IMMUNOLOGY SPECIAL SECTION

Bacterial chondronecrosis with osteomyelitis and lameness in broilers: a review

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ABSTRACT This review focuses on a specific cause of lameness known as bacterial chondronecrosis with osteomyelitis (**BCO**) in broilers. Rapid increases in body weight impose excessive torque and shear stress on structurally immature epiphyseal and physeal cartilage, primarily in the proximal femora, proximal tibiae, and flexible thoracic vertebrae. Excessive mechanical stress creates osteochondrotic clefts among the chondrocytes of susceptible growth plates. These wound sites are colonized by hematogenously distributed opportunistic bacteria, culminating in the gross abscesses and necrotic voids that are pathognomonic for terminal BCO. Lameness attributable to characteristic BCO lesions can be reproduced by rearing broilers on wire flooring to create persistent footing instability and physiological stress, without the need to inoculate the birds with pathogenic bacteria that presumably are present but quiescent within the bird's microbial communities or in the environment. Experiments using the wire-flooring model revealed innate differences in the susceptibility of broiler lines to BCO, and demonstrated that BCO incidences can be reduced by prophylactically providing probiotics in the feed, by prophylactically adding 25-hydroxy vitamin D_3 to the drinking water, or by therapeutically adding the antibiotic enrofloxacin to the drinking water. Hatchery and chick quality issues clearly influence the susceptibility of broilers to BCO. When broilers remain in a sitting posture for prolonged periods, the major arteries supplying their legs may be compressed. These episodes of inadequate blood flow may prevent chondrocyte maturation and trigger focal necrosis, thereby making the epiphyseal and physeal cartilage highly susceptible to osteochondrosis and BCO. Much remains to be revealed regarding the pathogenesis of BCO. Further revelations will be facilitated by the availability of the now-validated wire-flooring models that consistently trigger high incidences of BCO in experimental flocks.

Key words: broiler, lameness, osteomyelitis, osteochondrosis, vertebrae

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OVERVIEW

This review focuses on a specific cause of lameness known as bacterial chondronecrosis with osteomyelitis (**BCO**) in recognition that bacterial infection and necrosis typically develop in rapidly growing bones that are subjected to repeated mechanical stress, including the femora and tibiae (Figure 1), and the flexible thoracic vertebrae (Figure 2). The incidence of lameness attributable to BCO has increased noticeably during the past two decades, with recent reports suggesting that over 1% of all broilers grown to heavy processing weights may be affected after 5 wk of age (Smith, 1954; Carnaghan, 1966; Nairn and Watson, 1972; McCaskey et al., 1982; Kibenge et al., 1983; Mutalib et al., 1983a,b; Griffiths et al., 1984; Duff, 1990a; Pattison, 1992; Riddell, 1992; Thorp et al., 1993; Thorp, 1994; Thorp and Waddington, 1997; McNamee et al., 1998; Butterworth, 1999; McNamee and Smyth, 2000; Bradshaw et al., 2002; Dinev, 2009; Stalker et al., 2010; Wideman et al., 2012; Wideman and Pevzner, 2012; Wideman and Prisby, 2013). Turkey poults also develop typical BCO lesions in their femora, tibiae, and flexible thoracic vertebrae (Wise, 1971; Nairn, 1973; Julian, 1985; Wyers et al., 1991). This review addresses several objectives. Our current hypothesis regarding the pathogenesis of BCO is summarized, experimental models that successfully trigger BCO are reviewed, and strategies for successfully reducing the incidence of BCO are outlined. Finally, because inadequate blood flow (ischemia) likely contributes to the pathogenesis of BCO (Prisby et al., 2014), we evaluated the vascular anatomy of the leg bones to identify potential sites of arterial compression in broilers that remain sitting for prolonged intervals.

PATHOGENESIS

Rapid Growth and Lameness

Broilers weigh 40 g at hatch and grow to over 4 kg by 8 wk of age. This rate of body weight gain cannot

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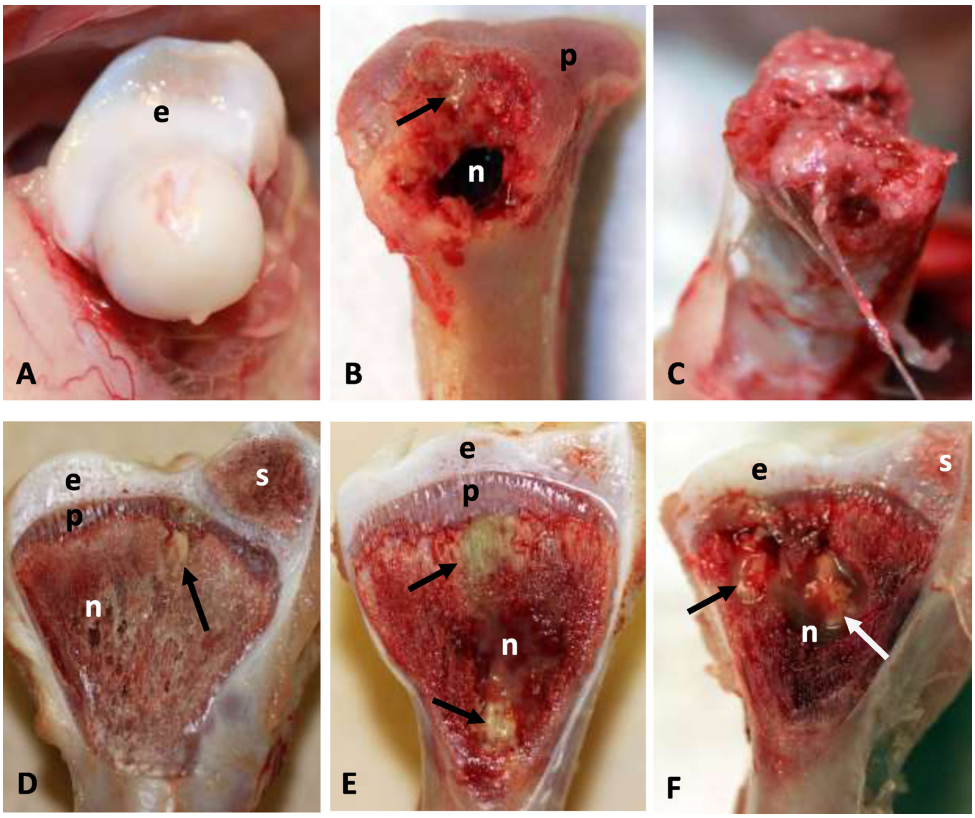

Figure 1. Stages of proximal femoral (A−C) or tibial (D−F) degeneration leading progressively to bacterial chondronecrosis with osteomyelitis: **A.** Normal proximal femoral head with white cap of epiphyseal cartilage (e); **B.** The femoral epiphysis remained in the acetabulum (epiphyseolysis) when the femur was disarticulated, revealing the underlying surface of the growth plate or physis (p), fibrinonecrotic exudate (arrow), and a large fracture revealing a necrotic void (n) in the metaphysis underlying the growth plate; **C.** The femoral epiphysis, physis and most of the metaphysis remained attached to the acetabulum when the diaphysis weakened by widely dispersed necrosis was fractured during disarticulation; **D.** Early proximal tibial BCO in which the physis (p) is irregular in width and has an underlying bacterial sequestrum (arrow), and small necrotic voids (n) in the metaphysis; secondary center of ossification (s); **E.** Tibial BCO showing caseous regions of bacterial infiltration (arrows), a large necrotic void (n) in the metaphysis, and a thickened (osteochondrotic) physis (p) above a metaphyseal zone of infection and vascular destruction; **F.** Gross infection of the metaphysis (arrows) surrounded by large necrotic voids (n) and severe disruption of the growth plate underlying the epiphysis (e).

be supported without equally dramatic increases in the size and structural integrity of the skeleton. Growth of the leg bones includes elongation accomplished via growth plates located at both ends of the shaft (diaphysis), as well as marked increases in the overall diameter attributable to highly dynamic remodeling of cortical bone (Wideman and Prisby, 2013). As demonstrated by Applegate and Lilburn (2002) the femora and tibiae increase approximately four-fold in length during the first 6 wk post-hatch, with mid-shaft diameters increasing three- to five-fold during the same interval. Similar estimates of rapid leg bone growth in broilers have been published by other investigators (Wise, 1970a; Riddell, 1975; Thorp, 1988d; Bond et al., 1991; Williams et al., 2000; Yalcin et al., 2001; Yair et al., 2012). The propensity for broilers to develop lameness when compared with egg laying strains was apparent more than 40 years ago and appears to be related to disproportions between the rate of body mass accretion versus the progress of skeletal maturation rather than to relative differences in skeletal morphometrics or a caudal-to-cranial redistribution of muscle mass and thus the center of gravity (Wise, 1970a; Williams et al., 2000). The highest incidences of lameness consistently occur in the fastest-growing broiler flocks. Management strategies that tend to reduce early growth rates also tend to reduce the incidence of skeletal disorders (Riddell, 1983; Havenstein et al., 1994; McNamee et al., 1999; Kestin et al., 2001; Bradshaw et al., 2002; Julian, 2005). These observations support a consensus hypothesis that in susceptible subsets of the population, the skeleton does not mature rapidly enough to support maximal rates of body mass accretion. However, absolute growth performance is not the sole trait that determines susceptibility; sequential body weights recorded prior to the onset of lameness clearly demonstrated it is not necessarily the fastest-growing individuals in a flock that ultimately succumb to BCO (Wideman et al., 2012).

Osteochondrosis of the Femora and Tibiae

Susceptibility of the femora and tibiae to BCO has been attributed to the presence of unusually long columns of chondrocytes extending from the proximal growth plate into the adjacent metaphysis. When

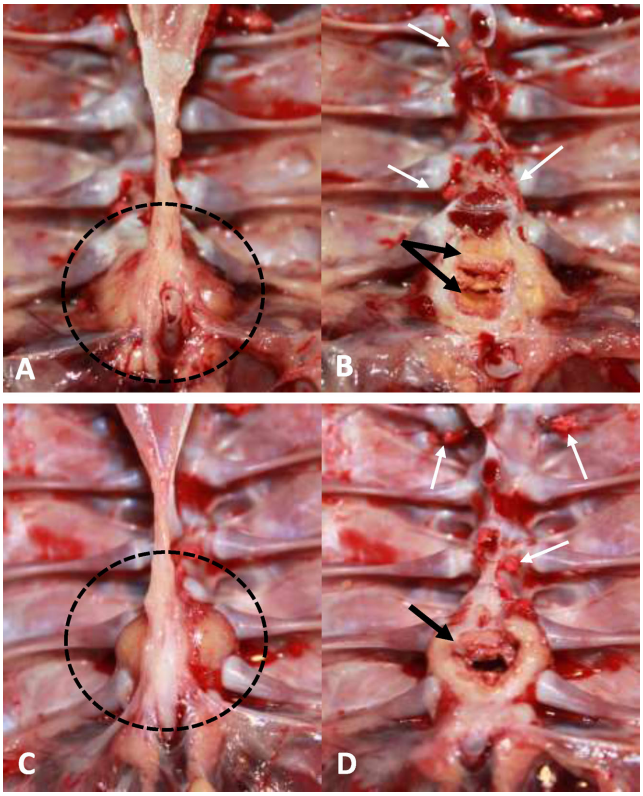

Figure 2. Two examples of vertebral BCO from 5 week old broilers (broiler 1 = panels A and B; broiler 2 = panels C and D) that exhibited spinal cord compression and paraplegic hock-resting posture due to grossly abscessed vertebral bodies. Externally the flexible thoracic vertebrae exhibited marked nodular swelling with a distinctive yellow discoloration of the translucent remnants of the vertebral bodies (panels A and C: dashed circles). Internally the abscessation involved at least two adjacent vertebrae (panels B and D; black arrows). Lung tissue was focally adherent to the intercostal spaces and to the abscessed vertebrae (white arrows).

compared with mammalian growth plates, the avian growth plate is much thicker and the chondrocyte columns are unevenly aligned. These differences have been attributed to high longitudinal growth rates associated with very rapid growth plate turnover times in birds (estimated at 21 h) when compared with rats (4 d) and humans (20 d). Indeed, the rate of avian leg bone elongation is positively correlated with thickness of the growth plate. The proximal ends of leg bones elongate more rapidly and have thicker growth plates than the distal ends of the same bone (Hales, 1727; Church and Johnson, 1964; Lutfi, 1970a; Kember and Kirkwood, 1987; Leach and Gay, 1987; Thorp, 1988c,d; Kirkwood et al., 1989a,b; Kember et al., 1990). Mechanical stress chronically exerted on the growth plates creates osteochondrotic clefts or microfractures between and within the cartilage layers (e.g., physeal osteochondrosis or osteochondrosis dissecans). Osteochondrotic clefts often transect local blood vessels, thereby causing focal ischemia and necrosis. Focal ischemia also has been attributed to sluggish blood flow and thrombosis caused by compression of the cartilage layers, the resting posture and inactivity of fully fed broilers, and an excessive resistance to flow through the long, narrow vascular channels that supply the growth plates (Wise, 1971; Riddell et al., 1983; Duff, 1984a–c, 1985, 1989a,b, 1990b; Julian, 1985; Duff and Randall, 1987; Thorp, 1988a–c, 1994; Thorp and Duff, 1988; Riddell, 1992; Thorp et al., 1993; Thorp and Waddington, 1997; Thorp, 1994; McNamee et al., 1998; Bradshaw et al., 2002; Dinev, 2009). Biomechanical stresses and impaired blood flow contribute to the pathogenesis of osteochondrosis in a variety of animal species (Trueta and Amato, 1960; Boss and Misselevich, 2003; Ytrehus et al., 2004a,b,c, 2007). Osteochondrosis dissecans has been observed in the leg bones and flexible thoracic vertebrae of apparently healthy broilers exhibiting no symptoms of infectious or traumatic lameness, suggesting that lameness is not necessarily caused by direct mechanical damage or osteochondrosis *per se* but rather by an ensuing bacterial infection (Wise, 1970b, 1973; Riddell et al., 1983; Duff, 1990b; McNamee et al., 1998; Wideman and Prisby, 2013; Wideman, 2014). Wise (1970b) noted that osteochondrotic clefts resemble the damage caused by feeding lathyrogenic compounds to poultry, with the caveat that in lathyrism the osteochondrotic clefts are widely distributed throughout the rapidly growing skeleton and are associated with generalized disruption and weakening of the cartilage matrix (Wise, 1970b; Barrow et al., 1974).

