DNA Relatedness among Established Ureaplasma Species and Unidentified Feline and Canine Serogroups

R. HARASAWA,1 E. B. STEPHENS,2 K. KOSHIMIZU,3 I.-J. PAN,3 AND M. F. BARILE*4

Department of Veterinary Microbiology, Miyazaki University, Miyazaki 889-21, Japan1; Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama 35294-2; Division of Animal Research, University of Tokyo, Tokyo 113, Japan1; and Laboratory of Mycoplasma, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, Maryland 20892*

The levels of DNA relatedness among two unclassified feline ureaplasma serogroups, four unclassified canine ureaplasma serogroups, and the three previously established Ureaplasma species were examined and compared. The strains examined included five feline strains representing two feline serogroups, four canine strains representing four canine serogroups, and the type strains of the three established species. Each strain representing each species or serogroup exhibited 78% or more actual DNA homology with its homologous DNA, but less than 10% DNA homology with DNAs from the heterologous strains. These findings indicate that each of these human, bovine, avian, feline, and canine strains is genomically distinct. In addition, the three previously recognized species (Ureaplasma urealyticum [human], Ureaplasma diversum [bovine], and Ureaplasma gallorale [avian]), which were established on the basis of phenotypic properties, were also shown to be genomically distinct. The three feline serogroup SI strains were genomically related (from 89 to 100% DNA homology) to each other but were unrelated (less than 10% DNA homology) to the feline serogroup SII strains, indicating that these two feline serogroups are also genomically distinct. Conversely, the two feline serogroup SII strains were genomically very similar (from 83 to 100% DNA homology) to each other but were unrelated (less than 10% DNA homology) to the three feline serogroup SI strains. However, canine serogroup SII strain DIM-C exhibited 77% DNA homology with serologically distinct canine serogroup SII strain D29M, indicating that these strains representing two separate serogroups belong to the same genomic species. Our findings indicate that the two feline ureaplasma serogroups and at least one canine ureaplasma serogroup are genomically distinct and are unrelated to the previously established species and that each serogroup represents a new species or subspecies within the genus Ureaplasma.

MATERIALS AND METHODS

Ureaplasmas. The species and strains used and their origins and sources of isolation are listed in Table 1. Each strain was filtered-cloned three times prior to use. Mycoplasma-hominis and Escherichia coli were used as the negative culture controls. The ureaplasmas were grown in 10-liter broth batches at 37°C in the 10-B medium described by Shepard and Luceford (21). The cells were sedimented by centrifugation at 15,000 × g for 60 min, and the pellets were washed in TE buffer (50 mM Tris [pH 8.0] and 10 mM disodium EDTA) and then stored at −70°C before they were used for DNA extraction.

Preparation of [3H]DNA probes and [3H]DNA-DNA hybridization procedure. A 2-μg portion of purified native DNA was labeled in vitro with [1',2',5'-3H]dCTP (Amersham Corp., Arlington Heights, Ill.) by the nick translation method (17) and was processed for hybridization as described previously (1). The specific activity of the resultant single-stranded [3H]DNA probes ranged from 1.0 × 107 to 2.0 × 107 cpm/μg of DNA, as determined by trichloroacetic acid precipitation. The [3H]-labeled DNA was sheared to approximately 400 base fragments by passing the probe through a French pressure cell. The sheared [3H]DNA was then extracted with phenol saturated with TNE buffer (10 mM NaCl, 1 mM EDTA, 10 mM Tris-hydrochloride; pH 7), dialyzed against TNE buffer, and concentrated by lyophili-

* Corresponding author.
TABLE 1. Strains of previously established *Ureaplasma* species and unclassified feline and canine strains used in this study

