Transmission of pathogenic respiratory bacteria to specific pathogen free pigs at slaughter.

Corinne Marois, Roland Cariolet, Hervé Morvan, Marylène Kobisch

Transmission of pathogenic respiratory bacteria to Specific Pathogen Free pigs at slaughter

Corinne Marois[1*], Roland Cariolet[2], Hervé Morvan[3], Marylène Kobisch[1]

[1] Agence Française de Sécurité Sanitaire des Aliments (AFSSA), Unité de Mycoplasmologie-Bactériologie, BP53, 22440 Ploufragan, France

[2] Agence Française de Sécurité Sanitaire des Aliments, Service de Production de Porcs Assainis et d'Expérimentation, BP53, 22440 Ploufragan, France

[3] Laboratoire de Développement et d'Analyses des Côtes d'Armor, BP54, 22440 Ploufragan, France

*Corresponding author: Tel: +33-2-96-01-01-72; Fax: +33-2-96-01-62-73

Email address: c.marois@ploufragan.afssa.fr

Abstract

The purpose of this study was to evaluate the transmission of pathogenic respiratory bacteria to thirteen 5 month-old Specific Pathogen Free (SPF) pigs, during the slaughtering process in a commercial slaughterhouse. Before transportation, the SPF pigs and the lorry were checked to confirm the absence of pathogenic respiratory bacteria.

Nine SPF pigs (group 1) were in contact in a conventional slaughterhouse with finishing pigs, during 4 hours before slaughtering. Four SPF pigs (group 2) were slaughtered immediately at arrival in the slaughterhouse.

Five bacterial pathogens (*Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Haemophilus parasuis* and *Streptococcus suis*) were detected by PCR, after slaughtering, from nasal cavities, tonsils and trachea in the two groups of pigs. Lung samples were PCR negative. Three and four bacterial species were isolated from the pigs of group 2 and group 1, respectively. Cultures were negative from the lungs.

All the bacterial species present in the SPF pigs were detected by PCR. *P. multocida* was isolated, from three samples of scalding water before the onset of slaughtering.

Our results suggest that the SPF pigs became contaminated mainly by the slaughterhouse environment and the scalding water. Histological examinations revealed that during scalding, contaminated water could reach the trachea and the lungs of pigs. Checks conducted at slaughter for respiratory disorders have to be carried on, but nasal cavities and tonsils are not appropriate for bacteriological investigations. Moreover, bacteriological results obtained from the lungs of slaughtered pigs have to be used with carefulness.

Keywords : SPF pigs, slaughterhouse, scalding tank water, pathogenic respiratory bacteria

1. Introduction

Mycoplasma hyopneumoniae, a widespread respiratory pathogen in pigs, is the primary agent of swine enzootic pneumonia. In combination with various other infectious agents, such as bacteria and viruses, this chronic disease is implicated in a syndrome known as the porcine respiratory disease complex (PRDC) causing substantial losses to the pig industry (Thacker et al., 1999; Thacker et al., 2001; Opriessnig et al., 2004; Thanawongnuwech et al., 2004). Among the pathogenic respiratory bacteria, *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Haemophilus parasuis* and *Streptococcus suis* are commonly associated with *M. hyopneumoniae* and can induce pathologic alterations in the thoracic organs of the pig. Lesions such as pneumonia, pleuropneumonia, pleuritis, abscesses and pericarditis can be observed in growing pigs during slaughter examinations that are an important diagnostic tool for surveillance of herd health at regular intervals. Microbiological investigations have been performed on slaughter. Detection of pathogenic bacteria involved in the lung lesions is the first step to control and to prevent respiratory disorders in pig herds (Pijoan and Fuentes, 1987; Andreasen et al., 2001). Pathogenic respiratory bacteria are generally detected by culture or PCR from the most effective samples carried out for each bacterial species, after macroscopic examinations and quantification of the extend of lung lesions (Calsamiglia et al., 1999; Schaller et al., 2001; Oliveira et al., 2001; Marois et al., 2004).

It is widely accepted that respiratory pathogens are transmitted by direct contact from pig to pig or by coughing and sneezing of infected pigs in close proximity. Pathogen circulation can also occur from infected to naïve pigs, in short distances by airborne transmission (Otake et al., 2002; Thacker, 2006; Gottschalk and Taylor, 2006).

At the end of the fattening period, pigs are collected from different farms and transported to the slaughterhouse, sometimes in the same vehicle. They wait in lairage at the slaughterhouse before slaughtering. The lairage duration is carried out during at least three hours, the time required before slaughtering for animal welfare and meat quality reasons.

