Genomic and Phenotypic Analyses of Avian *Ureaplasma* Strains

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ABSTRACT. *Ureaplasmas* isolated from avian sources were compared to the two established *Ureaplasma* species, *U. urealyticum* from humans and *U. diversum* from cattle, by the metabolic inhibition test, sodium dodecyl sulfate-polyacrylamide gel electrophoresis of cell protein patterns, restriction endonuclease cleavage patterns of genomic DNA and by [³H] DNA-DNA hybridization. All seven unclassified avian *ureaplasma* strains tested were serologically related to each other but were different from the two established bovine and human *Ureaplasma* species. The antigenic differences were also evident in Western blot analysis. The [³H] DNA-DNA hybridization data further confirmed these results by showing that avian *ureaplasmas* were genetically related to each other but distinct from the established *Ureaplasma* species. The restriction endonuclease cleavage patterns revealed variations among the avian strains that were not apparent by the serological and biochemical techniques. Our results support the notion that the avian *ureaplasmas* are distinct, are unrelated to the two established *Ureaplasma* species and merit consideration as a new species.—**KEY WORDS:** DNA-DNA hybridization, *Ureaplasma*, Western blot.

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The *ureaplasmas* comprise a large group of cell wall-less prokaryotes distinguished from other mycoplasmas by their ability to hydrolyse urea. At present only two species, *Ureaplasma urealyticum* with the type strain T960 (ATCC 27618) isolated from a patient with nongonococcal urethritis [21], and *U. diversum* with the type strain A417 (NCTC 10182) isolated from cattle with pneumonia [7], have been established within the genus *Ureaplasma*. There are at least 14 serovars of *U. urealyticum* and three serovars of *U. diversum*. In addition to human and bovine strains, *ureaplasmas* have been isolated from a variety of animal species, including nonhuman primates, goats, sheep, dogs, cats and birds. The avian *ureaplasmas* have been reported to be serologically distinct from the two established species [9–12], and the guanine-plus-cytosine content of deoxyribonucleic acid (DNA) was shown to be 27.6 mol% [5].

The present study was designed to compare the avian *ureaplasma* strains to the two type strains T960 and A417 of *U. urealyticum* and

U. diversum, respectively. The procedures used were the metabolism inhibition (MI) test, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of cell proteins, Western blot analysis, restriction endonuclease digestion of chromosomal DNA, and DNA-DNA hybridization.

MATERIALS AND METHODS

Ureaplasma strains: The strains used and their sources and origin are given in Table 1.

Cultivation and harvest of organisms: The organisms were grown at 37°C in the 10 B medium described by Shepard and Lunceford [20]. The organisms were harvested from five liters of broth culture by centrifugation at 9,000 rpm for 60 min in a Hitachi 20 PR centrifuge, and were washed once in TE buffer consisting of 50 mM tris(hydroxymethyl)aminomethane (Tris), pH 8.0 and 10 mM disodium ethylenediaminetetraacetate (EDTA).

Extraction of chromosomal DNA: The pel

Table 1. The ureaplasma strains used in the study

<i>Ureaplasma</i> species and strain	Origin	Source
D6-1	Leghorn chicken (Flock D)	Oropharynx
D23	Leghorn chicken (Flock D)	Oropharynx
F2-1	Japanese bantam (Flock F)	Oropharynx
F5	Japanese bantam (Flock F)	Oropharynx
Y4-2	Leghorn chicken (Flock Y)	Oropharynx
Y8-1	Leghorn chicken (Flock Y)	Oropharynx
T9-1	Red jungle fowl (Flock T)	Oropharynx
<i>U. diversum</i>		
A417	Bovine	Pneumonia
<i>U. urealyticum</i>		
T960	Human	Nongonococcal urethritis

All of the avian ureaplasma strains were isolated in the laboratory of K. Koshimizu. The type strain of *U. diversum* was obtained originally from R.N. Gourlay, ARC, Institute for Research on Animal Disease, Compton, England. The type strain of *U. urealyticum* was obtained originally from D.K. Ford, University of British Columbia, Vancouver, Canada. We received these two type strains from M. Ogata, University of Tokyo, Tokyo.

let was lysed in a solution containing 8 M urea, 1% SDS, 1 M sodium perchlorate, 1 mM EDTA and 0.24 M diphasic phosphate buffer (PB). The DNA was purified using the hydroxyapatite batch elution technique [3].