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>Serovar or serogroup</th>
<th>Host</th>
<th>Tissue</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>U. urealyticum</em></td>
<td>T960T</td>
<td>SVIII</td>
<td>Human</td>
<td>Urogenital tract</td>
<td>1</td>
</tr>
<tr>
<td><em>U. diversum</em></td>
<td>A417T</td>
<td>SI</td>
<td>Cattle</td>
<td>Lung</td>
<td>2</td>
</tr>
<tr>
<td><em>U. gallorale</em></td>
<td>D6-1T</td>
<td>SI</td>
<td>Chicken</td>
<td>Oropharynx</td>
<td>3</td>
</tr>
<tr>
<td>Ureaplasma sp.</td>
<td>F45d2</td>
<td>SI</td>
<td>Cat</td>
<td>Oral cavity</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>FT2-B</td>
<td>SI</td>
<td>Cat</td>
<td>Oral cavity</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>FT10-A</td>
<td>SI</td>
<td>Cat</td>
<td>Oral cavity</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>F2</td>
<td>SII</td>
<td>Cat</td>
<td>Oral cavity</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>F11</td>
<td>SII</td>
<td>Cat</td>
<td>Oral cavity</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>D1M-C</td>
<td>SII</td>
<td>Dog</td>
<td>Oral cavity</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>D29M</td>
<td>SII</td>
<td>Dog</td>
<td>Oral cavity</td>
<td>4</td>
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<tr>
<td></td>
<td>D11N-A</td>
<td>SIII</td>
<td>Dog</td>
<td>Nares</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>D6P-C</td>
<td>SIV</td>
<td>Dog</td>
<td>Prepuce</td>
<td>4</td>
</tr>
</tbody>
</table>

* U. *sp. F45d2, Feltine serogroup SI strain FT2-B, canine serogroup SII strain F2, and canine serogroup strain D1M-C.

The five feline strains fell into two genomically distinct groups, and these two genomic groups corresponded to the two distinct feline serogroups. The feline serogroup SI strain FT2-B DNA probe exhibited 89 and 92% DNA homology with feline serogroup SI strains F45d2 and FT-10, respectively, but less than 10% DNA homology with canine serogroup SII strains F2 and F11. Conversely, the canine feline serogroup SII strain F2 probe exhibited 83% DNA homology with canine serogroup SII strain F11, but less than 10% DNA homology with the canine serogroup SII strains. Thus, the three strains in canine serogroup SII were antigenically and genomically related to each other but distinct from strains in canine serogroup SII and from the established species, and each feline serogroup potentially represents a new species.

However, the canine serogroup SI strain D1M-C DNA probe strongly hybridized (73% DNA homology) with the serologically distinct canine serogroup SII strain D29M (13). These unexpected findings indicate that these canine strains belonging to two serologically distinct groups had considerable DNA homology; therefore, these strains belong to the same genomic species.

### DISCUSSION

Although only three species have been established previously within the genus *Ureaplasma*, the data presented here and elsewhere indicate that the two feline serogroups and at least one canine serogroup (serogroup SI) are each genomically distinct and are unrelated to the previously established species and that each taxon potentially represents a new species. In addition, unclassified ureaplasma serogroups have been described previously (1). Briefly, the reaction mixture contained 1 mg of unlabeled, sheared DNA per ml, 15,000 cpm of 3H-labeled DNA probe, 0.2% SDS, 1 mM EDTA, and 0.48 M phosphate buffer. Reaction mixtures were denatured at 105°C for 5 min and incubated at 65°C to a C0t value of more than 100 mol/l per liter (3). Hybridized DNA was adsorbed onto a hydroxyapatite column equilibrated at 60°C with 0.12 M phosphate buffer containing 0.2% SDS, and the radioactivity of each reaction mixture was measured by liquid scintillation spectrometry.

#### RESULTS

**Nucleic acid hybridization studies.** Each DNA probe prepared from either feline serogroup SI strain FT2-B, canine serogroup SII strain F2, and canine serogroup strain D1M-C, or the type strain of one of the three previously established *Ureaplasma* species exhibited significant actual hybridization with its homologous DNA (more than 79%), but not with the other heterologous ureaplasma or control strains tested (less than 10%) (Table 2). There was less than 10% DNA homology among *U. urealyticum* T960T (T = type strain), *U. diversum* A417T, *U. gallorale* D6-1T, feline serogroup SI strain FT2-B, canine serogroup SII strain F2, and canine serogroup SI strain D1M-C.

