

Diagnostic methods to detect *Mycoplasma hyopneumoniae* in pig farms - Klein C.S.^{1*}, Rebelatto R.¹, Morés N.¹, Locatelli C.², Bellaver F.A.V.¹, Morés M.A.¹, Gava D.¹, Silva G.B.², Kich J.¹, Coldebella A.¹, Silva V.S.¹, Zanella J.R.C.¹

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Mycoplasma hyopneumoniae (Mhyo), the primary agent of enzootic pneumonia (EP) in pigs, is distributed worldwide causing great economic losses in pig farming. The detection of Mhyo is essential to determine the health profile in pig herds, mainly to the certified pig-breeding farms (GRSC). The detection of Mhyo in a pig farm depends on the availability of appropriate diagnostic methods. The blood, oral fluid, nasal and tonsil swabs as well as lung samples were collected from 16 weaning-to-finish pig farms, including all production ages of swine. An indirect enzyme-linked immunosorbent assay (ELISA) for Mhyo was standardized and validated from serum. From the nasal and tonsil swabs, we performed PCR for Mhyo and bacteriological tests for other respiratory agents including *Pasteurella multocida*, *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis* and *Streptococcus suis*. Lungs with macroscopic lesions of EP of studied farms were evaluated at the slaughterhouse for pneumonia index. Multiple lung samples were also collected from pigs of each farm to perform the following tests: histopathological examination, immunohistochemistry for Mhyo, and bacteriological tests to detect other bacteria causing respiratory disease. Samples of oral fluid will be used to perform an indirect ELISA as well as PCR for Mhyo. The generated data of different performed tests will allow to recognize the health profile for Mhyo in the pig farm as well as to validate an indirect ELISA to screen affected pigs with Mhyo within a farm.

Key-words: *Mycoplasma hyopneumoniae*, enzootic pneumonia, indirect ELISA

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Diagnostic methods to detect *Mycoplasma hyopneumoniae* in pig farms

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INTRODUCTION

Mycoplasma hyopneumoniae (Mhyo), the primary agent of enzootic pneumonia (EP) in pigs, is distributed worldwide causing great economic losses in pig farming. Although EP is a disease of optional certification in pig breeding reproduction farms (GRSC), according to IN 19/2002/SDA-MAPA, the detection of Mhyo requires diagnostic methods still unavailable in the official laboratories in Brazil. Thus, in order to suggest diagnostic methods and control of EP, we have evaluated different diagnostic methods for detection of Mhyo in swine farms.

MATERIALS & METHODS

This study comprised the evaluation of 16 pig farms (farms 1 to 16). Previously, health status for *Mycoplasma hyopneumoniae* on these farms was based on absence of clinical signs for enzootic pneumonia (EP) and negative diagnostic tests for Mhyo in the previous years. Thus, farms were divided in health status for Mhyo in positive (11 farms) and negative (5 farms). Ten out of 11 positive farms were vaccinated for Mhyo, while all 5 negative farms were not vaccinated for Mhyo. We did not obtain the vaccination history in one farm (farm 15). Blood, oral fluid, nasal and tonsil swabs were sampled from 90 pigs on each farm (15 pigs/age group 3, 5, 10, 13, 17 and 20 week-old). ELISA for Mhyo was performed on serum samples. Nasal and tonsillar swabs were used for PCR for Mhyo, *Haemophilus parasuis* and *Actinobacillus pleuropneumoniae* as well as bacterial isolation of the main respiratory pathogens. Oral fluid has been stored at -70°C for later testing (ELISA and PCR).

Lungs of at least 30 finishing pigs at the respective farms were evaluated at the slaughterhouse. Lesions consistent with EP (to determine the pneumonia index - PI) as well as other significant gross lesions were recorded. Samples of affected lungs were collected for histopathology, immunohistochemistry (IHC) for Mhyo, and bacterial isolation. Samples and respective performed laboratory tests are summarized on figure 1.

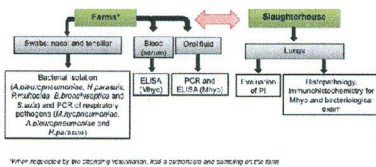


Figure 1. Sampling fluxogram and respective laboratory tests for *Mycoplasma hyopneumoniae* and other respiratory bacterial agents

RESULTS & DISCUSSION

The summarized data is shown on table 1.

Table 1. Results of different diagnostic methods for enzootic pneumonia and other respiratory bacterial agents in 16 farms.

Farm	Status	Vaccinated	B. Isolation			PCR			IHC			Bacterial isolation		
			Mhyo	H. parasuis	A. pleuropneumoniae	Mhyo	H. parasuis	A. pleuropneumoniae	Mhyo	H. parasuis	A. pleuropneumoniae	Mhyo	H. parasuis	A. pleuropneumoniae
1	+	No	+	+	+	+	+	+	+	+	+	+	+	+
2	+	No	+	+	+	+	+	+	+	+	+	+	+	+
3	+	Yes	+	+	+	+	+	+	+	+	+	+	+	+
4	+	No	+	+	+	+	+	+	+	+	+	+	+	+
5	+	No	+	+	+	+	+	+	+	+	+	+	+	+
6	+	No	+	+	+	+	+	+	+	+	+	+	+	+
7	+	No	+	+	+	+	+	+	+	+	+	+	+	+
8	+	No	+	+	+	+	+	+	+	+	+	+	+	+
9	+	Yes	+	+	+	+	+	+	+	+	+	+	+	+
10	+	No	+	+	+	+	+	+	+	+	+	+	+	+
11	+	No	+	+	+	+	+	+	+	+	+	+	+	+
12	+	No	+	+	+	+	+	+	+	+	+	+	+	+
13	+	No	+	+	+	+	+	+	+	+	+	+	+	+
14	+	No	+	+	+	+	+	+	+	+	+	+	+	+
15	-	No	+	+	+	+	+	+	+	+	+	+	+	+
16	-	No	+	+	+	+	+	+	+	+	+	+	+	+

ELISA and PCR tests were performed on all 16 evaluated pig farms. Comparing the results of these tests with IHC, PI and other gross lesions, the only negative health status farm for Mhyo was farm 3. Although table 1 shows a positive result for the ELISA test from farm 3, only 1,67% of finishing pigs were positive for this test on this farm. The farm 11 was not considered negative because PCR for Mhyo in all stages of production, indicating a recent infection, since IHC and ELISA tests were negative.

Pig lungs from 10 farms (farms 1, 2, 3, 9, 11, 12, 13, 14, 15, 16) were evaluated at the slaughterhouse; all farms had gross lesions typical of EP (cranioventral consolidation determined by PI). High PI index (in 7 farms) correlated with positive results of IHC for Mhyo, showing that the gross evaluation of lungs is a reliable test for predicting EP lesions in the slaughterhouse. Other significant gross lesions were also observed in 8 farms and included: pleurisy, pleural adhesions, focal abscesses, focal necrohemorrhagic pneumonia, and pericarditis. Out of these lesions other respiratory pathogens were isolated such as *Haemophilus parasuis*, *Pasteurella multocida*, and *A. pleuropneumoniae*. Although *Streptococcus suis* was isolated in 9 farms, it has not been serotyping; when *S. suis* had been isolated from nasal swab it was considered a bacterium of normal respiratory flora.

CONCLUSION

To determine the health status for *Mycoplasma hyopneumoniae* in a pig farm, multiple diagnostic tests should be applied and appropriately interpreted. Furthermore, infection with Mhyo in a pig farm is usually concurrent with other respiratory pathogens as part of porcine respiratory disease complex.

