ヤギ,ヒツジからのureaplasma の分離および血清学的比較

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Isolation and Serological Comparison of Ureaplasmas from Goats and Sheep

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Abstract. Cultures for *Ureaplasma* isolation were made from the oral cavity, nasal cavity, eye, rectum, prepuce and vagina of 62 goats of 3 separate herds and 11 sheep of one herd. All the animals were apparently healthy. Ureaplasmas were obtained from the prepuce (6/16, 37.5%) and vagina (18/46, 39.1%) of goats and from the prepuce (1/5, 20%) and vagina (2/6, 33.3%) of sheep, but not from any other organ. The caprine and ovine *Ureaplasma* strains were examined serologically by the growth inhibition and metabolism inhibition tests. Twenty-three caprine strains formed a single serological group, and 21 ovine strains were divided serologically into twenty forming a group and one distinct from these strains. Moreover, 2 groups of *Ureaplasma* strains isolated from the goats and sheep, but one ovine strain, were found to have a common antigen. Neither caprine nor ovine strains had any serological relationship with human, bovine, canine, simian or avian strains. The fact that the caprine and ovine strains have an antigenic similarity suggests that the host-specificity of *Ureaplasma* may be an exception for the caprine and ovine strains.

The occurrence of ureaplasmas has been recognized in man [24, 25, 34] and various animal species, such as cattle [3, 17, 22, 23, 28, 37, 38], dogs [14, 35], cats [6, 32, 33], monkeys [2, 7, 11, 15, 35], birds [12, 13, 30] and pigs [31]. Caprine and ovine ureaplasmas were first isolated from the urogenital tract by Gourlay et al. [4] in the United Kingdom in 1973 and from the urine by Livingston et al. [18] in the United States in 1975, respectively. Since then, they have been isolated only from the urogenital tract [1, 18, 19].

Experimentally, ureaplasmas induced mastitis in goats [4], and vulvitis [1] and abortion [19] in sheep.

Livingston et al. [18] mentioned that no serological relationship had been detected between caprine and ovine ureaplasmas by the mycoplasmacidal test. Then, they demonstrated the existence of 9 serotypes of ovine ureaplasmas by the same test [19]. The serological typing of caprine strains, however, has not been established, or that of ovine strains examined by any other serological method as yet.

This communication describes the isolation of ureaplasmas from goats and sheep, and the results of serological comparison between these organisms.

Materials and Methods

Ureaplasma strains: Two caprine and 15 ovine *Ureaplasma* strains were used as reference strains. They are listed in Table 1. Eight human strains of serotypes I to VIII [38], 7 bovine strains (C13, C24, C23, C17, C12, C7 and T44) [20], 4 canine

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Table 1. Origin of caprine and ovine ureaplasmas used as reference strains

Ureaplasma strain	Animal species	Source isolated	Supplied by
G1	Goat	Vagina	C.J. Howard*
G3		Vagina	
1611#1	Sheep	Vulva	H.L. Ruhnke**
1659#3		Vulva	
1655#1		Vulva	
1692#1d18		Vulva	
1643#1		Vulva	
1692A		Vulva	
1662#1		Vulva	
596		Urine	C.W. Livingston***
888		Urine	
823		Prepuce	
624		Urine	
961		Semen	
861		Prepuce	
854		Prepuce	
1202		Uterus	

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strains (D1M-C, D29M, D11N-A and D6P-C) [14], a simian strain (T167-2) [14], and an avian strain (D6-1) [14] were used for comparative study.

Goats and sheep: Sixty-two apparently healthy goats of 3 separate herds and 11 apparently healthy sheep of a herd were examined for the presence of ureaplasmas. All the sheep examined were maintained in one group of the same herd with some goats tested.

Media: The liquid medium (T-broth) and solid medium (T-agar), as described previously [14], were used for isolation and serological studies of ureaplasmas. In some experiments for ureaplasmal isolation, thallium acetate was omitted from T-broth and T-agar.

Isolation and cloning of ureaplasmas: Specimens were obtained with sterile cotton swabs from the oral cavity, nasal cavity, eye, rectum, prepuce and vagina of goats and sheep. They were cultivated in the same manner as mentioned before [14]. Of them, those from the rectum were filtrated through a 450 nm membrane filter (Sartorius) before inoculation into media.

