Isolation of *Mycoplasma meleagridis* from Turkeys in Japan

Takamasa SHIMIZU

Division of Veterinary Science, Faculty of Agriculture, Miyazaki University,
3-210 Funazuka, Miyazaki-shi, Miyazaki 880

Takeshi YAGIHASHI

Nippon Institute for Biological Science, 2221-1 Shin-machi, Ome-shi, Tokyo 198

(Received for publication June 29, 1979)

Abstract. Serological and biochemical studies were performed with 86 *Mycoplasma* strains isolated from airsacculitis, trachea, eye cavity, oropharynx and cloaca of a total of 50 turkeys raised in two farms in Miyazaki. In addition, 4 strains originating from the oropharynxes of 2 out of 3 turkeys raised in a farm in Tokyo and affected with airsacculitis were examined. Forty-six strains from Miyazaki and one strain from Tokyo were identified as *Mycoplasma meleagridis*. The remainder, 43 strains, was serologically identified as *M. gallinarum* (4 strains), serogroup D (5), serogroup I (18) and unidentified (16). Biochemically, *M. meleagridis* was characterized by being positive for the production of phosphatase and breakdown of arginine. Contamination with *M. meleagridis* among turkeys reared in many parts of Japan is suggested.

Mycoplasma meleagridis [16] was isolated for the first time from airsacculitis of turkey poult by Adler et al. [1] under the designation of N-strains. Since then, isolation of the same species has been reported from many parts of the world where abundant turkeys are raised [6, 9, 10–12, 18]. In Japan, however, no research for the infection with *M. meleagridis*, one of the important pathogens for respiratory diseases in turkey poult [2, 6–9, 11, 12, 15, 17, 19], has hitherto been performed. This paper describes the results of isolation and characterization of *M. meleagridis* and other mycoplasmas from turkeys raised in this country.

Materials and Methods

Turkeys: Five Broad-breasted Small White (Cannon) turkeys at 3 months of age suffering from airsacculitis and died of heat-attack as well as 5 normal farm-mates in Farm A in Miyazaki were subjected to the isolation of mycoplasmas and ureaplasmas. Eye cavities, oropharynxes and cloaca of 40 healthy turkeys of the same race raised in another farm

(Farm B) in Miyazaki and slaughtered at their 7 months of age were also examined for *Mycoplasma*-tales. Twenty-two air sac samples, 6 of which exhibited signs of airsacculitis such as exudate and thickening whereas the remainder, 16, was normal, and 40 tracheal samples were taken after the carcasses were soaked in hot water for tearing the feather off and then processed for meat. In addition, three 70-day-old Cannon turkeys raised in Farm C in Tokyo and affected with airsacculitis were involved in this study.

Media: The media used for isolation and cultivation of mycoplasmas were composed of 700 ml of PPLO-broth or PPLO-agar (Difco), 100 ml of 25% fresh yeast extract (Nitten), 100 ml each of horse and swine serums, 2 ml each of 1% diphosphopyridine nucleotide (Sigma) and 1% *l*-cystein-HCl, 5 ml of 5% thallium acetate and 125 mg of ampicillin. For the isolation of ureaplasmas, liquid medium of Taylor-Robinson et al. [14] was used, and solid medium of the same components was supplemented with 2.5 v/v% of a mixture of 7 volumes of 1.5 M KH_2PO_4 and 3 volumes of 1.5 M Na_2HPO_4 solutions.

Isolation: A small piece of either exudate from airsacculitis or air sac and tracheal mucosa was inoculated into liquid medium or streaked on the surface of a solid medium. Swab samples of eye

cavities, oropharynxes and cloaca were directly distributed on the solid medium. Inoculated liquid medium was incubated aerobically at 37°C, and an aliquot of the culture was distributed on a solid medium 2 to 3 days post-inoculation. The inoculated plates for ureaplasmas were incubated in jars in which the air was replaced by 95% N₂ and 5% CO₂. All of the plates were incubated at 37°C for 10 days before they were judged as negative for *Mycoplasma* species.