[³H]DNA probe synthesis: Purified native DNA was labeled *in vitro* with deoxy[1', 2', 5'-³H]cytidine 5'-triphosphate (Amersham Corp.) by nick translation method [15, 19] and processed for hybridization as described earlier [1]. The specific activity of the resultant single stranded DNA probe was approximately 1.5×10^7 cpm per μ g of DNA.

Nucleic acid hybridization procedure: The hybridization procedure used was as described earlier [1]. The hybridization mixture contained 100 μ g of unlabeled, sheared DNA per ml, 1.0×10^5 cpm [³H]DNA probe per ml, 0.2% SDS, 1 mM EDTA, and 0.48 M PB. Reaction mixtures were denatured at 105°C for 5 min and incubated at 65°C to a C_{0t} value of over 100 [2]. Hybridized DNA was adsorbed to a hydroxyapatite column equilibrated at 60°C with 0.12 M PB containing 0.2% SDS.

Thermal elution midpoint (te_{50}) determina-

tions: The thermal elution midpoint temperature (te_{50}), which is used as an indicator of the amount of base mismatching in DNA-DNA heteroduplex [14], was determined by preparing hydroxyapatite column as described above. The hybridized DNA absorbed onto the column at 60°C was then washed thoroughly with 0.21 M PB containing 0.2% SDS at 4°C increments up to 100°C. All measurements of radioactivity were performed by adding 10 ml ACSII (Amersham Corp.) to 4. ml of elute and counted in an Aloka model LSC903 liquid scintillation spectrometer (Tokyo).

DNA cleavage by restriction endonucleases: The restriction endonucleases, *SalI* and *PstI*, used were purchased from New England BioLabs. About 3 μ g of ureaplasma DNAs was digested with 10 to 20 units of each enzyme, according to the conditions suggested by the supplier. Digestions were carried out in 20 μ l reaction mixture at 37°C for 60 min and were subjected to 1.0% agarose gel electrophoresis. After electrophoresis, the gels were stained with ethidium bromide and were photographed as described previously

Table 2. Serologic relationships among the established *Ureaplasma* species and unclassified avian strains by metabolism inhibition test

Strain	Antiserum against ^{a)}											
	No.7	No.23	No.27	No.58	No.354	Pirillo	Cook	T960	A417	D6-1	Y8-1	T9-1
<i>U. urealyticum</i> T960	— b)	—	—	—	—	—	—	2560	—	—	—	—
<i>U. diversum</i> A417	—	—	—	—	—	—	—	—	640	—	—	—
<i>Ureaplasma</i> sp. D6-1	—	—	—	—	—	—	—	—	—	5120	5120	640
Y8-1	—	—	—	—	—	—	—	—	—	2560	10240	320
T9-1	—	—	—	—	—	—	—	—	—	2560	5120	2560
D23	—	—	—	—	—	—	—	—	—	5120	10240	320
F2-1	—	—	—	—	—	—	—	—	—	5120	10240	640
F5	—	—	—	—	—	—	—	—	—	5120	5120	1280
Y4-2	—	—	—	—	—	—	—	—	—	2560	10240	320

a) Antisera were prepared against eight serovars of *U. urealyticum* and type strain A417 of *U. diversum*, and three unclassified avian ureaplasma strains D6-1, Y8-1 and T9-1.

b) No inhibition at the serum dilution of 1:20.

[6].

