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1 Prospecting potential links between PRRSV infection susceptibility of alveolar macrophages and other
2 respiratory infectious agents present in conventionally reared pigs.

3

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9

10

11 Abstract

12 Porcine Reproductive and Respiratory Syndrome virus (PRRSV) is one of the main component of the
13 porcine respiratory disease complex (PRDC), which strongly impact the pig production. Although
14 PRRSV is often considered as a primary infection that eases subsequent respiratory coinfections, the
15 possibility that other PRDC components may facilitate PRRSV infection has been largely overlooked.
16 The main cellular targets of PRRSV are respiratory macrophages among them alveolar macrophages
17 (AM) and pulmonary intravascular macrophages (PIM). AM, contrarily to PIM, are directly exposed to
18 the external respiratory environment, among them co-infectious agents. In order to explore the
19 possibility of a co-infections impact on the capacity of respiratory macrophages to replicate PRRSV,
20 we proceed to *in vitro* infection of AM and PIM sampled from animals presenting different sanitary
21 status, and tested the presence in the respiratory tract of these animals of the most common porcine
22 respiratory pathogens (PCV2, *Actinobacillus pleuropneumoniae*, *Mycoplasma hyopneumoniae*,
23 *Mycoplasma hyorhinis*, *Mycoplasma flocculare*, *Pasteurella multocida*, *Bordetella bronchiseptica*,
24 *Streptococcus suis*). In this exploratory study with a limited number of animals, no statistic
25 differences were observed between AM and PIM susceptibility to *in vitro* PRRSV infection, nor
26 between AM coming from animals presenting very contrasting respiratory coinfection loads.

27

28 Introduction

29 Respiratory infections are one of the major cause of disease in pigs, leading to economic losses as
30 well as to antibiotics overuse. Because of its sanitary importance, this condition has been given a
31 name: porcine respiratory disease complex (PRDC). The major PRDC components described so far are
32 swine influenza A virus (swIAV), porcine reproductive and respiratory syndrome virus (PRRSV), and
33 porcine circovirus 2 (PCV2) as well as bacteria such as *Actinobacillus pleuropneumoniae*, *Mycoplasma*
34 *hyopneumoniae*, *Pasteurella multocida*, *Bordetella bronchiseptica* and *Streptococcus suis* (Opriessnig
35 et al., 2011). Among them, PRRSV, because of the duration of infection (more than a month), its
36 immunomodulatory properties as well as the emergence of highly pathogenic strains, is considering
37 as one of the pillars of PRDC (Montaner-Tarbes et al., 2019).

38 The pathogens constituting the PRDC are thought to interact with each other as well as with the host
39 tissues, in a complex manner (among others see (Ellis et al., 2004; Lévesque et al., 2014; Thacker et
40 al., 1999) (Saade et al., 2020)). Indeed, whereas the adaptive immune response can largely be
41 considered as pathogen-specific, the immediate inflammatory response and the countermeasures
42 triggered by the pathogens to quench this response may impact the infectious capacities of a second
43 pathogen. Moreover, some infections may trigger trained immunity (especially on monocytes and
44 macrophages, the main targets of PRRSV), and the persistent character of the majority of these
45 microbes may led to long term tissue remodelling.

46 In a previous study we observed that pulmonary intravascular macrophages (PIM) were equivalently
47 susceptible as alveolar macrophages (AM) to PRRSV infection, and this *in vitro* as well as *in vivo*
48 (Bordet et al., 2018). However, these preliminary data pointed on a lower variability between
49 animals in PIM PRRSV susceptibility compared with AM PRRSV susceptibility. Since AM, seated in the
50 airways, are more exposed to respiratory pathogens than PIM, which are protected from external
51 environment by three tissue layers (epithelium, interstitium and endothelium), we postulated that
52 the variability of AM susceptibility to PRRSV infection might be due to factors present in the airways
53 such as coinfectious agents. In order to test our hypothesis, we set up an original approach consisting
54 in monitoring ongoing *in vivo* recurrent respiratory infections and at the same time *in vitro* AM and
55 PIM capacities to replicate PRRSV, in animals presenting different sanitary status. Since this influence
56 between the pathogens and the components of the respiratory immune system might come from
57 interactions lasting several weeks, and that persistent infections are naturally much more susceptible
58 to be involved in coinfections situations, we focused on the following persistent PRDC components:

59 PCV2, *Actinobacillus pleuropneumoniae*, *Mycoplasma hyopneumoniae* and the related *Mycoplasma*
60 *hyorhinis* and *Mycoplasma flocculare*; *Pasteurella multocida*, *Bordetella bronchiseptica*, *Streptococcus*
61 *suis* and excluded *a priori* the short term swIAV infection that, moreover has been already shown to
62 downregulate PRRSV infection *ex vivo* (Dobrescu et al., 2014).

63

64 Materials and Methods

65 **Pig lung cells collection.** Lung tissue samples were obtained from 5- to 7-month-old Large White
66 conventionally bred sows coming from two origins: 6 animals from the Unité Experimentale de
67 Physiologie Animal de l'Orfrasière (UEPAO, Tours, France) and 6 from PORCI MAUGES slaughterhouse
68 (Beaupréau, France). Finally, 4 supplementary Large White specific pathogen free (SPF) 2 to 3-month-
69 old sows from the highly controlled SPF facility of ANSES, Ploufragan (France) free of numerous
70 respiratory pathogens such as PRRSV, swine Influenza virus, porcine circovirus type 2, *Mycoplasma*
71 *hyopneumoniae*, *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Bordetella bronchiseptica*
72 and *Haemophilus parasuis* were also tested. In order to reduce animal experiments, all the animals
73 were euthanized in the course of the regular management process of the herds, hence the absence
74 of trial number or experimental authorisation and the age difference according to the origin of the
75 animals. Tracheo-bronchial tissue and lymph nodes were sampled and directly frozen in dry ice for
76 subsequent pathogens detection (Zimmerman et al., 2012). A broncho-alveolar lavage (BAL)
77 procedure was then performed twice on the isolated left lung with 250 mL of PBS supplemented with
78 2mM EDTA (PBS/EDTA), to collect AM. Next, a 1-cm slice of external lung parenchyma was dissected
79 from the same lung. Tissues were minced and incubated in nonculture-treated Petri dishes, to avoid
80 plastic adherence of macrophages, for 2 h at 37 °C in complete RPMI, consisting of RPMI 1640
81 supplemented with 100 IU/mL penicillin, 100 mg/mL streptomycin, 2 mM L-glutamine, and 10%
82 inactivated foetal calf serum (FCS) (all from Invitrogen, Paisley, UK), containing 2 mg/mL collagenase
83 D (Roche, Meylan, France), 1 mg/mL dispase (Invitrogen), and 0.1 mg/mL Dnase I (Roche). Cells were
84 passed through 40 µm cell strainers and red blood cells lysed with erythrocytes lysis buffer (10 mM
85 NaHCO₃, 155 mM NH₄Cl, and 10 mM EDTA). Next, cells were washed with PBS/EDTA, counted, and
86 step-frozen in FCS/10% dimethyl sulfoxide (Sigma-Aldrich, St Louis, MO).

87 **Cell infection and viral titration.** Parenchymal and alveolar macrophages of one conventional and
88 one controlled animal were systematically thawed and infected in parallel. Because of technical
89 reasons, not enough PIM could be retrieved from animals 9, 11, 12 from Beaupréau slaughterhouse
90 (Fig 1A), leading to only 3 conventional PIM infection tests. Thawed cells were first enriched in
91 macrophages and depleted in dead cells by 1.065 density iodixanol gradient (Optiprep®, Nycomed
92 Pharma, Oslo, Norway). These gradient-enriched mononuclear phagocyte cell preparations were
93 further enriched in macrophages by 2 h plastic adherence, leading, from bronchoalveolar lavage to
94 an AM purity of 86% +/-8%, and for parenchyma to a PIM purity of 51%+/-20%. Enriched
95 macrophages were then cultured in complete RPMI for 24 h in flat-bottom 96-well plates at 3.10⁵
96 cells/well and then infected at a multiplicity of infection (MOI) of 10⁻³ with PRRSV virus Lena strain in
97 complete RPMI. At 24 hpi, plates were centrifuged and pelleted cells were lysed in 350 µL of RNA
98 extraction buffer (RLT Buffer, QIAGEN). These MOI and incubation time have been set to measure
99 the PRRSV titre during the linear phase of the infection, in order to observe weak infectivity
100 differences between the different macrophages. Total RNA were extracted using RNeasy Plus Mini kit
101 (QIAGEN) according to the manufacturer's instructions. RNA were reverse transcribed using oligo(dT)
102 and random primers (BioRad iScript Reverse Transcription supermix). RNA samples were treated with
103 DNase I Amp Grade (Invitrogen) (1 U/µg of RNA). The absence of genomic DNA contamination was
104 validated by the use of treated RNA as a template directly in PCR. Total RNA quantity and quality
105 were assessed using Nanophotometer (Implen, Munich, Germany). cDNA was generated with a virus
106 reverse transcriptase in the iScript Reverse Transcription Supermix for RT-qPCR (Bio-Rad, Hercules,