The isolates were cloned by transferring single colonies from T-agar to T-broth on three successive occasions in such manner as stated previously [14].

Preparation of antisera and serological tests: They were the same as described previously [20].

Table 2. Isolation of ureaplasmas from goats

Site tested	No. examined	No. positive (%)
Oral cavity	62	0 (0)
Nasal cavity	62	0 (0)
Eye	62	0 (0)
Rectum	62	0 (0)
Prepuce	16	6 (37.5)
Vagina	46	18 (39.1)
Total	310	24 (7.7)

Results

Isolation of ureaplasmas from goats

Table 2 presents the results of isolation of ureaplasmas from the 62 apparently healthy goats of 3 herds. The isolated ureaplasmas were identified by colony morphology, colony size, and urease activity.

They were derived from the prepuce of 6 animals (37.5%) and the vagina of 18 (39.1%). The number of organisms from one specimen ranged from 10^2 to 10^5 ccu/ml. No positive culture was obtained from the oral cavity, nasal cavity, eye or rectum.

The isolation rate of ureaplasmas from vaginal specimens in liquid or solid medium with or without thallium acetate was determined comparatively. It was not affected by the presence of thallium acetate at a concentration of 1:4,000.

Isolation of ureaplasmas from sheep

Eleven apparently healthy sheep were examined for the presence of ureaplasmas (Table 3). These organisms were isolated from the prepuce of one animal (20%) and the vagina of two (33.3%). The number of organisms isolated ranged from 10^2 to 10^5 ccu/ml. No ureaplasmas were isolated from any other site.

Table 3. Isolation of ureaplasmas from sheep

Site tested	No. examined	No. positive (%)
Oral cavity	11	0 (0)
Nasal cavity	11	0 (0)
Eye	11	0 (0)
Rectum	6	0 (0)
Prepuce	5	1 (20.0)
Vagina	6	2 (33.3)
Total	50	3 (6.0)

Serological comparison of caprine *Ureaplasma* strains

Table 4. Serological comparison between caprine and ovine ureaplasmas by growth inhibition test

Caprine strain	Geographic origin	Antiserum against					
		Caprine				Ovine	
		G3V–A	G9V	G10V	G39V–B	S8V–A	1202
G1V–A		1.5*	2	2	2	5	0
G2V–A		2	5	1.5	3	3	0
G3V–A		1.5	5	2	3	4	0
G9V		1.5	4	2	3	3	0
G10V		3	5	5	5	4	0
G20V–A		0	3	1.5	3	3	0
G29V–A		2	6	3	3	5	0
G30V–D		4	2	3	3	3	0
G39V–A		3	2	5	4	5	0
G39V–B		2	0	3	4	3	0
G42V–A	Japan	1.5	2	3	3	2	NT**
G44V–A		4	2.5	3	4	4	NT
G45V–B		3	3	5	3	3	0
G46P–C		2	4	3	3	3	0
G51V–A		2	4	5	2	5	NT
G53V–B		4	3	3	3	4	NT
G55V–A		4	3	3	3	4	0
G59V–A		2	4	5	4	5	0
G60V–A		0	3	3	3	3	0
G61V–A		2	3	5	3	5	0
G62P–A		2	3	2	3	5	NT
G1	England	2	2	2	3	5	0
G3		3	3	3	2	2	0

* The width (mm) of a zone of growth inhibition from the well edge.

** Not tested.

Table 5. Serological comparison between caprine and ovine ureaplasmas by metabolism inhibition test

Caprine strain	Geographic origin	Antiserum against					
		Caprine				Ovine	
		G3V-A	G9V	G10V	G39V-B	S8V-A	1202
G1V-A		1280*	5120	10240	2560	5120	—**
G2V-A		5120	≥40960	≥40960	640	2560	—
G3V-A		10240	≥40960	≥40960	10240	5120	—
G9V		2560	20480	≥40960	10240	20480	—
G10V		640	10240	20480	10240	20480	—
G20V-A		1280	10240	20480	≥40960	1280	—
G29V-A		2560	10240	5120	20480	1280	—
G30V-D		1280	1280	≥40960	5120	2560	—
G39V-A		5120	1280	≥40960	≥40960	5120	—
G39V-B		5120	640	20480	20480	1280	—
G42V-A	Japan	10240	20480	≥40960	20480	5120	NT***
G44V-A		2560	1280	20480	10240	5120	NT
G45V-B		1280	640	20480	10240	5120	—
G46P-C		1280	10240	10240	20480	20480	—
G51V-A		10240	1280	20480	2560	2560	NT
G53V-B		5120	1280	10240	5120	5120	NT
G55V-A		1280	320	5120	10240	10240	—
G59V-A		1280	320	2560	5120	20480	—
G60V-A		1280	1280	≥40960	10240	10240	—
G61V-A		5120	5120	10240	20480	2560	—
G62P-A		2560	10240	10240	5120	10240	NT
G1	England	5120	2560	10240	5120	2560	—
G3		2560	640	5120	10240	10240	—