SDS-PAGE of ureaplasma cell proteins: The washed ureaplasma cells were suspended in TE buffer, diluted to 1:1 with a solution containing 2% SDS, 5% mercaptoethanol, and 20% glycerol, and boiled for 3 min. SDS-PAGE of the boiled samples was carried out according to the method of Laemmli [13], using a vertical slab gels containing a 3% stacking gel and a 10% running gel. The molecular weight reference markers used were purchased from Bio-Rad Laboratories (Richmond, CA).

Immunoblotting: The Western blotting analysis was performed using the technique of Towbin *et al.* [23], with slight modifications. The boiled samples described above were run in duplicate on identical SDS-PAGE gels, one was stained with Coomassie brilliant blue and the other was electroblotted onto nitrocellulose filter (Schleicher & Schuell Inc.) using a Marysol electroblotting apparatus (Tokyo) at 60 volts for 3 hr. The rabbit hyperimmune serum prepared against the avian ureaplasma strain D6-1 was absorbed with the broth medium, diluted 1:100 and applied to the nitrocellulose filter impregnated with the protein antigens. The filter was

then incubated at 22°C overnight, rinsed in copious volumes of distilled water, and processed using the Bio-Rad Immuno-Blot system.

Serological procedures: The procedures used to obtain rabbit immune sera against the ureaplasma strains, including the eight serovars of human origin, are described elsewhere [10, 24]. The antigenic properties of the ureaplasma strains tested were examined by the MI test [17].

RESULTS

Serological properties of avian ureaplasmas: None of the seven avian ureaplasma strains tested reacted with the hyperimmune sera prepared against the eight established human *U. urealyticum* serovars nor with the bovine *U. diversum* type strain (Table 2). Conversely, the hyperimmune sera prepared against the three representative avian ureaplasma strains reacted with each other, but not with the human or bovine ureaplasma strains. The serological titers obtained with antisera prepared against the Leghorn chicken strains D6-1 and Y8-1 were identical with those for the other heterologous avian strains.

Fig. 1. SDS-PAGE of cell proteins from ureaplasma strains. Unclassified avian ureaplasmas: D6-1, A; D-23, B; F2-1, C; F5, D; Y4-2, E; Y8-1, F; T9-1, G; *U. diversum*: A417, H; *U. urealyticum*: T960, I. Molecular mass markers used: phosphorylase B (92.5 Kdal), bovine serum albumin (66.2 Kdal), ovalbumin (45 Kdal), carbonic anhydrase (31 Kdal), and soybean trypsin inhibitor (21.5 Kdal).

The MI titer of D6-1 antiserum against the rest of the avian strains was 2560 or 5120, and 5120 or 10240 for Y8-1 antiserum. However, the MI titers of T9-1 antiserum considerably varied from 320 to 2560.

Cell proteins and antigenic determinants: The polypeptide patterns of the ureaplasma cell lysates obtained by SDS-PAGE are shown in Fig. 1. Differences in stain intensity reflect differences in the protein concentrations used. A band with an approximate molecular weight of 55 kilodaltons (Kdal) was prominent and common in all the ureaplasmas tested regardless of animal origin. Although there were several other common protein bands, there were major differences in the cell protein patterns among the avian, human and bovine ureaplasmas. The Western blot analysis of the protein antigens of ureaplasma strains is presented in Fig. 2. The three major antigens among the seven avian

strains migrated at 76, 55 and 47 Kdal, and the minor antigens migrated at 25 and 22 Kdal. Antiserum prepared against the D6-1 strain reacted with each of the protein bands from the seven avian ureaplasmas with virtually identical results. Normal serum showed no reactivity. The antigenic polypeptides (with approximate molecular weights of 76, 47, 25 and 22 Kdal) specific for avian strains were not observed in cell protein patterns of established *U. urealyticum* and *U. diversum* type strains. These findings suggest that there are distinct antigenic determinants specific for the unclassified avian ureaplasmas.

Restriction endonuclease cleavage patterns of the genomic DNA: Cleavage patterns of the chromosomal DNAs from avian ureaplasmas after digestion with *SalI* are shown in Fig. 3. The DNA cleavage patterns produced were distinct from those of *U. urealyticum* and *U. diversum* strains (data not shown).

