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ORIGINAL ARTICLE

Enterobacteriaceae and *Enterococcaceae* are the dominant bacterial families translocating to femur heads in broiler chicks

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ABSTRACT

As a result of rapid growth of broilers, bacterial chondronecrosis with osteomyelitis has emerged in the last decade, with bacterial translocation from the gut to internal organs, including the femur head. In this study, we isolated translocated bacteria in femur heads, blood, and liver, during ageing of broilers, and identified the bacteria using 16S rDNA sequencing. We also provided histopathological descriptions of femur head lesions. Bacteria were isolated from blood, liver, and femoral head samples. In the femoral heads, an age-related presence of bacteria was observed, with high prevalence at 2 days post-hatch, and no bacteria isolated from femoral heads of broilers older than 14 days. Bacterial identification using 16S rRNA gene sequencing demonstrated that bacteria present in blood, liver, and femur belonged to different taxonomic groups. Bacteria isolated from blood and liver samples belonged predominantly to the *Enterobacteriaceae*, *Enterococcaceae*, and *Staphylococcaceae*, while *Escherichia/Shigella* and *Enterococcus* spp. were the most prevalent taxa in femoral head samples. No bacteria were isolated from the femoral head of any of the birds with histopathological lesions. All 19-day-old and 22-day-old birds in this study showed cartilage retention in both legs, and had signs of separation between the articular cartilage and the growth cartilage in one or both legs. This study shows that young clinically healthy broilers have a higher prevalence of bacteria in the femoral head compared to older broilers and that presence of bacteria in blood and liver is common during ageing of broilers.

RESEARCH HIGHLIGHTS

- Large number of bacteria isolated from femoral heads of clinically healthy broilers.
- The prevailing taxa in femoral heads were *Escherichia/Shigella* and *Enterococcus* spp.
- Continuous presence of bacteria in blood and liver of clinically healthy broilers.
- *Enterobacteriaceae*, *Enterococcaceae*, and *Staphylococcaceae* prevail in blood and liver.

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Broiler; bacterial chondronecrosis and osteomyelitis; *Enterococcaceae*; *Enterobacteriaceae*; bacterial translocation; gut health

Introduction

Modern broilers are the result of years of genetic selection directed at economic traits, including rapid weight gain, yield of breast meat, and feed use efficiency (Knowles *et al.*, 2008; Tallentire *et al.*, 2016). As a result of intensive genetic selection for these traits, broiler chicken growth has increased over 400% between 1950 and 2005 (Zuidhof *et al.*, 2014). The majority of broiler chickens are reared in intensive production systems (Knowles *et al.*, 2008), where they grow from approximately 40 g at hatch to over 3.5 kg by 5 weeks of age. As a consequence of optimizing traits for production, chickens may suffer reduced welfare, with poor walking ability being a major concern (Knowles *et al.*, 2008; Alrubaye *et al.*, 2020; Ferver *et al.*, 2021).

Bacterial chondronecrosis with osteomyelitis (BCO) is an important cause of severe lameness in fast-growing broilers (Wideman, 2016). Over the last two decades, the incidence of lameness caused by BCO has increased noticeably, with over 1% of all broilers grown to heavy weights currently being affected after 5 weeks of age (Wideman, 2016). The size and structural integrity of the broilers' skeletons are unable to keep up with the rapid increases in bodyweight currently expected in the broiler industry. Unsurprisingly, this has a detrimental effect on the developing cartilage, which results in cartilage micro-fractures in the growth plates of the proximal femur and tibia (Wideman *et al.*, 2015; Wideman, 2016). These microfractures can be colonized by haematogenous opportunistic bacteria that can enter the circulation via translocation through the integument, respiratory system, or gastro-intestinal tract. The colonization by bacteria can cause obstruction of the local blood flow, trigger osteomyelitis, and finally result in necrosis of the cartilage (Wideman *et al.*, 2015, 2012; Wideman, 2016). Bacterial translocation through the

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Table 1. Diet composition of the feed used throughout the study.

Base diet	Percentage (%)
Wheat	58.72
Soybean meal	26.4
Rapeseed meal	6
Sunflower oil	4.9
Monocalcium phosphate	1.65
Limestone	1.08
NaCl	0.38
Mineral premix	0.2
Vitamin premix	0.2
DL-Methionine	0.22
L-Lysine HCl	0.19
Threonine	0.06
Vitamin premix	Concentration (g/kg)
Calcium	317
All-rac-a-tocopheryl acetate	40
Niacin	30
Panthotenic acid	8.5
Riboflavin	4.3
Pyridoxine	2.7
Retinol	1.95
Menadione	1.6
Thiamine	1.6
Folic acid	1.1
Biotin	0.15
Cholecalciferol	0.0625
Cyanocobalamin	0.0085
Mineral premix	Concentration (g/kg)
Calcium	248
Zinc	55
Manganese	60
Iron	10
Copper	8
Iodine	0.625
Selenium	0.15
Magnesium	5
Sodium	1

epithelial lining of the gastro-intestinal tract, and bacteraemia, are of significant importance in the pathogenesis of BCO. Therefore, reducing the number of opportunistic pathogenic bacteria that can pass the intestinal barrier, improving the integrity of the intestinal barrier, and supporting the host's immune system in order to efficiently eliminate translocated bacteria from the systemic circulation are of importance in the prevention of this disease.

