

Isolation of *Mycoplasma* and *Ureaplasma* Species from Raccoon Dogs (*Nyctereutes procyonoides viverrinus*)

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(Received 9 December 1982; revised 10 February 1983)

Mycoplasma spp. were isolated from five wild raccoon dogs (*Nyctereutes procyonoides viverrinus*). On the basis of biochemical properties and serological tests, nine isolates were identified as *Mycoplasma edwardii* and four were similar to a possibly new *Mycoplasma* sp. represented by strain LM2 which is negative for both glucose fermentation and arginine hydrolysis. In addition, ureaplasmas were detected from these animals. Ureaplasmas were compared serologically with ureaplasma strains isolated from human, monkey, cattle, goat, sheep, cat, chicken and dog and cross-reacted with one of four serological groups of canine ureaplasmas.

INTRODUCTION

With regard to ecological studies on the distribution of mycoplasmas in various species of animals, a large number of reports have been published of mycoplasmas in domestic mammals and domestic fowls, but few surveys of these organisms have been made in wild animals.

Mycoplasmas have been isolated from wild cat (Hill, 1972), chamois (Nicolet & Freund, 1975), ground squirrel (Langford, 1977), hedgehog (Tan *et al.*, 1971), non-human primates (Barile, 1973), elephant (Clark *et al.*, 1980) and several species of wild birds (Koshimizu *et al.*, 1978; Shimizu *et al.*, 1979).

We have reported previously the isolation of mycoplasmas from wild raccoon dog (*Nyctereutes procyonoides viverrinus*), fox (*Vulpes vulpes japonica*) and Japanese badger (*Meles meles anakuma*) (Kanamoto *et al.*, 1981). This communication provides a more detailed assessment of the results of mycoplasmal isolation from raccoon dogs.

METHODS

Animals. Five raccoon dogs (*Nyctereutes procyonoides viverrinus*), four females and one male, caught in the wild from March to April 1981, were used for isolation of mycoplasmas. All of these animals were adult and appeared to be healthy.

Media. T-broth and T-agar (Ogata *et al.*, 1979) were used for the isolation of ureaplasmas. The composition of the medium (PPLO-broth, PPLO-agar) used for isolation of mycoplasmas has been described previously (Kanamoto *et al.*, 1981).

Isolation. The oral cavity, nasal cavity, external ear, rectum, vagina and prepuce of these animals were wiped with sterilized cotton swabs and the swabs thus obtained were immersed in test tubes containing 2 ml tryptic soy

Abbreviation: GI, Growth inhibition.

Table 1. *Strains of mycoplasma species or serovars used in comparative studies*

Strain	Serovar	Source	Strain	Source
Mycoplasma			Ureaplasma (continued)	
<i>M. edwardii</i>	PG24	Dog	D1M-C	Dog
<i>M. canis</i>	PG14		D29M	
<i>M. cynos</i>	H831		D11N-A	
<i>M. spumans</i>	PG13		D6P-C	
<i>M. maculosum</i>	PG15		My6690	Monkey
<i>M. molare</i>	H542	Sp1625A		
<i>M. opalescens</i>	MH5408	T167-2		
<i>M. feliminutum</i>	Ben	Cat	CH33	
<i>M. bovigenitalium</i>	PG11	Cattle	C13	Cattle
<i>M. pulmonis</i>	m53	Mouse	C24	
<i>M. neurolyticum</i>	PG28		C23	
Ureaplasma			C17	
No. 7	I	Human	C12	
No. 23	II		C7	
No. 27	III		T44	Goat
No. 58	IV		G10V	
No. 354	V		S8V-A	Sheep
Pirillo	VI		1202	
Cook	VII		F2	Cat
T-960	VIII		FT2-B	Chicken
			D6-1	

broth (BBL) supplemented with horse serum. The specimens were placed in a low temperature preservation box and transported to the laboratory, Hiroshima Prefectural Institute of Public Health, Japan. The specimen (1 ml) was inoculated into test tubes containing 1.8 ml T-broth and PPLO-broth, respectively, and 10-fold dilutions to 10^{-5} were made. These were incubated at 37 °C for a week. The cultures showing colour changes were subcultured on T-agar and PPLO-agar. Duplicate cultures were incubated at 37 °C in an atmosphere of aerobic condition and of 10% CO₂-90% N₂ (v/v).