Cloning of the isolates: At least two colonies were picked from an isolation plate inoculated with each material. These colonies were subjected to cloning by means of a single-colony picking repeated three times. A total of 90 strains thus collected were examined for their biochemical and serological properties.

Biochemical tests: The requirement for cholesterol was determined indirectly by the test for susceptibility to 1.5% digitonin (Merck and Co.) as described by Freundt et al. [4]. Other biochemical properties were examined by the methods described in a previous paper [13].

Serological identification: All of the 90 strains of mycoplasmas were subjected to the growth inhibition test (GIT) performed by the well method

of Black [3] by using antisera against avian mycoplasma reference strains recorded in Table 1 which had been prepared in rabbits and used in a previous study [13]. Strains not identified by the GIT were subjected to the metabolism-inhibition test (MIT) by the method described elsewhere [13].

Detection of serum agglutinin: Two strains of *M. meleagridis*, namely, TK-1, a new isolate from airsacculitis, and 886, the reference strain, were grown on the surface of solid medium for 3 days. The plates were then flooded with phosphate buffered saline (PBS, pH 7.0), and mycoplasma colonies were harvested. The suspension was centrifuged at 7,500 rpm for 30 minutes. The deposit of cells was washed twice in PBS and finally suspended in PBS so as to give a turbidity equivalent to ten times concentration of MacFarland tube No. 1. Antigen of strain O2, an isolate identified as serogroup I, was prepared by the same manner. Besides, commercial diagnostic antigens of *M. gallisepticum* and *M. synoviae* (Kitasato Institute) were used. A rapid plate agglutination of these antigens was tested with serum samples taken from 32 turkeys from Farm B at slaughter.

Results

Isolation and identification of mycoplasmas

Mycoplasmas were isolated from all of the 5 airsacculitis together with *Escherichia coli*. Three each of tracheal and cloacal samples and one each of eye and oral swabs of 5 dead poult were also positive for mycoplasmas. The same organisms were detected from an oropharynx and 3 cloacal swabs of 5 normal turkey poult reared in Farm A. Of 40 healthy turkeys raised in Farm B, 6 (15%) oropharynxes and 18 (45%) cloacal samples, but none of 40 pairs of eye cavities, were positive for mycoplasmas (Table 2). Twenty-two airsacs and 40 tracheas taken from 40 turkeys in Farm B after they were slaughtered and processed for meat were proved to be negative for mycoplasmas. No *Ureaplasma* was isolated from these materials mentioned above.

Three additional turkeys affected with airsacculitis were examined in Tokyo, and mycoplasmas were isolated from one each

Table 1. Avian mycoplasmas used for preparation of rabbit antisera

Species or serogroup	Strain	Source*
<i>M. meleagridis</i>	886	MLF
<i>M. gallisepticum</i>	801	MLF
<i>M. synoviae</i>	WVV1853	MLF
<i>M. gallinarum</i>	862	MLF
<i>M. iners</i>	640	MLF
<i>M. anatis</i>	1340	SC
<i>M. columbinum</i>	MMP1	TS
<i>M. columborale</i>	MMP4	TS
Group C	856	MLF
Group D	8	MLF
Group F	SA	MLF
Group I	695	MLF
	693	MLF
	1855	MLF
	PHN-D13	MLF
	L3-10	MLF
Group L	694	MLF
Group O	TC5	MLF
Group P	TC3	MLF

* MLF=M. L. Frey, Iowa State University, Ames, Iowa; SC=Sylvia Cunningham, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland; TS=the senior author, Miyazaki University, Miyazaki.

Table 2. Results of isolation of mycoplasmas from various sites of turkeys

Prefecture	Farm	Clinical sign of turkey	No. of turkeys examined	Site tested and No. positive for mycoplasmas				
				Air sac	Trachea	Eye	Oropharynx	Cloaca
Miyazaki	A	Dead with airsacculitis	5	5	3	1	1	3
		Healthy	5	NT	NT	NT	1	3
	B	Healthy	40	0/22*	0*	0	6 (15%)	18 (45%)
Tokyo	C	Airsacculitis	3	1	1	NT	2	NT

NT: not tested. * Samples were taken after the meat processing of turkeys.

