Systemic staphylococcosis in partridge chicks

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Keklik civcivlerinde sistemik staphylococcosis

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Öz

Gereç ve Yöntem: Alectoris chukar (18 gün) sistemik staphylococcosis, damar içi canlı Staphylococcus aureus (10⁶ cfu/mL) uygulaması ile gerçekleştirildi.


Öneri: Hayvanlarda gözlenen farklı klinik belirtiler ve lezyonlar keklik civcivlerinin sistemik staphylococcosise duyarlığını göstermektedir.

Anahtar kelimeler: Alectoris chukar, keklik, patoloji, staphylococcosis

Abstract
Aim: Staphylococcus infection is primarily caused by Staphylococcus aureus in poultry. Because little information was available in the literature about game birds experiencing staphylococcosis, this investigation was undertaken to evaluate the disease in partridge chicks.

Materials and Methods: Systemic staphylococcosis was induced in 18 days old Alectoris chukar by intravenous injection of a suspension containing 10⁶ cfu/mL of viable Staphylococcus aureus organisms.

Results: Injection resulted in a fever response after 30-hours post-inoculation. The disease rapidly developed over the 48-hour period with potentially devastating consequences, including huddling, lethargy, ruffled feathers, decreased activity, unable to stand, wing droop, decreased feed and water consumption, growth depression and lameness. Gross and histopathological examination of internal organs revealed that the injuries originally occurred in the kidneys, liver, spleen, lungs, alimentary tract, pancreas, heart, brain, joints and tendons sheaths. Lesions of systemic staphylococcal infection in partridge chicks consist of necrosis, vascular congestion, thrombosis, abscess formation, arthritis, synovitis and marked heterophilic infiltration in affected organs.

Conclusion: The diversity of clinical signs and lesions observed suggests the susceptibility of chukar partridge chicks to systemic staphylococcosis.

Keywords: Alectoris chukar, partridge, pathology, staphylococcosis
Staphylococcosis is common in human, animals and birds (Fitzgerald 2014, Peton et al 2014). The infection is primarily caused by *Staphylococcus aureus*. Infection of man by *Staphylococcus aureus* produces a variety of clinical diseases ranging from mild infection of the skin, to severe systemic diseases such as septicaemia and death. Staphylococcal food-borne disease is resulting from the production of heat-stable enterotoxins in uncooked or inadequately refrigerated foods (Hennekinne et al 2012).

In birds, the disease manifests itself as the septicaemia, gangrenous dermatitis, granulomas and localized infection in various organs in duding bones, tendons sheaths, joints, sternal bursa, skin, yolk sac, eyelid, heart, vertebrae, testes, liver and lungs (Monleon et al 2008, Chenier et al 2012, Andreasen 2013).

*Staphylococcus* spp. are ubiquitous and have been associated with poultry diseases throughout the world. The organisms are the normal inhabitant of skin and mucous membranes and are common environmental bacteria where poultry are hatched and reared. Some species are also considered to be normal flora. Infection can occur in birds following a breakdown in the natural defense mechanisms of the host such as a skin wound or inflamed mucous membrane. The open navel of newly hatched chicks, vaccinations and beak or toe trimming, may offer additional routes of entry for bacteria (Corrand et al 2012, Andreasen 2013). The game birds industry has experienced tremendous development and expansion during recent years (Tavakkoli et al 2013, Tavakkoli et al 2014a). The infectious diseases of these species have always been a major concern. Because little information was available in the literature about game birds experiencing staphylococcosis, this investigation was undertaken to evaluate inducing systemic staphylococcosis in young chukar partridge and to provide a description of the systemic form of the disease in this bird. We believe that the results of this study will contribute to our better understanding of the bacterial diseases of the game bird species.

**Materials and Methods**

**Birds**

Day-old chukar partridge chicks (*Alectoris chukar*) were obtained from a commercial breeder farm whose birds were kept and grown up under the standard condition of breeding. The birds were maintained in an electrically heated battery (Belderchin Damavand Co. PLC-DQSH, Iran) at 33°C with feed and water available ad libitum. The feed was formulated according to the nutritional requirements of the partridge chicks from which all medications had been omitted. At 18 days of age, 30 birds were selected for the experiment on the basis of their overall appearance and body weight uniformity. The sampled birds were in good health status. Furthermore, clinical examination of the bird showed no signs of other diseases.

