Mycoplasmosis is one of the most important porcine diseases. It has a great impact on the productivity and the economic performance in swine industry worldwide including Thailand where the pig production system has been becoming more industrialized. *Mycoplasma hyopneumoniae* is considered to play an essential role together with PRRS (porcine reproductive and respiratory syndrome) virus which escalates the economic losses induced by PRDC (porcine respiratory disease complex). *Mycoplasma hyosynoviae* causes nonpurulent polyarthritis in breeding and fattening pigs. The disease is also prevailed all over the world. Little is known on porcine mycoplasmas in Thailand because of their difficulties of isolation and cultivation of the organisms. The present investigations were carried out to get information which will improve the diagnostic techniques of porcine mycoplasmiosis and contribute to the control of the diseases in that country.

1. Antimicrobial susceptibilities of *Mycoplasma hyopneumoniae* field isolates and occurrence of enrofloxacin, macrolides and lincomycin resistance

To assess the in vitro susceptibilities of *M. hyopneumoniae* to antimicrobial agents is inevitable for the chemotherapy of MPS. A total of 159 Thai isolates of *M. hyopneumoniae* derived from pneumonic lungs of pigs in 41 farms during 2006-2011 were subjected for susceptibility testing against 12 antimicrobial agents widely used in Thailand; chlortetracycline (CTC), oxytetracycline (OTC), doxycycline (DOXY), lincomycin (LCM), josamycin (JM), kitasamycin (KT), spiramycin (SPM), tylosin (TS), erythromycin (EM), tiamulin (TM), florfenicol (FFC) and enrofloxacin (ERFX). TM showed the lowest minimal inhibitory concentrations (MICs) among drugs tested. TS, SPM, JM, KT and LCM showed high activities, however, two isolates were resistant to these drugs. EM showed exceptionally low activity. FFC showed moderately high activity whereas OTC and DOXY showed similar activities. On the other hand, activity of CTC to Thai isolates of *M. hyopneumoniae* was low. The MICs of ERFX distributed in a broad range and 76 of 159 (47.7 %) Thai isolates were regarded as ERFX resistant.

The comparison of susceptibilities of the present isolates with those of isolates obtained from 1997 to 1998 in Thailand revealed that susceptibilities of the present isolates to CTC, OTC, JM and TS were decreased considerably. The sequences of domain V of 23S rRNA of the two macrolides and LCM resistant isolates revealed a point mutation at
2. Antimicrobial susceptibilities of *Mycoplasma hyosynoviae* field isolates in Thailand during 2008-2011 and in vitro development of resistance to tylosin and lincomycin in type strain S16 of *M. hyosynoviae*

Forty one Thai isolates of *M. hyosynoviae* derived from tonsils, lungs and joint fluids of pigs from 9 farms during 2008-2011 were investigated for their in vitro susceptibilities against 10 antimicrobial agents widely used in Thailand, namely OTC, DOXY, LCM, JM, KT, SPM, TS, TM, FFC and ERFX. TM showed the lowest MICs against all *M. hyosynoviae* field isolates. LCM and JM showed high activities whereas KT and FFC showed moderately high activities and SPM and TS showed lower activities. Activities of OTC and DOXY to the isolates were low. Of the Thai isolates of *M. hyosynoviae* considerable portion was resistant to some of the drugs; 41 of 41 for OTC, 15 of 41 (36.6%) for ERFX and 13 of 41 (31.7%) for TS. An acquired G745A transition in domain II was found in 13 isolates by sequence analysis of domain II and V of 23S rRNA of all Thai isolates. The MICs of TS were of 4-8 µg/ml for these 13 isolates suggesting strongly that this G745A transition in *M. hyosynoviae* may confer a mild resistance to TS. MIC values of other macrolides and LCM were not correlated to this transition.

