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RESEARCH ARTICLE

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% (EI-nasser *et al*., 1994; Huang *et al*., 2002; Wladyka *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.

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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 reinoculated 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 1×10^{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 2.5×10^{11} cfu, medium dose of 2.5×10^{9} cfu, and low dose of 2.5×10^{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 centrifugation, and the sera were stored at -20°C for cytokine analysis. The remaining blood sample was set aside for analyzing 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 arthritis, 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, limpness, 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 (2.5×10^{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* ($n = 10$, %)

Inoculation routes		No. of inoculation bacteria (cfu)		
		2.5×10^{11}	2.5×10^{9}	2.5×10^{7}
Wing vein	Morbidity	0	20	10
	Mortality	100	50	10
Breast intramuscular	Morbidity	10	0	0
	Mortality	0	0	0
Intra-articular	Morbidity	100	60	30
	Mortality	60	10	0

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

Table 2: Findings in the joints of chickens after intra-articular injection of 2.5×10^9 *Staph. aureus* cells

Time after Inoculation (day)	No. of Chickens that Died (Total Mortality %)	Arthritis Morbidity (%)		Arthritis Coefficient		Joint HA Content		No. of Synovia with Bacteria		No. of Blood with Bacteria (Average No. 10^3 CFU/mL)	BW Changes (kg)	
		Right	Left	Right	Left	Right	Left	Right	Left		Test	Control
1	0 (0)	100	0	0.83±0.54**	0	-	-	-	-	9 (2.34)	2.12±0.17	2.10±0.25
3	0 (0)	88.89	26.67	1.20±0.75**	0.54±0.44	-	-	-	-	9 (2.66)	-	-
5	3 (6.67)	84.44	44.44	1.61±0.92*	1.12±0.77	1.67	1.00	3	3	5 (3.15)	-	-
7	4 (15.56)	77.78	51.11	2.28±1.03*	1.82±1.02	2.00	1.50	4	4	4 (1.43)	$2.02±0.26^{ΔΔ}$	2.45±0.28
14	5 (26.67)	71.11	55.56	2.33±0.97	1.89±0.88	1.60	1.00	5	4	2 (1.07)	$1.92±0.24^{ΔΔ}$	3.14±0.25
21	5 (37.78)	64.44	55.56	1.87±1.01	1.75±0.92	1.80	1.40	5	5	2 (0.57)	$2.11±0.45^{ΔΔ}$	3.35±0.49
28	5 (48.89)	55.56	48.89	1.55±0.83	1.34±0.61	1.33	1.90	5	4	2 (0.19)	$2.03±0.45^{ΔΔ}$	3.81±0.52
35	1 (51.11)	55.56	37.78	1.20±0.46*	0.84±0.33	3.00	2.00	1	1	0 (0)	$2.24±0.41^{ΔΔ}$	4.08±0.46

Note: "-" Not done. Comparison with the left joint ("**" P<0.01, "*" P<0.05); Comparison with the control group ("^{ΔΔ}" P<0.01).

displayed visible single or double joint limp at 3 DPI, especially at 5 DPI. The incidence of right arthritis was significantly different from that of left arthritis (P<0.05). The arthritis score on the right was higher than that on the left in all chickens at any time. At 14 DPI, the injury of the left joint healed, and a scab formed on the skin (Fig. 1-1). The right articular cartilage completely fell off, the medullary cavity became exposed, and the left articular cartilage suffered central necrosis at 21 DPI (Fig. 1-2).

The test chickens began to die at 5 DPI, the death peak was 4 wk post-injection (PI), and the death rate was 51.1%. Table 2 shows that the amount of synovial fluid HA of the test chickens was lower than that before the infection. All the dead chickens had inoculated bacteria synovial fluid HA contents higher in the left joint than in the right.

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 of the test chickens.

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 2.5×10^9 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 (EIasri *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 *Staphylococcus* 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,

