TÜBİTAK

Research Article

Turk. J. Vet. Anim. Sci.
2009; 33(1): 61-65
© TÜBİTAK
doi:10.3906/vet-0711-16

Detection of *Mycoplasma hyopneumoniae* in Pigs in Turkey

Arzu Funda BAĞCIGİL*, Serkan İKİZ , N. Yakut ÖZGÜR

Department of Microbiology, Faculty of Veterinary Medicine, İstanbul University, Avcılar, 34320, İstanbul - TURKEY

Received: 12.11.2007

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 Varlığının Araştırılması

Özet: *Mycoplasma hyopneumoniae* domuzlarda enzootik pneumoni'nin primer etkenidir. Bu araştırmada, Türkiye'de yetiştirilen domuzlarda *M. hyopneumoniae*'nın varlığının araştırılması amaçlandı. İki yüz seksen altı domuza ait akciğer örnekleri, kesim esnasında alındı. Her bir akciğer örneği 5 ml Friis sıvı besiyeri içerisinde homojenize edildi ve süspansiyonlardan yeni Friis besiyerlerine ekim yapıldı ve 37 ºC'de asidik renk değişikliği oluşana kadar ya da 21 gün süreyle inkube edildi. Kültürler *M. hyopneumoniae*-spesifik PCR ile identifiye edildi. *M. hyopneumoniae* 286 akciğer örneğinin 2 (% 0,70)'sinden izole edildi. Bu çalışmada Türkiye'de domuzlardan ilk *M. hyopneumoniae* izolasyonu bildirilmektedir.

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).

* E-mail: fucigil@istanbul.edu.tr

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 TCACCAAGA-3'; reverse primer 5'- TGTGTTAGTGACTTT TGCCACC-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 (1,4), bronchial swabs (5), and air samples (2) have been

described. Mattsson et al. (1) detected *M. hyopneumoniae* in nasal swabs from naturally infected pigs, but only during the limited time period of infection. Otagiri et al. (4) indicated that under experimental conditions the diagnosis of mycoplasmal pneumonia by nPCR in individual pigs with nasal swabs is reliable, but under field conditions it should only be used for monitoring the disease status of a herd. In the same study nPCR failed to detect the organism in the nasal samples of pigs with lung lesions or large numbers of organisms in the lung, which was associated with the presence of PCR inhibitors in the nasal samples. In the current study, lungs, which are the main colonization site of *M. hyopneumoniae*, were selected as investigation material. Instead of nPCR, we preferred cultivation in combination with PCR for detecting *M. hyopneumoniae*, so as to reduce the amount of PCR inhibitors that might be present in the lung tissues and to increase the possibility of PCR detection by cultivating a number of viable organisms (personal communication, Dr. Branko Kokotovic).

The role of environmental and management factors in the clinical outcome of *M. hyopneumoniae* infection is reported to be very important; it has been documented that the infection is detected frequently and is of a more severe nature on farms that house pigs of different ages together, use inadequate sanitation or disinfection methods, and where overcrowding and poor ventilation conditions are observed. Appropriate antibiotic treatment and vaccination have been proven to be effective in decreasing the rate of infection in a herd (11-13). However, Vicca et al. (11) reported that good conditions do not automatically restrict the course of EP and that poor conditions do not always result in severe disease. The pig farms investigated in the present study were most likely not under veterinary control, and general hygiene conditions were poor and vaccination against *M. hyopneumoniae* was not used at any of the farms. Despite these negative conditions, isolation of *M. hyopneumoniae* only from 2 (0.70%) of the 286 pig lung samples was surprisingly low.

It is clear that because *M. hyopneumoniae* is a pathogen only for pigs the agent requires pig populations to spread. The study farms, despite being overpopulated and lacking adequate hygiene conditions, were located in different geographic areas, which 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 this 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.

Acknowledgement

This study was supported by The Scientific and Technological Research Council of Turkey, project number: TOVAG 106 O 242.