107 CA, USA) from 100-200 ng of RNA free of genomic DNA per reaction. Diluted cDNA (4X) was
108 combined with primer/probe sets and IQ SYBR Green Supermix (Bio-Rad) according to the
109 manufacturer's recommendations. Real-time assays were run on a CFX Connect Real-Time PCR
110 Detection System (Bio-Rad). Samples were normalized internally by simultaneously using the average
111 Cycle quantification (*C_q*) of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) reference gene in
112 each sample. Then, qPCR data were expressed as relative values after Genex macro analysis (Bio-Rad)
113 (Vandesompele et al., 2002) using the *C_q* from the samples for the different transcripts. TTC AGT TCC
114 GGT GA); GAPDH (F: CTT CAC GAC CAT GGA GAA GG, R: CCA AGC AGT TGG TGG TAC AG), TBP1
115 (F: AACAGTTCAGTAGTTATGAGCCAGA, R: AGATGTTCTCAAACGCTTCG), ActB (F:
116 CACGCCATCCTGCGTCTGGA, R: AGCACCGTGTGGCGTAGAG), HPRT1 (F: GGAATGAAATCATGTTTGTG,
117 R: CAGATGTTTCCAACTCAAC), IFN- α (generic see (Sang et al., 2011)) (F: GGC TCT GGT GCA TGA GAT
118 GC, R: CAG CCA GGA TGG AGT CCT CC), IFN- β (F: GTT GCC TGG GAC TCC TCA A, R: CCT CAG GGA CCT
119 CAA AGT TCA T), Mx1 (F: AGT GTC GGC TGT TTA CCA AG, R: TTC ACA AAC CCT GGC AAC TC), Mx2 (F:
120 CCG ACT TCA GTT CAG GAT GG, R: ACA GGA GAC GGT CCG TTTA C) and PKR (F: CAC ATC GGC TTC
121 AGA GTC AG, R: GGG CGA GGT AAA TGT AGG TG). Glyceraldehyde 3-phosphate dehydrogenase
122 (GAPDH) was used as a reference gene since it was endowed with one of the most stable expression
123 in the whole porcine lung (Delgado-Ortega et al., 2011) as well as specifically in lung macrophages
124 (Maisonasse et al., 2016). Then, qPCR data were expressed as relative values after Genex macro
125 analysis (Bio-Rad) (Vandesompele et al., 2002). In all experiments other reference genes (TBP1 or
126 ActB and HPRT1) were used in parallel with GAPDH and gave comparable results. All the AM/PIM
127 were negative for PRRSV before *in vitro* infection.