Bacterial Translocation and Hematogenous Distribution

As summarized in Figure 3, the pathogenesis leading to BCO appears to be initiated by mechanical microfracturing of the growth plate, followed by colonization of osteochondrotic clefts or voids by hematogenously distributed opportunistic bacteria. Bacteria transmitted to chicks from breeder parents, contaminated eggshells or hatchery sources (Skeeles, 1997; McCullagh et al., 1998; Rodgers et al., 1999; McNamee and Smyth, 2000; Stalker et al., 2010; Kense and Landman, 2011), or that enter the chick's circulation via translocation through the integument, respiratory system or gastrointestinal tract (Mutalib et al., 1983a,b; Andreasen et al., 1993; Thorp et al., 1993; McNamee et al., 1999; Martin et al., 2011) spread hematogenously and can reach both sides of the growth plate via numerous terminal epiphyseal and physeal vascular plexuses (Figure 4). These plexuses are formed when the central arteriole within a cartilage canal divides into a tuft of capillary loops that make hairpin bends and reconverge as one or more venules carrying blood out of the canal. The capillaries have a discontinuous or fenestrated endothelium, with openings large enough to permit cellular elements in the blood to pass into the cartilaginous matrix (Beaumont, 1967; Lutfi, 1970b; Hunt et al., 1979; Howlett, 1980; Howlett et al., 1984; Emslie and Nade, 1983, 1985). Hematogenously distributed bacteria possessing the specific ability to bind to exposed bone collagen appear in some cases to be more virulent

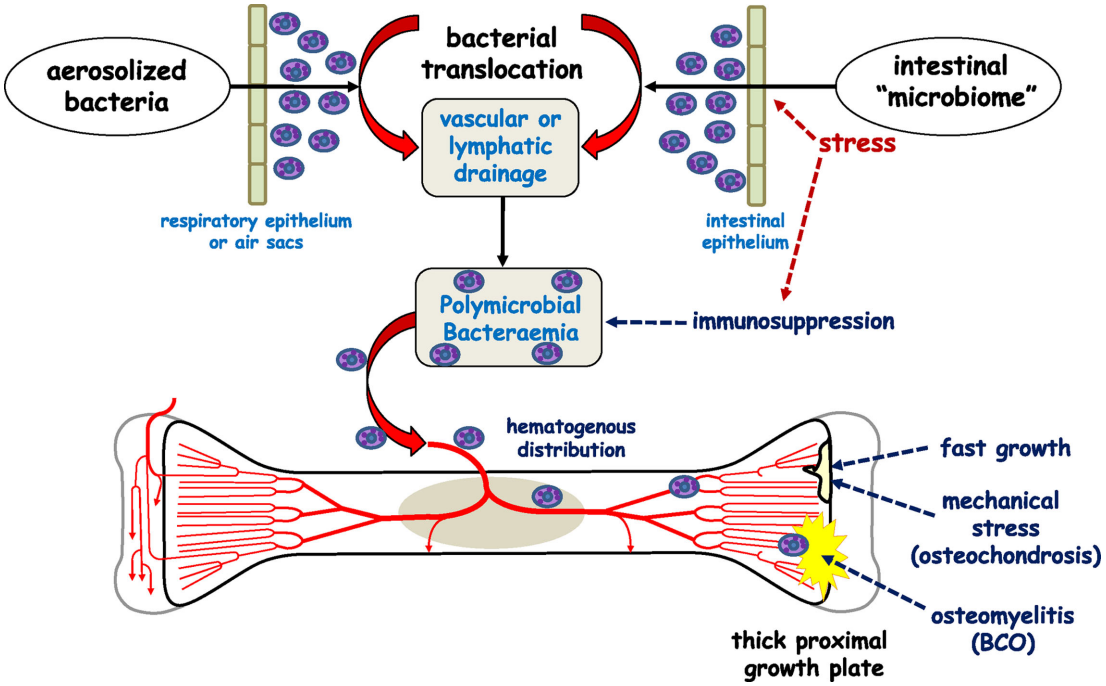

Figure 3. Fast growth is associated with thick growth plates containing poorly mineralized layers and columns of chondrocytes that are susceptible to the formation of microfractures (e.g., physeal osteochondrosis or osteochondrosis dissecans) in response to mechanical stress. Bacteria transmitted to chicks from breeder parents, contaminated eggshells or hatchery sources, or that enter the circulation via translocation through the integument, respiratory system or gastrointestinal tract, can spread hematogenously to colonize the osteochondrotic clefts. Translocated opportunistic pathogenic bacteria form bacterial foci and sequestrae within the growth plate and adjacent metaphysis, where they are notoriously inaccessible to antibiotics and cellular components of the immune system. Stress suppresses the immune system and facilitates bacterial translocation across the intestinal epithelium.

in their capacity to trigger osteomyelitis (Kibenge et al., 1983; Smeltzer and Gillaspy, 2000). The translocated bacteria can form obstructive bacterial emboli in the epiphyseal and metaphyseal vascular plexuses, adhere directly to the exposed cartilage matrix, and colonize osteochondrotic clefts and zones of necrosis. Bacterial foci and sequestrae within infected bone tissue are notoriously inaccessible to antibiotics and cellular components of the immune system (Emslie and Nade, 1983; Emslie et al., 1983, 1984; Kibenge et al., 1983; Speers and Nade, 1985; Alderson et al., 1986a,b; Alderson and Nade, 1987; Thorp, 1988b; Thorp et al., 1993; McNamee et al., 1998, 1999; McNamee and Smyth, 2000; Smeltzer and Gillaspy, 2000; Kense and Landman, 2011; Martin et al., 2011).

Terminal BCO and Clinical Lameness

Bacterial sequestrae rapidly expand into focal zones of necrosis or large fibrinonecrotic abscesses in the metaphysis of infected bones (Figure 1) (Emslie et al., 1983, 1984; Emslie and Nade, 1983; Alderson et al., 1986b; Daum et al., 1990; Thorp et al., 1993; Skeeles, 1997; Wideman et al., 2012). Lytic substances released at sites of bacterial colonization promote generalized necrosis within the calcifying zone of the metaphysis, destroying the vasculature and eliminating struts of metaphyseal trabecular bone that normally provide structural support to the growth plates (Figures 1 and 4) (Emslie and Nade, 1983; Emslie et al., 1984; Wyers et al., 1991; Wideman et al., 2012; Wideman and Prisby, 2013). Bacteria penetrating the epiphysis, perhaps via transphyseal vessels or perhaps directly via epiphyseal vascular complexes, trigger septic arthritis (Emslie et al., 1984; Emslie and Nade, 1985; Alderson et al., 1986b; Alderson and Nade, 1987; Thorp, 1988a; McNamee et al., 1998; Daum et al., 1990; Joiner et al., 2005). Terminal BCO presents as necrotic degeneration and bacterial infection primarily within the proximal ends of the femora and tibiae, as well as in the growth plates of the flexible thoracic vertebrae. High incidences of femoral, tibial and vertebral BCO lesions have been observed in lame broilers from commercial flocks. The distal ends of the femora and tibiae are affected less frequently (Emslie et al., 1983; Thorp and Waddington, 1997; McNamee et al., 1999). It is not unusual for BCO to affect only one leg while the contralateral leg appears macroscopically normal (McNamee et al., 1998; Dinev, 2009; Wideman, 2014). Femoral, tibial and vertebral BCO lesions usually occur together in commercial flocks, although in some outbreaks femoral and tibial lesions may be more common than vertebral lesions, whereas the opposite may be true in other outbreaks (Stalker et al., 2010; Kense and Landman, 2011; Wideman, personal observations). Multiple opportunistic organisms have been isolated from BCO lesions, including *Staphylococcus aureus*, *Staphylococcus* spp., *Escherichia coli*, and *Enterococcus cecorum*,

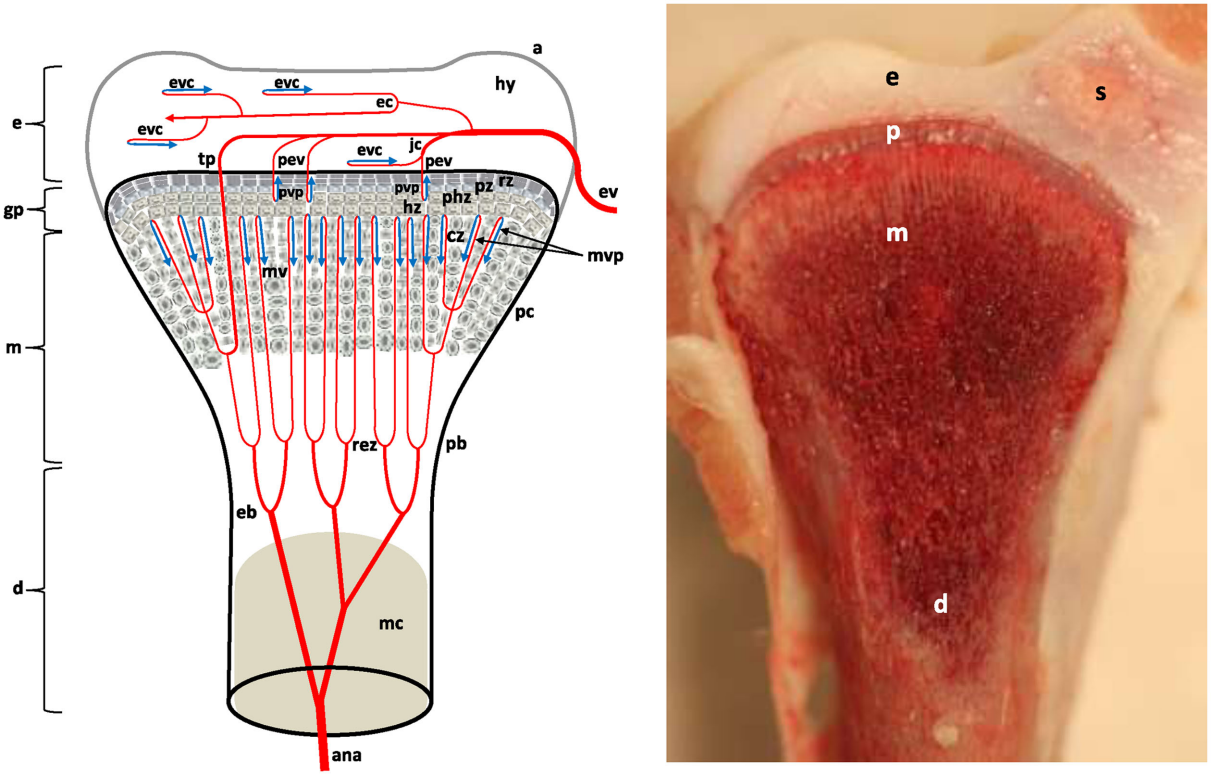

Figure 4. Diagram (left panel) and photograph (right panel) of the normal proximal tibial head from a clinically healthy broiler. The epiphyseal vascular supply (ev) courses through epiphyseal vascular canals (ec) within the hyaline zone (hy) of the epiphysis (e), or through junctional canals (jc) directed toward the growth plate or physis (p). Branches of the ev terminate as epiphyseal vascular capillary complexes (evc) within the hyaline zone, or become penetrating epiphyseal vessels (pev) that terminate as a penetrating vascular capillary plexus (pvp) supplying the resting zone (rz), the highly mitotic proliferating zone (pz) and prehypertrophic zone (phz, also known as the maturing zone) of the p. The ascending or proximal branch of the nutrient artery (ana) divides repeatedly inside the diaphysis (d) to form metaphyseal vessels (mv) within the metaphysis (m). The mv terminate as metaphyseal vascular capillary plexuses (mvp) supplying the calcifying zone (cz, also known as the degenerating hypertrophic zone) of the metaphysis. The hypertrophic zone (hz) normally is not penetrated by the pvp or mvp, but may rarely be penetrated by transphyseal vessels (tp). The pvp and mvp are formed by hairpin bends in fenestrated capillaries that return as venules coursing through the same canal (blue arrows). The metaphysis consists of calcifying (degenerating, apoptotic) chondrocytes and the newly formed osteoid of the calcifying zone (cz), spicules of trabecular bone that provide a support scaffolding for the growth plate, and the resorption zone (rez) in which the trabecular bone thins and ultimately is resorbed to form the medullary cavity (mc) of the diaphysis (d) Additional features include: (a) articular zone of epiphyseal cartilage; (eb) endosteal bone; (mc) medullary cavity; (pb) periosteal bone; (rez) resorption zone of the metaphysis; and, (s) secondary center of ossification. Adapted from Wideman and Prisby (2013), and Wideman (2014). Micro-anatomical terminology is consistent with the established anatomical nomenclature for avian bones (Beaumont, 1967; Lutfi, 1970a,1970b; Wise and Jennings, 1973; Howlett, 1979, 1980; Hunt et al., 1979; Duff, 1984a; Thorp, 1986, 1988a; Howlett et al., 1984; Ali, 1992; Farquharson and Jefferies, 2000).

often in mixed cultures with other bacteria including *Salmonella* spp. (Smith, 1954; Nairn and Watson, 1972; Andreasen et al., 1993; Tate et al., 1993; Thorp et al., 1993; McNamee et al., 1998; Butterworth, 1999; Smeltzer and Gillaspy, 2000; Joiner et al., 2005; Dinev, 2009; Stalker et al., 2010; Kense and Landman, 2011; Martin et al., 2011). Recently Jiang et al. (2015) used molecular profiling of 16S ribosomal RNA (**16S rRNA**) gene sequences to comprehensively analyze the structure and diversity of microbial communities in the proximal femora and tibiae from clinically healthy broilers and from lame broilers with obvious BCO lesions. Complex microbial communities were detected in all samples, including bones that appeared to be macroscopically normal. Further analyses demonstrated major differences in the microbial communities on different bones (femur versus tibia) and different lesion categories (macroscopically normal versus gross BCO lesions). The genus *Staphylococcus* was overrepresented in bones with BCO lesions, along with the genera *Enterobacter* and *Serrotia*. These results indicate that complex microbial communities exist in the growth plates and metaphyses of macroscopically normal proximal femora and tibiae, and that BCO lesions are associated with a less diverse community with overrepresentation by potentially pathogenic genera (Jiang et al., 2015). The likelihood exists that numerous bacterial species routinely translocate into the blood stream and are sequestered in the growth plates, where they form a complex microbial community. The obvious source of these translocating bacteria is likely to be the intestinal microbiome (Jiang et al., 2015), although complex microbial communities also are present within unabsorbed yolks, the conducting airways, and the lower respiratory tract of clinically healthy poultry (Cox et al., 2006; Shabbir et al., 2015; Sohail et al., 2015).

Vertebral Osteochondrosis and Spondylolisthesis (Kinky Back)

Modern veterinary anatomical terminology defines the first thoracic vertebra in birds as the cranial-most vertebra having a complete rib that articulates directly or indirectly with the sternum (Baumel and Witmer, 1993). Accordingly, only five thoracic vertebrae are formally recognized in fowl, and the freely movable thoracic vertebra separating the notarium and synsacrum is correctly identified as the fourth thoracic vertebra or **T4**. The cranial surface of the third thoracic vertebra fuses with the notarium whereas the caudal surface of the fifth thoracic vertebra fuses with the synsacrum (Duff, 1990a; Baumel and Witmer, 1993). Complete fusion is delayed until sexual maturity, presumably to permit ongoing longitudinal growth of the vertebral body in juvenile birds. Therefore, the third and fifth thoracic vertebrae retain partial mobility in immature broilers, and the epiphyses and growth plates of these vertebrae, along with those of the T4 vertebra, are subjected to repeated mechanical torsional and shear stresses (Duff, 1990a). These flexible thoracic vertebrae serve as the articulating fulcrum between the notarium supporting the cranial half of the bird and the synsacrum supporting the caudal half. Indeed, fast growing broilers have long been recognized for the susceptibility of their thoracic vertebrae to osteochondrosis (Figure 5) as well as to subclinical or clinical non-infectious non-inflammatory mechanical collapse, subluxation (downward rotation), or lateral displacement (scoliosis) of the T4 vertebral body. The clinical manifestation of T4 subluxation is known technically as spondylolisthesis (spine slippage) or as "kinky back," in which spinal cord compression leads to paraplegia, a hock- or tail-sitting posture, and an inability to stand (Osbaldiston and Wise, 1967; Wise, 1970b, 1973; Riddell and Howell, 1972; Riddell, 1973; Duff, 1990a). The peak onset of clinical spondylolisthesis typically occurs between 2 and 4 wk of age (Wise, 1970b, Riddell, 1973). Examinations of apparently normal broilers routinely reveal osteochondrotic clefts and subclinical deformations or "kinks" in the

