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Review

Vertebral Osteomyelitis in Broilers: A Review

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ABSTRACT

Locomotor diseases are still a challenge in modern poultry. Vertebral osteomyelitis (VO) is an emerging disease in broilers worldwide. The inflammatory process in the affected thoracic vertebra (T4) and subsequent spinal cord compression leads to clinical signs related to locomotor impairment, inadequate feeding and drinking, and increased mortality in the affected flocks. The pathogenesis of the disease is poorly understood and *Enterococcus cecorum* is the bacterium most frequently associated with the disease. However, other bacteria such as *E. faecalis*, *E. durans*, *Escherichia coli* and *Staphylococcus aureus* have been recently detected in cases of the disease, raising questions regarding its etiopathogenesis. As many questions about VO in broilers remain unanswered, knowledge on its prevention, control and treatment is limited. In this review, we compile and discuss the currently available information concerning VO in broilers and highlight important aspects of the disease.

INTRODUCTION

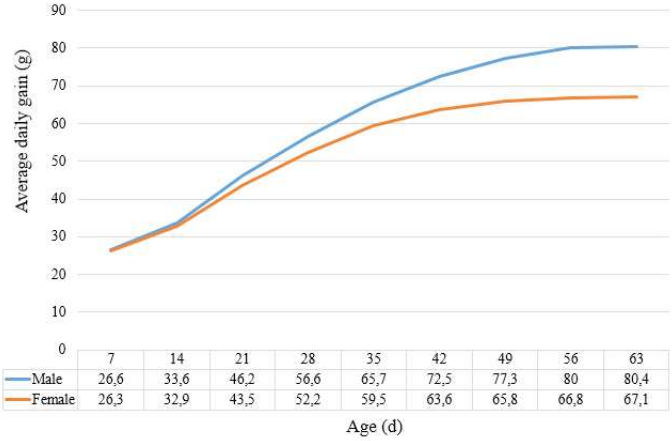
Vertebral osteomyelitis (VO), also known as spondylitis, is an emerging disease that affects broilers worldwide (Devriese *et al.*, 2002; Wood *et al.*, 2002; Herdt *et al.*, 2009; Aziz & Barnes, 2009; Gingerich *et al.*, 2009; Stalker *et al.*, 2010; Kense & Landman, 2011; Boerlin *et al.*, 2012). Outbreaks of this disease have been found in broilers and broiler breeders and associated with infection by *Enterococcus cecorum*, which is a normal inhabitant of the chicken intestinal tract. VO is characterized by infection causing inflammation and necrosis of the free thoracic (T4) vertebral body. This results in spinal cord compression and impaired mobility of the affected broilers, which often die from dehydration or starvation (Aziz & Barnes, 2007). The pathogenesis of the disease in chickens is still poorly understood (Martin *et al.*, 2011), although some progress has been made in recent years. In addition to the impact on animal health, it is important to note that enterococci have emerged as an important cause of nosocomial infections, with drug-resistant microorganisms largely involved in these cases (McGaw, 2013). This review aimed at compiling and discussing the current knowledge on vertebral osteomyelitis in broilers, as well as relevant aspects related to this disease.

Epidemiology of the disease

VO has been reported in poultry in different European countries, such as the United Kingdon (Wood *et al.*, 2002), The Netherlands (Devriese *et al*., 2002; Kense & Landman, 2011), Belgium (Herdt *et al.*, 2009), Hungary (Makrai *et al*., 2011), Norway (Kolbjørnsen *et al.*, 2011), and Bulgaria (Dinev, 2013). The disease was also described in South Africa (Aitchison *et al.*, 2014), Iran (Talebi *et al.*, 2016) and some

countries in North and South America, such as Canada (Stalker *et al.*, 2010), several US states (Pennsylvania, Washington, North Carolina, South Carolina, Arkansas, Mississippi, Alabama, and California) (Aziz & Barnes, 2009; Gingerich, 2009), and Brazil (Braga *et al.*, 2016b).

According to some reports, the disease occurs more frequently in males (Aziz & Barnes, 2007; Herdt *et al.,* 2009; Aitchison *et al.*, 2014) and may affect several strains (Wood *et al.*, 2002; Gingerich, 2009). The higher body weight normally observed in males (Figure 1) implies an increase in the weight needed to be supported by bones and joints and a greater possibility of trauma. The influence of high body weight has also been described in another condition that affects broilers named bacterial chondronecrosis with osteomyelitis (BCO) by Wideman & Prisby (2013).



	7	14	21	28	35	42	49	56	63
Male	26,6	33,6	46,2	56,6	65,7	72,5	77,3	80	80,4
Female	26,3	32,9	43,5	52,2	59,5	63,6	65,8	66,8	67,1

Figure 1 – Average daily weight gain (g) of male and female broilers from seven to 63 days-old. Adapted from Cobb manual (2015).

The broilers affected by VO are usually older than 30 days-old, and outbreaks of the disease are reported between three to 18 weeks-old (Herdt *et al.,* 2009; Armour *et al.*, 2011; Robbins *et al.*, 2012). However, there is a report in a flock as young as 15 days-old (Gingerich, 2009).

