Detection of *Mycoplasma hyopneumoniae* in Pigs in Turkey

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**Abstract:** *Mycoplasma hyopneumoniae* is the primary agent of enzootic pneumonia in pigs. The aim of this study was to investigate the occurrence of *M. hyopneumoniae* in pigs raised in Turkey. Samples were obtained from the lungs of 286 pigs, which had already been separated from the carcasses at slaughter. Lung tissues were homogenized in 5 ml of Friis medium, the suspensions were inoculated into Friis medium, and then incubated at 37 °C until they developed an acid color change or for 21 days. Cultures were identified by *M. hyopneumoniae*-specific PCR. *M. hyopneumoniae* was recovered from 2 (0.70%) of the 286 lung samples. This study is the first to report the isolation of *M. hyopneumoniae* in pigs in Turkey.

**Key Words:** *Mycoplasma hyopneumoniae*, pig, lung, Turkey

**Türkiye’de Domuzlarda *Mycoplasma hyopneumoniae*’nin Varlığını Araştırılması**


**Anahtar Sözcükler:** *Mycoplasma hyopneumoniae*, domuz, akciğer, Türkiye

**Introduction**

Enzootic pneumonia (EP) is a chronic respiratory disease characterized by a chronic cough and economic losses associated with retarded growth, poor feed conversion, and predisposition to bacterial pulmonary infections. The primary agent of the disease is *Mycoplasma hyopneumoniae*. Under field conditions the disease is characterized by high morbidity and low mortality (1-4). Furthermore, the agent is among the most prevalent and important infectious agents associated with the porcine respiratory disease complex (5,6).

Pigs are susceptible to the disease at any age; however, clinical signs are observed particularly in young animals (3,7,8). Infected animals shed the microorganism via respiratory exudates and transmission occurs by direct contact with respiratory tract secretions from infected pigs (1,2,7).

Conventional laboratory diagnosis of *M. hyopneumoniae* is based on cultivation of the organism and serological identification of the isolates. Although culturing is considered to be the “gold standard” due to its high specificity, it is rarely practiced because of the fastidious nature of the organism’s growth and the risk of overgrowth of other mycoplasmas (1,7,9,10). In the past 10 years several one-step or nested PCR (nPCR) protocols have been developed for the rapid and specific identification of *M. hyopneumoniae* (1-3,9).
In Turkey pig production is uncontrolled. There are very limited studies on contagious infectious diseases because of the limited number of breeding facilities, and inadequate knowledge and experience of veterinarians servicing the pig industry. The aim of the present study was to collect data on the status of EP in pigs in Turkey by investigating the presence of *M. hyopneumoniae*.

**Materials and Methods**

**Samples**

Lung samples of 286 pigs (103 pigs aged 3-11 months and 183 pigs 1-3 years of age) raised at 9 different farms were collected at slaughter. Lungs were examined grossly for the occurrence of lesions. Tissue samples were collected from sections with lesions and from the cranial lobes in the absence of lesions. The samples were transported to the laboratory in sterile bags via a cold chain.

Data concerning the general health status of the animals, antibiotic usage, and vaccination protocols at the farms were obtained from the farm owners or workers because no legal records of these farms existed.

**Cultivation**

Friis liquid medium (FLM) was prepared according to Kobisch and Friis (7). Each lung sample was homogenized in a Stomacher (IUL Instruments) using 5 ml of FLM. Lung suspensions were inoculated in 10-fold dilutions from $10^{-1}$ to $10^{-4}$ into FLM and incubated at 37 °C for 24-72 h. At the end of the incubation period if there was no color change in FLM, passages into a new FLM were performed 3 times. The cultures were incubated until a color change in the medium associated with pH change was visible or for 21 days. All the cultures in which color change was observed were identified by *M. hyopneumoniae*-specific PCR (6).

**PCR**

For each *M. hyopneumoniae*-suspected broth culture 1 ml was centrifuged, washed in phosphate buffered saline (PBS), resuspended in distilled water, and then lysed by heating at 100 °C for 5 min. Then, 5 μl of this DNA extract was used as a PCR template (1). The amplification was performed in a 50-μl reaction mixture that contained 4 μl of dNTP mixture (2.5 mM of each), 0.5 μl of 20 pmol of each primer (forward primer 5’-GAGCCTTCAAGCT TCACCAAG-3’; reverse primer 5’-TGTGTTATGTCATT TTGACC-3’), 5 μl of 10× PCR buffer, and 1.25 U of Taq DNA polymerase (TaKaRa Taq HS). Two droplets of mineral oil were layered on the reaction mixtures to prevent evaporation. The thermocycler (Biometra-Uno thermo block) was programmed for initial denaturation at 94 °C for 3 min, 35 cycles of denaturation at 94 °C for 1 min, primer annealing at 54 °C for 1 min, extension at 72 °C for 1.5 min, and final extension at 72 °C for 10 min. The amplified products were analyzed by electrophoresis in 1.5% agarose gel. The gel was stained with ethidium bromide (0.5 mg/ml) and visualized via a UV transilluminator. Lung homogenate samples with DNA bands at 649 bp were considered positive for *M. hyopneumoniae* (6).

*M. hyopneumoniae* and *M. hyorhinis* strains, which were obtained from Dr. Branko Kokotovic (National Veterinary Institute, New Technical University of Denmark, Copenhagen V, Denmark), were used as positive and negative controls for both isolation and PCR protocols.