To the best of our knowledge, there are no studies simultaneously investigating the translocation of bacteria to the blood and liver, and invading the bone marrow of the femoral heads at different timepoints in the early life of broilers as baseline data for further studies on the pathogenesis and control of BCO. We describe here the isolation of translocated culturable aerobic bacteria from femur heads, blood, and liver, during the rearing of broilers, and identification of the bacteria using 16 rDNA sequencing. In addition, histopathological descriptions of femur head lesions are provided.

Materials and methods

Ethics statement

The study was performed in accordance with the EU Directive 2010/63/EU and followed the guidelines of the ethics committee of the Faculty of Veterinary Medicine, Ghent University.

Birds and housing

One hundred and twelve one-day-old unvaccinated male Ross 308 broilers were obtained from a local hatchery (Vervaeke-Belavi, Tielt, Belgium) and housed at the Faculty of Veterinary Medicine, Ghent University. They were housed in floor pens on wood shavings, with water and feed (composition: see Table 1) provided *ad libitum*. The chicks were randomly divided over eight pens with 14 birds per pen. Temperature was adjusted with age, with target temperatures set at 32°C for day 2, gradually lowering to 23°C by day 22. The photoperiod was adjusted from 24 h light until day 3, to 23 h light: 1 h dark from day 3 until day 6, after which it was set to 18 h light: 6 h dark until the end of the trial.

Sample collection

During the trial birds were euthanized for sampling at 13 timepoints (days 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 19, and 22). On the first sampling day, two birds from each pen (16 birds) were euthanized. Subsequently, one bird per pen (eight birds) was euthanized at each timepoint.

On days 2–4 the chicks were euthanized by CO_2 gassing, while from day 5 on, euthanasia was by intravenous injection of an overdose of pentobarbital. After euthanasia, the birds were weighed, and samples were collected.

On days 2, 3, 4, 6, 7, 10, 14, and 19, both hip joints were exposed and disarticulated, after which the femoral heads were cut in half with a sterile knife and the cut surface was swabbed (Biolab Inc, Alpharetta, GA, USA) for microbiological culture. Thereafter the femoral heads were fixed in phosphate buffered formalin.

At five different timepoints (day 5, day 8, day 12, day 16, and day 22) the following samples were taken: first, 1 ml of blood was drawn from the jugular vein and added to 2 µl heparin (5000 U.I/ml stock) in a 2 ml Eppendorf tube, then mixed by inversion. Subsequently, the liver was carefully dissected from the digestive system and collected in its entirety. Finally, femoral head samples were collected as described above.

Samples were stored at 4°C after collection. Sample processing was performed the same day.

Sample processing

Characterisation of bacterial translocation

Blood, liver, and femoral head samples were plated for bacterial counts and bacterial identification.

Blood samples were cultivated on Colombia blood agar (Oxoid, Hampshire, UK) by spreading out 100 µl blood-on-heparin using a sterile L-shaped spreader, and incubated overnight at 37°C with 5% CO_2. After incubation, the colony forming units (CFU) on each plate were counted to determine the CFU/100 µl.

The livers were diluted with Hank's balanced salt solution (HBSS) (1 g liver/2 ml HBSS) and were homogenized. Subsequently a 1/10 dilution in HBSS was made. Six 20 µl drops (120 µl) of both the 10^0 and 10^{-1} dilutions were plated out on TSA agar (Sigma-Aldrich, Overijse, Belgium) and incubated overnight at 37°C with 5% CO_2. After the incubation, the total CFU in all six 20 µl drops were counted and added together. To determine CFU/g liver, the following calculations were made:

$$\mathrm{CFU/gram_{liver}}$$
$$\left(\frac{\text{total CFU}_{\text{counted}}}{6} * 50 * \text{dilution factor}\right) * 2$$

The swabs collected from the inside of the femoral head were immediately inoculated onto Colombia blood agar and incubated overnight at 37°C with 5% CO_2. After incubation the CFU of each side were counted and added together for each bird.

Identification of the bacterial isolates using Sanger Sequencing of the 16S rRNA gene

To identify the bacteria in the blood, liver, and femoral head samples, bacterial DNA was extracted and the 16S rRNA gene was sequenced. Bacterial colonies were harvested from their respective culture plates using a sterile 1 µl inoculation loop and DNA extraction was performed using 20 µl of lysis buffer (0.25% SDS and 1M NaOH diluted in distilled water), after which the mixtures were heated at 95°C for 5 min, and centrifuged for a short spin. Subsequently 180 µl deionized water (LiChrosolv®, Sigma-Aldrich) was added and the samples were centrifuged at 21,130× *g* for 5 min. The supernatant (DNA sample) was transferred and used in the following polymerase chain reaction (PCR).

