Distribution and aetiology of valvular endocarditis in suckling and weaned piglets in Serbia

Bozidar Savic1,2*, Oliver Radanovic1, Vesna Milicevic1, Branislav Kureljusic1, Nemanja Zdravkovic1, Ksenija Nesic1, Ognjen Stevancevic2

1Department for Pathology, Institute of Veterinary Medicine Belgrade, Belgrade, Republic of Serbia
2Department for Veterinary Medicine, Agriculture Faculty, University of Novi Sad, Novi Sad, Republic of Serbia

*Corresponding author: savic.bozidar@hotmail.com


Abstract: There are limited data available in the literature about the frequency and distribution of endocarditis in suckling and weaned piglets. The goal of this study was to investigate the frequency and distribution of lesions from cases of spontaneous valvular endocarditis in young piglets and to determine the bacterial agents involved in those lesions. Valvular endocarditis lesions in suckling piglets were detected in 21/454 cases (4.62%) and in 20/306 cases (6.53%) in weaned piglets. Streptococci were the dominant bacterial species isolated in 68% of the cases; S. suis was identified in 48.7% of the cases, S. pyogenes in 7.3% and S. spp., which could not be further identified, were cultured in 12.1% of the cases. E. rhusiopathiae was identified as the causal agent of endocarditis in four cases in the weaned piglets. In three cases, A. suis and S. aureus were isolated, and, in two cases, A. pleuropneumoniae was identified. Y. enterocolitica was isolated from one case of valvular endocarditis in a weaned pig. The finding of valvular endocarditis caused by Y. enterocolitica is an uncommon finding in pigs, and to the authors’ knowledge, there are no other reports of this agent being the cause of endocarditis in pigs. The involvement of Y. enterocolitica in endocarditis might have been assisted by the predisposing role of immunosuppressive viruses, such as porcine reproductive and respiratory syndrome.

Keywords: swine; piglets; endocarditis; bacteria

Valvular endocarditis is the most common acquired lesion of the porcine endocardium (Loynachan 2012). Such lesions are noted in clinically normal, slaughtered pigs at a level of 2% to 10% (Jones 1980). In many studies, it is established that Streptococcus suis and Erysipelothrix rhusiopathiae are the dominant agents of valvular endocarditis in pigs (Esgleas et al. 2005; Jensen et al. 2010; Loynachan 2012). However, from cases of spontaneous porcine endocarditis, other bacterial species have also been isolated, such as Streptococcus porcinus, Staphylococcus aureus, Staphylococcus hyicus, Trueperella pyogenes, Actinobacillus pleuropneumoniae, Actinobacillus suis, Escherichia coli, Pasteurella multocida, Listeria monocytogenes, Fusobacterium necrophorum and Enterococcus faecalis (Pedersen et al. 1984; Katsumi et al. 1997). Regardless of the aetiology, the mitral valve is most commonly affected and then, by the order of the frequency, the semilunar aortae, tricuspid and semilunar a. pulmonalis valves. Although it has been described that valvular endocarditis can occur in all age groups of pigs, ex-
cept in cases of experimental infections (Jones 1982; Johnson et al. 1986), there is very little information available about the naturally occurring distribution of lesions and causes of valvular endocarditis in young animals, particularly in suckling and weaned pigs. Therefore, in this retrospective study, we report the frequency and patterns of the distribution of the lesions from cases of spontaneous valvular endocarditis in suckling and weaned piglets and the results of the bacteriological examination of the corresponding lesions.

MATERIAL AND METHODS

In total, 454 suckling (from 2 kg to 10 kg) and 306 weaned (from 10 kg to 25 kg) dead piglets from conventional farrow-to-finish pig farms were necropsied at the Veterinary Institute Belgrade from January 2018 to March 2019. The animals were from seven farms originating amongst different regions in Serbia and were 1 to 12 weeks of age. All the farms were continuous flow operations, and had a poor management system of health issues, including biosecurity, inadequate sanitation and housing-environmental management shortcomings. All the farms included in the study vaccinated against erysipelas (sows in the first third of pregnancy; piglets at 85 days) and six farms vaccinated against porcine circovirus 2 (PCV2). The post-mortem findings for these submissions were varied and included systemic (porcine reproductive and respiratory syndrome – PRRS (84 cases), PCV2 – systemic disease (14 cases), Glässer’s disease (78 cases), respiratory [porcine respiratory disease complex – PRDC (125 cases)], swine pleuropneumonia (87 cases), enteric [colibacillosis (102 cases)], swine dysentery (33 cases) and skin [exudative epidermitis (67 cases)] diseases as well as other conditions caused by bacteria such as streptococci (134 cases), A. suis (19 cases) and S. aureus (17 cases). At necropsy, particular attention was paid to the inflammatory changes on the endocardium. The hearts were opened in the direction of the blood flow with an incision through the atrium and right ventricle to the right ventricular apex and then along the right ventricular wall adjacent to the interventricular septum into the pulmonary outflow tract. The left side was incised from the atrium to the left ventricular apex to expose the endocardial surface and mitral valve; subsequently, the mitral valve cusp was incised to expose the outflow tract into the aorta. The lesions were characterised by gross inspections.

