NOTE

Further Isolation of Mycoplasmas from Zoo-Animals

Kaoru KOSHIMIZU, Hitoshi KOTANI, Masahiro ITO, Kazuharu TASHIRO, Mitsuko MASUI,
Koki TANABE, Izumi KAWASAKI, Kazuo SAITO, and Hiroshi HIRAMATSU

*Division of Animal Research, Faculty of Medicine, University of Tokyo, Bunkyo, Tokyo 113,
Tama Zoological Park, Tokyo Metropolis, Hino, Tokyo 191, and Ueno Zoological Gardens,
Tokyo Metropolis, Taito, Tokyo 110, Japan*

(Received 30 September 1983/Accepted 17 November 1983)

Mycoplasmas were isolated from oral or nasal cavities of two lions, a Muscovy duck, a falcated teal, and three Indian white-backed vultures among zoo-animals comprising 19 mammals, six reptiles and 17 birds examined. The isolates from the lion were biochemically identical to those of *M. felis* but serologically different from them. No strains from the Indian white-backed vultures reacted with any antisera against the reference mycoplasma strains from birds.— *Key words*: Mycoplasma, Zoo-animals.

Jpn. J. Vet. Sci. 46(1): 129–132, 1984

In the previous report [7], we described the first isolation of *Acholeplasma laidlawii* from the oral and nasal cavities of mouflons (*Ovis musimon*) and from the prepuce of a Uganda giraffe (*Giraffa camelopardalis rothschildi*), which had been kept at zoological gardens. This report deals with the further isolation of mycoplasmas from various zoo-animals to obtain information concerning ecological situations of these microorganisms.

The animals examined were kept at the Tama Zoological Park and/or Ueno Zoological Gardens, Tokyo Metropolis. They were apparently healthy, and consisted of 19 mammals, six reptiles and 17 birds as shown in Table 1. Media used for the isolation of mycoplasmas and ureaplasmas were liquid and solid ones as described previously [5, 6]. Specimens were taken with sterile-cotton swabs from the oropharynx, vagina or cloaca, prepuce and eyes of each animal, and were cultured for the isolation of mycoplasmas as described previously [5, 6].

The isolates were cloned and subjected to biochemical and serological examinations. Digitonin sensitivity was tested by the method of Freundt *et al.* [3]. Fermentation of

glucose, hydrolysis of arginine and urea, reduction of tetrazolium chloride, and production of film and spots were tested by the procedures of Aluotto *et al.* [1]. Serological characterization of the isolated strains were performed by the growth inhibition test by the disc method of Clyde [2]. Antisera against mycoplasma strains were prepared by immunizing Japanese White rabbits by the procedure reported previously [4]. Antisera against avian mycoplasmas, *M. gallisepticum* PG31, *M. gallinarum* PG16, *M. iners* PG30, *M. synoviae* WVU1853, *M. meleagridis* 17529, *M. columbinum* MMP-1, *M. columborale* MMP-4, *M. pullorum* CKK, *M. gallinaceum* DD, *M. gallopavonis* WR1, *M. iowae* 695 and *M. columbinasale* 694 were provided by Prof. T. Shimizu of Faculty of Agriculture, Miyazaki University.

Mycoplasmas were isolated from the oral cavities of two lions (*Panthera leo*), the oral and nasal cavities of Muscovy duck (*Cairina moschata*), the nasal cavity of a falcated teal (*Anas falcata*), and the oral and nasal cavities of three Indian white-backed vultures (*Gyps bengalensis*), as shown in Table 1. No ureaplasma was detected in any sites of the ani-

