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Serological diagnosis of enzootic pneumonia of swine by a double-sandwich enzyme-linked immunosorbent assay using a monoclonal antibody and recombinant antigen (P46) of *Mycoplasma hyopneumoniae*

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Abstract

To facilitate the control of enzootic pneumonia (EP) of swine caused by *Mycoplasma hyopneumoniae*, the complement fixation (CF) test has been used for the detection of *M. hyopneumoniae* antibodies. However, the CF test is a cumbersome and time-consuming technique and cross-reactivity are major drawbacks associated with this method. To circumvent these drawbacks, we have developed a double-sandwich enzyme-linked immunosorbent assay (ELISA), consisting of purified monoclonal antibody (Mab) against the 46 kDa surface antigen (P46) of *M. hyopneumoniae* and recombinant P46 protein expressed in *Escherichia coli*, for the detection of antibodies to *M. hyopneumoniae* in serum samples from pigs experimentally inoculated with *M. hyopneumoniae* and from naturally infected pigs, and compared the practical usefulness of ELISA using the CF test. In experimentally inoculated pigs, the CF and ELISA antibodies were detected at almost the same time, and a good correlation was demonstrated between the CF test and the ELISA. In a survey conducted on field samples, the seropositivity by ELISA in pigs of age 2–6 months was increased. At the time of slaughter, approximately 80% of the animals were seropositive for ELISA. However, a gradual decrease in the prevalence of ELISA positive samples was observed in sows with increasing parity. No correlation was seen between the results obtained with the two methods in the clinical samples. The CF test appears to have limited value for the diagnosis of EP in conventional herds because nonspecific reactions were frequently observed. Therefore, this ELISA is a useful alternative to the CF test currently used for the diagnosis of EP.

Keywords: *Mycoplasma hyopneumoniae*; ELISA; Monoclonal antibody; Recombinant antigen; Pig; Bacteria

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1. Introduction

Enzootic pneumonia (EP) of swine, also called mycoplasmal pneumonia, caused by *Mycoplasma hyopneumoniae* is one of the most common, economically significant diseases of pigs. It has low mortality but high morbidity rates. Most field cases of EP are chronic, complex infections caused by *M. hyopneumoniae* coupled with secondary pathogens. Due to the slow growth of *M. hyopneumoniae* and the risk of overgrowth of other mycoplasmas (e.g., *Mycoplasma hyorhinis*), isolation and cultivation of this organism are laborious and are not suitable for routine diagnosis of EP (Ross, 1999). Therefore, serological methods detecting antibodies to *M. hyopneumoniae* are important for diagnosis of EP. To facilitate the control of the disease, the complement fixation (CF) test has been routinely used in Japan for the detection of *M. hyopneumoniae* antibodies (Mori et al., 1983). However, the CF test is a cumbersome and time-consuming technique, which is by no means optimal for a large number of diagnostic samples. Also, the anticomplementary activities of swine serum and cross-reactivity in the CF test are major drawbacks associated with this method (Hodges and Betts, 1969). To circumvent these drawbacks, several more sensitive and specific enzyme-linked immunosorbent assays (ELISA) have been developed (Nicolet et al., 1980a; Kazama et al., 1989; Feld et al., 1992; Djordjevic et al., 1994). These ELISAs are sensitive, but are based on crude antigen preparations, which may result in cross-reactivity (Ro and Ross, 1983). A double-sandwich ELISA using monoclonal antibody (Mab) against the 46 kDa surface antigen (P46) of *M. hyopneumoniae* and Tween-20 solubilized antigen has been developed for the detection of antibodies against *M. hyopneumoniae* (Mori et al., 1987). Futo et al. (1995) suggested that the recombinant P46 protein expressed in *Escherichia coli* could be used in an ELISA for the specific detection of antibodies against *M. hyopneumoniae*. On the basis of this knowledge, we have developed and evaluated a double-sandwich ELISA based on purified Mab against P46 and recombinant P46 antigen for the detection of antibodies to *M. hyopneumoniae*. The aims of the present work were to assess the application of this ELISA to the detection of antibody against *M. hyopneumoniae* serum from pigs with experimental and natural infections, and its practical usefulness in routine diagnosis as compared with the CF test.

2. Materials and methods

2.1. Mycoplasma strains

M. hyopneumoniae strain E-1, which was used for the experimental infection of pigs, was isolated from lung lesions of a pig with EP (Mori et al., 1988). To assess the specificity of the ELISA, *M. hyopneumoniae* strain J, *M. hyorhinis* BTS-7, *M. flocculare* Ms 42, and *M. hyosynoviae* S16 were employed for preparing antiserum.

2.2. Monoclonal antibody used in the ELISA

Purified Mab 14-4-4 (IgA isotype) against the 46 kDa surface antigen of *M. hyopneumoniae* was used in double-sandwich ELISA. The hybridoma used in this study did not easily adapt to low-serum or serum-free conditions. Mab could not be adequately purified from culture fluids, so it was produced by the ascites method. The preparation of Mab from ascitic fluids in mice was performed in accordance with the guidelines on antibody production, which has been developed by the Canadian Council on Animal Care. The methods used in the production of Mab have been described previously (Mori et al., 1987). Purified IgA was obtained from ascitic fluid by using a hydroxyapatite (HA) column. Briefly, ascitic fluid containing Mab was dialyzed against 0.001 M phosphate buffer (pH 6.8) and applied to an HA column (TSK gel HA-1000, Tosoh Co., Tokyo, Japan) equilibrated with the same buffer. Antibody was eluted with a linear concentration gradient of sodium phosphate ranging from 0.001 to 0.5 M at pH 6.8. The first peak was found to contain the IgA fractions and was used for the ELISA.

2.3. Recombinant P46 antigen expressed in E. coli

Purified recombinant P46 antigen was prepared from *E. coli* JM109 transformed with the plasmid vector pQE9 as previously described (Futo et al., 1995). Briefly, the coding region of the P46 gene was amplified by PCR. The amplified fragment was inserted into the *Bam*HI and *Pst*I sites of plasmid