PCR was performed to amplify the 16S rRNA gene aiming to characterize the taxonomic identity of the translocated bacteria. The PCR mix contained 650 µl Polymerase Taq platinum (Biomix®, Biomix, Fisher Scientific, Merelbeke, Belgium), 17.5 µl of both the forward (fD1; 5'-AGAGTTTGATCCTGGCTCAG-3') and the reverse primer (rD1; 5'-AAGGAGGTGATCCAGCC-3') (Weisburg *et al.*, 1991), and 488 µl 180 µl water for chromatography (LiChrosolv®, Sigma-Aldrich, Overijse, Belgium). Each reaction contained 2 µl of the PCR mix and 18 µl DNA sample. A negative control was included. The PCR amplification consisted of an initial denaturation at 95°C for 5 min, followed by three cycles of 45 s at 95°C, 2 min at 55°C and 1 min at 72°C and 30 cycles of 20 s at 95°C, 1 min at 55°C and 1 min at 72°C. The final extension was done at 72°C for 7 min.

DNA samples were diluted in aqua HPLC (1/2 dilution) with an end volume of 25 µl for Sanger sequencing. The 16S gene database of the Basic Local Alignment Search Tool (BLAST®, National Center for Biotechnology Information, Bethesda MD, USA) was used to determine the taxonomic group for each bacterial isolate.

Femoral head histology

To evaluate the morphology of the femoral heads, formalin-fixed femoral head samples from both the left and the right leg, derived from 19-day-old and 22-day-old chickens, were decalcified with a decalcification solution based on ethylenediaminetetraacetic acid (EDTA) (pH 8) for 2 weeks, after which they were placed in 70% ethanol solution. Subsequently, the femurs were embedded in paraffin, sectioned at 5 µm, and stained with haematoxylin and eosin for microscopic evaluation. The evaluation of femoral morphology was based on a scoring system quantifying separation of the articular cartilage from the growth cartilage (0 – no cleft; 1 – minimal cleft formation; 2 – moderate cleft formation; 3 – partial separation; 4 – complete separation), degeneration of the articular cartilage and the growth cartilage, scored separately (0 – no sign of degeneration; 1 – signs of degeneration/ necrotic lesions), cartilage retention (0 – no cartilage retention; 1 – cartilage retention), cellular depletion within the bone marrow (0 – no areas of cellular depletion; 1 – area(s) of cellular depletion), lymphoid hyperplasia within the bone marrow (0 – no lymphocyte follicles, 1 – focal presence of lymphocyte follicles, 2 – multifocal presence of lymphocyte follicles), and vascular regression within the growth plate (0 – no sign of regression; 1 – fibrin in vascular lumen). To obtain one value per chicken, the average score from the left and the right femoral leg samples was used. The sum of the scores for degeneration of articular cartilage and degeneration of growth cartilage was used as a measure of cartilage degeneration. The scoring system was based on lesions described by Wijesurendra *et al.* (2017).

Results

Bacterial translocation

At all five sampling points, bacteria could be detected in the blood and in the liver of some, but not all, chickens. No age-related effects were observed in bacterial counts derived from blood and liver samples (Figure 1(a and b)). The chicken's age affected the CFUs obtained from femoral head samples. On day 2, 87.5% of the chicks had ≥ 1 CFU in the femoral

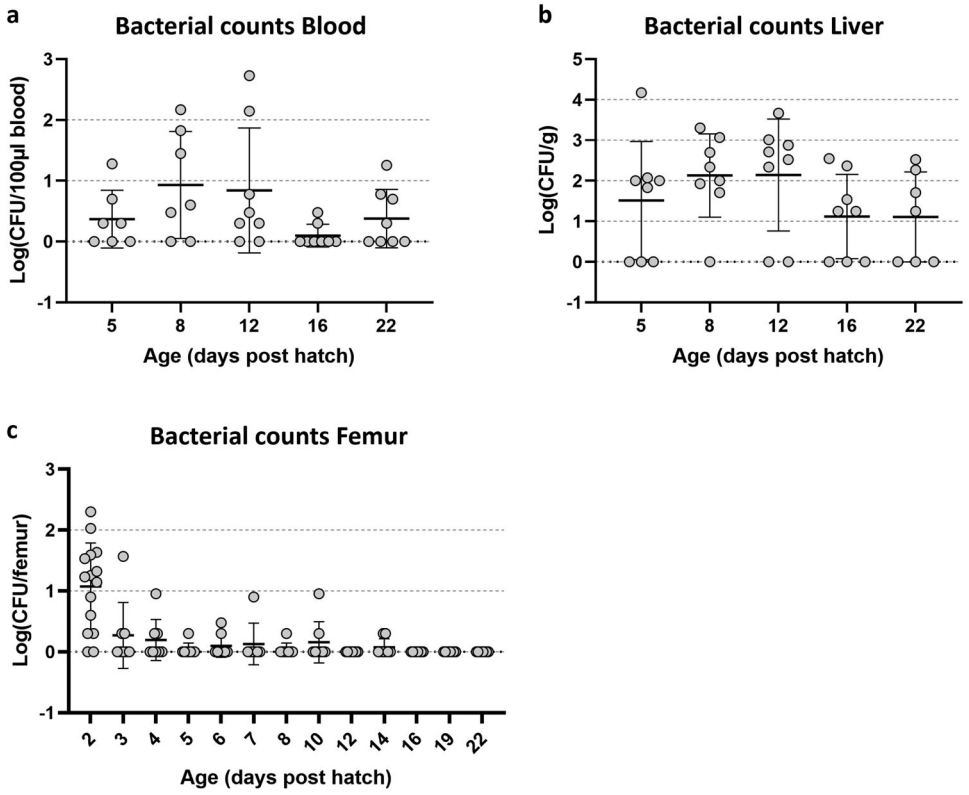

Figure 1. CFUs counted in samples from blood, liver, and femur at different timepoints. Samples were collected from blood (a), liver (b), and femur (c) at different timepoints, and were cultivated overnight. Bacterial CFUs were counted for each bird.

samples, with a subset of birds having high counts (Figure 1(c)). After day 12, not more than 1 CFU per bird was counted. On days 16, 19, and 22 all samples were free of bacteria.