To our knowledge, unlike bacterial species isolated from the digestive tract, no information is available about the risk factors influencing the spread of respiratory pathogens at slaughter. The main objectives of this work were to study (1) the impact of contact between pigs before slaughtering on transmission of pathogenic bacteria from the respiratory tract of conventional finishing pigs to Specific Pathogen Free (SPF) pigs, (2) the samples allowing the recovery of the pathogenic respiratory bacteria from the SPF pigs and (3) the role of the slaughterhouse environment and of the scalding tank water.

2. Materials and methods

2.1. Animals

Thirteen 5 month-old hysterectomy derived SPF pigs were obtained from the porcine experimental unit of AFSSA Ploufragan (Agreement B-22-745-1). Very strict biosecurity measures were implemented in order to avoid undesirable contamination of the pigs: air filtration system and airlocks for each unit, unit-specific clothes and compulsory showering before and after visiting the pigs (Cariolet et al., 1994).

The selected SPF pigs were ear tagged and carried to the slaughterhouse in a cleaned and disinfected conventional lorry. Forty-eight hours before departure, bacteriological analyses of the respiratory tract were performed on the animals. Drag-swab samples were also taken from different places in the lorry.

A first group of nine SPF pigs (average weight: 114.6 kg (± 9.9 kg)) was brought, very early in the morning, just before the onset of slaughter, to a cleaned commercial slaughterhouse,

where they were in contact during 4 h with finishing pigs coming from several conventional commercial herds. One week later, a second group of four SPF pigs (average weight: 111 kg (± 16.5 kg)) was slaughtered 4 h after the onset of slaughter. Pigs of group 2 were slaughtered immediately at arrival, without any contact with conventional finishing pigs.

2.2. Samples

2.2.1. SPF pigs

Before departure for the slaughterhouse, the SPF pigs were swabbed. CytoBrushs (VWR International, Fontenay-sous-Bois, France) were used for nasal cavities and palatine tonsils. Sterile catheters (Laboratoire Euromedis, Neuilly sous Clermont, France) were used for trachea swabbing (Marois et al., 2007). Drag-swabs (Sodibox, La Forêt Fouesnant, France), humidified with 5 mL of Buffered Pepton Water Broth (BP), were also taken from different places in the lorry.

At the end of the slaughterline, the thoracic organs of the SPF pigs were checked, especially for respiratory disorders. Nasal cavities and tonsils were swabbed. Lungs with trachea and whole palatine tonsils were recovered and carried to our laboratory. Trachea was swabbed with a CytoBrush. Whole tonsils and pieces of the right middle lobe of lungs were cauterised on the surface with a hot spatula and swabbed after several parallel incisions. Each swab was placed in 2 mL of BP (Initial Suspension: IS). The drag-swabs were placed in 10 mL of BP and the supernatant were centrifuged at 5.000 x *g* for 45 min. The pellet was resuspended into 2 mL of BP (IS).

2.2.2. Scalding tank water

Just before slaughtering of the SPF pigs, 50 mL water samples from the scalding tank were collected, with sterile bottles, thrice at a depth of 0.20 m, before the onset of slaughter (T0), as soon as the SPF pigs arrived (T+4 h) and 4 h later (T+8 h), at 5 different places (every 10 m) in the tank (50 m long and 1 m wide). In this slaughterhouse, the scalding tank was washed and cleaned every day. The temperature of the scalding water was measured during each sampling.

116

117 *2.3. Post-mortem examinations of finishing pigs from conventional herds*

118 In order to have some information about the score of lung lesions during the day of slaughter,
119 post-mortem examinations were carried out on 320 finishing pigs slaughtered one hour
120 before the SPF pigs. The lung lesions were scored according to the method previously
121 described by Madec and Derrien (1981). According to this method, the maximum total score
122 possible for each lung was 28.

