Veterinary Microbiology 203 (2017) 1–5

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Occurrence of *Mycoplasma hyorhinis* infections in fattening pigs and association with clinical signs and pathological lesions of Enzootic Pneumonia

Adrian Luehrs[a,b], Salome Siegenthaler[a], Niels Grützner[a], Elisabeth grosse Beilage[b], Peter Kuhnert[c], Heiko Nathues[a,*]

[a] Clinic for Swine, Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, Bremgartenstr. 109a, 3001 Bern, Switzerland
[b] Field Station for Epidemiology, University of Veterinary Medicine Hannover, Foundation, Bueschelerstr. 9, 49456 Bakum, Germany
[c] Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Länggassstr. 122, 3001 Bern, Switzerland

ARTICLE INFO

Article history:
Received 21 September 2016
Received in revised form 1 February 2017
Accepted 1 February 2017

Keywords:
Pig disease
Enzootic Pneumonia
Coughing
Prevalence study

ABSTRACT

Respiratory disorders in fattening pigs are of major concern worldwide. Particularly Enzootic Pneumonia remains a problem for the pig industry. This chronic respiratory disease is primarily caused by *Mycoplasma hyopneumoniae (M. hyopneumoniae)*. However, more recently it was hypothesised that *M. hyorhinis* can also cause similar lung lesions.

To investigate the relevance of *M. hyorhinis* as a cause of pneumonia in fattening pigs 10 farms in Switzerland (considered free of Enzootic Pneumonia) and 20 farms in Germany (regarded as endemic for Enzootic Pneumonia) with a history of chronic and/or recurrent respiratory diseases were included in the study. During a one-time farm visit the coughing index was determined in the batch of oldest fattening pigs in each farm before submission to slaughter. In total, 1375 lungs from these pigs were collected at the abattoir and individually scored for lesions. Furthermore, 600 lungs with, if present, indicative lesions for Enzootic Pneumonia (purple to grey areas of tissue consolidation in the cranio-ventral lung lobes) were tested for mycoplasma species by culture and by real-time PCR for the presence of *M. hyorhinis* and *M. hyopneumoniae*.

In total, 15.7% of the selected lungs were tested positive for *M. hyorhinis* by real-time PCR. The prevalence of *M. hyorhinis* was 10% in Switzerland and 18.5% in Germany and differed significantly between these two countries ($p = 0.007$). *M. hyorhinis* was detected significantly more often in pneumonic lungs ($p = 0.004$) but no significant association was found between *M. hyorhinis* and the coughing index or the *M. hyopneumoniae* status of the pig. *M. hyopneumoniae* was detected in 0% and 78.5% of the selected lungs in Switzerland and Germany, respectively.

We found no evidence that *M. hyorhinis* alone can lead to similar lung lesions as seen by an infection with *M. hyopneumoniae* in fattening pigs. In addition, a simultaneous infection with both *M. hyorhinis* and *M. hyopneumoniae* did not aggravate the observed lung lesions. Moreover, the presence of *M. hyorhinis* showed no clinical effect in terms of coughing at least at the end of the fattening phase. However, different levels of virulence of *M. hyorhinis* isolates as well as interactions with viral pathogens like porcine reproductive and respiratory syndrome virus (PRRSV) or porcine circovirus type 2 (PCV2) were reported in the literature and need to be further investigated.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

M. hyorhinis is a commensal inhabitant on the mucosa of the upper respiratory tract and tonsils (Mare and Switzer, 1965). Most infections with this bacterium are subclinical and infected pigs show no clinical signs (Friis, 1971). However, when *M. hyorhinis* becomes systemic and spreads haematogenously, it can cause different diseases in pigs. The main clinical disorders are polyserositis and arthritis in swine of various ages, but primarily during nursery period (Thacker and Minion, 2012). The role of *M. hyorhinis* in the pathogenesis of porcine pneumonia is discussed controversially for decades. It has been shown that *M. hyorhinis*

* Corresponding author.
E-mail address: heiko.nathues@vetsuisse.unibe.ch (H. Nathues).

http://dx.doi.org/10.1016/j.vetmic.2017.02.001
0378-1135/© 2017 Elsevier B.V. All rights reserved.

infection can cause pneumonia in gnotobiotic piglets (Gois et al., 1971). Additionally, authors of different studies performed on conventionally raised piglets found indicative (catarrhal to suppurative pneumonia) pneumonic lesions after inoculation with *M. hyorhinis*, although the observed lesions were often mild, showed a trend to restitution and were only observed in a small percentage of the infected pigs (Gois et al., 1968; Kinne et al., 1991). However, in recent investigations it was hypothesised that lung lesions typical for Enzootic Pneumonia can also be provoked by an infection with *M. hyorhinis* without involvement of *M. hyopneumoniae* (Assuncao et al., 2005; Lin et al., 2006). These interpretations contrast strongly with expert opinions and current knowledge about Enzootic Pneumonia (Maes et al., 2008; Sibila et al., 2009). It is one of the most common and economically important disease in the pig industry worldwide and primarily caused by *M. hyopneumoniae* (Maes et al., 2008). This chronic respiratory disease leads to retarded growth, poor feed conversion, predisposition to bacterial pulmonary infections and increasing use of antibiotics. Enzootic Pneumonia is characterized by a chronic, dry and non-productive coughing and occurs in fattening pigs that are 16 to 22 weeks of age with a high morbidity but low mortality (Thacker and Minion, 2012). In Switzerland, the Swiss Pig Health Services carried out a nation-wide eradication programme between 1999 and 2003 in all pig farms. By then Enzootic Pneumonia became a notifiable disease in Switzerland and vaccination against *M. hyopneumoniae* is no longer allowed. In contrast to the *M. hyopneumoniae*-free pig population in Switzerland several countries, including Germany, are endemically infected with this bacterium (Thacker et al., 1999; grosse Beilage et al., 2009).