Table 3. Serological identification of mycoplasmas from turkeys

Prefecture	Source	Farm	No. of strains	<i>M. meleagridis</i>		<i>M. gallinarum</i>	Group D GIT	Group I GIT	Not identified
				MIT	GIT	GIT			
Miyazaki	Airsacculitis	A	10	10	(5)	—	—	—	—
	Trachea	A	6	6	(4)	—	—	—	—
	Eye cavity	A	2	2	(2)	—	—	—	—
	Oropharynx	A	4	4	(1)	—	—	—	—
		B	18	6	(1)	—	—	7	5
	Cloaca	A	10	3	(2)	—	5	2	—
		B	36	15	(2)	4	—	6	11
	Total		86	46	(17)	4	5	15	16
Tokyo	Oropharynx	C	4	1	(1)	—	—	3	—
Grand total			90	47	(18)	4	5	18	16

MIT, metabolism inhibition test; GIT, growth inhibition test.

of air sac and trachea as well as from 2 oropharynxes (Table 2).

A total of 86 cloned isolates were obtained in Miyazaki, while 4 strains originating from oropharynxes of turkeys in Farm C in Tokyo were subjected to the identification. All of the 90 strains thus collected required serum for growth and were sensitive to digitonin and incapable of decomposing urea; accordingly, they were classified into genus *Mycoplasma*.

Results of serological identification of these strains are given in Table 3. Forty-seven strains including one from Tokyo were identified as *M. meleagridis* by the MIT. Confirmation of these results by the GIT was possible with only 18 of the 47 strains, the remainder, 29 strains, being in-

capable of growing so markedly as to form lawn on the solid medium. Both *M. gallinarum* and serogroup D mycoplasmas were detected only from the cloacal samples, whereas mycoplasmas belonging to serogroup I, or serogroup I-J-K-N-Q-R, were isolated from oropharynxes and cloacal swabs. Sixteen strains have been remained unidentified.

Colonial and biochemical properties of the isolates

Colonies of *M. meleagridis* were flat and lack of definite central growth (Fig. 1), in contrast to those of other species or serogroups (Figs. 2-4), at the initial isolation and subsequent passages for more than 15 generations. Main biochemical properties

Table 4. Main biochemical properties of *M. meleagridis* and other avian mycoplasmas

Species or serogroup	No. of strains tested	Breakdown of arginine	Breakdown of glucose	Phosphatase production	Film-spots formation	Colonial adsorption of chicken red cells	Transfer in liquid medium	Requirement of DFN for growth
<i>M. meleagridis</i>	46	+	—	+	—	—	d	—
<i>M. gallinarum</i>	5	+	—	—	+	—	e	—
<i>M. iners</i>	2	+	—	—	v	—	e	—
<i>M. gallisepticum</i>	3	—	+	—	—	+	e	—
<i>M. synoviae</i>	3	—	+	—	+	+	e	+
<i>M. anatis</i>	1	—	+	+	+	—	e	—
<i>M. columbinum</i>	18	+	—	—	+	—	e	—
<i>M. columborale</i>	32	—	+	—	—	—	e	—
Serogroup C	1	—	+	—	—	—	e	—
Serogroup D	6	—	+	—	—	—	e	—
Serogroup F	1	—	+	—	—	—	e	—
Serogroup L	1	+	—	—	+	—	e	—
Serogroup I	21	+	+	—	—	—	e	—

d, difficult; e, easy to perform; v, variable.

of the new isolates and other avian mycoplasmas were examined, and the results obtained are shown in Table 4. *M. meleagridis* was biochemically characterized by being positive for phosphatase production and breakdown of arginine, and negative for both decomposing glucose and formation of film and spots. It should be noted that repeated transfers in the liquid medium of *M. meleagridis* have been difficult, and that they usually required much longer period of incubation before decomposing arginine at the initial tests than other species of mycoplasmas did.