Taxonomic description of translocated bacteria

Nucleotide BLAST analyses revealed 22 bacterial taxa in the blood, liver, and femur. For some isolates it was not possible to differentiate between the species based on 16S rDNA sequencing, which is why they are grouped together (separated by a division sign or backslash) in the following results. Table 2 displays the identified bacterial species and the tissue from which they were isolated.

Figure 2 shows the fraction of the birds from which the bacteria were isolated at the different timepoints,

Table 2. Twenty-two different bacterial taxa were isolated from blood, femur, and liver.

Bacterial taxa	Phylum	Family	Blood	Femur	Liver
Acinetobacter lwoffii	Proteobacteria	Moraxellaceae			x
Acinetobacter Johnsonii/ A. haemolyticus	Proteobacteria	Moraxellaceae	x		
Acinetobacter radioresistens	Proteobacteria	Moraxellaceae	x		
Bacillus spp.	Firmicutes	Bacillaceae	x	x	
Corynebacterium glyciniphilum	Actinobacteria	Corynebacteriaceae		x	
Enterococcus faecium/E. hirae/E. durans/E. pseudoavium/E. lactis	Firmicutes	Enterococcaceae	x	x	x
Enterococcus gallinarum/ E. casseliflavus	Firmicutes	Enterococcaceae	x	x	x
Enterobacter spp./ *Citrobacter* spp.	Proteobacteria	Enterobacteriaceae	x		
Enterobacter spp.	Proteobacteria	Enterobacteriaceae	x		
Enterococcus avium	Firmicutes	Enterococcaceae			x
Enterococcus faecalis	Firmicutes	Enterococcaceae	x	x	x
Enterococcus pallens	Firmicutes	Enterococcaceae	x		x
Escherichia/Shigella	Proteobacteria	Enterobacteriaceae	x	x	x
Klebsiella pneumoniae	Proteobacteria	Enterobacteriaceae			x
Lactobacillus spp.	Firmicutes	Lactobacillaceae	x		
Macrococcus caseolyticus	Firmicutes	Staphylococcaceae	x		x
Microbacterium spp.	Actinobacteria	Microbacteriaceae	x		
Neobacillus spp.	Bacillota	Bacillaceae	x		
Pedobacter spp.	Bacteroidota	Sphingobacteriaceae	x		
Peribacillus spp.	Bacillota	Bacillaceae	x	x	
Proteus mirabilis	Proteobacteria	Enterobacteriaceae			x
Staphylococcus warneri/S. pasteuri	Firmicutes	Staphylococcaceae	x		x

Notes: in the first three columns the taxonomy is shown, and in columns 4, 5, and 6 an "x" marks whether that species was isolated from blood, femur, or liver, respectively.