123

124 *2.4. Bacteriological investigations*

125 Samples were prepared for PCR assays as described by Kellog and Kwok (1990).
126 Briefly, 1 mL of each IS was centrifuged (12.000 × *g*, 4°C, 20 min) and the pellets were
127 resuspended in 800 µL of lysis solution. Samples were incubated for 1 h at 60°C, 10 min at
128 95°C and kept at -20°C. When an inhibition of the PCR reaction was observed, DNA was re-
129 extracted with phenol/chloroform/isoamyl(ic) alcohol (25/24/1) (Marois et al., 2004). PCR
130 assays were carried out as previously described for *M. hyopneumoniae* (Calsamiglia et al.,
131 1999), *A. pleuropneumoniae* (Schaller et al., 2001), *Heamophilus parasuis* (Oliveira et al.,
132 2001) and *Streptococcus suis* (Marois et al., 2004). A new PCR test was developed for *P.*
133 *multocida* detection. PCR mixture contained PCR buffer (67 mM Tris-HCl, 16 mM $(NH_4)_2SO_4$,
134 0.01% Tween 20, 2.5 mM $MgCl_2$ [pH 8.8]), 500 µM of each deoxyribonucleoside triphosphate
135 (Eurobio, Les Ulis, France), 600 nM of Pm16S/3-f (AAGGGATGTTGTTAAATAGATAGC)
136 and Pm16S/6-r (GCTTCGGGCACCAAGCATAT) primers, 1.5 units of *Taq* DNA polymerase
137 (Eurobio), and 5 µl of the DNA template. Amplification was performed in GeneAmp PCR
138 system 9700 (Applied Biosystem). The reaction procedure consisted of initial denaturation at
139 94°C for 5 min, 40 cycles of denaturation at 94°C for 30 s, primer annealing at 60°C for 30 s,
140 and extension at 72°C for 60 s and final elongation at 72°C for 10 min. The amplified product
141 (411 pb) was revealed in a 2% agarose gel in TBE buffer (90 mM Tris, 90 mM borate, 2.5
142 mM EDTA [pH 8]) for 1 h at a constant voltage of 125 V. The specificity of this PCR assay
143 was tested with a collection of 75 strains representing 33 toxigenic and non toxigenic *P.*
144 *multocida* subsp. *multocida* as well as seven *P. multocida* subsp. *septica* or *gallicida* and 35

other bacterial species isolated from the respiratory tract of pigs, among them *A. pleuropneumoniae*, *H. parasuis*, *M. hyopneumoniae* and *S. suis*. The detection threshold was 3 *P. multocida* CFU/assay.

Firstly, all the samples were analysed by PCR, then the positive samples were cultured. In order to isolate *A. pleuropneum*oniae, *P. multocida, H. parasuis* and *S. suis*, 10 µL of each IS were placed onto PPLO agar (Difco, Cergy Pontoise, France), supplemented with 10 µg/ml of nicotinamide dinucleotide (β-NAD), 1 mg/ml of glucose and 5% of decomplemented horse serum agar (Savoye et al., 2000). Media were incubated overnight at 37°C in 5% CO_2 and all bacterial colonies were identified by PCR. *M. hyopneumoniae* was cultivated on modified Friis medium (Marois et al., 2007).

2.5. Microscopic examinations

Microscopic lung examinations were conducted on each SPF pig. Lung samples (a piece of the right middle lobe) were fixed in 10% buffered formalin: paraffin-embedded sections were cut at 5 µm, stained by a trichrome coloration (hematoxylin, eosin and safran) and examined by light microscopy.

3. Results

3.1. Post-mortem examinations of the lungs at slaughter

No macroscopic lung lesions were observed in the SPF pigs.

During the four-hour wait of SPF pigs of group 1, 3.828 finishing pigs were slaughtered and post-mortem examinations of lungs were carried out on 160 randomly selected pigs originating from 7 herds, slaughtered one hour before the SPF pigs. Pneumonia was observed in 70 % of lungs and the mean of pneumonic scores was 3.30 (± 4.20). Recovering lesions (corresponding to early infections) were also noticed in 34 % of lungs. Pleuritis and abscesses were observed in 5.6 % and 3.2 % of the pigs, respectively.

Post-mortem examinations were also carried out on 160 randomly selected lungs of 3.627 conventional finishing pigs from 8 farms, slaughtered one hour before the arrival of the second group of SPF pigs (4 SPF pigs without any contact with conventional finishing pigs).

Pneumonia was observed in 80 % of lungs and the mean score was 5.10 (± 5.30). Thirteen per cent of them had also recovering lesions. Pleuritis and abscesses were observed in 11.3 % and 1.3 % of the pigs, respectively.

3.2. Detection of pathogenic respiratory bacteria

3.2.1. Animals and lorry

Using culture and PCR tests, pathogenic respiratory bacteria were neither detected from the SPF pigs nor from the lorry before departure for the slaughterhouse.

Results of the bacteriological investigations performed on the SPF pigs at the slaughterhouse, are presented in Table 1. Major pathogenic respiratory bacteria (*M. hyopneumoniae*, *A. pleuropneumoniae*, *P. multocida*, *H. parasuis*, and *S. suis*) were detected in the SPF pigs by PCR, whatever the group of SPF pigs (group 1: with contact or group 2: without any contact, with finishing pigs at slaughter). One to five bacterial species were detected in each SPF pig. Except for *M. hyopneumoniae*, the percentages of positive samples were higher in pigs of group 2. The total number of positive results, obtained with tracheal swabs, was higher than with the other samples. Nevertheless, *M. hyopneumoniae*, A*. pleuropneumoniae, H. parasuis* and *S. suis*, were also detected from nasal cavities and palatine tonsils, particularly from the pigs of group 1. PCR assays were negative from the right middle lobe of the lungs.

