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Endocarditis-associated Brain Lesions in Slaughter Pigs

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Summary

Left-sided valvular endocarditis (LSVE) is a common finding in slaughter pigs. The lesion is often associated with renal thromboembolism, but information on embolization to other organs is sparse. This study focuses on the presence and type of endocarditis-associated brain lesions (EABLs). The brains of 20 slaughter pigs with spontaneously arising LSVE and 11 controls were examined by sectioning half of a formalin-fixed brain into 4 mm slices for histological examination. The aetiology of the endocarditis was determined by bacteriological and, in some cases, by fluorescence in-situ hybridization examinations. These examinations identified 11 cases of *Streptococcus suis*, six cases of *Erysipelothrix rhusiopathiae*, one *Streptococcus* spp. and two cases that remained aetiologically undetermined. One of the *S. suis* cases had a dual infection with *S. suis* in the aortic valve lesions and *Streptococcus dysgalactiae* subsp. *equisimilis* in the atrioventricular valve lesions. Renal infarcts were present in eight cases. Focal encephalitis was found in 12 cases, with the number of lesions ranging from one to 11. Most pigs had less than four microscopical lesions. Acute lesions were characterized by focal microabscesses without observable bacteria. Chronic lesions were characterized by astrocytosis and focal accumulation of mononuclear leucocytes. An infarct was observed in one animal. Perivascular inflammation was seen in 14 cases, mostly as two or three lesions, while focal leptomeningitis was found in eight cases. EABLs are therefore common in slaughter pigs with LSVE. The number of lesions per animal is small, which may explain the limited attention paid to this sequela of LSVE. EABLs have rarely been reported in domestic animals and mostly in patients with neurological signs. The frequent occurrence of EABLs in slaughter pigs suggests that this pathology should be investigated in other animal species with LSVE.

Keywords: brain; encephalitis; *Erysipelothrix rhusiopathiae*; infarct; pig; *Streptococcus suis*

Introduction

Endocarditis is a common finding in slaughter pigs. The lesions are mostly diagnosed at the time of post-mortem inspection and usually present as valvular vegetation in the left side of the heart, mainly involving the cusps of the atrioventricular (AV) valve (Maxie and Robinson, 2007; Jensen *et al.*, 2010). Several studies of porcine endocarditis have documented that *Streptococcus suis* and *Erysipelothrix rhusiopathiae* are the most prevalent agents, although a wide range of other opportunistic bacteria (e.g. *Pasteurella multocida*, *Staphylococcus aureus* and *Arcanobacterium pyogenes*) have been isolated (Geissinger, 1968; Narucka and Westendorp, 1973; Pedersen *et al.*, 1984; Katsumi *et al.*, 1997).

Endocarditis is generally accepted to be a sequela to bacteraemia with bacteria lodging on the valvular endothelium (Jones, 1981; Auclair, 1995). Superficial parts of the lesion may be detached following vegetation of the lesion by accumulation of fibrin, infiltration by inflammatory cells, trapping of blood components and bacterial proliferation. Such embolization is often reflected by the presence of embolic lesions in other organ systems (Jones, 1981, 1982). Endocarditis of the right side of the heart may

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be associated with embolization of the lungs, while left-sided endocarditis is mostly associated with renal infarcts or abscesses, although other lesions (e.g. myocardial abscesses) may develop (Thiene and Basso, 2006).

Embolic spread from inflamed cardiac valves to the central nervous system (CNS) seems to be uncommon in animals in contrast to the situation in man, where endocarditis-associated brain lesions (EABLs; e.g. infarcts and abscesses) are common (Tunkel and Kaye, 1993; Mathisen and Johnson, 1997). In domestic animals, EABLs have only been reported sporadically. EABLs have been described in a limited number of dogs (Calvert, 1982; Sisson and Thomas, 1984; Cook *et al*., 2005; Bach *et al*., 2007), but pathological examination of the brain has not always been performed in cases of endocarditis, mostly due to absence of neurological signs (Shouse and Meier, 1956; Ellison *et al*., 1988). In a study of sporadic porcine endocarditis, brain abscesses were seen in one animal and such lesions were also frequently observed in experimental infections with *Streptococcus* spp., but details were not provided (Jones, 1980). The aim of the present study was to provide detailed information on the prevalence and type of brain lesions in a group of slaughter pigs with spontaneous valvular endocarditis.

