

Isolation and Rapid Identification of *Mycoplasma* Species from Canine Tissues by Plate Immunofluorescence¹ (34747)

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In 1951, Edward and Fitzgerald (1) reported a study of the mycoplasmal flora of dogs and described three species which were designated as α , β , and γ . Subsequently, Edward and Freundt (2) named the three species *Mycoplasma spumans*, *M. canis*, and *M. maculosum*. One strain PG-24 was not named. In our study, four laboratories collaborated in the examination of the mycoplasmal flora in a large number of tissues from dogs. Two hundred and forty-nine mycoplasmas were isolated from 87 of 185 laryngeal and urogenital canine tissues. A plate immunofluorescence procedure (3) facilitated the detection of species in mixed culture. The findings extend the information on the mycoplasma infection of dogs.

Materials and Methods. Dogs. Ninety-three purebred beagle (60) and mongrel dogs (33) examined were purchased from Marshall Research Animals, Inc., North Rose, N. Y. and Lone Trail Kennels, Friedensburg, Pa., respectively, and were maintained by IITRI or Melpar. Young beagles age 2 to 3 months in good health at time of delivery were caged individually and quarantined from 4 to 6 weeks before used. Adult mongrel dogs when received were usually in poor physical condition and some showed signs of illness; they were housed overnight and used the next day.

Specimens. The surgical procedures used to obtain tissues for examination were similar to those reported in our previous studies (4). In brief, the dog was sacrificed, an appropriate midline incision was made and the entire

larynx and the entire cervix or prostatic-urethral tissues were removed. The mucosal linings of these tissues were cultured for the presence of mycoplasma by Melpar and IITRI. Duplicate tissue aliquots were sent frozen at -70° to HRC for additional isolation studies. All mycoplasmas were sent to HRC for identification of *Mycoplasma* species by the plate immunofluorescence procedure (3, 4).

Isolation and identification of *Mycoplasma* species. The procedures used were reported earlier (3, 4). In brief, tissues received at HRC were thawed; washed in phosphate buffered saline, pH 7.4, containing 0.05% thallium acetate and 0.02% gelatin; and inoculated, in duplicate, directly onto agar and into liquid media. The media were incubated at $36 \pm 1^{\circ}$ aerobically or in an atmosphere of 5% carbon dioxide in nitrogen (5). Liquid media were incubated for 2 to 4 days and subcultured to agar plate media. Agar media were incubated for 2 weeks and examined periodically for mycoplasma colonies by bright-field microscopy. Isolation plates showing mycoplasmas were stained directly with a series of fluorescein-conjugated antisera prepared against the 4 known canine mycoplasma serotypes, *M. spumans*, *M. canis*, *M. maculosum*, and strain PG-24. Additional plate cultures were prepared as required from the primary broth cultures. Plates were examined for the presence of mycoplasma colonies and each colony was examined for immunofluorescence. Occasionally, there were colonies which did not react with the 4 conjugated antisera. Attempts to isolate these unrelated mycoplasmas in pure culture were hampered at times by the presence of heavy

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TABLE I. Isolation and Identification of Mycoplasmas from Dogs.

Tissues	No. of tissues examined	No. of tissues positive	No. of mycoplasma strains isolated	No. of mycoplasma strains isolated per tissue				No. of mixed cultures	Immunofluorescent identification of mycoplasma serotypes				Unidentified	
				0	1	2	3		4	<i>M. spumans</i>	<i>M. canis</i>	<i>M. maculosum</i>		Strain PG-24
				27	9	10	15		32					
Larynx	93	66	202	27	9	10	15	32	53	55	45	48	1 ^a	
Cervix	35	13	27	22	6	3	1	3	10	4	6	7	—	
Prostatic-urethral	57	8	20	49	2	2	2	2	5	7	4	4	—	
Total	185	87	249	98	17	15	18	37	70	66	55	59	1	

^a Strain HRC 689.

and mixed mycoplasma growth. One strain, HRC 689, was successfully isolated, cloned, grown in pure culture, and found unrelated to the 4 canine mycoplasma serotypes (Table I).

Results. The results obtained on the isolation and identification by immunofluorescence of mycoplasmas from the laryngeal and urogenital tissues of beagle and mongrel dogs are summarized in Table I. Two hundred and forty-nine mycoplasmas were isolated from 87 of 185 (47%) laryngeal and urogenital tissues from the 93 dogs. The species were identified by plate immunofluorescence and the number of strains per species follows: *M. spumans* 68 (27%), *M. canis* 66 (26%), *M. maculosum* 55 (22%), and PG-24 related mycoplasmas 59 (24%). The remaining strain HRC 689 was not identified and may represent a new *Mycoplasma* species.

The mycoplasmal flora of laryngeal and urogenital tissues of purebred beagle and mongrel dogs were similar and each of these mucosal tissues contain each of the 4 canine mycoplasma serotypes. Each serotype comprised approximately one-fourth of the mycoplasmas isolated. Most of the mycoplasmas were isolated from laryngeal tissues; 66 of 93 laryngeal (71%), 13 of 35 cervical (35%) and 8 of 57 prostatic-urethral (14%) tissues were positive. Of the 249 isolated mycoplasmas, 202 were from laryngeal (81%), 27 from cervical (11%) and 20 from prostatic-urethral (8%) tissues. Mixed cultures containing 2 or more *Mycoplasma* species were found in cultures of 57 laryngeal (86%), 7 cervical (54%) and 6 prostatic-urethral (75%) tissues.

Discussion. Edward and co-workers (1, 2) first reported that dogs contain a mixed mycoplasmal flora. In our study, 85% of the positive laryngeal and 63% of the positive urogenital tissues contained a mixed mycoplasma flora. The usefulness of the plate immunofluorescence procedure for detecting and identifying mycoplasmas in mixed cultures was reported earlier (3), and is confirmed in this study. Whereas Edward and Fitzgerald (1) isolated *M. spumans* only from vaginal tissues, we were able to isolate

M. spumans from both the vaginal-cervical and the laryngeal tissues.

The original strain (C-21) designated PG-24 (2) was isolated by Edward and Fitzgerald (1) from the throat of a normal dog. A second mycoplasma related to strain PG-24 was isolated by Razin and Rottem (6) from lung tissues of a dog with pneumonia. In our study, 59 of the mycoplasmas isolated (24%) were related to the unclassified dog strain PG-24. These findings indicate that the strain PG-24 group of mycoplasmas are part of the normal dog flora, that these strains frequently occur and that they merit species designation. In collaboration with other investigators [Tully *et al.* (7)] we have studied the growth, serologic and biochemical characteristics of this group and have designated the species epithet, *Mycoplasma edwardii*.

Summary. Two hundred and forty-nine mycoplasmas were isolated from 87 of 185 laryngeal and urogenital tissues of 93 normal purebred beagle and mongrel dogs and were identified by the plate immunofluorescence procedure. There were 68 *M. spumans*, 66 *M. canis*, 55 *M. maculosum*, and 59 mycoplas-

mas related to strain PG-24 (recently named *Mycoplasma edwardii*) and one unidentified strain HRC 689 which may represent a new *Mycoplasma* species. Mixed mycoplasmal flora were found in cultures of 57 laryngeal, 7 cervical, and 6 prostatic-urethral tissues. The findings indicate that four species of *Mycoplasma* are commensal inhabitants of dogs.

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