Observations on Arthritis in Broiler Breeder Chickens Experimentally Infected with Staphylococcus aureus

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ABSTRACT
Staphylococcus aureus is the most common cause of bacterial arthritis in broiler breeder chickens. In this study, we established a model of broiler breeder chicken arthritis inoculated with Staph. aureus isolated from a spontaneously occurring bacterial arthritis in chickens. We evaluated the model by bacteriology, serology, pathology, and immunology. The results showed that 2.5 × 10^9 cfu Staph. aureus injected into the right joint cavity can successfully induce a chicken arthritis model. The majority of the infected chickens suffered lameness and joint swelling at 3 days post-inoculation (DPI). The death peak time was on 7 DPI and the mortality rate was 51.1%. Staph. aureus can be continuously isolated from the blood and left joint synovial fluid of the infected chickens. Lesions found on the infected chickens consisted of swollen joints full of caseous exudates, cartilage injury, and synovial membrane thickening with infiltration of inflammatory cells. The center of the lesion contained many round bacterial cocci. With joint injury aggravation, intra-articular hyaluronic acid gradually decreased, and serum interleukin-6 became significantly higher compared with the control (P<0.01) from 3 DPI. The results indicated that the chicken models of Staph. aureus-mediated arthritis were successful, and can be used to gain a better understanding of the host-bacterium relationship.

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INTRODUCTION

Cases of staphylococcosis in chickens are extensively reported worldwide since 1972. The incidence rate ranges from 0.5 to 20% (El-nasser et al., 1994; Huang et al., 2002; Wladyska et al., 2011). In 6-wk-old to 12-wk-old chickens, Staphylococcus arthritis caused by trauma is a chronic or secondary disease (Andreasen, 2003). Recent commercial and experimental flock analyses have revealed an increasing incidence of staphylococcal chondronecrosis with osteomyelitis in broilers (McNamee and Smyth, 2000; Weese et al., 2010; Fitzgerald, 2012). Other studies have also demonstrated that 20.4% of lame individuals in two commercial flocks had typical bacterial skeletal lesions that were primarily associated with Staphylococcus aureus (Joiner et al., 2005). Generalized leg weakness and lameness are the predominant economic and welfare concerns among poultry producers (McNamee and Smyth, 2000; Fitzgerald, 2012) and in large animals (Haerdi-Landerer et al., 2010). Studies on this disease report numerous cases, but its pathogenesis remains unclear. Despite the prevalence of the natural disease, staphylococcosis is difficult to reproduce experimentally. A recent successful model has used ad libitum feeding protocols and immunosuppression induced by the chicken infectious anemia virus and infectious bursal disease virus (McNamee et al., 1999). Wei et al. (1995) have suggested that skin injury around the joint may play a very important role in the development of bacterial chondronecrosis or arthritis. Hence, the current research aimed to develop a chicken model of Staph. aureus arthritis as similar as possible to its natural occurrence. An arthritis scale for pathogenesis research in future was also established.

§These authors contributed equally to this study.
**MATERIALS AND METHODS**

**Bacterial strains:** The *Staph. aureus* strain used in the present study was originally isolated from the joints of a spontaneously arthritic broiler (Huang *et al.*, 2002). The bacteria were cultured on blood agar for 24 h at 37°C, and then re inoculated on nutrient agar medium for 18 h at 37°C. From the culture, a bacterial suspension was prepared in sterile PBS at a concentration of $1 \times 10^{12}$ cfu/mL. Viable counts were used to check the concentration of the injected bacteria.

**Birds and experimental protocol:** Seven-wk-old broiler breeder chickens were obtained from Chia Tai China, Ltd. (Wuhan), and maintained in the animal facility of the Department of Veterinary Medicine, Huazhong Agricultural University. They were housed in isolation in wire-floored cages under standard conditions of light and temperature. They were fed with standard laboratory chow and water *ad libitum* for 1 wk.

Two independent in vivo animal experiments were performed in chronological sequence. Ninety animals (8 wk old) were randomly divided into nine groups by three injection methods (intramuscular, intravenous, and intra-articular). Each injection method contained three different dosages of the *Staph. aureus* suspension (high dose of $2.5 \times 10^{11}$ cfu, medium dose of $2.5 \times 10^9$ cfu, and low dose of $2.5 \times 10^7$ cfu). Occurrences of arthritis and mortality were monitored during the course of the experiment until the chickens were sacrificed after 21 days. The best inoculation condition was determined according to the first experiment results. In the second experiment, 70 broiler breeder chickens (8 wk old) were divided into a test (60 chickens) and control (10 chickens) group. The animals were weighed and inoculated with the *Staph. aureus* suspension selected by the above and an equivalent dose of sterile PBS, respectively. Weight changes, arthritic index, and mortality were monitored for 35 d. Blood samples (3 mL per chicken) were obtained via the wing-web at selected intervals (1, 3, 5, 7, 14, 21, 28, and 35 days post-inoculation, DPI) at 10 chickens per time point). Part of the blood sample was set aside for analyzizing bacterial load. The joint fluid of the chickens that was excised postmortem from dying or dead chickens was collected and examined for bacterial load and hyaluronic acid (HA). The surviving chickens were euthanized, and all joints were fixed for histopathological examination.