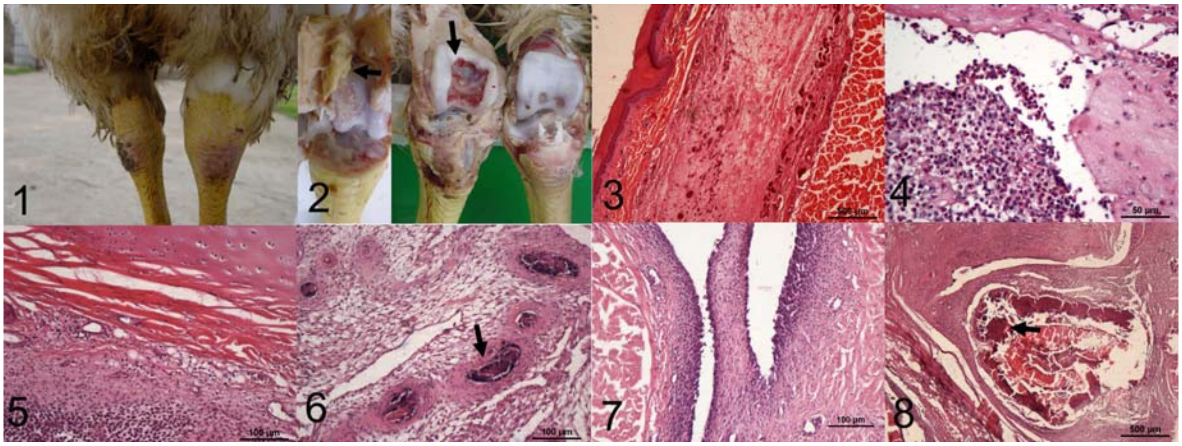

Fig. 1: Articular lesions in chickens inoculated with *Staph. aureus* by intra-articular injection. The right joint swelled and the left joint compensatory injury resulted in scar formation on the skin at 14 days post-inoculation (DPI; Fig. 1-1). Left articular cartilage injury and caseous exudatein (arrow) occurred in the joint cavity at 7 DPI. The middle articular cartilage completely fell off, the medullary cavity of bones became exposed (arrow) at 21 DPI, and the right articular cartilage was slightly injury in live chickens at 35 DPI (Fig. 1-2). HE staining in a light micrograph reveals chicken joint skin subcutaneous edema and intravascular congestion at 5 DPI. Bar = 500 μm (Fig. 1-3). Intra-articular serous effusion and a large number of heterophil infiltrations appeared at 7 DPI. Bar = 50 μm (Fig. 1-4). Marked proliferation of synovial tissues and those infiltrated with inflammatory cells. Bar = 100 μm (Fig. 1-5). Synovial cell necrosis accompanied with a lot of bacterial embolism (arrow) at 14 DPI. Bar = 100 μm (Fig. 1-6). A marked proliferation of synovial tissue appeared at 21 DPI. Bar = 100 μm (Fig. 1-7). Focal necrosis formed and large masses of bacteria diffused (arrow). Bar = 500 μm (Fig. 1-8).

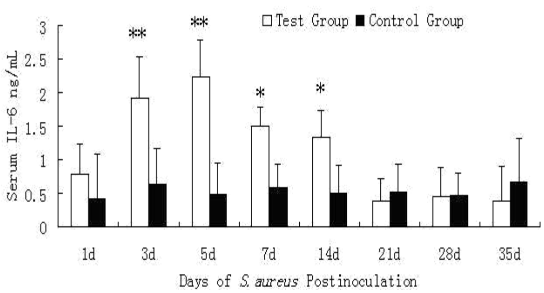

Fig. 2: Serum cytokine was determined by ELISA at different time points from chickens infected with *Staphylococcus aureus*. *P < 0.05 and **P<0.01 compared with the control.

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 blood changed progressively decreased. However, *Staph. aureus* can also reach the left joint via a hematogenous route. Once in the joint, the inflammatory response and resulting infiltration of leukocytes, as well as the swelling and degradation of cartilage were similar to those in septic arthritis caused by *Staph. aureus* in mice (Bremell *et al*., 1992; Johansen *et al*, 2012). Therefore, the bacteria isolated from both right and left joints may prove that *Staph. aureus* is drawn to joints (Wei *et al*., 1995). The presence of intra-articular bacteria is almost consistent with the symptoms of arthritis. Pathogens are often isolated from the joints of naturally infected chickens (Huang *et al*., 2002; Liu and Ning, 2006; Yang *et al*., 2006; Grahama *et al*., 2009; Wright *et al*., 2010).

Cytokines such as interleukin-6 play crucial roles in mice *Staphylococcus* arthritis (Bremell *et al*., 1992; Wright *et al*., 2010; Johansen *et al*, 2012). In the present study, the chicken interleukin-6 level at 3 DPI had a rapid growing trend and a certain relationship with arthritis development throughout the trial. However, the serum IL-6 level began to decrease from 14 DPI. Once the bacteria entered the body, 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 2.5×10^9 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

Andreasen CB, 2003. Staphylococcosis. In: Diseases of Poultry; 11th Ed, (Saif YM, ed), Iowa State Press, Ames, IA, 798-804.

Bremell T, A Abdelnour and A Tarkawski, 1992. Histopathological and serological progression of experimental Staphylococcus aureus arthritis. Infect immune, 60: 2976-2985.

Cai XY, C Yang, ZY Zhang, WL Qiu, Q Ha and M Zhu, 2008. A murine model for septic arthritis of the temporomandibular joint. J Oral Maxillofacial Surg, 66: 864-869.

Cheng Z, 1987. Synovial fluid laboratory examination. Chin J Clin Lab Sci, 1: 36-38.

Daum RS, W Hodges-Davis, K Barton-Farris, RJ Campeau, DM Mulvihill and SM Shane, 1990. A model of staphylococcus aureus bacteraemia, septic arthritis and osteomyelitis in chickens. J Orthop Res, 8: 804-813.

