Vertebral osteomyelitis associated with *Enterococcus faecalis* in Broiler Breeders in Chile

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**ABSTRACT.** Vertebral osteomyelitis is a re-emerging disease characterized by inflammation and necrosis of the thoracic vertebral body, caused by *Enterococcus cecorum*. Here, we report the first case of vertebral osteomyelitis caused by *Enterococcus faecalis* in Broiler Breeders, in Chile, which also causes infections in humans and is resistant to multiple antimicrobials, representing a risk to public health.

**Keywords:** poultry, public health, infections, zoonosis.

Vertebral osteomyelitis (VO), or enterococcal spondylitis, is a re-emerging disease that affects broilers and breeders worldwide (Souillard et al., 2022). Although there are several causative agents of VO, including *Escherichia coli* and *Staphylococcus aureus*, species of the genus *Enterococcus*, particularly *E. cecorum*, are the most commonly identified (Braga et al., 2018). VO is characterized by inflammation and necrosis of the free thoracic vertebral body (T4). As a result, there is compression of the spinal cord and an alteration in the mobility of affected birds, which eventually die because of dehydration or starvation. The posture described as “sitting on their hocks,” characterized by the extension of legs cranially and support from the tibiotarsal-metatarsal joints, is the classic clinical sign of this disease (Borst et al., 2017), which occurs more frequently in males than in females (Braga et al., 2018).

The genus *Enterococcus* belongs to the *Enterococcaceae* family. It is composed of ubiquitous gram-positive cocoid bacteria, catalase-negative, non-spore-forming, facultative anaerobes frequently found in the environment, and is part of the normal gastrointestinal (GI) tract microbiota in humans and animals, including birds and poultry. The main *Enterococcus* species associated with VO in poultry are *E. cecorum*, *E. faecium*, *E. hira*, and *E. faecalis*. The incidence of *Enterococcus*-associated diseases in poultry has increased in recent years, causing significant economic losses to the poultry industry (Souillard et al., 2022).

The first cases of VO due to *E. cecorum* infection were described in 2002 in farms in the Netherlands (Devriese et al., 2002) and the United Kingdom (Wood et al., 2002). Subsequently, cases have been reported in Belgium (De Herdt et al., 2009), Hungary (Makrai et al., 2011), and Germany (Jung & Rautenschlein, 2014). The disease has also been reported in North America, several US states (Pennsylvania, Washington, North Carolina, South Carolina, Arkansas, Mississippi, Alabama, and California) (Borst et al., 2012), and Canada (Stalker et al., 2010). In South America, vertebral osteomyelitis due to infection by other pathogens such as *E. coli*, *S. aureus* and *E. faecalis* has been reported in broilers in Brazil (Braga et al., 2016). *E. faecalis* is associated with several diseases affecting the poultry industry, including ophalitis, endocarditis, septicemia, and amyloidosis (Souillard et al., 2022). Here, we report a case of vertebral osteomyelitis caused by *Enterococcus faecalis* infection in broiler breeders in Chile based on pre- and post-mortem findings, bacterial cultures, biochemical characteristics, and molecular analysis.

Sudden onset of lameness and subsequent complete paralysis was reported in 67 male broiler breeders between six and 11 weeks of age on a farm with approximately 4,000 birds in Chile’s Valparaíso Region. Nine live birds were obtained from Universidad de Chile’s Avian Pathology Laboratory. They were prostrate, depressed, and sitting on their hocks (figure 1). The birds were euthanized by cervical dislocation, and post-mortem examination revealed a nodular mass in the free thoracic vertebral body (T4) (figure 2A). Sagittal section of the spine revealed vertebral osteomyelitis with necrosis and caseous material in the vertebral body (figure 2B). Some birds also presented with detachment of the coxofemoral articular cartilage, femoral head necrosis, femur osteomyelitis, and pericarditis.

Samples from vertebral lesions and femoral head necrosis were obtained aseptically, plated on tryptone soy agar with 5% blood and MacConkey agar, and incubated for 24 hours at 37°C under microaerophilic conditions. At 24 h post-incubation, cultures from the vertebral lesions and...
Figure 1.
Broiler breeder prostrate, depressed and sitting on its hocks.
femoral head necrosis presented cream-colored, smooth-edged, very small, non-hemolytic colonies of up to 1.5 mm on blood agar plates (figure 3). No bacterial growth was observed on MacConkey agar plates.

Subsequently, pure subcultures were generated from single colonies on blood agar plates. The following day, the subcultures were processed for Gram staining, catalase reaction, and biochemical tests using the Vitek® 2 Compact identification system, according to the manufacturer’s instructions (BioMerieux, Marcy-l’Étoile, France). Based on the characteristics of the colonies, Gram staining, and biochemical tests, the isolates obtained from vertebral lesions and femoral head necrosis corresponded to *E. faecalis*, which was confirmed by PCR. For this purpose,
Table 1. Antibiogram results for *Enterococcus faecalis* isolates from vertebral lesions.