**Preparation of bacterial suspension**

The inoculum was prepared from *Staphylococcus aureus* (ATCC 33591) strain in Luria-Bertani broth (Invitrogen, Paisley, Scotland) at 37°C for 24 hours. The broth culture was centrifuged (2 min at 2500 g) and the remaining pellet was washed three times with sterile phosphate buffered saline. After the last washing, the cell suspension was adjusted by optical density to 10⁶ cfu/mL (Thammavongsa et al 2009).

**Experimental design**

The partridge chicks were randomly assigned to two groups, of 15 birds each, as follows: One group served as the control, and the other group was inoculated with *Staphylococcus aureus*. At 18 days of age, the birds were injected intravenously via the left jugular vein with 0.5 mL of prepared suspension of *Staphylococcus aureus*, while the control birds received 0.5 mL of sterile phosphate buffered saline. The experiment was performed according to the suggested European ethical guidelines for the care of animals in experimental investigations and approved (No: 425-PAD) by the Animal Ethics Committee of the Research Council of Shahid Bahonar University, Iran.

**Measurements**

Body temperature (cloacal temperature), body weight and mortality were recorded daily. The birds were also monitored daily for clinical signs, abnormal behavior and gross symptoms during the experimental period.

**Pathology**

Five days after inoculation, the birds were euthanized by a lethal dose of thiopental sodium (Sandoze GmbH, Kundl-Austria) and necropsies were performed. Gross pathological lesions were recorded. Samples of kidneys, liver, spleen, lungs, alimentary tract, pancreas, heart, neural tissue, joints and tendons sheaths were removed and fixed in 10% neutral buffered formalin. Following routine preparation of tissues, serial sections of paraffin-embedded tissues of 5 μm thicknesses were cut using a microtome (Slee-Germany), then stained with hematoxylin and eosin (H&E) and studied under a light microscope.

**Microbiological examination**

Prior to euthanasia, blood samples were collected aseptically from the brachial vein and 50 µL of peripheral blood
plated on blood agar. Immediately after euthanasia, samples of liver, kidneys, spleen and joints (tibiotarsal joints) were taken by searing the tissue surfaces with a hot spatula and inserting a sterile metal loop through the seared area. The collected samples were also cultured on the above-mentioned medium. Plates were incubated at 37°C for 48 hours and examined for bacterial growth. The tests for detection and confirmation of *Staphylococcus aureus* were Gram staining, hemolysis, catalase production, coagulase and mannitol fermentation tests (Markey et al 2013).

**Statistical analysis**

The analysis of variance was used to determine the significant differences in body weight between experimental groups (SPSS version 20.0). A P-value of <0.05 was considered as statistically significant (Tavakkoli et al 2014b).

**Results**

**Body temperature**

The normal body temperature of partridge chicks, before the inoculation of bacteria, was 41.3±0.3°C. The initial effect following intravenous injection of *Staphylococcus aureus* was a marked increase in the body temperature, which was occurred 30-hours after injection. Body temperature continued to increase for another 24 hours (≥ 42°C in some cases). Body temperature was decreased, in cases where the death occurred, several hours before the death.

**Body weight**

A marked depression in the growth rate of the inoculated bird occurred for 5 days after injection. Table 1 shows the body weight of the injected and control birds. The statistical analysis revealed that *Staphylococcus aureus* caused severe depression in the growth of infected birds throughout the 5 days of the experimental period (P<0.05).

**Clinical signs**

All of the inoculated partridges exhibited clinical signs by the second day post-inoculation. The early symptoms manifested themselves as huddling, lethargy, ruffled feathers, and decreased activity. Feed and water consumption were significantly reduced. Following the progression of the disease, some affected birds were unable to stand and maintained their posture with drooping of one or both wings (Figure 1). Lameness in one or both legs was also seen in 27% of sick birds. The sensory organs appeared to be unaffected, although it was difficult to evaluate the auditory response. No mortality was occurred during the experimental period in the inoculated partridges. No clinical signs were observed in any of the control group birds.

### Table 1: Effect of intravenous injection of *Staphylococcus aureus* on body weight of chukar partridge chicks (Mean±SEM).

<table>
<thead>
<tr>
<th>Day</th>
<th>Control group</th>
<th>Infected group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>52.2±0.88a</td>
<td>52.5±1.01a</td>
</tr>
<tr>
<td>5</td>
<td>81.8±0.81a</td>
<td>57.5±1.6b</td>
</tr>
</tbody>
</table>

a,b: Statistically significant in the column (P<0.05).