128 **PCR for pathogens detection.** Tracheobronchial tissue and lymph node were thawed and
129 mechanically (Lysing matrix E FastPrep24, MpBio) disrupted before DNA extraction using QIAamp
130 cadior Pathogen kit (INDICAL BIOSCIENCE). A proteinase K enzymatic lysis was then performed. A
131 PCR was then run using previously described primers for PCV2 (F: TTT AGG GTT TAA GTG GGG
132 GGT C, R: CCG GAT CCA TGA CGT ACC CAA GGA GGC G, 470 bp fragment expected) *Pasteurella*
133 *multocida* (F: AAG GGA TGT TGT TAA ATA GAT AGC, R: GCT TCG GGC ACC AAG CAT AT, 410
134 bp fragment expected) *Haemophilus parasuis* (F: GTG ATG AGG AAG GGT GGT GT, R: GGC TTC GTC
135 ACC CTC TGT, 820 bp fragment expected), *Streptococcus suis* (F: TTC TGC AGC GTA TTC TGT CAA
136 ACG, R: TGT TCC CTG GAC AGA TAA AGA TGG, 700 bp fragment expected) (Cheong et al., 2017)
137 or for *Mycoplasma* species (*M. hyopneumoniae* F: TTCAAAGGAGCCTTCAAGCTTC, 1,000 bp fragment
138 expected; *M. flocculare* F: GGGAAGAAAAAATTAGGTAGGG, 754 bp fragment expected; *M.*
139 *hyorhinis*, F: CGGGATGTAGCAATACATTGAG, 1129 bp fragment expected, and a common reverse
140 primer R: AGAGGCATGATGATTTGACGTC) (Stakenborg et al., 2006). The PCR was performed in a
141 20 μ L reaction mixture containing 4 μ L of extracted DNA (containing a minimum of 25 ng of DNA),
142 0,5 μ M of each primer, 1,5 to 2,5 mM of MgCl₂, 0,1 mM of deoxynucleoside triphosphate and 0,02 U of
143 GoTaq G2 Flexi DNA Polymerase (Promega). The PCR was carried out for 40 cycles consisting of
144 denaturation for 20 seconds at 94°C, annealing for 30 seconds at T_m and extension for 30 seconds at
145 72°C using thermal cycle (BioRad). Then, qPCR data were expressed as relative values after Genex
146 macro analysis (Bio-Rad) (Vandesompele et al., 2002) using the *C_q* from the samples for the different
147 transcripts.

148
149 **Statistical analysis.** Data were analysed using Graph Pad Prism (version 6) and Mann-Whitney test
150 was used to compare relative expressions. Non-parametric approaches were chosen due to the few
151 number of samples available. The Mann-Whitney's test was used to compare unpaired samples
152 based on ranking. The AM and PIM from the same animal were considered as paired samples. For
153 paired samples, the Wilcoxon matched-pairs signed rank test was used. When scatter plots are used,
154 the mean is depicted by a horizontal bar. Factor Analysis for Mixed Data (FAMD) was performed
155 using FactoMineR package of RStudio (version 3.6.1) in order to investigate the relation between

156 isolated pathogens and gene expression.

157

158

159 Results and Discussion

160 AM and PIM were first collected from animals coming from 2 facilities presenting distinct health
161 status: the controlled pig breeding facility from the UEPAO (INRA, Nouzilly, France) and the PORCI
162 MAUGES slaughterhouse (Beaupréau, France) which processes animals from the conventional farms
163 of the surrounding area, known from the regional veterinary services as a PRRSV-free area
164 (Blanquefort and Benoit, 2000). The AM and PIM were infected *in vitro* at a multiplicity of infection
165 (MOI) of 0.001. Twenty-four hours later the cellular viral load was measured using RT-qPCR. No
166 significant differences were observed neither between AM from the two different origins nor
167 between AM and PIM from the same animals (Fig 1A). Conversely, we could observe a correlation
168 ($r=0.78$, $p=0.02$) of *in vitro* PRRSV infections between AM and PIM from the same animal (Fig 1B), in
169 contradiction with the hypothesis that AM exposed to airways co-infections would be differentially
170 susceptible to PRRSV than PIM. Finally, standard deviations of PRRSV titres presented no differences
171 between AM and PIM from the two origins (Fig 1C).