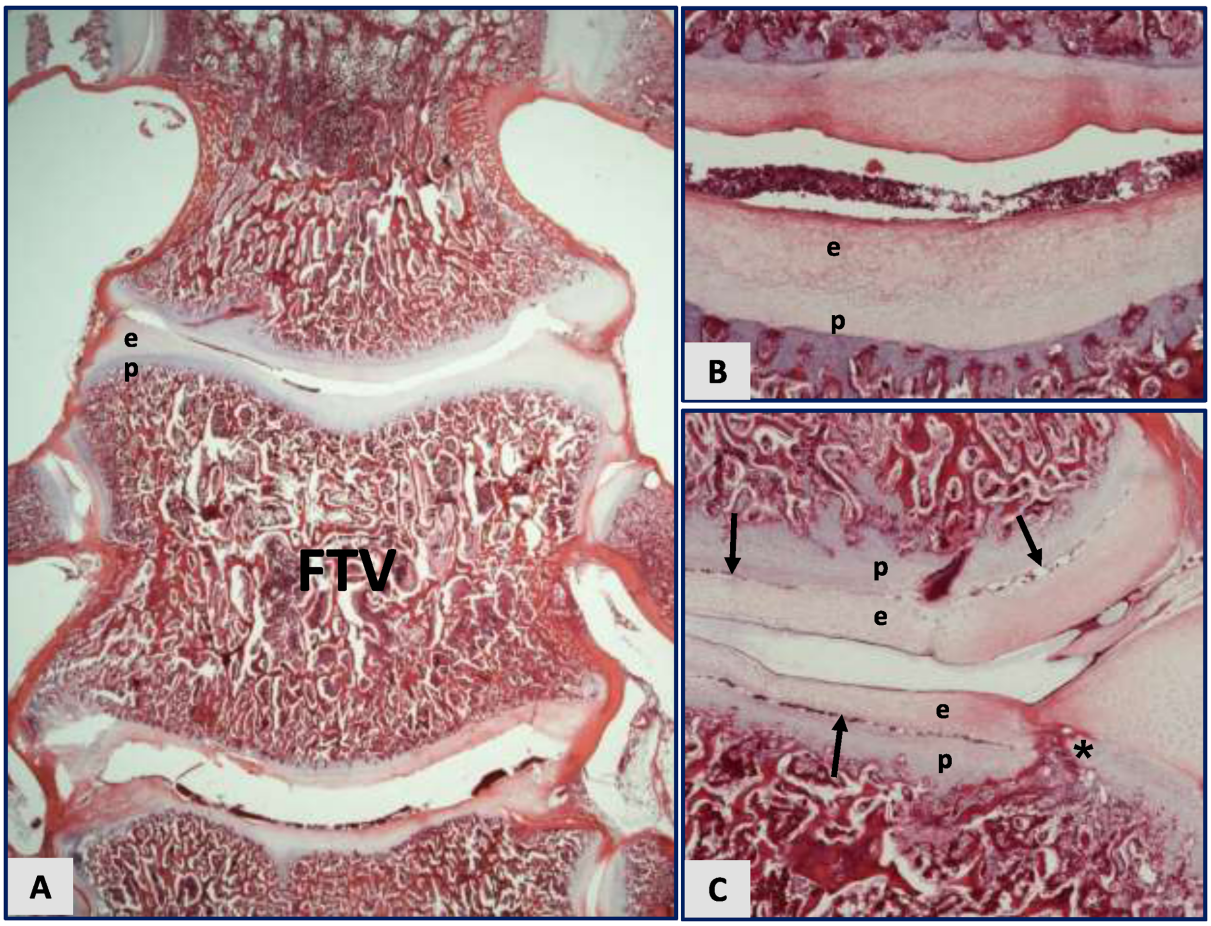

Figure 5. Broilers exhibiting no clinical symptoms of lameness were euthanized at 5 wk of age, and the thoracic vertebrae and synsacrum were fixed by immersion in 10% buffered formalin. After fixation the thoracic vertebrae and synsacrum were decalcified, embedding in paraffin, sectioned at 5 μM thickness, and stained with hematoxylin and eosin. (**1A**) Flexible thoracic vertebrae (**FTV**) have a thin growth plate or physis (p) underlying the articular and hyaline cartilage of the epiphysis (e) at the cranial and caudal ends of the vertebral body. (**1B**) The boundary between the epiphysis (e) and physis (p) normally should be seamless. (**1C**) In broilers that appear to be clinically healthy narrow osteochondrotic clefts or voids (arrows) containing cellular debris can be detected at the boundary between the epiphysis (e) and the physis (p). Osteochondrotic clefts may interrupt the local vasculature, cause distortions in the epiphyseal-physeal cartilage (*), and constitute wound sites that are preferentially colonized by opportunistic bacteria (similar to Figure 6 in McCaskey et al., 1982).

thoracic vertebrae. For example, Wise (1970b) observed osteochondrotic clefts and kinks in the thoracic vertebrae in 15% of the clinically normal broilers examined, with only 2% exhibiting clinical signs of spondylolisthesis. McCaskey et al. (1982) examined 200 fast-growing broilers and reported that nearly half exhibited evidence of minor microfractures or clefts in the epiphyseal and physeal cartilage layers of the thoracic vertebrae, and ten exhibited major epiphyseal or physeal osteochondrotic lesions often associated with focal necrosis of the adjacent cartilage. Twenty-six broilers also exhibited subclinical spondylolisthesis of the flexible thoracic vertebrae (McCaskey et al., 1982). Duff (1990a) evaluated the thoracic vertebrae of 40 broilers with clinical symptoms of spondylolisthesis and 20 clinically normal flock mates. Eight of the symptomatic birds had bacterial infections (gram positive cocci) of the thoracic vertebral bodies, articular surfaces or synovial spaces (e.g., vertebral osteomyelitis or BCO). The remaining symptomatic birds exhibited noninfective subluxation or lateral displacement of the T4 vertebra and a high incidence of physeal osteochondrosis. Osteochondrotic lesions also were commonly observed in the physeal plates of the thoracic vertebrae from clinically normal broilers (Duff, 1990a). Riddell (1973) collected 18 commercial broilers with clinical symptoms of spondylolisthesis and verified radiographically the deformation of their thoracic vertebrae. Eleven of these birds were nursed back to health, and by the age of sexual maturity they were able to walk normally, although their spinal column still exhibited deformation. These survivors were mated and after two generations of selection, the resulting susceptible line produced progeny exhibited a higher incidence of spondylolisthesis than was observed in chicks from a control population (13% vs. 0%, respectively) (Riddell, 1973). A similarly high genetic contribution to spondylolisthesis susceptibility was demonstrated by mating commercial broiler breeders that were purposefully selected for vertebral column abnormalities (Khan et al., 1977). The cumulative evidence therefore indicates that the thoracic vertebrae of rapidly growing broilers are subjected to mechanical stresses that can cause severe misalignments of the vertebral bodies and high incidences of osteochondrotic microfractures within the epiphyseal-physeal cartilages. Vertebral misalignments can remain subclinical or may lead to clinical spondylolisthesis that appears to be non-infectious and highly heritable. Osteochondrotic lesions of the vertebrae are a common non-infective non-inflammatory skeletal disorder that typically persist subclinically and have not, *per se*, been considered a primary cause of lameness in broilers (McCaskey et al., 1982; Duff, 1990a; Thorp, 1994; Martin et al., 2011). However, osteochondrotic clefts and associated regions of focal necrosis clearly constitute convenient wound sites with exposed collagen matrix that facilitates colonization by opportunistic bacterial pathogens, thereby contributing to the onset of osteomyelitis (Carnaghan, 1966; Wise, 1971; McNamee et al., 1998; Wideman and Prisby, 2013).

Vertebral BCO

Vertebral BCO also is known as vertebral osteomyelitis, spondylitis, spondylopathy, and more recently albeit incorrectly as "kinky back." Although the latter term currently is in common usage to describe paraplegic broilers exhibiting a hock-sitting posture due to bacterial infection and necrotic degeneration of the thoracic vertebrae, caution must be taken to differentiate the noninfectious (e.g., spondylolisthesis) from the infectious (e.g., vertebral BCO, spondylitis) pathogenesis for the "kinky back" symptomology. Furthermore, some broilers exhibiting the typical "kinky back" posture have severe bilateral femoral BCO with no macroscopic pathology of their flexible thoracic vertebrae (Wideman et al., 2014). The pathogenesis for vertebral BCO is presumed to be similar to that summarized for the leg bones in Figure 3, including the typical pathology shown in Figures 2 and 5. Mechanical stress creates microfractures in the epiphyseal and physeal cartilage of the flexible thoracic vertebrae, and these osteochondrotic clefts subsequently are susceptible to colonization by hematogenously distributed opportunistic bacteria. Aerosolized pathogens also potentially may gain access to these vertebrae via a diverticulum from the abdominal air sac (Nickel et al., 1977). Various bacterial species have been associated with vertebral BCO lesions. Carnaghan (1966) isolated *Staphylococcus pyogenes* from vertebral BCO abscesses in commercial fowl exhibiting hock-resting posture, and recreated spondylitis by inoculating the staphylococcal isolate intravenously into experimental groups of broilers. The resulting infection appeared to be specific for the flexible thoracic vertebrae, histopathology revealed aggregates of gram positive cocci in the caseous vertebral abscesses, and staphylococci were isolated from the affected vertebrae (Carnaghan, 1966). Wise (1971) isolated *S. aureus* and *Staphylococcus albus* from vertebral BCO abscesses in a turkey and a broiler, respectively. When these isolates were inoculated intravenously into broilers, only the *S. albus* isolate triggered vertebral BCO lesions, spinal cord compression and paraplegic symptoms. No other bone or joint abnormalities were observed. Histological sections revealed the presence of osteochondrotic clefts in the thoracic vertebrae that provided obvious sites for colonization by the *S. albus* isolate (Wise, 1971). Kibenge et al. (1983) and Griffiths et al. (1984) isolated several strains of *S. aureus* from lame commercial broilers. When inoculated intravenously into healthy broilers these isolates exhibited a broad tropism for rapidly growing bone and tendons, and triggered persistent bacteremia and vertebral BCO as well as proximal femoral and tibial BCO (Kibenge et al., 1983; Griffiths et al., 1984). During recent outbreaks of lameness in broiler and broiler breeder flocks in the European Union and North America, *Enterococcus cecorum* was implicated as the pathogen responsible for causing arthritis, tenosynovitis, and femoral and vertebral BCO (Stalker et al., 2010;

Kense and Landman, 2011; Martin et al., 2011; Wijetunge et al., 2012). Necropsies of lame broilers exhibiting a hock sitting posture revealed nodular swelling and abscessation of the T4 vertebral body with osteomyelitis and compression of the spinal cord. Lung tissue was focally adherent to the abscessed vertebrae. *E. coli* and gram-positive cocci were isolated from the spinal abscesses, and the cocci were identified by 16S rRNA sequencing as *Enterococcus cecorum* (Stalker et al., 2010). Martin et al. (2011) used *Enterococcus cecorum* isolated from a commercial outbreak of vertebral BCO to inoculate male broiler breeder chicks via the air sac route, the intravenous route, and the oral route. Oral transmission of *E. cecorum* was reported by these investigators to be the most productive method for reproducing the characteristic gross and microscopic lesions of vertebral BCO (Martin et al., 2011). Orally administered pathogenic bacteria clearly do readily cross the intestinal barrier and travel hematogenously to the vertebral growth plates to trigger osteomyelitis.

EXPERIMENTAL MODELS FOR TRIGGERING BCO

Models of Pathogen Exposure

The etiology, pathogenesis, and treatment strategies for BCO have been difficult to investigate because the incidence can be low or nonexistent in research flocks. Experimental models for triggering BCO are needed to confirm key triggering mechanisms and stressors, reveal innate limitations or susceptibilities, and provide a reliable test bed for evaluating practical prophylactic and therapeutic strategies. Attempts to initiate BCO via a respiratory challenge have been marginally successful (Devriese et al., 1972; Nicoll and Jensen, 1986; Jensen et al., 1987; Mutalib et al., 1983b; McNamee et al., 1999; Martin et al., 2011), whereas BCO has repeatedly been reproduced by injecting broilers and turkeys intravenously with appropriate strains of *Staphylococcus* spp. in quantities sufficient to cause sustained bacteremia. The artificially induced bacteremia resembles naturally occurring sepsis. Terminal metaphyseal and epiphyseal blood vessels rapidly become occluded by bacterial emboli and phagocytic cells, leading to focal necrosis and bacterial colonization of the ischemic cartilage. Intravenous pathogen injections typically trigger high incidences of lameness attributable to synovitis and BCO within 24 to 72 hours post-injection (Smith, 1954; Carnaghan, 1966; Wise, 1971; Nairn, 1973; Emslie and Nade, 1983, 1985; Emslie et al., 1983; Kibenge et al., 1983; Mutalib et al., 1983a; Griffiths et al., 1984; Alderson et al., 1986b; Daum et al., 1990; Martin et al., 2011). Smith (1954) induced bacteremia and lameness by injecting *Staphylococcus* spp. intravenously but none of the strains produced any effect when injected intraperitoneally or subcutaneously. Alderson and Nade (1987) recreated septic arthritis and localized osteomyelitis by injecting the hock joints of 4 week old broilers with a strain of *S. aureus* isolated from lame chickens. As summarized above, Martin et al. (2011) indicated that the oral route of inoculation was superior to air sac or intravenous inoculation for recreating *Enterococcus cecorum* BCO in broilers. Al-Rubaye et al. (2014) isolated *Staphylococcus agnetis* from bone and blood samples of broilers with clinical BCO. Administering this isolate in the drinking water to broilers reared on wire flooring (*vide infra*) increased the incidence of BCO three-fold when compared with BCO incidences in broilers that also were reared on wire flooring but only received tap water to drink (Al-Rubaye et al., 2014).

Mechanical Models

We developed mechanical models for reliably triggering BCO without the need to purposefully inoculate the birds with known pathogens. Our assumption is that the offending pathogens already are present but quiescent within the bird or in the environment, waiting for an effective triggering scenario (e.g., stress, immunosuppression) and attainment of sufficient body mass to create a conducive wound site (e.g., osteochondrotic clefts, focal necrosis). Pathogenic bacteria may be vertically transmitted from breeder hens, introduced in the hatchery, or transmitted as an aerosol or via the drinking water or feed, and then harbored subclinically at sites that have yet to be identified (e.g., intestinal, respiratory or physeal-metaphyseal microbial communities) until appropriate predisposing conditions converge (Nairn, 1973; Vela et al., 2012). Our mechanical models were designed to create sustained footing instability and thereby persistently exert excessive mechanical stresses on susceptible leg and vertebral joints. Based on our current understanding, these amplified forces cause micro-trauma and osteochondrosis of the epiphyseal-physeal cartilage, accompanied by mechanical truncation or thrombotic occlusion of metaphyseal blood vessels, followed by hematogenous bacterial colonization. For example, ramps can be placed under nipple waterers to force broilers to walk up a slope to drink. The incidence of lameness induced by a 20% slope did not differ from the spontaneous occurrence of lameness on litter alone (6%), whereas a 30% slope induced significantly higher incidence of lameness (27%). Clearly the steeper slope triggered greater footing insecurity and mechanical stress, presumably leading to amplified micro-trauma to the broilers' proximal leg joints (Wideman, 2014). We also developed portable obstacles known as "speed bumps" that consistently trigger moderate incidences of BCO in broilers (Gilley et al., 2014). The speed bumps are constructed in the shape of an isosceles triangular prism, and the sides have slopes ranging from 33% to 66%. Speed bumps are designed to be installed between feeders and waterers in litter flooring facilities, thereby forcing the birds to climb up and then down the slopes as they move back and forth to eat and drink. Speed bumps trigger 3-fold higher

incidences of BCO when compared with broilers reared on litter flooring without speed bumps (Gilley et al., 2014).

In the majority of our experiments the entire surface of the pen floor was covered with flat wire panels, thereby denying the birds access to litter and subjecting them to chronic footing instability as well as to behavioral stress (*vide infra*). Incidences of lameness between 20% and 60% are reliably induced by this challenge, depending on the genetic susceptibility and hatchery source of the broilers being evaluated (Wideman et al., 2012, 2013, 2014, 2015a,b; Gilley et al., 2014; Prisby et al., 2014; Jiang et al., 2015). This model consistently triggers pathognomonic BCO lesion progression, primarily within the proximal femora and proximal tibiae. Vertebral BCO was rarely observed until a recent study in which the chicks and feed were obtained from commercial sources. In that experiment 80% of the lameness was attributed to proximal femoral and tibial BCO and 20% of the lameness was attributed to typical vertebral BCO lesions (Figures 2 and 5). Most of the lameness triggered by wire flooring develops after 5 wk of age, as has been reported for field outbreaks of BCO (McNamee et al., 1998; Dinev, 2009). Females tend to be marginally less susceptible to lameness than males when both genders are reared together (Wideman et al., 2014). Lameness progresses very rapidly in broilers that appeared to be healthy during the preceding 24 to 48 h, as previously reported by Joiner et al. (2005). Broilers tend to exhibit relatively mild BCO lesion when they are euthanized at the earliest onset of clinical symptoms (hesitancy to stand, eagerness to sit, slight wing-tip dipping), whereas birds permitted to live until they become fully immobilized (unable to eat or drink) exhibit much more severe lesions. It also is apparent from necropsying survivors of a wire flooring experiment that severe lesions may occasionally be present in very large, apparently robust individuals that exhibit no signs of lameness or leg weakness (Wideman et al., 2012, 2014). We speculate that broilers may purposefully avoid exhibiting overt symptoms of lameness in order to avoid being victimized by the predatory behavior of their flock mates. Indeed, gait scoring does not accurately predict obvious skeletal pathologies or abnormalities detectable at postmortem examination (Naas et al., 2009; Sandilands et al., 2011; Wideman, unpublished observations). The pathogenesis of BCO cannot be instantaneous and therefore apparently healthy broilers often possess subclinical lesions primarily consisting of the earliest macroscopic BCO lesion pathology. Subclinical lesions are equally likely to develop in males and females, in left and right legs, and the status of the proximal femur does not determine the status of the ipsilateral or contralateral proximal tibia and *vice versa* (Wideman et al., 2012, 2013, 2014, 2015a,b; Gilley et al., 2014). These observations are consistent with the interpretation that subclinical mechanical damage to one or more proximal leg bones need not trigger overt lameness until the damaged area becomes infected. The resulting bacterial proliferation, immunological assault by responding phagocytes (macrophages and heterophils), and widespread lysis and necrosis of the metaphyseal trabecular bone and vasculature then culminate in intolerable discomfort and terminal lameness (Howlett, 1980; Duff, 1984b; Thorp et al., 1993; Wideman and Prisby, 2013).