Fig. 2. Immunoblot analysis of ureaplasma antigens with anti-D6-1 rabbit serum. Lanes A through I are the same as in Fig. 1.

Fig. 3. Cleavage patterns of the chromosomal DNA of avian ureaplasmas with *SalI*. Lane λ indicates molecular mass references of the phage λ DNA cleaved with *HindIII*. Lane A through G are the same as in Fig. 1.

Table 3. Hybridization of [³H] DNA from *U. urealyticum*, *U. diversum* and unclassified avian strains with unlabeled DNA of the human, bovine, and avian strains

Source of unlabeled DNA	Source of [³ H] DNA probe					
	T960	A417	D6-1	F2-1	Y8-1	T9-1
<i>U. urealyticum</i>	[81.4] ^{a)}					
T960	100.0 ^{b)}	18.5	21.5	24.4	20.6	27.3
<i>U. diversum</i>		[84.8]				
A417	21.0	100.0	25.6	26.3	26.8	25.1
<i>Ureaplasma</i> sp.			[81.5]			
D6-1	24.5	23.0	100.0	70.1 (79.0) ^{c)}	56.8 (78.7)	82.8
				[75.9]		
F2-1	18.6	19.3	74.8	100.0 (80.3)	67.4 (78.1)	78.6
					[89.7]	
Y8-1	20.9	18.5	52.9	58.1 (78.4)	100.0 (80.1)	57.0
						[82.2]
T9-1	18.7	22.3	75.3	75.8 (78.1)	65.1 (78.4)	100.0
D23	20.3	26.4	87.6	80.6 (78.9)	68.8 (77.6)	84.2
F5	23.5	26.5	70.6	85.8 (79.4)	52.4 (78.0)	80.4
Y4-2	21.6	18.9	57.8	56.4 (78.4)	95.7 (79.1)	50.5
Avian control DNA	[6.1] 7.5	[8.2] 9.7	[5.6] 6.9	[9.8] 12.9	[9.3] 10.4	[9.7] 11.8

a) The values in square brackets show actual hybridization values.

b) Homologous values were normalized to 100% homology and represent the average from two separate experiments.

c) The values in parentheses represent the thermal elution midpoint of DNA-DNA duplex.

The similarity of the cleavage patterns indicates that there is very close relatedness between Leghorn chicken strains F2-1 and F5 and also between strains Y4-2 and Y8-1. Strains F2-1 and F5 were isolated from one flock and strains Y4-2 and Y8-1 from another, separate flock.

Characterization of [³H]DNA probes: The [³H]DNA probes prepared from avian ureaplasma strains D6-1, F2-1, Y8-1 and T9-1 hybridized 81.5, 75.9, 89.7 and 82.2% to their homologous DNAs, respectively. The hybridization values obtained in the homologous DNA reactions were normalized to 100% homology for comparison (Table 3).

The [³H]DNA probes derived from the T960 strain of *U. urealyticum* and the A417 strain of *U. diversum* hybridized 81.4 and 84.8% to their homologous DNAs, respectively. The six ureaplasma [³H]DNA probes hybridized only from 6.9 to 12.9% to the control DNA derived from chicken tissues.

Nucleic acid hybridization studies: The results of DNA hybridization studies using [³H]DNA probes prepared from four unclassified avian ureaplasma strains and the two type strains of *U. urealyticum* and *U. diversum* are presented in Table 3. The data clearly show that the human, bovine and avian ureaplasma strains tested are genomically dis-

