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Vertebral osteomyelitis caused by *Enterococcus faecalis* in broiler chickens from southern Brazil[1]

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ABSTRACT.- Menck-Costa M.F., Huijboom J.A.A., Souza M., Justino L., Costa A.R., Bracarense A.P.F.R.L., Pereira U.P. & Baptista A.A.S. 2024. **Vertebral osteomyelitis caused by *Enterococcus faecalis* in broiler chickens from southern Brazil**. *Pesquisa Veterinária Brasileira 44:e07317, 2024*. Universidade Estadual de Londrina, Rodovia Celso Garcia Cid, PR-445 Km 380, Campus Universitário, Londrina, PR 86057-970, Brazil. E-mail: anaangelita@uel.br

Enterococcal spondylitis affects poultry and causes progressive lameness. This study reports what seems to be the first case of vertebral osteomyelitis caused by *Enterococcus* in broiler chickens in southern Brazil. We also conducted an experimental infection to evaluate microorganismal characteristics and pathogenicity in broiler chickens. We performed bacterial isolation, identification, and histopathology. The isolates were tested for their growth and survival capacity at different temperatures, pH values, and antimicrobial resistance profiles. The experiment infection was conducted with broiler breeders (n=9). Group 1 = negative control, Group 2 = challenged orally, Group 3 = challenged via air sac. The autopsy was performed on the 50th day of life (DOL). The report showed spondylitis and fusion of thoracic vertebra, accompanied by spinal cord compression, and femoral head necrosis. We used the isolates (n=17) to test their growth at 10°C and 45°C, survival capacity for up to 60° for 30 min, and growth under pH levels from four to 12. Higher resistance was observed against macrolides and quinolones. On experimental infections, all animals expressed signs of lameness and "sitting on the hocks". *Enterococcus faecalis* is the causal agent of enterococcal spondylitis in broilers in southern Brazil, which is an underreported and emerging pathological condition that requires attention.

INDEX TERMS: *Enterococcus faecalis*, vertebral osteomyelitis, enterococcal spondylitis, locomotor disorders, "sitting on the hocks", antimicrobial resistance.

RESUMO.- [**Osteomielite vertebral causada por *Enterococcus faecalis* em frangos de corte no sul do Brasil.**] A espondilite enterocócica afeta aves e causa claudicação progressiva. Este estudo relata o primeiro caso de osteomielite vertebral causada por *Enterococcus* em frangos de corte no sul do Brasil. Também conduzimos uma infecção experimental para avaliar as características microbianas e a patogenicidade em frangos de corte. Realizamos isolamento bacteriano, identificação e histopatologia. Os isolados foram testados quanto ao seu crescimento e capacidade de sobrevivência em diferentes temperaturas, valores de pH e perfil de resistência antimicrobiana. O experimento de infecção foi conduzido com matrizes de corte (n=9). Grupo 1 = controle negativo, Grupo 2 = provocado oralmente, Grupo 3 = provocado via saco aéreo. A autópsia foi realizada no 50º dia de vida (DOL). O laudo mostrou espondilite e fusão de vértebra torácica, acompanhada de compressão da medula espinhal e necrose da cabeça do fêmur. Usamos os isolados (n=17) para testar seu crescimento a 10°C e 45°C, capacidade de sobrevivência até 60° por 30 min e crescimento em níveis de pH de quatro a 12. A maior resistência foi observada contra macrolídeos e quinolonas. Na infecção experimental, todos os animais manifestaram sinais de claudicação e postura sentada sobre os jarretes. *Enterococcus faecalis* é o agente causal da espondilite enterocócica em frangos de corte no sul do Brasil, que é uma condição patológica emergente e subnotificada que requer atenção.

TERMOS DE INDEXAÇÃO: *Enterococcus faecalis*, osteomielite vertebral, espondilite enterocócica, desordens locomotoras, "sentar sobre os jarretes", resistência antimicrobiana.

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INTRODUCTION

Locomotor problems have a great economic impact on poultry production worldwide (Muchon et al. 2009, Shim et al. 2012, Guevara-Torres et al. 2022) as they reduce zootechnical performance (Sakamoto et al. 2020), increase carcass condemnation (Szafraniec et al. 2022), and compromise animal welfare (Silva et al. 2021). Locomotor disorders can have different etiologies (Bello Gonzalez et al. 2017, Borst et al. 2012, 2017). Vertebral osteomyelitis appears to be a relevant cause (Braga et al. 2018a, Borst et al. 2019), mainly associated with infections caused by *Enterococcus cecorum* (Marcon et al. 2019, Bollam et al. 2021, Szafraniec et al. 2022). *E. cecorum* was first described as a pathogen in Europe in 2002 (Wood et al. 2002, Devriese et al. 2002). In Brazil, vertebral osteomyelitis was described only once, in 2018, and was associated with *Enterococcus faecalis* (Braga et al. 2016).

