各種鳥類からのマイコプラズマの分離･同定

誌名	日本獣醫學雑誌 = The Japanese journal of veterinary science
ISSN	00215295
著者	清水, 高正 沼野, 和彦 内田, 幸治
巻/号	41巻3号
掲載ページ	p. 273-282
発行年月	1979年6月

Jap. J. vet. Sci. **41**, 273～282 (1979)

Isolation and Identification of Mycoplasmas from Various Birds: An Ecological Study

Takamasa SHIMIZU, Kazuhiko NUMANO* and Koji UCHIDA**

Department of Veterinary Hygiene, Faculty of Agriculture, Miyazaki University, Miyazaki-shi, Miyazaki 880

(Received for publication November 10, 1978)

Abstract. A total of 850 birds belonging to 40 species within 13 orders were examined for mycoplasmas during 1968 through 1977. In addition, 2,848 dead-in-shell chicks, 275 pathological specimens and 285 normal semen samples of chickens were subjected to the study. It was revealed that birds belonging to either *Galliformes* or *Columbiformes* harbored mycoplasmas in high incidence. Serological identification of 440 isolates proved that both *Mycoplasma gallisepticum* and *M. synoviae* parasitized among chickens, with an exception of sparrows. Host range of *M. gallinarum* was the widest of all species or serogroups of avian mycoplasmas. *M. iners* and serogroups C, D, and I-J-K-N-Q-R were detected from various tissues and sperm samples of domesticated and wild birds belonging to *Galliformes*. Both *M. columbinum* and *M. columborale* were detected only from pigeons, which were considered as the common hosts for these two species of mycoplasmas.

Although 6 species and several serogroups [2, 3, 11, 25] of avian mycoplasmas have currently been recognized, the common reservoirs and host ranges of some of them are remained unclear. On the other hand, very little information is available concerning the parasitism of mycoplasmas among free-flying wild birds, although it has been reported that some non-domesticated birds harbor mycoplasmas [12, 15, 18].

We have continued a series of ecological study to determine the incidence of mycoplasmas in domesticated, pet and wild birds during 1968 through 1977. Two hitherto unrecognized species were detected from pigeons, for which names of *Mycoplasma columbinum* and *M. columborale* have been proposed [24]. This report contains the results of isolation and identification of mycoplasmas from various birds during these 10 years.

Materials and Methods

Birds: Various birds belonging to 40 species within 13 orders were subjected to the present study. All of them had been kept, caught or found dead within southern part of Kyushu, Japan. Sources and specimens as well as numbers of these birds examined are given in Table 2. Among chickens used, 78 spermducts and 110 each of uterine and vaginal mucosa were taken from a part of 580 normal chickens subjected to the examination of tracheas, whereas 55 legs with arthritis and 220 livers with abscess were taken at two meat processing factories. Samples of semen taken for the purpose of artificial inseminations were obtained from individual sires in 22 farms. The majority of wild birds were found dead in the field, but a part of game birds such as pheasants and mallards were shot during the hunting seasons. Thirty-five pigeons residing in the buildings of two schools and dropping ticks were caught under the recognition of harmful birds by the local environmental inspectors and submitted to this laboratory. Of 47 sparrows,

Present addresses: *Eastern Animal Health Institute, Yamaguchi and **Agricultural Technical Center, Pfizer-Taito Co., Ltd, Shizuoka

Table 1. Growth inhibition patterns of reference strains with antisera used

Antiserum against	*Mycoplasma* strain																				
	801	862	R49	640	867	886	WVU1853	1340	MMP1	MMP4	8	SA	694	859	TC5	TC3	695	693	1855	PHN–D13	L3–10	D2497
M. gallisepticum, 801	4*																					
M. gallinarum, 862		4	3																			
M. gallinarum, R49		3	3																			
M. iners, 640				5	5																	
M. iners, 867				3	3																	
M. meleagridis, 886						5																
M. synoviae, WVU1853							3															
M. anatis, 1340								2														
M. columbinum, MMP1									4													
M. columborale, MMP4										3												
D, 8											4											
F, SA												7										
L, 694													4									
C, 859														3	5	3						
O, TC5															5	2						
P, TC3															6	5						
I, 695																	3		2	1	2	2
J, 693																		4	1			
K, 1855																	3	1	6	2	2	2
N, PHN–D13																	1	1	1	10		1
Q, L3–10																	1		2		3	1
R, D2497																	1		3	1	1	3

Remarks.