Predisposing factors for VO are not well defined (Kense & Landman, 2011; Robbins *et al.*, 2012); however, immunosuppression and environmental conditions have been identified as factors that contribute to the occurrence of the disease (Stalker *et al.*, 2010; Armour *et al.*, 2011). Any immunosuppressive condition can naturally predispose to opportunistic infection by *E. cecorum*, which is a normal intestinal commensal. However, a study demonstrated differences in the pathogenicity among commensal and clinical cases isolates, raising the question whether the emergence of clones is most likely the cause for the increased occurrence of infections (Boerlin *et al.*, 2012).

Some evidences suggest that the higher incidence of enterococci-associated diseases in poultry may be due to horizontal spread of dominant *E. cecorum* clones that exhibit high pathogenicity (Kense & Landman, 2011; Boerlin *et al.*, 2012). However, the source of pathogenic *E. cecorum* remains unknown (Borst *et al.*, 2017). Moreover, bacterial strains with genotypes similar to those isolated from VO cases were rarely recovered from the ceca of birds with VO and the presence of these isolates was not statistically associated with a higher risk of disease (Borst *et al.*, 2012). These findings suggest that the long-term cecal carrier state of pathogenic clones may not be required for the pathogenesis of VO caused by *E. cecorum*. However, as the disease has a chronic character, taking weeks from the time of infection until the onset of clinical signs, pathogenic strains may be transient in the gastrointestinal tract and therefore not recoverable in the moment of clinical presentation (Borst *et al.*, 2012). Field observations showed that the disease occurred in successive flocks, suggesting the persistence of *E. cecorum* on the farm (Herdt *et al.*, 2009; Kense & Landman, 2011). According to Borst *et al.* (2017), persistence of *E. cecorum* in the environment between flocks is unproven, although the repeated outbreaks on affected farms may be associated to environmental contamination by pathogenic *E. cecorum*.

Despite their worldwide distribution, how pathogenic clones of *E. cecorum* spread remains undetermined. Epidemiological studies on different VO outbreaks suggest that mechanical spreading by biological vectors or inadequate biosafety may contribute to disease transmission, but horizontal transmission between geographically distant locations is considered unlikely (Borst *et al.*, 2012).

Kense & Landman (2011) demonstrated that vertical transmission does not occur. Recently, Borst *et al.* (2014) showed lower survival rate of SPF and non-SPF chicken embryos inoculated with *E. cecorum* isolated from vertebral lesions compared with those inoculated with *E. cecorum* isolated from the intestines of healthy birds. The embryos infected with pathogenic strains presented lesions of septicemia, such as hemorrhage and edema. In embryos inoculated with non-pathogenic strains, these lesions were found only 48 hours later.

Etiology

Enterococcus cecorum

Since 2002, *E. cecorum* has been found to be the bacterium most often involved in outbreaks of non-

vertebral and vertebral osteomyelitis and arthritis in broiler and broiler breeders (Aziz & Barnes, 2007; Herdt *et al.*, 2009; Aziz & Barnes, 2009; Gingerich, 2009; Stalker *et al.*, 2010; Martin *et al.*, 2011; Boerlin *et al.*, 2012; Aitchison *et al.*, 2014).

Enterococcus spp. are gram-positive and spherical bacteria, which occur alone, in pairs or short chains. They are non-motile, non-spore-forming, facultative anaerobic with diverse biochemical properties (Wages, 1998). However, the relationship between the biochemical characteristics and pathogenicity of this genus remains unknown (Thayer *et al.*, 2008). *Enterococcus* spp. are ubiquitous in nature, with worldwide distribution in avian species. They are considered part of the normal intestinal microbiota of chickens and commonly found in poultry environments. The frequency with different species of *Enterococcus* spp. are isolated from the intestinal tract of healthy birds may vary according to age, but only a limited number of species are commonly isolated. The species *E. faecium*, *E. cecorum*, *E. faecalis*, *E. hirae* and *E. durans* were regularly isolated in at least one of three different age groups (1 day-old, 3 to 4 weeks-old, and older than 12 weeks-old) examined by Devriese *et al.* (1991). Enterococci are also important as nosocomial pathogens that cause bacteremia, endocarditis and other infections. Some strains are resistant to many antibiotics and possess virulence factors, such as adhesins, invasins, hemolysin, and pili (Franz *et al.*, 2011).

E. cecorum occurs more frequently in the intestines of chickens older than 12 weeks of age (Devriese *et al.*, 1991) and has rarely been associated with clinical disease in these birds (Devriese *et al.*, 2002; Wood *et al.*, 2002; Chadfield *et al.*, 2004; Thayer *et al.*, 2008). This could explain the limited number of publications on its role in disease and its pathogenicity (Makrai *et al.*, 2011). Two main hypotheses were proposed to explain the recent increase in the incidence of *E. cecorum* infections: 1) changes in the host or in environmental factors; and 2) emergence of individual clones with increased pathogenicity (Boerlin *et al.*, 2012). In order to test the second hypothesis, those authors analyzed *E. cecorum* isolates recovered from the ceca of healthy chickens and those with VO by pulsed-field gel electrophoresis (PFGE). The *E. cecorum* genotypes isolated from the vertebral lesions were significantly more similar to each other than those isolated from the ceca of healthy birds and of birds with VO, regardless of the affected broiler flock.