**Gross and histopathology:** Each chicken was individually labeled and monitored. Arthritis was defined as a visible erythema and/or swelling of at least one joint. To evaluate the severity of arthritis, we used clinical scoring (Bremell *et al.*, 1992), in which macroscopic inspection yields a score of 0 to 3 for each joint (0, normal; 1, mild swelling and/or erythema; 2, moderate swelling and erythema; and 3, marked swelling and occasional ankylosis). The resulting arthritic score ranged from 0 to 3 for each chicken.

Histopathological examination of the joints was performed after routine fixation, decalcification, and paraffin embedding. The tissue sections were cut by Histotome (RM2135 LEICA GER) and stained with hematoxylin-eosin (HE). The joints were examined for synovial hypertrophy as well as cartilage and subchondral bone destruction.

**Hyaluronic acid in synovial fluid and serum IL-6 examination:** According to literature (Cheng, 1987), the agglutination of synovial fluid in 4% acetic acid can determine HA content. To evaluate the severity of arthritics, we used clinical scoring, in which macroscopic inspection yields a score of 1 to 4 for each joint (4, forming complete clots, surrounding a clear solution; 3, soft clots, mild solution turbidity; 2, loose clots, surrounding a turbid solution; and 1, no clot formation, turbid suspension). Serum IL-6 was measured using an ELISA kit (PD6050, R&D Company, AM) according to the manufacturer’s specifications.

**Statistical analysis:** The results are presented as mean±SEM. SPSS program version 12.0 was used for data analysis. Differences between two groups were compared between two groups by Tukey’s test.

**RESULTS**

**Determination of best inoculation route and dose:** After inoculation with *Staph. aureus*, the appetite of the chickens remained normal until 2 DPI, and then gradually declined. The chickens exhibited gloominess, fever, swelling of some parts of the tibial tarsal joint, limpniness, and reluctance to move 5 DPI. Most chickens had empty ingluvies as well as wet feces adhered around the anus and abdomens. The bellies of the dead chickens lied on the ground with fluffy feathers.

Table 1 summarizes the chicken arthritis occurrence infected by different routes and doses infecting. High and medium-dose intravenous injections of *Staph. aureus* led to 100% and 50% experimental chicken mortalities, respectively, on 7 DPI. The low-dose injection triggered no effective arthritis (10%). No clinical symptom appeared after breast intramuscular injection. On the other hand, arthritis can be simulated by joint cavity injection, and mortality increased by 60% after high-dose injection. The low dose induced minor arthritis symptoms. Therefore, middle-dose ($2.5 \times 10^9$ cfu) intra-articular bacteria injection was used in subsequent experiments to simulate the animal model.

**Table 1:** Occurrence of arthritis in broiler chickens infected with *Staph. aureus* ($n = 10, \%$)

<table>
<thead>
<tr>
<th>Inoculation routes</th>
<th>No. of inoculation bacteria (cfu)</th>
<th>2.5 x 10^7</th>
<th>2.5 x 10^8</th>
<th>2.5 x 10^9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wing vein</td>
<td>Morbidity</td>
<td>0</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Mortality</td>
<td>100</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>Breast</td>
<td>Morbidity</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>intramuscular</td>
<td>Mortality</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Intra-articular</td>
<td>Morbidity</td>
<td>100</td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Mortality</td>
<td>60</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

**Arthritis model evaluation:** Based on the above mentioned results, the definitive conditions for an animal model evaluation were determined. The evaluation results of the chicken arthritis are shown in Table 2. By right articular cavity bacteria injection, most chickens
Bacteriological examination: *Staph. aureus* was isolated from cultured samples of synovial fluid in the chickens. Table 2 shows that *Staph. aureus* was isolated from the right joints of all dying or dead chickens, and 91.3% was isolated from the left. The number of bacteria isolated from the left joint was higher than that from the right in the same chicken.

From 1 DPI to 28 DPI, the periphery blood samples from the experimental chickens can be examined for bacteria. At 3 DPI, 90% of the chickens carried the bacteria, gradually declining at 5 DPI. The total number of bacteria isolated from 1 DPI sharply increased, peaked at 5 DPI, and then gradually decreased. No bacterium was isolated from chickens in the control group.

