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Assessment of human health risk associated with pyaemia in Danish finisher pigs when conducting visual-only inspection of the lungs

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ABSTRACT

The most important lesion to be overlooked when performing visual-only inspection of the lungs is embolic pneumonia. The aim of the present study was to assess the additional human health risk represented by overlooking cases of pyaemia represented by embolic pneumonia in finisher pigs, when conducting visual-only compared to palpation of the lungs, as is the traditional meat inspection procedure.

An examination of bacteria isolated from 19 finisher pigs identified with embolic pneumonia at traditional meat inspection was undertaken. From each pig samples were taken from various organs (lungs, spleen, heart, liver and kidney), from the carpal joints (*A. carpi*) and flexor muscle (*M. flexor digitorum superficialis*) on the right foreleg. These data were included in a risk assessment following OIE guidelines.

Bacteria were isolated from 78 out of 127 tissue and swap samples taken (61% positive samples). *Staphylococcus aureus* (N = 37) was the most frequently isolated bacterium. The predominant site of *S. aureus* was the lung. *S. aureus* was detected although less frequently in low numbers in some organs (<100 CFU/sample) and muscle samples (<10 CFU/sample). Only one MRSA isolate was found.

Staphylococcus warneri (N = 24) was the second most commonly found bacterium. There was no predominant site and the number of *S. warneri* was less than 50 CFU per sample.

The risk of a food-borne intoxication from *S. aureus* in relation to pyaemia in pigs was considered very low due to the low quantitative numbers of *S. aureus* in muscle tissue samples. Implementing visual-only inspection will reduce the exposure of *S. aureus* due to less cross-contamination and handling of the plucks by the meat inspectors. The human health risk associated with *S. warneri* was considered very low, due to the limited zoonotic potential of this bacterium. In conclusion, the additional human health risk in relation to possibly overlooking pyaemia in Danish finisher pigs was considered negligible when conducting visual-only compared to traditional meat inspection.

1. Introduction

1.1. Background

Meat inspection has been conducted for more than 100 years. During that period, the hazards have changed and infectious diseases like bovine tuberculosis and brucellosis are no longer present in several parts of the world. The current meat inspection in the European Union (EU) is to a large extent based on the hazards of the past, whereas it does not eliminate the hazards of today, such as *Salmonella* spp. and *Yersinia enterocolitica*. In addition, incision and palpation of the organs and lymph nodes, which is part of the traditional meat inspection, may even increase the risk of cross contamination with human pathogens both within the plucks/carcass and between plucks/carcasses. Therefore, the European Food Safety Authority (EFSA) suggested that meat inspection for pigs should be visual (EFSA, 2011).

According to the EU Meat Inspection Regulation 854/2004 (valid until June 2014) modifications of the traditional meat inspection of finisher pigs raised under controlled housing condition (implying indoor with high biosecurity) can be implemented, if it can be documented by a risk assessment that the changes will not have a negative impact on human health (Anonymous, 2004). In Denmark, three risk assessments undertaken by the Danish Agriculture & Food Council have already dealt with the effect on human health associated with a change from traditional to visual-only inspection of finisher pigs in Denmark. The first dealt with omission of the routine opening of the heart and incisions into the mandibular lymph node (Alban et al., 2008). The second dealt with omission of routine palpation of the intestinal lymph nodes (Alban et al., 2010). And the third dealt with omission of palpation of

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the lungs and their associated lymph nodes as well as the liver and its lymph nodes (Pacheco et al., 2013). All three risk assessments showed that changing from traditional to visual-only inspection would not have negative impact on human health. Furthermore, visual-only was not expected to have a negative impact on the ability to identify notifiable, exotic animal diseases, nor on animal welfare. According to EU regulation 218/2014 amending annexes to the Meat Inspection Regulation, and coming into force in June 2014, routine palpation of the lungs is no longer required (Anonymous, 2014). Palpation shall now only take place upon suspicion. Suspicion could arise if any abnormalities are seen during ante mortem inspection, the post mortem inspection or if epidemiological data or food chain information indicates that palpation or incision is necessary. Hence, visual-only will be the customary way of conducting meat inspection for all pigs, sows and boars — irrespective of the housing and management.

In our risk assessment on visual-only inspection contra palpations of the lungs, we found that embolic pneumonia constitutes the most important lesion that might be overlooked if routine palpation of the lungs is omitted (Pacheco et al., 2013). Between 1080 and 1800 cases of embolic pneumonia are estimated to be missed per year in Denmark when slaughtering around 20 million finisher pigs. Embolic pneumonia is characterized by multifocal distribution of abscesses involving a varying part of the pulmonary lobes as a result of pyaemia (McGavin and Zachary, 2007). Pyaemia is a systemic infection, normally caused by pyogenic bacteria, such as *Staphylococcus aureus* and *Streptococcus* spp. The bacteria enter the body through skin infections, typically as a result of tail bites, fight wounds or castration wounds. If the bacteria manage to pass through the skin barrier they can spread throughout the body and enter different organs and tissues through the blood (McGavin and Zachary, 2007). Pyaemia can also lead to abscess formation in the bones (osteomyelitis).