Enterococcal spondylitis affects chickens and causes progressive lameness and paralysis, typically without swollen joints (Martin et al. 2011, Aitchison et al., 2014, Borst et al. 2017). Clinically, affected birds display one or more characteristic postures, including "sitting on the hocks" with the legs extended forward (Braga et al. 2018b) and arching their back or lying on their side (Martin et al. 2011, Talebi et al. 2016).

Enterococcus spp. is a Gram-positive and spherical bacterium that occurs alone, in pairs, or short chains (Zhong et al. 2017). It is a non-motile (Ramos & Morales 2019), non-spore-forming (Ramos et al. 2019), and facultative anaerobic (Lominski et al. 1958, Braga et al. 2018b) bacterium.

In recent years, *Enterococcus* spp. has shown a relevant increase in antimicrobial resistance (Talaga-Ćwiertnia & Bulanda 2018, Fatoba et al. 2022), which is aggravated by its survival capacity in extreme conditions of pH (Mubarak & Soraya 2018) and temperature, which makes it hard to eliminate this microorganism from poultry litter.

Enterococcus spp. is a relevant pathogen associated with healthcare-associated infections (HAI) and *E. faecalis* is the most prevalent species (García-Solache & Rice 2019). Thus, a One Health approach is critical to tackle the problem.

Few studies have investigated *Enterococcus* infections in poultry farms in Brazil and there is a lack of pathogen reproduction of experimental infections, responsible for osteomyelitis; therefore, knowledge of the disease causal agent is limited in the country. This study reports what seems to be the first detection and experimental reproduction of vertebral osteomyelitis caused by *E. faecalis* isolated from broiler chicken in southern Brazil.

MATERIALS AND METHODS

Animal Ethics. The experiment was previously approved by the institutional Ethics Committee to Use Animals (CEUA/UEL), under protocol number 056.2021.

This study was divided into two stages. First, we carried out a clinical report of the field case with the phenotypic and genotypic characterization of the isolates. Second, an experimental infection was conducted with the strains isolated from the case report.

Case report

Case presentation: Epidemiological and clinical signs. The affected broiler nucleus housed approximately 30,000 birds from the Cobb XL Male lineage. Antimicrobials were not used as growth promoters or therapeutic drugs in this batch. The poultry litter was reused after fermentation treatment.

From the 20th day of life (DOL) onwards, a mortality rate of approximately 0.5% per day was observed. The animals showed lameness, followed by paresis and/or paralysis of the legs, prostration, and support on the tibiotarsi metatarsal joint. Broiler chickens (n=10) on the 40th DOL with clinical signs were sent to the Avian Medicine Laboratory for diagnosis. The animals were euthanized by cervical dislocation and the standard autopsy was performed. We collected a pool of organ samples (spleen, liver, and heart), tibiotarsal and metatarsal synovial fluid, and swabs from the caseous exudate in the costochondral region (3rd to 5th thoracic ribs), femoral head, humeral head, and vertebral body (T3-T7) for bacterial isolation and identification. For histopathology, we collected the costochondral region, femoral head, vertebral body (T3-T7), and humeral body.

After removing the birds and performing the fermentation and alkalization processes, poultry litter samples (n=3) were collected with boot swabs, previously moistened in buffered peptone water (1%) to evaluate the survival capacity of the agent and consequently the possibilities of successive batches housed on the same poultry litter to present the disease.

Bacterial isolation, phenotypic and genotypic characterization. Organ samples, tibiotarsal and metatarsal synovial fluid, swabs from joints, and boot swabs were incubated in brain heart infusion (BHI) broth (CM1135; Oxoid) supplemented with 20µg/mL of kanamycin, in microaerophilic conditions, for 18 to 20 hours at 35°C and subsequently seeded on kanamycin agar sodium esculin azide plates (KAA, CM0591B; Thermo Scientific™) incubated at 35°C in microaerophilic conditions for 48 hours.

