BRIEF NOTE

Isolation of Ureaplasma (T-Mycoplasma) from the Chicken and Jungle-Fowl

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Ureaplasma (T-mycoplasma) was first isolated from the urogenital tract of men by Shepard [10] in 1954, and thereafter, isolation of this organism from various species of mammals such as the cattle [2, 6, 9, 11, 13, 15], dog [14], cat [12], subhuman-primates [1, 4, 5, 14], sheep [7] and goat [3, 7] has been reported. As yet, there is no report of demonstration of Ureaplasma in birds. Attempts have recently been made at this laboratory to isolate Ureaplasma from the oropharynx and cloaca of various gallinaceous birds, with the results demonstrating isolation of the microorganism from White Leghorn chickens (*Gallus gallus* var. *domesticus*) and from a red jungle fowl (*Gallus gallus*). This report presents the results including the findings noted as to characteristics of these isolates.

A total of 21 birds of nine different species, i.e. 9 White Leghorn chickens brought in this institute to be used as laboratory animals and 12 gallinaceous birds of various species maintained at the Tama Zoological Park, Tokyo, was studied (Table 1).

The media employed for ureaplasma isolation were a liquid medium composed

of 70 ml of PPLO broth (Difco), 20 ml of horse serum, 10 ml of 25% (w/v) yeast extract (Nitten Co.), 1,000 units of penicillin G per ml, 0.5 ml of 10% urea and 0.002% phenol red, with the pH adjusted to 6.0 ± 0.2 using 10% hydrochloric acid, and a solid medium consisting of same ingredients except that 66 ml of PPLO broth (Difco), 0.5 ml of 2.5% thallium acetate, 2.5 ml of phosphate buffer (1.5 M KH_2PO_4 : 1.5 M $\text{Na}_2\text{HPO}_4 = 7 : 3$), and 1.2% Special Agar Noble (Difco), with the pH adjusted to 6.5. To collect mucus specimens for ureaplasma isolation, sterile cotton swabs were passed over the oropharyngeal parts or into the cloaca of birds, and cultures were made in the liquid and on the solid media. The inoculated broth cultures were observed for a week, and tubes showing a significant rise in pH with the color of indicator turned from yellow to red or purplish red were subcultured. At the same time, a tenfold dilution of each broth culture showing a rise in pH was inoculated onto plates of solid medium, a drop to each plate, which were incubated at 37°C for 3 days in 90% N_2 and 10% CO_2 .

As can be seen from Table 1, of the 21

短報: ニワトリおよび野鶏からの Ureaplasma (T-mycoplasma) の分離: 興水 馨・曲淵輝夫 (東京大学医学部附属動物実験施設)

Table 1. Isolation of ureaplasmas from gallinaceous birds

Bird's name	No. examined	Isolation of organism	
		Oropharynx	Cloaca
White Leghorn chicken (<i>Gallus gallus</i> var. <i>domesticus</i>)	9	3	0
Japanese bantam (<i>Gallus gallus</i> var. <i>domesticus</i>)	1	0	0
Red jungle fowl (<i>Gallus gallus</i>)	1	1	0
Ceylon jungle fowl (<i>Gallus lafayetii</i>)	1	0	0
Green pheasant (<i>Phasianus versicolor</i>)	3	0	0
Vieillot's crested fireback (<i>Lophura ignita rufa</i>)	1	0	0
True silver pheasant (<i>Lophura nycthemerus</i>)	1	0	0
Indian peafowl (<i>Pavo cristatus</i>)	1	0	0
Lady Amherst's pheasant } Golden pheasant } (<i>Chrysolophus amherstiae</i>) * (<i>Chrysolophus pictus</i>) }	1	0	0
Guinea fowl (<i>Numida galeata</i>)	2	0	0
Total	21	4	0

Remarks.

*: Mixed breed of two species.

birds examined, ureaplasmas were isolated from the throat of 3 White Leghorn chickens and 1 red jungle fowl but none from the cloaca. The Mycoplasma-like colonies formed on the solid medium comprised two distinct types of colonies which were intermixed in distribution, i.e. the so-called fried-egg-shaped, large colonies and tiny colonies possibly of Ureaplasma (Fig. 1). Individual colonies of the latter type measured about 58 μ m in diameter. Biological and serological properties of eight strains cloned from these fine colonies were investigated. None of these strains were converted into bacterial form after 3 passages in liquid medium devoid of bacterial inhibitors. They also failed to grow in serum-free media and proved to actively utilize urea within 24 hours of incubation but not glucose or arginine.

