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CASE REPORT

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 coccoid 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 omphalitis, 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.

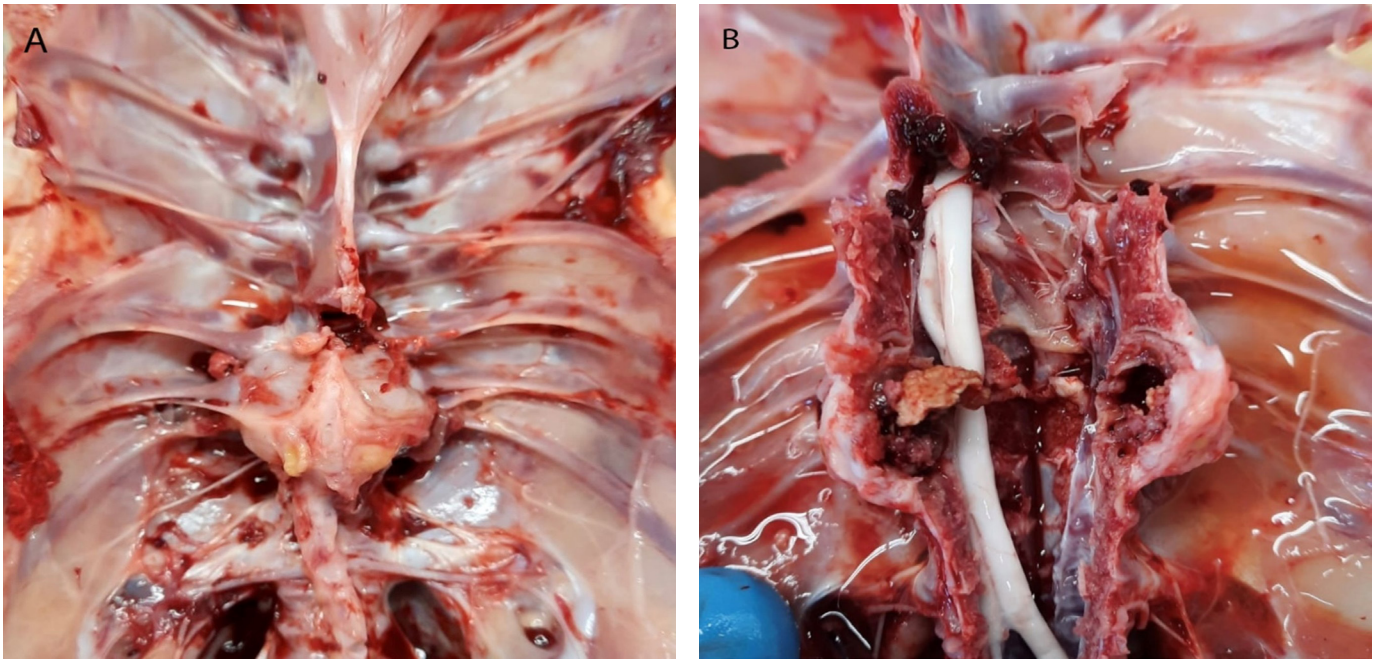

Figure 2.
A) Nodular mass in the free thoracic vertebral body (T4). B) Vertebral lesion with necrosis and caseous material after performing the sagittal section of the spine.

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,

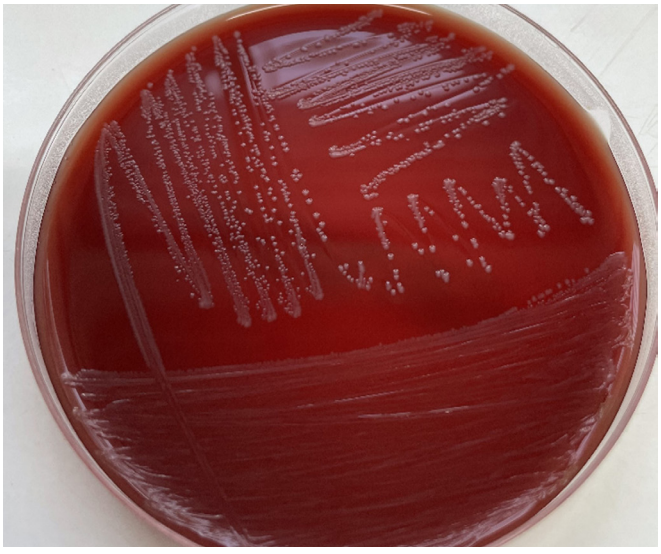

Figure 3.
Culture of *Enterococcus faecalis* on tryptone soy agar with 5% blood.

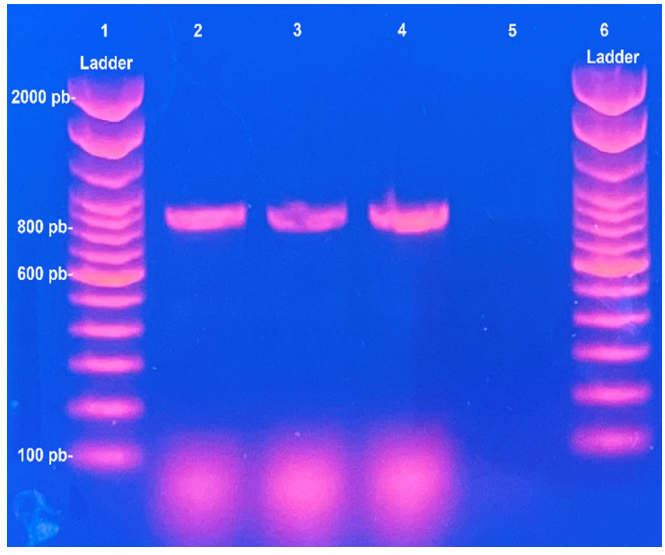

Figure 4.
Detection of *Enterococcus faecalis* by PCR. Lane 1:100 bp DNA Ladder. Lanes 2-4: *Enterococcus faecalis* DNA. Lane 5: Negative control. Lane 6:100 bp DNA Ladder.

Table 1.
Antibiogram results for *Enterococcus faecalis* isolates from vertebral lesions.

Antimicrobial	Inhibition halo (mm)	Interpretation
Amoxycillin	25	Sensitive
Colistin	0	Resistant
Enrofloxacin	21	Sensitive
Streptomycin	0	Resistant
Florfenicol	20	Sensitive
Fosfomycin	10	Resistant
Fosfomycin + Tylosin	0	Resistant
Lincomycin	0	Resistant
Lincomycin-Spectinomycin	10	Resistant
Norfloxacin	15	Intermediate
Oxytetracycline	0	Resistant
Sulfadoxine-Trimethoprim	22	Sensitive
Sulfisomidine	0	Resistant
Tiamulin	0	Resistant
Tylosin	0	Resistant

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.

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