tinct. The seven unclassified avian strains form a cluster of genetically related organisms but exhibit a moderate amount of genomic variation. Hybridization of the *U. urealyticum* T960 probe showed only 21% homology to the bovine strain and from 18.6 to 24.5% to the seven avian strains. The *U. diversum* A417 probe provided similar results, only 18.5% homology to the human strain and from 18.5 to 26.5% homology to the seven avian strains. The four avian ureaplasma strains were 20.6 to 27.3% homologous to the human and bovine strains but from 50.5 to 95.7% homologous to the avian strains. Thus, a moderate degree of heterogeneity was observed among the unclassified avian ureaplasmas. These strains were isolated from different birds, *i.e.*, Leghorn chickens, Japanese bantams and a red jungle fowl, which were maintained in separate flocks. It was of interest that strains isolated from the same type of bird maintained in the same flock showed the highest homologies; *e.g.*, strains D6-1 and D23 isolated from the Leghorn chicken flock D, strains F2-1 and F5 isolated from the Japanese bantam flock F and strains Y4-2 and Y8-1 isolated from the Leghorn flock Y showed 87.6%, 85.8% and 95.7% homology, respectively. The thermal elution midpoint (te_{50}) for the strain F2-1 homoduplex was 80.3°C and the heteroduplex value for strain F5 was 79.4°C. The homoduplex value for Y8-1 was 80.1°C and the heteroduplex value for Y4-2 was only 79.1°C. The heteroduplex values obtained with the strain F2-1 and Y8-1 probes for the other avian ureaplasmas range from 77.6 to 79.4°C, supporting the presence of heterogeneity among the avian strains tested.

DISCUSSION

The unclassified ureaplasmas isolated from birds have been shown to be serologically related to each other but distinct from the two established *Ureaplasma* species, *U. urealyti-*

cum and *U. diversum* using the growth inhibition and metabolism inhibition tests [9–12]. In this report, the metabolism inhibition studies were extended to examine additional avian strains. Our findings confirm earlier studies and support the notion that avian ureaplasmas are serologically related to each other but distinct from the human and bovine *Ureaplasma* species. A moderate amount of antigenic heterogeneity among the avian ureaplasmas was observed only when the antiserum prepared against the ureaplasma strain T9-1 isolated from the red jungle fowl was used. There was no difference in titer among the avian ureaplasma strains when antisera prepared against the Leghorn chicken strains D6-1 and Y8-1 were used. A similar amount of antigenic heterogeneity has been reported among strains of *U. urealyticum* [22] and *U. diversum* [7, 8].

The electrophoretic patterns of cell proteins showed that the seven avian ureaplasma strains have many polypeptides in common. Moreover, the immunoblotting analysis indicated that the polypeptides of similar size specific for avian strains shared common antigenic determinants. Although the protein patterns among the avian ureaplasmas are specific, there are a few polypeptides of avian strains which migrate along with the protein bands of human and bovine ureaplasma strains. In addition, the avian strain D6-1 antiserum reacted with the polypeptide bands of human and bovine strains not present in the protein patterns of avian strains. Thus, the findings indicate that the seven avian ureaplasmas tested have many common, antigenically similar proteins and that they also share a few antigenic determinants with human and bovine ureaplasma polypeptides which have distinctly different sizes.

DNA cleavage pattern analysis of mycoplasma chromosomes has been used effectively to establish genomic relatedness among a number of species and strains belonging to the class *Mollicutes* [5, 6, 18]. The DNA

cleavage patterns revealed close genomic relatedness among the avian ureaplasma strains but also show some variation among the strains. The genomic variation based on DNA cleavage patterns and DNA-DNA homology was more than was expected based on the earlier serologic findings. We observed that strains isolated from chickens maintained in the same flock appeared much more similar to each other than to strains isolated from birds maintained in different flocks. Whether the host or habitat influences the genotypic composition of these organisms remains to be determined. A similar amount of genomic heterogeneity was also seen among strains of *U. urealyticum* and strains of *U. diversum* [4, 8, 16, 18]. In fact, the cleavage patterns obtained using a series of restriction endonucleases separated the nine serovars of *U. urealyticum* into two definite clusters [18]. Thus, DNA cleavage pattern analysis has provided a useful means for determining genetic relatedness among the mycoplasmas.

