Etiology and Serological Diagnosis of Swine Enzootic Pneumonia

By ICHIRO TAKATORI

Researcher, 1st Virus Section, 2nd Research Division,
National Institute of Animal Health

Swine enzootic pneumonia (SEP) is probably the most prevalent and important disease of pigs in the world today. Economic loss due to growth retardation and low feed efficiency in the infected pigs is a serious problem for pig producers.

The occurrence of this disease has been known since the 1930's in European countries. At present this disease probably occurs everywhere swine are raised as domestic animals. In Japan this disease was first reported by Konno *et al.* (1963). However, it is not known whether the disease had existed in Japan before that time.

Swine enzootic pneumonia is a chronic pneumonia characterized by a non-productive cough, loss of condition, growth retardation, high morbidity and low mortality. The disease is spread by aerosol transmission. Our modern system of swine management in which a large number of pigs are confined in close contact may provide, therefore, an ideal environment for the rapid spread of this disease. Clinical signs usually appear 2 to 3 weeks after exposure. At necropsy, gross pneumonic lesions are found predominantly in the apical, cardiac and intermediate lobes, as well as on the anterior parts of the diaphragmatic lobes. They are usually well-demarcated, plum-colored or grayish lesions. Histological examination of the lesion reveals pronounced peribronchiolar and perivascular lymphoid hyperplasia, thickening of the alveolar walls, septal cell proliferation, and a moderate neutrophil infiltration.

Etiology of SEP

In the field of the etiological investigation for SEP, nearly three decades of confusion and contradiction were spent before a new species of Mycoplasma was recently isolated and proved to be a causative agent of SEP.

As early as the 1930's, Köbe first discovered that a chronic and enzootic pneumonia of swine, which had been prevalent in Germany, was a disease distinct from Shope's swine influenza. A name, Ferkelgrippe, was given to the disease. After Köbe's investigation, similar enzootic pneumonias under various names were reported from several European countries. However, the etiology of the disease was not elucidated during this period.

The first experimental work on the etiology of the disease was carried out by Gulrajani and Beveridge (1951) in England and by Hjärre *et al.* (1952) in Sweden. These investigators, however, concentrated mainly on the differentiation of this disease from swine influenza.

Throughout the 1950's, a number of research workers particularly in Sweden, England, Canada and the United States investigated to elucidate the etiology of this disease mainly from the virological point of view. However, the results obtained during this decade were often contradictory and confusing, although a lot of peripheral information was obtained.

These contradictions and confusions may have been due to a historical misleading preconception that the causative agent might be

a virus, or to the difficulty in obtaining respiratory disease-free pigs for experimental inoculation, or to an unfortunate stumbling block that their starting materials had often been contaminated with *Mycoplasma hyorhinis* (named by Switzer; 1955), which is the most common secondary contaminant to the SEP pneumonic lesions and of which propagation exceeds over that of the causative agent in question in both cell cultures and cell-free media.

The first reliable results for etiology of SEP were reported by Betts and Whittlestone (1963) and Goodwin and Whittlestone (1963) in England. Using starting materials free of secondary contaminants, such as *M. hyorhinis*, they could cultivate a pleomorphic organism in pig-lung cell cultures and reproduced the disease by inoculating the infected cell culture fluid into respiratory disease-free pigs.

The same organisms were also successfully propagated in cell-free medium, which consisted of 70 per cent Hanks' balanced salt solution, 20 per cent inactivated pig serum (from an SEP-free herd), 0.5 per cent lactalbumin hydrolysate, 0.01 per cent Difco yeast extract, 200 units per ml penicillin and 50 units per ml nystatin (Goodwin and Whittlesone; 1964). They suggested their organisms seemed most likely to fall into the PPLO group (Mycoplasma).

However, because of the lack of evidence that their organisms could form Mycoplasmal colony on solid medium, they could not conclude it. In the following year, Maré and Switzer (1965) in the United States isolated also the similar causative organisms in the cell-free medium essentially same as that reported by Goodwin and Whittlestone (1964) and also observed the formation of the Mycoplasmal colony on solid medium inoculated with the organisms grown in cell-free liquid medium. They concluded that their isolate was a new species of Mycoplasma and proposed a name, *M. hyopneumoniae*, for their isolate.

