

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

Abraham Loera-Muro^{1*}, Francisco J. Avelar-González¹, Víctor M. Loera-Muro¹, Mario Jacques², Alma L. Guerrero-Barrera³

¹Centro de Ciencias Básicas, Universidad Autónoma de Aguascalientes, Aguascalientes, México;

*Corresponding Author: alguerre@correo.uaa.mx

²Groupe de Recherche sur les Maladies Infectieuses du Porc, Faculté de Médecine Vétérinaire, Université de Montréal, St-Hyacinthe, Canada

³Laboratorio de Biología Celular y Tisular, Departamento de Morfología, Centro de Ciencias Básicas, Universidad Autónoma de Aguascalientes, Aguascalientes, México

Received 20 January 2013; revised 3 March 2013; accepted 7 April 2013

Copyright © 2013 Abraham Loera-Muro *et al.* This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

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, *Actinobacillus pleuropneumoniae*, *Streptococcus suis*, *Pasteurella multocida*, *Bordetella bronchiseptica*, *Haemophilus parasuis* and *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 *A. pleuropneumoniae*, *S. suis*, *P. multocida*, *B. bronchiseptica*, *H. parasuis* and *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 *A. pleuropneumoniae* (located in the 79% of farms); 17% were positive for *S. suis* (in 86% of farms), of these, 3% were *S. suis* serovar 2; 30% were positive for *H. parasuis*

(93% of farms); 23% of the samples to *P. multocida* (in 79% of farms); and 19% to *M. hyopneumoniae* (in 64% of farms). *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; *Actinobacillus pleuropneumoniae*; *Streptococcus suis*; *Pasteurella multocida*; *Bordetella bronchiseptica*; *Haemophilus parasuis*; *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 is 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 *A. pleuropneumoniae*, *S. suis*, *P. multocida*,

B. bronchiseptica, *H. parasuis* and *M. hyopneumoniae* are the main bacterial pathogens involved in causing great economic losses to the swine industry [3], and begin to be regarded as important zoonotic agents with significant increase of infectious in humans [4-6].

A. pleuropneumoniae causes hemorrhagic swine pleuropneumonia, this is a severe contagious and economically significant disease in the swine industry [7-9]. *S. suis* is recognized as a major respiratory pathogens of pigs and emerging zoonotic agent [4,5,10]. *P. multocida* caused the atrophic rhinitis in pigs [11,12], as well as *B. bronchiseptica* [13,14]. *H. parasuis* causes Glässer's disease or polyserositis, as well as pneumonia [15-17]. *M. hyopneumoniae* is the primary cause of enzootic pneumonia in pigs [18-20].

The objective of this work was to estimate the presence of *A. pleuropneumoniae*, *S. suis*, *P. multocida*, *B. bronchiseptica*, *H. parasuis* and *M. hyopneumoniae* in the upper respiratory tract of asymptomatic pigs in swine farms in Aguascalientes, Mexico, using PCR technique.

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 calculated according to Alvarez *et al.* [21], for a study in the State of Yucatan, Mexico, to sought *A. pleuropneumoniae* and *M. hyopneumoniae*. The nasal swabs were taken by inserting the swab in the nasal cavity of the pig softly, rotating the swab to easily enter the cavity, and withdrawing it in the same way. The samples were store in sterile tubes. The pigs were randomly selected, ensuring sampling of pigs of different ages and weights, covering the entire farm. Each tube was labeled with the number of the farm, followed by a period and the number of tube following a sequential order. The tubes were store in a cooler until reach the laboratory. In the laboratory, the tubes were store at 4°C until processed.

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* serovar 2-735, *H. parasuis* serovar 5 (Nagazaki), *P. multocida* 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-