172 Respiratory infections occurring *in vivo* at the time of AM and PIM collection were then detected by
173 PCR on tracheo-bronchial lymph nodes and bronchial epithelium tissue (Figure 2), using previously
174 described primers (Cheong et al., 2017; Stakenborg et al., 2006). Strikingly, none of the tested
175 pathogens were detected in controlled animals whereas all the conventional animals were positive
176 for at least one of the tested pathogens (Table 1), *P. multocida* (4 animals), *S. suis* (3 animals) and
177 PCV2 (3 animals) being the most frequent infections. One animal was detected positive for 5
178 pathogens simultaneously (animal n°3, for PCV2, *H. parasuis*, *M. flocculare*, *P. multocida* and *S. suis*).
179 However, no link could be inferred between PRRSV *in vitro* titre upon AM infection, and the *in vivo*
180 detection of one or more respiratory pathogens. To note, no animals presented *M. hyopneumoniae*
181 and *M. hyorhinis* infections.

182 Type I IFNs responses were then evaluated in respiratory macrophages upon *in vitro* PRRSV infection
183 by testing IFN α (generic), IFN β and three interferon stimulating genes Mx1, Mx2 and PKR transcripts.
184 No differences were observed between PRRSV mock or infected AM or between AM from the two
185 different origins (Figure 3A), neither in raw transcriptomic expression, nor in induction (fold increase)
186 upon PRRSV infection (data not shown). Once again, no consistent difference could be observed
187 between AM and PIM from the same animals.

188 Finally, in order to test the possibility of a non-identified respiratory infection occurring in both
189 controlled and conventional animals, we also tested AM from the highly controlled SPF facility of
190 ANSES, Ploufragan (France). The same infections and measures than with controlled and
191 conventional AM were performed. AM from SPF animals were as susceptible to PRRSV infection as
192 AM from controlled and conventional animals (Figure 3B). We observed, as expected, a clear
193 upregulation of IFN β upon PRRSV infection in all conditions (Figure 3C). More globally, the only
194 difference which can be observed for type I IFN and ISG responses (Figure 3D) was the lower IFN β
195 transcript level before PRRSV infection in AM from SPF animals, which might be due either to the SPF
196 status of the animals or to their lower ages (3-month-old compared with 5 to 7-month-old for
197 conventional and controlled animals).

198 In order to globally analyse our quantitative (PRRSV titre, IFN-I related genes fold change upon *in*
199 *vitro* PRRSV infection) as well as categorical (presence or not of other pathogens in the respiratory
200 tract) data, we proceeded to a Factor Analysis for Mixed Data (FAMD) (Fig 3E). According to the great
201 variability as well as to the small size of the samples, no significant links between AM origins, type of
202 respiratory infections and IFN-related genes induction could be observed. The analysis of the
203 quantitative variables however showed an interesting segregation on the first and second axis

204 (encompassing respectively 26% and 25% of the total variability of the sample) of PRRSV-Lena, the
205 three ISG transcripts measured (Mx1, Mx2, PKR) and IFN α , but not IFN β transcriptomic induction.
206 Thus ISG genes expressions correlation with PRRSV titre argues for the induction by PRRSV of type I
207 IFN protein(s) expression.

208 In conclusion, although the difference of pathogens load from conventional and controlled animals
209 was striking, no difference of respiratory macrophages PRRSV infection susceptibility could be
210 observed, neither according to the pathogens' identities nor to the tissue location of the
211 macrophages (AM and PIM), invalidating our hypothesis of a recurrent airways infection leading to
212 the onset of AM more susceptible to PRRSV infection. One possibility would be that the observed AM
213 susceptibility variation in our preliminary study was due to *M. hyorhinis* infections as reported by
214 others (Lee et al., 2016; Thacker et al., 1999), a pathogen that was not detected in the animal tested
215 in this study.

216 Despite this negative result, that might be confirmed by a larger scale campaign, our study allowed
217 us to highlight a clear correlation between AM and PIM PRRSV susceptibility *in vitro*, in agreement
218 with their high similarity (Bordet et al., 2018; Maisonnasse et al., 2016). Moreover, we could note
219 that in the *Pays de la Loire* area (France), conventional, commercial pigs presented systematically
220 one or several respiratory infections in perfect agreement with the concept of CRP, with 4 out of 6
221 (2/3) of the conventional pigs presenting at least two simultaneous infections, among them 3 out of
222 4 presented at least 3 pathogens.

