

Mycoplasma synoviae Infection in Chickens

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Disease caused by *Mycoplasma synoviae*, so far called infectious synovitis, shows symptoms such as joint swelling, lameness, pale comb, and retarded growth mainly of chicken and turkey. This disease, first noticed in a broiler raising area of U.S.A., was reported by Olson et al. and Wills in 1954. Since then the disease has been recognized in European countries and South Africa¹⁾. In Japan, although the occurrence of synovitis caused by this mycoplasma in field-raising chickens was not yet confirmed, two strains of *M. synoviae* were isolated from the material taken from a chicken processing plant in Kyushu district by Shimizu et al. (1971)²⁾.

On the other hand, Yoder (1970)³⁾ made clear that frequent occurrence of airsacculitis in the broiler flocks, not being infected by *M. gallisepticum*, in U.S.A. was a result of the infection of *M. synoviae*. At that time, number of broilers condemned at processing plants due to airsacculitis was reported to be 8-25%, and the antibody survey of breeding flock found out that about a half of the breeding flock showed positive reaction at a rate of as high as 80-90%.

As to the factors inducing airsacculitis by *M. synoviae*, it was experimentally proved that the airsacculities was caused when the chickens treated with the live-vaccine of infectious bronchitis or the combined live-vaccine of infectious bronchitis and New castle disease (ND) were subjected to aerosol-infection of *M. synoviae*²⁾. It was also known that the extent of causing airsacculitis and synovitis were different by strains of *M. synoviae*.

Thus, the recent trend is, with regard to the disease caused by *M. synoviae*, that more

interest is given in airsacculitis than in original infectious synovitis.

In Japan, although there seems to be no occurrence of synovitis as stated above, it is considered that the respiratory tract infection may exist as in U.S.A. In order to examine this aspect, study on method of serological diagnosis practically applicable was carried out. In the present paper, practical usefulness of the diagnostic antigen prepared for the test and isolation of *M. synoviae* from the respiratory tracts of field-raising chickens will be described briefly.

Serological reaction of *M. synoviae*

Olson et al. (1963)⁴⁾ established the agglutination reaction by means of plate test, and tube test and Vardaman & Yoder (1968)⁵⁾ developed the haemoagglutination inhibition test (HI test). In U.S.A. the antigen is on the market, but it is not manufactured in Japan.

The author, with co-workers, has prepared antigen to be used for the agglutination reaction and HI test by the method shown in Fig. 1, using a medium formulated by Frey et al.¹⁾ In this method of producing antigen, it has been a problem that the yield of antigen is low in relation to the amount of medium, but by increasing pH of the medium from the standard of 7.7 to about 8.5 the antigen yield was increased to 2% level from original 1-1.5%. Further study is needed to increase antigen yield.

1) Agglutination reaction

For the agglutination reaction, 1 drop

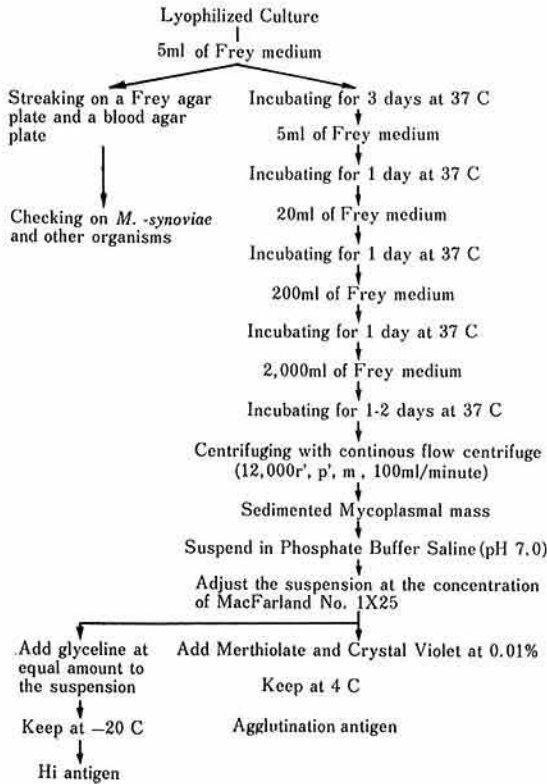


Fig. 1. Preparation method of *Mycoplasma synoviae* antigen

of antigen is used for 1 drop of serum in the serum plate test, and 2 drops of antigen are used for 1 drop of blood in the whole blood test. Under a temperature of 22–25°C, the antigen and serum or blood are thoroughly mixed by stirring on a glass plate, and then development of agglutination reaction was observed by slightly inclining the glass plate back and forth and side to side.