References

1. Mattsson, J.G., Bergström, K., Wallgren, P., Johansson, K.E.: Detection of *Mycoplasma hyopneumoniae* in nose swabs from pigs by in vitro amplification of the 16S rRNA gene. J. Clin. Microbiol., 1995; 33: 893-897.
2. Stärk, K.D.C., Nicolet, J., Frey, J.: Detection of *Mycoplasma hyopneumoniae* by air sampling with a nested PCR assay. Appl. Environ. Microbiol., 1998; 64: 543-548.
3. Caron, J., Quardani, M., Dea, S.: Diagnosis and differentiation of *Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis* infections in pigs by PCR amplification of the p36 and p46 genes. J. Clin. Microbiol., 2000; 38: 1390-1396.
4. Otagiri, Y., Asai, T., Okada, M., Uto, T., Yazawa, S., Hirai, H., Shibata, I., Sato, S.: Detection of *Mycoplasma hyopneumoniae* in lung and nasal swab samples from pigs by nested PCR and culture methods. J. Vet. Med. Sci., 2005; 67: 801-805.
5. Calsamiglia, M., Collins, J.E., Pijoan, C.: Correlation between the presence of enzootic pneumonia lesions and detection of *Mycoplasma hyopneumoniae* in bronchial swabs by PCR. Vet. Microbiol., 2000; 76: 299-303.
6. Assunção, P., De la Fe, C., Kokotovic, B., González, O., Poveda, J.B.: The occurrence of mycoplasmas in the lungs of swine in Gran Canaria (Spain). Vet. Res. Commun., 2005; 29: 453-462.
7. Kobisch, M., Friis, N.F.: Swine mycoplasmoses. Rev. Sci. Tech., 1996; 15: 1569-1605.
8. Sørensen, V., Ahrens, P., Barfod, K., Feenstra, A.A., Feld, N.C., Friis, N.F., Bille-Hansen, V., Jensen, N.E., Pedersen M.W.: *Mycoplasma hyopneumoniae* infection in pigs: duration of the disease and evaluation of four diagnostic assays. Vet. Microbiol., 1997; 54: 23-34.
9. Baumeister, A.K., Runge, M., Ganter, M., Feenstra, A.A., Delbeck, F., Kirchhoff, H.: Detection of *Mycoplasma hyopneumoniae* in bronchoalveolar lavage fluids of pigs by PCR. J. Clin. Microbiol., 1998; 36: 1984-1988.
10. Verdin, E., Saillard, C., Labbé, A., Bové, J.M., Kobisch, M.: A nested PCR assay for the detection of *Mycoplasma hyopneumoniae* in tracheobronchiolar washings from pigs. Vet. Microbiol., 2000; 76: 31-40.
11. Vicca, J., Stakenborg, T., Maes, D., Butaye, P., Peeters, J., de Kruif, A., Haesebrouck, F.: Evaluation of virulence of *Mycoplasma hyopneumoniae* field isolates. Vet. Microbiol., 2003; 97: 177-190.
12. Sibila, M., Calsamiglia, M., Vidal, D., Badiella, L., Aldaz, A., Jensen, J.C.: Dynamics of *Mycoplasma hyopneumoniae* infection in 12 farms with different production systems. Can. J. Vet. Res., 2004; 68: 12-18.

13. Vicca, J., Maes, D., Jonker, L., de Kruif, A., Haesebrouck, F.: Efficacy of in-feed medication with tylosin for the treatment and control of *Mycoplasma hyopneumoniae* infections. Vet. Rec., 2005; 156: 606-610.

14. Çayukli, S.: Serological and bacteriological investigation of *Mycoplasma hyopneumoniae* infection (Swine Enzootic Pneumonia) in Pigs. Master Thesis. İstanbul University, Institute of Health Science, Department of Microbiology, İstanbul, Turkey. 2007.

15. Huhn, R.G.: Swine enzootic pneumonia: age susceptibility and treatment schemata. Can. J. Comp. Med., 1971; 35: 77-81.

16. Jericho, K.W.F.: Pathogenesis of mycoplasma pneumonia of swine. Can. J. Vet. Res., 1986; 50: 136-137.