THE HARMONIZATION OF SANITARY DECISION CRITERIA FOR VERTEBRAL OSTEOMYELITIS IN PIG CARCASSES

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Introduction

Vertebral osteomyelitis (VO), a typically suppurative lesion, is the main cause of pig carcass condemnation during post mortem inspection in Portugal, being responsible, in 2015, for total condemnation of 0.1% of the slaughtered finishing pigs.

Sanitary decision of VO cases taken by official veterinarians should be based on Regulation (EC) N.º 854/2004 which defines that meat must be declared unfit for human consumption if it derives from animals affected by a generalized disease, such as septicemia, pyaemia, toxemia or viraemia. So, considering that presence of VO in pigs is indicative of pyaemia, it seems logical to declare VO cases unfit for human consumption. However, if the pyaemia has ended, is it then necessary to condemn the entire carcass?

For the cases not recognised in the live animal during ante mortem inspection, pyaemia may be detected during post mortem inspection by revealing suppuration, with or without abscesses, and haemorrhagic infarcts in different parts like the lung, mediastinum, pleural cavity, spleen, kidneys, joints and muscle. Those cases, with multiple suppurative findings, obviously require total condemnation. On the opposite, VO cases associated with prior pyaemia and no indication of current systemic changes, the judgment of meat inspection could be condemnation only of the affected parts. Nevertheless, in Portugal many of these VO cases are totally condemned in an undifferentiated way, because of the perceived risk related to pyaemia and due to the lack of objective criteria to support an alternative sanitary decision.

The objectives of this study were 1) to assess the need for total condemnation of carcasses with VO based on the health risk for the consumer and 2) to define harmonized criteria for the condemnation of these cases based upon objective macroscopic parameters, scientific-based. This would lead to avoidance of unnecessary condemnation of affected carcasses that do not represent a risk to the consumers.

Material and methods

During 17 weeks in the winter 2015/16, meat inspection of 211,159 pigs was undertaken in one Portuguese abattoir. All VO cases were declared unfit for human consumption by the official veterinarians. From those, 40 VO cases were selected at random and analysed in order to assess the need to apply this sanitary decision.
Based on Regulation (EC) N.º 854/2004, Gracey et al. (1999) and Ninios (2014) the following objective macroscopic parameters were used as criteria to classify VO cases with respect to need for condemnation:

**Total condemnation**

- **Generalized (G) cases:**
  - G1 - ≥1 Acute VO lesion;
  - G2 - 1 or 2 Chronic VO lesions in contiguous vertebrae with additional suppurative lesions in other parts of the carcass and/or respective viscera;
  - G3 - ≥ 2 Chronic VO lesions in separated vertebrae;
  - G4 - ≥ 3 Chronic VO lesions in contiguous vertebrae.
- **Extensive carcass contamination with purulent exudate.**

**Partial condemnation**

- **Localized (L) cases:**
  - L1 - 1 Chronic VO lesion without additional suppurative lesions in other parts of the carcass and/or respective viscera;
  - L2 - 2 Chronic VO lesions in contiguous vertebrae without additional suppurative lesions in other parts of the carcass and/or respective viscera.

In order to support the aforementioned criteria, VO lesions were classified as acute or chronic, based on the following objective macroscopic characteristics:

- **Acute:** Shiny and moist lesions with, sometimes, congested areas Evident bone destruction not circumscribed by adjacent remodelling tissue; presence of fluid purulent exudate;
- **Chronic:** Moderate bone destruction circumscribed by remodelling tissue; thickened exudate.

To evaluate the effectiveness of these macroscopic criteria, the same lesion was submitted to a histopathological analysis. For that, the column fragment with the VO lesion was cut and stored in a container with formalin (10%) for further evaluation. During post mortem inspection, each carcass and respective viscera were evaluated being registered the local (Cervical, Thoracic, Lumbar, Sacral, Coccygeal) number and contiguity of affected vertebrae, presence of perivertebral abscesses and other suppurative lesions beyond VO (muscle, joints, tail, lung…) in order to apply harmonizing criteria for the condemnation.