E. cecorum was also isolated from a broiler flock with pericarditis, hepatitis, femoral head necrosis and/or VO and the authors concluded that bacteremia and generalized infection seem to be important steps in the pathogenesis of the infection caused by this bacterium in broilers (Jung & Rautenschlein, 2014). This was also suggested by Borst et al. (2017), who observed that *E. cecorum* isolates recovered from the intestine, spleen, and free thoracic vertebra (FTV) of broilers in natural cases of VO had matching genotypes, confirming that intestinal colonization with pathogenic strains precedes bacteremia and infection of the FTV.

Other etiologic agents

Infections by *E. hirae* are relatively frequent in broilers, but its importance is not well understood. The incidence of diseases caused by this bacterium have increased in some countries, such as Norway, where *E. hirae* was isolated from cases of osteomyelitis in the proximal femur and endocarditis in broilers (Kolbjørnsen *et al.*, 2011).

Braga et al. (2016b) recently reported *E. hirae*, *E. faecalis*, *Escherichia coli*, and *Staphylococcus aureus* in single or mixed culture from VO cases in broilers. *E. coli*, which has also been isolated from VO cases in poultry (Dinev, 2013; Braga *et al.*, 2016a; Braga *et al.*, 2016b), is part of normal intestinal microbiota of humans and many animal species. *E. coli* is a gram-negative non-spore-forming bacillus, measuring 2-3 x 0.6 µm, and most strains are motile with peritrichous flagella (Barnes *et al.*, 2008).

Several *E. coli* strains are able to express virulence factors and cause intestinal or extra-intestinal diseases (Ambrozic *et al.*, 1998). *E. coli* is currently considered the most important gram-negative bacterium in poultry due to its different pathogenicity mechanisms and described diseases (Nakazato *et al.*, 2009). In avian species, *E. coli* pathogenic strains are named Avian Pathogenic *Escherichia coli* (APEC), which are responsible for extra-intestinal diseases generically known as colibacillosis (Ewers *et al.*, 2004). The presence of *E. coli* in bone and synovial tissues is a common consequence of colisepticemia and the affected birds probably were not able to have completely eliminated the bacterial infection (Barnes *et al.,* 2008).

Several virulence factors are associated with APEC, such as: F1 and P fimbrial adhesins, aerobactin iron acquisition system, k1 capsular antigen, complement resistance, and many proteins, such as Tsh autotransporter (Dho-Moulin & Fairbrother, 1999).

Staphylococcus pyogenes was isolated from VO cases in seven- to 16-weeks-old chickens (Carnaghan, 1966). Nairn (1973) reported the isolation of *S. aureus* from vertebral lesions in turkeys naturally affected with locomotor disorder, and that the experimental inoculation of turkeys resulted in osteomyelitis in the vertebral body and long bones. Van Veen (1999) detected the involvement of *Aspergillus fumigatus* in VO outbreaks in two flocks of 17- to 19-weeks-old broilers.

Pathogenesis

The pathogenesis of VO in birds remains largely unknown, although important progress has been made (Kense & Landman, 2011; Robbins *et al.*, 2012; Jung & Rautenschlein, 2014; Borst *et al.*, 2017). There are few publications on the pathogenicity of *E. cecorum* (Makrai *et al.*, 2011) and the knowledge of the genetic basis for the recently acquired pathogenicity of certain *E. cecorum* strains and the pathogenesis of vertebral lesions characteristic of the disease remains limited (Borst *et al.*, 2012; Borst *et al.*, 2015).

Recent molecular epidemiologic studies found that pathogenic *E. cecorum* strains were genetically clonal and suggested acquisition of specific virulence determinants by pathogenic *E. cecorum*. This study also showed that pathogenic isolates had smaller genomes with a higher guanidine-cytosine (GC) content and large regions of synteny compared with commensal *E. cecorum* isolates. Molecular phylogenetic analysis demonstrated that, at a threshold of 98% identity, 414 predicted proteins were highly conserved in pathogenic *E. cecorum* but not in commensal strains (Borst *et al.*, 2015).

A recent study compared pathogenic and non-pathogenic *E. cecorum* strains from different animal species. Pathogenic and commensal *E. cecorum* strains were not clearly separated from each other in a phylogenetic tree based on partial sequences of the 16S-rRNA-gene and their fatty acid profile. Also, it was noted that all pathogenic *E. cecorum* strains was not able to utilize mannitol, while 31.0% of the commensal strains were mannitol positive (Jung *et al.*, 2017). This characteristic was also observed in other studies regarding the phenotypic and genotypic characterization of *E. cecorum* associated with infections in poultry (Dolka *et al.*, 2016). Jung *et al.* (2017) did not find any significant difference in virulence factors between pathogenic and commensal isolates. On the other hand, mean embryo lethality of pathogenic *E. cecorum* isolates (39.7%) was significantly higher than that of commensal strains (18.9%). Those authors also reported that two different antisera were produced, although none of the serotypes were predominantly found either in pathogenic or commensal isolates.