brook and Russell [22], with Loera *et al.* [23] modifications. The swabs were cut in an Eppendorf tube with tweezers and scissors sterilized by flame. Subsequently, 1ml of lysis buffer (15% agarose, 0.03 mM Tris, 0.05 M EDTA, and 0.03 mg lisozyme·ml⁻¹, pH 8) was added directly to the tubes and then they were incubated for 30 minutes at 37°C. After, 100 µl SDS 10% (0.1 v/v), 100 µl NaCl 5M (0.1 v/v) and 5 µl Proteinase K (0.4 mg·ml⁻¹; USB Corporation) was added, and the tubes were mixed and incubated for 1 h at 50 °C. Then, 500 µl of the supernatant was taken, in which 200 µl of phenol-chloroform-isoamyl alcohol (24:24:1, Gibco BRL) was added. The mixture was vortexed (Fisher) and centrifuged for 5 min at 10,000 g. The supernatant was recovered and 200 µl ammonium acetate (7.5 M) and 200 µl absolute ethanol (1 v) was added, and mixed for 5 min by inversion and allowed to precipitate overnight at 4°C. Then, samples were centrifuged at 4°C for 15 min at 10,000 g. The supernatant was discarded and the pellet was washed several times with 100 µl ethanol (70%). Pellets were dried at 37°C, resuspended in distilled water and kept at -20°C until use for the PCR reactions.

2.4. PCR Reactions

PCR amplifications were carried out in a DNA thermal cycler (TECHNE T-412). PCR against *A. pleuropneumoniae* was performed as described by Schaller *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 developed based on the method of Marois *et al.* [25]. Multiplex 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 elongation step at 72°C for 5 min. PCR against toxigenic strains of *P. multocida* was developed under the conditions 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

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

Farm	Nasal Swab Samples	App	Ss	Ss ser 2	Hp	Pm	Bb	Mh
1	15	4 27%	4 27%	2 13%	6 40%	6 40%	0 0%	6 40%
2	15	0 0%	4 27%	2 13%	6 40%	6 40%	0 0%	6 40%
3	15	4 27%	4 27%	2 13%	6 40%	5 33%	0 0%	3 20%
4	15	4 27%	0 0%	0 0%	0 0%	6 40%	0 0%	3 20%
5	15	4 27%	3 20%	0 0%	6 40%	4 27%	0 0%	6 40%
6	15	4 27%	3 20%	0 0%	6 40%	6 40%	0 0%	3 20%
7	15	0 0%	2 13%	0 0%	6 40%	4 27%	0 0%	0 0%
8	17	4 24%	1 6%	0 0%	6 35%	3 18%	0 0%	6 35%
9	15	0 0%	2 13%	0 0%	4 27%	3 20%	0 0%	4 27%
10	15	3 20%	1 7%	0 0%	4 27%	0 0%	0 0%	0 0%
11	15	4 27%	1 7%	0 0%	3 20%	0 0%	0 0%	4 27%
12	15	4 27%	0 0%	0 0%	3 20%	0 0%	0 0%	0 0%
13	15	5 33%	6 40%	1 7%	3 20%	3 20%	0 0%	0 0%
14	15	2 13%	5 33%	0 0%	4 27%	2 13%	0 0%	0 0%
Total	212	42	36	7	63	48	0	41
Total Percentage (%)		19.8	16.9	3.3	29.7	22.6	0	19.3
Infected Farms (n)		11	12	4	13	11	0	9
Farm Distribution (%)		78.6	85.7	28.6	92.9	78.6	0	64.3

Ap, *A. pleuropneumoniae*; Ss, *S. suis*; Ss ser. 2, *S. suis* serotype 2; Hp, *H. Parasuis*; Pm, *P. multocida*; Bb, *B. bronchiseptica*; Mh, *M. hyopneumoniae*.

Table 2. Primers sequences used in this study.

Name	Sequence (5'-3')	Product size (pb)	Reference
APXIVANEST1L APXIVANEST1R	GGG GAC GTA ACT CGG TGA TT GCT CAC CAA CGT TTG CTC	377	Schaller <i>et al.</i> [23]
16S-195 (s) 16S-489 (as2)	CAG TAT TTA CCG CAT GGT AGA TAT GTA AGA TAC CGT CAA GTG AGA A	294	Marois <i>et al.</i> [25]
cps2J-s cps2J-as	GTT GAG TCC TTA TAC ACC TGT T CAG AAA ATT CAT ATT GTC CAC C	459	Marois <i>et al.</i> [25]
HPS-forward HPS-reverse	GTG ATG AGG AAG GGT GGT GT GGC TTC GTC ACC CTC TGT	821	Oliveira <i>et al.</i> [26]
TOXA set 1F TOXA set 1R	GGT CAG ATG ATG CTA GAT ACT CC CCA AAC AGG GTT ATA TTC TGG AC	338	Kamp <i>et al.</i> [27]
Fla 2 Fla 4	AGG CTC CCA AGA GAG AAA GGC TT TGG CGC CTG CCC TAT C	237	Hozbor <i>et al.</i> [28]
MH649-F MH649-R	GAG CCT TCA AGC TTC ACC AGG A TGT GTT AGT GAC TTT TGC CAC C	649	Cai <i>et al.</i> [30]