The biochemical tests were performed according to Manero & Blanch (1999) and García-Solache & Rice (2019) to confirm the bacterial genus, fermentation of sugars (arabinose, cellobiose, dulcitol, galactose, glucose, inulin, lactose, maltose, mannitol, raffinose, salicin, sucrose, sorbitol, trehalose, and xylose), amino acid decarboxylation (lysine, ornithine, and arginine), hemolytic activity, Gram stain, oxidase, catalase, pigment production, esculin hydrolysis, urease, motility, halotolerance, and L-pyrrolidonyl-beta-naphthylamide (PYR).

Two isolates, one from the vertebral lesion and one from osteonecrosis of the femoral head, were selected to extract genetic material using PureLink™ Genomic DNA Mini Kit (Invitrogen) and purification with PureLink™ PCR Purification Kit (Invitrogen). DNA was amplified according to Weisburg et al. (1991) using the primer sequence fD1 (5'–CCGAATTCGTCGACAACAGAGTTTGATCCTGGCTCAG–3') and rD1 (5'–CCCGGGATCCAAGCTTAAGGAGGTGATCCAGCC–3').

The amplification products were sequenced in the applied biosystem apparatus (AB-3500) and analyzed using the BLAST database and the evolutionary analysis was conducted by the maximum likelihood method (Kumar et al. 2018).

Histopathological analysis. Tissues were fixed in a 10% buffered formalin solution for 24 hours, followed by immersion in alcohol 70%. After, bone tissue and joints were submitted to a decalcification protocol (EDTA tetrasodium, sodium and potassium tartrate, sodium tartrate, hydrochloric acid, and distilled water), according to Venâncio (2021). The samples were immersed in the decalcifying solution for 8 hours/day, followed by 10 min of tap water washing. The samples were conserved in alcohol 70%. This process was repeated until the samples were properly decalcified. The decalcified samples were routinely processed (Venâncio 2021), and embedded in paraffin, and 5µm sections were cut and stained with hematoxylin and eosin (HE) and MacCallum Goodpasture Gram.

Growth capacity and time-kill assay at different temperatures and pH values. The isolates obtained from the clinical case were used to test the growth capacity at 10°C and 45°C, at time-kill at 60°C, and different pH values, following the technique described by Martínez et al. (2003) and Scandorieiro et al. (2016) respectively, with modifications.

The suspension of cells (10^6 CFU/mL) was carried out and incubated at 10°C and 45°C in BHI broth. After 24 hours, broth turbidity was considered positive for growth capacity.

The suspension of cells (10^8 CFU/mL) was incubated at 60°C for 15 and 30 min and each time 10µL of cultures were transferred to MHA to perform the time-kill assay.

For the pH test, cells (10^6 CFU/mL) were directly suspended in BHI broth with different pH levels (3 to 13) at 35°C. At four time points (12, 24, 48, and 72 hours) of incubation, 10µL of each assay were transferred to MHA (Weckwerth et al. 2013). To avoid cell mortality, extra BHI was added every 12 hours at a 1:10 ratio of the original amount for each test. After growing on BHI agar, isolates were considered positive for time-kill assays. All tests were performed in triplicate and the *Enterococcus faecalis* ATCC 29212 strain was used as a positive control.

Antimicrobial susceptibility profile. Sensitivity to antimicrobials was determined using the disc diffusion method (Bauer et al. 1966). The antimicrobials used were tetracycline (TE, 30µg), rifampicin (RD, 5µg), teicoplanin (TEC, 30µg), vancomycin (VA, 30µg), norfloxacin (NOR, 10 µg), levofloxacin (LEV, 5µg), linezolid (LZD, 30µg), ampicillin (AMP, 10µg), ciprofloxacin (CIP, 5µg), erythromycin (E, 15µg), and fosfomycin (FOT, 200µg) (Oxoid Ltda., Basingstoke, Hants, UK). *E. faecalis* strain ATCC 29212 was used as a positive control. The results were interpreted according to CLSI (2019) and BrCAST (2019). Isolates that showed resistance to three or more classes of antimicrobials were considered multidrug-resistant (MDR) (Magiorakos et al. 2012).

Experimental infection

One-day-old male broiler chicks (n=9), Ross lineage (male chicks from the female line), were housed in experimental cages, according to Martin et al. (2011), with adaptations. The animals received water and food *ad libitum*, heating, and lighting according to the Ross broiler guideline (Aviagen 2018). The animals were randomly divided into three groups. Group 1 (G1): Negative control – three unchallenged chickens, Group 2 (G2): Three chickens challenged with E. *faecalis*, orally (1mL), and Group 3 (G3): Three chickens challenged with *E. faecalis* via left abdominal air sac (100uL) (Table 1).