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Inhibition halo (mm)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxycillin</td>
<td>25</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Colistin</td>
<td>0</td>
<td>Resistant</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>21</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>0</td>
<td>Resistant</td>
</tr>
<tr>
<td>Florfenicol</td>
<td>20</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>10</td>
<td>Resistant</td>
</tr>
<tr>
<td>Fosfomycin + Tylosin</td>
<td>0</td>
<td>Resistant</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>0</td>
<td>Resistant</td>
</tr>
<tr>
<td>Lincomycin-Spectinomycin</td>
<td>10</td>
<td>Resistant</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>15</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>0</td>
<td>Resistant</td>
</tr>
<tr>
<td>Sulfadoxine-Trimethoprim</td>
<td>22</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Sulfisomidine</td>
<td>0</td>
<td>Resistant</td>
</tr>
<tr>
<td>Tiamulin</td>
<td>0</td>
<td>Resistant</td>
</tr>
<tr>
<td>Tylosin</td>
<td>0</td>
<td>Resistant</td>
</tr>
</tbody>
</table>

bacterial DNA was extracted according to the manufacturer’s instructions using the PureLink™ Genomic DNA Mini Kit (Invitrogen, Massachusetts, United States). An 803-bp portion of the tuf gene was amplified using the primers EfisF: ATGCCGACATTGAAAGAAAAAATT and EfisR: TCAATCTTTGGTTCCATCTCT under previously reported conditions (Mahmoudpour *et al.*, 2007) (figure 4). An antibiogram (Kirby-Bauer test) was performed to determine the antimicrobial susceptibility of *E. faecalis* isolated from the vertebral lesions. The results are presented in Table 1.

This case report describes for the first time a case of vertebral osteomyelitis associated with *E. faecalis* in broiler breeders in Chile. This result is interesting because most reports have shown an association between vertebral osteomyelitis and *E. cecorum* infection in broilers and broiler breeders (Braga *et al.*, 2018). The case reported here affects broiler breeders between six and 11 weeks of age, which coincides with previous reports (age range: 5-13 weeks) and correlates with a period of rapid skeletal development in birds (Braga *et al.*, 2018). The *E. faecalis* strain isolated in this study was resistant to 10 of the 15 antimicrobials tested, indicating multidrug resistance. Furthermore, given the difficulty in achieving adequate concentrations of these drugs in the spine, the therapeutic options for affected birds are clearly diminished and treatment is longer than usual (Pöntinen *et al.*, 2021).

The origin and pathogenesis of vertebral osteomyelitis due to *Enterococcus* infection remains largely unknown (Borst *et al.*, 2017). However, predisposing factors such as immunosuppression, rapid bird growth, stocking density, and heat stress are thought to contribute to the susceptibility of poultry to enterococcal infections (Schreier *et al.*, 2022). In *Gallus domesticus*, the last cervical vertebra and first three thoracic vertebrae are fused into a notarium structure. After notarium, the fourth thoracic vertebra (T4) was the only mobile thoracic vertebra. The remaining thoracic and lumbar vertebrae are fused with the sacral vertebrae in a structure known as the sinsacrum. As the only mobile vertebra, T4 is subjected to high mechanical stress and microtrauma, predisposing it to infection (Braga *et al.*, 2018). The most likely explanation is that *E. faecalis*, normally present in the intestine, enters through
hematogenous dissemination due to a compromised or damaged intestinal barrier resulting from infection with other infectious agents, such as *E. coli* or *Eimeria* species. Consequently, when combined with the aforementioned environmental factors, any condition that disrupts the intestinal barrier may predispose an *Enterococcus*-associated infection (Jung & Rautenschlein, 2014).

Vertebral osteomyelitis caused by *E. faecalis* could have a significant economic impact on the poultry industry, including increased mortality in breeders, poor feed conversion ratios, and increased slaughterhouse condemnation in broilers (De Herdt et al., 2008). Minimizing the predisposing factors described above appears to be critical for preventing this disease. *Enterococcus* species are zoonotic agents that play an important role in nosocomial diseases (Pöntinen et al., 2021). They easily acquire antimicrobial resistance (AMR) determinants and thus play a key role in the dissemination of AMR. Humans can acquire enterococci from several sources, including environmental and animal food sources, contaminated with intestinal microbiota. Antimicrobial use in animal production systems (poultry, cattle, and pigs) is a major risk factor in the selection of AMR, and the poultry industry contributes the most to the dissemination, selection, and persistence of antimicrobial resistance in human populations, thereby increasing the risk to public health (Abreu et al., 2023).

Studies that allow phenotypic and genetic characterization of this bacterium would make it possible to adequately understand and control the disease and, in turn, reduce the role of the poultry industry as a source of *E. faecalis* strains and AMR.

DEclarations

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Competing interests

The authors declare that they have no competing interests.

REFERENCES