Molecular studies on conserved genes to speciate enterococci suggested that pathogenic *E. cecorum* might be considered a subspecies in view of the divergence between commensal and pathogenic *E. cecorum* genomes (Borst *et al.*, 2015). In addition, pathogenic strains showed conserved potential mediators of virulence, including genes encoding predicted collagen-binding proteins, a capsular locus, and orthologs to the enterococcal polysaccharide antigen of *E. faecalis* and *E. faecium* (Borst *et al.*, 2015).

The disease was experimentally reproduced by Martin *et al.* (2011) by inoculating broilers with *E. cecorum* by oral and intravenous routes. Gross lesions were found five weeks after the experimental infection in 6.1% and 2.9% of the broilers orally and intravenously inoculated, respectively. However, histologic lesions were found in 30.3% of the orally-inoculated broilers, and the authors suggest that macroscopic evidence of disease would be greater if the broilers were older.

The FTV (T4) body is singly affected in VO and the reasons for this predilection are not fully understood. The FTV articulation is located between the immediately anterior fused thoracic vertebrae and the posterior synsacrum (Figure 2), enabling body position adjustments and flexibility during walk and flight, and is subject to greater biomechanical stress and microtraumas than any other vertebra. Excessive stress may lead to changes in blood flow, with the development of microthrombi, sequestrum and multiplication of bacteria present in the blood (Aziz & Barnes, 2007; Stalker *et al.*, 2010; Wideman & Prisby, 2013; Aitchison *et al.*, 2014). This corroborates with a recent study reporting that broilers with osteochondrosis dissecans (OCD) were more susceptible to VO development than birds with no FTV lesions, and that suggested that intestinal colonization, bacteremia, and OCD of the FTV in early life are crucial to the pathogenesis of the disease (Borst *et al.*, 2017).

According to Stashak & Mayhew (1984), VO is usually secondary to the hematogenous dissemination of a microorganism. However, other theories have been proposed to explain how the bacteria reach the FTV. One of these theories suggests that the agent has access to the bones via bloodstream due to disruption of the intestinal mucosal barrier (Stalker *et al.*, 2010; Martin *et al.*, 2011), as occurs in coccidiosis or bacterial enteritis (Gingerich, 2009). According to Martin *et al.* (2011), any factor that negatively interferes with intestinal health or disturbs intestinal microbiota

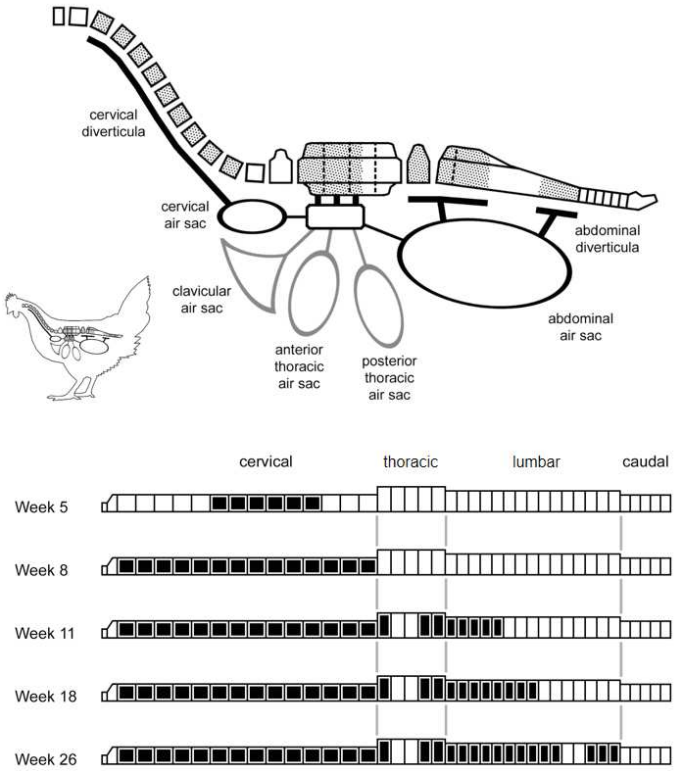


Figure 2 – Pneumatization of the vertebral column in the chicken (*Gallus gallus*). Pneumatic vertebrae are represented by grey (upper diagram) or black (lower diagram). The vertebral column is pneumatized by cervical diverticula and abdominal air sacs and lungs. Adapted from Wedel (2007).

balance may predispose broilers to the systemic dissemination of *E. cecorum*. On the other hand, Borst *et al.* (2017) observed that clinical intestinal disease was not required to cause *E. cecorum* bacteremia.