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 $\cdot\text{mL}^{-1}$. 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

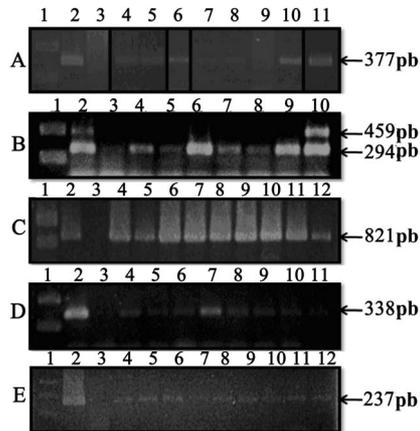


Figure 1. PCR products of the different PCR done; all were analyzed on 1.5% agarose gel electrophoresis, followed by ethidium bromide stain. (A) *A. pleuropneumoniae* PCR's: Lane 1—DNA ladder (Fermentas); Lane 2—*A. pleuropneumoniae* S-1-4074 (positive control); Lane 3—*E. coli* ATCC 25922 (negative control); Lane 4 to 11—Nasal swabs samples; (B) *S. suis* PCR's: Lane 1—DNA ladder (Fermentas); Lane 2—*S. suis* 735 serovar 2 (positive control); Lane 3—*E. coli* ATCC 25922 (negative control); Lane 4 to 10—Nasal swabs samples; (C) *H. parasuis* PCR's: Lane 1—DNA ladder (Fermentas); Lane 2—*H. parasuis* serovar 5 (Nagazaki) (positive control); Lane 3—*E. coli* ATCC 25922 (negative control); Lane 4 to 12—Nasal swabs samples; (D) *P. multocida* PCR's: Lane 1—DNA ladder (Fermentas); Lane 2—*P. multocida* 4-4056 (type D, DNT+) (positive control); Lane 3—*E. coli* ATCC 25922 (negative control); Lane 4 to 11—Nasal swabs samples; (E) *M. hyopneumoniae* PCR's: Lane 1—DNA ladder (Fermentas); Lane 2—*M. hyopneumoniae* ATCC 25095 (positive control); Lane 3—*E. coli* ATCC 25922 (negative control); Lane 4 to 11—Nasal swabs samples. No evidence of *B. bronchiseptica* in nasal swab samples was found in any of the farms sampled in this study (*B. bronchiseptica* serovar 276 was the positive control).

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

This project was supported by a grant from CONACYT, Mexico (No. 258863). We appreciate the support received by Dr. Ricardo Oropeza, Institute of Biotechnology, UNAM, Mexico, for their help in obtain the control strains. We thank Josée Labrie for critical reading of the manuscript. We also thank to Adriana C. Moreno Flores for her technical assistance.