Table 1. Examination of mycoplasmas in zoo-animals

Order	Animals	No. positive/ No. examined
Artiodactyla	Nilgai (<i>Boselaphus tragocamelus</i>)	0/1
	Sambar (<i>Cervus unicolor</i>)	0/1
	Yaku island deer (<i>Cervus nippon yakushimae</i>)	0/1
	Goat (<i>Capra hircus</i>)	0/1
	Llama (<i>Lama glama</i>)	0/1
Perissodactyla	Malayan tapir (<i>Tapirus indicus</i>)	0/1
	Great Indian rhinoceros (<i>Rhinoceros unicornis</i>)	0/4
	Donkey (<i>Equus asinus domesticus</i>)	0/1
	Mongolian horse (<i>Equus przewalskii</i>)	0/1
Proboscidae	Asian elephant (<i>Elephas maximus</i>)	0/1
Carnivora	Lion (<i>Panthera leo</i>)	2/2
	Jaguar (<i>Panthera onca</i>)	0/1
Primates	Thick-tailed bushbaby (<i>Galago crassicaudatus</i>)	0/1
	Orang-utan (<i>Pongo pygmaeus</i>)	0/1
Squamata	Green python (<i>Chondropython viridis</i>)	0/1
	African rock python (<i>Python sebae</i>)	0/1
Testudines	Reeve's turtle (<i>Chinemys reevesi</i>)	0/4
Anseriformes	Muscovy duck (<i>Cairina moschata</i>)	1/1
	Falcated teal (<i>Anas falcata</i>)	1/6
	Mallard (<i>Anas platyrhynchos</i>)	0/1
Gruiformes	Stanley crane (<i>Anthropoides paradisea</i>)	0/1
	Red crowned crane (<i>Grus japonensis</i>)	0/1
Gariformes	Rock partridge (<i>Alectoris graeca</i>)	0/1
Passeriformes	Grey-backed myna (<i>Sturnus sinensis</i>)	0/1
Falconiformes	King vulture (<i>Sarcorhamphus papa</i>)	0/1
	Indian white-backed vulture (<i>Gyps bengalensis</i>)	3/3
Struthioniformes	Ostrich (<i>Struthio camelus</i>)	0/1

mals examined. No strains were converted into bacterial form after three serial passages in liquid medium devoid of bacterial inhibitors. They required serum but no urea for growth.

Mycoplasma strain LO-12 isolated from a lion was similar to *M. felis* CO and *M. felis* 51Nd2 in the biochemical tests, but serologically different from them as shown in Tables 2 and 3. Consequently, glucose-fermenting mycoplasma strains of feline origins were divided into four sero-groups by the growth inhibition test. Strains LL2 and LX1 suppli-

ed by Dr. A. Hill were identical to each other biochemically and serologically, but different from the other feline mycoplasmas.

The strains isolated from Indian white-backed vultures comprized both glucose-fermenting mycoplasmas and arginine-hydrolyzing mycoplasmas, although they did not react with any reference antisera against avian mycoplasmas in the growth inhibition test.

The isolates from the Muscovy duck and the falcated teal were lost in their passages and could not be characterized.

Table 2. Biochemical properties of mycoplasmas originated from feline animals

Strains	Sources	Serum require- ment	Digitonin sensiti- vity	Glucose fermen- tation	Arginine break down	Tetra- zolium reduction	Film & spot
<i>M. felis</i> CO	S. Rosendal ^{a)}	+	+	+	—	—	—
<i>M. felis</i> 51Nd2	Our laboratory	+	+	+	—	—	—
<i>M. sp.</i> LY	A. Hill ^{b)}	+	+	+	—	—	—
<i>M. sp.</i> LA	A. Hill	+	+	+	—	—	—
<i>M. sp.</i> LP	A. Hill	+	+	+	—	—	—
<i>M. sp.</i> LC	A. Hill	+	+	+	—	—	—
<i>M. sp.</i> LO-12	Our laboratory	+	+	+	—	—	—
<i>M. gateae</i> Mart	S. Rosendal	+	+	—	+	—	—
<i>M. gateae</i> C2	S. Rosendal	+	+	—	+	—	—
<i>M. arginini</i> G230	S. Rosendal	+	+	—	+	—	—
<i>M. feliminutum</i> Ben	S. Rosendal	+	+	—	—	—	—
<i>M. feliminutum</i> C1-1	Our laboratory	+	+	—	—	—	—
<i>M. sp.</i> LL2	A. Hill	+	+	(+) ^{c)}	—	+	+
<i>M. sp.</i> LX1	A. Hill	+	+	(+)	—	+	+

a) The Institute of Medical Microbiology, University of Aarhus, Denmark.

b) Medical Research Council, England.

c) Questionably positive.