The [³H]DNA-DNA hybridization data indicate that the seven avian ureaplasmas belong to a genomically related cluster of organisms and that they are genomically different from the established species *i.e.*, *U. urealyticum* and *U. diversum*. To the best of our knowledge, this is the first report to show a lack of genomic relatedness between *U. urealyticum* and *U. diversum* as determined by DNA-DNA hybridization. The difference in DNA homology between *U. urealyticum* and *U. diversum* (18.5–21.0%) was similar to differences between the avian ureaplasmas and *U. urealyticum* or *U. diversum* (18.5–27.3%). Whereas the DNA-DNA homology among the avian ureaplasmas ranges from 50 to 95%, strains isolated from birds in the same flock showed the highest amount of homology. These findings confirm the close genomic relatedness among strains isolated from the same type of bird maintained in the same flock. The degree of DNA homology among the avian ureaplasmas correlated well

with their respective te_{50} values. There was no more than 5% mismatching in base pairing among the common sequence in the seven avian ureaplasmas tested. Thus, the accumulative data from serology, SDS-PAGE protein patterns, DNA-DNA hybridization and DNA cleavage patterns indicate that the seven avian ureaplasma strains tested form a cluster of related organisms, are quite distinct from the established strains of *U. urealyticum* and *U. diversum* and merit species designation.

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REFERENCES

1. Aulakh, G. S., and Gallo, R.C. 1977. Rauscher-leukemia virus related sequence in human DNA: presence in some tissue of patients with hematopoietic neoplasia and absence in DNA from other tissues. *Proc. Natl. Acad. Sci. U.S.A.* 74: 353–357.
2. Britten, R.J., Graham, D.E., and Neufeld, D. 1974. Analysis of repeating DNA sequences by reassociation. *Methods in Enzymology*, 24: 363–418.
3. Britten, R.J., Pavich, M., and Smith, J. 1968. A new method for DNA purification. *Ann Rep. Carnegie Inst.*, Washington, D.C. pp. 400–403.
4. Christiansen, C., Black, F.T., and Freundt, E.A. 1981. Hybridization experiments with deoxyribonucleic acid from *Ureaplasma urealyticum* serovars I to VIII. *Int. J. Syst. Bacteriol.* 31: 259–262.
5. Harasawa, R., Koshimizu, K., Pan, I.-J., Stephens, E.B., and Barile, M.F. 1984. Genomic analysis of avian and feline ureaplasmas by restriction endonucleases. *Isr. J. Med. Sci.* 20: 942–945.
6. Harasawa, R., Kotani, H., Razin, S., and Koshimizu, K. 1984. Genomic analysis of animal ureaplasmas by restriction endonucleases. *Jpn. J. Vet. Sci.* 46: 737–740.
7. Howard, C.J., and Gourlay, R.N. 1982. Proposal for a second species within the genus *Ureaplasma*, *Ureaplasma diversum* sp. nov. *Int. J. Syst. Bacteriol.* 32: 446–452.
8. Howard, C.J., Pocock, D.H., and Gourlay, R.N. 1981. Polyacrylamide gel electrophoretic comparison of the polypeptides from ureaplasmas isolated from cattle and humans. *Int. J. Syst. Bacteriol.*