223 This study is a first step showing the feasibility and interest of testing in parallel, on conventional
224 animals, the *in vivo* presence of respiratory pathogens as well as the *in vitro* sensitivity, response and
225 functions of AM in presence of different respiratory mimicking conditions such as PRRSV, PCV2 or
226 Influenza infections as well as TLR-ligand stimuli or oxidative stress, for instance, allowing to explore
227 the possible impact of long term respiratory infections on AM, one of the primary respiratory barrier.
228

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234

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241
242

243 Figures Legends

244 Figure 1: AM and PIM from different animals' origins were not distinctly susceptible to PRRSV
245 infection.

246 Gradient and plastic-adherence enriched alveolar macrophages (AM) and pulmonary intravascular
247 macrophages (PIM) were infected by Lena strain PRRSV at an MOI of 10^{-3} for 24h. PRRSV titre was
248 measured by RT-qPCR. Each symbol represents one animal. Macrophages from controlled animals
249 (Ctred), macrophages from conventional animals (Conv). A) PRRSV titre using GAPDH as reference
250 gene (Genex macro analysis (Bio-Rad)). The correspondence between animal identity numbers (for
251 correspondence with Table 1) and symbols are indicated in the cartouche. B) Correlation plot of AM

252 and PIM PRRSV titre according to data depicted in A) using Spearman test. C) Standard deviations of
253 data depicted in A).

254

255 Figure 2: Detection of respiratory pathogens.

256 Example of positive samples detected by PCR of PCV2 (PCV2 - 470 bp), *Streptococcus suis* (*S. suis* -
257 700 bp), *Haemophilus parasuis* (*H. parasuis* - 820 bp), *Pasteurella multocida* (*P. multocida* - 410 bp),
258 and *Mycoplasma* species (*M. hyopneumoniae* - 1,000 bp, *M. flocculare* - 754 bp, *M. hyorhinis* - 1,129
259 bp) from Tracheal epithelium and trachea-bronchial lymph node. The results for all pigs are shown in
260 Table 1.

261

262 Figure 3: AM and PIM from different animals' origins did not distinctly respond to PRRSV infection.

263 A) Transcriptomic expression of type I IFN α and β genes and three representative interferon
264 stimulated genes Mx1, Mx2 and PKR from *in vitro* infected AM and PIM from different origins. The
265 samples are the same, with the same identification as in Figure 1. B) Comparison of *in vitro* PRRSV
266 infection of AM from SPF animals with the previously depicted (Figure 1) PRRSV infected AM from
267 controlled and conventional animals. C) Transcriptomic expression of type I IFN α and β genes and
268 three representative interferon stimulated genes Mx1, Mx2 and PKR from *in vitro* infected AM from
269 SPF animals. The samples are the same, with the same identification as in Figure 3B. D) Factor
270 Analysis for Mixed Data (FAMD) plotting together data from Figure 1, Table 1 and Figure 3.