From the forty-one hearts with macroscopically visible valvular endocarditis lesions, avoiding contamination, the material was sampled for a histological and bacteriological examination.

Table 1. Primers used for the detection of the pathogens studied in this work

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Oligonucleotides</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. rhusiopathiae</td>
<td>F: AGATGCCATAGAAACTGGTA R: CTGTATCCGCCCATAACTA</td>
<td>Makino et al. (1994)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>F: ATAGAGATGCTGTGGTACAGG R: GCITTCGATTGTTCCGATGC</td>
<td>Hookey et al. (1998)</td>
</tr>
<tr>
<td>S. pyogenes</td>
<td>F: AAAAGACGCCTTAACCCACCT R: TGGCAAGGTAAACTTCAAGCA</td>
<td>Liu et al. (2005)</td>
</tr>
<tr>
<td>A. suis</td>
<td>F: GAGCTGGGAAGCCTGACATAT R: CCCCACATCTTCAAACAGGAT</td>
<td>Kariyawasam et al. (2011)</td>
</tr>
<tr>
<td>Y. enterocolitica</td>
<td>F: AATACCGCATAACGCTCTCG R: CTCTCTTTCTGCAGGTA ACGTTC</td>
<td>Wannet et al. (2001)</td>
</tr>
<tr>
<td>Multiplex PCR S. suis serotype 2 (plus 1/2)</td>
<td>S. suis F: CAGTATTTACCCCGCATGGTGATAT S. suis R: GTAAGATACCGCTCAAGTGGAGAA serotype 2 (plus 1/2) F: GTTGAGCTTCTTTACACCTGTT serotype 2 (plus 1/2) R: CAGAAATTTCATATTTGCTCCACC</td>
<td>Marois et al. (2004)</td>
</tr>
</tbody>
</table>
Histopathology

Samples of the valves’ tissue were fixed by immersion in 10% buffered formalin. The fixed tissue was dehydrated, embedded in paraffin wax, sectioned at 5 µm, and stained with haematoxylin and eosin (H&E). The slides were examined under a light microscope.

Bacteriology

For the bacterial isolation, Columbia agar with 5% defibrinated ovine blood and MacConkey agar were used (Hi Media, Mumbai, India). For the A. pleuropneumoniae isolation, an S. aureus culture as the source of the V factor and a chocolate agar with a Poly Vitex supplement (BioMerieux, Marcy l’Etoile, France) were used.

The plates were incubated in an aerobic condition on 37 °C for 24 to 48 hours. The bacteria were identified using the colony morphology, haemolytic pattern on the blood agar and a further microscopic examination (Gram staining), standard biochemical methods, a serogrouping agglutination test of streptococci of Lancefield Groups A, B, C, D, F and G (Microgen Bioproduct Ltd., Camberley, U.K.) and a commercial test (BBL – Crystal, Becton Dickinson, Cockeysville, USA). The bacterial isolates were further tested by PCR (polymerase chain reaction) using a DreamTaq PCR Master Mix kit (Thermo Scientific, Vilnius, Lithuania) and the primers described for the DNA detection of E. rhusiopathiae, S. aureus, S. pyogenes, A. suis, A. pleuropneumoniae and Y. enterocolitica (Makino et al. 1994; Hookey et al. 1998; Schaller et al. 2001; Wannet et al. 2001; Liu et al. 2005; Kariyawasam et al. 2011) (Table 1). For the detection of S. suis serotype 2 (plus 1/2), a multiplex PCR (Multiplex PCR kit; Qiagen GmbH, Hilden, Germany), as described previously, was applied (Marois et al. 2004).

RESULTS

The incidence and distribution of the lesions from the cases of naturally-occurring valvular endocarditis in the piglets studied is presented in Table 2. Of the 760 (454 suckling and 306 weaned) piglets, examined macroscopically, visible valvular endocarditis was diagnosed in 5.39% (41/760) of the

