The Occurrence of Mycoplasmas in the Lungs of Swine in Gran Canaria (Spain)

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ABSTRACT

The study was conducted to investigate the mycoplasmal flora in the lungs of pigs with enzootic pneumonia at Gran Canaria (Spain). From 54 pneumatic lungs collected at an abattoir, 85 isolates were cultivated. On the basis of cultural and biochemical characteristics, the isolates were preliminarily identified as Mycoplasma species. Using different species-specific PCRs, 40, 27, 11 and 7 of the isolates were identified as M. hyorhinis, M. hyopneumoniae, M. hyosynoviae and M. floculare, respectively. Nine of the M. hyopneumoniae cultures were found to be in mixed culture with M. floculare as demonstrated by PCR. By use of a M. floculare antiserum it was possible to eliminate M. floculare from M. hyopneumoniae mixed cultures. This study is the first report on isolation of porcine mycoplasmas at Gran Canaria (Spain).

Keywords: PCR, isolation, mycoplasma, diagnostic, porcine enzootic pneumonia

Abbreviations: dNTP, deoxynucleoside triphosphatase; EP, enzootic pneumonia; NCTC, National Collection of Type Cultures; PCR, polymerase chain reaction; PRDC, porcine respiratory disease complex

INTRODUCTION

Enzootic pneumonia (EP) is a chronic respiratory disease that causes considerable economic losses to the pig industry worldwide (Kobisch and Friis, 1996). EP has been described as a multifactorial disease the consequences of which are greatly influenced by environmental and management factors (Done, 1991). Owing to the description of a new respiratory syndrome in which several infectious agents are involved, the tendency now is to speak more of a porcine respiratory disease complex (PRDC), in which Mycoplasma hyopneumoniae is one of the most important pathogens involved. (Calsamiglia et al., 2000; Thacker, 2001)

Conventional laboratory diagnosis of M. hyopneumoniae is based on cultivation of the organism in artificial media and serological identification of the isolates. However,
the primary isolation of *M. hyopneumoniae* is tedious and time-consuming, mainly because of the fastidious nature of the organism, and may be complicated further by the presence of *Mycoplasma hyorhinis* and *Mycoplasma flocculare* in the investigated samples (Kobisch, 2000). *M. hyorhinis* is associated with different disorders, including pneumonia, polyserositis and arthritis (Friis and Feenstra, 1994), but can also frequently be found in the respiratory tract of healthy pigs. *M. flocculare* can be found in nasal cavities and normal and pneumatic lungs of pigs, and has no apparent pathogenic capabilities (Friis, 1974; Strasser et al., 1992). In addition to these mycoplasmas, *M. hyosynoviae*, which is the primary agent of non-purulent arthritis in growing pigs (Ross and Duncan, 1970), can occasionally be found in the lower respiratory tract as a secondary invader of pre-existing pneumatic lesions (Friis, 1971).

With the advent of PCR technology, new approaches to diagnosis of porcine mycoplasmas have emerged. In the past few years, several one-step or nested PCR protocols have been described for rapid and specific identification of *M. hyopneumoniae* (Mattsson et al., 1995; Baumeister et al., 1998; Calsamiglia et al., 1999; Verdin et al., 2000; Kurth et al., 2002) as well as *M. hyorhinis*, *M. flocculare* (Stenke et al., 1994; Caron et al., 2000) and *M. hyosynoviae* (Ahrens et al., 1996).

The aim of this study was to isolate and to identify mycoplasmas from lungs of pigs with EP and thereby shed light on occurrence of porcine mycoplasmas in Gran Canaria (Spain).

**MATERIAL AND METHODS**

**Samples and mycoplasma isolation**

A total of 54 lungs that showed lesions consistent with EP were collected from 54 pigs, 20–24 weeks old, being slaughtered at a Gran Canaria abattoir in the period from January to August 2002. The animals were derived from at least 10 different herds. From each of the lungs, several samples were taken with sterile swabs; the number of samples taken was related to the extension of the EP lesions. The total number of samples processed was 142. A culture medium was prepared according to Friis (1975a) with the modifications that the medium contained exclusively horse serum (20%) and ampicillin (0.5 mg/ml) was used instead of penicillin. The culturing procedure was as follows. Samples were inoculated in a liquid medium and incubated at 37°C in a rotatory shaker at 12 rpm for 24 h. The cultures were then filtered through 0.45 μm filters (Pall, Ann Arbor, MI, USA) and incubated further until a pH change in the medium was visible. Subsequently, 200 μl aliquots of the cultures were grown on a solid medium without antibiotics in an aerobic atmosphere in order to observe development of colonies. The isolates were cloned three times prior to further examination. At the same time, an aliquot of each culture was passaged to Columbia agar (Oxoid) to check the absence of bacterial growth.