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Application of acute phase protein measurements in meat extract collected during routine veterinary inspection at abattoirs

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ABSTRACT

The possible application of acute phase protein measurements of meat extract in porcine carcass inspection at abattoir, under routine conditions, was studied. Concentrations of two acute phase proteins, C-reactive protein and haptoglobin, were quantified in 357 samples from carcasses subjected to official veterinary inspection at slaughterhouses. Carcasses were classified according to their sanitary status in five groups of animals ranging from healthy animals, without any organ alteration (group 1), to completely condemned carcasses with gross alteration in several organic systems (group 5). The concentration of both acute phase proteins appeared significantly higher in groups 2 to 5 in comparison to group 1. Sensitivity of these proteins to detect animals with organ alterations was 86% when the values of both proteins were taken into account. The quantification of the levels of acute phase proteins could be of help during routine veterinary meat inspection by offering an objective tool for active disease detection.

1. Introduction

Slaughterhouses are intermediates between the animal production system and the distribution of food of animal origin, making abattoirs an ideal point for sanitary and productive evaluation. Furthermore, its value has been recently recognized as an animal health surveillance tool (Harley et al., 2012) as well as its traditional function to reduce food-borne risks to public health (Edwards et al., 1997). However, the meat inspection process needs to be standardized and reformed before it can be reliably utilized in large-scale pig welfare surveillance schemes (Harley et al., 2012).

From a practical veterinary perspective, it has been reported that meat inspection in general has high specificity but only acceptable sensitivity in respiratory disorders (Bonde et al., 2010). Thus an individual farmer or veterinarian might be confident that a report of one or more problems at the meat inspection reflects a true presence of disease; but an absence of problems might not guarantee that the pigs slaughtered were truly healthy (Bonde et al., 2010). In this context, additional sensitive tools such as acute phase protein (APP), that veterinary inspectors could implement at the time of slaughter, could be of great value.

APPs have been defined as valuable predictive indicators for risk assessment in meat inspection since health and welfare could be monitored by their levels (Klauke et al., 2013). Several APPs have been used for animal health monitoring in the porcine production chain such as C-reactive protein (CRP) and haptoglobin (Hp) (Gutiérrez et al., 2012), Serum amyloid A (Soler et al., 2012) or pig-MAP (Piñeiro et al., 2013). Moreover the quantification of acute phase proteins in meat extract samples has been used for the assessment of animal health in pig production with implications for food safety and meat quality (Piñeiro et al., 2009). Meat extract has been postulated as an alternative matrix to serum at post mortem examinations where serum is usually not available. Into this context, several infectious agents have been detected in slaughtered pigs using meat extract samples. Moreover, meat extract is an established matrix to assess food safety parameters like salmonella antibodies for quality management systems in the pork sector in Germany (Fischer et al., 2012), Austria (Köfer et al., 2006) and Denmark (Nielsen et al., 2001).

The present study aimed, for the first time, to investigate the possible usefulness of the quantification of APPs in meat extract samples for the detection of generalized pathology in carcasses during routine veterinary inspection at slaughterhouses. The results of this study would provide important objective information for the possible implementation of APPs as a veterinary tool during slaughter inspections.

2. Material and methods

2.1. Animal origin, sampling and classification

A total of 357 finishing pigs (conventional Duroc × (Landrace × Large White)) of mixed sex, from seven different slaughterhouses located in Murcia, in the southeast of Spain, were sampled at two different

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periods. The first period included 177 animals sampled, from five abattoirs, between May and December 2010 on nine different days, while the second consisted of 180 animals sampled, from three abattoirs, between December 2012 and September 2013 on eight different days. Animals originated from one herd per abattoir expect in two abattoirs in the first period in which two different herds were sampled in each abattoir.

An average of twenty one samples per day was collected during routine official sanitary meat inspections. Portions of diaphragm ($2 \times 2 \times 2$ cm approximately) were collected from the suspended porcine carcasses, deposited in meat extract collectors (Sarstedt, Nümbrecht, Germany) and stored at −20 °C until analysis.

Ante-mortem and post-mortem examinations of animals and carcasses were carried out by the same Official Sanitary Meat inspectors from the Autonomic Community of Murcia, Spain (a total of five inspectors). Parameters investigated during ante-mortem and post-mortem examinations are described in Table 1. Ante-mortem inspection was performed visually at the reception pen in the slaughterhouses and only those animals suspected to be diseased were separated for further clinical examination including mobility inspection or/and temperature. Post-mortem inspection included carcass examination and inspection of all organs using the standard protocol for routine meat control from the Sanitary and Consumption Office (Murcia Town Council). Organ lesions were scored according to the estimated percentage of organ affected and indicators of acuteness such as degree of vascularisation and regional ganglion affection were also taken into account for carcass classification.