The aim of the present study was to determine the prevalence and relevance of infections with *M. hyorhinis* in the lower respiratory tract of fattening pigs in two different pig populations.

2. Methods

2.1. Selection of herds and coughing index

Ten farms in Switzerland and 20 farms in Germany with a history of chronic and recurrent respiratory disease in fattening pigs and current coughing in the oldest batch of pigs were selected. No antimicrobial treatment should be applied to this batch of fattening pigs two weeks before expected slaughter. The Swiss pig population served as the 'negative control' in terms of infections with *M. hyopneumoniae* as it has successfully passed an eradication programme. The effects of the infection level of *M. hyorhinis* by the eradication programme in this country were unknown. The pig population in Germany was expected to be positive for *M. hyopneumoniae* and *M. hyorhinis*. A batch of fattening pigs was clinically examined three to twelve days prior to slaughter at the slaughterhouse. During the examination the coughing index was determined in at least three groups (≥60 pigs in total) as described elsewhere (Nathues et al., 2012). The coughing index represents the average percentage of pigs coughing within one minute.

2.2. Sampling and lung scoring

At the slaughterhouse 50 lungs were randomly collected of pigs belonging to the group of which the coughing index was determined previously on farm. In Switzerland, in case that less than 50 pigs were submitted to slaughter all lungs of the batch were collected (median: 34.5, range: 21–50). All lungs were scored by the first author for lesions according to the scoring scheme of Madec and Kobisch (1982). This scheme is based on the evaluation of each individual lung. Thereby each of the seven lobes is divided into quarters and scored with points from 0, which means 'no lesions visible on the surface' to 4, which is given when ≥three-quarters of the surface of the lung is covered by lesions. Then the total individual lung score is counted up and ranges between 0 (no lesions) and 28 (whole lung affected).

Twenty lung samples out of the pool of up to 50 lungs per farms with, if present, indicative lesions for Enzootic Pneumonia (purple to grey areas of tissue consolidation in the cranio-ventral lung lobes) were selected and a lung section of 1.5 cm length of the edge was frozen in small aluminium containers (first liquid nitrogen, then −70 °C) until further laboratory examination (in total 600 lungs, Switzerland: 200, Germany: 400).

2.3. Isolation of M. hyorhinis

The frozen samples were thawed and a section of 0.5 cm length of the edge was brought into 2 ml of Mycoplasma Liquid Medium® (MLM, Mycoplasma Experience Ltd., UK). Then 100 μl were transferred to 900 μl MLM (dilution 10^{-1}). These diluted samples were incubated aerobically at 37 °C. If a colour change occurred during the first week, 10 μl of the broth culture were passaged and streaked onto Mycoplasma Solid Medium® (Mycoplasma Experience Ltd., UK) and incubated again aerobically at 37 °C. The plates were checked daily for colony growth by stereomicroscopy. If typical colonies were detected, three different looking single colonies suspected to be *M. hyorhinis* were picked and passaged to 1 ml MLM each, which were subsequently incubated at 37 °C air atmosphere. After colour change, cultures were tested for purity of *M. hyorhinis* by 16S-23S PCR (Nathues et al., 2011).

All samples from the initial culture (diluted sample) were tested by 16S-23S PCR regardless of whether a colour change occurred or not after 21–28 days of incubation.

2.4. PCR identification of Mycoplasma isolates

For DNA template preparation 50 μl of broth cultures were transferred into 450 μl lysis buffer (pH 8.5). The samples were lysed for one hour at 60 °C followed by 15 min at 97 °C. Lysed samples were used immediately or stored at −70 °C until further analysis.

The amplification of the 16S-23S IGS region was performed according to Nathues et al. (2011). The total of 25 μl reaction mixture contained 12.5 μl of ReadyMix™ Taq PCR Reaction Mix, Sigma-Aldrich, Austria), 10 pmol of each primer F2A and R2, and 3 μl DNA template. After a first denaturation step at 94 °C for 180 s. 35 cycles were run on a thermocycler (Mastercycler® pro S, Eppendorf AG, Germany) using the following conditions: denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 60 s. A final extension step was implemented at 72 °C for 300 s.

PCR products were analysed on 1.5% agarose gel and 1× Tris-Acetate-EDTA buffer at 170 V for 70 min. Amplification products were visualized by in-gel staining with Midori Green Advance® (Biozym Scientific GmbH, Germany) according to manufacturer's instructions and photographed under UV light using a UV transilluminator (AlphaImager® EC, ProteinSimple™, USA). Based on the size of the generated amplicons the PCR allowed a differentiation into *M. hyosynoviae* (260 bp), *M. hyorhinis* (320 bp), *M. hyopharyngis* (360 bp), *M. flocculare* (450 bp) and *M. hyopneumoniae* (580 bp).

2.5. M. hyorhinis *and* M. hyopneumoniae *real-time PCR*

For the direct purification of DNA from 10 mg of each lung tissue sample the DNeasy® Blood & Tissue Kit (Qiagen GmbH, Germany) was used according to manufacturer's instructions. The elution step was performed with 200 μl elution buffer (provided by the manufacturer). The DNA eluate was used immediately or stored at −70° until further analysis.

Download English Version:

https://daneshyari.com/en/article/5545316

Download Persian Version:

https://daneshyari.com/article/5545316

Daneshyari.com