* Reciprocal of the highest dilution of serum showing metabolism inhibition.
** No inhibition at the dilution of 1 : 20.
*** Not tested.

Twenty-three caprine *Ureaplasma* strains consisting of 21 cloned strains isolated in this study and 2 strains of England origin were tested against 4 caprine and 2 ovine antisera by the growth inhibition test (Table 4). Most of them were inhibited by antisera to strains G3V-A, G9V, G10V and G39V-B. A few of them, however, did not react with either of the 4 antisera. All the caprine strains were inhibited in growth by antiserum to ovine strain S8V-A, but never by antiserum to ovine strain 1202.

As shown in Table 5, the metabolism inhibition test revealed results similar to those of the growth inhibition test. Almost all the caprine strains had higher titers against antiserum to ovine strain S8V-A than against antisera to the caprine strains, but all those strains formed a single serological group. These findings suggest that the caprine strains tested might be antigenically interrelated with one another and might possess a common antigen with the ovine strain.

Serological comparison of ovine *Ureaplasma* strains

Twenty-one ovine strains were tested against 2 ovine and 3 caprine antisera by the growth inhibition test, as shown in Table 6. They were composed of 6 strains obtained by the authors, 7 from sheep in

Table 6. Serological comparison between ovine and caprine ureaplasmas by growth inhibition test

Ovine strain	Geographic origin	Antiserum against				
		Ovine		Caprine		
		S8V–A	1202	G3V–A	G10V	G39V–B
S8V–A	Japan	5*	0	0	0	0
S8V–B		6	0	3	3	3
S10P–A		5	0	2	3	3
S10P–C		2	0	4	3	3
S11V–A		5	0	4	3	3
S11V–B		5	0	2	2	2
1611♯1	Canada	6	0	4	5	4
1659♯3		7	0	3	7	7
1655♯1		7	0	5	5	3
1692♯1d18		7	0	5	6	5
1643♯1		6	0	5	5	5
1692A		7	0	4	6	6
1662♯1		6	0	4	5	5
596	U. S. A.	3	0	2	3	2
888		5	0	4	5	3
823		6	0	3	4	5
624		4	0	4	4	4
961		2	0	2	2	2
861		5	0	4	5	2
854		5	0	3	4	3
1202		0	6	0	0	0

* The width (mm) of a zone of growth inhibition from the well edge.

Canada, and 8 from sheep in the United States. Of them, 20 strains, S8V-A to 854, were inhibited in growth by antiserum to ovine strain S8V-A, but had no reaction against antiserum to strain 1202. This strain reacted only with homologous antiserum. Nineteen strains, S8V-B to 854, were then inhibited by antisera to the 3 caprine strains, G3V-A, G10V and G39V-B. Strains S8V-A and 1202, however did not react with any of the 3 caprine antisera. Therefore, S8V-A was found to have an antigenicity cross-reacting one-way with the caprine strains. Thus, the 21 ovine strains tested were divided serologically into one group consisting of 20 strains and one distinct strain.

The result of the metabolism inhibition test is given in Table 7. The ovine strains were separated into one serological group and one unique strain. All of them, except strain 1202, had the same antigenicity as the caprine strains.

These results indicate that there are at least 2 antigenically distinct serogroups represented by strains S8V-A and 1202, respectively, in the ovine ureaplasmas.

Serological relationship of caprine and ovine ureaplasmas to those from the other animal species

Caprine strain G10V and ovine strains S8V-A and 1202 were tested by the metabolism inhibition test with antisera to human, bovine, canine, simian and avian strains (Table 8). These strains had no serological relationship with any of the reference strains from the other host species.