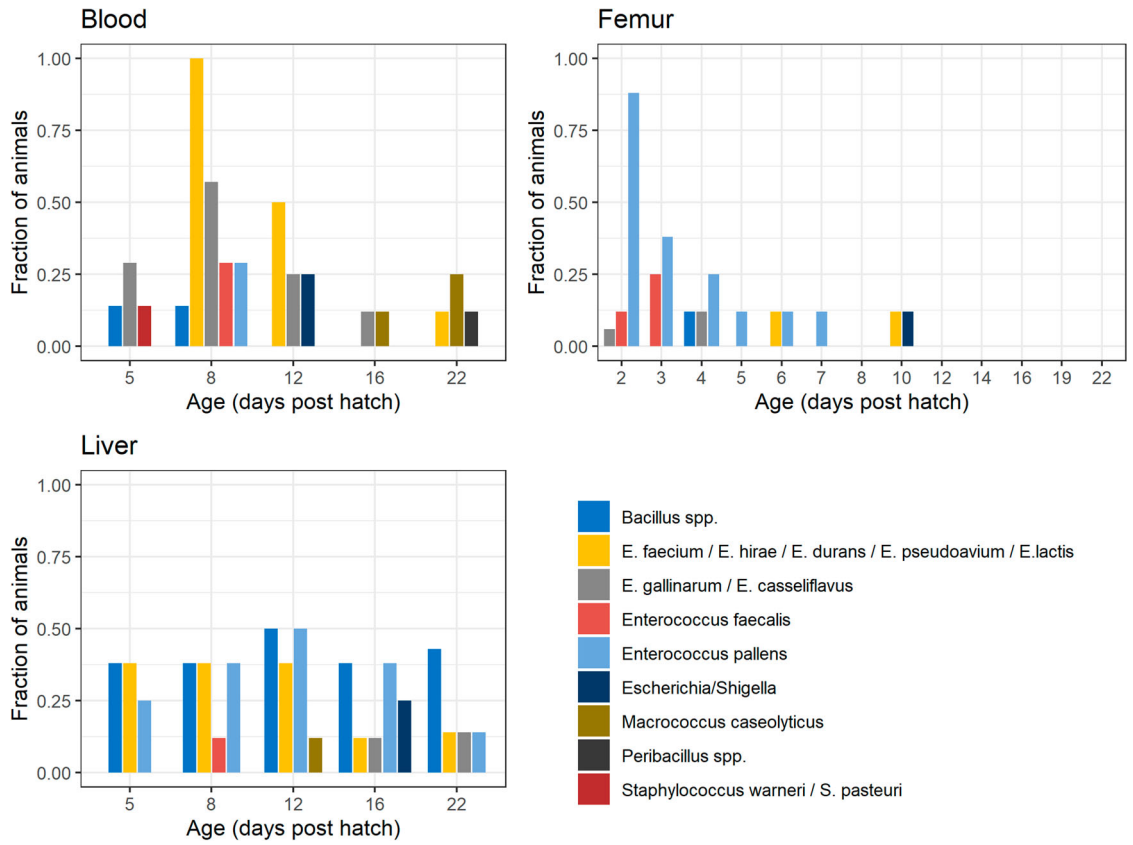

Figure 2. Fraction of birds with *Macrococcus caseolyticus*, *Bacillus* spp., *E. faecium/E. hirae/ E. durans/ E. pseudoavium/ E. lactis*, *Enterococcus gallinarum/E. casseliflavus*, *Enterococcus faecalis*, *Enterococcus pallens*, *Enterococcus* spp., *Escherichia/ Shigella*, *Staphylococcus hominis/ S. petrasii/ S. haemolyticus*, *Staphylococcus warneri/ S. pasteuri*, and *Peribacillus* spp. in blood cultures.

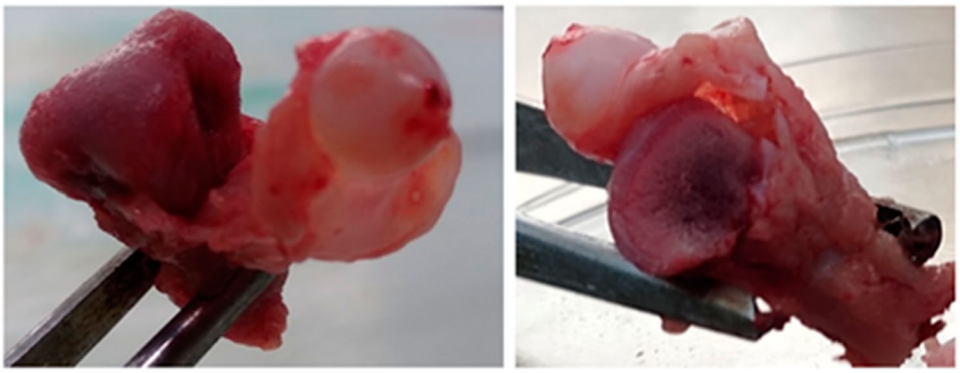

Figure 3. Femoral head separation in a 12-day-old bird.

but not the quantity in which the bacteria were present.

Femoral head morphology

In two birds (one 12-day-old and one 22-day-old bird) femoral head separation (FHS) was detected macroscopically (Figure 3).

Microscopically, all birds showed cartilage retention in both legs at both timepoints (Figure 5). Separation of the articular cartilage from the growth cartilage, degeneration of the articular cartilage and the growth cartilage, cartilage retention, cellular depletion within the bone, lymphoid hyperplasia within the bone marrow, and vascular regression within the growth plate are reported in Figure 4.

On both day 19 and day 22, 100% of the birds showed signs of separation between the articular cartilage and the growth cartilage in one or both femoral heads (Figure 6). The presence of necrotic lesions (Figure 7) or a more severe form of separation between the articular and the growth cartilage in a sample was not associated with the presence of lymphocyte follicles (Figure 8(a)).

Figure 8 shows the formation of follicles of lymphocytes (Figure 8(a)), areas of cellular depletion in the bone marrow (Figure 8(b)), and fibrin in the blood vessels of the growth plate (Figure 8(c)).

