Preventive Veterinary Medicine 145 (2017) 67–72

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

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

Persistence of *Mycoplasma hyopneumoniae* sequence types in spite of a control program for enzootic pneumonia in pigs

Gudrun Overesch, Peter Kuhnert*

Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Länggasstrasse 122, 3012 Bern, Switzerland

ARTICLE INFO

Article history:
Received 5 January 2017
Received in revised form 10 May 2017
Accepted 16 June 2017

Keywords:
Epidemiology
Genotyping
Swine disease
Eradication
MLST
p146

ABSTRACT

Enzootic pneumonia (EP) in pigs caused by *Mycoplasma (M.) hyopneumoniae* has successfully been combatted in Switzerland. A control program was fully implemented in 2004 which is based on total depopulation strategies of affected fattening farms as well as partial depopulation on breeding farms. Thereby, the number of cases has dropped drastically from more than 200 in 2003 to two cases in 2013. Currently monitoring is done based on clinical observation and subsequent diagnostic of coughing pigs. Moreover, in case of more than 10% gross pathological lesions per slaughter batch laboratory confirmation for EP is compulsory. Despite these strict measures it was not possible to eliminate *M. hyopneumoniae* from Swiss pig production. In fact, during the last few years the number of EP cases has slightly increased. Therefore, genotyping of the involved *M. hyopneumoniae* strains was conducted in order to elucidate possible sources and routes of infection. All available and typeable samples from totally 22 cases during the period 2014–2016 were investigated by extended multilocus sequence typing (MLST). A total of 16 cases, including eight from 2014, five from 2015 and three from 2016 could thereby be included in the study. MLST revealed that the majority of cases in 2014/2015 were due to two major spread scenarios, i.e. two *M. hyopneumoniae* sequence types, each scenario involving six individual production farms in five to six different Cantons (states), respectively. Moreover, by comparison of archived sequences some sequence types were observed over ten years demonstrating their persistence over a long time and the possible partial failure of elimination measures in Switzerland. Insufficient sanitation on affected farms and subsequent animal transport of symptomless infected pigs could lead to recurrent cases. Wild boar harbor identical strains found with EP but solid data are missing to assign a role as reservoir to this wild animal. Implementing a monitoring scheme for *M. hyopneumoniae* in wild boar in combination with genotyping of all available samples from domestic pigs could direct responsible authorities to possible gaps and deficiencies of control measures taken for combating enzootic pneumonia. With the newly installed PubMLST database sequence types for *M. hyopneumoniae* are now available and allow tracing back strains on the international level.

1. Introduction

Enzootic pneumonia (EP) in domestic pigs is endemic in countries with pig production and caused by *Mycoplasma (M.) hyopneumoniae* (Simionatto et al., 2013). The disease shows low mortality but high morbidity thereby having a high economic impact for producers (Sibila et al., 2009). In 1992 64% of Swiss swine lung at slaughter showed lesions consistent with EP. Therefore, in Switzerland a national control program was initiated in 1996 and fully implemented by 2004 (Stark et al., 2007). Since then EP is a notifiable and no longer endemic disease in Switzerland (Luehrs et al., 2017). The aim of this program is not eradication of the agent per se but absence of disease. Cantonal veterinary services take cost of diagnostics and are allowed to take measures against infected farms. Vaccination is forbidden in Switzerland. The program is based on total depopulation strategies of affected fattening farms as well as partial depopulation on breeding and breeding to finishing farms. During partial depopulation young animals are removed from the farm, whereas adult breeding pigs are medicated. In 2010 more detailed directives concerning possible measures to be taken in case of an outbreak were implemented. These directives comprise possible antimicrobial treatment options for fattening herds until slaughter and it is not mandatory to isolate affected pigs in special quarantine stables if no vulnerable farms are in the vicinity (Swiss ordinance of epizootics). Surveillance of

* Corresponding author.
E-mail addresses: gudrun.overesch@vetsuisse.unibe.ch (G. Overesch), peter.kuhnert@vetsuisse.unibe.ch (P. Kuhnert).