Table 7. Serological comparison between ovine and caprine ureaplasmas by metabolism inhibition test

Ovine strain	Geographic origin	Antiserum against				
		Ovine		Caprine		
		S8V–A	1202	G3V–A	G10V	G39V–B
S8V–A	Japan	≥40960*	—**	640	640	640
S8V–B		20480	—	2560	≥40960	5120
S10P–A		10240	—	2560	10240	5120
S10P–C		10240	—	10240	10240	1280
S11V–A		10240	—	10240	20480	20480
S11V–B		20480	—	10240	2560	5120
1611♯1	Canada	5120		640	5120	1280
1659♯3		20480	—	1280	10240	10240
1655♯1		10240	—	1280	10240	2560
1692♯1d18		10240	—	1280	10240	2560
1648♯1		20480	—	2560	20480	2560
1692A		1280	—	640	640	10240
1662♯1		10240	—	1280	10240	10240
596	U. S. A.	10240	—	5120	10240	5120
888		20480	—	320	640	20480
823		10240	—	5120	5120	10240
624		320	—	2560	640	640
961		1280	—	1280	20480	5120
861		2560	—	1280	5120	2560
854		1280	—	2560	20480	5120
1202		40	≥40960	40	—	—

* Reciprocal of the highest dilution of serum showing metabolism inhibition.
** No inhibition at the dilution of 1 : 20.

Discussion

Ureaplasmas have been isolated from the urogenital tract of goats in England [4], the United States [18] and Japan [21], and from the same tract of sheep in the United States [18, 19] and Canada [1]. Spradbrow and Marley [29] in Australia reported the isolation of organisms resembling ureaplasmas from the conjunctival sac of sheep with kerato-conjunctivitis and normal sheep. They could not demonstrate, however, the urease activity of these isolates. Therefore, the organisms remained unrecognized correctly as ovine ureaplasmas. In the present study, ureaplasmas were isolated only from two anatomical regions, the prepuce and vagina, of each of the 2 animal species. As shown previously, however, they have been isolated from a variety of anatomical sites, such as the respiratory and urogenital tracts of man [24, 34], cattle [3, 34, 36], dogs [14, 21], cats [6, 32, 33] and monkeys [7, 11, 15]. Recently, Koshimizu et al. (personal communication) have mentioned that these organisms were isolated from the oral cavity of a goat kept in their laboratory. So, the authors' failure in isolating these organisms from any other site of these animals may be due to their infrequent occurrence or absence. It seems that the frequency of their isolation may reflect the difference in herd in which animals were maintained. In the future, a more extensive survey on these animals is needed to verify this difference.

Several investigators [16, 25, 37, 38] de-

Table 8. Serological relationships of ureaplasmas of caprine and ovine origins to those of human, bovine, canine, simian and avian origins by metabolism inhibition test

Antiserum		Origin	Homologous titer	*Ureaplasma* strain		
Strain	Serotype			Caprine G10V	Ovine S8V–A	Ovine 1202
No. 7	I	Human	10240*	—**	—	—
No. 23	II		2560	—	—	—
No. 27	III		20480	—	—	—
No. 58	IV		20480	—	—	—
No. 354	V		10240	—	—	—
Pirillo	VI		10240	—	—	—
Cook	VII		10240	—	—	—
T-960	VIII		10240	—	—	—
C13		Bovine	10240	40	80	—
C24			10240	—	—	—
C23			≥40960	—	—	—
C17			5120	—	—	—
C12			≥40960	—	—	—
C7			≥40960	—	—	—
T44			≥40960	—	—	—
D1M–C		Canine	10240	—	—	—
D29M			20480	—	—	—
D11N–A			≥40960	—	—	—
D6P–C			≥40960	—	—	—
T167–2		Simian	2560	—	—	—
D6–1		Avian	10240	—	—	—

* Reciprocal of the highest dilution of serum showing metabolism inhibition.

** No inhibition at the dilution of 1 : 20.

scribed that the presence of thallium acetate in medium inhibited the growth of ureaplasmas, and that this chemical should not be added to primary cultures. The authors also found that the concentration of this chemical used had an initial inhibitory effect to induce delay in growth. The final titers of those organisms, however, did not significantly change with or without thallium acetate. The addition of this antibacterial agent usually inhibited the growth of fungi.