The culture of the microorganisms was fastidious, nevertheless, three and four bacterial species were isolated in group 2 and in group 1, respectively. *H. parasuis* and *S. suis* were the most frequent bacteria in the two groups. Except for *M. hyopneumoniae*, never isolated from the respiratory tract of the SPF pigs, the most appropriate sites for bacteria cultivation were nasal cavities, tonsils and trachea. Respiratory bacteria were not isolated from the lungs.

3.2.2. Scalding tank water

During the collection of samples A (corresponding to group 1) and samples B (corresponding to group 2), the average temperatures inside the scalding tank water were 61.1°C (± 0.4°C) and 60.7°C (± 0.3°C), respectively. The pigs stayed in the water for about 7 min.

Bacteriological results are shown in Table 2. All bacterial species, followed during this study, were detected in the scalding tank water by PCR tests. Before the onset of slaughter, *M. hyopneumoniae*, *A. pleuropneumoniae* and *S. suis* were detected in five samples A, while samples B were PCR negative at the same moment. The following samples (T+4 h and T+8 h) were PCR positive for the five bacterial species, except for *H. parasuis* at T+8 h.

P. multocida was isolated from samples B, in four cases (T+4 h and T+8 h).

3.3. Microscopic examinations

Microscopic lung examinations of the SPF pigs at the end of the slaughter process, revealed severe alterations of the respiratory tract of 4/9 pigs of group 1 (Fig.1). In these pigs, an exfoliation of the bronchial epithelial cells and a dilation of the capillary vessels were observed (Fig.1 B). Moreover, the examinations showed cellular debris, scales and bacterial accumulations in the bronchial lumen (Fig. 1 C and D). In 9/13 pigs, the structure of the respiratory tract was normal (Fig. 1 A).

4- Discussion

In the conditions described in this study, major pathogenic respiratory bacteria (*M. hyopneumoniae*, *A. pleuropneumoniae*, *P*. *multocida*, *H. parasuis* and *S. suis*) were detected in the respiratory tract of SPF pigs slaughtered in a conventional slaughterhouse. It is widely accepted that transportation of pigs, from farms to slaughterhouses, is a crucial risk factor of cross-contaminations between pigs. In our study, transportation could not have any influence on the transmission of respiratory pathogens to the SPF pigs.

The bacteriological results suggest that the SPF pigs became contaminated (1) by the slaughterhouse environment or (2) by contact with infected finishing pigs during the lairage period (pigs of group 1 only) or (3) by the scalding water.

A rapid contamination occured in the two groups of SPF pigs in the slaughterhouse. PCR, more sensitive than culture, was able to detect five bacterial species. Trachea was the most appropriate site to detect these pathogens. Moreover, four and three species were isolated in groups 1 and 2, respectively. One cause of contamination of SPF pigs was probably the microbial flora present in the slaughterhouse environment. Previous studies indicated that *M. hyopneumoniae* and *A. pleuropneumoniae* could be transmitted by aerosols over short distances (Jobert et al., 2000; Otake et al., 2002; Gottschalk and Taylor, 2006; Thacker, 2006). The potential role of air for dissemination of pathogens is accepted, for instance, *M. hyopneumoniae* was detected in air samples from pig herds (Stärk et al., 1998). Effective control of respiratory pathogens depends on an optimal environment (air quality, temperature, etc) but factors, such as high relative humidity, mixed infections and stress may encourage the development and the spread of respiratory pathogens in pigs and in their environment (Gottschalk and Taylor, 2006; Pijoan, 2006).

The main route of spread of respiratory pathogens is by direct contact from pig to pig (Thacker, 2006). The time spent in lairage at the slaughterhouse (four hours in our study) might be a risk factor for naïve pigs, as described for *Salmonella* (Beloeil et al., 2004). In experimental conditions, *M. hyopneumoniae* was quickly transmitted to sentinel pigs and was detected in nasal, tracheal and bronchial swabs (Meyns et al., 2004; Fano et al., 2005; Marois et al., 2007). Similar results were described with *S. suis* and *A. pleuropneumoniae.* (Jobert et al., 2000; Berthelot-Hérault et al., 2001, Marois et al., 2004) and with *P. multocida* (de Jong, 2006). Nevertheless, our results showed that, except for *M. hyopneumoniae*, percentages of PCR positive samples were higher in pigs of group 2. Percentages of positive cultures were similar in the two groups, but *A. pleuropneumoniae* was not isolated from pigs of group 2. These results suggest that a contact between the SPF pigs and the finishing pigs, during 4 h in the slaughterhouse, had little or no influence on the transmission of respiratory pathogens.