Antisera. These were prepared as described by Ogata *et al.* (1979).

Reference strains. Mycoplasma reference strains used for biochemical and serological tests are shown in Table 1. In addition, mycoplasma strain LM2 isolated previously from a raccoon dog by the present authors (Kanamoto *et al.*, 1981) was included in the present study.

Ureaplasma reference strains (Table 1) used for serological tests were of human, monkey, cattle, goat, sheep, cat, chicken and canine origins, described previously (Kotani *et al.*, 1980).

Biochemical and serological tests. Biochemical tests were carried out by the methods reported by Aluotto *et al.* (1970). The serological test used for mycoplasmas was the growth inhibition (GI) test (Clyde, 1964), and for ureaplasmas was the GI test (Ogata *et al.*, 1979).

RESULTS

The number of isolates of mycoplasmas from raccoon dogs is shown in Table 2. Nineteen isolates were cloned three times or more (Koshimizu & Magaribuchi, 1978) and examined further. Nine of the isolates were identified as *Mycoplasma edwardii*. They had the same biochemical properties, i.e. they were digitonin sensitive, did not reduce tetrazolium chloride, fermented glucose but did not hydrolyse arginine or possess urease activity. These strains were finally identified as *M. edwardii* by the GI test.

Four of the isolates appeared similar to a previously isolated but unnamed *Mycoplasma* sp. strain LM2 (Kanamoto *et al.*, 1981). These strains cross reacted with LM2 antisera in the GI test. They also failed to ferment glucose, hydrolyse arginine or urea, to reduce tetrazolium and produce film and spots. All were inhibited by digitonin.

Six of the isolates were identified as ureaplasmas. They were found to cross react in the GI test with antisera to ureaplasma strain D1M-C, one of the representative serological types from dogs. They did not react with antisera to any of the other ureaplasma strains listed in Table 1.

Table 2. Isolation of mycoplasmas from raccoon dogs

The results are expressed as (No. of specimens positive for mycoplasma or ureaplasma)/(No. of specimens examined). Each specimen was from a different raccoon dog.

Site tested	Mycoplasma	Ureaplasma
Oral cavity	5/5	5/5
Nasal cavity	2/5	4/5
External ear	0/5	0/5
Rectum	3/5	1/5
Vagina	0/4	0/4
Prepuce	0/1	0/1
Total	10/25	10/25

DISCUSSION

Since ureaplasmas were first isolated from the urinary tract of man by Shepard (1954), these micro-organisms have been isolated from many mammals such as cattle (Taylor-Robinson *et al.*, 1967), dog (Taylor-Robinson *et al.*, 1971), cat (Tan & Markham, 1971), monkey (Brown *et al.*, 1976), sheep (Livingston & Gauer, 1975), goat (Gourlay *et al.*, 1973), mink (Friis *et al.*, 1980) and pig (Stipkovits *et al.*, 1978). Most of these animals were domestic animals or pets, and there have been no reports of ureaplasmas in wild animals.

Attempts were made in the present study to isolate mycoplasmas from wild raccoon dogs. Ureaplasma spp. and *Mycoplasma edwardii* were isolated and identified for the first time from this source. Ureaplasma strains isolated cross-reacted with antiserum to one of the four serological groups originated from dogs. Nine mycoplasma strains were identified as *M. edwardii* whose host was originally dogs (Rosendal, 1975).

Four other isolates had properties similar to strain LM2 which had been isolated previously from a raccoon dog. These strains seem to be a new species of mycoplasma, and identification is under investigation.

We wish to thank Mr Y. Fukumoto, Asa Zoological Park, for allowing us to obtain samples from the raccoon dogs.

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