mycoplasmas in respiratory organs of Group A

Case No.	Organ ^a	<i>M. hyorhinis</i>	<i>M. hyosynoviae</i>	Others
A5	N	— ^b	+ ^c	—
	P	—	+	—
	T	—	—	—
A6	N	+	+	+ ^d
	P	+	+	—
	T	—	—	—
A7	N	—	+	—
	T	—	+	—
	PL	—	—	—
A8	N	+	—	—
	T	+	—	—
	PL	+	—	—
A9	N	+	+	+ ^d
	T	+	+	—
	PL	+	—	—
A10	N	+	—	—
	T	+	—	—
	PL	—	—	—
A11	N	+	+	—
	T	—	+	—
	PL	—	+	—
Total	N	5/7 ^e	5/7	2/7
	P	1/2	2/2	0/2
	T	3/7	3/7	0/7
	PL	2/5	1/5	0/5

^a, N: Nasal cavities. P: Pharynx. T: Trachea.

PL: Pneumonic lungs.

^b, Negative isolation. ^c, Positive isolation.

^d, *Acholeplasma laidlawii*.

^e, Number of pigs positive/number of pigs examined.

cavities was as high as 50% throughout the survey. On the contrary, the isolation rates of *M. hyorhinis* and *M. hyosynoviae* from pneumonic lungs were relatively low, and they were about 30% and 10%, respectively.

Other mycoplasmas were isolated only from the nasal cavities; *A. laidlawii* from nine pigs (9%) in three different groups, *M. salivarium* from six pigs (6%) in two groups, and *M. arginini* from one pig. The remaining three strains, which were all digitinin-sensitive and arginine-hydrolyzers, were not identified by the antisera against the reference strains used in this study and type strains of following seven species used in the previous study [11]; *M. spumans*, *M. opalescens*, *M. gateae*, *M. arthritidis*, *M. gallinarum*, *M. hominis* and *M. orale*.

All strains identified as *M. salivarium* were isolated only by anaerobic cultivation and strains of *M. hyosynoviae* and *M. arginini* and three unidentified strains were isolated more easily by anaerobic than by aerobic cultivation. *A. laidlawii* was isolated by either cultivation.

Relatively high incidence of *M. hyosynoviae* and its frequent coexistence with *M. hyorhinis* in the nasal cavities of pigs in this survey, compared to another report [12], can be attributed partly to the im-

Table 3. Isolation of mycoplasmas from nasal cavities and pneumonic lungs of Group A-D

Mycoplasma	Positive isolation from nasal cavities						Positive isolation from pneumonic lungs			
	A/7 ^a	BN/17	CN/20	D/60	Total/104		A/5	BL/18	CL/18	Total/41
<i>M. hyorhinis</i>	5 ^b (71%)	10 (59%)	17 (85%)	41 (68%)	73 (70%)		2 (40%)	6 (33%)	4 (22%)	12 (29%)
<i>M. hyosynoviae</i>	5 (71%)	11 (65%)	14 (70%)	41 (68%)	71 (68%)		1 (20%)	2 (11%)	1 (6%)	4 (10%)
<i>A. laidlawii</i>	2 (29%)	3 (18%)	0	4 (7%)	9 (9%)		0	0	0	0
<i>M. salivarium</i>	0	1 (6%)	0	5 (8%)	6 (6%)		0	0	0	0
<i>M. arginini</i>	0	1 (6%)	0	0	1 (1%)		0	0	0	0
<i>M. spp.</i> ^c	0	0	2 ^d (10%)	1 ^e (2%)	3 (3%)		0	0	0	0
None	0	2 (12%)	1 (5%)	5 (8%)	8 (8%)		2 (20%)	11 (61%)	13 (72%)	26 (63%)
Coexistence of <i>M. hyorhinis</i> and <i>M. hyosynoviae</i>	3 (43%)	7 (41%)	12 (60%)	30 (50%)	52 (50%)		0	1 (6%)	0	1 (2%)

^a, Group/number of cases examined. ^b, Number of positive cases.

^c, Arginine hydrolysing and digitonin-sensitive strains.

^d, Strains CN16f and CN17a. ^e, Lost during identification.

proved isolation of the organism by anaerobic cultivation.

M. salivarium has been regarded as resident flora of human oropharynx [14], and isolated also from nonhuman primates [2, 3, 7, 9, 10]. Friis [4] reported isolation of a *M. salivarium*-related strain from the nasal mucosa of a pig. In the present survey, *M. salivarium* was isolated from six pigs in two separated groups by anaerobic cultivation, suggesting a prevalence of the organism also among pigs. Further investigation is required to find the distribution of *M. salivarium* among pigs and other animals.

The present study has revealed the usefulness of anaerobic cultivation in isolation of mycoplasmas. Both anaerobic and aerobic cultivation should be used for isolating unspecified mycoplasmas as well as *M. hyosynoviae*.

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要 約

好気および嫌気培養を用いた豚の呼吸器からの *Mycoplasma hyosynoviae* その他のマイコプラズマの分離(短報): 河村晴次・山本孝史・尾形 学(東京大学農学部家畜微生物学教室)——*M. hyosynoviae* を主対象として4群の豚について呼吸器マイコプラズマの検索を嫌気培養を併用して行った。*M. hyorhinis* と *M. hyosynoviae* は鼻腔に高率(約70%)に常在, 共存(約50%)していたのに対して, 両菌種の肺炎病巣部からの分離率はそれぞれ30%, 10%に過ぎなかった。また, *M. salivarium* は鼻腔から嫌気培養によってのみ分離されたことが注目される。