BCO has been proposed to develop as an "ascending" infection that somehow is transmitted from a leg or foot lesion through the tissues (presumably via lymph ducts or the ascending veins) to the ipsilateral proximal tibial and femoral joints. Indeed, Alderson and Nade (1987) triggered septic arthritis and bacterial colonization of the localized epiphyseal and physeal vasculature by injecting *S. aureus* directly into broilers' hock joints. However, the presumed anatomical basis or route for a bacterial infection to "ascend" directly from dermal lesions to the proximal growth plates remains specious. If this ascending route of infection is to be considered plausible, then infection of one joint should predispose toward infection of the other joints in the same (ipsilateral) leg. Accordingly, the tibial and femoral diagnostic data for all birds developing lameness on wire flooring were pooled as the basis for a meta-analysis of ipsilateral leg health. When the proximal femur appeared to be normal nevertheless relatively few ipsilateral tibiae remained normal. Similarly, when the proximal tibia appeared normal, nevertheless many of the ipsilateral proximal femora developed lesions. This meta-analysis failed to support the "ascending infection" hypothesis with regard to the pathogenesis of BCO (Wideman, 2014). Evaluations of foot pad health in clinically lame broilers across multiple experiments revealed no consistent or predictive relationship between the presence of foot pad dermatitis and BCO. Many broilers reared on floor litter develop foot pad dermatitis but do not exhibit clinical lameness. Perhaps immunosuppression predisposes broiler flocks to the independent onset of both foot pad dermatitis and BCO in stressed flocks. Dermal lesions and omphalitis can lead to generalized sepsis and thus secondarily to broadly distributed BCO. For example, infected toes that were trimmed after hatching provided a portal of entry for *S. aureus* to initiate bilateral osteomyelitis and synovitis in the femora and tibiae of turkey poults (Alfonso and Barnes, 2006).

Stress-Mediated Immunosuppression Models

Immunosuppression caused by chicken anemia virus, infectious bursal disease virus, or environmental stressors can facilitate microbial proliferation. Immunosuppression has been implicated in the etiology of spontaneous BCO outbreaks in commercial poultry flocks (Mutalib et al., 1983a,b; Andreasen et al., 1993; Butterworth, 1999; McNamee et al., 1998, 1999; Huff et al., 2000; McNamee and Smyth, 2000).

Environmental stressors and immunosuppression contribute to the eruption of opportunistic pathogens harbored subclinically in the proximal tibial joints of turkeys that develop turkey osteomyelitis complex (**TOC**). Bacterial arthritis and infection of the proximal tibiae are characteristic symptoms of TOC, and in an experimental setting TOC can be triggered by injecting turkey poults with repeated immunosuppressive doses of dexamethasone, a synthetic glucocorticoid (Wyers et al., 1991; Huff et al., 1998, 1999, 2000, 2005, 2006). Glucocorticoid injections also trigger femoral head necrosis in adult Leghorn hens (Cui et al., 1997) and epiphyseolysis in broilers (Durairaj et al., 2012). Based on the pathogenic similarities between BCO and TOC, we conducted three experiments to determine if dexamethasone injections might be used as a model for triggering BCO in broilers. In all three experiments, dexamethasone-injected broilers developed lameness primarily associated with increased incidences of BCO-like lesions of the proximal tibiae (Wideman and Pevzner, 2012). Within this context, it is highly likely that, in addition to creating footing instability, wire-flooring models *per se* also elicit stress-mediated immunosuppression. Elevated flooring systems that deprive birds of access to floor litter stimulate chronic stress, including immunosuppression (El-Lethey et al., 2003). In the same context, stress promotes the translocation of indigenous bacteria from the G.I. tract into the circulation of mammals (Berg, 1992), and acute and chronic stress responses are amplified in mammals when orally administered or indigenous bacteria are translocated into the circulation (Ando et al., 2000).

SUSCEPTIBILITY OF BROILER LINES

Genotype and selection for growth performance have been implicated in the susceptibility of broilers and turkeys to impaired walking ability (Wise, 1970a,b; Nestor, 1984; Kestin et al., 1999, 2001; Nestor and Anderson, 1998) as well as to a variety of leg disorders including tibial dyschondroplasia (Leach and Nesheim, 1965; Riddell, 1976; Sheridan et al., 1978; Walser et al., 1982; Zhang et al., 1995; Kuhlers and McDaniel, 1996), spondylolisthesis (Riddell, 1973, 1976; Wise, 1973; Khan et al., 1977), valgus-varus deformities (Mercer and Hill, 1984; Sorensen, 1992), and twisted leg or perosis (Somes, 1969; Haye and Simons, 1978; Mercer and Hill, 1984; Sorensen, 1992). In some but not all comparisons, faster growing lines or crosses tend to exhibit higher incidences of leg disorders and impaired walking ability when compared with slower growing lines (Nestor, 1984; Sorensen, 1992; Kuhlers and McDaniel, 1996; Kestin et al., 1999, 2001). The wire-flooring model enabled us to compare for the first time the potential impact of genotype on the susceptibility of commercial broilers to BCO (Wideman et al., 2013). Four broiler crosses were evaluated, including two standard crosses that grow rapidly at an early age, and two high-yield crosses that initially tend to grow more slowly. These crosses are commercially available to broiler integrators worldwide. In two independent experiments, the standard broiler crosses developed higher incidences of lameness than the high-yield crosses, implicating an association between rapid early growth and susceptibility to BCO (Wideman et al., 2013), results that match the experience of broiler integrators who have used these crosses. Integrators subsequently suggested that, regardless of initial growth performance, broilers derived from crossing sires from line "A" on dams from line "C" are more likely to develop higher incidences of BCO than broilers derived from crossing sires from line "B" on dams from line C. Accordingly, two independent experiments were conducted to evaluate the influence of the sire line on the susceptibility of broiler crosses to BCO (Wideman et al., 2014). The sire line did significantly influence the susceptibility of the resulting progeny to BCO, with broilers derived from sire line A being more likely to develop higher incidences than broilers derived from sire line B. The biological basis for this sire contribution remains to be determined. It is clear that integrators can choose appropriate broiler crosses and sires to reduce the incidence of BCO when broilers are grown to heavy yield weights (Wideman et al., 2014).

PROPHYLACTIC AND THERAPEUTIC TREATMENTS

Prophylactic Probiotic Treatments

Bacterial translocation and bacteremia are essential features of our hypothesis for the pathogenesis of BCO (Figure 3). Tight junctional complexes comprise a key component of the intestinal barrier by sealing the apical surfaces of adjacent epithelial cells. "Leaky" tight junctions provide paracellular portals through which pathogenic bacteria can cross the gastrointestinal epithelium and ultimately enter the systemic arterial circulation. This process of bacterial leakage across the intestinal epithelial barrier, known as bacterial translocation, can lead to the hematogenous distribution of pathogenic bacteria that infect the bone (Figure 4). Factors known to modulate the integrity of existing tight junctions and influence the dynamic synthesis of new tight junction proteins include physiological stress and "crosstalk" (direct cell-to-cell signaling) between gastrointestinal epithelial cells and commensal or pathogenic bacteria of the intestinal microbial community (Saunders et al., 1994; Ando et al., 2000; Steinwender et al., 2001; Ulluwishewa et al., 2011; Pastorelli et al., 2013). Heat stress and enhanced intestinal microbial challenges can impair the integrity of tight junctions and facilitate bacterial translocation across the epithelium of the small intestine in broilers (Quinteiro-Filho et al., 2010, 2012a,b; Murugesan et al., 2014). It also has been demonstrated that probiotics alone or in combination with prebiotics can attenuate intestinal barrier dysfunction in broilers

challenged by heat stress or pathogenic bacteria (Sohail et al., 2010, 2012; Murugesan et al., 2014; Song et al., 2014). Commensal and probiotic bacterial species that enhance intestinal barrier integrity by stimulating tight junction protein expression and the formation of occlusive tight junctional complexes also are effective in preventing bacterial translocation (Ulluwishewa et al., 2011; Pastorelli et al., 2013). In view of concerns regarding the development of antibiotic resistance in bacteria commonly associated with osteomyelitis (McNamee and Smyth, 2000; Waters et al., 2011), probiotics potentially can provide a plausible alternative for prophylactically reducing the incidence of BCO. Probiotics may interfere with the development of osteomyelitis by attenuating intestinal populations of pathogenic bacteria, by improving gut health and integrity to reduce bacterial leakage (translocation), or by priming the immune system to better eliminate translocated bacteria. Probiotics are not antibiotics and are unlikely to be effective if administered therapeutically only after lameness has developed in a flock. Indeed, administering probiotics in the feed beginning at 1 d of age, but not after the onset of BCO lameness, significantly reduced the incidence of lameness attributable to BCO in five independent experiments conducted over the course of two years and using four different broiler lines (Wideman et al., 2012). The first four of these experiments evaluated a proprietary probiotic containing *Enterococcus faecium*, *Bifidobacterium animalis*, *Pediococcus acidilactici*, and *Lactobacillus reuteri*. The fifth experiment evaluated a proprietary single-microbe probiotic containing *Enterococcus faecium*. Prophylactically providing these probiotics in the feed consistently reduced the incidence of BCO lameness by at least 50% and without attenuating growth performance when compared with broilers that also were reared on wire flooring but were not provided probiotics in their feed (Wideman et al., 2012). In subsequent studies a proprietary probiotic containing *Bacillus subtilis* significantly delayed the age of onset and reduced the cumulative incidence of BCO lameness in broilers reared on wire flooring, whereas experiments conducted with a different proprietary *B. subtilis* probiotic had no significant impact on the incidence of BCO lameness (Wideman et al., 2015a). Accordingly, although the specific biological mechanism remains to be determined, these experiments provide the first evidence that some, but not all, probiotics can significantly interrupt the pathogenesis of lameness attributable to BCO. Trials conducted on accumulated litter in commercial broiler facilities also have demonstrated the practical efficacy of probiotics for reducing the incidence of BCO (Wideman, personal observations). It is our hypothesis that susceptibility to BCO is minimized by probiotics that attenuate the translocation of pathogenic bacteria into the blood stream. Based on recent evidence that the proximal femora and tibiae harbor complex microbial communities in their growth plates and metaphyses, and that major differences exist in the microbial communities in different bones (femur versus tibia) and different lesion categories (macroscopically normal versus gross BCO lesions), it is intriguing to speculate that effective probiotics may improve bone health by modulating the composition and diversity of the microbial communities within the growth plates (Jiang et al., 2015). Probiotics potentially might influence growth plate microbial communities by reducing pathogen translocation, by enhancing the immune response to translocating microbial species, or via translocation of the probiotic species to the growth plates followed by direct modulation of a local microbial community *in situ*.

Therapeutic Antimicrobial (Enrofloxacin) Treatments

Based on our hypothesis for the pathogenesis of BCO (Figure 3), lameness incidences should be reduced if therapeutic levels of antibiotics are capable of attenuating the bacteremia and abscess development in broilers reared on wire flooring. Accordingly, an experiment was conducted to determine the extent to which administering an antibiotic therapeutically (after the initiation of BCO) could reduce the incidence of BCO lameness in broilers reared on wire flooring (Wideman et al., 2015a). Enrofloxacin is a potent fluoroquinolone antimicrobial that is used routinely outside of the United States to treat bacterial or mycoplasmal diseases of the respiratory and alimentary tracts in poultry. We administered enrofloxacin via the drinking water at recommended therapeutic levels starting when the cumulative incidence of BCO had reached an average of 3%. Throughout the entire interval of enrofloxacin administration, twice as many birds developed clinical lameness in the control group when compared with the enrofloxacin group. After enrofloxacin was withdrawn equal numbers of birds developed lameness in both groups, although the incidence of cumulative lameness at the end of the experiment remained higher in the control group than in the enrofloxacin group (Wideman et al., 2015a). The mechanical and physiological stresses that are imposed chronically by wire flooring can be very difficult for broilers to survive, regardless of the efficacies of prophylactic or therapeutic treatment regimens. Our experiments clearly demonstrate that both therapeutic antibiotic administration and prophylactic probiotic administration can only partially surmount the severe, chronic mechanical and physiological stresses imposed by rearing broilers on wire flooring. These studies further imply that probiotics have the potential to provide reasonably effective alternatives to antibiotics for reducing BCO lameness (Wideman et al., 2015a).

Prophylactic Vitamin D_3 Treatments

Turkey osteomyelitis complex (TOC) and BCO in broilers share a similar pathogenesis, including the translocation of opportunistic bacteria across the

intestinal and respiratory epithelial barriers, and the onset of lameness attributable to osteomyelitis within the physis and metaphysis of rapidly growing proximal tibiae. Stress and immunosuppression also contribute to the pathogenesis of both BCO and TOC. Indeed, BCO and TOC are readily induced by injecting broilers or poults with repeated immunosuppressive doses of the synthetic glucocorticoid dexamethasone (Wyers et al., 1991; Huff et al., 1998, 1999, 2000, 2006; Wideman and Pevzner, 2012). Huff et al. (2000) demonstrated that the immunosuppression and incidence of TOC induced by dexamethasone injections could be attenuated by prophylactically supplementing the drinking water with vitamin D_3, in spite of the presence of vitamin D_3 levels in the poult starter feed that met National Research Council (1994) requirements. It is within this context that an experiment was conducted to determine if prophylactic administration of a proprietary 25-hydroxy vitamin D_3 (**25-OHD$_3$**) product via the drinking water might reduce the incidence of BCO in broilers reared on wire flooring (Wideman et al., 2015b). Broiler chicks reared on wire flooring were supplied with either tap water alone or with 25-OHD$_3$ in their drinking water. Feed was provided ad libitum and was formulated to meet or exceed minimum standards for all ingredients, including 5,500 IU vitamin D_3/kg. The cumulative incidence of BCO was higher in the tap water group than in the 25-OHD$_3$ group (34.7% vs. 22.7%, respectively; $P = 0.03$) (Wideman et al., 2015b). The mechanisms by which 25-OHD$_3$ supplementation reduced lameness remain to be identified. Vitamin D_3 plays a key role in regulating calcium and phosphorus metabolism and thus bone mineralization, however no overt symptoms of a primary vitamin D_3 deficiency (e.g., growth depression, skeletal deformity, bone fractures, rickets) were detected in the present or previous studies in which