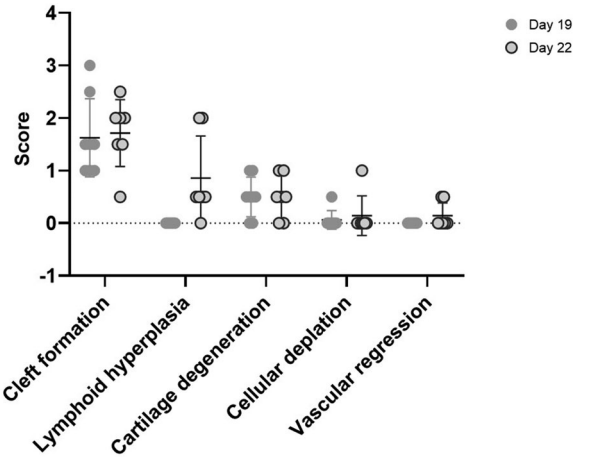

Figure 4. Morphology of the femur in 19-day-old and 22-day-old broilers. Data are presented as the mean ± standard deviation. Femur tissue was evaluated based on a scoring system quantifying cleft formation between the articular cartilage and the growth cartilage (0 – no cleft; 1 – minimal cleft formation; 2 – moderate cleft formation; 3 – partial separation; 4 – complete separation), lymphoid hyperplasia within the bone marrow (0 – no lymphocyte follicles, 1 – focal presence of lymphocyte follicles, 2 – multifocal presence of lymphocyte follicles), articular and growth cartilage degeneration (0 – no sign of degeneration; 1 – signs of degeneration/ necrotic lesions), cellular depletion within the bone marrow (0 – no areas of cellular depletion; 1 – area(s) of cellular depletion), and vascular regression within the growth plate (0 – no sign of regression; 1 – fibrin in vascular lumen).

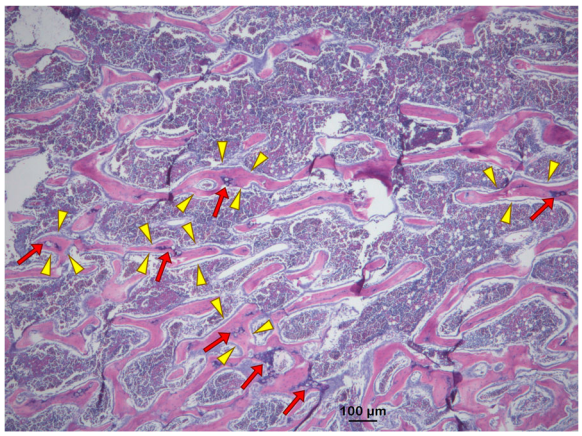

Figure 5. Retention of cartilage in osteoid. HE stained sample of a 22-day-old bird. Several areas of retained cartilage (red arrows) can be observed in within the osteoid (yellow arrowheads).

Discussion

Lameness in broilers is one of the main concerns in the poultry industry, resulting in reduced welfare and major economic losses (Hul *et al.*, 2021). BCO is recognized as an important cause of lameness that may affect over 1% of broilers grown to heavy processing weights after 5 weeks of age (Wideman, 2016). In our study, we examined the early life bacterial translocation and femoral head morphology to better understand the underlying causes of BCO. The trial was performed with male broilers, as they tend to be more prone to developing osteomyelitis and chondronecrosis because of rapid growth.

Relatively large numbers of bacteria were present in the femoral heads of birds at 2 days post hatch compared to older birds. BCO is the result of bacteria that enter the circulation by translocation through the respiratory and/or intestinal mucosal barrier. Subsequently, these bacteria spread haematogenously and colonize the small blood vessels in the cartilage suffering from microtrauma in the long bones, most commonly the femur and the tibia (Wideman, 2016). In the present study, several chickens of all ages had bacteria in the blood and/or in the liver; however, this was not the case for the femoral samples. An age-related effect on the presence of bacteria in the femoral heads was observed. Almost all 2-day-old chicks had bacteria present in the femoral heads. While some only had a few colonies, others had over 100 CFUs in the femoral samples. This number decreased drastically as the broilers got older and, after the age of 2 weeks, no colonies were found in the femoral samples. These results indicate that bacteria may be present in the circulation, without noticeable colonization of the femoral head at later ages.

Bacteria present in blood, liver, and femur belonged to different taxonomic groups. Mandal *et al.* (2016) investigated the blood microbiota and argued that, despite the current opinion that blood is sterile, there might be dormant and not-immediately-culturable forms of microbes present in the blood. In their study, they found that chicken blood microbiota was dominated by Proteobacteria, followed by Bacteroidota, Firmicutes, Actinobacteria, and Cyanobacteria. The discrepancy in the abundance of these phyla in the blood and gut led to the view that the blood microbiota may not be the result of bacteria passively present in the blood after translocation from the gut. Similar to Mandal *et al.* (2016), the bacteria isolated from blood samples in the present study belonged predominantly to the phyla Proteobacteria, Firmicutes, and Actinobacteria. The study of Mandal *et al.* (2016) was based on deep sequencing of 16S RNA genes on blood samples using a relatively high number of PCR amplification cycles. PCR is unable to differentiate live from dead bacteria. Therefore, the results may be an overestimation of the number of bacteria present in the samples. The presence of bacteria in the blood without any obvious clinical manifestation was also observed in the present study. The origin of the bacteria present in the blood is unclear. It should be considered that there is a possibility that the growth of microorganisms may have been inhibited by the use of heparin as anticoagulant (Rosett and Hodges, 1980). The liver serves as a filter that clears bacteria that have successfully penetrated the intestinal barrier (Balmer *et al.*, 2014). Therefore, it could be expected that the