* Zone of growth inhibition in mm.

22 were caught in a chicken pen while they invade it to pick feed, whereas the remainder was caught or found dead in the field. Dead or diseased birds from pet shops and 4 parks including a zoo were subjected to this study for diagnostic purpose.

Media: The base medium employed during 1968 and 1972 was composed of 2.5% heart infusion broth base (Eiken), 1.0% phytone (Difco), 0.5% yeast extract (Difco), 0.16% disodium phosphate, and 0.01% monopotassium phosphate in deionized water. This was substituted by 2.32% Mycoplasma broth base (Pfizer Diagnostic Division) during 1973 through 1977. A 850 m*l* each of base medium was supplemented with 150 m*l* of heat-inactivated swine serum, 2 m*l* of 1% diphosphopyridine nucleotide (Sigma) mixed with the same amount of 1% l-cysteine-HCl, 2 m*l* of 1% phenol red, 5 m*l* of 5% thallium acetate and 1,000 U/m*l* of penicillin. In addition, 10 m*l* of Eagle vitamine solution (Difco 100X) was added to the Pfizer medium. The pH was always adjusted to 7.8. When a solid or semi-solid medium was required, 1 g and 0.2 g of J-agar (Sanko-Junyaku) was added to the base medium, respectively.

Isolation of mycoplasmas: A small piece of trachea, liver abscess, spermduct, uterine and vaginal mucosa of a bird or pipped chick embryo, and a loopful of yolk from non-developed chick embryo was inoculated into 2 to 5 m*l* of liquid medium. After 2 to 3-day incubation at 37°C, a loopful of each culture was streaked on a solid medium. Oropharyngeal and cloacal swabs and one drop each of sperm samples were directly distributed on a plate medium. All the inoculated plates were contained in candle jars and incubated at 37°C, and growth of colonies was observed every 3 days for a period of 15 days.

Cloning of the isolates: When a large number of isolates were obtained at one time, as in the case of sperms and dead-in-shell chicks, several well-isolated colonies were picked from a plate and inoculated into 1 m*l* each of liquid medium. A loopful of 2-day culture was distributed on a plate so as to obtain well-isolated colonies. This was re-

peated three times, and each strain was subjected to the preliminary identification. All of the isolates from wild and pet birds were filter-cloned 2 to 3 times through millipore filters with average porosity size of 450 nm. Several strains from a sample were routinely subjected to the preliminary test, and if they were revealed to be serologically identical, only one strain was employed for the confirmation test. When two or three serogroups were detected from a material, one each strain of different serogroup was incorporated for further studies. A total of 440 strains selected by this manner were used in this study.

Antisera: Antisera were prepared by immunizing rabbits with 19 reference strains of Dierks et al. [6] obtained through the courtesy of Professor M. L. Frey, Iowa State University, Ames., Iowa. *M. anatis* strain 1340 was gifted by Mrs. Sylvia Cunningham, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland. Antigens of these strains were prepared by the methods of Dierks et al. [6]. Rabbit antisera against *M. columbinum* MMP1 and *M. columborale* MMP4 were also prepared and employed in this study. Growth inhibition of these antisera against the reference strains are shown in Table 1.

Serological test: Growth inhibition test was employed for the identification of new isolates. The dried paper disc technique of Dighero et al. [7] was used for the preliminary identification, and the disc method of Clyde [5] and well method of Black [4] were employed in the confirmation test.