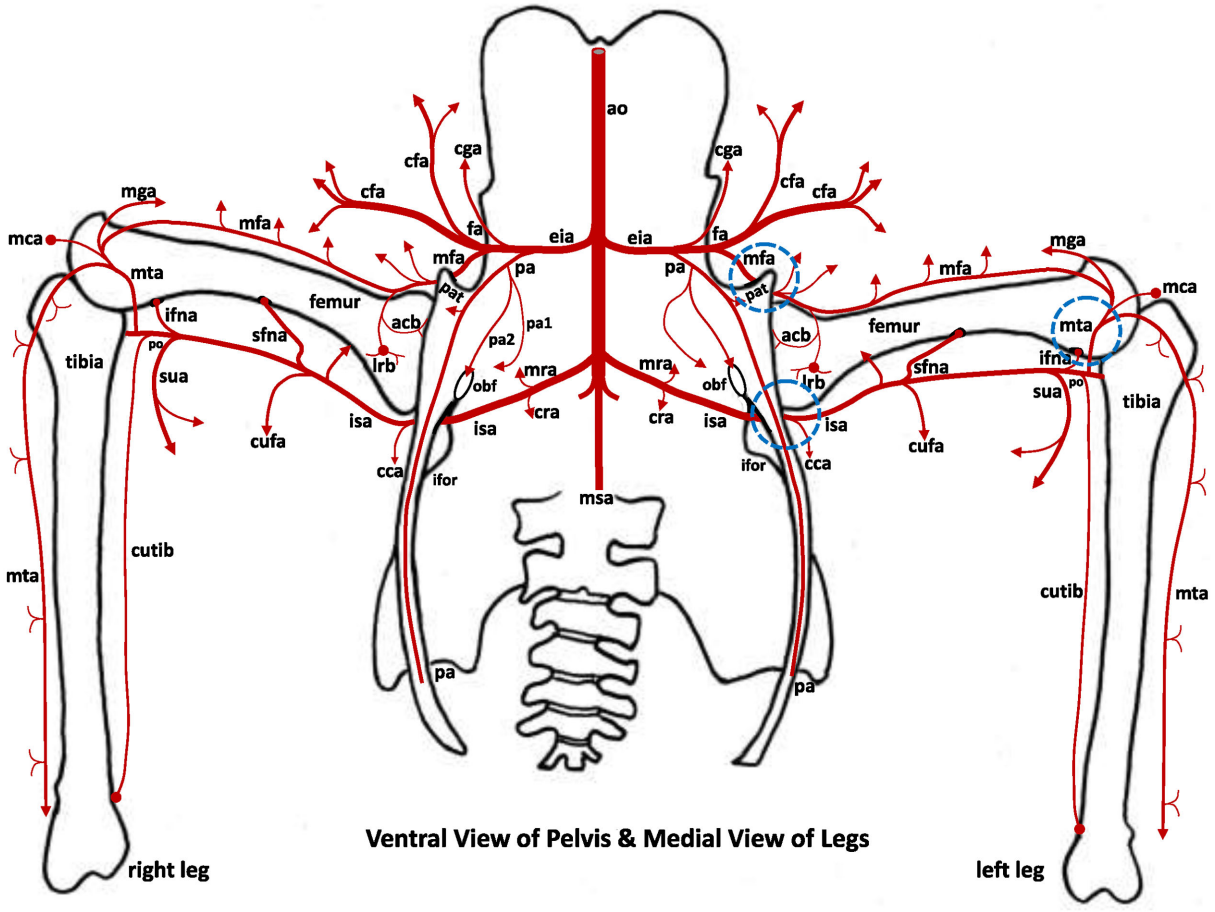

Figure 6. Arterial supply to the pelvis, femora and tibiae based on vascular castings. Choke points (dashed blue circles) identified as sites where an artery is likely to be compressed when broilers are in a sitting posture include: the medial femoral artery (mfa) as it passes dorsal to the preacetabular tuberculum (pat); the ischiadic artery (isa) as it passes through the ischiadic foramen (isa) of the pelvis caudal to the head of the femur; and, the medial tibial artery (mta) passing behind the "knee" (coxofemoral) joint of the distal femur and proximal tibia. Sporadic episodes of ischemia due to localized compression of the mfa or the isa may be circumvented by retrograde blood flow through the anastomosis ischiofemoralis that provides a route for artery-to-artery communication between the mfa and isa via the mta. Additional labels include: acb: acetabular branch of the mfa; ao: aorta (descending aorta, abdominal aorta); cca: caudal coxal artery; cfa: cranial femoral artery (circumflex femoral); cga: cranial gluteal artery; cra: cranial renal artery; cufa: caudal femoral artery; cutib: caudal tibial artery; eia: external iliac artery; fa: femoral artery (eia becomes fa on leaving the pelvis); ifna: inferior femoral nutrient artery; iom-i: internal obturator pars iliaca muscles; lrb: lateral retinacular branch of the mfa; mca: medial crural artery; mga: medial genicular artery; mra: medial renal artery; msa: medial sacral artery; obf: obturator foramen; pa: pubic artery; pa1: pubic artery branch 1; pa2: pubic artery branch 2; po: popliteal artery; sfna: superior femoral nutrient artery; sua: suralis artery.

high incidences of BCO were induced on wire flooring. The 25-OH D_3 metabolite is water soluble and better absorbed than lipid soluble vitamin D_3. Administering supplemental 25-OHD_3 also bypasses the 25-hydroxylation step in the liver, thereby directly providing additional substrate for the synthesis of biologically active 1,25-$(OH)_2$ vitamin D_3. Higher circulating levels of 1,25-$(OH)_2$ vitamin D_3 may in turn modulate the response of the immune system to counteract stress-mediated immunosuppression, inhibit bacterial translocation across the gastrointestinal tract, and attenuate the microbial infection and pathological deterioration of proximal growth plates associated with BCO in broilers and TOC in turkeys (Huff et al., 2000; Wideman et al., 2015b). The encouraging response to 25-OHD_3 supports a potential role for 25-OHD_3 supplementation in preventing BCO outbreaks in commercial poultry.

BREEDER FLOCKS AND HATCHERIES

Breeder flocks and hatcheries repeatedly have been suspected of being the source of *Staphylococcus* spp. and *Enterococcus* spp. that subsequently are isolated from lame broilers (Skeeles, 1997; McCullagh et al., 1998; Rodgers et al., 1999; McNamee and Smyth, 2000; Stalker et al., 2010; Kense and Landman, 2011). Various bacterial species, including *Staphylococcus*, *Enterococcus* and *Salmonella* spp. have been isolated from spleens harvested from broiler embryos at d 18 of incubation. Broiler breeders are continuously subjected to stresses such as feed restriction, aggressive males, slat (non-litter) flooring, and oviposition. Sustained stress may promote bacterial translocation and bacteremia sufficient to cause vertical transmission of the resident microbial communities from breeder hens to their progeny (Liljebjelke et al., 2005; Funkhouser and Bordensteim, 2013). Certain hatcheries, particularly older hatcheries, are notorious for producing chicks that are susceptible to BCO. Incidences of femoral, tibial and vertebral BCO can vary widely among separate barns on the same farm, with some barns exhibiting little or no lameness while other barns develop high BCO incidences. Different barns on the same farm typically are affected during sequential flock cycles, strongly implicating the breeder flock, hatchery, or post-hatch stress as potential factors contributing to early bacterial contamination.

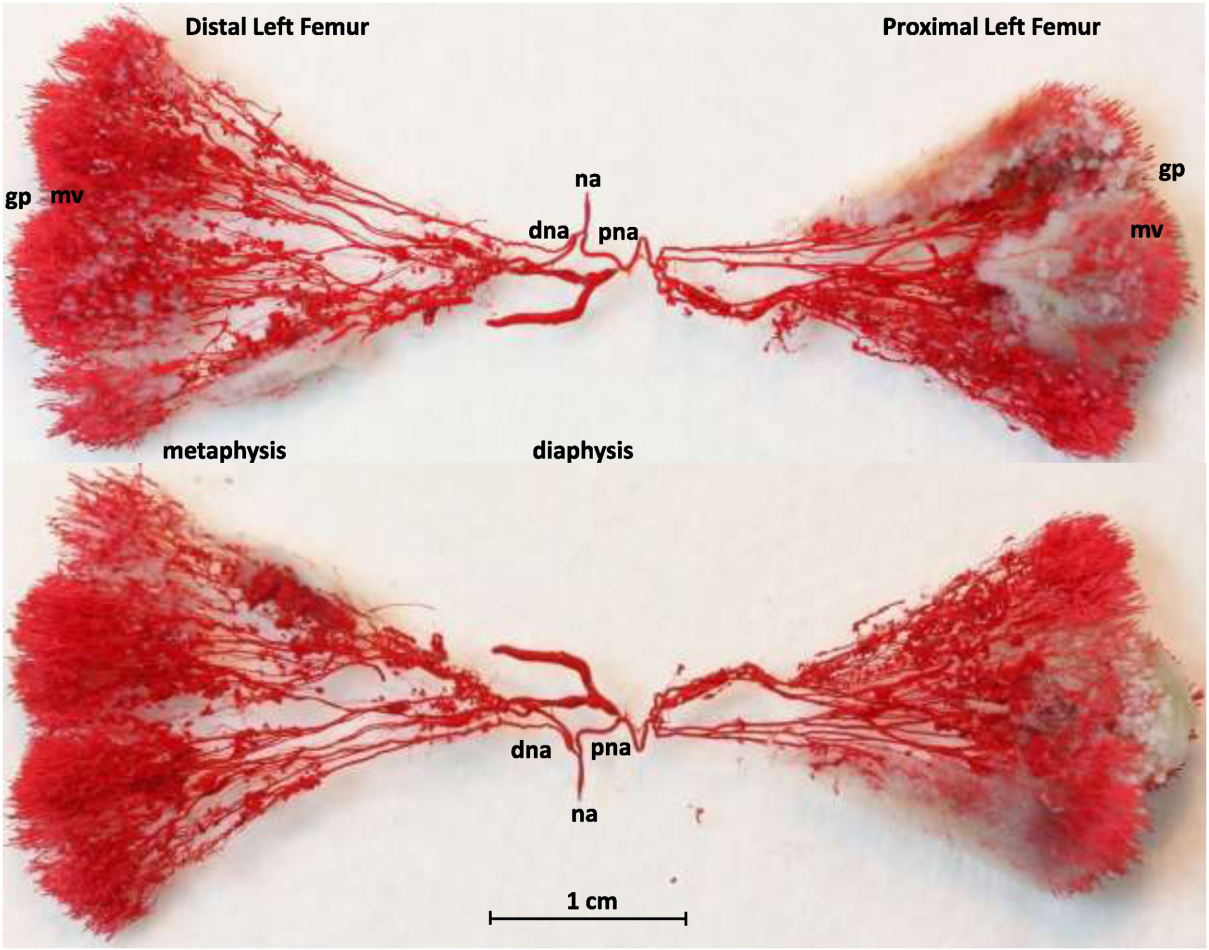

Figure 7. Vascular cast of the dense arterial supply to the femur. Blood flow to the metaphysis is supplied by a nutrient artery (na) that penetrates the diaphysis or shaft of the bone via a nutrient foramen. Within the marrow the na divides to form proximal and distal nutrient arteries (pna, dna) that branch repeatedly to form metaphyseal vessels (mv) that terminate as metaphyseal vascular plexus (not shown) at the interface between the calcifying zone of the metaphysis and the hypertrophic zone of the physis or growth plate (gp).

A similar epidemiology was documented for the vertical transmission of *Campylobacter jejuni* in broilers (Pearson et al., 1996). Our ongoing research using the wire-flooring model repeatedly has implicated breeder flocks, hatchery sources or chick quality in the susceptibility of broilers to BCO. Indeed, we routinely cull our experimental chicks on d 14 because necropsies of runts and culls during the first 2 wk post-hatch often reveal evidence of systemic bacterial infection including omphalitis, sepsis and BCO. Placing chicks on wire flooring on the day of hatch followed by heavy culling on d 14 currently is our standard experimental protocol for delaying the onset of clinical BCO until d 35 to 40 (Wideman et al., 2012, 2013, 2014, 2015a,b). Current industry efforts to reduce the incidence of BCO in problematic (repeater) broiler complexes include improving hatchery sanitation, minimizing heat stress in the hatchery and during brooding, prescribing antibiotic treatments for chicks immediately post-hatch, and reducing the photoperiod to modestly reduce early growth performance. For these approaches to be effective they must be addressing triggering mechanisms or early bacterial colonization that confer subclinical susceptibility at hatch or even prior to hatching. Indeed, we demonstrated that to be effective probiotics must be provided prophylactically beginning on the day of hatch (Wideman et al., 2012). Al-Rubaye et al. (2014) administered *Staphylococcus agnetis* in the drinking water to broiler chicks during two of the first 10 d post-hatch, after which the birds consumed only tap water. Enhanced lameness attributable BCO was not observed until four wk post-inoculation, and by 8 wk of age BCO incidences in the inoculated broilers subsequently escalated three-fold higher than incidences in un-inoculated controls (Al-Rubaye et al., 2014). When combined, these observations suggest that pathogens responsible for causing BCO may be present but quiescent very early pre- or post-hatch, waiting for the creation of a conducive wound site in larger heavier birds before clinical lameness attributable to BCO emerges. In that case, it would be extremely difficult to deduce the epidemiology of a BCO outbreak in 40- to 60-day-old broilers if the primary source of their bacterial inoculation occurred *in ovo* or in the hatchery.

BLOOD FLOW AND EXERCISE

The pathogenesis of BCO is complex and may be initiated by mechanical means, including mechanical interference with blood flow. An insufficient or obstructed blood supply to epiphyseal and physeal cartilage has been implicated as the primary cause of osteochondrosis in numerous animal species (Ytrehus et al., 2007). Examples of inadequate blood flow leading to bone

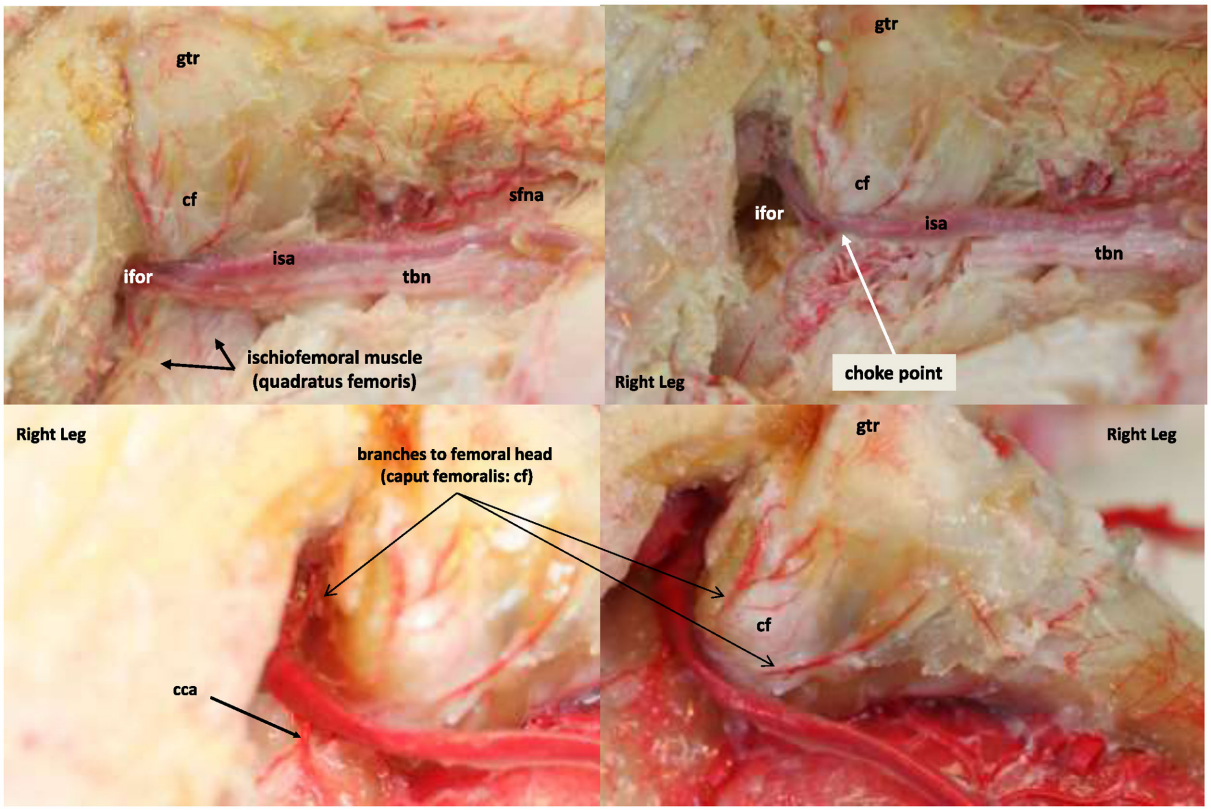

Figure 8. Vascular casts of the arterial supply to the proximal femur, showing a potential choke point where the ischiadic artery (isa) exits the pelvis through the ischiadic foramen (ifor) between the ischiofemoral muscle and the caput femoralis (cf; ball protuberance of the proximal femur) along with the tibial nerve (tbn). Casts of the isa consistently flatten and narrow as the vessel curves around the CF. Branches of the isa penetrating into the cf are indicated by arrows. Additional labels include: cca: caudal coxal artery; and, gtr: greater trochanter of the proximal femur.