The scalding tank appeared to be a critical point of the slaughtering process. Before the onset of slaughter, DNA from three major respiratory pathogens was detected in water of the scalding tank (samples A). After the passage of finishing pigs in the scalding water (more

than 3.600 pigs in each case), and before the slaughtering of SPF pigs, the number of positive samples of water increased and five bacterial species were recovered by PCR. Four hours later, *H. parasuis* was not detected from the water samples. Post-mortem examinations of 320 lungs of finishing pigs, before the slaughtering of SPF pigs, showed lung lesions in a large proportion of them. No bacteriological analysis was carried out from these lungs. Nevertheless, it is well known that the bacterial pathogens involved in respiratory diseases of pigs are the species detected from pigs and scalding water in the present study. Thus, the scalding tank water was contaminated by infected finishing pigs during the day of slaughter. Interestingly, viable *P. multocida* were detected from four samples of water. In the scalding tank water, *P. multocida* was probably surrounded by organic material and was protected against a high temperature. According to de Jong, (2006), *P. multocida* can survive 10 min at 60°C. During the scalding, some water contaminated by respiratory pathogens, can reach the trachea and the lungs of recently killed pigs. Histological results were in agreement with this hypothesis. Contaminated water from the scalding tank water invaded bronchi of some SPF pigs and bacterial accumulations were observed in the bronchial lumen.

To conclude, this experimental study showed that bacterial population present in slaughterhouses might contribute to transmit pathogens between pigs. Nevertheless, the scalding tank water was the critical point of the slaughtering process and an important source of contamination for pigs. Checks routinely conducted at slaughter, for respiratory disorders in finishing pigs, are very useful for veterinarians and have to be carried on, but nasal cavities, tonsils and trachea are not appropriate for bacteriological investigations. Despite we could not detect pathogenic respiratory bacteria from the lungs of the SPF pigs, bacteriological investigations at slaughter have to be used with carefulness.

Acknowledgement

The authors thank Pr. M. Gottschalk, Dr A. Bouchardon and Dr. F. Madec for helpful discussions and Pierre Ecobichon, André Kéranflec'h and Jean-Claude Rault and the slaughter staff for skilled technical assistance.

287 *References*

288 Andreasen, M., Mousing, J., Krogsgaard Thomsen, L., 2001. No simple association between
289 time elapsed from seroconversion until slaughter and the extent of lung lesions in Danish
290 swine. Prev. Vet. Med. 52, 147-161.

291 Berthelot-Herault, F., Cariolet, R., Labbe, A., Gottschalk, M., Cardinal, J.Y., Kobisch, M.,
292 2001. Experimental infection of specific pathogen free piglets with French strains of
293 *Streptococcus suis* capsular type 2. Can. J. Vet. Res. 65, 196-200.

294 Calsamiglia, M., Pijoan, C., Trigo, A., 1999. Application of a nested polymerase chain
295 reaction assay to detect *Mycoplasma hyopneumoniae* from nasal swabs. J. Vet. Diagn.
296 Invest. 11, 246-251.

297 Cariolet, R., Marie, P., Moreau, G., Robert, H., 1994. Rappel des différentes méthodes
298 d'obtention de porcelets assainis : conditions de maintient du statut sanitaire et
299 valorisation de ces animaux. Journées Rech. Porcine en France. 26, 1-12.

300 de Jong, M.F., 2006. Progressive and non progressive atrophic rhinitis. In: Straw, B.E.,
301 Zimmerman, J., D'Allaire, S., Taylor, D.J. (Eds), Diseases of swine, 9th ed. Iowa State
302 University Press, Ames, IA, pp. 577-597.

303 Fano, E., Pijoan, C., Dee, S., 2005. Evaluation of the aerosol transmission of a mixed
304 infection of *Mycoplasma hyopneumoniae* and porcine reproductive and respiratory
305 syndrome virus. Vet. Rec. 157, 105-108.

306 Gottschalk, M., Taylor, D.J., 2006. *Actinobacillus pleuropneumoniae*. In: Straw, B.E.,
307 Zimmerman, J., D'Allaire, S., Taylor, D.J. (Eds), Diseases of swine, 9th ed. Iowa State
308 University Press, Ames, IA, pp. 563-576.

