

## Evaluation of Antiseptic Role of One Nanosilver Based Drug as a New Therapeutic Method for Treatment of Bumblefoot in Pheasant (*Phasianus colchicus*)

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**Abstract:** Bumblefoot is a general term for any inflammation or degenerative condition of the avian foot. The infection is usually chronic and responds poorly to antimicrobial therapy or immunization. For study of nanosilver effect on treatment of these lesions, 200 pheasants (*Phasianus colchicus*) with clinically lameness and footpad inflammation were randomly selected from different farms of pheasants in central of Iran. 90 out of 200 pheasants were affected to grade 3 of bumblefoot with positive *S. aureus* in blood and ulcer culture. This 90 pheasants divided into 3 groups with 3 replicate. Group1 treated with no antibiotic (as control), group2 treated with systemic piperacillin and group 3 treated with systemic piperacillin with a local spray of one nanosilver (SilvoSept). Results represented, after treatments, blood cultures in groups 1, 2 and 3 were 100%, 63.3% and 23.3% positive for *Staphylococcus aureus*, respectively and ulcer cultures in groups 1, 2 and 3 were 100%, 83.3% and 16.6% positive for *S. aureus*, respectively. The difference between before treatment and after treatment was significant. Also, after treatment, the difference between groups 2 and 3 was significant. The results demonstrate that nanoparticle of Silver can be have antimicrobial effect on *S. aureus in vitro* and *in vivo* condition and can be useful for treatment of *S. aureus* infection in skin and local lesions in birds. It was concluded that systemic piperacillin with local SilvoSept can be effective in control of bumblefoot caused with *S. aureus* infection in pheasant.

**Key words:** Pheasant • Bumblefoot • *Staphylococcus aureus* • Nanosilver

### INTRODUCTION

Bumblefoot or pododermatitis has been reported in many species of birds [1] and occurs frequently in the birds [2]. Bumblefoot is a general term for any inflammation or degenerative condition of the avian foot and may range from very mild redness or swelling to chronic, deep-seated abscesses and bony changes [3]. It is believed to be caused by rough and hard surfaces that caused by trauma to the bottom of the bird's feet [2]. *Staphylococcus* species especially *Staphylococcus aureus* are frequently isolated from the lesions [1]. *S. aureus* may successfully invade through the body surfaces

(Skin and mucous membranes) because of tissue damage or because of their own ability to colonize and multiply in the presence of predisposing factors such as viral infections, stress, Immunosuppression or starvation [4]. The infection can extend directly to deep support structures, such as bones and joints, leading to primary osteomyelitis and synovitis [5]. Staphylococcal septicemia causes acute death in birds [6]. The infection is usually chronic and responds poorly to antimicrobial therapy or immunization [6]. The aim of this study is to evaluate and compare indication of antibiotic with nanosilver in treatment of pheasant (*Phasianus colchicus*) affected to bumblefoot with *S. aureus* infection.

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## MATERIALS AND METHODS

Two hundred pheasants (*Phasianus colchicus*), 16 weeks of age, with clinically mild lameness and swelling of footpad were randomly selected from some commercial pheasant farms in central areas of Iran. 90 out of 200 pheasants selected for examination by same grade lesions (grade 3) [3]. The birds were housed on floor standard management procedure and feed were provided based on NRC requirement (1994). Feed and water was provided *ad libitum*.

In start of experiment 0.5 ml of heparinized blood was collected from brachial vein of each bird to determine if *S.aureus* was present in the blood. For this, 0.5 ml of heparinized blood was inoculated into sterile tubes containing 4.5 ml of TSB. The tubes were incubated at 37°C for 24 hours. A loop of culture was streaked onto Bacto Baird-parker's selective agar (Bacto is a trademark of Difco Laboratories, division of Becton, Dickinson and Company). Agar plates were incubated aerobically at 37°C and examined after 24 hours and again at 48 hours for black shiny convex colonies with narrow white margins surrounded by clear zones. The colonies by this property defined as egg yolk positive [7]. Also, these colonies were Gram stained and tested for production of coagulase. Egg yolk positive, coagulase positive and Gram positive isolates were classified as *S. aureus* [4]. Furthermore, all birds that were positive in blood culture were examined for ulcer culture of footpad. The pheasants that were positive for *S. aureus* in blood culture and ulcer culture, selected for examination and divided to 3 groups and in each group 3 replicates were assigned (9 pens, with 10 pheasants on each). Group 1 treated with no antibiotic, group 2 and 3 treated with systemic antibiotic (Intramuscular injection of piperacillin sodium (150 mg/kg b.wt. for 10 days), only group 3 was given one local spray of nanosilver (SilvoSept, ChitoTech company, Iran) on infectious area for 3 times per day, for 10 days, 0.5 µg/ml. After 10 days, blood and ulcer culture from 3 groups were examined for *S. aureus*, as above described. Furthermore, a control *in vitro* test for effectiveness of SilvoSep was done.