The challenged groups received the inoculum at the 10th, 11th, 12th, and 30th DOL (Al-Rubaye et al. 2017, Jung et al. 2018) The control group received sterile BHI broth at all challenge points. The inoculum was prepared with isolates of *E. faecalis* ENTLMA27.3 and ENTLMA42.2 previously isolated from the case report.

The animals were autopsied on the 50th DOL. First, blood was collected for microbiological analysis (Al-Rubaye et al. 2017), and then the animals were euthanized by cervical dislocation. The macroscopic evaluation of the lesions was performed and samples of organs (spleen, liver, heart), hip joint, and vertebral body (T3-T7) were collected for bacterial isolation and identification. The biological material was processed as described in the item "Bacterial isolation, phenotypic and genotypic characterization" for *E. faecalis* isolates.

RESULTS

Case report

Gross and histopathological findings. Birds expressed signs of "sitting on the hocks" (Fig.1). The main finding was a whitish mass on the spinal column involving T5, T6, and T7 at different severity degrees (Fig.2). The sagittal section displayed vertebral canal compression by a mass (Fig.3). Spondylitis and fusion of the free thoracic vertebra with the adjacent ones were observed in six animals, accompanied by spinal cord compression (Fig.2 and 3).

Femoral alterations were also observed with epiphyseolysis on the growth plate, fracture, and femoral head necrosis (Fig.4) in four animals, while caseous exudate in the costochondral region associated with humeral head necrosis affected one bird.

Splenomegaly and catarrhal enteritis were also observed in nine and seven animals, respectively. The histopathological tests of all vertebral body sections analyzed showed spondylitis, with medullary compression by a mass of necrotic material and the presence of caseous exudate in the lesion. On the Gram Goodpasture, staining Gram-positive cocci in the necrotic tissue was observed (Fig.4-6). In the femoral and costochondral cartilage, a myriad of bacterial colonies also occurred. The colonies were also formed by Gram-positive cocci.

Bacterial isolation and identification. Small circular colonies with an entire margin capable of hydrolyzing esculin were observed on KAA agar. Regarding the Gram stain, Gram-positive cocci occurred in clusters, short chains, diplococci, and single cocci.

The biochemical profile was compatible with the genus *Enterococcus* spp. in 100% of the isolates (Manero & Blanch 1999) and positive to produce α hemolysis, esculin hydrolysis, production of L-pyrrolidonyl-beta-naphthylamide (PYR), arginine decarboxylation and fermentation of sugars: cellobiose, galactose, glucose, lactose, maltose, mannitol, salicin, sucrose, and trehalose.

However, the biochemical profile showed negative results in producing catalase, oxidase, and pigments, growth in NaCl (6.5%), presence of motility, urease production, fermentation of sugars: arabinose, dulcitol, inulin, raffinose, xylose, and lysine decarboxylation and ornithine. In Sorbitol fermentation, 82% of the isolates were positive.

In total, 17 isolates of *Enterococcus* spp. were obtained, with a recovery rate of 83.3% (5/6) from vertebral lesions, 25% (1/4) from necrosis of the femoral head, 60% (6/10) from the pool of organs (liver, spleen, and heart). In addition,

Table 1. Description of treatments in experimental infection trial of broiler breeder chickens with *Enterococcus faecalis*

Group	Description of treatments	Challenge route	n	Challenge days	CFU/mL
G1	Negative control – Unchallenged chickens	-	3	-	-
G2	Chickens challenged with *Enterococcus faecalis*	Orally (1mL)	3	10th, 11th, 12th and 30th	109
G3	Chickens challenged with *Enterococcus faecalis*	Left abdominal air sac (0.1mL)	3		109
	TOTAL		9		

Inoculum: 10th day of life (DOL) = 1.66x 10^9 CFU/mL; 11th DOL = 1.18x 10^9 CFU/mL; 12th DOL = 1.5x 10^9 CFU/mL; 30th DOL = 4.2x 10^9 CFU/mL.

Enterococcus spp. was isolated from caseous exudate in the costochondral region (1/1), humeral head necrosis (1/1), and fermented poultry litter (3/3).