<table>
<thead>
<tr>
<th>Production stage</th>
<th>Positive Mitral and aortic valves</th>
<th>Positive Mitral and atrial valves</th>
<th>Positive Mitral and tricuspid valves</th>
<th>Positive Mitral and aortic valves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suckling piglets (2–10 kg)</td>
<td>17/454 (3.74%)</td>
<td>Streptococcus spp. (3)</td>
<td>2/454 (0.44%)</td>
<td>S. suis (3)</td>
</tr>
<tr>
<td></td>
<td>21/454 (4.62%)</td>
<td>S. pyogenes (2)</td>
<td>1/454 (0.22%)</td>
<td>S. suis (1)</td>
</tr>
<tr>
<td></td>
<td>20/454 (4.22%)</td>
<td>A. pleuropneumoniae (1)</td>
<td>1/454 (0.22%)</td>
<td>S. suis (1)</td>
</tr>
<tr>
<td>Weaned piglets (10–25 kg)</td>
<td>13/306 (4.24%)</td>
<td>Streptococcus spp. (2)</td>
<td>2/306 (0.65%)</td>
<td>S. suis (1)</td>
</tr>
<tr>
<td></td>
<td>20/306 (6.53%)</td>
<td>S. pyogenes (1)</td>
<td>3/306 (0.98%)</td>
<td>E. rhusiopathiae (2)</td>
</tr>
<tr>
<td></td>
<td>3/306 (0.98%)</td>
<td>A. pleuropneumoniae (1)</td>
<td>2/306 (0.65%)</td>
<td>E. rhusiopathiae (2)</td>
</tr>
<tr>
<td>Total (760)</td>
<td>41/760 (5.39%)</td>
<td>S. suis (1)</td>
<td>3/760 (0.39%)</td>
<td>E. rhusiopathiae (1)</td>
</tr>
<tr>
<td>Positive</td>
<td>41/41 (100%)</td>
<td>S. suis (1)</td>
<td>2/41 (4.87%)</td>
<td>E. rhusiopathiae (1)</td>
</tr>
<tr>
<td></td>
<td>5/41 (12.1%)</td>
<td>Y. enterocolitica (1)</td>
<td>3/41 (7.31%)</td>
<td>Y. enterocolitica (1)</td>
</tr>
<tr>
<td></td>
<td>1/41 (2.4%)</td>
<td>S. suis (2)</td>
<td>1/41 (2.4%)</td>
<td>S. suis (1)</td>
</tr>
</tbody>
</table>

Table 2. Incidence and distribution of the valvular endocarditis in the suckling and weaned piglets
Valvular endocarditis lesions in the suckling piglets were detected in 21 cases (21/454, 4.62%). The mitral valve was involved in 20 of the cases and was the only valve affected in 17 of the cases, the mitral valve and the semilunar aortae valve were affected in two of the cases, the mitral valve and tricuspid valve were affected in one of the cases, and the tricuspid valve and semilunar aortae valve were affected in one of the cases. In the weaned piglets, valvular endocarditis was diagnosed in 20 cases (20/306, 6.53%) with only the mitral valve being affected in 13 of the cases, the mitral valve and the semilunar aortae valve were affected in three of the cases, the mitral valve and tricuspid valve were affected in two of the cases and the tricuspid valve and semilunar aortae valve were affected in one of the cases.

In the mild cases, the endocardial lesions were characterised by the presence of solitary yellow-red nodules on the leaflets’ ending. Severe lesions were characterised by deep valve tissue damage with the formation of nodous, mushroom or cauliflower thrombotic vegetations (vegetative endocarditis) (Figure 1).

Thrombotic masses were found on the atrioventriculares valves on the atrial surface of the valves and on the semilunar valves on the ventricular surface, near to the cusps’ endings. In the cases in which thrombotic deposits were situated on one valve, they had a verrucous shape only ( verrucous endocarditis), whereas, in the cases of the extended and intensively inflammatory processes, mushroom and cauliflower shapes were dominant. In one case (a weaned pig), all the valves were affected by inflammatory processes.

The main findings in the histopathological examination of the affected valves were the partial replacement of valve tissue by a mixture of fibrin, neutrophils and mononuclear inflammatory cells, necrotic debris, mineralised foci and plaques. The thickened valves had a notably increased fibroblastic proliferation.

In the lesions, accumulated layers of fibrin with numerous embedded bacterial colonies underlaid by a zone of infiltrated leucocytes and granulation tissue were noted (Figure 2).

The bacterial agents isolated and information about the co-morbidities in the 41 cases of naturally-occurring valvular endocarditis in suckling and weaned piglets are presented in Table 3.

Streptococci were the dominant bacterial species isolated in 68% (28/41) of the cases; S. suis was identified in 48.7% of the cases (20/28), S. pyogenes in 7.3% (3/28) and S. spp., which could not be further identified, were cultured in 12.1% (5/28) of the cases. E. rhusiopathiae was identified as the causal agent of endocarditis in four of the cases in the weaned piglets.