REFERENCES

- [1] Brogden, K.A. and Guthmiller, J.M. (2002) Polymicrobial diseases. ASM Press, Washington DC.
- [2] Pósa, R., Donkó, T., Bogner, P., Kovács, M., Repa, I. and Magyar, T. (2011) Interaction of *Bordetella bronchiseptica*, *Pasteurella multocida*, and *fumonisin B1* in the porcine respiratory tract as studied by computed tomography. *Canadian Journal of Veterinary Research*, **75**, 176-182.
- [3] MacInnes, J., Gottschalk, M., Lone, A., Metcalf, D., Ojha, S., Rosendal, T., Watson, S. and Friendship, R. (2008) Prevalence of *Actinobacillus pleuropneumoniae*, *Actinobacillus suis*, *Haemophilus parasuis*, *Pasteurella multocida*, and *Streptococcus suis* in representative Ontario swine herds. *Canadian Journal of Veterinary Research*, **72**, 242-248.
- [4] Hao, N., Nga, T. and Schultsza, C. (2011) Slaughterhouse pigs are a major reservoir of *Streptococcus suis* serotype 2 capable of causing human infection in Southern Vietnam. *PLoS*, **6**, Article ID: e17943. [doi:10.1371/journal.pone.0017943](https://doi.org/10.1371/journal.pone.0017943)
- [5] Kim, H., Lee, S., Moon, H., Kim, Y., Lee, S., Hur, M. and Yun, Y. (2011) *Streptococcus suis* causes septic arthritis and *Bacteremia*: Phenotypic characterization and molecular confirmation. *The Korean Journal of Laboratory Medicine*, **31**, 115-117. [doi:10.3343/kjlm.2011.31.2.115](https://doi.org/10.3343/kjlm.2011.31.2.115)
- [6] Nga, T., Nghia, H. and Schultsza, C. (2011) Real-time PCR for detection of *Streptococcus suis* serotype 2 in cerebrospinal fluid of human patients with meningitis. *Diagnostic Microbiology and Infectious Disease*, **70**, 461-467. [doi:10.1016/j.diagmicrobio.2010.12.015](https://doi.org/10.1016/j.diagmicrobio.2010.12.015)
- [7] Buettner, F., Konze, S., Maas, A. and Gerlach, G. (2011) Proteomic and immunoproteomic characterization of a DIVA subunit vaccine against *Actinobacillus pleuropneumoniae*. *Proteome Science*, **9**, 23. [doi:10.1186/1477-5956-9-23](https://doi.org/10.1186/1477-5956-9-23)
- [8] Tobias, T.J., Boum, A., Klinkenberg, D., Daemen, A.J., Stegeman, J.A., Wagenaar, J.A. and Duim, B. (2012) Detection of *Actinobacillus pleuropneumoniae* in pigs by real time quantitative PCR for the *apxIVA* gene. *The Veterinary Journal*, **193**, 557-560. [doi:10.1016/j.tvjl.2012.02.004](https://doi.org/10.1016/j.tvjl.2012.02.004)
- [9] Sadilkova, L., Nepereny, J., Vrzal, V., Sebo, P. and Osicka, R. (2012) Type IV fimbrial subunit protein ApfA contributes to protection against porcine pleuropneumonia. *BMC Veterinary Research*, **43**, 2. [doi:10.1186/1297-9716-43-2](https://doi.org/10.1186/1297-9716-43-2)
- [10] Wertheim, H., Nghia, H., Taylor, W. and Schultsz, C. (2009) *Streptococcus suis*: An emerging human pathogen. *Emerging Infections*, **48**, 617- 625.
- [11] Tang, X., Zhao, Z., Hu, J., Wu, B., Cai, X., He, Q. and Chen, H. (2009) Isolation, antimicrobial resistance, and virulence genes of *Pasteurella multocida* strains from swine in China. *Journal of Clinical Microbiology*, **47**, 951-958. [doi:10.1128/JCM.02029-08](https://doi.org/10.1128/JCM.02029-08)