No bacterial growth was observed from the synovial fluid collected from the tibiotarsal and metatarsal joints. Both isolates of *Enterococcus* spp. submitted to sequencing showed 100% similarity with *E. faecalis* (Fig.7). GenBank accession number: SUB11090579 Enterococ-cus_*faecalis*_strain_42_2_16S_rRNA OM689135 (ENTLMA42.2) and SUB11090579 En-terococcus_*faecalis*_strain_27_3_16S_rRNA OM689136 (ENTLMA27.3).

Temperature resistance and pH tolerance. The *Enterococcus* isolates showed growth (turbidity) at temperatures of 10°C and 45°C and survival capacity of up to 60°C for 30 min. Regarding halotolerance, microorganismal growth occurred in pH levels from four to 12. The time-kill was observed after 72 hours of incubation at pH 3 and at pH 13 in any of the time points evaluated.

Antimicrobial susceptibility profile. Higher resistance was observed between the isolates against macrolides (100%) and quinolones (98%) (Fig.8). Eighty-two percent (14/17) of the evaluated isolates presented MDR.

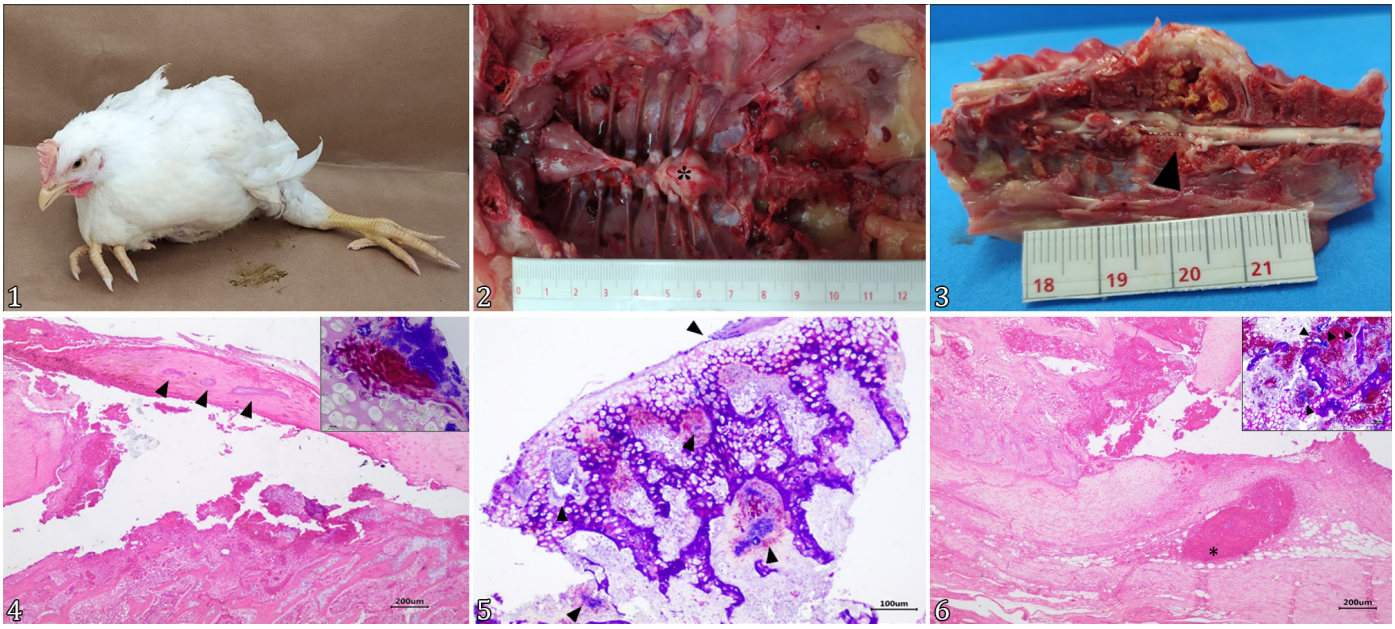

Fig.1-6. Gross and histopathological findings in broiler chickens. (**1**) Broiler chicken with lameness. (**2**) Gross lesion characterized by a whitish, soft mass (1cm diameter) on the spinal column involving T5-T7. (**3**) Gross lesion, sagittal section of the vertebral column with a significant caseous material that compresses the spinal cord (arrowhead). A fibrous layer covers the caseous material. (**4**) Femur: Myriad of bacteria in the articular layer. HE, bar = 200µm. Inset: Femur. Gram-positive cocci colonies in the articular layer. Gram Goodpasture, bar = 10µm. (**5**) Rib: A myriad of Gram-positive cocci colonies (arrowhead). Gram Goodpasture, bar = 200µm. (**6**) Vertebral body: Necrotic mass (*) in the vertebral canal, compressing the spinal cord. HE, bar = 200µm. Inset: Vertebral body. Necrotic mass with a myriad of Gram-positive cocci colonies (arrowhead). Gram Goodpasture, bar = 10µm.