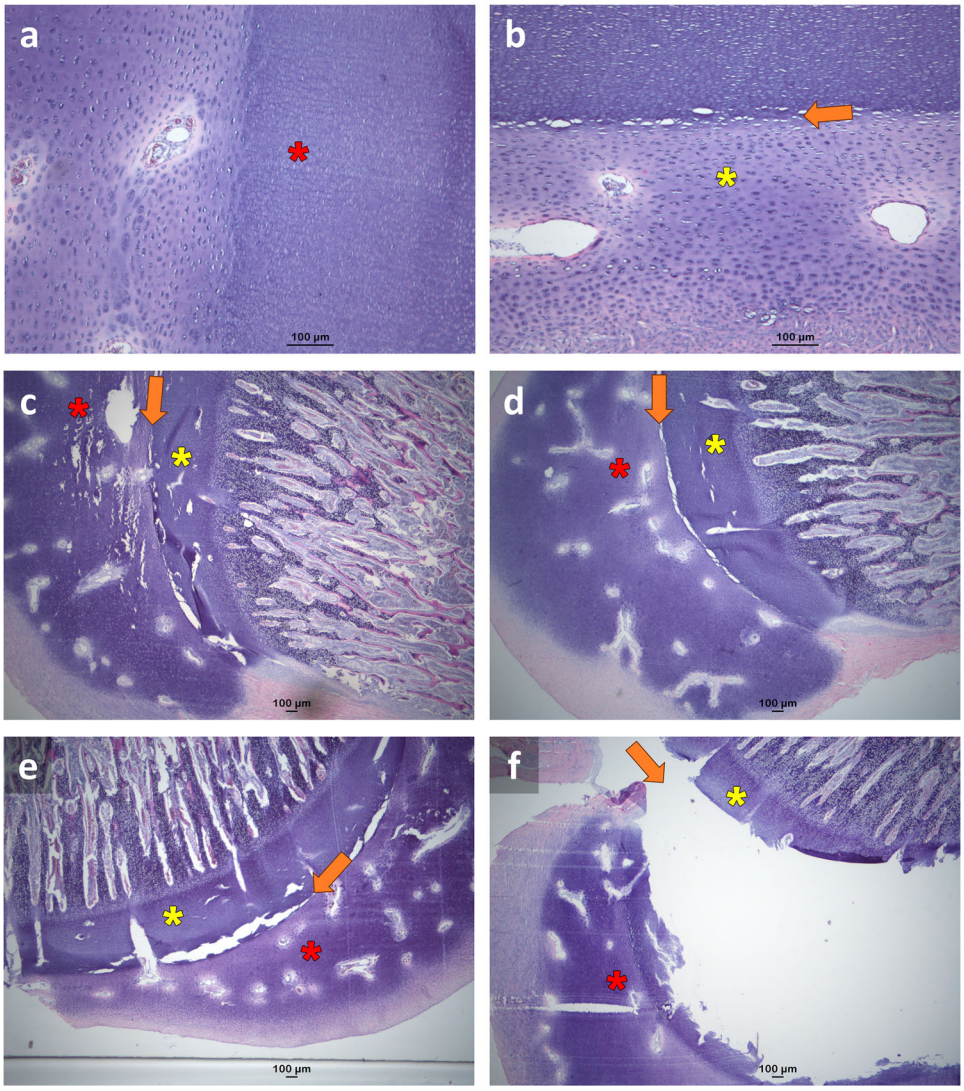

Figure 6. Degrees of separation between the articular cartilage and the growth cartilage in 22-day-old broilers. (a) No signs of separation (orange arrow) between the articular cartilage (red *) and the growth cartilage (yellow *). (b) Formation of small clefts between the articular and the growth cartilage. (c) Larger clefts between the articular and the growth cartilage. (d) Larger clefts between the articular and the growth cartilage over an extended area. (e) Partial separation between the articular and the growth cartilage. (f) Complete separation of the articular cartilage.

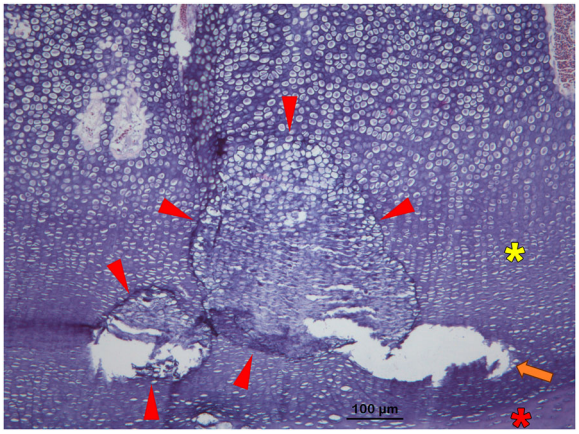

Figure 7. Necrotic areas in the growth cartilage of a 22-day-old bird. Red arrowheads indicate two areas of necrotic cells. The orange arrow shows a cleft between the articular cartilage (red *) and the growth cartilage (yellow *).

taxa found in the blood could also be found in the liver samples. However, not all bacteria found in the blood samples were found in liver samples, indicating that bacteria translocating through the intestinal barrier either did not pass through the liver, were not removed by the liver, or had a different origin.