To apply the antigen to the tube agglutination test, the antigen was diluted to 12.5 times by using phosphate buffer solution (PBS) of pH 7.0. The diluted antigen (0.25 ml) was added to an equal volume (0.25 ml) of the serum diluted with PBS, mixed well, and incubated at 37°C for 2 hr. After the mixture was kept standing still overnight in a refrigerator, reaction was observed.

When stored at 4°C, the antigen was found to maintain its antigenicity for at least 7

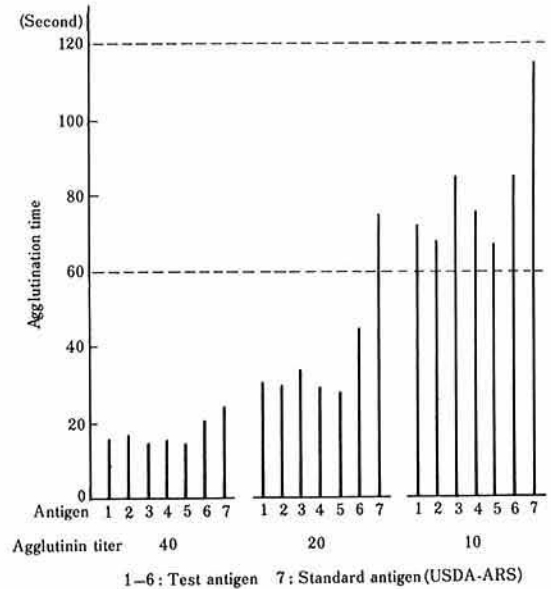


Fig. 2. Agglutinability of test antigens in serum plate test

months.

Sensitivity of the antigen was examined with several lots of the antigen thus prepared. As given in Fig. 2, positive reaction was shown within 1 min by serum plate method with positive serum having agglutination value more than 20 times. The agglutinability was more or less higher than that of the standard antigen of USDA, given to the author by courtesy. In case of whole blood test, positive reaction was observed within 1 min when the serum agglutination titer was more than 40 times.

It was reported that non-specific reaction in serum plate agglutination test with *M. gallisepticum* antigen, recently observed in England and other countries, was attributable to the inoculation of killed virus polyvalent vaccine or infection of staphylococcus or streptococcus. Therefore, serum plate test was carried out by using serums taken from chickens inoculated with inactivated polyvalent vaccine or suffered from synovitis caused by staphylococcus. As given in Table 2, all results were negative. In view of this result, serious consideration on the non-specific reaction may not be necessary for the field use of this

Table 1. Formula and preparation method of Frey's medium

Formulá :	
Mycoplasma broth base (Albimi)	22.5 g
Eagle essential vitamins (100×)	25.0 ml
Dextrose	10 g
Swine serum	120 ml
Oxidized NAD (Coenzyme I)	0.1 g
Cysteine · HCl	0.1 g
Phenol red	25 mg
Distilled H ₂ O	1,000 ml

Procedure for preparation:

Dissolve Frey's medium base in 970 ml distilled water. Add phenol red at a 0.25% concentration. Add Eagle basal medium vitamins, dextrose, and swine serum which has been previously inactivated at 56°C for 35 min. Dissolve NAD and cysteine · HCl in the remaining 30 ml of distilled H₂O. (NAD must be in a reduced form and this procedure insures reduction.) After 10 min add this solution to the medium. With 0.1 N NaOH, adjust the pH of the medium to 7.7. All glassware, filter pad, tubes, and pipettes employed for the cultivation of *M. synoviae* in this medium must be prerinsed with demineralized or distilled water to prevent inhibition of growth by inorganic impurities. This medium must be sterilized by filtration.

Preparation of broth base by heat sterilization: Mycoplasma broth base, thallium acetate and phenol red were dissolved into distilled water and sterilized for 15 min at 120 C. Other components were added to cooled broth base.