In order to evaluate the risk to the consumer related to the presence of pyaemia, paired samples of VO purulent exudate and muscle (diaphragm) were aseptically collected in sterile containers and sent to the laboratory under refrigerated conditions for further microbiological analysis. The presence of the same bacteria in both samples was considered as indicative of pyaemia related to VO. Additionally, from each batch of VO cases provenance, a sample of diaphragmatic muscle was collected from one carcass fit for consumption (with no lesions) to be used as controls in the microbiological analysis.

At the laboratory, microbiological analyses were carried out according to standard techniques targeting the main etiological agents responsible for these lesions: *Staphylococcus, Trueperella pyogenes, Streptococcus and Pasteurella*. Presumptive *Staphylococcus* spp. isolates (Coccus Gram +, DNase and catalase +) were identified by molecular methods (multiplex PCR 16S rDNA, mecA, nuc).
For histopathology examination, specimens were fixed in 10% neutral-buffered formaldehyde and routinely processed for histological examination, embedded in paraffin wax and sectioned at 3 μm. One section was stained with HE (haematoxylin - eosin) and the other with Gram coloration. Histopathological classification of VO lesions was based in the following criteria:

- **Acute:**
  - **Osseous changes:** Osteonecrosis; Irregular contours and fragmentation of bone trabeculae, with bone sequester formation; There are intramedullary granulocyte infiltrates and fibrin exudates; Reduced or complete lack of haemopoiesis.
  - **Soft tissue changes:** necrosis;
  - **Inflammatory infiltrate pattern:** neutrophilic granulocyte infiltrate diffuse.

- **Chronic:**
  - **Osseous changes:** Bone neogenesis, medullary space tissue with granulation tissue formation;
  - **Soft tissue changes:** Granulation tissue formation and fibrosis;
  - **Inflammatory infiltrate pattern:** Lymphocyte/macrophage/plasma cell infiltrate, with a few neutrophilic granulocytes.

The statistical significance of the association between macroscopic and histopathological classification of VO lesions was assess by Phi test for paired samples, using IBM SPSS Statistics® 20 software. Phi test was also applied in order to evaluate the association between pyaemia and the macroscopic parameters used to classify VO cases. Differences were considered significant at \( P < 0.05 \).

**Results**

From the 211.159 pigs slaughtered during this study period, 240 carcasses (0.11%) were totally condemned. From those, all VO cases (n=152) were declared unfit for human consumption by the official veterinarians, representing 0.08% of the slaughtered pigs and the main cause of total condemnation (152/240; 63%).

From the 40 randomly selected VO cases, using the objective macroscopic parameters pre-determined, 20 were classified as chronic and 20 as acute. Results from histopathological analyses revealed a highly significant association (Fi=0.804, p<0.001) with the macroscopic results allowing to validate the proposal for VO classification in acute and chronic during post mortem inspection contributing to its harmonisation.

In this study, lesions indicative of VO were more commonly observed in only one location (35/40, 87.5%) than in two or more different locations (5/40, 12.5%). From the cases found in only one location, the majority were (F=2.19, p<0.05) observed in the thoracic vertebrae (n=19).

Additionally, post mortem inspection of the carcass and viscera allowed to identify other suppurative lesions beyond VO. In the 40 VO cases, the majority (23/40, 57.5%) did not present additionally suppurative lesions observed during post mortem inspection. The remaining 17 (42.5%) presented additionally diverse suppurative lesions (Table 1). Pleuropneumonia was found in 12 cases. Suppurative exudate or abscesses were not found in any of these cases, and therefore, these lesions were not considered as related to VO but rather an independent finding of post mortem inspection reflecting
that chronic pleuritis is the most common finding in post mortem inspection of finishing pigs.

Microbiological analysis revealed 13 (32.5%) positive pyaemic cases (presence of the same bacteria in both samples: muscle and VO), No bacteria were isolated from the 36 control muscle samples.

