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Bacteriological and Histopathological Evaluation of Articulations of Chickens Diagnosed with Arthritis

ABSTRACT

Arthritis implies inflammation of the intra-articular structures and is one of the causes of condemnation in broilers. The disease is associated with infectious agents such as *Staphylococcus aureus*, which may pose a potential public health risk. This work was conducted with the objective to determine the occurrence of arthritis in broiler chickens, predominantly of bacterial staphylococcal origin and if there is a difference between the degrees of injury regarding the presence of the bacterium and the histological lesion. Sixty samples of chickens diagnosed with arthritis by Federal Inspection Service from commercial slaughterhouse were collected according to their severity (of mild and severe degree) and submitted to bacteriological and histopathological analysis. There was bacterial growth of *Staphylococcus aureus* in 3.3% of the samples classified as mild degree in 10% of the samples classified as severe grade of lesion ($p=0,29$). For histopathological evaluation, 16.67% and 70% of the samples presented inflammatory infiltrate in mild and severe degrees, respectively ($p=0,0001$). Mild degree arthritis is related to non-infectious lesions in their higher prevalence while severe-grade arthritis has infectious causes. The condemnation criteria were efficient since *S. aureus* could be present regardless of the degree of the lesion diagnosed in arthritis.

INTRODUCTION

Locomotive disorders can be classified according to the underlying pathology as infectious, developmental and degenerative. Infectious disorders are related to necrosis of the femur head of bacterial origin, tenosynovitis, arthritis, and virus-induced neoplasia. There are numerous causes that lead to skeletal deformities in chickens, such as nutritional, genetic, pathogenic and management practices hampering the growth and development of poultry (Cook, 2000).

Infectious arthritis in broilers is of paramount importance because it causes economic losses of great impact due to the reduction of productivity, besides representing a sanitary problem (Reck *et al.*, 2012) and is one of the main causes of condemnation of birds in slaughterhouses (Moura *et al.* 2012; Oliveira *et al.*, 2016). *Staphylococcus aureus* is known to cause septic arthritis in poultry with significant economic losses and may pose a potential public health risk (Nazia *et al.*, 2015). This agent has high affinity for collagen-rich surfaces, such as the articular surfaces of joints, and synovial sheaths around joints and tendons (Joiner *et al.*, 2005). Arthritis or polyarthritis is defined in the condemnation as swelling of one (arthritis) or both (polyarthritis) of the knees (Lupo *et al.*, 2008).

Meat sanitary inspection aims to protect public health by ensuring that minimal hazardous material enters in the food chain through carcasses rejected (Huneau-Salaun *et al.*, 2015).



These carcasses, which are rejected as unfit for human consumption, are detected on the basis of macroscopic visual criteria and the condemnation operation is the responsibility and under the supervision of the official veterinary services (Lupo *et al.*, 2008). The identification of the etiological agents involved in the infectious arthritis facilitates preventive and control measures in the farms to reduce this pathology, avoiding economic losses due to the partial or total condemnation of the carcasses in the slaughterhouses (Costa *et al.*, 2016).

The aim of this study was to verify if the occurrence of arthritis in broilers is predominantly of bacterial staphylococcal origin and if there is a difference between the degrees of macroscopic lesion as to the presence of the bacterium and the histological lesion, thus providing subsidies to those involved in the work inspection.

MATERIAL AND METHODS

The study was conducted in a commercial slaughterhouse in the state of Santa Catarina, with a daily slaughter of 180.000 broilers/day 60 broiler carcass samples with partial arthritic condemnation were used, in which the evaluation and condemnation of the carcasses were carried out by the Federal Inspection Service in accordance with the criteria for carcass condemnation of poultry established by brazilian legislation (Brasil, 1998). The carcasses samples were collected from slaughtered broilers with 32 days of age and an average weight of 1.5 kg with macroscopic alterations in condemned tibiotarsial joints.