necrosis have been noted in obese humans, such that repetitive mechanical strain on weight bearing joints is associated with femoral head necrosis and avascularity of subchondral bone. Studies in broilers suggested the early BCO pathology appears to be initiated by transient or sustained ischemia in the proximal growth plates or within the underlying metaphyses of susceptible femora and tibiae (Prisby et al., 2014). To begin addressing this possibility, vascular casts were used to map the arteries supplying the femora and tibiae of broilers. Arterial blood flow to the leg bones is primarily supplied by branches of the ischiadic artery, with a minor contribution from the medial femoral artery (Figure 6) (Nishida, 1963; Xu et al., 2010). Blood vessels enter the epiphysis at several sites (Howlett et al., 1984; Thorp, 1986; 1988a), and this epiphyseal vasculature subdivides into central arterioles coursing through blind-ended epiphyseal vascular canals within the hyaline zone of the epiphysis, or through junctional canals angled toward the growth plate (Figure 4). Blood flow to the metaphysis is supplied by nutrient arteries that penetrate the diaphysis or shaft of the long bones via a nutrient foramen (Figures 6 and 7). Within the marrow cavity, proximal (ascending) and distal (descending) nutrient arteries branch repeatedly to form a dense profusion of metaphyseal vessels, each of which terminates in vascular plexuses at the interface between the calcifying zone of the metaphysis and the hypertrophic zone of the growth plate (Figures 4 and 7). Vascular casts of the blood vessels supplying the femora and tibiae of broilers revealed three potential "choke points" or points of compression within the arterial tree (Figure 6). When broilers remain in a sitting posture (i.e., sternal recumbency with the legs in full flexion) the major arteries supplying the femora and tibiae potentially may be compressed at these choke points, thereby creating episodes of ischemia to the growth plates. For example, casts of the ischiadic artery consistently flatten and narrow as the artery exits the pelvis through the ischiadic foramen and curves around the caput femoralis (ball protuberance of the proximal femur) (Figure 8). Vascular casts also reveal compression and discontinuities in the medial tibial artery as it passes inside the "knee" joint. When a cast of the medial tibial artery exhibits discontinuities at this location, nevertheless upstream and downstream from the discontinuity the cast remains uninterrupted, suggesting the vessel is subject to compression behind the knee joint (Figure 9). These observations indicate that prolonged sitting may be detrimental to the normal development, maturation and strengthening of the highly vascularized bones and joints of broilers being pushed to

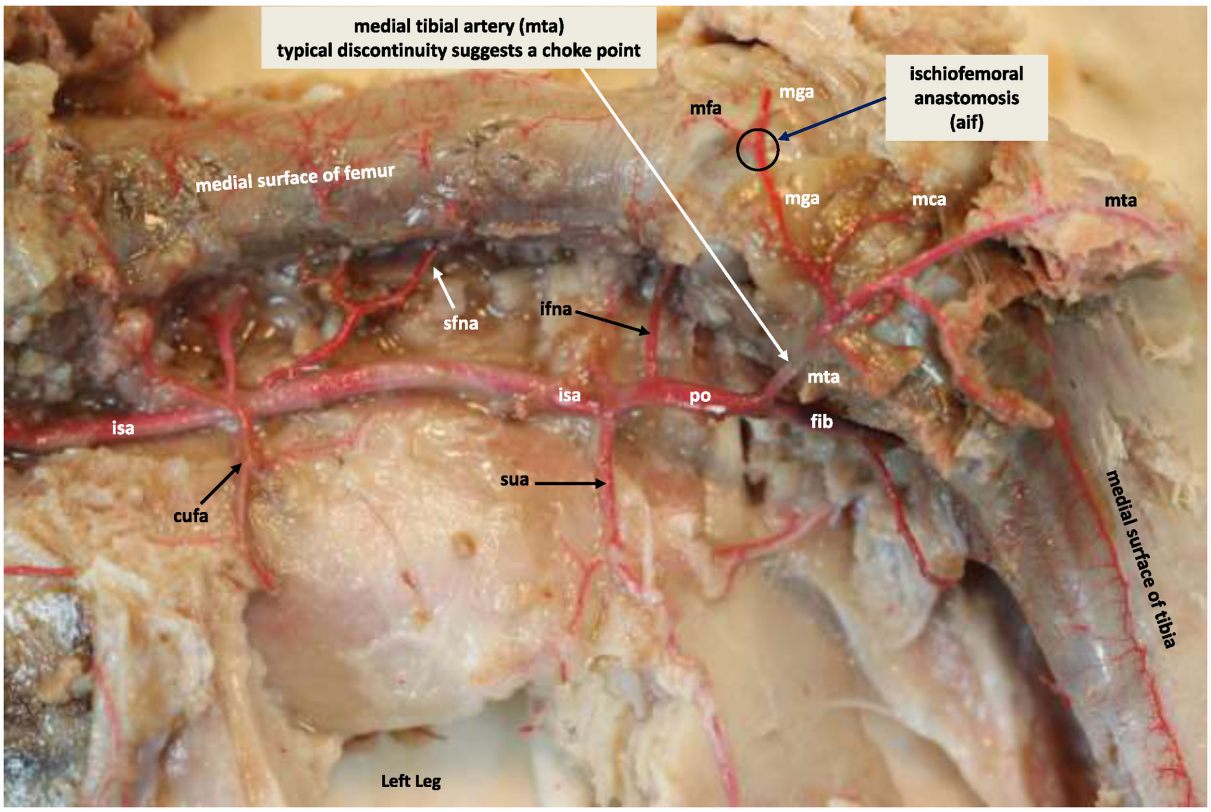

Figure 9. Vascular casts of the arterial supply to the distal femur and proximal tibia, showing a potential choke point where the medial tibial artery (mta) branches from the popliteal artery (po) and traverses behind the "knee" joint. Casts of the mta consistently exhibit discontinuities at this location, but upstream and downstream casts are uninterrupted, suggesting the mta is compressed behind the knee joint. The po is the continuation of the ischiadic artery (isa) beginning at the root of the suralis artery (sua) and ending at the origin of the fibular artery (fib). Note that distal to the potential point of compression the mta provides a direct route for artery-to-artery communication between the medial femoral artery (mfa) and the isa through the anastomosis ischiofemoralis (aif; circled). Additional labels include: ifna: inferior femoral nutrient artery; mca: medial crural artery; mga: medial genicular artery; and, sfna: superior femoral nutrient artery.

achieve their maximal growth potential. Current broiler management practices promote inactivity through dim light intensities, high stocking densities, and easy access to closely positioned drinkers and feeders (Hester, 1994; Foutz et al., 2007; Ruiz-Feria et al., 2014). Inactivity and a resting posture at night has been correlated with significant reductions in blood flow to the breast of SCWK chickens (Nishihara et al., 2013).

CONCLUSIONS AND PERSPECTIVES

Over the past several decades, investigators have reported the presence of osteochondrotic microfractures in the epiphyseal and physeal cartilage of the leg bones and vertebrae of apparently healthy broilers and turkey poults. Accordingly, mechanical damage and osteochondrosis *per se* cannot be directly correlated with clinical symptoms of lameness. However, osteochondrotic clefts do expose the collagen matrix and also transect terminal epiphyseal and metaphyseal vascular plexuses that are essential for the survival and maturation of chondrocytes. The consequences of osteochondrosis therefore include the creation of wound sites (clefts within and between the chondrocyte columns and layers) and microscopic zones of focal ischemia and necrosis, all of which constitute ideal niches for colonization by hematogenously distributed opportunistic bacteria. Pathogens that reach these niches are able to proliferate outside of the surveillance of circulating leukocytes or antibiotics. Osteochondrotic lesions are presumed to develop with increasing frequency after broilers accumulate sufficient body mass to impose excessive mechanical force on the thick cartilage layers of proximal femora, tibiae, and thoracic vertebrae. Therefore an innate susceptibility to osteochondrosis would be expected to predispose rapidly growing birds to also exhibit innate susceptibility to BCO. Research focused on understanding the fundamental causes of epiphyseal-physeal susceptibility to osteochondrosis clearly will help poultry geneticists select lines that are robustly resistant to BCO.

As a caveat to the usefulness of direct pathogen exposure models for triggering BCO, preexisting osteochondrotic microfracturing of the epiphyseal-physeal cartilage may not be an essential permissive condition when naturally occurring sepsis or experimental pathogen inoculations cause acute bacteremia. For example, intravenously injecting effective doses of *Staphylococcus* spp. typically trigger sustained bacteremia that causes bacterial emboli to form quite rapidly in the terminal epiphyseal and metaphyseal vascular plexuses throughout the skeleton, leading to widely dispersed osteomyelitis and a rapid (within 1 to 3 d) onset of lameness. This form of generalized/septic osteomyelitis apparently does not depend on the creation of osteochondrotic clefts (wound sites) as niches for bacterial colonization. Models of pathogen inoculation that very quickly overwhelm the body's defense mechanisms and trigger generalized septicemia are unlikely to be useful for detecting innate differences in BCO susceptibility among broiler lines that may primarily depend on susceptibility to osteochondrosis, or for demonstrating relative efficacies of practical prophylactic treatments that improve the barrier integrity at sites of bacterial translocation.

Before bacteria can be distributed hematogenously, they first must penetrate the body's defenses and then persist within the body's fluids and tissues. The ease with which viable bacteria can be routinely isolated from the blood and tissues (e.g., spleen, liver and bones) of broilers pre- and post-hatch readily discredits the common misconception that blood and tissues are essentially sterile in clinically healthy birds, or that the immune system efficiently destroys invading pathogenic bacteria. In fact, broadly diverse microbial communities are now known to persist quiescently in the respiratory tract and bones of apparently healthy broilers, and these communities appear to be tolerated by the immune system. Potential routes of bacterial penetration include vertical transmission from the breeder hen, or translocation from the environment (e.g., the hatchery, growout facilities, accumulated litter, feed, water, intestinal microbiome) through the integument, respiratory epithelium, or intestinal epithelium. Experiments have repeatedly demonstrated the ease with which orally administered or indigenous enteric bacteria can be translocated across the gastrointestinal epithelium to be carried by the circulatory system (adherent to or engulfed by phagocytes) to organs and tissues throughout the body. The efficacy of feeding probiotics prophylactically to reduce the incidence of BCO implicates the gastrointestinal tract as a major avenue of bacterial translocation. Many poultry health professionals consider BCO to be a secondary infection that develops several weeks after a severe respiratory challenge (e.g., mycoplasma infection; poor hatchery sanitation; rough response to respiratory vaccine), an enteric challenge (e.g., coccidiosis; enteritis), an immunosuppressive challenge (e.g., stress; chicken anemia virus; infectious bursal disease virus), or infection of the integument (e.g., wet navels; foot pad or hock lesions). The epidemiology of BCO outbreaks in 40- to 60-day-old broilers would be extremely difficult to unravel if the primary pathogen inoculation occurred *in ovo*, in the hatchery, or during the earliest weeks of a chick's life. The fact that pathogenic bacteria apparently are routinely able to translocate into the blood stream of broilers, and the fact that clinically healthy broilers can harbor microbial communities and bacterial abscesses in their femora, tibiae and flexible thoracic vertebrae, firmly positions BCO as a food safety issue in addition to its obvious detrimental impact on broiler production (economic losses due to lameness) and animal welfare (lameness and stress).

Our wire-flooring model induces fast growing broilers to spontaneously develop high incidences of lameness attributable to pathognomonic BCO lesions. Wire flooring imposes persistent footing instability to create mechanical damage to proximal growth plates, and also

constitutes a significant chronic stressor that promotes indigenous bacterial translocation and proliferation attributed to stress-mediated immunosuppression. This model provides an important opportunity to conduct research focused on understanding the multi-factorial pathogenesis of BCO and clinical lameness in broilers. Mechanisms of bacterial translocation remain to be revealed, particularly including the specific role of probiotics in improving the barrier function of the intestinal epithelium. It is intriguing to speculate that probiotic species may translocate into the blood stream to modulate internal microbial communities. We do not understand how pathogenic bacteria circulating in the blood avoid elimination by the immune system, or how complex microbial communities are allowed to persist in apparently healthy growth plates without triggering an inflammatory response. Research focused on understanding the fundamental role of the immune system during the pathogenesis of BCO clearly will help poultry geneticists select lines that are robustly resistant to bacteremia, systemic colonization and infection. Highly significant research opportunities abound!

REFERENCES

Alderson, M., and S. Nade. 1987. Natural history of acute septic arthritis in an avian model. J. Orthopaed. Res. 5:261–274.

Alderson, M., D. Speers, K. Emslie, and S. Nade. 1986b. Acute haematogenous osteomyelitis and septic arthritis - a single disease. J. Bone Joint Surg. 68B:268–274.

Alderson, M., K. Emslie, D. Speers, and S. Nade. 1986a. Transphyseal blood vessels exist in avian species. J. Anat. 146:217–224.

Alfonso, M., and H. J. Barnes. 2006. Neonatal osteomyelitis associated with *Staphylococcus aureus* in turkey poults. Avian Dis. 50:148–151.

Ali, S. Y. 1992. Matrix formation and mineralisation in bone. Pages 19–38 in *Bone Biology and Skeletal Disorders in Poultry*, C. C. Whitehead ed. Carfax Publishing Company, Abingdon, UK.

Al-Rubaye, A.A., K. Estill, R.F. Wideman, and D.D. Rhoads. 2014. 16S rRNA-based diagnosis and whole-genome sequencing of bacteria cultured from lame broilers with osteomyelitis. Poult. Sci. 93:314P.

Ando, T., R. F. Brown, R. D. Berg, and A. J. Dunn. 2000. Bacterial translocation can increase plasma corticosterone and brain catecholamine and indoleamine metabolism. Am. J. Physiol. Reg. Integr. Comp. Physiol. 279:R2164–R2172.

Andreasen, J.R., C.B. Andreasen, M. Anwer, and A.E. Sonn. 1993. Heterophil chemotaxis in chickens with natural staphylococcal infections. Avian Dis. 37:284–289.

Applegate, T. J., and M. S. Lilburn. 2002. Growth of the femur and tibia of a commercial broiler line. Poult. Sci. 81:1289–1294.

Barrow, M. V., C. F. Simpson, and E. J. Miller. 1974. Lathyrism: a review. Quarterly Rev. Biol. 49:101–128.

Baumel, J. J., and L. M. Witmer. 1993. Osteologia. Pages 45–132 in Handbook of Avian Anatomy: Nomina Anatomica Avium, second edition, J. J. Baumel, A.S. King, J. E. Breazile, H. E. Evans, and J. C. Vanden Berge eds. Nuttall Ornithological Club, c/o Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts.

Beaumont, G. D. 1967. The intraosseous vasculature of the ulna of *Gallus domesticus*. J. Anat. 101:543–554.

Berg, R. D. 1992. Bacterial translocation from the gastrointestinal tract. J. Med. 23:217–244.

Bond, P. L., T. W. Sullivan, J. H. Douglas, and L. G. Robeson. 1991. Influence of age, sex, and method of rearing on tibia length and mineral deposition in broilers. Poult. Sci. 70:1936–1942.

Boss, J. H., and I. Misselevich. 2003. Osteonecrosis of the femoral head of laboratory animals: the lessons learned from a comparative study of osteonecrosis in man and experimental animals. Vet. Pathol. 40:345–354.

Bradshaw, R. H., R. D. Kirkden, and D. M. Broom. 2002. A review of the aetiology and pathology of leg weakness in broilers in relation to welfare. Avian Poult. Biol. Rev. 13:45–103.

Butterworth, A. 1999. Infectious components of broiler lameness: a review. World's Poult. Sci. J. 55:327–352.

Carnaghan, R. B. A. 1966. Spinal cord compression due to spondylitis caused by *Staphylococcus pyogenes*. J. Comp. Pathol. 76:9–14.

Church, L. E., and L. C. Johnson. 1964. Growth of long bones in the chicken. Rates of growth in length and diameter of the humerus, tibia, and metatarsus. Am. J. Anat. 114:521–538.

Cox, N. A., L. J. Richardson, R. J. Buhr, J. K. Northcutt, P. J. Fedorka-Cray, J. S. Bailey, B. D. Fairchild, and J. M. Mauldin. 2006. Natural occurrence of Campylobacter species, Salmonella serovars, and other bacteria in unabsorbed yolks of market-age commercial broilers. J. Appl. Poult. Res. 15:551–557.