Metabolism inhibition tests [8] conducted to investigate serological characteristics of the isolates revealed no antigenic relationship to Black's serotypes I through VIII which were the ureaplasma type strains from human clinical specimens, Strains T167-1, T167-2 and T163 isolated from subhuman-primates, or Strain C2 from the cattle.

Ecological or etiological significance of these ureaplasmas isolated from birds is entirely unknown and yet to be clarified. Characteristics of the large colony-forming mycoplasmas isolated concomitantly with Ureaplasma will be described elsewhere.

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Table 2. Serological relationship of isolated strains to human, simian and bovine ureaplasmas as determined by metabolism inhibition test

Antisera		Origin	Homologous titers	Ureaplasmas isolated from gallinaceous birds							
Strain	Serotype			D6-1	D6-2	D7	D8-1	D8-2	D8-3	T9-1	T9-2
No. 7	Black I	Human	>20,480*	—**	—	—	—	—	—	—	—
No. 23	II		1,280	—	—	—	—	—	—	—	—
No. 27	III		>20,480	—	—	—	—	—	—	—	—
No. 58	IV		10,240	—	—	—	—	—	—	—	—
No. 354	V		>20,480	—	—	—	—	—	—	—	—
Pirillo	VI		>20,480	—	—	—	—	—	—	—	—
Cook	VII		>20,480	—	—	—	—	—	—	—	—
T-960	VIII		>20,480	—	—	—	—	—	—	—	—
T167-1		Simian	1,280	—	—	—	—	—	—	—	—
T167-2			10,240	—	—	—	—	—	—	—	—
T163			>20,480	—	—	—	—	—	—	—	—
C2		Bovine	>20,480	—	—	—	—	—	—	—	—

Remarks.

*: Reciprocal of the highest dilution of serum showing metabolic inhibition.

**: No inhibition at the dilution of 1:40.

bovine type strains, and to Dr. K. Tashiro, of the Tama Zoological Park, for sampling materials examined.

References

- [1] Brown, W. J., Jacobs, N. F., Jr., Arum, E. S., and Arko, R. J. (1976). *Lab. Anim. Care*, **26**, 81-83.
- [2] Gourlay, R. N. (1968). *Res. vet. Sci.*, **9**, 376-378.
- [3] Gourlay, R. N., Brownlie, J., and Howard, C. J. (1973). *J. gen. Microbiol.*, **76**, 251-254.
- [4] Koshimizu, K., Magaribuchi, T., Yamamoto, K., and Ogata, M. (1975). *Exp. Anim.*, **24**, 95-101.
- [5] Kundsins, R. B., Rowell, T., Shepard, M. C., Parreno, A., and Lunceford, C. D. (1975). *Lab. Anim. Sci.*, **25**, 221-224.
- [6] Livingston, C. W. (1972). *Amer. J. vet. Res.*, **33**, 1925-1929.
- [7] Livingston, C. W., and Gauer, B. B. (1975). *Amer. J. vet. Res.*, **36**, 313-314.
- [8] Purcell, R. H., Taylor-Robinson, D., Wong, D., and Chanock, R. M. (1966). *J. Bacteriol.*, **92**, 6-12.
- [9] Ruhnke, H. L., and Van Dreumel, A. A. (1971). *Canad. J. comp. Med.*, **36**, 317-318.
- [10] Shepard, M. C. (1954). *Amer. J. Syph. Gonorr. ven. Dis.*, **38**, 113-124.
- [11] Shimizu, T., Nosaka, D., and Nakamura, N. (1975). *Jap. J. vet. Sci.*, **37**, 121-131.
- [12] Tan, R. J. S., and Markham, J. G. (1971). *Jap. J. exp. Med.*, **41**, 247-248.
- [13] Taylor-Robinson, D., Haig, D. A., and Williams, M. H. (1967). *Ann. N. Y. Acad. Sci.*, **143**, 517-518.
- [14] Taylor-Robinson, D., Martin-Bourgon, C., Watanabe, T., and Addey, J. P. (1971). *J. gen. Microbiol.*, **68**, 97-107.
- [15] Yamamoto, K., Harasawa, R., Ogata, M., Miura, T., and Nakane, H. (1976). *Jap. J. vet. Sci.*, **38**, 7-14.

Ureaplasma and Mycoplasma colonies from fowl origin,
developing on solid medium.

Arrows a and b show classical Mycoplasma colony and
Ureaplasma one, respectively.