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**EFFECT OF SOW VACCINATION AGAINST *MYCOPLASMA***

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***HYOPNEUMONIAE* ON SOW AND PIGLET COLONIZATION AND**

4

**SEROCONVERSION, AND PIG LUNG LESIONS AT SLAUGHTER**

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24 **Abstract**

25 The objectives of the present study were to compare *Mycoplasma hyopneumoniae* (Mh)  
26 colonization and serologic status on Mh vaccinated and non-vaccinated sows and to  
27 assess the effect of sow vaccination on colonization and serologic status of their piglets  
28 at weaning as well as presence of Enzootic Pneumonia (EP) lung lesions at slaughter.  
29 Fifty sows (25 vaccinated and 25 unvaccinated) as well as 5 of their piglets were  
30 included in the study. Blood samples and nasal swabs from sows at 7 weeks pre-  
31 farrowing and 1 week post-farrowing and from piglets at 3-4 weeks of age were taken.  
32 Nasal swabs and sera were tested by a nested Polymerase Chain Reaction (nPCR) and  
33 by an enzyme-linked immunosorbent assay (ELISA) test to detect Mh DNA and  
34 antibodies to the pathogen, respectively. Finally, at 23 weeks of age, pigs were sent to  
35 the slaughter where the extension of EP-compatible gross lesions was assessed.  
36 Vaccination with two doses of Mh vaccine resulted in a significantly higher ( $p<0.05$ )  
37 percentage of seropositive sows than in the non-vaccinated group at 1 week post-  
38 farrowing. On the contrary, no statistical significant differences were found in the  
39 number of nasal nPCR positive sows among different treatments ( $p>0.05$ ). At 3-4 weeks  
40 of age, a significantly higher percentage ( $p<0.001$ ) of seropositive piglets came from  
41 vaccinated than from non-vaccinated sows. Although the number of Mh infected piglets  
42 coming from non-vaccinated sows was higher than the one from vaccinated sows, the  
43 difference was not statistically significant ( $p>0.05$ ). Overall, piglets from vaccinated  
44 sows had a significant lower ( $p<0.05$ ) mean of EP-compatible lung lesions ( $1.83\pm 2.8$ )  
45 than piglets from non-vaccinated sows ( $3.02\pm 3.6$ ). Under the conditions described in  
46 this study, sow vaccination did not affect sow or piglet colonization but increased the  
47 percentage of seropositive sows and piglets at weaning and reduced significantly the  
48 mean EP-compatible lung lesion scoring at slaughter.

49 Key words: *Mycoplasma hyopneumoniae*, vaccination, colonization, serology, lung  
50 lesions

51

## 52 **1. Introduction**

53 *Mycoplasma hyopneumoniae* (Mh) is the causative agent of enzootic pneumonia  
54 (EP), a chronic respiratory disease that affects mainly growing and finishing pigs  
55 (Thacker, 2006).

56 Vaccination strategies against Mh vary depending on the type of herd,  
57 production system, Mh infection dynamics and number of vaccine doses applied.  
58 Although efficacy of commercial vaccines in reducing EP lung lesions has been  
59 extensively proven, protection conferred seems to be incomplete since vaccinated  
60 animals can be also infected (Haesebrouck et al., 2004). Another putative strategy to  
61 ameliorate the effects of Mh infection is to reduce the vertical transmission of the  
62 pathogen and to elicit and to transfer strong colostrum derived immunity to the piglets  
63 by means of sow vaccination. Under field conditions, Mh sow vaccination is not  
64 frequently included in vaccination strategies, with the exception of gilts in  
65 acclimatisation programmes (Bargen, 2004). So far, sow vaccination has been applied  
66 to assess its effects on serologic and colonization status in vaccinated (Grosse Beilage  
67 and Schreiber, 2005; Jayappa et al., 2001; Martelli et al., 2006) and non-vaccinated  
68 (Calsamiglia and Pijoan, 2000; Ruiz et al., 2003) weaning piglets. On the other hand,  
69 Diaz et al., (2004) evaluated the effect of sow vaccination on lung lesion development  
70 in Mh vaccinated piglets. Nevertheless, the effect of Mh vaccine-induced passive  
71 immunity on EP lung lesions development in non-vaccinated slaughtered pigs has not  
72 been described.

73 Therefore, the objectives of the present study were 1) to compare Mh  
74 colonization and serologic status on Mh vaccinated and non-vaccinated sows and 2) to  
75 assess the effect of sow vaccination on colonization and serologic status of their piglets  
76 at weaning as well as presence of EP lung lesions at slaughter.