Agar medium base was prepared by adding 1.5% agar to the above broth base. After sterilization by autocraving, other sterilized components were added to cooled agar base.

antigen, but as various factors such as age of chicken, progress after infection and difference of antigen lots may influence the reaction, further examination will be needed on these particular aspects.

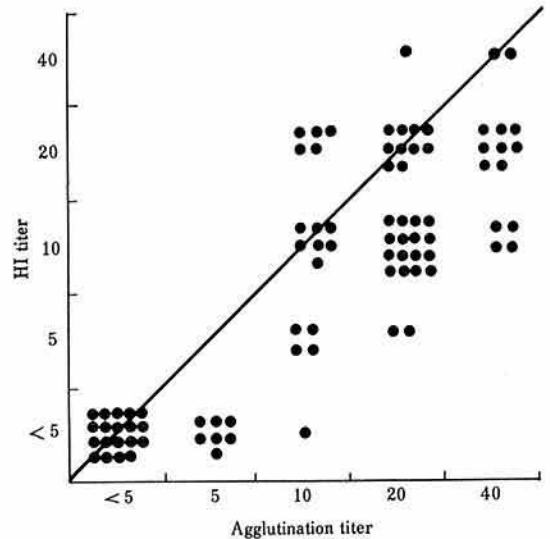
2) HI test

The antigen for HI test was prepared by mixing with an equal volume of glycerol, and when stored at a temperature lower than -20°C it maintained haemoagglutination (HA) titer for at least 6 months. The HA

Table 2. Specificity of antigen

Origin of serum	Number of positive cases
	Number of tested cases
Chickens inoculated with ND, IB, IC combined vaccin	0/15
Chickens inoculated with ND, IC combined vaccin	0/30
Chickens inoculated with <i>Haemophilus gallinarum</i>	0/5
Chickens infected with <i>Staphyrococcus aureus</i>	0/19
Chickens infected with <i>Salmonella pulloum</i>	0/7
Chickens inoculated with <i>Mycoplasma gallisepticum</i>	0/20
Chickens inoculated with <i>M. synoviae</i>	20/20

ND: Newcastle disease, IB: Infectious bronchitis
IC: Infectious colizae

**Fig. 3. Relationship between agglutination titer and HI titer against *Mycoplasma synoviae***

titer of the test antigen was 160–320 times, and 4 units (40–80 times) were used for the HI test. Method of HI test followed the method reported by Kuniyasu³⁾ with *M. gallisepticum*.

When test serum was treated with 5% chicken blood cell suspension in order to remove normal haemoagglutinin by absorption, low HI titor such as 5–10 times could be detected. In case of the Vardaman and Yoder method⁸⁾ HI titor higher than 80 times were regarded as positive, presumably in order to

avoid the misinterpretation due to HI reaction caused by normal haemoagglutinin at a low dilution, because no absorption procedure was included.

Non-specific cross reaction, as was found with plate agglutination reaction, was not reported with HI test. Relationship between agglutination value and HI titer, examined by the author with chicken groups infected by *M. synoviae*, is shown in Fig. 3. Fairly good consistence was observed, except agglutination value of about 5 times showed negative HI titer (lower than 5 times).

3) Fluctuation of antibody titer in artificially infected chickens in a course of time

Intranasal inoculation of *M. synoviae* was applied to baby chick, and they were killed periodically to determine the fluctuation of antibody titer by agglutination test and HI test, as well as to recover inoculated organisms. Antigen began to be recognized at 6 weeks of age, reaching a peak at 12 weeks of age. As the increase of HI antigen tended to delay as compared to that of agglutinin, positive reaction with agglutination and negative reaction with HI test may occur at an early stage after infection. Although the antigen titer were generally low, only less than 20 times in a geometrical mean titer, the inoculated organism was isolated from nasal cavity, infraorbital sinus or trachea at 19 weeks of age, indicating a long-lasting existence of the organism.

4) Serological reaction of field-raising chickens

By using the antigen prepared by the author, a survey was conducted with 10 groups each of broilers and that brought into a processing plant. Antibody for *M. synoviae* was found in 4 groups of the former, 2 group of them showed also the antibody for *M. gallisepticum*. Among the latter groups, one group showed antibody for both *M. synoviae* and *M. gallisepticum*, and 2 groups showed antibody for either one.