- [12] Hotchkiss, E., Hodgson, C., Lainson, A. and Zadoks, R. (2011) Multilocus sequence typing of a global collection of *Pasteurella multocida* isolates from cattle and other host species demonstrates niche association. *BMC Microbiology*, **11**, 115. [doi:10.1186/1471-2180-11-115](https://doi.org/10.1186/1471-2180-11-115)
- [13] Kaut, C., Duncan, M., Kim, J., Maclaren, J., Cochran, K. and Julio, S. (2011) A novel sensor kinase is required for *Bordetella bronchiseptica* to colonize the lower respiratory tract. *Infection and Immunity*, **79**, 3216-3228. [doi:10.1128/IAI.00005-11](https://doi.org/10.1128/IAI.00005-11)
- [14] MacArthur, I., Jones, J., Goodlett, D., Ernst, R. and Preston, A. (2011) Role of *pagL* and *lpxO* in *Bordetella bronchiseptica* lipid biosynthesis. *Journal of Bacteriology*, **193**, 4726-4735. [doi:10.1128/JB.01502-10](https://doi.org/10.1128/JB.01502-10)
- [15] Mullins, M., Register, K., Bayles, D., Dyer, D., Kuehn, J. and Phillips, G. (2011) Genome sequence of *Haemophilus parasuis* strain 29755. *Standards in Genomic Sciences*, **5**, 61-68. [doi:10.4056/sigs.2245029](https://doi.org/10.4056/sigs.2245029)
- [16] Zhang, N., Chu, Y., Gao, P., Zhao, P., He, Y. and Lu, Z. (2012) Immunological identification and characterization of extracellular serine protease-like protein encoded in a putative *espP2* gene of *Haemophilus parasuis*. *Journal of Veterinary Medical Science*, **74**, 983-987. [doi:10.1292/jvms.11-0260](https://doi.org/10.1292/jvms.11-0260)
- [17] Zhou, M., Zhang, Q., Zhao, J. and Jin, M. (2012) *Haemophilus parasuis* encodes two functional cytolethal distending toxins: CdtC contains an atypical cholesterol recognition/interaction region. *PLoS One*, **7**, Article ID: e32580. [doi:10.1371/journal.pone.0032580](https://doi.org/10.1371/journal.pone.0032580)
- [18] Liu, W., Feng, Z., Fang, L., Zhou, Z., Li, Q., Li, S., Luo, R., Wang, L., Chen, H., Shao, G. and Xiao, S. (2011) Complete genome sequence of *Mycoplasma hyopneumoniae* strain 168. *Journal of Bacteriology*, **193**, 1016-1017. [doi:10.1128/JB.01305-10](https://doi.org/10.1128/JB.01305-10)
- [19] Villarreal, I., Vranckx, K., Calus, D., Pasmans, F., Haesebrouck, F. and Maes, D. (2012) Effect of challenge of pigs previously immunized with inactivated vaccines containing homologous and heterologous *Mycoplasma hyopneumoniae* strains. *BMC Veterinary Research*, **8**, 2. [doi:10.1186/1746-6148-8-2](https://doi.org/10.1186/1746-6148-8-2)
- [20] Vranckx, K., Maes, D., Marchioro, S., Villarreal, I., Chiers, K., Pasmans, F. and Haesebrouck, F. (2012) Vaccination reduces macrophage infiltration in bronchus-associated lymphoid tissue in pigs infected with a highly virulent *Mycoplasma hyopneumoniae* strain. *BMC Veterinary Research*, **193**, 557-560. [doi:10.1016/j.tvjl.2012.02.004](https://doi.org/10.1016/j.tvjl.2012.02.004)