77

## 78 **2. Materials and methods**

### 79 **2.1. Farm**

80 A 150 sow, farrow-to-finish farm with EP-related respiratory problems in  
81 finishing pigs was selected to carry out this study. No Mh-vaccination was used in the  
82 farm before the starting of the present field study. Mh infection was confirmed one  
83 month before the initiation of the study by nested PCR (nPCR) in nurseries in 55 3-  
84 week-old piglets (7 out of 55 [12.8%] were positive by nPCR in tonsillar swabs), in 20  
85 slaughtered animals by serology (17 out of 20 [85%] were seropositive to Mh) and by  
86 EP-compatible lung lesion scoring (mean gross lung lesion score of 6.5 based on the  
87 classification by Hannan et al., [1982]).

### 88 **2.2. Study design**

89 Fifty sows were selected 7 weeks pre-farrowing; nasal swab and blood were  
90 taken from all of them. Parity distribution among these 50 sows were 2-4 (n=21, 42%),  
91 5-7 (n=24, 48%) and more than 7 (n=5, 10%). At 5 weeks pre-farrowing, sows were  
92 divided into two groups (vaccinated and non-vaccinated) taking into account the Mh  
93 ELISA and nPCR results of the samples taken at 7 weeks pre-farrowing (Table 1). At 5  
94 and 3 weeks pre-farrowing, 25 sows received 2 ml of an intramuscular injection of  
95 MYPRAVAC-SUIS® (HIPRA, Spain) and the rest 25 sows received a 2 ml  
96 intramuscular injection of PBS. This vaccine is based on a suspension of inactivated Mh  
97 strain J with a carbomer/levamisol adjuvant. At 1 week post-farrowing, nasal swab and

98 blood were taken from each sow and a mean of 5 piglets per sow were randomly  
99 selected and ear-tagged. From the 246 piglets included in the study, 122 and 124 came  
100 from vaccinated and non-vaccinated sows, respectively. Animals were weaned at 17 to  
101 23 days of age and transferred to the nursery units, where litters from different sows  
102 were mixed. At 3-4 weeks of age, nasal swabs and blood were taken from all these  
103 piglets. At 9 weeks of age, pigs were moved to the growing-finishing units and they  
104 were distributed taking into account the body condition, following the farmer criterion.  
105 Finally, at 23 weeks of age, pigs were sent to the slaughter and extension of EP-  
106 compatible gross lesions (craneo-ventral pulmonary consolidation) was assessed as  
107 previously described (Hannan et al., 1982).

108         Treatments, housing, husbandry and slaughtering conditions conformed to the  
109 European Union Guidelines and Good Clinical Practices.

### 110         **2.3. DNA extraction and nPCR procedures**

111         DNA extraction from nasal swabs and Mh nPCR were performed as previously  
112 described (Sibila et al., 2004). Mh antibodies in serum samples were detected using a  
113 monoclonal blocking ELISA test (CIVTEST suis® MYCOPLASMA  
114 HYOPNEUMONIAE, HIPRA, Girona, Spain) following manufacturer's instructions.  
115 The inhibition percentage (IP) was calculated taking into account the optical densities  
116 (OD) of each sample as well as the one of the negative control (negative value). ELISA  
117 results interpretation, based on IP values, was as follows: IP < 50%, negative; IP > 50%,  
118 positive.

### 119         **2.4. Statistical Analyses**

120         Bivariate analyses using contingency tables (Chi-square statistics or Fisher's  
121 exact test for 2x2 tables) were used to compare the following parameters among  
122 different treatment groups: 1) number of Mh seropositive sows at 7 weeks pre- and 1

123 week post-farrowing; 2) number of nPCR positive sows at nasal cavity at 7 weeks pre-  
124 and 1 week post-farrowing; 3) number of Mh seropositive piglets at 3-4 weeks of age;  
125 and 4) number of nPCR positive piglets at 3-4 weeks of age. In order to study the  
126 influence of parity number on several parameters, sows were divided into previously  
127 described groups: 2 to 4, 5 to 7 and higher than 7 (Calsamiglia and Pijoan, 2000).  
128 Contingency tables were also used to analyze the effect of parity number on sow and  
129 piglet serologic and colonization status. Non-parametric statistical analysis (Kruskal-  
130 Wallis statistic) was used to test differences in gross lung lesion scoring among animals  
131 from different treatments groups. On the other hand, Wilcoxon statistics were used to  
132 assess the relationship between gross lung lesion scoring at slaughter with nPCR  
133 positive and negative pigs at the weaning age. Statistical analyses were performed with  
134 the SAS system for Windows version 8.0 (SAS Institute Inc, Cary, North Carolina,  
135 USA). Statistical significance level was set at  $\alpha=0.05$ .

136 Vaccine efficacy in reducing lung lesions measured at slaughter was calculated  
137 using group median lung scores (Jones et al., 2005), as follows: Vaccine efficacy =  
138 [(median non-vaccinated group score – median vaccinated group score)/ median non-  
139 vaccinated group score] x 100.