Three groups of different age were ex-

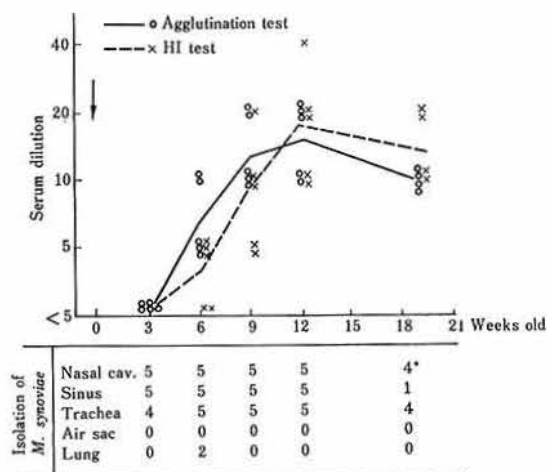


Fig. 4. Serological tests and isolation of mycoplasma in chickens inoculated intranasally with *Mycoplasma synoviae*

amined in other breeding farm of broilers. Nearly 100% of positive reaction were found with aged group, and about half of the positive chickens showed antibody for both *M. synoviae* and *M. gallisepticum* (Table 3).

Isolation of *M. synoviae* from field-raising chickens

1) Method of isolation and culture

Method of isolation from respiratory tracts is almost similar to that of *M. gallisepticum*. Samples were taken from the inner surface of nasal cavity, infraorbital sinus, trachea and airsac by sterilized cotton swab. For the sampling from lung, a small piece, about the top of a little finger in size, was cut off from the central portion of the lung. Sampling from infected joint was made by a surgical operation to expose the joint and taking exudative liquid by a syringe or cotton swab, after sterilizing the skin with heated sperture or removing the skin aseptically.

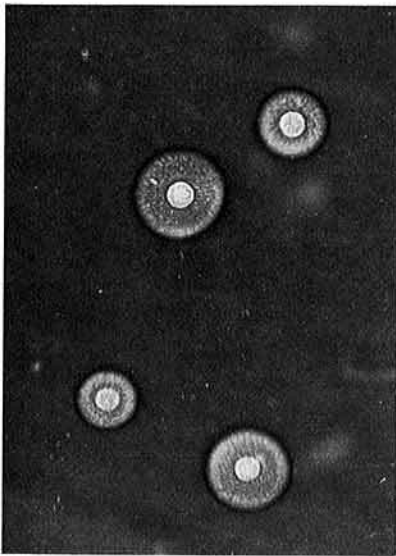
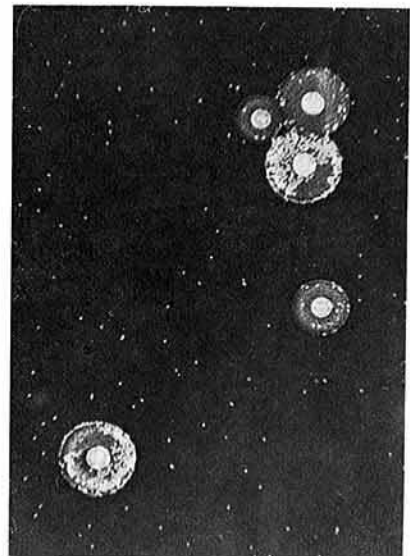
The samples thus obtained were smeared to on the Frey's agar plate (see Table 1) or, in order to detect other bacteria, smeared on the blood agar or selective media for staphylococcus or enteric bacteria and then transplanted to test tubes containing 2-3 ml of the Frey's broth (see Table 1). The test tubes

Table 3. Serological test for mycoplasma in a broiler breeding farmA: Rate of reactor*¹ in flocks basis

No. of flock	2	3	4
Age (days old)	118	230	314
Antigen	<i>M. synoviae</i>	14.8%	94.3%
	<i>M. gallisepticum</i>	40.7	45.7
			96.3%
			92.6

B: Rate of reactor*¹ in chickens basis

Number of tested	Results of test* ²	Number of positive (%) ^{*3}	Results of test		Number of positive	(%)* ³
			MS	MG		
90	MS+	64 (71.1)	+	-	22	(24.4)
	MG+			+	42	(46.7)
	MS, MG-	52 (57.8)	-	+	10	(11.1)
			-	-	16	(17.8)