- nary Research, **8**, 24. doi:10.1186/1746-6148-8-24
- [21] Álvarez, M., Rodríguez, J., Ciprián, A., Rodríguez, L., Ayora, G. and Segura, J. (2004) Serological profile of porcine influenza virus, *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae*, in farms of Yucatán, México. *Veterinaria México*, **35**, 296-305.
- [22] Sambrook, J. and Russell, D. (2001) Molecular cloning, a laboratory manual. 3rd Edition, Cold Spring Harbor Laboratory Press, New York.
- [23] Loera, V., Jacques, M., Tremblay, Y., Avelar-González, F., Loera, A., Ramírez-López, E., Medina-Figueroa, A., González-Reynaga, H. and Guerrero-Barrera, A. (2013) Detection of *Actinobacillus pleuropneumoniae* in drinking water from pig farms. *Microbiology*, **159**, 535-543. doi:10.1099/mic.0.057992-0
- [24] Schaller, A., Djordjevic, S.P., Eamens, G.J., Forbes, W.A., Kuhn, R., Kuhnert, P., Gottschalk, M., Nicolet, J. and Frey, J. (2001) Identification and detection of *Actinobacillus pleuropneumoniae* by PCR based on the gene *apx-IVA*. *Veterinary Microbiology*, **79**, 47-62. doi:10.1016/S0378-1135(00)00345-X
- [25] Marois, C., Bougeard, S., Gottschalk, M. and Kobisch, M. (2004) Multiplex PCR assay for detection of *Streptococcus suis* species and serotypes 2 and 1/2 in tonsils of live and dead pigs. *Journal of Clinical Microbiology*, **42**, 3169-3175. doi:10.1128/JCM.42.7.3169-3175.2004
- [26] Oliveira, S., Galina, L. and Pijoan, C. (2001) Development of a PCR test to diagnose *Haemophilus parasuis* infections. *Journal of Veterinary Diagnostic Investigation*, **13**, 495-501. doi:10.1177/104063870101300607
- [27] Kamp, E., Bokken, G., Vermeulen, T., Jong, M., Buys, H., Reek, F. and Smits, M. (1996) A specific and sensitive PCR assay suitable for large-scale detection of toxigenic *Pasteurella multocida* in nasal and tonsillar swabs specimens of pigs. *Journal of Veterinary Diagnostic Investigation*, **8**, 304-309. doi:10.1177/104063879600800305
- [28] Hozbor, D., Fouque, F. and Guiso, N. (1999) Detection of *Bordetella bronchiseptica* by the polymerase chain reaction. *Research in Microbiology*, **150**, 333-341. doi:10.1016/S0923-2508(99)80059-X
- [29] Register, K. and DeJong, K. (2006) Analytical verification of a multiplex PCR for identification of *Bordetella bronchiseptica* and *Pasteurella multocida* from swine. *Veterinary Microbiology*, **117**, 201-210. doi:10.1016/j.vetmic.2006.05.003
- [30] Cai, H., Dreumel, T., McEwen, B., Hornby, G., Bell-Rogers, P., McRaid, P., Josephson, G. and Maxie, G. (2007) Application and field validation of a PCR assay for the detection of *Mycoplasma hyopneumoniae* from swine lung tissue samples. *Journal of Veterinary Diagnostic Investigation*, **19**, 91-95. doi:10.1177/104063870701900115
- [31] INEGI (2012) www.inegi.org.mx
- [32] Negrete-Abascal, E., Tenorio, V., Serrano, J.J., García, C. and de la Garza, M. (1994) Secreted proteases from *Actinobacillus pleuropneumoniae* serovar 1 degraded porcine gelatin, hemoglobin and immunoglobulin A. *Canadian Journal of Veterinary Research*, **58**, 83-86.
- [33] Negrete-Abascal, E., Tenorio, V., Guerrero, A.L., García, R.M., Reyes, M. and de la Garza, M. (1998) Purification and characterization of a protease from *Actinobacillus pleuropneumoniae* serovar 1, an antigen common to all the serovars. *Canadian Journal of Veterinary Research*, **62**, 183-190.
- [34] Negrete-Abascal, E., Reyes, M., García, R., Vaca, S., Giron, J., García, O., Zenteno, E. and de la Garza, M. (2003) Flagella and motility in *Actinobacillus pleuropneumoniae*. *Journal of Bacteriology*, **185**, 664-668. doi:10.1128/JB.185.2.664-668.2003
- [35] Enríquez-Verdugo, I., Guerrero, A.L., Serrano, J.J., Godínez, D., Rosales, J.L., Tenorio, V. and de la Garza, M. (2004) Adherence of *Actinobacillus pleuropneumoniae* to swine-lung collagen. *Microbiology*, **150**, 2391-2400. doi:10.1099/mic.0.27053-0
- [36] García-González, O., García, R.M., de la Garza, M., Vaca-Pacheco, S., Paniagua, G.L., Mejía, R., *et al.* (2004) *Actinobacillus pleuropneumoniae* metalloprotease: Cloning and *in vivo* expression. *FEMS Microbiology Letters*, **234**, 81-86. doi:10.1111/j.1574-6968.2004.tb09516.x
- [37] Hamer-Barrera, R., Godínez, D., Enríquez, V., Vaca-Pacheco, S., Martínez-Zúñiga, R., Talamás-Rohana, P., Suárez-Güemez, F. and de la Garza, M. (2004) Adherence of *Actinobacillus pleuropneumoniae* serotype 1 to swine buccal epithelial cells involves fibronectin. *Canadian Journal of Veterinary Research*, **68**, 33-41.
- [38] Serrano, L., Tenorio-Gutiérrez, V., Suárez, F., Reyes-Cortés, R., Rodríguez-Mendiola, M., Arias-Castro, C., Godínez-Vargas, D. and de la Garza, M. (2008) Identification of *Actinobacillus pleuropneumoniae* biovars 1 and 2 in pigs using a PCR assay. *Molecular and Cellular Probes*, **22**, 305-312. doi:10.1016/j.mcp.2008.09.001
- [39] Gutiérrez, L., Vargas, D., Ocampo, L., Sumano, H., Martínez, R. and Tapia, G. (2011) Plasma concentrations resulting from florfenicol preparations given to pigs in their drinking water. *Journal of Animal Science*, **89**, 2926-2931. doi:10.2527/jas.2010-3576
- [40] Williams, J.J., Torres-León, M., Echeverría-Coello, P. and Matos-Medina, M. (2000) Aislamiento e identificación de *Actinobacillus pleuropneumoniae* en pulmones de cerdos con pleuroneumonía crónica sacrificados en el rastro municipal de Mérida. *Revista Biomédica*, **11**, 175-181.