The birds were electrically stunned (50Hz, 100 mA for 12 s), bled out, scalded (60.5°C, 2 min), plucked, gutted automatically and picked in the federal inspection department (DIF). Each sample consisted of a single leg, classified by macroscopic changes, staining and volume of the lesion as described in table 1, after condemnation of the sanitary inspection (Barcelos *et al.*, 2006).

Table 1 – Classification macroscopic of the lesions of arthritis.

| | Staining | Volume |
|----------------------|----------------------------|------------------|
| Mild degree lesion | Light red | low volume |
| Severe degree lesion | Dark red to greenish color | increased volume |

The legs were stored in identified sterile bags and kept refrigerated (2 to 8°C) in thermal boxes for a maximum period of 24 hours.

The bacteriological and histopathological analyzes were performed at the Mercolab laboratory (Cascavel - PR). For bacteriological evaluation, the external surface of the joint was flamed, performing a cut above the joint and collecting with a swab for determination of *Staphylococcus aureus*. The swab was scored on blood agar plates. The already seeded plates were incubated inverted in a bacteriological oven at 37°C for 24-48 hours (Quinn *et al.*, 2005). The plaques that presented bacterial growth were smeared on glass slides for microscopy and stained for verification of the morphotintorial characteristics by the Gram method (Quinn *et al.*, 2005). The biochemical test of catalase was carried out from the colonies. After that, the biochemical tests were read and interpreted according to (Quinn *et al.*, 2005).

The epidermis, articular capsule, tendons and adjacent tissues were collected for histopathological processing. The samples were fixed in 10% buffered formalin solution, included in liquid paraffin, then cut into a microtome (5µm), stained with hematoxylin and eosin (H & E), and finally analyzed by an optical microscope (McManus & Mowry, 1965).

For the statistical analysis, the frequency of positive tests in the bacteriological and histopathological studies in relation to the total of samples tested was analyzed by the binary logistic regression test using Minitab software version 17.3.

RESULTS AND DISCUSSION

Of the 60 samples analyzed, there was bacterial growth for *Staphylococcus aureus* in 3.3% of the mild degree and 10% in the severe degree. There was no difference for *S. aureus* growth between the samples classified in mild and severe degree arthritis ($p=0.29$) (Table 2). However, lesions and heterophilic inflammatory infiltrates in the histopathological analyzes were significantly higher ($p=0.0001$) in the samples classified as severe degree arthritis (Table 2).

Table 2 – Binary logistic regression of number and percentage (%) of positive samples for bacterial growth and histopathological changes in arthritis samples diagnosed by sanitary inspection.

| | Mild degree lesion | | Severe degree lesion | | Value - P |
|-------------------|--------------------|---------|----------------------|---------|-----------|
| | N | % | N | % | |
| Bacteriological | 1 | 3.3% | 3 | 10.0% | 0,29 |
| Histopathological | 5 | 16.67 % | 21 | 70.00 % | 0,0001 |

The prevalence of *S. aureus* in the present study concur with Tsai *et al.* 2015, in which 15,5% isolates



from the arthritic joint of chicken were identified as *S. aureus* collected from chicken. However, it was lower than in previous studies varying according to the study. Rasheed (2011) and Joiner *et al.* (2005) detected isolates of *S. aureus* in 50.98% and 45,3% respectively from broilers with arthritis. Kibenge *et al.* (1982) and Nazia *et al.* (2015) reported that *S. aureus* was recovered in about 70% of the tendon fluid samples examined with macroscopic changes and hock joints, respectively. In animals, as in humans, *S. aureus* is found even in healthy carriers, and can induce a broad panel of infections ranging from superficial, such as mild to severe arthritis to deep infections and septicemia Peton & Le Loir (2014).