A possible link of the VO with air sacs and pneumatic vertebra could exist (Aziz & Barnes, 2007). However, the pneumatization of the vertebra where the disease occurs (T4) begins only after eight weeks of age (Figure 2). The experimental inoculation of *E. cecorum* in two-weeks-old broilers by air sac route did not result in VO (Martin *et al.*, 2011), suggesting that older broilers should be inoculated for experimental studies. In a study conducted by Tankson *et al.* (2002), *E. faecalis*, *E. durans*, and *E. coli* were isolated from the heart and lungs in 15% of healthy chicks. However, there are no studies that provide this information for *E. cecorum*.

Some aspects of the pathogenesis of BCO may aid the understanding of VO pathogenesis. This disease commonly affects the femur and the tibiotarsus, but it may affect the FTV. BCO begins with the degeneration and necrosis of the cartilage, followed by bacterial invasion, and it is mainly associated with *S. aureus*, *E. coli* and *E. cecorum*, often in mixed infections, as well as with other bacteria (Wideman & Prisby, 2013). Also, a recent study (Borst *et al.*, 2017) demonstrated that pathogenic *E. cecorum* was observed within OCD lesions in the FTV in 1- to 3-weeks-old broilers with VO, and suggested that intestinal colonization, bacteremia, and OCD of the FTV in early life seem to be crucial for the pathogenesis of VO.

It is believed that the BCO begins with mechanical damage to the poorly-mineralized columns of chondrocytes present mainly in the proximal growth plate of fast-growing bones, such as the femur and tibia, and is followed by the colonization of the chondronecrotic clefts by opportunistic hematogenous bacteria (Carnaghan, 1966; McNamee & Smyth, 2000; Dinev, 2009; Wideman Jr *et al.*, 2012), which was recently described in VO cases by Borst *et al.* (2017). Terminal BCO presents itself as degeneration, necrosis and bacterial infection at the proximal ends (epiphyseal and metaphyseal growth plates) of the femur and tibiotarsus. A similar process may occur in the growth plates of other bones that are subject to severe torque and shear stresses, as occur in the FTV, which functions as a flexible pivot between the cranially-fused vertebrae of the notarium and the caudally-fused vertebrae of the synsacrum (Carnaghan, 1966; McNamee & Smyth, 2000; Dinev, 2009; Wideman Jr *et al.*, 2012).

According to Barnes *et al.* (2008), the involvement of *E. coli* in infectious processes of the bone and synovial tissues is a common consequence of colisepticemia. Osteomyelitis caused by the hematogenous spread of *E. coli* after infection by the hemorrhagic enteritis virus was experimentally reproduced in turkeys (Droual *et al.*, 1996). Some authors report that, although the intravenous inoculation of *E. coli* promoted its hematogenous spread to the bones and joints and reproduction of lesions, bird mortality caused by initial sepsis is usually high (Bayyari *et al.*, 1997). According to Bayyari *et al.* (1997), that bacterium colonizes the vascular branches that invade the growth plate of growing bones, causing an inflammatory response that results in osteomyelitis. The transphyseal vessels in birds may possibly serve as conduits for the spread of bacteria to the joint and surrounding soft tissues.

Clinicopathological changes

Clinical signs

The clinical signs are similar in all VO cases reported (Gingerich, 2009), although the age of onset of clinical presentation may vary. In cases of osteomyelitis and arthritis caused by *E. cecorum*, Herdt *et al.* (2009) reported that the clinical signs started in the first and second weeks of age, with a mortality rate of 7%. In the osteomyelitis cases studied by Makrai *et al.* (2011), the clinical signs started between 5-9 weeks up to

10-13 weeks of age, with a mortality rate ranging from 8% to 30%, which was higher than previously reported (Wood *et al.*, 2002; Herdt *et al.*, 2009).

The major clinical sign observed is the limited mobility, with birds presenting mild to severe lameness. The affected birds frequently acquire the posture described as "sitting on their hocks", characterized by cranially extended legs and support given by the tibiotarsus-metatarsus joints (Figure 3a) (Gingerich, 2009; Braga *et al.*, 2016b). This is considered the classic clinical presentation of the disease, which is similar to that observed in birds with spondylolisthesis (Wood *et al.*, 2002; Gingerich, 2009).

Severely affected broilers may remain in lateral recumbency (Gingerich, 2009). They occasionally use their wings to help locomotion, which may result in wing laceration and hematomas (Makrai *et al.*, 2011). One of the consequences of impaired locomotion is the difficulty to access water and feed, resulting in reduced growth rate and ultimately in death due to dehydration or starvation (Barnes *et al.*, 2008; Borst *et al.*, 2017).