Biochemical test: The test for cholesterol requirement was performed by inoculating a small block of plate culture of a test strain into 2 m*l* of liquid medium containing no serum, and a loopful of 2-day-old culture was streaked on a solid medium containing no serum. The control medium was supplemented with 15% swine serum. Strains grew with serum but did not without serum after incubating the inoculated plate at 37°C for 7 days were regarded as requiring cholesterol for growth. Besides, strains isolated from pigeons and other pet- and wild-birds were tested for the sensitivity to digitonin by the method of Freundt et al. [10]. Breakdown of glucose and arginine was examined with all of the test strains grown in a liquid medium containing 0.5% of either of the two substrates. Production of film and spots was determined on a solid medium containing 20% horse serum instead of 15% swine serum. Colonial hemoadsorption (CHA) of chicken erythrocytes was tested by a modified method of Sato et al. [23]. Two- to 3-day-old broth culture of a test strain was diluted 10- and 100-fold in broth, and a loopful of each dilution and undiluted culture was spotted on the surface of solid medium. Three strains were inoculated on a plate of 6 cm diameter. After incubating the inoculated plate at 37°C for 3 days, approximately 3 m*l* of 0.3% washed chicken erythrocytes suspended in physiological saline was flooded on the plate, which was then left at room temperature for 10 minutes. Surface of the plate was calmly washed with saline three times, and colonial hemoadsorption was observed under microscope at ×50 magnification.

Results

1. Incidence of mycoplasmas in birds

A total of 850 birds, 2,848 dead-in-shell chicks, 275 pathological specimens and 285 normal semen samples of chickens were examined for mycoplasmas. Results of isolation of mycoplasmas from these materials are given in Table 2. Mycoplasmas were detected from birds belonging to 11 species in 6 orders, namely, chicken and Japanese bantam, green pheasant, bamboo partridge, swan, white-fronted goose, sparrow, brown-eared bulbul, demoisella crane, adjutant, night heron, and pigeon. Among them, birds belonging to either *Galliformes* or *Columbiformes* harbored mycoplasmas in high incidence (Table 2).

Except for pathological specimens of chickens, both tracheal and oropharyngeal mucosa were more frequently infected with mycoplasmas than any other tissues examined; they were exceptionally isolated from a cloacal swab of a swan, and eye swabs of a swan in a zoo and 4 pigeons found dead in the field. Usually, domesticated birds such as chickens and bantams were more commonly parasitized by these organisms, but some wild birds like green pheasants and pigeons also harbored mycoplasmas in high rate. All of the 3 sparrows from which mycoplasmas were detected had been caught in a chicken pen with other 19 sparrows.

2. Identification of the isolates

Results of serological identification of the

Table 2. Materials examined and incidence of mycoplasmas

Bird (Latin name)	Source	No. of bird examined	Specimen	No. pos. for myco. (%)
Galliformes				
Chicken (*Gallus gallus domesticus*)	Farm	580	T*	87 (15%)
	Factory	55	Arthritis, leg	3 (5%)
	Factory	220	Liver abscess	23 (10%)
	Farm	78	Spermduct	11 (14%)
	Farm	110	Uterus & vagina	4 (4%)
	Farm	280	Sperm	121 (42%)
Dead-in-shell chick	Hatchery	1,626	Pipped, T	57 (4%)
	Hatchery	1,222	Non-developed, yolk	0
Japanese bantam (*G. gallus domesticus*)	Farm	10	Or	10 (100%)
Green pheasant (*Phasianus colchicus*)	Wild	21	T	8 (38%)
Copper pheasant (*P. soemmerringii*)	Wild	4	T	0
Bamboo partridge (*Bambusicola thoracica*)	Wild	8	T,Or	1 (13%)
Golden pheasant (*Chrysolophus pictus*)	Farm	9	T,Or	0
Indian peafowl (*Pavo cristatus*)	Zoo	1	T	0
Anseriformes				
Swan (*Cygnus bewickii*)	Zoo	5	T,Or,E,C	2 (40%)
White-fronted goose (*Anser albifrons frontalis*)	Farm	1	T,Or,E,C	1
Mallard (*Anas platyrhynchos platyrhynchos*)	Wild	17	T	0
Duck (*A. platyrhynchos domesticus*)	Farm	2	T,Or,E,C	0
Falcated teal (*A. falcata*)	Wild	2	T	0
Teal (*A. crecca*)	Wild	3	T	0
Shoveler (*A. clypeata*)	Wild	1	T	0
Mandarin duck (*Aix galericulata*)	Farm	1	T	0
Passeriformes				
Sparrow (*Passer montanus*)	Wild	47	T,Or	3 (6%)
Brown-eared bulbul (*Hypsipetes amaurotis*)	Wild	1	T	1
Jungle crow (*Corvus macrorhynchos*)	Wild	1	T,Or,E,C	0
Canary (*Serinus canarinus*)	Farm	12	T	0
Mina (*Gracula religiosa*)	Shop	3	T,Or	0
Emeraldbird (*Larvivora cyane*)	Shop	5	T,Or	0
Lovebird (*Uroloncha striata domestica*)	Shop	3	T	0
Dusky thrush (*Turdus naumanii*)	Wild	1	T	0
Gruiformes				
Demoisella crane (*Anthropoides virgo*)	Zoo	1	T,Or	1
Coot (*Fulica atra*)	Wild	1	T	0
Moorhen (*Gallinula chloropus*)	Wild	3	T	0
Watercock (*Gallicrex cinerea*)	Wild	1	T	0
Stork (*Balearica pavonina*)	Zoo	1	T,Or,E,C	0
Ciconiiformes				
Adjutant (*Leptoptilus dubius*)	Zoo	2	T,Or	2
Night heron (*Nycticorax nycticorax*)	Wild	9	T,Or,E,C	2 (22%)
Purple heron (*Ardea purpurea*)	Zoo	2	T,Or	0
Little egret (*Egretta garzetta*)	Wild	1	T,Or,C	0
Columbiformes				
Pigeon (*Columba livia domestica*)	Zoo, school, field	65	T,Or,E	45 (69%)
Psittaciformes				
Parakeet (*Psittacula* sp.)	Shop	6	T	0
Budgerigar (*Melopsittacus* sp.)	Shop	2	T	0
Macaw (*Ara macas*)	Zoo	4	T,Or,E,C	0