Bacterial species most commonly associated with BCO lesions are *Enterococcus cecorum*, *Staphylococcus aureus*, and *Escherichia coli*, often in combination with other microorganisms (McNamee and Smyth, 2000; Wideman *et al.*, 2012; Ferver *et al.*, 2021). The retrospective study of Souillard *et al.* (2022) indicated that 77.9% of the locomotor diseases in France associated with *Enterococcus* spp. involved *E.* cecorum, many of which were associated with other bacteria such as *E. coli* and *Staphylococcus* spp. Jiang *et al.* (2015) analysed the structure and diversity of

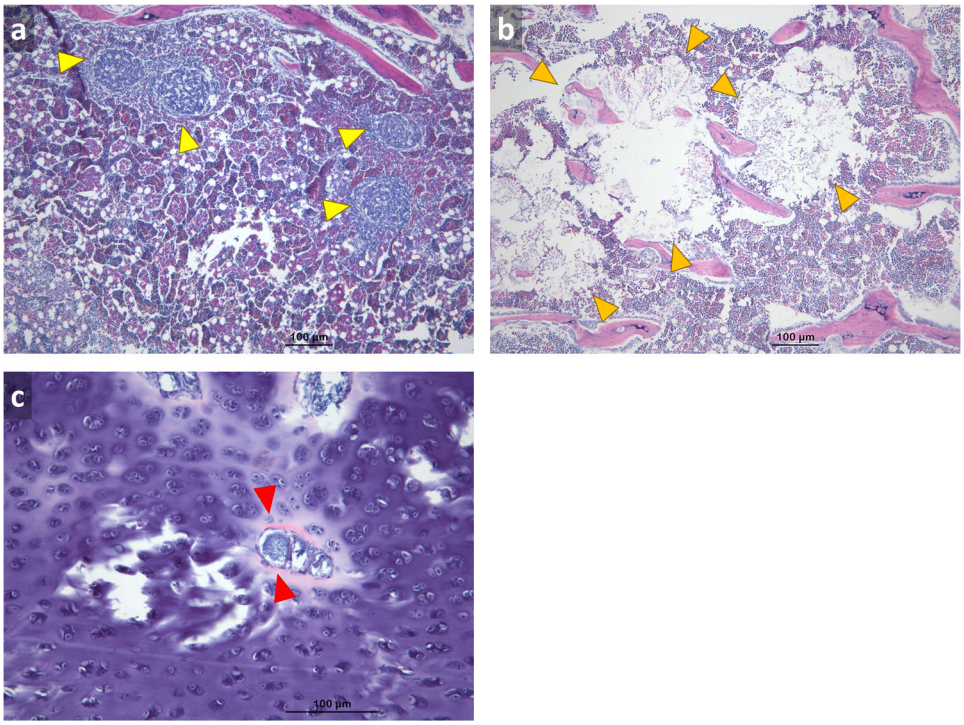

Figure 8. HE stained femur samples of 22-day-old broilers. (a) Lymphoid follicles in bone marrow (yellow arrowheads). (b) Cellular depletion in the bone marrow (orange arrowheads). (c) Fibrin in the blood vessels (red arrowheads).

microbial communities in the proximal femora and tibia from both clinically healthy broilers and from lame broilers with BCO lesions, through molecular profiling of 16S ribosomal RNA. They detected complex microbial communities in all samples, even in those that appeared to be macroscopically normal. Interestingly, they were able to demonstrate major differences in the microbial communities between microscopically normal bones and bones with BCO lesions. In the bones with BCO lesions, the genera *Staphylococcus, Enterobacter, and Serratia* were overrepresented. In the current study, *Escherichia/Shigella* and *Enterococcus* spp. were the most prevalent taxa in femoral samples. No *Enterobacter* spp. were isolated from femoral samples; however, *Enterobacter* spp. were found in some blood samples. It is important to note that the absence of culturable bacteria in the present study compared to the findings of Jiang *et al.* (2015) could be associated with the bacterial culture methodology, while the identification methodology in Jiang *et al.* (2015) bypassed bacterial culture. Interestingly, in none of the birds where FHS was observed macroscopically were any bacteria isolated from the femoral samples. These findings are in accordance with the results of Wilson *et al.* (2020), whose study was also based on bacterial culture methodology. They found that in most femoral head alterations (FHS, femoral head transitional changes, and femoral head necrosis) no bacteriological evidence of BCO was present. Even in the most severe lesion category (severe femoral head necrosis), only 33% of samples contained culturable bacteria.

The causes of translocation of the bacterial agents are not fully understood, but early post-hatch intestinal permeability or loss of intestinal epithelial integrity because of intestinal pathogens at the later age, could be causative. Also, factors that affect intestinal integrity, such as heat stress, can increase *E. cecorum* translocation in an infection model (Schreier *et al.*, 2022). While early post-hatch permeability is a normal physiological process to enable yolk antibodies to reach the bloodstream, intestinal integrity losses at later ages can be prevented in various ways, including dietary additives that support the intestinal microbiota or mucosal integrity (Alrubaye *et al.*, 2020).

In conclusion, this study shows that the femoral heads of young broilers have a higher prevalence of bacteria, mainly belonging to *Enterococcaceae* and *Enterobacteriaceae*. The results of bacterial isolates from blood and liver samples indicate the presence of bacteria in the blood and liver without an obvious clinical manifestation. Histopathological lesions indicating some level of FHS were seen in all 19-day-old and 22-day-old birds in this study, not associated with bacterial colonization.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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