309 Jobert, J.L., Savoye, C., Cariolet, R., Kobisch, M., Madec, F., 2000. Experimental aerosol
310 transmission of *Actinobacillus pleuropneumoniae* to pigs. Can. J. Vet. Res. 64, 21-26.

311 Kellog, D.E., Kwok, S., 1990. Detection of human immunodeficiency virus. In*:* Innis, M.A.,
312 Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds), PCR protocols: A guide to methods and
313 applications. Academic Press, San Diego. pp. 339-343.

314 Kobisch, M., Friis, N.F., 1996. Swine mycoplasmoses. Rev. Sci. Tech. Off. Int. Epiz. 15,
315 1569-1605.

316 Madec, F., Derrien, H., 1981. Fréquence, intensité et localisation des lésions pulmonaires
317 chez le porc charcutier. Journées Rech. Porcine en France. 231-236.
318 Marois, C., Bougeard, S., Gottschalk, M., Kobisch, M., 2004. Multiplex PCR assay for
319 detection of *Streptococcus suis* species and serotypes 2 and 1/2 in tonsils of live and
320 dead pigs. J. Clin. Microbiol. 42, 3169-3175.
321 Marois, C., Le Carrou, J., Kobisch, M., Gautier-Bouchardon, A.V., 2007. Isolation of
322 *Mycoplasma hyopneumoniae* from different sampling sites in experimentally infected and
323 contact SPF piglets. Vet. Microbiol. 120, 96-104.
324 Meyns, T., Maes, D., Dewulf, J., Vicca, J., Haesebrouck, F., de Kruif, A., 2004. Quantification
325 of the spread of *Mycoplasma hyopneumoniae* in nursery pigs using transmission
326 experiments. Prev. Vet. Med. 66, 265-275.
327 Oliveira, S., Galina, L., Pijoan, C., 2001. Development of a PCR test to diagnose
328 *Haemophilus parasuis* infections. J. Vet. Diagn. Invest. 13, 495-501.
329 Opriessnig, T., Thacker, E.L., Yu, S., Fenaux, M., Meng, X.J., Halbur, P.G., 2004.
330 Experimental reproduction of postweaning multisystemic wasting syndrome in pigs by
331 dual infection with *Mycoplasma hyopneumoniae* and porcine circovirus type 2. Vet.
332 Pathol. 41, 624-640.
333 Otake, S., Dee, S.A., Jacobson, L., Torremorell, M., Pijoan, C., 2002. Evaluation of aerosol
334 transmission of porcine reproductive and respiratory syndrome virus under controlled field
335 conditions. Vet. Rec. 150, 804-808.
336 Pijoan, C., Fuentes, M., 1987. Severe pleuritis associated with certain strains of *Pasteurella*
337 *multocida* in swine. J. Am. Vet. Med. Assoc. 191, 823-826.
338 Pijoan, C. 2006. Pneumonic Pasteurellosis. In: Straw, B.E., Zimmerman, J., D'Allaire, S.,
339 Taylor, D.J. (Eds), Diseases of swine, 9th ed. Iowa State University Press, Ames, IA, pp.
340 719-725.
341 Savoye, C., Jobert, J.L., Berthelot-Herault, F., Keribin, A.M., Cariolet, R., Morvan, H., Madec,
342 F., Kobisch, M., A PCR assay used to study aerosol transmission of *Actinobacillus*
343 *pleuropneumoniae* from samples of live pigs under experimental conditions. Vet.
344 Microbiol. 73, 337-347.

345 Schaller, A., Djordjevic, S.P., Eamens, G.J., Forbes, W.A., Kuhn, R., Kuhnert, P., Gottschalk,
346 M., Nicolet, J., Frey, J., 2001. Identification and detection of *Actinobacillus*
347 *pleuropneumoniae* by PCR based on the gene apxIVA. Vet. Microbiol. 79, 47-62.
348 Stark, K.D., Nicolet, J., Frey, J., 1998. Detection of *Mycoplasma hyopneumoniae* by air
349 sampling with a nested PCR assay. Appl. Environ. Microbiol. 64, 543-548.
350 Thacker, E.L., Halbur, P.G., Ross, R.F., Thanawongnuwech, R., Thacker, B.J., 1999.
351 *Mycoplasma hyopneumoniae* potentiation of porcine reproductive and respiratory
352 syndrome virus-induced pneumonia. J. Clin. Microbiol. 37, 620-627.
353 Thacker, E.L., Thacker, B.J., Janke, B.H., 2001. Interaction between *Mycoplasma*
354 *hyopneumoniae* and swine influenza virus. J. Clin. Microbiol. 39, 2525-2530.
355 Thacker, E.L., 2006. Mycoplasmal Diseases. In: Straw, B.E., Zimmerman, J., D'Allaire, S.,
356 Taylor, D.J. (Eds), Diseases of swine, 9th ed. Iowa State University Press, Ames, IA, pp.
357 701-717.
358 Thanawongnuwech, R., Thacker, B., Halbur, P., Thacker, E.L., 2004. Increased production of
359 proinflammatory cytokines following infection with porcine reproductive and respiratory
360 syndrome virus and *Mycoplasma hyopneumoniae*. Clin. Diagn. Lab. Immunol. 11, 901-
361 908.