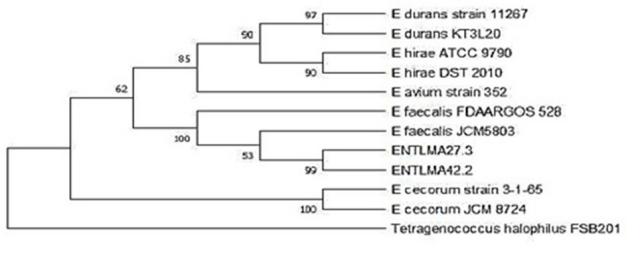

Fig.7. Phylogenetic analysis by maximum likelihood method. The evolutionary history was inferred by using the Kimura 2-parameter model. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 0.1102)]. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved nine *Enterococcus* nucleotide sequences and one *Tetragenococcus halophilus* as an outgroup. There were a total of 1,000 positions.

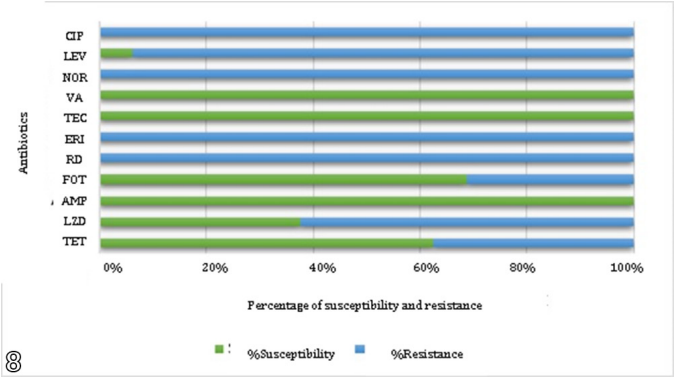

Fig.8. Antimicrobial susceptibility profile of *Enterococcus faecalis* isolated from broilers. Tetracycline (TE, 30µg), rifampicin (RD, 5µg), teicoplanin (TEC, 30µg), vancomycin (VA, 30µg), norfloxacin (NOR, 10µg), levofloxacin (LEV, 5µg), linezolid (LZD, 30µg), ampicillin (AMP, 10µg), ciprofloxacin (CIP, 5µg), erythromycin (E, 15µg) and fosfomycin (FOT, 200µg).

Experimental infection

Two chickens challenged via the air sacs (G3) died within three days after the first challenge. From the 11th DOL onwards, profuse diarrhea was observed for five consecutive days in all animals, while, from the 35th DOL onwards, all birds expressed signs of lameness and "sitting on the hocks", regardless of the challenge route.

In G2, femoral head necrosis was detected in two birds (2/3) and the presence of an inflammatory process covering the vertebrae (T5-T7), was observed in one bird (1/3). There was a slight bulging of the vertebral column (T5-T7) in two broiler breeder chicks (2/3). In G3, a slight bulging of the vertebrae (T5-T7) was observed in one animal (1/1) since the other two died shortly after the challenge, which compromised the evaluation. Animals of the negative control group did not show any lesions (Fig.9-16).

In the group challenged via gavage (G2), *Enterococcus faecalis* was isolated from the vertebrae and blood of all broiler breeder chickens (3/3) and the hip joints and organs of two animals (2/3). The microorganism was recovered only in vertebrae and organs in the birds challenged via the air sac (G3). All isolates grew in pure culture.

DISCUSSION

Enterococcus spp. is a causal agent of vertebral osteomyelitis in broilers (Aitchison et al. 2014, Borst et al. 2017, Braga et al. 2018a, Jung et al. 2018). *Enterococcus cecorum* occurs in different countries and is considered the main causal agent of enterococcal spondylitis (Devriese et al. 2002, Borst et al. 2012). However, in Brazil, only *Enterococcus faecalis* has been described (Braga et al. 2016) and associated with the lesions of enterococcal spondylitis.

Our study seems to be the first experimental reproduction of vertebral osteomyelitis by *E. faecalis* isolated from a clinical case in broilers in Brazil and the first description of the disease in southern Brazil.