**Body weight (BW) changes:** The BW changes of the chickens following inoculation with *Staph. aureus* remained the same as that pre-inoculation (Table 2). No significant difference was observed between the pre- and post-experimental BWs (P>0.05). However, the BW gain of the control chickens showed a high significant difference (P<0.01) at 7 DPI compared with that pre-inoculation (Table 2). No significant difference was observed in the BW changes of the test chickens (P>0.05).

**Histological findings:** Microscopic examination showed joint skin subcutaneous edema, intravascular congestion at 5 DPI (Fig. 1-3), serous effusion with a large number of heterophil infiltration (Fig. 1-4), as well as necrotic cartilage cells on the articular surface that fell off and were replaced with accumulating inflammatory cells at 7 DPI (Fig. 1-5). Synovial hyperplasia or degeneration caused the necrosis to fall off, and infiltration with inflammatory cells at 14 DPI was observed (Figs. 1-6 and 1-7). Necrotic foci were found in the bone marrow cavity and ligaments. Scattered in the foci were a large number of degenerating or necrotic heterophils and bacteria cocci. Inflammatory cells infiltrated the blood vessels and elastic fibers, seriously gathering into large septic foci in the articular capsule wall at 28 DPI (Fig. 1-8).

**Inflammatory response:** The serum IL-6 levels of the infected chickens 1 wk PI rapidly increased from 0.37 μg/mL to 2.24 μg/mL. Compared with the control group, there were only significant differences (P<0.01) at 3 and 5 DPI, as well as from 7 to 14 DPI (Fig. 2).

**DISCUSSION**

Chicken *Staphylococcus* arthritis often occurs in 6-wk-old to 8-wk-old broiler breeder chickens (Huang et al., 2002; Liu and Ning, 2006; Wladyka et al., 2011). Most models of *Staphylococcus* septic arthritis in rabbit (Hamel et al., 2008), mouse (Gjerstsson et al., 2005; Sakiniene and Takowski, 2002), swine (Johansen et al., 2012) and chicken (Daum et al., 1990) have been established by intravenous inoculation with *Staph. aureus*. In the present study, the inoculated pathogen *Staph. aureus* was also isolated from the swollen joint of a naturally infected chicken (Huang et al., 2002). Based on preliminary trials, the best simulated chicken arthritis model for intra-articular injection with 2.5 × 10⁹ cfu per chicken was determined. Joint cavity infection can be concluded as more closely resembling natural infection, scilicet, as well as mechanical injury on joints that lead to bacterial adhesion and invasion (Elasri et al., 2002; Weese et al., 2010), which is mostly due to the large weights of broiler breeder chickens (Liu and Ning, 2006).

The artificial infection and spontaneous outbreak of arthritis in the animals showed consistent clinical symptoms (Cai et al., 2008; Wladyka et al., 2011). Upon autopsy, intra-articular exudates appeared early including pus which related to the Panton–Valentine leukocidin secreted by *Staph. Aureus* (Nguyen et al., 2010), and synovial hyperplasia appeared later. The right arthritis index was significantly higher than the left in the chickens inoculated with *Staph. aureus*. This finding was different from that in a mouse model that had four limp consistent lesions of *Staphyllococcus* arthritis by intravenous inoculation (Gjerstsson et al., 2005; Sakiniene and Takowski, 2002) owing to the two different inoculation routes. HA in the joint cavity is a polymeride secreted by synovial cells, and HA content in synovia can indicate synovial injury (Qing et al., 2008). In the present study,
The intra-articular HA content in the dead and dying chickens inoculated with *Staph. aureus* were obviously lower than that in the control group, which may have a direct correlation with the arthritic score.

The number of bacteria in the blood of the chickens gradually increased. At 7 DPI, the number of bacteria in the joint cavity was slightly lower than that in the left joint, which may have a direct correlation with the arthritic score.

The number of bacteria in the blood of the chickens changed progressively decreased. However, at 14 DPI, the inflammatory-related cells in the chicken were activated, subsequently releasing inflammatory substances that lead to joint damage. Moreover, lipoteichoic acid of *Staph. aureus* enhances IL-6 expression in activated human basophils (Jeona et al., 2012). This phenomenon requires confirmation by further research based on the present model.

**Conclusion:** The present work demonstrated that $2.5 \times 10^9$ cfu *Staph. aureus* injected into the right joint cavity can successfully establish an arthritis model in chicken. Injury to the joint directly injected with bacteria is more serious than that by other injection routes.

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**REFERENCES**


