Presence of \textit{Actinobacillus pleuropneumoniae}, \textit{Streptococcus suis}, \textit{Pasteurella multocida}, \textit{Bordetella bronchiseptica}, \textit{Haemophilus parasuis} and \textit{Mycoplasma hyopneumoniae} in upper respiratory tract of swine in farms from Aguascalientes, Mexico

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ABSTRACT
Respiratory diseases are one of the most important health problems in pig herds. The porcine respiratory disease complex (PRDC) is the term used to describe pneumonic diseases caused by multiple infectious agents that provoke weight loss in animals or death. In the PRDC multiple pathogens (bacteria and/or viruses) work in combination to induce this respiratory disease. Within this complex, \textit{Actinobacillus pleuropneumoniae}, \textit{Streptococcus suis}, \textit{Pasteurella multocida}, \textit{Bordetella bronchiseptica}, \textit{Haemophilus parasuis} and \textit{Mycoplasma hyopneumoniae} are the main bacterial pathogens involved in great economic losses to the swine industry. The aim of this work was to estimate the presence of \textit{A. pleuropneumoniae}, \textit{S. suis}, \textit{P. multocida}, \textit{B. bronchiseptica}, \textit{H. parasuis} and \textit{M. hyopneumoniae} in the upper respiratory tract of pigs in representative swine farms in Aguascalientes, Mexico, using PCR technique. The study was performed in 14 swine farms. We obtained a total of 212 nasal swabs. Near 20\% of samples were positive for \textit{A. pleuropneumoniae} (located in the 79\% of farms); 17\% were positive for \textit{S. suis} (in 86\% of farms); of these, 3\% were \textit{S. suis} serovar 2; 30\% were positive for \textit{H. parasuis} (93\% of farms); 23\% of the samples to \textit{P. multocida} (in 79\% of farms); and 19\% to \textit{M. hyopneumoniae} (in 64\% of farms). \textit{B. bronchiseptica} was not detected in this study. The results obtained show that bacterial pathogens of PRDC were present in the upper respiratory tract of pigs in all farms studied; therefore, these pathogens are widely disseminated in pig farms of Aguascalientes, Mexico.

Keywords: Porcine Respiratory Disease Complex; \textit{Actinobacillus pleuropneumoniae}; \textit{Streptococcus suis}; \textit{Pasteurella multocida}; \textit{Bordetella bronchiseptica}; \textit{Haemophilus parasuis}; \textit{Mycoplasma hyopneumoniae}

1. INTRODUCTION
Respiratory diseases are one of the most important health problems in pig herds. Due to the multifactorial nature of these diseases, they are considered as a porcine respiratory disease complex (PRDC). The PRDC is the term used to describe pneumonic diseases caused by multiple infectious agents, which produce weight loss in animals or death. The PRDC is a major health problem in the current production of pigs [1,2]. Within this complex, the bacteria \textit{A. pleuropneumoniae}, \textit{S. suis}, \textit{P. multocida},
2.3. DNA Isolation

was performed as described by Sam-

2.2. Control Strains

The objective of this work was to estimate the pres-

2.1. Sampling Procedures

For this study were selected 14 farms producing pigs

2.4. PCR Reactions

PCR amplifications were carried out in a DNA thermal
cycler (TECHNE T-412). PCR against *A. pleuropneu-
moniae* was performed as described by Schäller et al. [24],
with modifications for MacInnes et al. [3] and Loera et al. [23]. The PCR run conditions were: 95°C for 1 min
followed by 30 cycles of 94°C for 30 s, 54°C for 30 s and
72°C for 1 min with a final elongation step at 72°C for 5
min. Multiplex PCR for detection of *S. suis* was devel-
oped based on the method of Marois et al. [25]. Multi-
plex PCR permitted the simultaneous detection of the *S.
 suis* species (primers 16S-195 [s] and 16S-489 [as2]) and
serotypes 2 and 1/2 (primers cps2J-s and cps2J-as).
The amplification conditions were: 1 cycle at 95°C for 1
min, 40 cycles at 94°C for 30 s, 60°C for 30 s, 72°C for 1
min and a final elongation cycle at 72°C for 10 min. The
PCR protocol for detection of *H. parasuis* was used like
previously described Oliveira et al. [26] with modifications
for MacInnes et al. [3]. The PCR run conditions were:
95°C for 5 min followed by 30 cycles of 94°C for 30 s, 59°C for 30 s and 72°C for 2 min with a final elonga-
tion step at 72°C for 5 min. PCR amplification of toxigenic
strains of *P. multocida* was developed under the condi-
tions of Kamp et al. [27]. The amplification conditions
were: 1 cycle at 95°C for 1 min, 32 cycles at 95°C for 30
s, 65°C for 60 s, 72°C for 2.5 min and a final elongation
cycle at 72°C for 20 min. The PCR for *B. bronchiseptica*
was performed using the method Hozbor et al. [28], with
modifications by Resgister and DeJong [29]. The PCR run conditions were: 1 cycle at 95°C for 1 min, 35 cycles
at 94°C for 60 s, 53°C for 30 s, 72°C for 20 s and a final