Table 2. (Continued)

Other Orders				
Woodcock (*Scolopax rusticola*)	Wild	7	T	0
Hodgson's hawk eagle (*Spizaetus nipalensis*)	Wild	2	T	0
Japanese green woodpecker (*Picus awokera*)	Wild	1	T	0
Ural owl (*Strix uralensis*)	Wild	1	T	0
Flamingo (*Phoenicopterus roseus*)	Zoo	2	T,Or,E,C	0
Ostrich (*Struthio camelus*)	Zoo	1	T,Or,E,C	0

Remarks.

Latin names of birds were followed after the "Check-List of Japanese Birds", 5th ed., by the Ornithological Society of Japan, published by Gakken Co., Ltd., Tokyo (1974).

* Abbreviations: T, trachea; Or, oropharynx; E, eye cavity; C, cloaca. These apply to the Table 3.

Table 3. Results of serological identification of avian isolates

Source	No. of strains isolated	Species or serogroup										
		M. gallisepticum	*M. synoviae*	*M. gallinarum*	*M. iners*	C	D	1–J–K–N–Q–R	*M. columbinum*	*M. columborale*	*A. laidlawii*	Unidentified
Chicken												
Trachea, affected	59	48	4	6	—	—	—	1	—	—	—	—
Trachea, normal	31	—	—	28	2	1	—	—	—	—	—	—
Arthritis	3	2	1	—	—	—	—	—	—	—	—	—
Liver abscess	23	—	1	18	4	—	—	—	—	—	—	—
Spermduct	11	—	—	4	—	7	—	—	—	—	—	—
Sperm	129	1	—	66	19	26	10	5	—	—	—	2
Uterus and vagina	4	—	—	2	—	2	—	—	—	—	—	—
Dead-in-shell chick	57	50	2	3	—	1	—	1	—	—	—	—
Japanese bantam (Or*)	10	—	—	1	—	9	—	—	—	—	—	—
Green pheasant (T)	8	—	—	—	—	8	—	—	—	—	—	—
Bamboo partridge (Or)	1	—	—	1	—	—	—	—	—	—	—	—
Sparrow (T)	4	2	—	2	—	—	—	—	—	—	—	—
Swan (C,E)	2	—	—	1	—	—	—	—	—	—	—	1
Demoisella crane (Or)	1	—	—	1	—	—	—	—	—	—	—	—
Pigeon(T,Or,E)	93	—	—	—	—	—	—	—	40	51	—	2
Adjutant (Or)	1	—	—	—	—	—	—	—	—	—	1	—
White-fronted goose (Or)	1	—	—	—	—	—	—	—	—	—	—	1
Night heron (T)	2	—	—	—	—	—	—	—	—	—	—	2
Total	440	103	8	133	25	54	10	7	40	51	1	8

Remarks.