Gross changes

The macroscopic examination of the thoracolumbar region of vertebral column of the affected broilers reveals gross changes in the FTV (T4), characterized by a palpable whitish to yellowish enlargement (Figure 3b). The sagittal section of this lesion shows caseonecrotic material inside the vertebral body characterized by yellow to gray, granular and friable exudate, which is surrounded by a thick whitish capsule of fibrous connective tissue (Figure 3b, inset) (Gingerich, 2009; Martin *et al.*, 2011; Robbins *et al.*, 2012, Braga *et al.*, 2016b). The most pronounced lesion is characterized by the increased size of the vertebral body as a result of infection, which results in narrowing of the overlying spinal canal and compression of the spinal cord (Makrai *et al.*, 2011; Aitchison *et al.*, 2014, Braga *et al.*, 2016b). In the early stages of the disease, there is no obvious increase of the vertebral body and spinal compression of the sagittal section is absent or mild. The body condition of the affected birds varies from good to cachectic (Braga *et al.*, 2016b).

Histopathology

The microscopic changes of VO observed with the experimental reproduction of the disease by *E. cecorum* infection were detailed by Martin *et al.*(2011) and were similar to those reported in natural cases of the disease (Stalker *et al.*, 2010; Robbins *et al.*, 2012; Aitchison *et al.*, 2014, Braga *et al.*, 2016b). On the histopathologic examination, the FTV body and occasionally the adjacent vertebrae of notarium and synsacrum, presented necrotic tissue and exudate composed of fibrin, hemorrhage, and heterophils (Figure 3e and 3f). The bone tissue that forms the basis of the spinal canal is replaced by fibrous connective tissue and exudate, leading to spinal canal stenosis and spinal cord compression. In addition, there is fibrous connective tissue proliferation and bone remodeling in the areas surrounding the lesion. There are also areas of bone and cartilage tissue sequestrum within the exudate. When bacterial colonies are present (Figure 3e, inset), they are numerous and associated with the sequestrated areas (Aitchison *et al.*, 2014; Braga *et al.*, 2016b). In addition to these changes, Aitchison *et al.* (2014) and Braga *et al.* (2016b) described reactive osteoid formation and cartilaginous metaplasia in the areas where there was severe thickening of the vertebral body, resulting in areas of spinal cord compression. In these areas, there was axonal loss and degeneration, and the neuropil was disorganized and vacuolated, indicating a compressive effect.

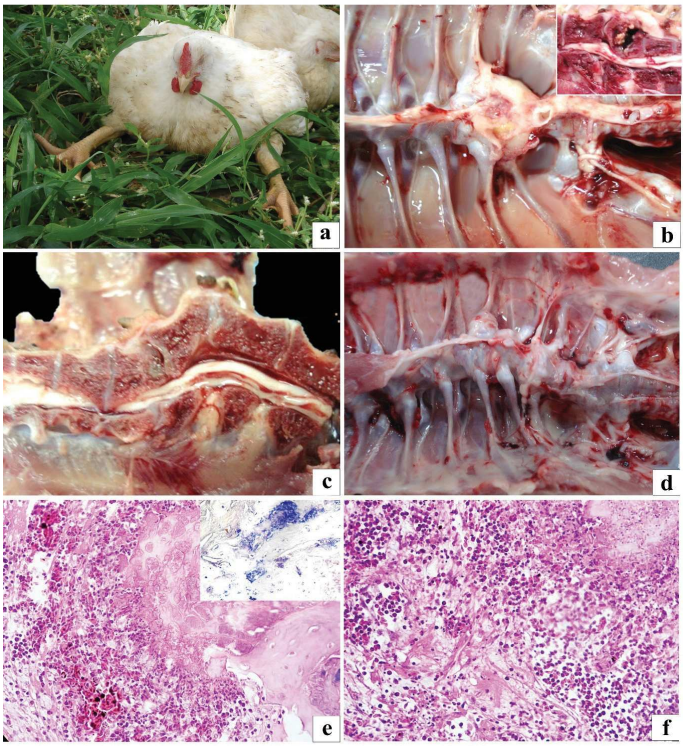


Figure 3 – Clinicopathological changes of vertebral osteomyelitis and differential diagnosis in the broilers. (a) A broiler presenting the classical clinical sign of vertebral osteomyelitis. (b) Gross changes in the vertebral osteomyelitis showing enlargement of affected vertebral body (T4). Inset: sagittal section with caseonecrotic material in the T4 vertebra and spinal cord compression. (c) Displacement of T4 vertebral body, characteristic of spondylolisthesis with spinal cord compression. (d) Scoliosis characterized by lateral deviation of vertebral column. (e, f) Histological changes of vertebral osteomyelitis. Necrotic tissue, cell debris, heterophils, hemorrhage, and fibrin are present. HE stain, bar = 50 µm. Inset: Gram-positive bacteria associated with vertebral lesion. Goodpasture stain, bar = 50 µm.

Martin *et al.* (2011) reported histologic changes in broilers in the absence of macroscopic lesions, including mild histologic lesions in the subchondral vertebral areas, with no extension to the articular cartilage or adjacent vertebrae. Moderate to severe lymphocyte infiltration and diffuse fibroplasia in the affected vertebra with intralesional bacteria was confirmed in half (4/8) of the cases. Braga *et al.* (2016b) detected also lesions in adjacent vertebrae, characterized by degeneration and necrosis of the articular cartilage (T4/T5), and occasional presence of clefts associated or not with hemorrhages and bacterial colonies. In the study of Martin *et al.* (2011), osteochondrosis was present in all birds, some of which presenting different degrees of subluxation FTV. A recent study on VO pathogenesis also described histologic lesions consistent with OCD, characterized by variably large cartilaginous clefts, which were often partially to completely filled with thrombocytes, fibrin, and erythrocytes. In some cases, there were cocci consistent with *E. cecorum* colonizing some of these clefts (Borst *et al.*, 2017).