2. MATERIALS AND METHODS

2.1. Sampling Procedures

For this study were selected 14 farms producing pigs
in the State of Aguascalientes, Mexico, for convenience
and willingness of producers. The sampling period was
from June to October 2011. The total number of pigs
sampled was 212 (Table 1). The sample size was calcu-
lated according to Alvarez et al. [21], for a study in the
State of Yucatan, Mexico, to sought *M. hyopneumoniae*, *H. parasuis* and *M. hyopneumoniae* in the upper respira-
tory tract of asymptomatic pigs in swine farms in Aguascalientes, Mexico, using PCR technique.

2.2. Control Strains

All strains used as positive control in this study are
from laboratory of Dr. Mario Jacques. The control strains
were: *A. pleuropneumoniae* serovar 1-4074, *S. suis* ser-
rovar 2-735, *H. parasuis* serovar 5 (Nagazaki), *P. multo-
cida* 4-4056 (type D, DNT+), *M. hyopneumoniae* ATCC
25095 and *B. bronchiseptica* 276. Negative control used
was *Escherichia coli* ATCC 25922.

2.3. DNA Isolation

DNA isolation was performed as described by Sam-

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### Table 1. Presence of swine respiratory pathogens in upper respiratory tract of swine and its distribution per farm.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Nasal Swab Samples</th>
<th>App</th>
<th>Ss</th>
<th>Ss ser 2</th>
<th>Hp</th>
<th>Pm</th>
<th>Bb</th>
<th>Mh</th>
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<td>Total</td>
<td>212</td>
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<td>36</td>
<td>7</td>
<td>63</td>
<td>48</td>
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<td>Infected Farms (n)</td>
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<td>11</td>
<td>12</td>
<td>4</td>
<td>13</td>
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<td>Farm Distribution (%)</td>
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<td>78.6</td>
<td>85.7</td>
<td>28.6</td>
<td>92.9</td>
<td>78.6</td>
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<td>64.3</td>
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### Table 2. Primers sequences used in this study.

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<thead>
<tr>
<th>Name</th>
<th>Sequence (5’-3’)</th>
<th>Product size (pb)</th>
<th>Reference</th>
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<td>APXIVANEST1</td>
<td>GGG GAC GTA ACT CGG TGA TT</td>
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<td>APXIVANEST1R</td>
<td>GCT CAC CAA CGT TT CTC</td>
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<td>16S-195 (s)</td>
<td>CAG TAT TTA CCG CAT GTG AGA</td>
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<td>Marois et al. [25]</td>
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<td>16S-489 (as2)</td>
<td>TAT GTA AGA TAC CGT CAA GTG AGA A</td>
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<td></td>
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<td>cps2J-s</td>
<td>GTG GAC TCC TTA TAC ACC TGT T</td>
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<td>Marois et al. [25]</td>
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<td>cps2J-as</td>
<td>CAG AAA ATT CAT ATT GTC CAC C</td>
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<tr>
<td>HPS-forward</td>
<td>GTG ATG AGG AAG GTG GTG GT</td>
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<td>HPS-reverse</td>
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<td>TOXA set 1F</td>
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<td>338</td>
<td>Kamp et al. [27]</td>
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<td>TOXA set 1R</td>
<td>CCA AAC AGG GTT ATA TTC TGG AC</td>
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<td></td>
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<td>Fla 2</td>
<td>AGG CTC CCA AGA GAG AAA GGC TT</td>
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<td>Fla 4</td>
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<td>MH649-F</td>
<td>GAG CCT TCA AGC TTC ACC AGG A</td>
<td>649</td>
<td>Cai et al. [30]</td>
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<td>MH649-R</td>
<td>TGT GTT AGT GAC TTT TGC CAC C</td>
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</table>