* Abbreviations are same as those in Table 2.

new isolates are given in Table 3. Of 440 strains isolated, 439 did not grow on a solid medium containing no serum and were identified as *Mycoplasma* spp.. Among them, 431 strains were serologically identified while 8 strains were remained unidentified.

The majority of 103 strains of *M. gallisepticum* and all of 8 strains of *M. synoviae* were isolated from diseased chickens and dead-in-shell chicks (Table 3). The former species was also isolated from tracheas of

Table 4. Main biochemical properties of mycoplasmas from various birds

Biopattern		1	2	3	4	5	6
Property	CHA	+	−	−	−	−	−
	Glucose	+	+	+	+	−	−
	Arginine	−	+	−	−	+	+
	Film-spots	−	−	+	−	+	−
M. gallisepticum		103*					
M. synoviae		8					
I-J-K-N-Q-R			7				
M. anatis				(1)			
M. columborale					51		
C					54		
D					10		
F					(1)		
M. gallinarum						133	
M. columbinum						40	
L						(1)	
M. iners						7	18
M. meleagridis							(1)

Remarks.
* No. of new isolates.
(1) : Exhibited only by a reference strain.

2 sparrows. It should be noted that a strain of *M. synoviae* detected from a liver abscess of a chicken in 1968 was the first isolate of this species in Japan.

It was revealed that *M. gallinarum* distributed in the normal tissues of birds of various species, whereas *M. iners* and serogroups D and I-J-K-N-Q-R were commonly detected from normal specimens of chickens. Growth of 43 strains were inhibited by antisera C and O, and 11 strains were inhibited by antisera C, O and P. These strains were similarly classified as serogroup C in this study. This serogroup was detected from domesticated and wild birds belonging to *Galliformes*.

All but 2 of 93 pigeon isolates were identified as either *M. columbinum* or *M. columborale*, and these two species were never isolated from other birds.

Only one strain from the oropharynx of an adjutant was identified as *Acholeplasma* sp. as it grew without serum and was resistant to digitonin. Its growth was inhibited by antiserum against *A. laidlawii* PG8 obtained through the courtesy of Dr. R. H. Leach, Mycoplasma Reference Laboratory, London. No strain identified as *M. meleagridis, M. anatis,* serogroup F or L was isolated in the present study.

3. Biochemical patterns of the isolates

Four main biochemical properties were examined with all of the test strains and reference strains. As the results, 6 biochemical patterns (biopatterns) were recognized, as are shown in Table 4.

All of the strains exhibiting biopattern 1, in which reactions of CHA, glucose, arginine and film-spots formation were expressed as + + − −, were either *M. gallisepticum* or *M. synoviae,* and no strains within these two species demonstrated other biopatterns. Biopattern 2 was characteristic to 7 isolates and 6 reference strains of serogroup I-J-K-N-Q-R. Biopattern 3 was demonstrated only by *M. anatis* strain 1340. On the other hand, biopattern 4 was exhibited by strains identified as either *M. columborale,* serogroup C

or D and strain SA of serogroup F as well. Similarly, biopatterns 5 and 6 were demonstrated simultaneously in two or three species. In general, it was found that each species or serogroup consisted of strains with a homologous biochemical properties as far as these 4 reactions were concerned, though 7 of 25 *M. iners* produced film without remarkable spots while the remainer did not (Table 4).