Economic losses caused by infection with *Enterococcus* spp. are attributed to the lack of uniformity of the flocks and mortality (Jung & Rautenschlein 2014), due to the difficulty of locomotion of birds to reach water and food (Muchon et al. 2019). Birds affected by enterococcal spondylitis lean on their hocks ("sitting on their hocks") and display a swelling of the tibiotarsal and metatarsal joint (Braga et al. 2018b), a clinical sign commonly observed in other diseases, which compromises the diagnosis of the disease. Dissemination of information on the causal agent and disease can contribute to identifying the pathological condition.

Enterococcus spp. is a microorganism with a great capacity to survive adverse conditions and remain viable in extreme temperatures and pH (Jackson et al. 2005, García-Solache & Rice 2019), which helps the survival of the microorganism in poultry litter (Braga et al. 2018b, Fatoba et al. 2022), compromising the birds in the next cycle. Poultry litter treatment by alkalization, acidification, or fermentation may not be effective in eliminating the agent. Grund et al. (2021) performed survival tests of *E. cecorum* in poultry litter exposed to different conditions and proved the survival capacity of the microorganism. *E. faecalis* isolates evaluated in this study showed a survival capacity until 60°C for 30 min and resisted the fermentation and alkalization treatment of poultry litter.

Enterococcus spp. can present intrinsic resistance to cephalosporins, aminoglycosides, aztreonam, and oxacillin (Gilmore et al. 2020). The potential capacity of transferring resistance genes from *Enterococcus* spp. to other microorganisms (Pöntinen et al. 2021, Fatoba et al. 2022) aggravates the