elongation cycle at 72°C for 5 min. Last, PCR for detection of *M. hyopneumoniae* was developed using the method of Cai et al. [30]. The amplification conditions were: 1 cycle at 95°C for 1 min, 35 cycles at 94°C for 20 s, 60°C for 30 s, 72°C for 40 s and a final elongation cycle at 72°C for 7 min. All PCR reactions contained 0.25 μl 5 U·μl⁻¹ Taq DNA polymerase (Fermentas), and 2 μl template. All amplified products were observed by elec-
trophoresis in 1.5% agarose gel, stain with 1 μg ethidium bromide ml⁻¹. Images of the gels were captured using the Chemi Doc (Bio-Rad), image analyzer and the software Quantity One (Bio-Rad, California, USA). The primer sequences are shown in Table 2.

3. RESULTS AND DISCUSSION

As a representative sample, 14 pig farms distributed in all the area of the State of Aguascalientes, Mexico, were selected for this study. This region, located at central of the country, is semi-arid (average annual precipitation of 550 mm, 80% in the rainy season in summer), with an average annual temperature of 17°C - 18°C; however, in spring and summer the average daily temperature is above 30°C [31]. A total of 212 pigs were sampled, with around 15 pigs per farm (Table 1). Pigs were sampled randomly; they had no apparent signs of illness and were in normal production process on farms. Most pigs sampled were 1 - 2 years old; even though, reproductive sows with different ages were also sampled.

Of the 212 nasal swabs, 19.8% were positive to A. pleuropneumoniae and distributed in 78.6% of the farms sampled. Almost 17% of the samples were positive for S. suis, with a distribution in 85.7% of farms; of these, 3.3% were S. suis serovar 2 (the most virulent serovar). In the case of H. parasuis, 29.7% of the samples were positive and distributed in 92.9% of the farms. For P. multocida, 22.6% were positive, with a distribution in the 78.6% of the farms. For M. hyopneumoniae, 19.3% were positive, with a distribution in 64.3% of the farms sampled. Finally, no evidence of B. bronchiseptica in nasal swab samples was found in any of the farms studied (Table 1 and Figure 1).

Furthermore, 35.7% of farms showed the presence of five bacterial pathogens of PRDC; the same frequency was observed by the jointly presence of four of them. In 21.4% of farms were found three pathogens; and two pathogens were observed in 7.1% of farms. Farms affected by six PRDC bacterial pathogens were not observed, as well as farms free of these pathogens. Like wise, the bacterial pathogens pairs found most frequently in the same farm were the following: S. suis and H. parasuis (85.7% of farms), and with the same frequency (71.4%) the pairs A. pleuropneumoniae and H. parasuis, H. parasuis and P. multocida, and the pair P. multocida and S. suis.

In Mexico, there are several studies on different pathogens of PRDC, mainly on A. pleuropneumoniae [23,32-39], but there are few field studies on the bacterial pathogens distribution that make up this complex [21,40], which is the biggest health problem faced by pork producers today. One of these studies, conducted by Álvarez et al. [21], estimate the frequency of A. pleuropneumoniae, M. hyopneumoniae and swine influenza virus (SIV) in the State of Yucatan, region located at the Southeast of the country. They found a frequency of 100% for A. pleuropneumoniae and M. hyopneumoniae. Although in our study we found a lower frequency of both pathogens, probably due to regional differences, it is remarkable the high percentage of farms affected by PRDC pathogens observed in both studies, which clearly show the severe respiratory health problems facing pig production in our country.
4. CONCLUSION

Bacterial pathogens of porcine respiratory disease complex were present in the upper respiratory tract of pigs in all swine farms sampled, in the State of Aguascalientes, Mexico. These results show the great respiratory health problems present in our swine farms, which is a reflection of the current situation of the Mexican field.

5. ACKNOWLEDGEMENTS

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