Later in the same year, Goodwin *et al.* (1965) also observed the formation of Mycoplasmal colony on solid medium inoculated with their organisms. In addition, they could reproduce the disease by inoculating pigs with their colonized organisms.

They concluded, consequently, that their colonized organism was a pure causative agent of SEP and proposed a name, *M. suipneumoniae*, for the organism. Thereafter evidences have been reported that these two isolates, *M. hyopneumoniae* and *M. suipneumoniae*, are serologically indistinguishable from each other (Goodwin *et al.*; 1967, Hodges *et al.*; 1969).

Isolation of the Mycoplasma, which is believed to be *M. hyopneumoniae* (or. *M. suipneumoniae*), has recently been reported also in Canada (L'Ecuyer; 1969) and Japan (Ogata *et al.*; 1968, Fujikura *et al.*; 1968).

Both the pneumonia-producing ability and serological distinction may be the most important indexes for distinguishing *M. hyopneumoniae* (or *M. suipneumoniae*) from other Mycoplasma species of swine-respiratory organ-origin so far. The use of respiratory disease-free pigs is the essential requirement for examination of pneumonia-producing ability of the agent in question.

On the other hand, metabolic inhibition test and growth inhibition test are available for immune rabbit sera as the differential serological tests. Complement-fixation (CF) test, indirect hemagglutination test and tube agglutination test are available for immune swine sera. These tests for immune swine sera will precisely be explained in the next section.

Many other auxiliary indexes for distinguishing *M. hyopneumoniae* (or *M. suipneumoniae*) have been known. For example, fastidiousness about growth factors or formation of small colony without nipple has been known for this Mycoplasma species. Takatori *et al.* (1964, 1967) have reported that floating crystals soluble in organic solvents (FCSOS) were produced on the surface of the medium of swine kidney cell cultures inoculated with *M. hyorhinis*. Such FCSOS-production is not observed for the cell cultures infected with

M. hyopneumoniae.

On the other hand, Switzer (1967) referred to a pronounced film formation on solid medium by *M. granularum* (named by Switzer; 1964) and no film formation by *M. hyopneumoniae*. These findings may also be utilized as auxiliary indexes for distinguishing *M. hyopneumoniae* from *M. hyorhinis* or *M. granularum*. Dinter *et al.* (1965) discovered the existance of four additional serotypes of swine Mycoplasma besides *M. hyorhinis* and *M. granularum*. The relationship of these Mycoplasma to *M. hyopneumoniae*, however, is not known.

Serological diagnosis of SEP

Since 1936 many attempts have been made to develop a serological test for demonstrating immune antibody in the sera of pigs affected with SEP (Köbe; 1936, Hjärre *et al.*; 1952, Wesslén and Lannek; 1954, Lannek and Börnfors; 1957, Börnfors and Lannek; 1958). The results have been, however, discouraging. These failures are probably due to the inability to obtain specific antigen for the tests, since the causative agent of this disease has only recently been cultivated.

This success in cultivation of the causative agent in artificial medium has made it possible to obtain quantities of specific antigen. By use of such an antigen, which consisted of washed and concentrated organisms of *M. hyopneumoniae* or such species of Mycoplasma believed to be *M. hyopneumoniae*, Roberts (1968), Boulanger and L'Ecuyer (1968) and Takatori *et al.* (1968) first demonstrated an immune antibody against the Mycoplasma in the sera of pigs infected with SEP by using the CF test. Recently, an indirect hemagglutination test was reported by Goodwin *et al.* (1969). More recently, Fujikura *et al.* (1970) have reported a tube agglutination test.

1) CF test

The antigen for the CF test is prepared by inoculating seed culture of *M. hyopneumoniae* into a large quantity of cell-free liquid medium, followed by concentration, washing and resuspension of the Mycoplasmal bodies grown in the medium. High-speed centrifugation is used for the concentration and washing. Since the antigen is usually anticomplementary and there is not a wide margin between the anticomplementary activity and specific antigenicity, the amount of antigen to be used for antibody detection should be determined strictly in the preliminary test.

Procomplementary activity of swine sera can be reduced to some extent by storing at −20°C or lower temperature, heating at 60°C for 30 minutes and filtration through filter pads. One per cent of fresh bovine serum is added to complement diluent as a supplementary factor. The veronal-bicarbonate buffer solution serves as diluent or washing solution.