- 31: 128-130.
9. Koshimizu, K., Kotani, H., Yamamoto, K., Magaribuchi, T., Harasawa, R., Ito, M., and Ogata, M. 1984. Serological analysis of ureaplasmas isolated from various animals. *Isr. J. Med. Sci.* 20: 950-953.
 10. Koshimizu, K., Kotani, H., Magaribuchi, T., Yagihashi, T., Shibata, K., and Ogata, M. 1982. Isolation of ureaplasmas from poultry and experimental infection in chickens. *Vet. Rec.* 110: 424-429.
 11. Koshimizu, K., and Magaribuchi, T. 1977. Isolation of ureaplasma (T mycoplasma) from the chicken and jungle-fowl. *Jpn. J. Vet. Sci.* 39: 195-199.
 12. Koshimizu, K., and Magaribuchi, T. 1978. Biological and serological characterization of ureaplasmas isolated from domestic fowls and red jungle fowls. *Jpn. J. Vet. Sci.* 40: 719-727.
 13. Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* 227: 680-685.
 14. Laird, C.E., McConaughy, B.L., and McCarthy, B.J. 1969. Rate of fixation of nucleotide substitutions in evolution. *Nature (London)* 224: 149-154.
 15. Maniatis, T., Jeffrey, A., and Kleid, D.G. 1975. Nucleotide sequence of the rightward operator of phage λ . *Proc. Natl. Acad. Sci. U.S.A.* 72: 184-1188.
 16. Mouches, C., Taylor-Robinson, D., Stipkovits, L., and Bové, J.M. 1981. Comparison of human and animal ureaplasmas by one and two-dimensional protein analysis on polyacrylamide slab gel. *Ann. Microbiol. (Paris)* 132B: 171-196.
 17. Purcell, R.H., Taylor-Robinson, D., Wong, D., and Chanock, R.M. 1966. Color test for the measurement of antibody to T-strain mycoplasmas. *J. Bacteriol.* 92: 6-12.
 18. Razin, S., Harasawa, R., and Barile, M.F. 1983. Cleavage patterns of the mycoplasma chromosome, obtained by using restriction endonucleases, as indicators of genetic relatedness among strains. *Int. J. Syst. Bacteriol.* 33: 201-206.
 19. Rigby, P.W., Dieckermann, M., Rhodes, C., and Berg, P. 1977. Labeling DNA to high specific activity *in vitro* by nick translation with DNA polymerase I. *J. Mol. Biol.* 113: 237-251.
 20. Shepard, M.C., and Lunceford, C.D. 1978. Serological typing of *Ureaplasma urealyticum* isolated from urethritis patients by an agar growth inhibition method. *J. Clin. Microbiol.* 8: 566-574.
 21. Shepard, M.C., Lunceford, C.D., Ford, D.K., Purcell, R.H., Taylor-Robinson, D., Razin, S., and Black, F.T. 1974. *Ureaplasma urealyticum* gen. nov., sp. nov.: proposed nomenclature for the human T(T-strain) mycoplasmas. *Int. J. Syst. Bacteriol.* 24: 160-171.
 22. Taylor-Robinson, D., and Furr, P. 1973. The distribution of T-mycoplasmas within and among various animal species. *Ann. N.Y. Acad. Sci.* 225: 108-117.
 23. Towbin, H., Staehelin, T., and Gordon, J. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. U.S.A.* 76: 4350-4354.
 24. Yamamoto, K., Harasawa, R., Ogata, M., Miura, T., and Nakane, H. 1976. Bacteriological examination of bovine pneumonic lungs in Japan. *Jpn. J. Vet. Sci.* 38: 7-14.

要 約

核酸相同性と抗原性からみた鳥類由来ウレアプラズマの分類: 原澤 亮・興水 馨¹⁾・潘 英仁¹⁾・Barile, M. F.²⁾ (宮崎大学農学部家畜微生物学教室, ¹⁾東京大学医学部動物実験施設, ²⁾米国食品医薬品検査局)——鳥類由来ウレアプラズマとヒトおよびウシ由来の2基準株とを代謝阻止(MI)試験, 細胞タンパク質のドデシル硫酸ナトリウム—ポリアクリルアミド・ゲル電気泳動(SDS-PAGE), 染色体DNAの制限酵素による切断像, およびDNA-DNA交雑試験で比較した。MI試験では, 鳥類由来株は血清学的に均一で, ウシおよびヒト由来の基準株とは区別された。この抗原的差異はウェスタン・ブロット法でも確認された。鳥類由来株の染色体DNAの切断像の分析により, 細胞タンパク質のSDS-PAGEやMI試験では検出困難な生態学的な変化を知ることができた。DNA-DNA交雑試験では, 鳥類由来株が遺伝的に均一で, 2種の基準株とは異なることが示された。これらの成績は, 鳥類由来ウレアプラズマが新種であることを支持するものであった。