### 140 **3. Results and Discussion**

141 Vaccination with two doses of vaccine resulted in a significantly higher ( $p<0.05$ )  
142 percentage of seropositive sows at 1 week post-farrowing than in the non-vaccinated  
143 group (Table 1). While in the non-vaccinated group, percentage of seropositive sows  
144 varied from 24% (6 out of 25) pre-treatment to 36% (9 out of 25) post-treatment  
145 administration ( $p>0.05$ ), the figures changed from 28% (7 out of 25) to 96% (22 out of  
146 23; serum from two sows was lacking) for vaccinated animals ( $p<0.05$ ). Percentage of  
147 seropositive vaccinated sows at 1 week post-farrowing was similar to the ones obtained

148 in previously reported studies where sows were double vaccinated at 5 and 3 weeks pre-  
149 farrowing (Ruiz et al., 2003) or single vaccinated at 2 weeks pre-farrowing (Martelli et  
150 al., 2006). No statistical significant differences between vaccinated and non-vaccinated  
151 groups were found in the number of nasal nPCR positive sows at 1 week post-farrowing  
152 ( $p=0.14$ ). However, it is noteworthy that the percentage of nPCR positive sows at 7  
153 weeks pre-farrowing and at 1 week post-farrowing showed a more marked decrease in  
154 the vaccinated sows group (from 28% to 0%) compared to that of the non-vaccinated  
155 sows (from 12% to 8%).

156 At 3-4 weeks of age, a significantly higher percentage ( $p<0.001$ ) of seropositive  
157 piglets came from vaccinated sows (60 out of 118 [51%]) compared to non-vaccinated  
158 sows (3 out of 119 [3%]) (Table 2). Martelli et al. (2006) and Ruiz et al. (2003),  
159 reported also higher percentage of seropositive piglets coming from vaccinated (80%  
160 and 81-82%, respectively) than from non-vaccinated (0% and 5-21%, respectively)  
161 sows. On the other hand, Kristensen et al. (2004) and Grosse Beilage and Shreiber  
162 (2005) described a significantly higher level of Mh antibodies in pigs from vaccinated  
163 sows compared to control ones up to 3 and 4 weeks of age, respectively. Although, in  
164 the present study there were 37 out of 58 (64%) and 75 out 116 (64.5%) seronegative  
165 piglets that came from seropositive vaccinated and non-vaccinated sows, respectively.  
166 This observation may be due to the half-life of maternal antibodies to Mh, calculated as  
167 15.8 days (Morris et al., 1994), and therefore at 3-4 weeks of age they might be very  
168 low or have already disappeared. On the other hand there were 23 seropositive piglets  
169 (20 and 3 from vaccinated and non-vaccinated sows, respectively) that came from  
170 seronegative sows. The latter ones may be explained by cross-fostering practice,  
171 although this practice was seldom performed in this farm.

172 On the other hand, only 8 out of 244 (3.3 %; nasal swab from two piglets was  
173 lacking) piglets were Mh nPCR positive at 3-4 weeks of age. This low percentage of  
174 Mh detection at nasal cavity in nursery pigs is similar to the one reported by another  
175 field study in a farrow-to-finish operation (Sibila et al., 2007a) Although number of  
176 piglets infected by Mh coming from non-vaccinated sows was higher than the one from  
177 vaccinated sows (5 versus 3), the difference was not statistically significant ( $p>0.05$ ).  
178 These low numbers may have been the cause of no statistical significant differences  
179 found between piglets from vaccinated versus non-vaccinated sows. However, this low  
180 percentage of Mh infected pigs may be explained by a low infectious pressure present in  
181 the farm at that moment or by a, less probably, limited sensitivity of the nPCR used. It  
182 is generally believed that vaccination against Mh does not prevent from infection  
183 (Thacker, 2006) and, therefore, it has not a clear effect on sow or piglet colonization  
184 (Calsamiglia and Pijoan, 2000). More recently, however, it has been described that  
185 piglet vaccination may help in reducing the prevalence of Mh infected animals (Baccaro  
186 et al., 2005; Ruiz et al., 2003; Sibila et al., 2007b). Therefore, the present study does no  
187 help in elucidating the effect of Mh vaccination on sow and piglet colonization.

188 It is worthy to remark that 6 (75%) of these 8 nPCR positive piglets were  
189 seronegative, supporting the idea that passive immunity may decrease the percentage of  
190 infected piglets (Ruiz et al., 2003; Sibila et al., 2007a).

