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Genetic diversity of *Mycoplasma hyopneumoniae* isolates of abattoir pigs☆

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ABSTRACT

Mycoplasma hyopneumoniae, the causative agent of porcine enzootic pneumonia, is present in swine herds worldwide. However, there is little information on strains infecting herds in Canada. A total of 160 swine lungs with lesions suggestive of enzootic pneumonia originating from 48 different farms were recovered from two slaughterhouses and submitted for gross pathology. The pneumonic lesion scores ranged from 2% to 84%.

Eighty nine percent of the lungs (143/160) were positive for *M. hyopneumoniae* by real-time PCR whereas 10% (16/160) and 8.8% (14/160) were positive by PCR for *M. hyorhinis* and *M. flocculare*, respectively. By culture, only 6% of the samples were positive for *M. hyopneumoniae* (10/160). Among the selected *M. hyopneumoniae*-positive lungs ($n = 25$), 9 lungs were co-infected with *M. hyorhinis*, 9 lungs with PCV2, 2 lungs with PRRSV, 12 lungs with *S. suis* and 10 lungs with *P. multocida*. MLVA and PCR-RFLP clustering of *M. hyopneumoniae* revealed that analyzed strains were distributed among three and five clusters respectively, regardless of severity of lesions, indicating that no cluster is associated with virulence. However, strains missing a specific MLVA locus showed significantly less severe lesions and lower numbers of bacteria. MLVA and PCR-RFLP analyses also showed a high diversity among field isolates of *M. hyopneumoniae* with a greater homogeneity within the same herd. Almost half of the field isolates presented less than 55% homology with selected vaccine and reference strains.

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1. Introduction

Mycoplasma hyopneumoniae is present in the majority of swine herds around the world (Kobisch and Friis, 1996). It is the primary agent involved in porcine enzootic pneumonia (EP). This condition is associated with respiratory disease and reduced productivity in pigs causing severe economic losses to the swine industry. The importance of *M. hyopneumoniae* is also linked to its ability to increase the severity of infections caused by viruses (Opriessnig et al., 2004), as well as bacteria (Marois et al., 2009; Palzer et al., 2008). When these pathogens are in co-infection with *M. hyopneumoniae*, the severity of the respiratory lesions is increased. Moreover, *M. hyopneumoniae* can enhance the quantity and the persistence of PVC2

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antigens and can increase the incidence of postweaning multisystemic wasting syndrome (PMWS) in swine (Opriessnig et al., 2004; Thacker et al., 2001).

Isolation of *M. hyopneumoniae* is known to be fastidious due to the long incubation period needed for its culture (Friis, 1975; Marois et al., 2007) and to the frequent co-isolation of *Mycoplasma hyorhinis*, a normal flora inhabitant of the upper respiratory tract of young pigs (Kobisch and Friis, 1996). *M. hyorhinis* has also been involved in a variety of diseases in swine including enzootic pneumonia and respiratory disease in general (Kawashima et al., 1996; Kobisch and Friis, 1996; Lin et al., 2006).

At the genomic level, high heterogeneity has been demonstrated between *M. hyopneumoniae* isolates throughout the world using various typing techniques such as random amplified polymorphic DNA (RAPD) (Artiushin and Minion, 1996), amplified fragment length polymorphism (AFLP) (Kokotovic et al., 1999) and pulsed-field gel electrophoresis (PFGE) (Stakenborg et al., 2005). However, the RAPD technique and the analysis of polyserine repeat have weak reproducibility rates among different laboratories, and the AFLP and PFGE techniques are considered fastidious. Thus, new techniques based on DNA amplification have been developed in the last few years. The multiple loci variable number of tandem repeats (VNTR) analysis (MLVA) and the PCR combined with restricted fragments length polymorphism (PCR-RFLP) are two methods that can be easily performed, are reproducible and have a high discriminatory power (Marois-Créhan et al., 2012; Stakenborg et al., 2006b; Vranckx et al., 2011). Recently, a MLVA assay was described as a tool to differentiate *M. hyopneumoniae* strains in samples from the respiratory tract without prior cultivation (Vranckx et al., 2011). Previous studies have shown genetic heterogeneity between isolates from different farms (Mayor et al., 2007; Nathues et al., 2011; Stakenborg et al., 2005). However, other reports have shown both genetic heterogeneity and homogeneity between isolates from the same herds (Maes et al., 2008; Marois-Créhan et al., 2012). Field isolates of *M. hyopneumoniae* have also shown virulence variability (Vicca et al., 2003).