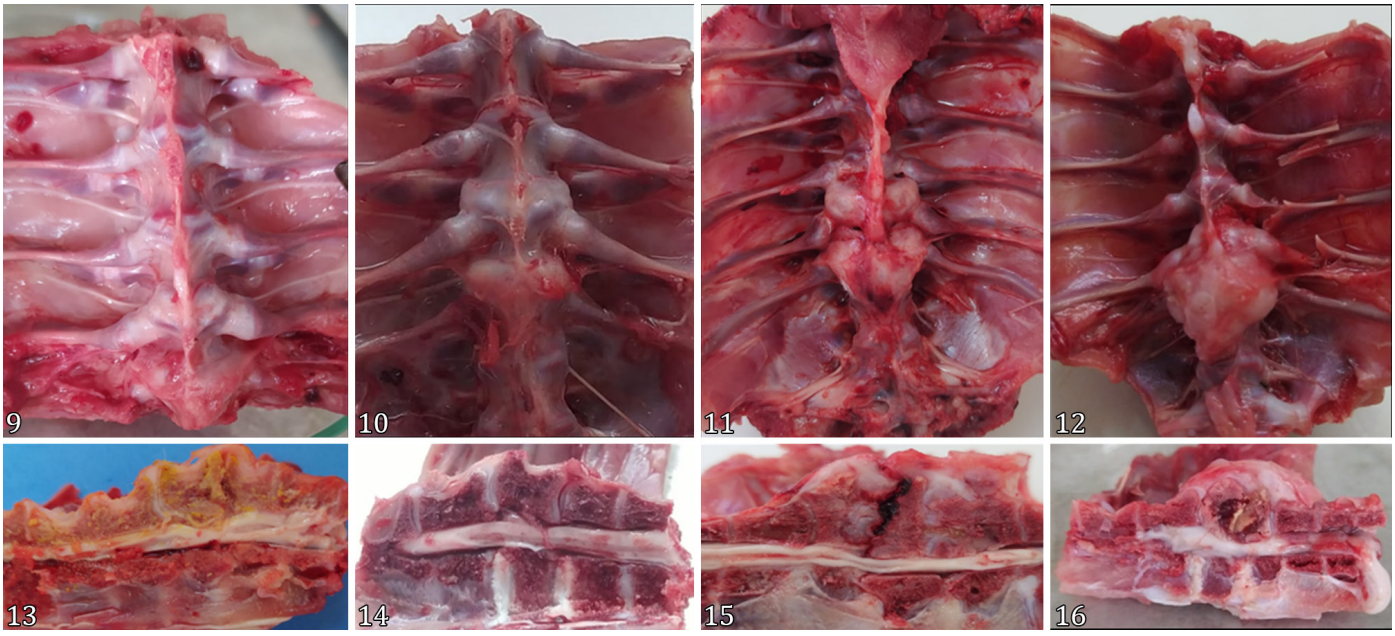

Fig.9-16. Macroscopic lesions in vertebrae of broiler breeders after experimental infection with *Enterococcus faecalis*. (**9**) No lesions, negative control. (**10**) Slight bulging of the vertebrae (T5-T6). (**11**) Moderate inflammatory process in the vertebral bodies (T5-T6-T7). (**12**) Severe inflammatory process covering the thoracic vertebrae (T6-T7). (**13**) No lesions, negative control. (**14**) Mild spinal cord compression. (**15**) Dorsal expansion of the spondylitis lesion and slight spinal canal compression. (**16**) Caseonecrotic exudate in the vertebral body and spinal cord compression. (**13-16**) Sagittal section of the thoracic vertebrae.

phenomenon of antimicrobial resistance and raises public health concerns (Bello Gonzalez et al. 2017, Pöntinen et al. 2021). In this study, we observed a high percentage of MDR profile isolates (82%); which is similar to reports by Sanlibaba et al. (2018), who detected more than 90% of MDR profiles in *E. faecalis* and *Enterococcus faecium* isolates from chicken meat.

The scientific opinion published by the European Food Safety Authority (EFSA et al. 2021) underscored that *E. faecalis* and *E. cecorum* isolated from chickens in different countries have a high sensitivity to penicillin. This is similar to the results found in our study, where we detected 100% sensitivity to ampicillin.

It is important to note that the pathogenesis of vertebral osteomyelitis is still not completely understood (Braga et al. 2018b, Souillard et al. 2022). It is believed that microorganisms have access to bones via the bloodstream, by translocation, due to disruption of the intestinal mucosal barrier or respiratory barriers (Thorp et al. 1993, McNamee & Smith 2010, Braga et al. 2018b). Wideman & Prisby (2013) pointed out that wire flooring contributes to leg instability and consequently increases joint torque, promoting micro lesions that favor colonization by *Enterococcus* spp. This is especially important in poorly mineralized chondrocytes of the proximal growth plate in fast-growing bones (femur and tibia), which can grow more than 70-fold between the first and 42nd day of life in broilers (Applegate & Lilburn 2002). Additionally, bacteria that can translocate the natural barriers adhere to the cartilage matrix leading to the degeneration of the epiphyseal cartilages, with the formation of bacterial colonies at the proximal ends (Wideman & Prisby 2013).

Concerning pathological changes, similar gross and microscopical lesions were observed in natural and experimental infections. In both infections femoral head necrosis, spondylitis, caseous exudate, and necrosis resulting in medullar compression were detected. The lesions affected the T5, T6, and T7 vertebrae. Previous reports have associated these lesions with *E. cecorum* and *E.* faecalis infection in broiler chickens (Borst et al. 2017, Braga et al. 2018b). Epidemiological surveillance study of the last 15 years conducted in France (Souillard et al. 2022) demonstrated a predominance of these two *Enterococcus* species in *Enterococcus*-associated diseases (EAD), suggesting that both species may have co-emerged, as a result of changes in the production system (e.g. ban on antimicrobials).

Dysbiosis is believed to favor translocation of *Enterococcus* present in the gastrointestinal tract of birds (Martin et al. 2011, Borst et al. 2017). The spread of the agent to bone tissue can promote femoral head necrosis and inflammation-causing bulging of the thoracic vertebrae (T5-T7), resulting in spinal cord compression. The experimental infection performed in this study showed profuse watery diarrhea and catarrhal enteritis in animals challenged orally. We hypothesized that the inoculum of *E. faecalis* (10^9 CFU/mL) may have caused a disbalance in the intestinal microbiota that allowed the disruption of the intestinal barrier allowing for bacteremia and future migration of the microorganism to bone tissue.

In the challenge route by the left abdominal air sac, two of the three birds died after the challenge, indicating the virulence of the strains. The agent was isolated from the surviving animal, demonstrating that the respiratory tract can also be a gateway for the pathogen, leading to the development of clinical signs of spondylitis.

In this study, we highlight two important routes of natural infection that can occur in broiler farms. We detected that *E. faecalis* can survive after the treatment of poultry litter with fermentation, before the housing of a new batch, capable of remaining on the farm, leading to new contamination cycles.

CONCLUSION

Enterococcus faecalis is the causal agent of enterococcal spondylitis in broilers in southern Brazil. It is an underreported and emerging pathological condition that requires attention due to the antimicrobial resistance profile of the microorganism and risks to public health.

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