191 Due to loss of the ear-tag at the slaughterhouse, lung lesion scoring was only  
192 assessed on 198 (100 from vaccinated and 98 from non-vaccinated sows) out of 246  
193 initially included pigs. Seventy six out of 198 (38,3%) did not show any EP-compatible  
194 lung lesion (Table 3). The highest lung lesion score observed was 16 (from a maximum  
195 of 35) in one piglet in each treatment. Overall, piglets from vaccinated sows had a  
196 significant lower ( $p<0.05$ ) mean of EP-compatible lung lesions ( $1.83\pm 2.8$ ) than piglets

197 from non-vaccinated sows ( $3.02\pm 3.6$ ). Vaccine efficacy in reducing lung lesions score  
198 was 50%. Díaz et al. (2004), reported a low incidence and grade of lung lesions in  
199 vaccinated piglets coming from vaccinated sows compared with the ones from non-  
200 vaccinated sows. In this latter study the effect of sow vaccination on their non-  
201 vaccinated piglets was not truly assessed since lung lesion development was compared  
202 between double Mh vaccination (sow and piglets) versus piglet vaccination at different  
203 ages. Therefore and as far as the authors' knowledge, this is the first description of the  
204 effect of Mh sow vaccination in reducing EP-compatible lung lesions in their non-  
205 vaccinated piglets at slaughter. It is known that Mh vaccine effect are greater with  
206 higher Mh infectious pressure (Maes et al., 1999); and therefore, the relatively low  
207 levels of infection and of EP lung lesions observed in the present study might have  
208 prevented to see a more significant effect of the vaccine used.

209 An interesting point to remark was the significant relationship ( $p<0.05$ ) between  
210 positivity by nPCR at 3-4 weeks of age and lung lesion scoring at slaughter (Fano et al.,  
211 2006a; Sibila et al., 2007b). Specifically, piglets infected by Mh at 3-4 weeks of age had  
212 greater mean lung lesion score ( $7.00\pm 5.2$ ) than non-infected piglets ( $2.28\pm 3.0$ ).  
213 Therefore, our results further support that the detection of Mh at nasal cavity of weaning  
214 pigs can be used as an indicator of EP-compatible lung lesions at slaughter-age (Fano et  
215 al., 2006b).

216 In summary, under the conditions described in this study, sow vaccination did  
217 not affect piglet colonization but increased the percentage of seropositive piglets at  
218 weaning and reduced significantly the mean EP-compatible lung lesion scoring at  
219 slaughter.

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- 281

**Table 1:** Number (and percentage) of positive sows by ELISA and nPCR at 7 weeks pre- and 1 week post-farrowing in both treatments (Mh vaccinated and non-vaccinated) and their parity distribution. Different superscripts within a column mean statistical significant differences.

Sows	7 weeks pre-farrowing		1 week post-farrowing		Parity distribution			
	ELISA + (%)	nPCR + (%)	ELISA + (%) <sup>a</sup>	nPCR + (%)	2-4	5-7	>7	Total
Vaccinated	7 (28)	7 (28)	22 (96) <sup>a</sup>	0 (0)	9	13	3	25
Non-vaccinated	6 (24)	3 (12)	9 (36) <sup>b</sup>	2 (8)	11	11	3	25
Total	13 (26)	10 (20)	31 (65)	2 (4)	20	24	6	50

**Table 2:** ELISA and nPCR results of piglets at 3-4 weeks of age taking into account the treatment received by the sows (Mh vaccinated and non-vaccinated) as well as their serologic and infectious status at 1 week post-farrowing.

				Piglets (3-4 weeks of age)					
				ELISA			nPCR		
				Positive	Negative	Total	Positive	Negative	Total
Sows (1 week post-farrowing)	ELISA	Vaccinated (n=23)	Positive (n=22)	40	37	77	2	74	76
			Negative (n=1)	20	21	41	1	40	41
			Total	60	58	118	3	114	117
		Non-vaccinated (n=25)	Positive (n=9)	0	75	75	5	69	74
			Negative (n=16)	3	41	44	0	44	44
			Total	3	116	119	5	113	118
	nPCR	Vaccinated (n=25)	Positive (n=0)	0	0	0	0	0	0
			Negative (n=25)	63	59	122	3	118	121
			Total	63	59	122	3	118	121
		Non-vaccinated (n=25)	Positive (n=2)	0	10	10	0	10	10
			Negative (n=23)	3	106	109	5	103	108
			Total	3	116	119	5	113	118

**Table 3:** EP lung lesion scoring at slaughter in pigs from vaccinated and non-vaccinated sows.

<b>Lung lesions scoring</b>	<b>Piglets from vaccinated sows</b>	<b>Piglets from non-vaccinated sows</b>	<b>Total (%)</b>
0	48	28	76 (38)
1-5	41	47	88 (44)
6-10	7	19	26 (13)
11-15	3	3	6 (3)
16-20	1	1	2 (1)
Total	100	98	198 (100)

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