Diagnosis

VO may be suspected in birds sitting on their hocks (McNamee & Smyth, 2000). It should be emphasized that high-quality veterinary support and thorough pathological and bacteriological examinations are indispensable for the early diagnosis of VO in broiler flocks. This is essential for the success of antibiotic therapy in the early stages of the disease (Jung & Rautenschlein, 2014).

Broilers presenting locomotor disorders should be placed in orthostatic (standing) position and encouraged to move to assess gait and posture changes. These birds should be then euthanized and necropsied to examine possible gross changes in the locomotor system (axial and appendage skeleton), and the vertebral column sectioned along the longitudinal midline to examine the vertebral body and assess the degree of spinal cord compression (Gingerich, 2009; Braga *et al.*, 2016b). At necropsy, samples for bacterial isolation and identification, polymerase chain reaction (PCR) tests, and histopathology should be collected.

For bacterial culture and isolation, swabs from caseonecrotic material of vertebral lesions should be aseptically collected and inoculated onto blood agar (BA) and MacConkey agar. It is recommended to incubate one BA plate under microaerophilic conditions (37 °C, 24 to 72 h) and the other plates under aerobic conditions at the same temperature and time to enable the isolation of different etiologic agents (Braga *et al.*, 2016b). After initial bacterial growth, the colonies must be Gram stained and submitted to catalase and oxidase tests. Bacterial isolates may be identified by biochemical tests, rapid ID 32 STREP (BioMerieux), VITEK 2 system (bioMérieux), and MALDI-TOF (AXIMA Assurance).

For DNA detection of the etiologic agent, vertebral lesion samples should be collected in sterile microtubes and frozen at -20 °C for DNA extraction and subsequent PCR testing. A multiplex PCR assay was described to enable the detection of different species of *Enterococcus* sp. (Jackson *et al.*, 2004). Recently, Jung *et al.* (2017) reported the use of a newly-developed quantitative TaqMan real-time PCR (qPCR) assay based on the 16S–rRNA-gene for the detection of *E. cecorum*. According to those authors, this new qPCR is highly specific, more sensitive than classical cultivation, and was able to determine colonization differences between a broiler flock presenting a late outbreak of *E. cecorum* disease compared to a healthy flock.

After sample collection for bacteriology and PCR, vertebral columns with gross lesions must be preserved in 10% neutral buffered formalin (48 to 72 h) for histopathological analysis. Vertebral columns should be decalcified in 24% formic acid before routine histological processing. The slides should be stained with hematoxylin-eosin and Goodpasture stains (Luna, 1968) to search for VO characteristic lesions (previously described in the histopathology subsection) and intralesional bacteria under a light microscope (Braga *et al.*, 2016b).

The differential diagnosis of VO includes other pathologies that may cause spinal cord compression or changes in nerves causing impaired mobility. One of these conditions is spondylolisthesis ("kinky back"), characterized by subluxation of the FTV (Armour *et al.*, 2011; Robbins *et al.*, 2012). Grossly, these cases show varying extents of ventral dislocation of the FTV, whose posterior end raises the 5th thoracic vertebra. The dislocation may produce kyphotic angulation of the spinal canal and varying degrees of spinal cord compression (Figure 3c). Necrotic and inflammatory lesions of the vertebral body in broilers with spondylolisthesis may not be present (Dinev, 2012). Scoliosis characterized by lateral deviation of the spine (Figure 3d) should also be considered for differential diagnosis of the aforementioned conditions (Droual *et al.*, 1991). Proper monitoring of the flock may aid the early detection of these conditions, facilitating their diagnosis (Gingerich, 2009). Birds with the paralytic or the neurological form of Marek's disease may present clinical signs similar to VO and it should be considered

in differential diagnosis. In Marek's disease, no changes in the vertebral column are observed, but there is enlargement of peripheral nerves, which become greyish or yellowish with loss of striations, acquiring an edematous appearance in some cases (Schat & Nair, 2008).

Prevention, treatment and control

Information on prevention, treatment and control of the disease is limited, as the etiopathogenesis of VO remains unclear (Aitchison *et al.*, 2014), and most studies are related only to infections caused by *E. cecorum*. For the prevention of VO, recommendations on management practices to reduce the risk of developing the disease have been made, such as: 1) avoiding excessive feed restriction; 2) following the suggested weight gain standards and nutritional recommendations (Martin *et al.*, 2011); 3) promoting adequate control of coccidiosis (Stalker *et al.*, 2010); 4) avoiding high housing density; 5) ensuring adequate access to feeders; and 6) preventing respiratory diseases (Aitchison *et al.*, 2014). All practices to prevent bacterial infections that may cause bacteremia would probably also help to avoid bone and articular inflammation.