Elasri MO, JR Thomas, RA Skinner, JS Blevins, KE Beemken, CL Nelson and MS Smeltzer, 2002. Staphylococcus aureus collagen adhesin contributes to the pathogenesis of osteomyelitis. Bone 30: 275-280.

El-nasser AA, FM Mohmoud and LM El-shabzny,1994. Studies on major bacterial agents causing arthritis in chickens in kaluobia province. Vet Med J Giza, 13: 277-285.

Fitzgerald JR, 2012. Livestock-associated *Staphylococcus aureus*: origin, evolution and public health threat. Trends Microbiol 20: 192-198.

Grahama JP, SL Evans, LB Price and EK Silbergeld, 2009. Fate of antimicrobial-resistant enterococci and staphylococci and resistance determinants in stored poultry litter. Environmental Research, 109: 682–689

Gjertsson I, M Innocenti, LM Matrisian and A Tarkowski, 2005. Metallopoteinase-7 contributes to joint destruction in staphylococcus aureus induced arthritis. Microbiol Pathogen 38: 97-105.

Haerdi-Landerer MC, J Habermacher, B Wenger, MM Suter and A Steiner, 2010. Slow release antibiotics for treatment of septic arthritis in large animals. Vet J, 184: 14-20.

Hamel A, J Caillon, C Jacqueline, E Batard and G Potel, 2008. Efficacy of quinupristin/dalfopristin versus vancomycin, clone or in combination with rifampicin, against methicillin-resistant Staphylococcus aureus in a rabbit arthritis model. Int J Antimicrob Agents 31: 158-160.

Huang JG, XY Hu, GF Cheng, SQ Zhou and NH Song, 2002. The diagnosis of staphylococcus arthritis in breeding broilers. Hubei Agric Sci 4: 78-79.

Jeona JH, SK Kimb, JE Baikb, SS Kangb, CH Yuna, DK Chung and SH Hanb, 2012. Lipoteichoic acid of *Staphylococcus aureus* enhances IL-6 expression in activated human basophils. Comp Immunol Microbiol Infect Dis, 35: 363-374.

Johansen LK, J Koch, D Frees, B Aalbæk, OL Nielsen, PS Leifsson, TM Iburg, E Svalastoga, LE Buelund, T Bjarnsholt, N Høiby and HE Jensen, 2012. Pathology and Biofilm Formation in a Porcine Model of Staphylococcal Osteomyelitis. J Comp Path, 147: 343-353.

Joiner KS, FJ Hoerr, E van Santen and SJ Ewald, 2005. The avian major histocompatibility complex influences bacterial skeletal disease in broiler breeder chickens. Vet Pathol, 42: 275-281.

Liu XM and GB Ning, 2006. Investigation on staphylococcus arthritis of chicken. Beifang Muye, 8: 68-69.

McNamee PT, JJ McCullagh, JD Rodgers, BH Thorp, HJ Ball, TJ Connor, D McConaghy and JA Smyth, 1999. Development of an experimental model of bacterial chondronecrosis with osteomyelitis in broilers following exposure *Staphylococcus aureus* by aerosol and inoculation with chicken anaemia and infectious bursul disease viruses. Avi Pathol, 28: 26-35.

McNamee PT and JA Smyth, 2000. Bacterial chondronecrosis with osteomyelitis ('femoral head necrosis') of broiler chickens: a review. Avi Pathol 29: 253-270.

Nguyen HM, MA Rocha, KR Chintalacharuvu and DO Beenhouwer, 2010. Detection and quantification of Panton-Valentine leukocidin in *Staphylococcus aureus* cultures by ELISA and Western blotting: Diethylpyrocarbonate inhibits binding of protein A to IgG. J Immunol Methods 356: 1-5.

Qing XN, X Long and JR Li, 2008. Clinical study of sodium hyaluronate acid injection for treatment of temporomandibular joint osteoarthrosis. J Oral Sci Res, 24: 845-846.

Sakiniene E and A Tarkowski, 2002. Low molecular weight heparin aggravates infectious arthritis triggered by *staphylococcus aureus*. J Orthop Res, 20: 198-203.

Weese JS and E van Duijkeren, 2010. Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in veterinary medicine. Vet Microbiol, 140: 418-429.

Wei HJ, KY Cheng, CP Cheng and ZL Nin, 1995. Experiments on portal of infection of arthritis type staphyloccosis in chicken. Journal of Hunan Agricultural College, 21: 89-92.

Wladyka B, G Dubin and D Adam, 2011. Activation mechanism of thiol protease precursor from broiler chicken specific *Staphylococcus aureus* strain CH-91. Vet Microbiol 147:195-199.

Wright JA and SP Nair, 2010. Interaction of staphylococci with bone. Int J Med Microbiol, 300: 193-204.

Yang QM, XC Yang, F Wang, YL Sun, XH Deng, 2006. Bionomics of staphylococci inducing arthritis in chicken. Vet Res, 192: 19-21.