Cui, Q., G.-J. Wang, C.-C. Su, and G. Balian. 1997. Lovastatin prevents steroid induced adipogenesis and osteonecrosis. Clin. Orthopaed. Rel. Res. 344:8–19.

Daum, R. S., W. H. Davis, K. B. Farris, R. J. Campeau, D. M. Mulvihill, and S. M. Shane. 1990. A model of staphylococcus aureus bacteremia, septic arthritis, and osteomyelitis in chickens. J. Orthopaed. Res. 8:804–813.

Devriese, L. A., A. H. Devos, and J. Beumer. 1972. *Staphlococcus aureus* colonization on poultry after experimental spray inoculations. Avian Dis. 16:656–665.

Dinev, I. 2009. Clinical and morphological investigations on the prevalence of lameness associated with femoral head necrosis in broilers. Br. Poult. Sci. 50:284–290.

Duff, S. R. I. 1984a. Dyschondroplasia of the caput femoris in skeletally immature broilers. Res. Vet. Sci. 37:293–302.

Duff, S. R. I. 1984b. Capital femoral epiphyseal infarction in skeletally immature broilers. Res. Vet. Sci. 37:303–309.

Duff, S. R. I. 1984c. Consequences of capital femoral dyschondroplasia in young adult and skeletally mature broilers. Res. Vet. Sci. 37:310–319.

Duff, S. R. I. 1985. Hip instability in young adult broiler fowls. J. Comp. Pathol. 95:373–382.

Duff, S. R. I. 1989a. Physeal clefts and disturbed endochondral ossification in broiler fowl. J. Comp. Pathol. 101:75–86.

Duff, S. R. I. 1989b. Disturbed endochondral ossification in the axial skeleton of young broiler fowls. J. Comp. Pathol. 101:399–409.

Duff, S. R. I. 1990a. Do different forms of spondylolisthesis occur in broiler fowls? Avian Pathol. 19:279–294.

Duff, S. R. I. 1990b. Diseases of the musculoskeletal system. Pages 254–283 in Poultry Diseases, F.T.W. Jordan Ed., 3rd ed. Bailliere Tindall, London, UK.

Duff, S. R. I., and C. J. Randall. 1987. Observations on femoral head abnormalities in broilers. Res Vet. Sci. 42:17–23.

Durairaj, V., F. D. Clark, C. C. Coon, W. E. Huff, R. Okimoto, G. R. Huff, and N. C. Rath. 2012. Effects of high fat diets or prednisolone treatment on femoral head separation in chickens. Br. Poult. Sci. 53:198–203

El-Lethey, H., B. Huber-Eicher, and T. W. Jungi. 2003. Exploration of stress-induced immunosuppression in chickens reveals both stress-resistant and stress-susceptible antigen responses. Vet. Immunol. Immunopathol. 95:91–101.

Emslie, K. R., and S. Nade. 1983. Acute hematogenous staphylococcal osteomyelitis: A description of the natural history in an avian model. Am. J. Pathol. 110:333–345.

Emslie, K. R., and S. Nade. 1985. Acute hematogenous staphylococcal osteomyelitis. Comp. Pathol. Bulletin 17:2–3.

Emslie, K. R., L. M. Fenner, and S. M. L. Nade. 1984. Acute haematogenous osteomyelitis: II. The effect of a metaphyseal abscess on the surrounding blood supply. J. Pathol. 142:129–134.

Emslie, K. R., N. R. Ozanne, and S. M. L. Nade. 1983. Acute hematogenous osteomyelitis: an experimental model. J. Pathol. 141:157–167.

Farquharson, C., and D. Jefferies. 2000. Chondrocytes and longitudinal bone growth: The development of tibial dyschondroplasia. Poult. Sci. 79:994–1004.

Foutz, T., A. Ratterman, and J. Halpert. 2007. Effects of immobilization on the biomechanical properties of the broiler tibia and gastrocnemius tendon. Poult. Sci. 86:931–936.

Funkhouser, L. J., and S. R. Bordensteim. 2013. Mom knows best: The universality of maternal microbial transmission. PLoS Biol. 11:e1001631. doi:10.1371/journal.pbio.1001631.

Gilley, A. D., H. Lester, I. Y. Pevzner, N. B. Anthony, and R. F. Wideman, Jr. 2014. Evaluating portable wire flooring models for inducing bacterial chondronecrosis with osteomyelitis (BCO) in broilers. Poult. Sci. 93:1354–1367.

Griffiths, G. L., W. L. Hopkinson, and J. Lloyd. 1984. Staphlococcal necrosis in the head of the femur in broiler chickens. Austral. Vet. J. 61:293.

Hales, S. 1727. Static Essays, Vol I: Vegetable Staticks, W. Innys & Woodward, London.

Havenstein, G. B., P. R. Ferket, S. E. Scheidler, and B. T. Larson. 1994. Growth, livability and feed conversion of 1957 vs. 1991 broilers when fed "typical" 1957 and 1991 broiler diets. Poult. Sci. 73:1785–1794.

Haye, U., and P. C. M. Simons. 1978. Twisted legs in broilers. Br. Poult. Sci. 19:549–557.

Hester, P. Y. 1994. The role of environment and management on leg abnormalities in meat-type fowl. Poult. Sci. 73:904–915.

Howlett, C. R. 1979. The fine structure of the proximal growth plate of the avian tibia. J. Anat. 128:377–399.

Howlett, C. R. 1980. The fine structure of the proximal growth plate and metaphysis of the avian tibia: endochondral osteogenesis. J. Anat. 130:745–768.

Howlett, C. R., M. Dickson, and A. K. Sheridan. 1984. The fine structure of the proximal growth plate of the avian tibia: vascular supply. J. Anat. 139:115–132.

Huff, G. R., W. E. Huff, J. M. Balog, and N. C. Rath. 1998. Effects of dexamethasone immunosuppression on turkey osteomyelitis complex in an experimental *Escherichia coli* respiratory infection. Poult. Sci. 77:654–661.

Huff, G. R., W. E. Huff, J. M. Balog, and N. C. Rath. 1999. Sex differences in the resistance of turkeys to *Escherichia coli* challenge after immunosuppression with dexamethasone. Poult. Sci. 78:38–44.

Huff, G. R., W. E. Huff, N. C. Rath, and J. M. Balog. 2000. Turkey osteomyelitis complex. Poult. Sci. 79:1050–1056.

Huff, G. R., W. E. Huff, J. M. Balog, N. C. Rath, N. B. Anthony, and K. E. Nestor. 2005. Stress response differences and disease susceptibility reflected by heterophil to lymphocyte ratio in turkeys selected for increased body weight. Poult. Sci. 84:709–717.

Huff, G., W. Huff, N. Rath, J. Balog, N. B. Anthony, and K. Nestor. 2006. Stress-induced colibacillosis and turkey osteomyelitis complex in turkeys selected for increased body weight. Poult. Sci. 85:266–272.

Hunt, C. D., D. A. Ollerich, and F. H. Nielsen. 1979. Morphology of the perforating cartilage canals in the proximal tibial growth plate of the chick. Anat. Rec. 194:143–157.

Jensen, M. M., W. C. Downs, J. D. Morrey, T. R. Nicoll, S. D. Lefevre, and C. M. Meyers. 1987. Staphylococcosis of turkeys. 1. Portal of entry and tissue colonisation. Avian Dis. 31:64–69.

Jiang, T., R. K. Mandal, R. F. Wideman, Jr., A. Khatiwara, I. Pevzner, and Y. M. Kwon. 2015. Molecular survey of bacterial communities associated with bacterial chondronecrosis with osteomyelitis in broilers. PLOS ONE DOI:10.1371/journal.pone.0124403 April 16, 2015. (http://www.ncbi.nlm.nih.gov/pubmed/25881241).

Joiner, K. S., F. J. Hoerr, E. van Santen, and S. J. Ewald. 2005. The avian major histocompatibility complex influences bacterial skeletal disease in broiler breeder chickens. Vet. Pathol. 42:275–281.

Julian, R. J. 1985. Osteochondrosis, dyschondroplasia, and osteomyelitis causing femoral head necrosis in turkeys. Avian Dis. 29:854–866.

Julian, R. J. 2005. Production and growth related disorders and other metabolic diseases of poultry - A review. Vet. J. 169:350–369.

Kember, N. F., and J. K. Kirkwood. 1987. Cell kinetics and longitudinal bone growth in birds. Cell Tissue Kinet. 20:625–629.

Kember, N. F., J. K. Kirkwood, P. J. Duignan, D. Godfrey, and D. J. Spratt. 1990. Comparative cell kinetics of avian growth plates. Res. Vet. Sci. 49:283–288.

Kense, M. J., and W. J. M. Landman. 2011. *Enterococcus cecorum* infections in broiler breeders and their offspring: molecular epidemiology. Avian Pathol. 40:603–612.

Kestin, S. C., G. Su, and P. Sorensen. 1999. Different commercial broiler crosses have different susceptibilities to leg weakness. Poult. Sci. 78:1085–1090.

Kestin, S. C., S. Gordon, G. Su, and P. Sorensen. 2001. Relationship in broiler chickens between lameness, liveweight, growth rate and age. Vet. Rec. 148:195–197.

Khan, M. A., N. O. Olson, and D. O. Overman. 1977. Spontaneous spondylolisthesis in embryonic and adult chick. Poult. Sci. 56:689–697.

Kibenge, F. S. B., G. E. Wilcox, and D. A. Pass. 1983. Pathogenicity of four strains of *Staphylococcus aureus* isolated from chickens with clinical tenosynovitis. Avian Pathol. 12:213–220.

Kirkwood, J. K., D. M. J. Spratt, and P. J. Duignan. 1989b. Patterns of cell proliferation and growth rate in limb bones of the domestic fowl (*Gallus domesticus*). Res. Vet. Sci. 47:139–147.

Kirkwood, J. K., P. J. Duignan, N. F. Kember, P. M. Bennett, and D. J. Price. 1989a. The growth of the tarsometatarsus bone in birds. J. Zool., Lond. 217:403–416.

Kuhlers, D. L., and G. R. McDaniel. 1996. Estimates of heritabilities and genetic correlations between tibial dyschondroplasia expression and body weight at two ages in broilers. Poult. Sci. 75:959–961.

Leach, R. M., Jr., and C. V. Gay. 1987. Role of epiphyseal cartilage in endochondral bone formation. J. Nutr. 117:784–790.

Leach, R. M., and M. C. Nesheim. 1965. Nutritional, genetic and morphological studies of an abnormal cartilage formation in young chicks. J. Nutr. 86:236–244.

Liljebjelke, K. A., C. L. Hofacre, T. Liu, D. G. White, S. Ayers, S. Young, and J. J. Maurer. 2005. Vertical and horizontal transmission of *Salmonella* within integrated broiler production system. Foodborne Pathogens Dis. 2:90–102.

Lutfi, A. M. 1970a. Study of cell multiplication in the cartilagenous upper end of the tibia of the domestic fowl by tritiated thymidine autoradiography. Acta Anat. 76:454–463.

Lutfi, A. M. 1970b. The mode of growth, fate and function of cartilage canals. J. Anat. 106:135–145.

Martin, L. T., M. P. Martin, and H. J. Barnes. 2011. Experimental reproduction of enterococcal spondylitis in male broiler breeder chickens. Avian Dis. 55:273–278.

McCaskey, P.C., G.N. Rowland, R.K. Page, and L.R. Minear. 1982. Focal failures of endochondral ossification in the broiler. Avian Dis. 26:701–717.

McCullagh, J.J., P.T. McNamee, J.A. Smyth, and H.J. Ball. 1998. The use of pulsed-field gel electrophoresis to investigate the epidemiology of *Staphylococcus aureus* infection in commercial broiler flocks. Vet. Microbiol. 63:275–281.

McNamee, P. T., and J. A. Smyth. 2000. Bacterial chondronecrosis with osteomyelitis ('femoral head necrosis') of broiler chickens: a review. Avian Pathol. 29:253–270.

McNamee, P. T., J. J. McCullagh, B. H. Thorp, H. J. Ball, D. Graham, S. J. McCullough, D. McConaghy, and J. A. Smyth. 1998. Study of leg weakness in two commercial broiler flocks. Vet. Rec. 143:131–135.

McNamee, P. T., J. J. McCullagh, J. D. Rodgers, B. H. Thorp, H. J. Ball, T. J. Connor, D. McConaghy, and J. A. Smyth. 1999. Development of an experimental model of bacterial chondronecrosis with osteomyelitis in broilers following exposure to *Staphlococcus aureus* by aerosol, and inoculation with chicken anemia and infectious bursal disease viruses. Avian Pathol. 28:26–35.

Mercer, J. T., and W. G. Hill. 1984. Estimation of genetic parameters for skeletal defects in broiler chickens. Heredity 53: 193–203.

Murugesan, G. R., N. K. Gabler, and M. E. Persia. 2014. Effects of direct-fed microbial supplementation on broiler performance, intestinal nutrient transport and integrity under experimental conditions with increased microbial challenge. Br. Poult. Sci. 55:89–97.

Mutalib, A., C. Riddell, and A. D. Osborne. 1983a. Studies on the pathogenesis of staphylococcal osteomyelitis in chickens. I. Effect of stress on experimentally induced osteomyelitis. Avian Dis. 27:141–156.

Mutalib, A., C. Riddell, and A. D. Osborne. 1983b. Studies on the pathogenesis of staphylococcal osteomyelitis in chickens. II. Role of the respiratory tract as a route of infection. Avian Dis. 27: 157–160.

Naas, I. A., I. C. L. A. Paz, M.S. Baracho, A. G. Menezes, L. G. F. Bueno, I. C. L. Almeida, and D. J. Moura. 2009. Impact of lameness on broiler well-being. J. Appl. Poult. Res. 18:432–439.

Nairn, M. E. (1973). Bacterial osteomyelitis and synovitis in the turkey. Avian Dis. 17:504–517.

Nairn, M. E., and A. R. A. Watson. 1972. Leg weakness of poultry - a clinical and pathological characterisation. Aust. Vet. J. 48:645–656.

National Research Council (NRC). 1994. Nutrients Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington DC.

Nestor, K. E. 1984. Genetics of growth and reproduction in the turkey. 9. Long-term selection for increased 16-week body weight. Poult. Sci. 63:2114–2122.

Nestor, K. E., and J. W. Anderson. 1998. Effect of crossing a line selected for increased shank width with two commercial sire lines on performance and walking ability of turkeys. Poult. Sci. 77:1601–1607.

Nickel, R., A. Schummer, E. Seiferle, and W. G. Siller. 1977. Anatomy of the Domestic Birds. Pages 62–69, W. G. Siller, and P. A. L. Wight ed. Springer-Verlag, New York.

Nicoll, T. R., and M. M. Jensen. 1986. Preliminary studies on bacterial interference of staphylococcosis of chickens. Avian Dis. 31:140–144.

Nishida, T. 1963. Comparative and topographical anatomy of the fowl. The blood vascular supply of the hind limb in the fowl. Part I. The artery. Jap. J. Vet. Sci. 25:103–111.

Nishihara, K., W. Iwasaki, M. Nakamura, E. Higurashi, T. Soh, T. Itoh, H. Okada, R. Maeda, and R. Sawada. 2013. Development of a wireless sensor for the measurement of chicken blood flow using the laser Doppler blood flow meter technique. IEEE Trans. Biomed. Eng. 60:1645–1653.

Osbaldiston, G. M., and D. R. Wise. 1967. Spondylolisthesis and leg weakness in the chicken – a common aetiology. Vet. Rec. 80:320–322.

Pastorelli, L., C. De Salvo, J. R. Mercado, M. Vecchi, and T. T. Pizarro. 2013. Central role of the gut epithelial barrier in the pathogenesis of chronic intestinal inflammation: lessons learned from animal models and human genetics. Frontiers in Science (Front. Immunol.) 4:280. doi:10.3389/fimmu.2013.002080.

Pattison, M. 1992. Impacts of bone problems on the poultry meat industry. Pages 329–338 in Bone Biology and Skeletal Disorders in Poultry, C. C. Whitehead, ed. Carfax Publishing Company, Abingdon, United Kingdom.