The test is performed in small glass tubes (10.5 × 76.0 mm) utilizing a total reaction volume of 0.5 ml, which consisted of 0.1 ml of twofold serial dilution of test serum, 0.1 ml of 2 units of antigen, 0.1 ml of 2 exact units of complement and 0.2 ml of fully sensitized sheep erythrocyte suspension. Appropriate controls are included in the test. Serum, antigen and complement are mixed and incubated overnight at 4°C, after which the hemolytic system is added and incubation continued for 30 minutes in a water bath at 37°C. Tests in which 50 per cent or more inhibition of hemolysis are observed are considered positive.

Slight differences can be seen among the techniques of CF tests hitherto reported to SEP. However, these differences do not seem to be considerably affecting the results obtained, since all the techniques are giving the results which do not come into conflict with each other.

Evidences have been reported that relatively good correlation exists between development of SEP lesion and occurrence of detectable CF antibody levels. For example, it is shown in Table 1. The CF antibody against *M. hyopneumoniae* was demonstrated in the post-inoculation sera of 12 of the 13 pigs, which were supplied from a respiratory disease-free herd and

Table 1. Demonstration of CF antibody in pigs experimentally infected with *M. hyopneumoniae*

Pig No.	inoculum	Days*1 after inoculation	SEP*2 pneumonic lesion	Recovery of *M. hyopneumoniae*	CF antibody titer of Preinoc. serum	CF antibody titer of Postinoc. serum
3770G			+	NT*3	<2	4
3774G		24	+	NT	<2	32
3803G			+	NT	<2	16
3773G	Pig passaged *M. hyopneumoniae*		+	NT	<2	<2
2931G			+	NT	NT	64
2942G		53	+	+	NT	512
2942B			+	+	NT	128
3990G			+	+	<4	16
4001B		35	+	+	<4	16
4010G	Fluid-culture *M. hyopneumoniae*		+	+	<4	64
3992G			+	+	<4	64
4000G		63	−	−	<4	32
4010B			+	+	<4	128
3951G			−	NT	<2	<2
3952G			−	NT	<2	<2
3970G			−	NT	<2	<2
2930B			−	NT	NT	<2
2932B	(Normal controls)		−	NT	NT	<2
2940B			−	NT	NT	<2
2943B			−	NT	NT	<2
3991G			−	−	<4	<4
4001G			−	−	<4	<4
4003B			−	−	<4	<4

*1 The day when necropsy was performed and postinoculation serum was collected. (Takatori et al.; 1968)
*2 Swine enzootic pneumonia.
*3 Not tested.

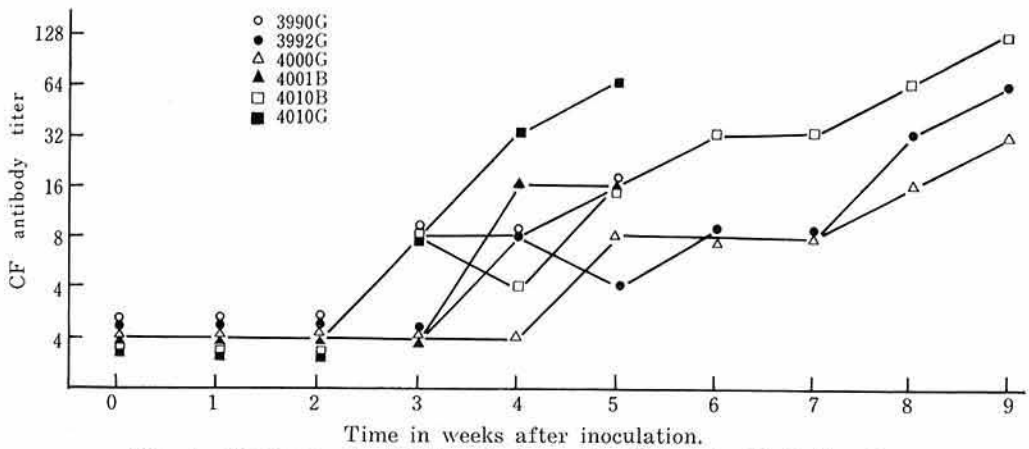

Fig. 1. Development of CF antibody in pigs infected with fluid-culture *M. hyopneumoniae*.

experimentally infected with SEP by inoculation of *M. hyopneumoniae*. Neither the pre-inoculation sera of the inoculated pigs nor the sera of the normal control pigs contained a similar CF antibody.