271

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331

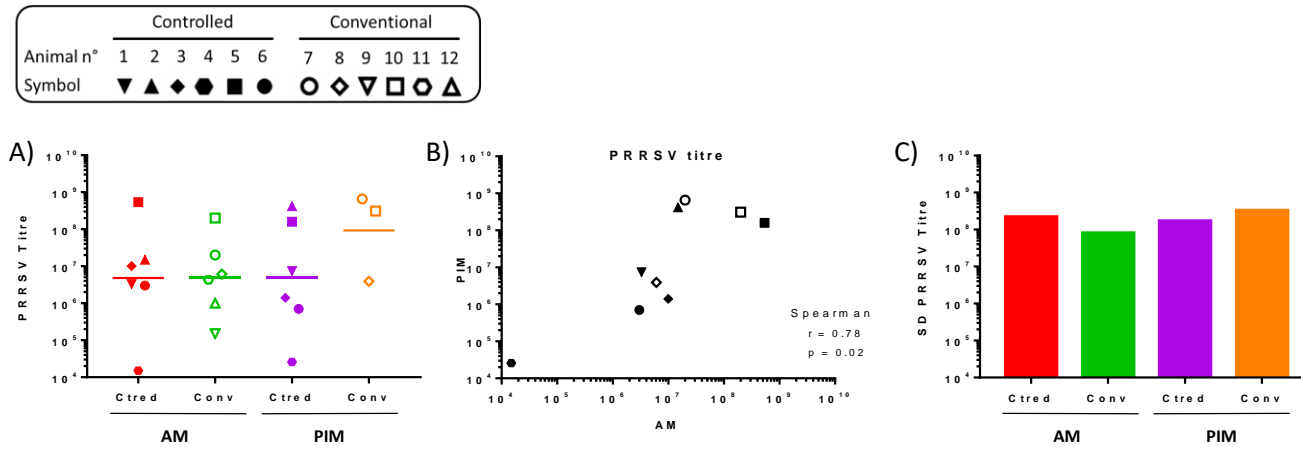


Figure 1: AM and PIM from different animals' origins were not distinctly susceptible to PRRSV infection