Discussion

After the intensive works of Edward and Freundt [8], Yamamoto and Adler [29], Fabricant [9], Kleckner [17], Kelton and Van Roekel [16], Roberts [22] and Yoder and Hofstad [31], Dierks et al. [6] reported 19 serotypes among the reference strains of the previous workers and new strains of avian mycoplasmas. Their serotypes were later reclassified into 11 serogroups by growth inhibition test and 9 serogroups by the gel-diffusion test by Aycardi et al. [2]. Frey et al. [11] also reclassified them into 9 serogroups by the micro-complement fixation test while Barber and Fabricant [3] divided them into 10 serogroups by the metabolism-inhibition test. Stipkovits and El-Ebeedy [25] re-tested these reference strains biochemically and serologically, and, at present, 6 species and 5 serogroups have been recognized within genus *Mycoplasma* of avian origin.

Both *M. gallisepticum* and *M. synoviae* have been known as the pathogens for the chicken and turkey [14]. The former was isolated from a bobwhite quail [19] and partridges [28, 31], whereas the latter, from a natural synovitis of a guinea-fowl [21]. Isolations of *M. gallisepticum* from 2 sparrows caught in a chicken pen in the present study suggest that this *Mycoplasma* may invade and multiply in some wild birds provided that it has an opportunity of contacting with them. Host ranges of both *M. meleagridis* and *M. anatis* seem to be limited to the turkey and duck, respectively [22, 30]; no isolate of these species was obtained from the present materials.

Both *M. gallinarum* and *M. iners* have been isolated from the chicken, turkey, red jungle fowl [1, 6, 9, 18, 31] and even from swine tissues [27]. The former has been isolated from goose-embryo tissue culture as well [26]. Results of the present study expanded the host range of *M. gallinarum* to bamboo partridge, sparrow, swan and demoisella crane.

Mycoplasmas belonging to serogroup C-D-O-P [11] or groups C and D [2, 3] have been detected from the chicken and turkey. Koshimizu et al. [18] recently isolated these serogroups mostly from oropharynxes of gallinaceous birds kept in laboratories and zoological gardens. All of the 64 strains of these serogroups isolated in the present study were also from gallinaceous birds. They were commonly isolated from the reproductive tissues and sperm samples of chickens as well as from tracheas and oropharynxes of birds. It is of interest to note that 8 out of 21 green pheasants, a free-flying wild bird, harbored mycoplasmas of serogroup C in the tracheal tissue. Frequency of isolation of serogroup I-J-K-N-Q-R was very low, and its host range was limited to chickens.

Besides various birds in *Galliformes*, pigeons have been revealed to harbor mycoplasmas in high incidence; 45 out of 65 (or 69%) of pigeons were infected with these microorganisms. In literature, Gianforte et al. [13] isolated a mycoplasma strain from air sac disease of a pigeon. It hemagglutinated chicken erythrocytes and is considered as *M. gallisepticum*. Mathy et al. [20] isolated a strain from a pigeon with mild respiratory disease, but its characteristics

were not examined. Jordan et al. [15] also isolated unidentified mycoplasmas from oesophagus of a pigeon. Yoder and Hofstad [31] firstly isolated the serogroup L *Mycoplasma* strains from the pigeon. Later, strains of this serogroup were detected from the turkey [6] and chicken [1]. Accordingly, it has not been apparent whether this serogroup is a host-specific parasite for pigeons. Recently, however, Gerlach [12] isolated many strains of mycoplasmas from pigeons, and a part of her isolates were identified as serogroup L, while the remainer was divided into as many as 5 'biotypes'. It is strange that none of pigeon strains isolated in the present study was identified as serogroup L; 91 out of 93 strains were serologically and biochemically divided into two groups. Strains MMP1 and MMP4, the representatives of both serogroups, did not cross-react with any of the type or reference strains of currently accepted *Mycoplasma* species and serogroups including serogroup L by the indirect immunofluorescence and growth inhibition tests [24]. With morphological and biochemical properties, both strains have been proposed to be the type strains of two new *Mycoplasma* species [24]. These species, *M. columbinum* and *M. columborale,* seem to parasitize specifically in pigeons, since no isolate of them was obtained from other birds.

It should be investigated further if geographical difference will result in varied mycoplasmal flora in the pigeon and other birds.

A test for biochemical patterns expressed by CHA, glucose, arginine and film-spots formation seems to be useful for the preliminary division of isolates from birds, since these properties are stable even after many passages of strains and easy to perform, and well correlated with species or serogroups.