The antibody can also be demonstrated in the sera of pigs naturally infected with SEP. Development of the CF antibody in the infected pigs is rather slow. The antibody appears first 2 to 3 weeks after infection and tends to increase for at least 9 weeks after infection (Fig. 1).

The results of investigations on the specificity of the CF test for *M. hyopneumoniae* infection in pigs revealed the fact that no cross reaction could be obtained between *M. hyopneumoniae, M. hyorhinis,* or *M. granularum* and their heterologous antisera derived from pigs specifically infected with the respective organism, although positive reactions were obtained between the respective organism and their homologous antisera (Takatori *et al.*; 1968). This indicates that the CF test may be of value for detection of specific antibody for not only *M. hyopneumoniae* but also *M. hyorhinis* or *M. granularum* infection in pigs.

A question has been prevalent among research workers whether the same causative agent is involved in the chronic pneumonia called under different names, such as swine enzootic pneumonia (SEP), virus pneumonia of pigs (VPP) or infectious pneumonia of pigs. However, the evidences that the CF antibody against *M. hyopneumoniae* (or such species of Mycoplasma believed to be *M. hyopneumoniae*) was demonstrated in the sera of pigs infected with the pneumonia in England (Roberts; 1968, Goodwin *et at.*; 1969), Canada (Boulanger & L'Ecuyer; 1968), the United States (Takatori *et at.*; 1968) and Japan (Takatori; 1969, Fujikura *et at.*; 1970) may indicate the involvement of the same causative Mycoplasma species in this chronic pneumonia in the world.

2) Other serological tests

Goodwin *et al.* (1969) demonstrated immune antibody against *M. suipneumoniae* by use of an indirect hemagglutination test. The test was made in 12 × 75-mm. Pyrex tubes. Sheep erythrocytes, which were tanned and sensitized with the Mycoplasmal antigen, were used in the test. Doubling serum dilutions, starting at 1/5, were made in 1 per cent protein in 0.5 ml volumes. To each tube 0.05 ml of sensitized cells was added. After thorough shaking, the tubes were incubated at 37°C for 2 hours; the results were then noted, the tubes re-shaken and left at room temperature overnight before making the final reading. The end-point was taken as the highest serum dilution to give a clearly positive agglutination.

They reported, in a comparative study with the CF test, that the indirect hemagglutination titers appeared later and lasted longer than the CF titers.

Fujikura *et al.* (1970) have reported a demonstration of the antibody against *M. hyopneumoniae* in the infected pigs by use of a tube agglutination test. This test was performed in glass tubes (13 × 100 mm) utilizing a total reaction volume of 0.5 ml, which consisted of 0.25 ml of twofold serial dilution of test serum and 0.25 ml of antigen. The mixture of antibody and antigen was put into the water bath at 37°C for 2 hours for sensitization. Then it was placed in a cold room at 4 to 5°C for further 18 hours. The reading of the test was carried out macroscopically. The end-point was taken as the highest serum dilution to give a clearly positive agglutination.

In this test, they found a relatively good correlation between presence of SEP lesion and detectable antibody levels, as well as no cross reaction between *M. hyopneumoniae* and *M. hyorhinis*.

The antigen for the indirect hemagglutination test and tube agglutination test was prepared essentially in the same manner as that described for the CF test.

Metabolic inhibition test is usually recognized as an encouraging test for detection of Mycoplasmal antibodies in various kinds of animals. For detection of *M. suipneumoniae* (or *M. hyopneumoniae*) antibody in the infected pigs, however, this test seems to be of little value. Goodwin *et al.* (1969) could not detect

specific metabolic inhibition antibody against *M. suipneumoniae* in the infected swine sera. Instead, they found the presence of non-specific inhibitory substances in some normal pigs, as well as some infected pigs. Takatori and Switzer (1967) have also made an unsuccessful attempt to demonstrate metabolic inhibition antibody against *M. hyopneumoniae* in the sera of experimentally infected pigs.

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