Detection of serum agglutinin

Agglutinin against strain TK-1 of *M. meleagridis* was positive for 18 of 32 turkey serum samples with titers ranging from 1:1 to 1:16. With antigen of strain 886 of the same species, agglutinin was detected in only 12 of the 18 sera positive with the

former strain, with the maximum titer of 1:4. No positive agglutinin was detected against antigen of either *M. gallisepticum*, *M. synoviae*, or strain O2 of serogroup I.

Discussion

In Japan, turkey-raising farms have been increasing only for these years, and this is one of the main reason why research on the *Mycoplasma* infection among turkeys has not been carried out previously.

In the present study, infection with *M. meleagridis* was detected among turkeys reared in Miyazaki as well as in Tokyo. Both of the two farms in Miyazaki had purchased day-old poults from a hatchery in Tokyo from which turkey poults have been supplied to many parts of Japan. Both farms in Miyazaki were completely isolated from the residential area and from each other, and no farms raising either turkeys or chickens located within the areas in

which they were situated. Under these circumstances, there was little possibility of the inter-farm transmission of *M. meleagridis*, and, therefore, introduction of infected poultts would have resulted in the intra-flock distribution of this organism. If this is the case, turkey-raising farms in various parts of Japan may have been contaminated with *M. meleagridis*.

At any rate, *M. meleagridis* was revealed to parasitize in various sites of turkeys, as has been reported by the previous workers [6, 10, 18].

The role of *M. meleagridis* in airsacculitis of turkey poultts has been confirmed [2, 7-9, 11, 12, 15, 17, 19]. The lesion in the air sac of the infected turkey poultt has been reported to be similar to that described for infection with *M. gallisepticum* and *M. synoviae* but less severe [2, 12]. In the present study, neither isolation nor serological examination proved association of these two *Mycoplasma* species. Occurrence of genital infection and transmission to nest eggs of *M. meleagridis* have been indicated [7, 8]. Although differences in pathogenicity among strains have also been reported [5], strains originating from oviduct and semen of turkeys produced airsacculitis in the poultts after artificial inoculation [15]. Consequently, examination of cloaca and oral cavity as well as upper respiratory tract of the turkey for mycoplasmas seems to be useful for detecting a flock contamination with this organism [8, 18].

Among the avian mycoplasmas examined in the present study, *M. meleagridis* was the only species, with an exception of *M. anatis* strain 1340, that was positive for the production of phosphatase. These two species could be discriminated from each other in that the former was positive for arginine breakdown but negative for decomposing glucose and formation of film

and spots, whereas the latter was positive for glucose breakdown and film and spots formation but negative for arginine breakdown. Thus, test for the production of phosphatase seems to be useful for the screening of *M. meleagridis* at the initial isolation.

References

- [1] Adler, H. E., Fabricant, J., Yamamoto, R., and Berg, J. (1958). Isolation and identification of new serotype "N" from turkeys. *Am. J. Vet. Res.* 19, 440-447.
- [2] Arya, P. L., Asutler, J. H., and Pomeroy, B. S. (1971). Pathogenesis and histopathology of air-sacculitis in turkeys produced by experimental inoculation of day-old poultts with *Mycoplasma meleagridis*. *Avian Dis.* 15, 163-176.
- [3] Black, F. T. (1973). Modification of the growth inhibition test and its application to human T-mycoplasmas. *Appl. Microbiol.* 25, 528-533.
- [4] Freundt, E. A., Andrews, B. E., Ernø, H., Kunze, M., and Black, F. T. (1973). The sensitivity of *Mycoplasma* species to sodium polyanethol sulfonate and digitonin. *Zentralbl. Bakteriologie, Parasitenkunde, Infektionskrankheiten, Hygiene, I, Original* 225, 104-112.
- [5] Ghazikhanian, G., and Yamamoto, R. (1974). Characterization of pathogenic and nonpathogenic strains of *Mycoplasma meleagridis*: *In ovo* and *in vitro* studies. *Am. J. Vet. Res.* 35, 425-430.
- [6] Jordan, F. T. W., Rashid, R. A., and Uthman, A. U. (1978). The recovery of mycoplasma from the avian oesophagus. *Vet. Rec.* 102, 403-404.
- [7] Matzer, N., and Yamamoto, R. (1970). Genital pathogenesis of *Mycoplasma meleagridis* in virgin turkey hens. *Avian Dis.* 14, 321-329.
- [8] Mohamed, Y. S., and Bohl, E. H. (1967). Studies on the transmission of *Mycoplasma meleagridis*. *Avian Dis.* 11, 634-641.
- [9] Mohamed, Y. S., and Bohl, E. H. (1968). Serological studies on *Mycoplasma meleagridis* in turkeys. *Avian Dis.* 12, 554-566.
- [10] Mohamed, Y. S., Chema, S., and Bohl, E. H. (1966). Studies on *Mycoplasma* of the H serotype (*M. meleagridis*) in the reproductive and respiratory tracts of turkeys. *Avian Dis.* 10, 347-352.
- [11] Rhoades, K. R. (1971). *Mycoplasma mele-*

