Isolation of Mycoplasmas from Fantail Pigeons

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ABSTRACT. Isolation of mycoplasmas from the oropharynxes of 60 fantails reared under natural conditions at different zoological parks in Miyazaki prefecture was carried out. *Mycoplasma columbinum*, *M. columborale* and *M. columbinasale* were isolated from 28 (46.7%), 22 (36.7%), and 1 (1.7%) of 60 oropharynxes, respectively, but no *Mycoplasma* was isolated from 5 cloacas tested. *Ureaplasma* was not isolated from any of the 28 oropharynxes or 5 cloacas examined. We report that 41 (68.3%) of 60 fantails had one or two species of mycoplasmas in their oropharynxes, and make the first confirmation of *M. columbinasale* inhabiting a Japanese pigeon. — **KEY WORDS:** fantail pigeon, mycoplasma.

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In 1978, two new species of *Mycoplasma*, *M. columbinum* and *M. columborale* were isolated from feral pigeons and designated [10]. Thereafter, it was confirmed that these species of *Mycoplasma* were specific to pigeons, as reported by Shimizu *et al.* [11]. In 1982, another strain which had previously been identified as a serotype-L of avian mycoplasmas [4, 5, 8, 10–13], was designated as *M. columbinasale* [3]. *M. columbinasale* was isolated from pigeons in the United States of America [5], England [6, 12] and Australia [9], and from chickens in India [1]. Sinclair [12] reported a respiratory disease associated with *M. columbinasale* in racing (homing) pigeons. However, reports concerning the isolation of this organism have not been published in Japan till this study.

In this study, we tried to isolate mycoplasmas from 60 oropharynxes and 5 cloacas, as well as *Ureaplasma* from 28 oropharynxes and 5 cloacas of 60 healthy fantail pigeons which had been introduced to parks through bird shops in this country 20 to 25 years ago. Fantail pigeons naturally inhabit two large scale zoological parks in Miyazaki city. Located by the seashore, the climate is mild, and the fantail pigeons are freely in contact with hens, turkeys, parakeets, peafowls and galeenies through feeds and drinking water. All the birds are fed with market feeds for the hen and the drinking water contained no antimicrobial agents. Swab samples were taken when the fantail pigeons returned to rest in their hut at night. Edward's liquid and solid media were used for the isolation, subculture and biochemical tests. The liquid medium was composed of *Mycoplasma* broth base (Difco), 20% fresh horse serum, 10% yeast extract, 0.5% glucose, 0.25% L-arginine, 0.025% thallium acetate, 200 mg AB-PC (Toyo Jyozo Co., Ltd.) and 0.002% phenol-red, and its pH was adjusted to 7.0. Solid medium (agar plate) without phenol-red was made from the liquid medium supplemented with 1% agar (Noble agar, Difco). For the isolation of *Ureaplasma*, Taylor-Robinson's liquid medium [7] was used. Each swab sample was put into a test tube containing 2 ml of Edward's liquid medium, and was then well shaken by a test tube mixer (Iuchi Co., Ltd.). A portion of 25 μ l from each tube was inoculated and streaked on the solid medium. The agar plate was incubated at 37°C for 10 days in a 5% CO₂ atmosphere (Forma Scientific 3158), and

the colonies developed were observed daily from the 3rd to the 10th day postincubation by microscopy (40 × or 100 ×). Colonies showing differences in shape were subjected to cloning by picking up a single colony with an agar-block, and transplanting into fresh liquid medium. After purification, the isolated strains were identified with routine biochemical tests and serological tests of the metabolism inhibition (MI) and growth inhibition (GI) [10, 11].

Three reference strains, *M. columbinum* MMP1, *M. columborale* MMP4, and *M. columbinasale* 694 (by courtesy of Dr. Kelton, W. H., Univ. of Massachusetts, Amherst U.S.A.) together with the isolated 3 strains, 28L, 20P and 30L were used in the tests. Rabbit antisera against the 3 reference strains and 1 of the isolated strains, 30L, were prepared with suspensions of whole cells and Freund's complete adjuvant (Iatron Laboratories, Tokyo) via S.C.

Isolation of *Ureaplasma* was carried out by diluting a swab-broth mixture into 10 fold serial dilutions from 10⁻¹ to 10⁻⁵, and then incubated at 37°C for 7 days. Color change of the liquid medium following the growth of *Ureaplasma* was observed daily.