*¹: Positive reaction was observed within 1 minute in serum plate agglutination test.*²: MS; *Mycoplasma synoviae*, MG; *Mycoplasma gallisepticum*.*³: Number of positive/Number of tested.Plate 1. Colonies of *Mycoplasma synoviae* on Frey's agar plate incubated at 37°C for 5 days ($\times 160$)Plate 2. Colonies of *Mycoplasma synoviae* adsorbed chicken erythrocytes ($\times 160$)

were incubated at 37°C with rubber stoppers. Immediately after the yellow color developed, one loopful of content was smeared to the Frey's agar plate. Additional use of agar plate of Frey's formulation from which β -NAD was removed facilitates identification. These agar plate cultures were incubated under high

humidity and 5–10% of CO₂ (in CO₂-incubator or candle jar) at 37°C for 3–10 days. With liquid medium cultures, observation has to be made at least for 10 days, and blind passage to new medium every 3–5 days accelerates separation.

Colonies developed on agar plates were ex-

Table 4. Isolation of mycoplasma from chickens naturally infected

(Sato et al. 1974)

Respiratory disease ¹⁾	Flock No.	Days old	Examined	Number of chickens			
				positive in			
				serological test for		isolation of	
MS ²⁾	MG ³⁾	MS	MG				
—	1 - B ⁴⁾	151	12	12	0	10	0
	4 - B	70	4	2	0	3	0
	5 - B	555	10	10	10	4	4
+	9 - L ⁵⁾	160	5	5	5	5	5
	10-1-B	67	5	0	0	3	2
	10-2-B	117	4	0	3	1	0
	10-3-B	124	6	1	0	4	3
	12-1-L	47	2	0	0	1	0
	12-2-L	81	2	2	1	1	1
			50	32	19	32	15

1) Chickens showed some respiratory signs or airsacculitis.

2) MS: *Mycoplasma synoviae*.

3) MG: *Mycoplasma gallisepticum*.

4) B: Broiler.

5) L: Layer.

amined under microscope with magnification of 20–50 X. *M. synoviae* is able to grow only on the media containing β -NAD, and forms small colony with diameter of about 0.2 mm (Plate 1, left). A central nipple-shaped portion looks to be larger than that of *M. gallisepticum*.

Within about 5 days of incubation, some colonies were able to absorb chicken erythrocytes (Plate 1, right). The absorbing capacity differed with strains, and it decreased with aging of the culture.

With liquid medium cultures, coccobacilli-like form of 0.2–0.5 μ of size appeared.

Based on these characters, *M. synoviae* can be identified, but further confirmation is made by agglutination reaction with immune serum and specific fluorescence of the colony by fluorescent conjugated antiserum.

2) Results of isolation from field-raising chickens

Isolation of Mycoplasma was carried out with chickens received by the Poultry Disease Laboratory for diagnosis. As shown in

Table 4, *M. synoviae* was isolated at relatively high frequency from nasal cavity, infraorbital sinus, trachea and airsac, irrespective with or without airsacculitis or infected respiratory organ. With fairly many cases, *M. gallisepticum* was also found in addition to *M. synoviae*. This result accorded well with the result of serological examinations, confirming the reliability of the serological reaction. The fact that *M. synoviae* was actually isolated from airsac suggests the possibility of the occurrence of airsacculitis due to *M. synoviae* to a considerable extent in Japan.

3) Inoculation test of isolated strains

Each of 3 strains of *M. synoviae* isolated from trachea of infected chickens (at latent period) was inoculated to airsac of 3 chickens taken from an uncontaminated group. After 2 weeks, occurrence of airsacculitis was examined. In all inoculated chickens, severe airsacculitis showing thickening of airsac and accumulation of mucous or cheese-like exudative liquid was observed. Inoculated organism was recovered and antibody in blood

was also detected. This result indicates the possibility of causing airsacculitis by *M. synoviae* isolated in Japan.

In view of these results, it is necessary to carry out a survey on the extent of contamination of *M. synoviae* in this country, as well as to make clear the role of this mycoplasma for the occurrence of the diseases in field and to establish countermeasures to mycoplasma including *M. synoviae*.

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