Acknowledgments: We are grateful to Mr. Y. Nakashima of this Faculty for help in identification and supply of a part of wild birds. We thank Mr. T. Murakami of this Faculty, Mr. N. Katayama of Miyazaki Natural Zoological Park, Mr. Y. Kubo of the Minami-Kyushu Food Industry Co., Ltd., and many other people who submitted materials used in the present study. Kind supply of Mycoplasma broth base by Mr. M. Tsukaguchi, Taito-Pfizer Co., Tokyo, is greatly acknowledged.

This study was supported in part by a Grant-in-Aid for Scientific Research on Mycoplasmas (1975–76, with Prof. M. Ogata, University of Tokyo, as the representative) from the Ministry of Education, Science and Culture of Japan.

A part of this study was presented in the 2nd Conference of the International Organization of Mycoplasmology held in Freiburg in 1978.

References

[1] Asnani, P. J., and Agarwal, S. C. (1974). Serotyping of avian mycoplasma species in India. *Appl. Microbiol.* **27**, 997–1000.

[2] Aycardi, E. R., Anderson, D. P., and Hanson, R. P. (1971). Classification of avian mycoplasmas by gel-diffusion and growth inhibition tests. *Avian Dis.* **15**, 434–447.

[3] Barber, T. L., and Fabricant, J. (1971). A suggested reclassification of avian mycoplasma serotypes. *Avian Dis.* **15**, 125–138.

[4] Black, F. T. (1973). Modification of the growth inhibition test and its application to human T-mycoplasmas. *Appl. Microbiol.* **25**, 528–533.

[5] Clyde, W. A., Jr. (1964). Mycoplasma species identification based upon growth inhibition by specific antisera. *J. Immunol.* **92**, 958–965.

[6] Dierks, R. E., Newman, J. A., and Pomeroy, B. S. (1967). Characterization of avian mycoplasma. *Ann. N. Y. Acad. Sci.* **143**, 170–189.

[7] Dighero, M. W., Bradstreet, C. M. P., and Andrews, B. E. (1970). Dried paper discs for serological identification of human mycoplasmas. *J. appl. Bact.* **33**, 750–757.

[8] Edward, D. G. ff., and Freundt, E. A. (1956). The classification and nomenclature of or-

ganisms of the pleuropneumonia group. *J. gen. Microbiol.* **14**, 197–207.

[9] Fabricant, J. (1960). Serological studies of avian pleuropneumonia-like organisms (PPLO) with Edward's technique. *Avian Dis.* **4**, 505–514.

[10] Freundt, E. A., Andrews, B. E., Ernø, H., Kunze, M., and Black, F. T. (1973). The sensitivity of Mycoplasmatales to sodium polyanethol sulfonate and digitonin. *Zbl. Bakt., I. Abt., Orig.* **225**, 104–112.

[11] Frey, M. L., Hawk, S. T., and Hale, P. A. (1972). A division by micro-complement fixation tests of previously reported avian mycoplasma serotypes into identification groups. *Avian Dis.* **16**, 780–792.

[12] Gerlach, H. (1977). Über das Vorkommen von Mykoplasmen bei Tauben. *Berl. Münch. Tierärztl. Wschr.* **90**, 140–143.

[13] Gianforte, E. M., Jungherr, E. L., and Jacobs, R. E. (1955). A serologic analysis of seven strains of pleuropneumonia-like organisms from air sac infection in poultry. *Poult. Sci.* **34**, 662–669.

[14] Jordan, F. T. W. (1975). Avian mycoplasma and pathogenicity—A review. *Avian Pathol.* **4**, 165–174.

[15] Jordan, F. T. W., Rashid, R. A., and Uthman, A. U. (1978). The recovery of mycoplasma from the avian oesophagus. *Vet. Rec.* **102**, 403–404.

[16] Kelton, W. H., and Van Roekel, H. (1963). Serological studies of mycoplasma (PPLO) of avian origin. *Avian Dis.* **7**, 272–286.

[17] Kleckner, A. L. (1960). Serotypes of avian pleuropneumonia-like organisms. *Amer. J. vet. Res.* **21**, 274–280.