The biochemical and serological properties of the 3 reference strains and the 3 isolated strains are given in Table 1. Strain 28L was identified as *M. columbinum*, strain 20P as *M. columborale* and strain 30L as *M. columbinasale*. In total, 74 isolates were identified by the same methods, and the results are summarized in Table 2. *M. columbinum* alone was isolated from 5 (16.7%) of 30 fantails at park F, and 13 (43.3%) of 30 at park K. *M. columborale* alone was isolated from 4 (13.3%) and 9 (30%) at park F and K, respectively. *M. columbinum* and *M. columborale* together were isolated concurrently from 6 (20%) at park F and 3 (10%) at park K. Additionally, *M. columbinum* and *M. columbinasale* together were isolated from only 1 fantail at park K. *Mycoplasma* was not isolated from 15 (50%) oropharynxes at park F and 4 (13.3%) at park K. *Mycoplasma* was negative in 5 cloacas tested. *Ureaplasma* was not isolated from any of the 28 oropharynxes or 5 cloacas examined. In total, *M. columbinum* existed in 28 (46.7%) and *M. columborale* in 22 (36.7%) of 60 fantails. *M. columbinasale* was harbored in one (1.7%) fantail only. Therefore, 41 (68.3%) of the 60 fantails had one or two

Table 1. Serological and biochemical properties of reference and isolated strains

Species	Strain	Biochemical test			Serological test							
		Glucose	Arginine	Film & spots	GI				MI			
					MMP1 ^{a)}	MMP4	694	30L	MMP1	MMP4	694	30L
<i>M. columbinum</i>	MMP1	—	+	+	4.0 ^{b)}	—	—	—	256 ^{c)}	. ^{d)}	.	.
	28L	—	+	+	4.0	—	—	—	256	.	.	.
<i>M. columborale</i>	MMP4	+	—	—	—	3.0	—	—	.	128	.	.
	20P	+	—	—	—	3.5	—	—	.	64	.	.
<i>M. columbinasale</i>	694	—	+	+	—	—	3.0	4.0	.	.	2048	102
	30L	—	+	+	—	—	5.0	5.0	.	.	1024	512

a) Antiserum. b) Growth inhibition zone (mm). c) MI titer. d) MI titer <2.

Table 2. *Mycoplasma* species isolated from oropharynxes of 60 fantails at 2 parks

Species isolated	Samples (%)		Total (%)
	Park F	Park K	
<i>M. columbinum</i>	5 (16.7)	13 (43.3)	18 (30.0)
<i>M. columborale</i>	4 (13.3)	9 (30.0)	13 (21.7)
<i>M. columbinum</i> & <i>M. columborale</i>	6 (20.0)	3 (10.0)	9 (15.0)
<i>M. columbinum</i> & <i>M. columbinasale</i>	0	1 (3.3)	1 (1.7)
None	15 (50.0)	4 (13.3)	19 (31.7)
Total	30 (100)	30 (99.9)	60 (100.1)

species of *Mycoplasma*. Regarding the number of viable cells, 38 (93%) of 41 samples were in 10² colony forming unit (CFU) or more per swab, and the remaining 3 (7%) samples were less than 10² CFU per swab.

It was reported in Japan [11] that *M. columbinum* and *M. columborale* were isolated from 45 (69%) of 65 feral pigeons, but no *M. columbinasale* was detected. These data for feral pigeons are similar in isolation rate to those of the present study of fantails. However, the isolation of *M. columbinasale* reported in the present study is confirmed for the first time in Japan. *Ureaplasma gallorale* [7] was not detected in this study. Additionally, the prevalence of mycoplasmas between the fantails and the other birds with which they were in close contact was not determined.

Countries other than Japan: Sinclair [12], in England, reported the isolation of *M. columbinasale* from 10 racing pigeons suffering from respiratory disease, and the organisms were eradicated from the pigeons as a result of the administration of tiamurine, an antibiotic. Jordan *et al.* [4], also in England, isolated *M. columbinum* and *M. columborale* from the esophagus, lung, air sac and brain of feral pigeons, but not *M. columbinasale*. However, other reports from Europe [2, 6, 12] and Australia [9] did reveal the inhabitation of *M. columbinasale* in pigeons. These facts suggest that the prevalence of *M. columbinasale* in pigeons may be different depending on the country or the area.

MacOwan *et al.* [8], in England, isolated *M. columborale* from a pigeon infected with a respiratory disease, and the disease was cured by administering an antibiotic. Moreover, the antibody against *M. columbinum* was detected in 11% of 37 racing pigeons suffering from respiratory disease, but not the antibody against *M. columborale* [6]. These reports suggest that these three species of *Mycoplasma* are possibly associated with some diseases of pigeons. However, the fact that *M. gallinarum* and *M. synoviae*, two usual inhabitants of chickens, have been isolated from 2 tracheas out of 20 pigeons suffering from respiratory disease [9], and that *M. columbinasale* has been isolated from chickens [1] suggest that pigeons may serve as carriers of poultry diseases. The present and the past reports encourage the etiological investigation of mycoplasmal flora of pigeons in poultry diseases.

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