Table 1: Detection of pathogenic respiratory bacteria from 13 SPF pigs, at the end of the slaughter process.

NS: nasal swabs; TOS: tonsillar swabs; WT: whole tonsils; TS: tracheal swabs; LS: lung swabs

*4 h before slaughtering

Mhp: *Mycoplasma hyopneumoniae*, App: Actinobacillus *pleuropneumoniae*, Pm: *Pasteurella multocida*, Hps: *Haemophilus parasuis*, S. suis : *Streptococcus suis.*

Table 2: Detection of pathogenic respiratory bacteria from the scalding tank water (water samples taken thrice (T0, T+4, T+8) at 5 different places in the tank: A (9 SPF pigs), B (4 SPF pigs).

Mhp: *Mycoplasma hyopneumoniae*, App: Actinobacillus *pleuropneumoniae*, Pm: *Pasteurella multocida*, Hps: *Haemophilus parasuis*, S. suis : *Streptococcus suis.*

Figure 1: Microscopic examinations in the lungs of three SPF pigs (group 1) checked at the end of the slaughter process.

A: Normal structure of the bronchial epithelium (x200)

B: Exfoliation of the bronchial epithelial cells, dilated capillary vessels (x200)

C: Cellular debris, scales and bacterial accumulations in the bronchial lumen (x25)

D: Cellular debris, scales and bacterial accumulations in the bronchial lumen (x100).

386

387 Table 1: Detection of pathogenic respiratory bacteria from 13 SPF pigs, at the end of the

388 slaughter process.

389

Groups of SPF pigs	Samples from SPF pigs	Positive PCR					Positive cultures				
		Mhp	App	Pm	Hps	S. suis	Mhp	App	Pm	Hps	S. suis
1 (n=9) waiting in lairage *	NS	3	1	0	3	7	0	0	0	3	7
	TOS	2	1	0	8	3	0	0	0	8	2
	WT	1	4	3	0	0	0	0	1	0	0
	TS	7	7	1	6	3	0	2	1	5	2
	LS	0	0	0	0	0	0	0	0	0	0
	Total (n=45)	13 28.9%	13 28.9%	4 8.9%	17 37.8%	13 28.9%	0 0%	2 4.4%	2 4.4%	16 35.6%	11 24.4%
2 (n=4)	NS	1	2	1	3	3	0	0	0	2	3
	TOS	0	0	1	3	2	0	0	1	2	1
	WT	0	1	1	1	1	0	0	0	1	1
	TS	2	3	4	3	4	0	0	0	2	4
	LS	0	0	0	0	0	0	0	0	0	0
	Total (n=20)	3 15%	6 30%	7 35%	10 50%	10 50%	0 0%	0 0%	1 5%	7 35%	9 45%

390

391 NS: nasal swabs; TOS: tonsillar swabs; WT: whole tonsils; TS: tracheal swabs; LS: lung

392 swabs

393 *4 h before slaughtering

394 Mhp: *Mycoplasma hyopneumoniae*, App: *Actinobacillus pleuropneumoniae*, Pm: *Pasteurella*

395 *multocida*, Hps: *Haemophilus parasuis*, S. suis : *Streptococcus suis.*

396

397

Table 2: Detection of pathogenic respiratory bacteria from the scalding tank water (water samples taken thrice (T0, T+4, T+8) at 5 different places in the tank: A (9 SPF pigs), B (4 SPF pigs).