Pearson, A. D., M. H. Greenwood, R. K. A. Feltham, T. D. Healing, J. Donaldson, D. M. Jones, and R. R. Colwell. 1996. Microbial ecology of *Campylobacter jejuni* in a United Kingdom supply chain: Intermittent common source, vertical transmission, and amplification by flock propagation. Appl. Environ. Microbiol. 62:4614–4620.

Prisby, R., T. Menezes, J. Campbell, T. Benson, E. Samraj, I. Pevzner, and R. F. Wideman, Jr. 2014. Kinetic examination of femoral bone modeling in broilers. Poult. Sci. 93:1122–1129.

Quinteiro-Filho, W. M., A. Ribeiro, V. Ferraz-de-Paula, M. L. Pinheiro, M. Sakai, L. R. M. Sa, A. J. P. Ferreira, and J. Palermo-Neto. 2010. Heat stress impairs performance parameters, induces intestinal injury, and decreases macrophage activity in broiler chickens. Poult. Sci. 89:1905–1914.

Quinteiro-Filho, W. M., A. V. S. Gomes, M. L. Pinheiro, A. Ribeiro, V. Ferraz-de-Paula, C. S. Astolfi-Ferreira, A. J. P. Ferreira, and J. Palermo-Neto. 2012b. Heat stress impairs performance and induces intestinal inflammation in broiler chickens infected with *Salmonella* Enteritidis. Avian Pathol. 41:421–427.

Quinteiro-Filho, W. M., M. V. Rodrigues, A. Ribeiro, V. Ferraz-de-Paula, M. L. Pinheiro, L. R. M. Sa, A. J. P. Ferreira, and J. Palermo-Neto. 2012a. Acute heat stress impairs performance parameters and induces mild intestinal enteritis in broiler chickens: Role of acute hpothalamic-pituitary-adrenal axis of activation. J. Anim. Sci. 90:1986–1994.

Riddell, C. 1973. Studies on spondylolisthesis (kinky-back) in broiler chickens. Avian. Pathol. 2:295–304.

Riddell, C. 1975. Studies on the pathogenesis of tibial dyschondroplasia in chickens. II. Growth rate of long bones. Avian Dis. 19:490–496.

Riddell, C. 1976. Selection of broiler chickens for high and low incidence of tibial dyschondroplasia with observations on spondylolisthesis and twisted legs (perosis). Poult. Sci. 55:145–151.

Riddell, C. 1983. Pathology of the skeleton and tendons of broiler chickens reared to roaster weights. I. crippled chickens. Avian Dis 27:950–962.

Riddell, C. 1992. Non-infectious skeletal disorders of poultry - an overview. Pages 119–145 in Bone Biology and Skeletal Disorders in Poultry, C. C. Whitehead, ed. Carfax Publishing Company, Abingdon, United Kingdom.

Riddell, C., and J. Howell. 1972. Spondylolisthesis (kinky-back) in broiler chickens in Western Canada. Avian. Dis. 16:444–452.

Riddell, C., M. W. King, and K. R. Gunasekera. 1983. Pathology of the skeleton and tendons of broiler chickens reared to roaster weights. II. Normal chickens. Avian Dis. 27:980–991.

Rodgers, J.D., J.J. McCullagh, P.T. McNamee, J.A. Smyth, and H.J. Ball. 1999. Comparison of *Staphylococcus aureus* recovered from personnel in a poultry hatchery and in broiler parent farms with those isolated from skeletal disease in broilers. Vet. Microbiol. 69:189–198.

Ruiz-Feria, C. A., J. J. Arroyo-Villegas, A. Pro-Martinez, J. Bautista-Ortega, A. Cortes-Cuevas, C. Narciso-Gaytan, A. Hernandez-Cazares, and J. Gallegos-Sanchez. 2014. Effects of distances and barriers between resources on bone and tendon strength and productive performance of broiler chickens. Poult. Sci. 93:1608–1617.

Sandilands, V., S. Brocklehurst, N. Sparks, L. Baker, R. McGovern, B. Thorp, and D. Pearson. 2011. Assessing leg health in chickens using a force plate and gait scoring: how many birds is enough? Vet. Rec. 168:77–83.

Saunders, P. R., U. Kosecka, D. M. McKay, and M. H. Perdue. 1994. Acute stressors stimulate ion secretion and increase epithelial permeability in rat intestine. Am. J. Physiol. 267:G794–G799.

Shabbir, M. Z., T. Malys, Y. V. Ivanov, J. Park, M. A. B. Shabbir, M. Rabbani, T. Yaqub, and E. T. Harvill. 2015. Microbial communities present in the lower respiratory tract of clinically healthy birds in Pakistan. Poult. Sci. 94:612–620.

Sheridan, A. K., C. R. Howlett, and R. W. Burton. 1978. The inheritance of tibial dyschondroplasia in broilers. Br. Poult. Sci. 19:491–499.

Skeeles, K. J. 1997. Staphylococcosis. Pages 247–253 in Diseases of Poultry 10th Edition, Chapter 11. B.W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald, and Y. M. Saif, Eds., Iowa State University Press, Ames, IA, USA.

Smeltzer, M. S., and A. F. Gillaspy. 2000. Molecular pathogenesis of staphylococcal osteomyelitis. Poult. Sci. 79:1042–1049.

Smith, H. W. 1954. Experimental staphylococcal infection in chickens. J. Pathol. Bacteriol. 67:81–87.

Sohail, M. U., A. Ijaz, M. S. Yousaf, K. Ashraf, H. Zaneb, M. Aleem, and H. Rehman. 2010. Alleviation of cyclic heat stress in broilers by dietary supplementation of mannan-oligosaccharide and *Lactobacillus*-based probiotic: Dynamics of cortisol, thyroid hormones, cholesterol, C-reactive protein, and humoral immunity. Poult. Sci. 89:1934–1938.

Sohail, M. U., M. E. Hume, J. A. Byrd, D. J. Nisbet, A. Ijaz, A. Sohail, M. Z. Shabbir, and H. Rehman. 2012. Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. Poult. Sci. 91:2235–2240.

Sohail, M. U., M. E. Hume, J. A. Byrd, D. J. Nisbet, M. Z. Shabbir, A. Ijaz, and H. Rehman. 2015. Molecular analysis of the caecal and tracheal microbiome of heat stressed broilers supplemented with prebiotic and probiotic. Avian Pathol. 44:67–74.

Somes, R. G., Jr. 1969. Genetic perosis in the domestic fowl. J. Hered. 60:163–166.

Song, J., K. Xiao, Y. L. Ke, L. F. Jiao, C. H. Hu, Q. Y. Diao, B. Shi, and X. T. Zou. 2014. Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress. Poult. Sci. 93:581–588.

Sorensen, P. 1992. The genetics of leg disorders. Pages 213–229 in Bone Biology and Skeletal Disorders in Poultry, C. C. Whitehead, ed. Carfax Publishing Company, Abingdon, United Kingdom.

Speers, D. J., and S. M. L. Nade. 1985. Ultrastructural studies of adherence of Staphylococcus aureus in experimental acute hematogenous osteomyelitis. Infection and Immunity 49:443–446.

Stalker, M. J., M. L. Brash, A. Weisz, R. M. Ouckama, and D. Slavic. 2010. Arthritis and osteomyelitis associated with *Enterococcus cecorum* infection in broiler and broiler breeder chickens in Ontario, Canada. J. Vet. Diag. Invest. 22:643–645.

Steinwender, G., G. Schimpl, B. Sixl, and H. H. Wenzl. 2001. Gut-derived bone infection in neonatal rat. Pediatr. Res. 50:767–771.

Tate, C. R., W. C. Mitchell, and R. G. Miller. 1993. *Staphylococcus hyicus* associated with turkey stifle joint osteomyelitis. Avian Dis. 37:905–907.

Thorp, B. H. 1986. Vascular pattern of the developing proximal femur in the domestic fowl. Res Vet. Sci. 40:231–235.

Thorp, B. H. 1988a. Vascular pattern of the developing knee joint in the domestic fowl. Res Vet. Sci. 44:89–99.

Thorp, B. H. 1988b. Vascular pattern of the developing intertarsal joint in the domestic fowl. Res Vet. Sci. 44:100–111.

Thorp, B. H. 1988c. Pattern of vascular canals in the bone extremities of the pelvic appendicular skeleton in broiler type fowl. Res. Vet Sci. 44:112–124.

Thorp, B. H. 1988d. Relationship between the rate of longitudinal bone growth and physeal thickness in the growing fowl. Res. Vet Sci. 45:83–85.

Thorp, B. H. 1994. Skeletal disorders in the fowl: a review. Avian Pathol. 23:203–236.

Thorp, B. H., and D. Waddington. 1997. Relationships between the bone pathologies, ash and mineral content of long bones in 35-day-old broiler chickens. Res. Vet. Sci. 62:67–73.

Thorp, B. H., C. C. Whitehead, L. Dick, J. M. Bradbury, R. C. Jones, and A. Wood. 1993. Proximal femoral degeneration in growing broiler fowl. Avian Pathol. 22:325–342.

Thorp, B.H., and S.R.I. Duff. 1988. Effect of unilateral weight-bearing on pelvic limb development in broiler fowls: vascular studies. Res. Vet. Sci. 44:164–174.

Trueta, J., and V. P. Amato. 1960. The vascular contribution to osteogenesis III. Changes in the growth cartilage caused by experimentally induced ischaemia. J. Bone Joint Surg. 42B:571–587.

Ulluwishewa, D., R. C. Anderson, W. C. McNabb, P. J. Moughan, J. M. Wells, and N. C. Roy. 2011. Regulation of tight junction permeability by intestinal bacteria and dietary components. J. Nutr. 141:769–776.

Vela, J., K. Hildebrandt, A. Metcalfe, H. Rempel, S. Bittman, E. Topp, and M. Diarra. 2012. Characterization of *Staphylococcus xylosus* isolated from broiler chicken barn aerosol. Poult. Sci. 91:3003–3012.

Walser, M. M., F. L. Cherms, and H. E. Dziuk. 1982. Osseous development and tibial dyschondroplasia in five lines of turkeys. Avian Dis. 26:265–270.

Waters, A. E., T. Contente-Cuomo, J. Buchhagen, C. M. Liu, L. Watson, K. Pierce, J. T. Foster, J. Bowers, E. M. Driebe, D. M. Engelthaler, P. S. Keim, and L. B. Price. 2011. Multi-drug resistant *Staphylococcus aureus* in US meat and poultry. Clin. Infect. Dis. 52:1227–1230.

Wideman, R. F., Jr. 2014. Bacterial chondronecrosis with osteomyelitis and lameness in broilers: pathogenesis, experimental models, and preventative treatments. Pages 91–128 in Probiotics in Poultry Production, Concept and Applications. W. H. A. Abdelrahman, ed. 5m Publishing Ltd., Sheffield, UK.

Wideman, R. F., Jr., A. Al-Rubaye, A. Gilley, D. Reynolds, H. Lester, D. Yoho, J. D. Hughes, Jr., and I. Y. Pevzner. 2013. Susceptibility of four commercial broiler crosses to lameness attributable to bacterial chondronecrosis with osteomyelitis. Poult. Sci. 92:2311–2325.

Wideman, R. F., Jr., A. Al-Rubaye, D. Reynolds, D. Yoho, H. Lester, C. Spencer, J. M. Hughes, and I. Y. Pevzner. 2014. Bacterial chondronecrosis with osteomyelitis (BCO) in broilers: Influence of sires and straight-run vs. sex-separate rearing. Poult. Sci. 93:1675–1687.

Wideman, R. F., Jr., A. Al-Rubaye, Y. M. Kwon, J. Blankenship, H. Lester, K. N. Mitchell, I. Y. Pevzner, T. Lohrmann, and J. Schleifer. 2015a. Prophylactic administration of a combined prebiotic and probiotic, or therapeutic administration of enrofloxacin, to reduce the incidence of bacterial chondronecrosis with osteomyelitis in broilers. Poult. Sci. 94:25–36.

Wideman, R. F., Jr., J. Blankenship, I. Y. Pevzner, and B. J. Turner. 2015b. Efficacy of 25-OH vitamin D_3 prophylactic administration for reducing lameness in broilers grown on wire flooring. Poult. Sci. 94:1821–1827.

Wideman, R. F., and I. Pevzner. 2012. Dexamethasone triggers lameness associated with necrosis of the proximal tibial head and proximal femoral head in broilers. Poult. Sci. 91:2464–2474.

Wideman, R. F., and R. D. Prisby. 2013. Bone circulatory disturbances in the development of spontaneous bacterial chondronecrosis with osteomyelitis: a translational model for the pathogenesis of femoral head necrosis. Frontiers in Science (Front. Endocrin.) 3:183. doi:10.3389/fendo.2012.00183.

Wideman, R.F., K.R. Hamal, J.M. Stark, J. Blankenship, H. Lester, K.N. Mitchell, G. Lorenzoni, and I. Pevzner. 2012. A wire flooring model for inducing lameness in broilers: Evaluation of probiotics as a prophylactic treatment. Poult Sci. 91:870–883.

Wijetunge, D. S., P. Dunn, E. Waller-Pendleton, V. Lintner, H. Lu, and S. Kariyawasam. 2012. Fingerprinting of poultry isolates of *Enterococcus cecorum* using three molecular typing methods. J. Vet. Diag. Invest. 24:1166–1171.

Williams, B., S. Solomon, D. Waddington, B. Thorp, and C. Farquharson. 2000. Skeletal development in meat-type chickens. Br. Poult. Sci. 41:141–149.

Wise, D. R. 1970a. Carcass conformation comparisons of growing broilers and laying strain chickens. Br. Poult. Sci. 11:325–332.

Wise, D. R. 1970b. Spondylolisthesis ('kinky back') in broiler chickens. Res. Vet. Sci. 11:447–451.

Wise, D. R. 1971. Staphylococcal osteomyelitis of the avian vertebral column. Res. Vet. Sci. 12:169–171.

Wise, D. R. 1973. The incidence and aetiology of avian spondylolisthesis (kinky-back). Res. Vet. Sci. 14:1–10.

Wise, D. R., and A. R. Jennings. 1973. The development and morphology of the growth plates of two long bones of the turkey. Res. Vet. Sci. 14:161–166.

Wyers, M., Y. Cherel, and G. Plassiart. 1991. Late clinical expression of lameness related to associated osteomyelitiand tibial dyschondroplasia in male breeding turkeys. Avian Dis. 35:408–414.

Xu, J., A. Wang, C. B. Toney, J. Seamon, and Q. Cui. 2010. Blood supply to the chicken femoral head. Comp. Med. 60:295–299.

Yair, R., Z. Uni, and R. Shahar. 2012. Bone characteristics of late-term embryonic and hatchling broilers: Bone development under extreme growth rate. Poult. Sci. 91:2614–2620.

Yalcin, S., S. Ozkan, E. Coskuner, G. Bilgen, Y. Delen, Y. Kurtulmus, and T. Tanyalcin. 2001. Effects of strain, maternal age and sex on morphological characteristics and composition of tibial bone in broilers. Br. Poult. Sci. 42:184–190.

Ytrehus, B., C. S. Carlson, N. Lundeheim, M. Mathisen, F. P. Reinholt, J. Teige, and S. Ekman. 2004b. Vascularisation and osteochondrosis of the epiphyseal growth cartilage of the distal femur in pigs - Development with age, growth rate, weight and joint shape. Bone 34:454–465.

Ytrehus, B., C.S. Carlson, and S. Ekman. 2007. Etiology and pathogenesis of osteochondrosis. Vet. Pathol. 44:429–448.

Ytrehus, B., H. H. Andreas, C. N. Mellum, C. S. Carlson, S. Ekman, J. Teige, and F. P. Reinholt. 2004a. Experimental ischemia of porcine growth cartilage produces lesions of osteochondrosis. J. Orthop. Res. 22:1201–1209.

Ytrehus, B., S. Ekman, C. S. Carlson, J. Teige, and F. P. Reinholt. 2004c. Focal changes in blood supply during normal epiphyseal growth are central in the pathogenesis of osteochondrosis in pigs. Bone 35:1294–1306.

Zhang, X., G. R. McDaniel, Z. S. Yalcin, and D. L. Kuhlers. 1995. Genetic correlations of tibial dyschondroplasiaincidence with carcass traits in broilers. Poult. Sci. 74:910–915.