- gridis* infections: Development of lesions and distribution of infection in turkey embryos. *Avian Dis.* 15, 910-922.
- [12] Rosenfeld, L. E., and Grimes, T. M. (1972). Natural and experimental cases of airsacculitis associated with *Mycoplasma meleagridis* infection in turkeys. *Aust. Vet. J.* 48, 240-243.
- [13] Shimizu, T., Ernø, H., and Nagatomo, H. (1978). Isolation and characterization of *Mycoplasma columbinum* and *Mycoplasma columborale*, two new species from pigeons. *Int. J. Syst. Bacteriol.* 28, 538-546.
- [14] Taylor-Robinson, D., Haig, D. A., and Williams, M. H. (1967). Bovine T-strain mycoplasma. *Ann. New York Acad. Sci.* 143, 517-518.
- [15] Yamamoto, R., and Bigland, C. H. (1965). Experimental production of airsacculitis in turkey poults by inoculation with the "N" type mycoplasma. *Avian Dis.* 9, 108-118.
- [16] Yamamoto, R., Bigland, C. H., and Ortmyer, H. B. (1965). Characteristics of *Mycoplasma meleagridis* sp. n., isolated from turkeys. *J. Bacteriol.* 90, 47-49.
- [17] Yamamoto, R., and Ortmyer, H. B. (1966). Pathogenicity of *Mycoplasma meleagridis* for turkey and chicken embryos. *Avian Dis.* 10, 268-272.
- [18] Yamamoto, R., Ortmyer, H. B., Bigland, C. H., Seely, M. A., and Corstvet, R. E. (1965). Isolation of "N" mycoplasma from different sites of the turkey. *Poult. Sci.* 44, 732-736.
- [19] Yoder, H., Jr., and Hofstad, M. S. (1964). Characterization of avian mycoplasma. *Avian Dis.* 8, 481-512.

要 約

本邦の七面鳥からの *Mycoplasma meleagridis* 分離：清水高正（宮崎大学農学部），八木橋 武（日本生物科学研究所）——宮崎県下2か所の飼育場における七面鳥計50羽の気嚢炎・気管・眼窩・口腔・総排泄孔から，総計86株のマイコプラズマが分離された。これらは血清学的に，*M. meleagridis* 46株，*M. gallinarum* 4株，D群5株，I群15株に同定され，16株は同定不能であった。さらに東京都下の一飼育場の3羽中2羽の七面鳥の口腔から分離されたマイコプラズマ4株中1株は *M. meleagridis*，3株はI群と同定された。*M. meleagridis* はフオスファターゼ産生能とアルギニン分解能が陽性で，これらの性状は他の鳥類由来マイコプラズマとの識別上重要である。ひなの流通経路から，*M. meleagridis* は本邦において広範囲に飼育場を汚染していることが推定される。

Explanation of Figures

Figures exhibit colonies of the new isolates of turkey mycoplasmas grown on the solid medium at 37°C for 3 days. Enlarged approximately 30 times of the original size.

- Fig. 1. *M. meleagridis* TK-1.
 Fig. 2. *M. gallinarum* 19B.
 Fig. 3. Strain TK-11 (Serogroup D).
 Fig. 4. Strain O2 (Serogroup I).