[18] Koshimizu, K., Magaribuchi, T., Tanabe, K., and Kono, N. (1978). Isolation and characterization of mycoplasmas from gallinaceous birds. *Jap. J. vet. Sci.* **40**, 445–449.

[19] Madden, D. L., Henderson, W. H., and Moses, H. E. (1967). Case report: Isolation of *Mycoplasma gallisepticum* from bobwhite quail (*Colinus virginianus*). *Avian Dis.* **11**, 378–380.

[20] Mathey, W. J., Jr., Adler, H. E., and Siddle, P. J. (1956). Isolation of a pleuropneumonia-like organism from pigeons. *Amer. J. vet. Res.* **17**, 521–522.

[21] Pascucci, S., Maestrini, N., Govoni, S., and Prati, A. (1976). *Mycoplasma synoviae* in the guinea-fowl. *Avian Path.* **5**, 291–297.

[22] Roberts, D. H. (1964). Serotypes of avian mycoplasma. *J. comp. Pathol.* **74**, 447–456.

[23] Sato, S., Matsui, K., and Yoshida, Y. (1965). Chicken red blood cell adsorption test for detection of colonies of *Mycoplasma gallisepticum* developed on agar media. *Natl. Inst. Anim. Hlth Quart.* **5**, 45–46.

[24] Shimizu, T., Ernø, H., and Nagatomo, H. (1978). Isolation and characterization of *Mycoplasma columbinum* and *Mycoplasma columborale*, two new species from pigeons. *Int. J. syst. Bact.* **28**, 538–546.

[25] Stipkovits, L., and El-Ebeedy, A. A. (1977). Biochemical and serological studies of avian mycoplasmas. *Zbl. Vet. Med., B* **24**, 218–230.

[26] Stipkovits, L., El-Ebeedy, A. A., Kisary, J., and Varga, L. (1975). Mycoplasma infection of geese. I. Incidence of mycoplasmas and acholeplasmas in geese. *Avian Path.* **4**, 35–43.

[27] Taylor-Robinson, D., and Dinter, Z. (1968). Unexpected serotypes of mycoplasmas from pigs. *J. gen. Microbiol.* **53**, 221–229.

[28] Wichmann, R. W. (1957). Case report—PPLO infection in chukar partridges (*Alectoris graeca*). *Avian Dis.* **1**, 222–226.

[29] Yamamoto, R., and Adler, H. E. (1958). Characterization of pleuropneumonia-like organisms of avian origin. II. Cultural, biochemical, morphological and further serological studies. *J. inf. Dis.* **102**, 243–250.

[30] Yamamoto, R., Ortmayer, H. B., Bigland, C. H., Seely, M. A., and Corstvet, R. E. (1965). Isolation of "N" mycoplasma from different sites of the turkey. *Poult. Sci.* **44**, 732–736.

[31] Yoder, H. W., and Hofstad, M. S. (1964). Characterization of avian mycoplasma. *Avian Dis.* **8**, 481–512.

要　約

各種鳥類からのマイコプラズマの分離・同定：一生態学的研究：清水高正・沼野和彦・内田幸治（宮崎大学農学部家畜衛生学研究室）——1968年から1977年にかけて，13目40種に属する850羽の家禽・愛玩鳥・野鳥を対象に，マイコプラズマの生態学的研究を行なった．同時に死ごもり卵2,848例，鶏の病的材料275例及び正常精液285例についてもマイコプラズマの検査を実施した．その結果，キジ目及びハト目に属する鳥類が高率にマイコプラズマを保有することが判明した．総計440株の血清学的同定を行ない，宿主域を調べたところ，*M. gallisepticum* は例外的に2羽のスズメから分離された他は，*M. synoviae* 同様すべて鶏由来であった．*M. gallinarum* の宿主域は既知の鳥類マイコプラズマのいずれよりも広く，また *M. iners* や血清群 **C, D, I-J-K-N-Q-R** などは，キジ目に属する家禽や野鳥に限定して検出された．先に新種として提案した *M. columbinum* と *M. columborale* は，ハトからのみ分離され，逆にハト由来株93株中91株はこれらのいずれかに同定されたことから，これら2種マイコプラズマは，ハトを本来の宿主とすることが証明された．