A random sampling procedure was used during the first period. Two veterinary inspectors randomly selected animals at ante-mortem examination. Afterwards, animals were included into one sanitary group according to carcass classification (Table 2), which was performed according to the absence or presence and degree of pathological findings. Due to the low number of carcasses classified into groups 1 and 5, the second sampling period was focussed on an increase in the number of sampled animals included in those sanitary groups corresponding to healthy pigs and pigs with either acute pathological processes and/-or bad carcass conformation. Therefore, in the second sampling, condemned animals and at least one healthy animal from the same batch were sampled by three different veterinary inspectors, one per abattoir.

Data of carcass weight and fat thickness were only recorded in the second set of samples. Fat thickness was measured by Fat-O-Meat'er I (FOM I) devices at 6 cm of the midline and between the 3rd and 4th last ribs and perpendicular to the skin as indicated by Official European Authorities (Commission Decision 2012/384/EU).

Table 1
Variables investigated during ante-mortem and post-mortem official veterinary inspections.

Ante-mortem symptoms	*Post-mortem lesions*
Congestion/fever/trembling	Subcutaneous congestion
External injuries/fracture	Enlarged lymphatic ganglion
Skin hemorrhage	Lesions in skin and mucosa
Skin injuries/dermatitis	Vascular lesion
Cachexia/emaciation	Cachexia/emaciation
Cough/sneeze	Pneumonia/pleuritis
Atrophic rhinitis	Pericarditis/peritonitis
Diarrhea/vomit	Digestive disorders
Abscesses	Splenic alteration
Prolapse	Liver alteration
Lameness (arthritis)	Polyarthritis
Bedsore	Urinary lesion
Others	Genital alteration

2.2. Acute phase protein determinations

The concentrations of CRP and Hp in meat extract samples were measured by using a specific simultaneous time-resolved immunofluorimetric assay. The assay is a non-competitive sandwich immunoassay that allows the quantification of both proteins in 90 min with high levels of precision and accuracy as previously reported (Gutiérrez et al., 2012).

The concentrations of CRP and Hp were compared in animals of the five different groups by using the Kruskal–Wallis non-parametric statistical tests with Dunn's multiple comparison in order to study if the concentration of APPs in healthy animals (group 1) differs from any other pathological group. The statistical analysis was carried out by using the Graph Pad Prism 6 statistical program. The level of significance was set at $p < 0.05$. Moreover, the Spearman's correlation coefficient between CRP and Hp was calculated.

2.3. Diagnostic performance of acute phase protein measurements for disease diagnosis in meat extract samples

The concentration of acute phase proteins obtained in animals of group 1, healthy pigs without any lesion at ante-mortem and post-mortem inspection, was used as "controls" ($n = 69$) for the diagnostic performance of APPs. Animals included in groups 2 to 5, from animal's carcasses with mild lesions to partially or totally condemned, were classified as "patients" ($n = 288$). Selection of cut-off values for CRP and Hp was undertaken using receiver operating characteristic curve (ROC) analysis.

ROC analysis plots a curve of sensitivity versus specificity for all possible threshold values of the parameter to be evaluated. Sensitivity is defined as the fraction of animals with any lesion at post-mortem inspection that the measurement of APPs correctly identifies as positive. Specificity is defined as the fraction of healthy animals without any lesion at post-mortem inspection that the test correctly identifies as negative. The area under the curve (AUC) indicated the diagnostic accuracy of the examined parameter, with an AUC of 1 being meaningful and 0.5 being meaningless.

ROC analysis was carried out for CRP and Hp separately and then for the combination of both proteins as reported before (Gutiérrez et al, 2012) by using the Graph Pad Prism 6 statistical program.

3. Results

3.1. Ante-mortem and post-mortem examination

The distribution of animals according to sex was similar in the two series of samplings performed and was 58.2% females, 34.8% castrated males and 7% entire males in the first set and 56.2% females, 27.3% castrated males and 16.4% entire males in the second sampling interval.

Average values of carcass weight between animals of group 1, without any clinical sign of disease and without anatomical alterations at post-mortem examination (85.5 kg ± 8.2) and group 5, which included animals with clinical symptoms and/or acute pathological processes and/or bad carcass conformation (76.68 kg ± 15.1), from the second sampling period, showed statistical significant differences ($p < 0.0001$). Statistical significant differences ($p < 0.0004$) were also found in the values of fat thickness between those groups of animals with higher average values of 18 mm in group 1 in comparison to the 13.8 mm of group 5.

The clinical signs recorded during ante-mortem examination were diverse and widely distributed between the different sanitary groups used to classify carcasses. Those carcasses classified into the sanitary group 1 and 2 did not show any ante-mortem alteration. In contrast, carcasses classified in groups 3 to 5 had variable percentages of ante-mortem alterations. Only an 8% of animals from those