Also, in three cases, A. suis and S. aureus were isolated, and in two cases A. pleuropneumoniae was identified. Y. enterocolitica was isolated from the one case of valvular endocarditis in a weaned pig.
The results of the frequency and distribution of the lesions in spontaneous valvular endocarditis reported in the literature for older pigs (Guarda and Negro 1989; Bauman and Bilkei 2002; Loynachan 2012). However, the predilection of the mitral and semilunar aortae valves for valvular endocarditis, regardless of the aetiology in these age groups of pigs are not described as strongly as in this study.

In addition, the analysis of the distribution of the lesions showed that tricuspid and semilunar a. pulmonalis valves were affected in two cases (one case in a suckling pig and one case in a weaned pig), and from the author’s knowledge, this is the first description of such patterns of the distribution of the lesions of valvular endocarditis in suckling and weaned piglets. For the localisation of the observed thrombotic masses (on the atioventricular and semilunar valves on the atrium and ventricular surface), we have no further explanation than that what was proposed earlier for slaughtered pigs, that the solid and less flexible dense connective tissue (through its content of collagen fibres) which supports the endocardium on the ventricular side, forms a barrier that forces the inflammation within a valve toward the atrial chamber (Jarplid et al. 1997).

The multiple valvular endocarditis lesions observed in both age groups of pigs were a part of the primary diseases in which these lesions represent an accompanying finding. In all the related cases, according to the clinical background, post-mortem findings and bacteriological results, a streptococcal disease/infection was most frequently diagnosed. Thus, the damaged gingival epithelium (associated with a poor teeth clipping technique) with the subsequent gingivitis and periodontal inflammation (traumatic stomatitis), and the inflammatory lesions of the joints (arthritis) in suckling piglets, and skin wounds (lesions and abrasive skin damage) arising from injuries from poor pen structure and/or fighting, combined with the probable persistent streptococcal colonisation of the tonsils in the weaned piglets, were the most important entry sites for the streptococci. The subsequent valvular endocarditis is a sequel to an extracardiac streptococcal infection associated with one or more episodes of bacteraemia (Kumar et al. 1992; VanVleet and Ferrans 2007). Consequently, streptococci were most frequently isolated (21/48, 68%) from the valvular endocarditis lesions with S. suis.
being the main infectious agent of valvular endocarditis in both age groups of the piglets.

Although naturally occurring bacterial valvular endocarditis in pigs is generally known to be caused by \textit{E. rhusiopathiae} (Bauman and Bilkei 2002; Jensen et al. 2010; Loyanchan 2012), the bacteriological results from our study showed a lower incidence than expected in the aethiopathogenesis of the valvular endocarditis in the suckling piglets (valvular endocarditis caused by \textit{E. rhusiopathiae} was not detected in suckling piglets), in contrast to its detection rate in the weaned piglets (9.7%). The reasons that contribute to the considerable incidence of \textit{E. rhusiopathiae} valvular endocarditis in weaned piglets, despite the vaccination programme against erysipelas, may be: affecting the shortening duration of the maternal immunity, a greater impact on the conditions that can lead to \textit{Y. enterocolitica} bacteraemia, several may have particular significance, such as a PRRS active infection, PCV2 – a systemic or enteric disease and stress-induced immunosuppression (Butts and Sternberg 2008).

In this case, it could be speculated that numerous underlying conditions can facilitate a \textit{Y. enterocolitica} infection followed by colonisation of the tonsils and/or intestines. The endocardium may have been infected during a subsequent episode of the bacteraemia. Among many underlying diseases and conditions that can lead to \textit{Y. enterocolitica} bacteraemia, several may have particular significance, such as a PRRS active infection, PCV2 – a systemic or enteric disease and stress-induced immunosuppression (Butts and Sternberg 2008).

In conclusion, the spontaneous valvular endocarditis in the sucking and weaned piglets, reported here, represents the accompanying lesions associated with the underlying diseases.

Thus, in most cases (68%), a streptococcal disease/infection has been diagnosed, and streptococci, particularly \textit{S. suis} (48.7%), were the dominant bacterial species isolated in both age groups of the piglets. \textit{E. rhusiopathiae} was detected as a causative agent of valvular endocarditis in the weaned piglets only (4). The other cases were associated with \textit{A. suis} and \textit{S. aureus} (3) or \textit{A. pleuropneumoniae} (2). In general, the frequency and patterns of the distribution of the lesions on the heart valves are very similar to those reported in older pigs, however, lesions on the tricuspid and semilunar aortae pulmonalis valves only, in cases of naturally occurring valvular endocarditis, have not been reported on earlier in either sucking or weaned piglets.

Although most of the infections by \textit{Y. enterocolitica} in pigs have been reported as being asymptomatic, to our knowledge, this is the first reported case of valvular endocarditis caused by this bacterium in pigs.

**Conflict of interest**

The authors declare no conflict of interest.
REFERENCES


Received: April 28, 2020
Accepted: October 9, 2020

https://doi.org/10.17221/99/2020-VETMED