http://dx.doi.org/10.1016/j.prevetmed.2017.06.007
0167-5877/

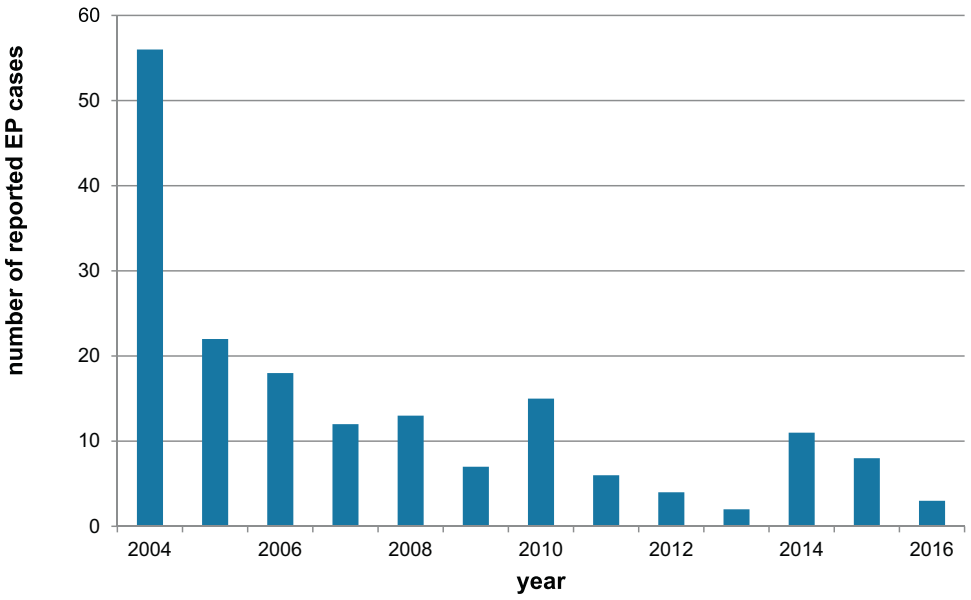

Fig. 1. Number (n) of enzootic pneumonia (EP) cases in Switzerland 2004–2016. The number of yearly reported and/or diagnosed cases since full implementation of the EP sanitation program is shown. Data were taken from the infoSM database at the FSVO (infosm.blv.admin.ch, 31.12.2016) and the diagnostics from the Swiss national reference laboratory for EP.

EP is implemented based on clinical observation of infection with subsequent diagnostic on nasal swabs from coughing individuals (Zeeh et al., 2005). Moreover, if more than 10% gross pathological lung lesions per slaughter batch are observed suspicious lungs have to undergo EP diagnostics by licensed laboratories. For diagnostics a real-time PCR assay was evaluated and prescribed showing 100% specificity and 85% sensitivity on herd level for lung samples (Dubosson et al., 2004). For nasal swabs this real-time PCR turned out to be 100% specific and 100% sensitive on herd level when at least 10 pigs, showing coughing, are analyzed (Zeeh et al., 2005). By implementing the EP sanitation program in Switzerland the number of cases has dropped drastically from more than 200 in 2003 to two cases in 2013 (Fig. 1), and the incidence decreased from more than 60% to less than 1% (Stark et al., 2007). However, although measures were implemented, it was not possible to completely combat EP from Swiss pig production. In contrast, since 2014 the number of EP cases slightly increased to 11 in 2014, eight cases in 2015 and decreased again to three in 2016 (Fig. 1). The control is challenging if the sources of infection are not identified, especially when the disease is endemic outside the national borders. Defining *M. hyopneumoniae* strains by multilocus sequence typing (MLST) extended with p146 typing was shown to be crucial for knowledge on the epidemiology of EP in the past (Kuhnert and Overesch, 2014). Although *M. hyopneumoniae* exhibits high strain variability, it was shown in Switzerland that identical strains can be identified within an affected farm and that geographically closely linked cases are indeed caused by the same genotype (Mayor et al., 2007, 2008). The same was true for operationally linked production sites, whereas unrelated farms also had unrelated *M. hyopneumoniae* strains responsible for the disease. We recently investigated a possible role of wild boar as a reservoir for *M. hyopneumoniae* (Kuhnert et al., 2011; Kuhnert and Overesch, 2014). Indeed, wild boar do harbor *M. hyopneumoniae* and sometimes even identical strains that can be found in domestic pigs. However, such identical genotypes were only found in wild boar in the vicinity of a recent EP-case after but never before clinical manifestation on the farm. Wild boar seems to be more likely recipient rather than transmitter of *M. hyopneumoniae*. This observation was later corroborated by a risk-factor study (Batista Linhares et al., 2015).

In the present study the long term persistence of certain *M. hyopneumoniae* sequence types in the Swiss pig population for more than ten years could be demonstrated for the first time. Moreover, possible linkage of Swiss EP genotypes to *M. hyopneumoniae* strains abroad were not under investigation up to now, as the typing data were not available on an internationally linked database. By establishing a PubMLST database (Jolley and Maiden, 2010) the epidemiology of *M. hyopneumoniae* could be elucidated for the first time on the level of defined sequence types. Implementation of molecular typing reveals the importance of possible risk factors and thereby supports authorities to take effective control measures.

2. Materials and methods

All samples received for routine diagnostics of EP by the national reference laboratory for enzootic pneumonia at the Institute of Veterinary Bacteriology, University of Bern, Switzerland were analyzed (Table 1). Suspicious lungs were sent in from slaughterhouses, if more than 10% of a slaughter batch showed typical pulmonic lesions. Nasal swabs were taken from life pigs if coughing was observed at farm. Lungs from at least 3 suspicious animals and nasal swabs from at least 10 coughing animals from a farm were tested. Lysates were prepared and tested for the presence of *M. hyopneumoniae* by real-time PCR as previously described (Dubosson et al., 2004). Samples being strongly positive (Ct-value < 30) could further be genotyped directly (Mayor et al., 2007, 2008). The three genes used for MLST as well as the p146 gene were combined in an extended MLST scheme. The resulting four DNA sequences were entered into the *M. hyopneumoniae* database (Bionumerics v.7.6, Applied Maths, Sint-Martens-Latem, Belgium) containing archived sequence data of >300 *M. hyopneumoniae* from EP cases as well as from > 100 wild boar samples over a time span of more than 10 years. A UPGMA cluster analysis was performed using a pairwise alignment based on default Bionumerics similarity calculation with no correction to recognize corresponding genotypes. Moreover, sequence data were entered to and analyzed in the new PubMLST database (http://pubmlst.org/mhyopneumoniae) for designation of sequence type (ST). The *adk, rpoB, tpiA and* p146 genes were used for the PubMLST database established in the framework of this project.