Samples of water	Time (h)	Positive PCR					Positive cultures				
		Mhp	App	Pm	Hps	S. suis	Mhp	App	Pm	Hps	S. suis
A	T0	2	2	0	0	1	0	0	0	0	0
	T+4	5	4	5	3	1	0	0	0	0	0
	T+8	5	5	2	0	1	0	0	0	0	0
Total (n=15)		12	11	7	3	3	0	0	0	0	0
B	T0	0	0	0	0	0	0	0	0	0	0
	T+4	5	5	5	2	5	0	0	3	0	0
	T+8	5	2	5	0	4	0	0	1	0	0
Total (n=15)		10	7	10	2	9	0	0	4	0	0

Mhp: *Mycoplasma hyopneumoniae*, App: Actinobacillus *pleuropneumoniae*, Pm: *Pasteurella multocida*, Hps: *Haemophilus parasuis*, S. suis : *Streptococcus suis.*

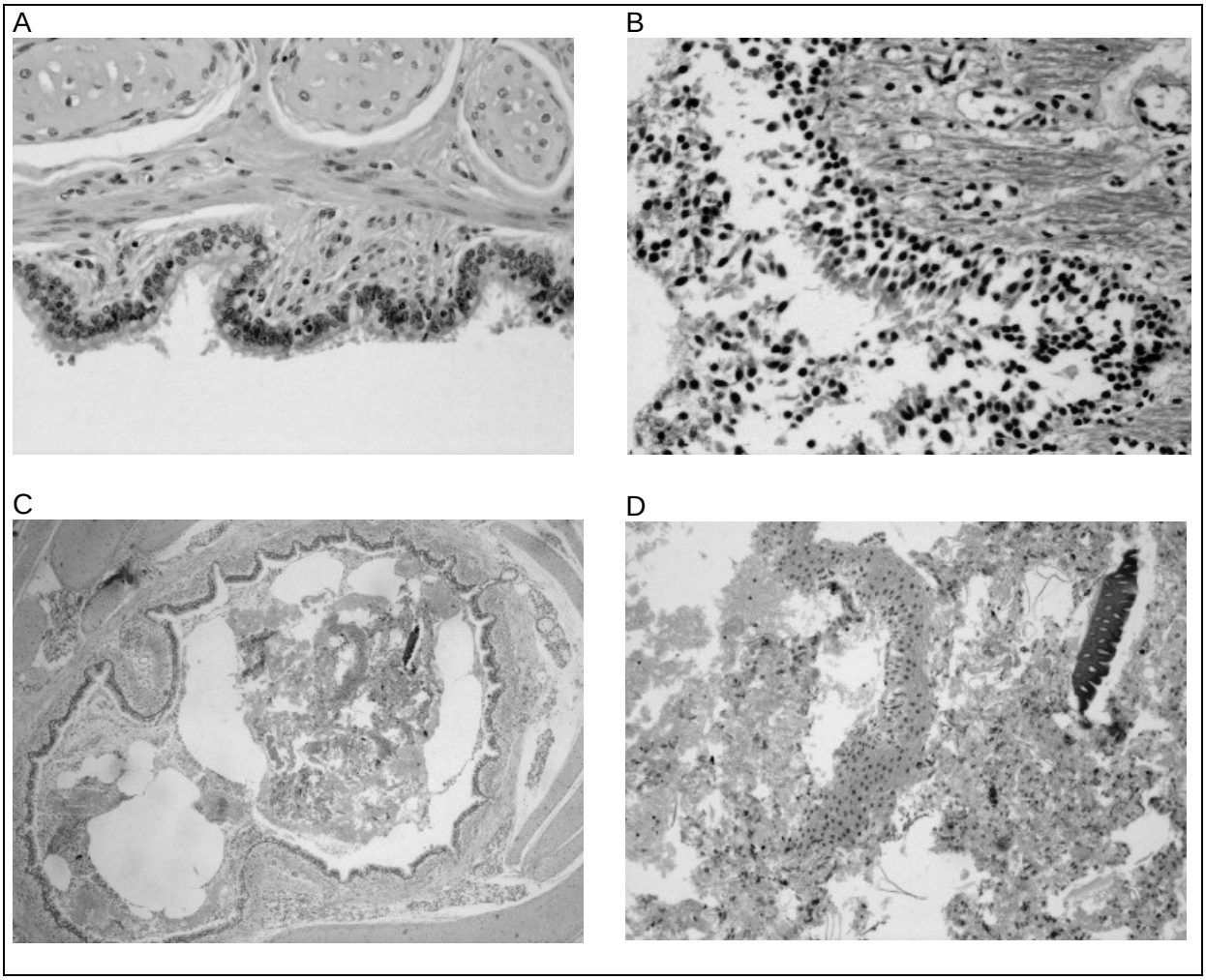

Figure 1: Microscopic examinations in the lungs of three SPF pigs (group 1) checked at the end of the slaughter process.

A: Normal structure of the bronchial epithelium (x200)

B: Exfoliation of the bronchial epithelial cells, dilated capillary vessels (x200)

C: Cellular debris, scales and bacterial accumulations in the bronchial lumen (x25)

D: Cellular debris, scales and bacterial accumulations in the bronchial lumen (x100).