According to EU Regulation (EC) No 854/2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption, meat is to be declared unfit for human consumption if it derives from animals affected by a generalized disease, such as pyaemia (Anonymous, 2004). In Denmark, suspect cases of embolic pneumonia detected during meat inspection will be investigated further in the rework area. Here it will be determined whether the processes in the lungs are reflecting a chronic or acute stage of infection. In a chronic stage, a capsule is developed, enclosing the abscesses, which is not the case for an acute stage (Jensen et al., 2010). The destiny of the carcass depends on this judgement. If the processes are indicative of a chronic stage of infection, the carcass is sent for de-boning according to current Danish legislation (Anonymous, 2005). Contrary, if the lesions indicate an acute stage of infection, the whole carcass will be condemned (Jensen et al., 2010). The organs (plucks and intestines) will in all cases be condemned due to logistic reasons.

The aim of this study was to assess the additional human health risk associated with pyaemia in pigs, detected as embolic pneumonia when conducting visual-only compared to palpation of the lungs, which is the traditional meat inspection procedure. This includes identification of bacteria associated with embolic pneumonia.

The data collection undertaken in relation to a previous risk assessment showed that the most commonly found bacteria in the lungs of pigs with embolic pneumonia were *S. aureus* and *Escherichia coli* (Pacheco et al., 2013). In that project, only presence of bacteria in the lungs was examined. However, from a food safety perspective it is important to examine whether the bacteria causing the pyaemia also can be found in the muscles or other edible tissues. Therefore, in the present study we investigated the prevalence of bacteria in the organs, joint and muscle of pigs given the code pyaemia during post mortem inspection. Muscle tissue samples from carcasses approved with no comments served as control group. Based on the microbiological findings we performed an assessment of the human health risk associated with pyaemia in pigs.

2. Materials and methods

2.1. Sample collection

Tissue and swap samples from pigs were collected at a Danish pig abattoir, for 1 week in both April and May 2013. All pigs initially identified as possibly suffering from embolic pneumonia during ordinary inspection and subsequently given the meat inspection code *Pyaemia, blood poisoning* by the veterinarian after an intensive inspection at the rework were included in the study (N = 19).

Pigs were included independent of the final destiny of the carcass (condemned or accepted for de-boning). Samples were taken aseptically from the lungs, heart, liver, kidney, and the spleen from each pig. Samples were also taken aseptically from the carpal joints (*A. carpi*) and flexor muscle (*M. flexor digitorum superficialis*) on the right foreleg. Signs of tail bites or wounds on the skin were recorded for each pig.

Muscle tissue samples from 60 healthy pigs were used as controls. These were collected at the same slaughterhouse, also from the flexor muscle on the right foreleg, and examined as described for the muscle tissue samples from pigs identified with embolic pneumonia. These control samples were collected during November 2013.

All carcasses underwent bacteriological investigation no later than 1 h after slaughter. For the lungs, a swap sample was taken from an abscess in the depth of the lung tissue. After decontamination of the surface with a branding iron, a cut was made with a sterile scalpel and a swap was taken with a sterile 1 μl loop from the abscess. For the heart, a swap was taken from the heart valves with a sterile 1 μl loop, after making a cut with a sterile scalpel. For the joint, swap samples were taken directly after cutting and opening into the joint where a swap was taken with a sterile 1 μl loop (estimated sample sizes of lung and joint swap samples are 10 μl and of heart swap sample 15 μl due to differences in viscosity). Swap samples from the lung, heart and joint were subsequently streaked on blood agar plates. Tissue samples from the muscle, liver, spleen, and kidney were cut out from the depth of the tissue with a sterile scalpel, after decontamination of the surface. Approximately 1 g of tissue was cut out and streaked to the bottom of an empty petri dish (sample size corresponded to a surface area of approximately 6 cm^2). The tissue sample was left in the petri dish, and blood agar was poured until the tissue sample was half-covered. Blood agar was prepared from blood agar base (Oxoid, UK) supplemented with 5% sterile bovine blood.

All plates were incubated at 37 °C under aerobic conditions for 24 to 48 h. After incubation bacteria were counted as number of colony forming units (CFU). For some samples, it was impossible to count an exact number of colonies due to smear observed on the agar plate. This was especially the case for the lungs where swap samples were taken from the abscesses containing an expected high number of bacteria. For these cases, results are given in semi-quantitative numbers, e.g. approximately >200 CFU per streak with the inoculation needle. From each plate, one colony of each type of colonies present on the plates (based on colony morphology) was sub-cultured on blood agar plates at 37 °C to obtain pure cultures.

2.2. Identification of bacteria

Bacteria detected in the present study were identified using *Matrix-assisted laser desorption-ionization–time of flight mass spectroscopy* (MALDI-TOF-MS) in a VITEK ® MS MALDI-TOF machine (bioMérieux, NC). Bacteria were sub-cultured twice on blood agar plates before analysis with MALDI-TOF. One single colony was taken directly from the blood agar plates. The colony material was spread onto a slide and mixed with a UV-light absorbing matrix consisting of Acetonitrile 28% (VITEK MS-CHCA, bioMérieux, NC). Analysis by MALDI-TOF MS was done according to the manufacturer's protocol and matched with reference spectra by the computer software. *E. coli* was used as control strain in all analysis. A satisfactory result was demanded implying a match