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isolated from nonhuman primate, ovine, and caprine tissues are also phenotypically distinct, and each of these may represent a new species. Each of these unclassified serogroups is readily separated from the others and from the previously established species by antigenic properties, SDS-polyacrylamide gel electrophoresis profiles, guanine-plus-cytosine (G+C) values, DNA homologies, and DNA restriction endonuclease cleavage patterns (8, 11-13, 16, 19, 20, 22, 28).

Canine ureaplasma strains were first isolated from the urogenital tracts of dogs by Taylor-Robinson et al., who separated these organisms into two serogroups (26). Subsequently, Kotani and Ogata isolated, cloned, and analyzed 36 additional strains (16) and separated them into four canine serogroups, represented by strains D1M-C (serogroup SI), D29M (serogroup SII), D11N-A (serogroup SIII), and D6P-C (serogroup SIV). In addition, we (15) also isolated a number of ureaplasmas from the oral cavities of cats and separated them into two feline serogroups, represented by strains FT2-B (serogroup SI) and F2 (serogroup SII). The *Ureaplasma* species and serogroups have also been separated on the basis of the G+C contents of their DNAs. The values reported for the previously established *Ureaplasma* species range from 26.9 to 31.6 mol%. In an extensive study, Howard et al. (11) reported G+C values of 27.2 to 27.8 mol% for canine strains, 27.6 mol% for a serovar VI strain of *U. urealyticum*, 27.9 mol% for feline strains, 28.3 mol% for squirrel monkey strain SP1625A, 28.7 to 30.2 mol% for several bovine serovars of *U. diversum*, 29.6 mol% for marmoset strain My6690, and 30.6 to 31.6 mol% for ovine-carpine strains. We reported G+C values of 27.6 mol% for *U. gallorale* and 27.1 mol% for an oral feline strain (8). Thus, the G+C contents of DNAs from the unclassified feline, canine, ovine-carpine, and nonhuman primate ureaplasma serogroups are within the range of values obtained for the previously established *Ureaplasma* species. In addition, the marked differences in G+C values can readily distinguish *U. diversum* from *U. urealyticum*. Moreover, the marked differences in G+C values between some of the unclassified serogroups and the previously established species provide further support that additional species or subspecies exist within the genus *Ureaplasma*.

A major contribution of DNA homology studies has been to provide a more unifying concept of species within the class *Mollicutes*. The proposed taxonomic groupings based upon DNA homology data suggest that strains belonging to the same species should exhibit 60% or more DNA homology (27) and that strains belonging to different species should exhibit less than 20% homology. In this study, we have shown for the first time that the three previously recognized species (*U. urealyticum* [human], *U. diversum* [bovine], and *U. gallorale* [avian]), which were established initially on the basis of phenotypic properties, are also genomically distinct, providing additional evidence in support of separating them into distinct species.

We have also shown that two feline serogroups and at least one canine serogroup are genomically unrelated to the type strains of the three previously established *Ureaplasma* species; thus, these three serogroups merit species status. There was 89 to 100% DNA homology among the three feline serogroup S1 strains, but less than 10% homology with the serologically distinct feline serogroup SII strains. Conversely, the serogroup SII strains were genomically distinct from the serogroup S1 strains. However, the canine serogroup S1 strain exhibited 73% DNA homology with a canine serogroup SII strain, indicating that these two serologically distinct strains belong to the same genomic species. Thus, phenotypic data alone, including antigenically distinct profiles, are not always adequate for identification of species. Species definition and classification based on serology have limitations, and genomic relatedness based on DNA cross-hybridization data should be used whenever possible.

Our DNA homology data and data reported elsewhere on the antigenic properties (13-16), SDS-polyacrylamide gel electrophoresis profile patterns (19, 20), G+C values, and DNA restriction cleavage patterns (8) indicate that the two feline ureaplasma serogroups and at least one canine serogroup (serogroup SI) represent new species or subspecies within the genus. In an accompanying paper (6), we present further characterizations of the two feline serogroups and propose that each of the feline serogroups (serogroups SI and SII) be given species status.

**LITERATURE CITED**