Table 3. Growth inhibition test of glucose-fermenting mycoplasmas from feline animals

Species	Strains	Growth inhibition with antiserum								
		CO	51Nd2	LY	LA	LP	LO-12	LC	LL2	LX1
<i>M. felis</i>	CO	3 ^{a)}	1	2	— ^{b)}	—	—	—	—	—
<i>M. felis</i>	51Nd2	2	1	—	—	—	—	—	—	—
<i>M. sp.</i>	LY	—	—	1	—	—	—	—	—	—
<i>M. sp.</i>	LA	—	—	—	4	3	2	—	—	—
<i>M. sp.</i>	LP	—	—	—	—	—	—	—	—	—
<i>M. sp.</i>	LO-12	—	—	—	—	—	2	—	—	—
<i>M. sp.</i>	LC	—	—	—	—	—	—	2	—	—
<i>M. sp.</i>	LL2	—	—	—	—	—	—	—	2	2
<i>M. sp.</i>	LX1	—	—	—	—	—	—	—	2	2

a) The width (mm) of growth inhibitory zone.

b) No growth inhibition.

In the present study, mycoplasmas were isolated only from a few animals but not from any of the many other animal species examined. All the animals examined were those for exhibition at the zoological gardens under extraordinary environments. Therefore, it should be noted that the findings obtained in this study may not depict true ecological features or normal flora of mycoplasmas in whole population of these wild animals.

REFERENCES

1. Alluotto, B. B., Wittler, R. G., Williams, C. O., and Faber, J. E. 1970. *Int. J. Syst. Bacteriol.* 20: 35–58.
2. Clyde, W. A., Jr. 1964. *J. Immunol.* 92: 958–965.
3. Freundt, E. A., Andrews, B. E., Ernfø, H., Kunze, M., and Black, F. T. 1973. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg., 1, Orig. A* 225: 104–112.
4. Koshimizu, K. and Ogata, M. 1974. *Jpn. J. Vet. Sci.* 36: 391–406.

5. Koshimizu, K., Magaribuchi, T., Tanabe, K., and Kono, N. 1978. *Jpn. J. Vet. Sci.* 40: 445–499.
6. Kotani, H., and Ogata, M. 1979. *Jpn. J. Vet. Sci.* 41: 639–646.
7. Kotani, H., Ogata, M., Koshimizu, K., Tashiro, K., and Masui, M. 1982. *Vet. Rec.* 110: 16.

要 約

動物園で飼育されている動物からのマイコプラズマ分離—続報（短報）：奥水 馨・小谷 均・伊藤 正博・田代和治¹⁾・増井光子²⁾・田辺興記²⁾・川崎 泉²⁾・斎藤和夫²⁾・平松 廣²⁾（東京大学医学部附属動物実験施設，東京都¹⁾多摩動物公園，²⁾恩賜上野動物園）——動物園で飼育されている各種哺乳類19匹，爬虫類6匹，鳥類17匹からマイコプラズマの分離をこころみたところ，ライオンの口腔（2/2），バリケンの口腔（1/1）および鼻腔（1/1），ヨシガモの鼻腔（1/6），ベンガルハゲワシの口腔（3/3）および鼻腔（3/3）から分離された。ライオンから分離された株はネコ由来の *M. felis* と生化学的性状は一致したが，発育阻止試験により血清学的に区別された。ベンガルハゲワシから分離された株は鳥類由来の参照株の抗血清と反応しなかった。