**Results**

Based on the information received from the owners of the farms, no clinical signs of *M. hyopneumoniae* were recorded in any of the slaughtered pigs; however, continuous antibiotic administration was used on the farms. Based on examination of the lungs for gross lesions, cyst-like lesions were observed in 2 pigs and mild hemorrhagic areas were observed in 2 other pigs; no lesions were observed in the remaining pigs.

pH color changes were observed in 40 of the 286 lung samples inoculated into FLM. As a result of PCR performed with 40 suspected isolates, PCR products 649 bp in molecular weight were detected in 2 samples, which were identified as *M. hyopneumoniae*; however, no clinical signs or pathological findings were observed in these 2 pigs.

**Discussion**

*M. hyopneumoniae* colonizes the surface of the ciliated epithelium of the trachea, bronchi, and bronchioles of infected pigs. Additionally, although it does not normally colonize the nasal cavity of pigs, it has been isolated or detected with nasal swabs from affected pigs (4,10). Several PCR protocols to detect *M. hyopneumoniae* from lung samples (8), bronchoalveolar lavage fluids (9), tracheobronchial lavage fluids (10), nasal swabs (3,4), bronchial swabs (5), and air samples (2) have been...
conditions were poor and vaccination against M. hyopneumoniae was not under veterinary control, and general hygiene conditions do not always result in severe disease. The pig farms investigated in the present study were most likely to be overcrowded and lack ventilation and adequate hygiene conditions, were located in different geographic areas, and made it difficult for the organism to spread from one farm to another. Furthermore, as some of the breeders usually stop production for a few months after the slaughter and then restart production, this intermittent type of breeding management limits the microorganism’s ability to find a host to provide it durability, which in turn might limit the development of outbreaks. As pig breeding is not well developed in Turkey, it is limited in terms of both geography and herd density, the occurrence and spread of M. hyopneumoniae infection are limited. Although M. hyopneumoniae was detected in only 2 samples in the present study, because of the very high seroprevalence rate reported in another recent study (14) we suggest that spread of the infection among farms and the occurrence of more serious problems with pig production will be unavoidable if pig production becomes widespread and if breeders do not adhere to general hygiene and proper management methods.

The most typical clinical sign of EP is a dry, non-productive cough, which develops within 2 weeks of infection and is observed for a few weeks or months (2,7,10). Reduced rates of growth and feed efficiency could be observed in infected herds (7). On the other hand, in some cases no clinical signs are observed (2,7,10). Sibila et al. (12) reported that although M. hyopneumoniae was detected in all of the farms tested, the percentage of infected animals was relatively low, and they indicated that wider use of antibiotics in feed in the Spanish herds tested caused this low isolation rate. Vicca et al. (13) reported that although the signs of respiratory tract disease and lung lesions were observed in both the tylosin-treated and untreated infected groups, they were less severe in the tylosin-treated group. In the present study it was not possible to collect reliable data concerning the health status of the sampled pigs, because there was no data recording at the pig farms. Although the workers or owners of the farms stated that there were no health problems with the pigs, they said that antibiotics were continuously administered to the animals. The low isolation rate obtained in the current study might have been associated with uncontrolled antibiotic use.

Huhn (15) reported that under experimental conditions antibiotic treatment partially or completely inhibited the formation of lesions, but did not prevent infection, because pigs developed lesions when the drug was removed. Because of the distances between the farms and slaughterhouses in that study, and because there was no systematic slaughter schedule, the pigs were transported sometimes for 2 days and waited for slaughter for varying amounts of time; because of these difficulties antibiotic administration stopped. In this case, it is possible that...
existing infections within the herds intensified as a result of pre-slaughter stress factors, such as transportation, poor ventilation, and crowding, and these infected pigs were slaughtered before the lesions developed.

No macroscopic pathological findings were observed in the lung samples in the present study, except in 4 of the 286 pigs. The lack of pathological signs in 2 lung samples from which *M. hyopneumoniae* was isolated supports the suggestion by Calsamiglia et al. (5) that diagnosis based solely on macroscopic and histological changes, especially in the early or late stages of the disease, might be misleading; therefore, techniques such as PCR should be used for confirmation. Jericho (16) determined that it is possible to interpret the isolation of *M. hyopneumoniae* from non-pneumonic lungs, as the infection has occurred and pneumonic changes are possibly in early stages of the disease’s development. In the present study, at one of the *M. hyopneumoniae*-positive farms, it is possible that the early stage of infection was detected. In contrast, on the other *M. hyopneumoniae*-positive farm there were also pigs with lung lesions, but no isolation. We considered that these animals probably had already been infected and that the lack of isolation from these 2 pigs was associated with intensive antibiotic usage.

In conclusion, the present study is the first to report the detection of *M. hyopneumoniae* in pigs in Turkey. As the culturing technique was followed by PCR in this study, we had an opportunity to detect viable microorganisms; this indicates the occurrence of *M. hyopneumoniae* infection in pigs in these 2 herds, even though the isolation rate was very low. The high seropositivity rates for *M. hyopneumoniae* detected in a previous study in Turkey also support this status. Because of the lack of antibiotic administration data for the study farms it was difficult to interpret whether infection was in the early stage of development or if had been already started within the herds. Currently, as the number of farms is small in Turkey, it is necessary to improve hygiene conditions at the farms and put into practice essential veterinary control protocols to prevent serious health problems in the pig industry in the future.

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**References**