In vitro development of resistance to TS and LCM were investigated by passaging this strain in broth medium containing various concentrations of TS or LCM. At the sixth passage the highest concentration of the drug that S16 mutant strain could grow was 4,000 or 1,000 times higher for TS or LCM respectively. In LCM selection, the A2058C/G or A2059C transitions were obtained in S16 mutants. This mutant was resistant not only to LCM but also to all macrolide drugs tested. In TS selection the A2062G transition was obtained which conferred the resistance to macrolides but not to LCM.

3. Development of semi-nested PCR for detection of 16S rRNA gene of *Mycoplasma hyosynoviae*

Successful treatment of arthritis due to *M. hyosynoviae* in pigs is best achieved by injecting effective antimicrobials as early as possible. It needs several days, however, to diagnose the disease since the isolation of mycoplasmas is time-consuming. The semi-nested PCR to detect *M. hyosynoviae* using three oligonucleotide primers was developed for the rapid diagnosis of the disease. The primers were designed based on 16S rRNA gene of *M. hyosynoviae*. Using the adequate concentration of the *M. hyosynoviae* DNA, the first round of semi-nested PCR could generate an amplified fragment about 649 bp followed by 295 bp for the second round of semi-nested PCR. The semi-nested PCR developed here detected as little as $10^{-14}$ g of purified *M. hyosynoviae* DNA in a reaction and the limit of detection for clinical materials was at least $10^3$ CFU (colony forming unit) per gram of the lung samples. To evaluate the applicability of this method a total of 300 tonsillar samples were collected and compared the detection rate with that by cultivation. As a result 45 of 300 (15%) samples were positive by semi-nested PCR while 17 of 300 (5.7%) were positive by cultivation. Thus the semi-nested PCR was shown to be a useful tool for rapid detection of *M. hyosynoviae* in clinical materials in pig herds.

4. Genetic diversity of *Mycoplasma hyosynoviae* field isolates in Thailand

Genetic characterization of field isolates of *M. hyosynoviae* was investigated by pulsed-field gel electrophoresis (PFGE) and random amplified polymorphic DNA (RAPD) analyses. Well-separated DNA fragments were obtained from
37 of 41 Thai isolates and type strain S16 with PFGE and 22 different patterns were detected. Among isolates derived from the same farm, two to five different profiles were also detected in addition to the same profiles. Though different profiles were usually detected among isolates derived from different farms, one common profile was detected in two isolates from two different farms on an occasion. These two farms were located in the same province, suggesting ‘farm to farm’ transmission of these isolates with the same profile. The PFGE technique had a high reproducibility with identical banding patterns for replicate samples. Thus it revealed that *M. hyosynoviae* had high genetic heterogeneity both within a pig farm and among the farms by PFGE. It was suggested that PFGE might be a useful tool for the epidemiological studies of *M. hyosynoviae* infection. In contrast, RAPD analyses were carried out after various preliminary experiments. The clear patterns were obtained by using RAPD primer 6 and RAPD beads. Thirty-nine different patterns were detected among 42 strains and two isolates with the same profile by PFGE derived from different farms showed different pattern by RAPD. On the other hand, the same banding patterns were not necessarily obtained by repeated analyses of the same strains. Due to this low reproducibility RAPD analyses were not considered appropriate to obtain epidemiological information of the disease despite its high discriminatory power.

The results on antimicrobial susceptibilities of *M. hyopneumoniae* described in this work have great importance for controlling not only MPS but also PRDC in which *M. hyopneumoniae* plays an important role. Rapid detection of *M. hyosynoviae* enables the rapid diagnosis and earlier medication of arthritis due to *M. hyosynoviae* and improves the treatment together with the results of antimicrobial susceptibilities. The elucidation of the mechanisms of macrolide and LCM resistances of *M. hyosynoviae* will contribute to establish the prudent use of antimicrobials in animal husbandry. Lastly, PFGE analyses of *M. hyosynoviae* isolates in Thailand will facilitate the molecular epidemiological study of animal mycoplasmosis in that country.