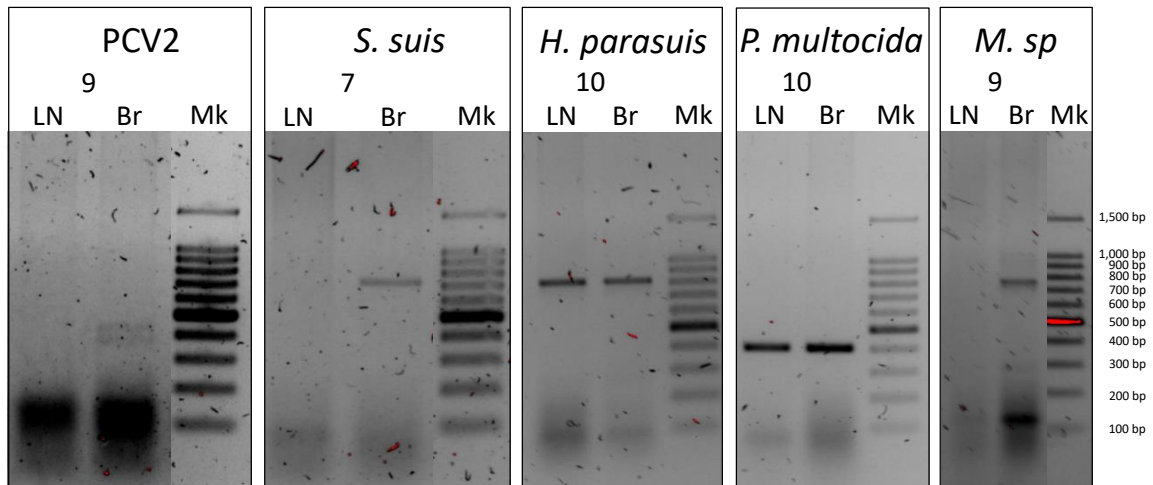


Figure 2: Detection of respiratory pathogens

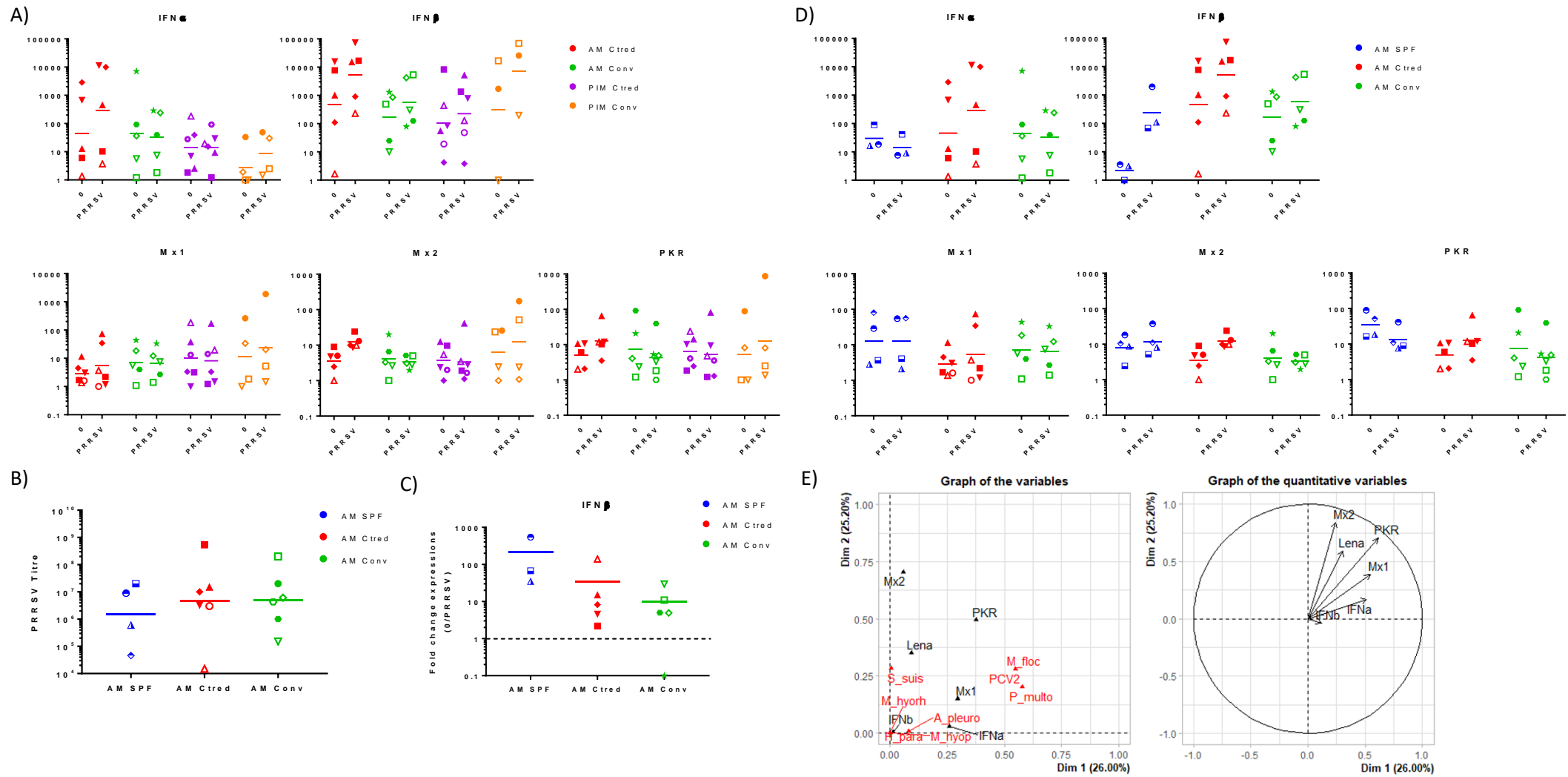


Figure 3: AM and PIM from different animals' origins did not distinctly respond to PRRSV infection.

		Controlled												Conventional											
		1		2		3		4		5		6		7		8		9		10		11		12	
		Br	LN	Br	LN	Br	LN	Br	LN	Br	LN	Br	LN	Br	LN	Br	LN	Br	LN	Br	LN	Br	LN	Br	LN
<i>in vivo</i> infection (whole tissues PCR detection)	PCV2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	-
	<i>A. pleuro</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
	<i>H. para</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
	<i>M. floc</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-
	<i>M. hyop</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	<i>M. hyorh</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	<i>P. multo</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	+	+	-
<i>S. suis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-	-	-	-	-	
<i>in vitro</i> titre (AM)	PRRSV	3,3E+06		1,5E+07		1,E+07		1,5E+04		5,4E+08		3,E+06		2,0E+07		6,1E+06		1,5E+05		4,3E+06		2,0E+08		1,E+06	

Table 1: Conventional but not controlled animals presented viral and bacterial respiratory infections.

Bronchial epithelium (Br) and trachea-bronchial lymph node (LN) of animals from different sanitary status (Controlled, from UEPAO, INRA and Conventional, from commercial slaughterhouse) were tested by PCR for the presence of different respiratory pathogens as illustrated in Figure 2. The titre of *in vitro* PRRSV-infected AM (MOI 0,01, 24h post-infection, RT-qPCR-relative expression) from the same animals are depicted in the last row (see Figure 1). The second row depict the identification number of each animal as identified in figure 1).