Microbiological results showed that *T. pyogenes* (32) was the most frequently isolated bacteria from both VO and muscle samples, followed by *Streptococcus* spp. (16) and *S. aureus* (6).

Table 1 resumes the main results reached in this study.

### Table 1. Sanitary decision and judgment criteria of VO cases, characteristics of lesions and presence of pyaemia.

<table>
<thead>
<tr>
<th>Judgment criteria</th>
<th>VO lesion</th>
<th>Additional suppurative lesions</th>
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<td>Criteria reference</td>
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A/C – Acute/Chronic; SD – Sanitary decision; Criteria reference – Reference used for judgment criteria: G1 – Generalized1, G2 – Generalized2, G3 – Generalized3, G4 – Generalized4, L1 – Localized 1, L2 – Localized 2; V1, V2, V3 – One, two and tree affected vertebrae with osteomyelitis, respectively; n.i. – No bacterial isolation

The data presented in Table 1 shows that both 13 active pyaemia cases were classified as generalized (totally condemned), being this association very significant (Fi=0.4543; p<0.01). Also, acute cases revealed a very significant association (Fi=0.6547; p<0.01) with pyaemia in opposite to the other macroscopic parameters used to classify VO cases that did not shown any association.

Using the criteria proposal to classify VO cases with respect to need for condemnation, 11 carcasses out of 40 (27.5%) could have been spared from total condemnation. Those were chronic cases that matched 100% with HP evaluation, and did not reveal any signs of pyaemia, meaning that the criteria used to judge VO cases represent no risk to the consumer concerning to the presence of pyaemia.
Conclusions

Total condemnation of VO cases are mainly due to the perceived risk related to pyaemia, which is one of the criteria defined in the Regulation (EC) No. 854/2004 to declare meat unfit for consumption. Based on this it was important to understand and objectively detect, under meat inspection conditions, the cases of VO that are not related to pyaemia and could be spared from a total condemnation. In this study, the harmonized criteria proposal for condemnation of VO cases, defined by objective macroscopic parameters, allowed to allocate them in generalized (pyaemia) and localized cases leading, respectively, to total and partial condemnation. Under this harmonised protocol, classification of VO in acute or chronic was determinant, especially in single cases. The highly significant association found between macroscopic and histopathologic results allows to validate the proposal for VO classification in acute and chronic during post mortem inspection that strengthens this harmonized protocol.

The application of the proposed objective criteria to support sanitary decisions for VO, could have spared the rejection of 27.5% of the affected carcases, not constituting any risk to consumers concerning to the presence of pyaemia and fitting the objective of Regulation (EC) No. 854/2004. Also, using these harmonized criteria, an approximate loss of €1,309 could have been avoided, considering a production cost of €109/pig with approximately 107 kg live weight (SIP, 2015). This result is of extremely importance in Portugal since VO is the main cause of condemnation of finishing pigs at slaughterhouse (In 2015, 4,267 carcasses were totally condemned by VO, corresponding to 0.1% of the slaughtered pigs).

Considering the microorganisms isolated, the majority (T. pyogenes and Streptococcus) should be considered as a potential occupational risk for slaughterhouse worker. Only S. aureus, isolated from two cases, should be considered as potential foodborne pathogen and a potential risk to consumers, if carcases reach to the market under conditions that favour the production of enterotoxins.

As final remark we would like to underline the importance of VO lesion in pork production chain that requires a multidisciplinary approach in order to mitigate the economic loss at the slaughterhouse level that, according to the authors, should include the revision of condemnation judgment during post mortem inspection. In fact, the inexistence of objective criteria to classify VO lesions may favour some subjectivity judgments concerning to carcasses condemnation, leading to unnecessary economic losses and to uncomfortable and inconvenient perceptions by food business operators concerning to official veterinarians, that must be avoid.

These results allows to define objective macroscopic criteria, scientific based, to support the harmonization of sanitary decision procedures associated with VO cases, reducing economic losses for industry without compromising food safety and public health.