Actually, little is known about *M. hyopneumoniae* isolates found in Canada. The aim of this study was to evaluate the genetic diversity of *M. hyopneumoniae* isolated from single or mixed infections from abattoir pigs.

2. Materials and methods

2.1. Sample collection and histopathology

A total of 160 swine lungs presenting gross lesions suggestive of porcine enzootic pneumonia, originating from 48 farms, were recovered from two slaughterhouses (#1, $n = 110$; #2, $n = 50$) located in the province of Quebec (Canada) from October 2008 to March 2009. The lungs were all scored for macroscopic pneumonic lesions as previously described by Straw et al. (1986). For *M. hyopneumoniae* isolation, swabs from the trachea and lungs were resuspended in 1 mL of buffered peptone water. A subset of 25 *M. hyopneumoniae*-positive lungs by real-time PCR (Table 1) were further analyzed for the detection

Table 1
Severity of lesions, quantification of *M. hyopneumoniae* in lungs with lesions suggestive of EP with or without other pathogens in abattoir pigs.

Lung identification number	Severity of lesions (%)	*M. hyopneumoniae* culture[a]	*M. hyopneumoniae* quantification (genome/mL)	*M. hyorhinis*	PRRSV	PCV 2	*S. suis*	*P. multucida*	*H. parasuis*	*APP*	*A. suis*
#101	69	*Mhp*/*Mhr*	9.20×10^6	+	–	–	+	+	–	–	–
#105	60	*Mhp*/*Mhr*	1.20×10^9	+	+	–	–	–	–	–	–
#112	57	*Mhp*/*Mhr*	4.16×10^8	+	–	–	+	+	–	–	–
#122	41	*Mhp*	3.01×10^8	–	–	–	–	+	–	–	–
#119	20	*Mhr*	1.14×10^6	–	–	–	+	+	–	–	–
#120	5	*Mhr*	1.41×10^6	–	–	–	+	–	–	–	–
#123	17	*Mhp*/*Mhr*	3.25×10^7	+	–	–	+	+	–	–	–
#125	24	*Mhp*/*Mhr*	1.59×10^9	+	–	–	+	–	–	–	–
#127	23	*Mhp*/*Mhr*	9.87×10^7	+	–	–	–	+	–	–	–
#132	14	*Mhp*/*Mhr*	1.90×10^8	+	–	+	+	–	–	–	–
#135	22	*Mhp*/*Mhr*	9.45×10^8	+	–	+	–	–	–	–	–
#149	17	*Mhp*/*Mhr*	5.49×10^8	+	–	+	–	+	–	–	–
#007	14	–	3.01×10^7	–	–	–	–	–	–	–	–
#014	44	–	1.82×10^6	–	–	–	–	–	–	–	–
#021	25	–	6.74×10^7	–	–	+	–	–	–	–	–
#028	33	–	3.16×10^8	–	–	+	+	+	–	–	–
#035	22	–	1.26×10^7	–	–	–	+	–	–	–	–
#042	27	–	5.09×10^7	–	–	+	–	–	–	–	–
#049	22	–	1.02×10^8	–	–	–	+	–	–	–	–
#056	45	–	1.03×10^8	–	+	+	–	+	–	–	–
#063	8	–	4.40×10^8	–	–	–	–	–	–	–	–
#070	42	–	4.62×10^8	–	–	+	–	+	–	–	–
#077	25	–	2.02×10^6	–	–	+	+	–	–	–	–
#084	62	–	1.12×10^8	–	–	–	–	–	–	–	–
#091	48	–	2.10×10^8	–	–	–	+	–	–	–	–

Results for *M. hyorhinis*, PRRSV and PCV2 are from PCR testing whereas those for *S. suis*, *H. parasuis*, *P. multocida*, *A. suis* and *A. pleuropneumoniae* are from traditional bacteriological culture.
PRRSV: Porcine reproductive and respiratory syndrome virus; PCV2: Porcine circovirus type 2; *APP*: *Actinobacillus pleuropneumoniae*.
[a] *Mhp*: *M. hyopneumoniae*; *Mhr*: *M. hyorhinis*; shifted cultures were confirmed by multiplex PCR.

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