For this, 45 Bacto Baird-parker's selective agars were sprayed with SilvoSept and 45 same agars were not sprayed with SilvoSept. The entire medium have cultured with *S. aureus* that were isolated from blood of pheasant at start of experiment. After incubation on 37°C for 48 hours, the growth of *S. aureus* on Baird-parker was investigated.

The positivity percent of ulcer culture and blood culture in before and after treatment was compare with McNemar test and the differences were significant at the level of P value less than 0.05. The mean differences in all groups were analysed with one way ANOVA test. The SPSS 16.0 statistical software (SPSS, Chicago, IL), were used and differences were considered significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

Before treatment, 90 out of 200 pheasants with lameness and swelling of footpad by grade 3 lesion were positive for *S. aureus* in blood and ulcer culture simultaneously. Therefore before treatments, all pheasants (100%) were positive in blood and ulcer culture. After different treatments of 90 pheasants in 3 groups, blood cultures in groups 1, 2 and 3 were 100%, 63.3% and 23.3% positive for *S. aureus*, respectively and ulcer cultures in groups 1, 2 and 3 were 100%, 83.3% and 16.6% positive for *S. aureus*, respectively (Table 1).

The difference between before treatment and after treatment was significant ( $p < 0.05$ ). These findings indicate that treatment with piperacillin alone and piperacillin with SilvoSept can be effective in bumblefoot caused with *S. aureus* infection.

After treatment, the difference between groups 2 and 3 was significant ( $p < 0.05$ ). The finding indicate treatment by piperacillin simultaneous with SilvoSept can reduce the load of *S. aureus* in blood and foot ulcer but treatment with piperacillin alone can decrease *S. aureus* population from blood and treatment with SilvoSept alone can reduce local *S. aureus* colonization.

Table 1: The number and Percentage of positive *S.aureus* culture, after treatment.

Groups (Total culture)	Positive Ulcer Culture		Positive Blood Culture	
	Number	Percent	Number	Percent
1 (n=30)	30	100	30	100
2 (n=30)	25	83.33	19	63.33
3 (n=30)	5	16.66	7	23.33

In this study, in all cases of *S. aureus* isolation, only coagulase positive strains were investigated because only coagulase positive strains isolated from clinical material are considered pathogenic [8].

In this study did not occur any death in period of experiment. Although, mortality and morbidity rates of bumblefoot caused by *S. aureus* are usually low but in acute cases and in the absence of treatment, mortality rates of up to 60% have been reported [9]. Antimicrobial agents are, in fact, widely used in the treatment and control of Staphylococcal infection in poultry [10]. Treatment of avian *S. aureus* skin infections required a long term approach, often 4 to 6 weeks [11]. One report explained, oxacillin suspension administered 100 mg/kg orally 3 times a day provides better results than quinolones or amoxicillin/clavulanic acid combination [11]. Erythromycin was reported as the choice treatment for the effective control of the disease, administered in water for 3 to 5 days or in feed at the rates of 200 g/ton [12]. However, the disease is usually chronic and responds poorly to antimicrobial therapy [6]. One common treatment is a topically applied combination of dimethylsulfoxide (DMSO) (30ml), dexamethasone (2mg) and appropriate antibiotics based on sensitivity, every 8 hours for 3 to 6 weeks [2]. As far as aware, no information is available concerning the nanotherapy in *S. aureus* infection in pheasant.

Our results shown no bacterial growth occurred in any culture that were sprayed with silvosept. This study clearly demonstrate that nanoparticle of Silver can be have antimicrobial effect on *S. aureus in vitro* and *in vivo* condition and can open a way to nanotherapy of *S. aureus* infection in skin and local lesions in birds.

It was concluded that local nanosilver compound and systemic antibiotic e.g. local SilvoSept plus systemic piperacillin can be effective in control of bumblefoot caused with *S. aureus* infection in pheasant and same effect in other birds must be investigated.

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