Antibiotics have been used to treat the bacterial infection in VO. Although several antibiotics have shown efficacy against the commonly described bacteria, it is difficult to achieve adequate concentrations of the antibiotics in the vertebral column. In the reported outbreaks of the disease, antibiotics were ineffective in reducing mortality possibly due to the inability of the antibiotic to effectively penetrate the anatomical areas where the bacterium is located or antimicrobial resistance of E. cecorum (Kense & Landman, 2011). The antimicrobial susceptibility profiles of *E. cecorum* isolated from outbreaks in different countries were similar (Herdt *et al.*, 2009; Aitchison *et al.*, 2014). Aitchison *et al.* (2014) reported that, after isolation and identification of *E. cecorum*, it was difficult to perform the antibiotic susceptibility test due to the growing conditions of the bacterium. Makrai *et al.* (2011) reported that, after the onset of the outbreak, broilers presenting clinical signs were separated from those clinically normal and the clinically normal were treated with different antibiotics (amoxycillin, amoxycillin with clavulanic acid, lincomycin or doxycycline), resulting in no new clinical case of the disease in the flock.

After the occurrence of the disease, the prevention of new cases requires repeated cycles of disinfection and usually occur after a single cleaning and disinfection procedure. Increased efforts in subsequent flocks are required to eliminate the disease. Some practices that may reduce the risk of VO in subsequent flocks include: 1) emptying and completely disinfecting the broiler house; 2) changing or composting the litter; 3) adequate cleaning of water lines; and 4) continuously sanitizing the water (Gingerich, 2009; Stalker *et al.*, 2010; Armour *et al.*, 2011; Martin *et al.*, 2011).

Antimicrobial resistance and public health

As previously mentioned, enterococci are normal bacteria of the gastrointestinal tract of animals and humans and are often considered as beneficial commensal organisms (Tannock, 1995). However, they may also be opportunistic pathogens, responsible for serious systemic infections and for the spread of antimicrobial resistance and virulence determinants (Wisplinghoff *et al.*, 2004; Heuer *et al.*, 2006). In recent years, enterococci have emerged as a major cause of nosocomial infections, particularly *E. faecalis* (Kola *et al.*, 2010), causing extraintestinal infections in humans (Creti *et al.*, 2004). These bacteria have intrinsic resistance to many antibiotics and have acquired new resistance genotypes, out of which vancomycin-resistant enterococci (VRE) are of special concern (Cetinkaya *et al.*, 2000; Willems & Bonten, 2007). VRE have become a major problem in nosocomial infections. A retrospective study of ten human patients with osteomyelitis showed that eight cases were due to infection by *E. faecalis* resistant to vancomycin, with one death reported due to bacteremia (Holtom *et al.*, 2002).

The contamination of animals and their by-products by resistant bacteria and their possible foodborne transmission to humans are an animal and public health concern (Foulquié-Moreno *et al.*, 2006). Enterococci may not only contaminate raw meat, but may also be present in processed meat products, such as cured raw sausages or cooked products (Martin *et al.*, 2005; Barbosa *et al.*, 2009; Ruiz-Moyano *et al.*, 2009). Although no food poisoning associated with *E. faecalis* has been described in humans, a recent study performed in Brazil showed the presence of this bacterium in 42% of the chicken carcasses tested (Campos *et al.*, 2013). All strains were resistant to at least one of the antibiotics tested, with the detection of the antimicrobial resistance genes *erm(B)*, *vanC-1*, *aph(3')-IIIa, ant(6)-Ia, vanB, vanA, aac(6')-Ie-aph(2")-Ia, erm(A)*e *tet(M)*. The relevance of *E. faecalis* in public health is also highlighted by its ability to transfer antimicrobial resistance genes to other organisms present in the intestinal tract of humans and animals, limiting the utilization of antibacterial drugs (Campos

et al., 2013). Hayes *et al.* (2003) analyzed 981 raw meat samples of various species (chicken, turkey, swine and bovine) obtained in grocery stores and isolated 1,357 *Enterococcus* spp. strains, including *E. faecalis* (29%) and *E. hirae* (5.7%). Those authors also detected gentamicin resistance in 4% of the strains, most of which isolated from chicken meat. Braga *et al.* (2017), analyzing *E. faecalis* isolates from VO in broilers, demonstrated that the highest level of antibiotic resistance was against aminoglycosides, particularly gentamicin (40%).

CONCLUSION

VO is an emerging disease in poultry worldwide that still needs to be further elucidated. Many aspects of the etiopathogenesis of the disease remain unclear, limiting its prevention and control. Most reports associate the disease to the infection by *E. cecorum*, probably related to emerging clones with higher pathogenicity. However, other bacteria have been isolated from VO in broilers, raising questions about the etiology and pathogenesis of the disease. This highlights the need for isolation and identification of the etiologic agent in cases of VO, as well as studies for molecular characterization and antimicrobial resistance of the involved bacterium.

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