Materials and Methods

Specimens

Sampling of the brain and kidneys from cases of endocarditis was undertaken as part of a study of the pathology and aetiology of endocarditis in slaughter pigs (Jensen *et al*., 2010). The brain was sampled by the meat inspection staff after sagittal bisection of the carcass. The brain was either removed from the skull at the abattoir before submission or the two parts of the head were submitted. In addition to cases of endocarditis, the brain was sampled from pigs without endocardial lesions (control group).

The brain surface was evaluated at arrival and the brain was fixed in 1 l of 10% neutral buffered formalin for at least 3 weeks. Twenty brains from pigs with endocarditis were selected for this study according to the following criteria: (1) presence of left-sided valvular endocarditis (LSVE), (2) one undamaged half of a brain available for histology and (3) the kidneys had also been submitted. Eleven control brains were included. Only half of each brain was examined and an attempt was made to balance the number of right and left brain halves for both cases and controls (cases, nine left and 11 right; controls, six left and five right).

Histopathology

A casting mould with walls made of 2 mm metal plates was filled with aqueous agar gel (A1296, Sigma–Aldrich, St Louis, Missouri) at 60°C. Half of a formalin-fixed brain was placed at the bottom of the mould with the medial surface downwards and the longitudinal axis of the brain parallel to the walls of the mould. The mould with the agar-embedded brain was covered by moist wadding and left overnight at 5°C in a plastic bag. The brain was subsequently cut in 4 mm slices (i.e. between every second metal plate). In this way, half of each brain was cut into 16–22 transverse slices (mean $n = 19$). The slice numbers varied due to various amounts of the most rostral or caudal parts of the brain being included. The cut surfaces of each slice were examined for gross lesions before being placed into a cassette, processed through graded concentrations of alcohol and xylene and embedded in paraffin wax. Tissue sections (3–4 μm) were stained with haematoxylin and eosin (HE). Selected sections were stained by Mallory's phosphotungstic acid haematoxylin method (PTAH) or Luna's method for erythrocytes and eosinophil granules (Bancroft and Stevens, 1996). Immunohistochemistry (IHC) for labelling of glial fibrillary acidic protein (GFAP) was performed on selected sections with a rabbit polyclonal antibody (Z0334, Dako, Glostrup, Denmark) as previously described (Nielsen *et al*., 2007).

Examination of the Heart

Cardiac lesions were sampled for bacteriological, histological and fluorescence in-situ hybridization (FISH) examinations as reported elsewhere (Jensen *et al*., 2010). In brief, lesions were characterized by gross inspection and samples were taken from diseased cusps. Samples for bacteriology were collected with a sterile loop and transferred to blood agar plates (Oxoid Blood Agar Base; Oxoid, Basingstoke, England) supplemented with 5% sterile bovine blood and incubated at 37°C for 2 days. Colonies were subcultured and identified biochemically using standard phenotypical methods (Barrow and Feltham, 1993). Streptococcal isolates were further characterized through partial 16S rRNA gene sequencing. Gene sequencing was accomplished using universal eubacterial primers 37F (5′-GGC TCA GRW YGA ACG C-3′) and 519R (5′-GTR TTA CCG CGG CTG CTG-3′). The PCR products were purified using the PCR clean-up gel extraction kit (NucleoSpin® Extract II, Macherey-Nagel, Düren, Germany) and were submitted to Macrogen Inc. (Seoul, South Korea) for sequencing.

Samples of affected valves were fixed in 10% neutral buffered formalin and processed as described