Table 3 – Histopathological features present in arthritic lesions diagnosed by Federal Inspection Service.

| Lesion characteristics | Mild level | Severe Level |
|---|-------------------|-------------------|
| | Number of samples | Number of Samples |
| Absence of significant lesions and infiltration | 25 | 9 |
| Discrete heterophilic inflammatory infiltrate | 2 | 9 |
| Moderate heterophilic inflammatory infiltrate | 3 | 5 |
| Intense heterophilic inflammatory infiltrate | 0 | 5 |
| Severe heterophilic inflammatory infiltrate | 0 | 2 |

Table 3 shows the distribution of the characteristics of the lesions present in articulations diagnosed with arthritis and Figure 1 shows the histopathological findings. All samples that were positive for *S. aureus* in the bacteriological study also had heterophilic inflammatory infiltrate, which confirms according to Daum *et al.* (1990) that septic arthritis is defined bacteriologically when *S. aureus* is isolated from the synovial surface and when heterophilic infiltrates are present in the synovial surface.

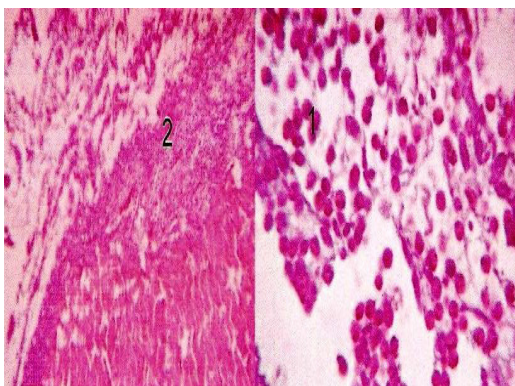


Figure 1 – Peritendinous area presenting mild (1) moderate (2) heterophilic inflammatory infiltrate.

The histological result of the severe degree samples agrees with the findings of Tsai *et al.* (2015) which detected alterations of heterophilic infiltration of the

synovial membrane, synovial fold, and tendon collagen in half of the arthritic cases. As in this study, Joiner *et al.* (2005) demonstrated the histomorphologic criteria confirmed for staphylococcal osteomyelitis and tenosynovitis of the lame chickens that had either negative bacterial cultures and / or no significant gross lesions.

The positive histopathological result along with the bacteriological absence may suggest that an infection by another etiological agent that was not detected in the bacteriological examination may have thus prompted a type of heterophilic inflammatory response in articulations with arthritis in severe degrees. It is hypothesized that arthritis and tenosynovitis may also be due to an early viral infection that usually remains subclinical and that secondary infection with bacteria, particularly *S. aureus*, may be responsible for the development of clinical signs (Kibenge *et al.*, 1982). Arthritis is also predisposed by infectious agents such as Reovirus (artrotropic) or *Mycoplasma synoviae*. This would justify the absence of bacteriological positivity. These agents have already been recovered from chicken tendon tissue with a concomitant bacterial infection. Although the pathology observed in chickens clinically affected is suggestive of a bacterial etiology (Kibenge *et al.*, 1982; Whitehead, 1992).

An absence of infectious agents is still possible, as Carlton & McGavin (1998) reported that sterile lesions of the articular cartilage may be a consequence of trauma, since the articular cartilage and the subchondral bone deform under pressure and the synovial membrane may respond to aggression by hypertrophy and hyperplasia of villi and lining cells. The mild injuries could be caused by traumas associated with rapid growth rates of modern chickens and thus not infectious (Julian, 1998, 2005). Tendon ruptures due to over weight results in damaging the affected part in the processing because the tip of the ruptured tendon exhibits grossly visible hemorrhage macroscopically with red, blue or green coloration on the tissue above the knee (Julian, 2005).

CONCLUSION

The mild lesions due to the low incidence of heterophilic and bacteriological infiltrates are non-infectious in their higher prevalence. The condemnation criteria was efficient, discarding legs with potential risk of transmission of diseases for consumers since *S. aureus* could be present regardless of the degree of the lesion diagnosed in arthritis.



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