In the specimens as mentioned above, *Acholeplasma* was also isolated from the oral cavity, nasal cavity, eye and urogenital tract of goats, but not from any organ of sheep.

Livingston et al. [18] found previously in the mycoplasmacidal test that 2 ovine and a caprine ureaplasmas were serologically distinct. Recently, they distinguished 9 serological groups by the same test in 19 urogenital isolates from sheep [19]. In the present study by 2 serological methods, 23 caprine strains, including 2 from England, belonged to a serologically similar group, and all the ovine strains that had been isolated from sheep in Japan, Canada and the United States formed one group, except one strain which was antigenically distinguished from the others. Moreover, these 2 groups of ureaplasmas from goats

and sheep were found to have a common antigen to each other.

This finding suggests that caprine and ovine ureaplasmas may be classified into one and the same group. It seems to support the result that caprine and ovine strains had the guanine- plus- cytosine contents of deoxyribonucleic acids which were similar within the range from 31.6 to 30.6 mol% [10]. The results of the present investigation seem to indicate that the caprine ureaplasmas may be mostly homogeneous antigenically, but that the ovine ureaplasmas have heterogeneous antigens as stated previously [19], as well as the human [27], bovine [8, 9, 20] and canine [14] ones. Of the 9 ovine serotypes which had been identified by Livingston et al. [19] by the mycoplasmacidal test, 6 reacted with 3 caprine antisera as well as one ovine antiserum, in the growth inhibition and metabolism inhibition tests. They belonged to one serogroup. Reference strain 854 which had been reported as a multiple reactor to the established serotypes [19] was included in this serogroup. Thus, it is presumed that the mycoplasmacidal serotyping technique may be more sensitive than the growth inhibition and metabolism inhibition tests, and that it may reveal the presence of multiple serogroups in ureaplasmal isolates.

As for the relationship between disease and serotype, Shepard and Lunceford [26] reported that a specific serotype of *U. urealyticum* was associated most frequently with some human disorders of the urogenital tract. Ovine strain 1202 was reported by Livingston et al. [19] to be associated with such disorders as abortion and infertility. In the present study, it is of interest to note that this strain was distinguished antigenically from the other ovine strains studied. This result may serve as a new piece of information on the epizootiology of ovine diseases associated with ureaplasmas.

Caprine and ovine ureaplasmas had no antigenical relationship to those originated from man, cattle, dogs, monkeys or birds. These results suggested that those ureaplasmas might be independent serologically from the other members of the genus *Ureaplasma*. The finding, however, that caprine and ovine ureaplasmas have a serological homogeneity is an important evidence. It suggests that since ureaplasmas usually exhibit a strict host-specificity, those originated from goats and sheep may be an exception of this property.

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要　約

ヤギ，ヒツジからの *Ureaplasma* の分離および血清学的比較：小谷　均・永友寛司・尾形　学（東京大学農学部家畜微生物学教室）——外見上健康なヤギ62頭（3飼育場）およびヒツジ11頭（1飼育場）の口腔，鼻腔，眼，直腸，包皮，膣からの *Ureaplasma* の分離をおこなった．*Ureaplasma* は，ヤギの包皮（6/16, 37.5%），膣（18/46, 39.1%）から，ヒツジの包皮（1/5, 20%），膣（2/6, 33.3%）から分離されたが，その他の検索部位からは分離されなかった．発育阻止試験ならびに代謝阻止試験により，分離株の性状を血清学的に比較したところ，ヤギ由来23株は単一の血清群を形成し，ヒツジ由来21株は20株を含む1群と，それとは抗原的に異なる1株に分かれた．さらに，ヤギおよびヒツジ由来の両血清群は共通抗原を持つことが判明した．ヒツジ由来の特異な1株は，ヤギ由来株とも血清学的に異なっていた．一方，ヤギおよびヒツジ由来 *Ureaplasma* は，ヒト，ウシ，イヌ，サルならびにトリ由来 *Ureaplasma* と血清学的関係が認められなかった．ヤギとヒツジ由来株が抗原的類似性を示したことは，ヤギおよびヒツジにおいて，*Ureaplasma* の宿主特異性は例外であることを示唆している．