**Gross lesions**

Gross lesions were observed in kidneys, liver, spleen, lungs, alimentary tract, heart, joints and tendons sheaths (Table 2). In all birds inoculated with *Staphylococcus aureus*, vascular congestion in many internal organs including the kidneys, liver, spleen, and lungs was noticed. The kidneys were swollen and dark, the liver and spleen exhibited a dark discoloration and, in some instances, green liver discoloration with multiple, small foci of necrosis were seen (Figure 2). Involvement of the lungs was indicated by congestion. The alimentary tract was often devoid of food and most frequently, congestion was also seen on the alimentary tract surfaces. In some affected birds, petechial hemorrhages were occurred in epicardial fat (Figure 3). Swollen hock joint with the extension of inflammatory yellow-exudates along tendons sheaths were common in chickens exhibited signs of lameness (Figure 4). Carcasses of affected birds were generally dark-colored. On the brain surfaces, no gross lesions were observed.

**Microscopic lesions**

Microscopic lesions at the level of light microscopy originally occurred in the kidneys, liver, spleen, lungs, alimentary tract, pancreas, heart, brain, joints and tendons (Table 3). In affected birds, congestion, hemorrhage and thrombosis in many internal organs including the kidneys, liver, spleen, lungs, pancreas and brain were common. In some instances, microabscesses were evident in the kidneys, lungs, the muscular layer of the gizzard and intestinal mucosa (Figures 5-8). Histologically, microabscess composed of large numbers of dark purple bacterial colonies, areas of necrosis and inflammatory cell infiltration (particularly the heterophils). When the joints and tendons (particularly the tibiotarsal joints) were affected, synovitis, abscess formation with bacterial colonies and dense infiltration of heterophils were seen (Figure 9). Microbiological examination Cultures of blood, liver, kidneys, and spleen were positive for *Staphylococcus aureus* growth. Cultures of joints were positive for *Staphylococcus aureus* in birds experiencing lameness. The blood and tissue samples of the control birds were negative for microbial contamination.

**Discussion**

The present findings indicate that systemic staphylococc-
Staphylococcosis in partridges

Andreasen et al. (1991) showed that the incubation period of systemic staphylococcosis is short in chickens. Following experimental infection by intravenous inoculation of susceptible chickens, clinical signs were evident 48-72 hours post-inoculation. Continuous monitoring of the body temperature revealed that it significantly increased after 30-hours post-inoculation. Based on this result, it is concluded that the incubation period for systemic staphylococcosis in partridge chicks is about 30-hours following infection by intravenous route. It is reported that an acute disease can be caused by an organism with a short incubation period and high replication rate in a susceptible bird (Collett 2013). Therefore, systemic staphylococcosis in partridge chicks is an acute disease due to its short incubation period. Furthermore, in our study, the disease was characterized by a rapid progression of clinical signs over a 48-96 hour period. Under such period, the disease rapidly developed with potentially devastating consequences, including decreased activity, lethargy, ruffled feathers, huddling, unable to stand, wing droop and decreased feed and water consumption (Figure 1).

**Table 2. Gross lesions in the various tissues of the chukar partridge chicks following intravenous injection of Staphylococcus aureus.**

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Gross lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidneys</td>
<td>Congestion, swollen, dark discoloration</td>
</tr>
<tr>
<td>Liver</td>
<td>Congestion, dark discoloration, small foci of necrosis</td>
</tr>
<tr>
<td>Spleen</td>
<td>Congestion, dark discoloration, small foci of necrosis</td>
</tr>
<tr>
<td>Lungs</td>
<td>Congestion</td>
</tr>
<tr>
<td>Alimentary tract</td>
<td>Petechial hemorrhages in epicardial fat</td>
</tr>
<tr>
<td>Heart</td>
<td>Swollen hock joint, extension of exudates along tendons sheaths</td>
</tr>
<tr>
<td>Joint</td>
<td></td>
</tr>
</tbody>
</table>
inoculation.

The second indicator of the systemic staphylococcosis in chukar partridge was the severe growth depression in birds as young as 18 days old. After 5 days post-inoculation, an analysis of variance of body weights revealed that *Staphylococcus aureus* caused severe growth depression (Table 1).

In the present study, the induced staphylococcosis was characterized by septicaemia in the inoculated partridge chicks. Various gross abnormalities (Figures 2-4) and histopathological lesions (Figures 5-9) were observed in multiple tissues of the birds. Lesions of systemic staphylococcal infection in partridge chicks consist of necrosis, vascular congestion, thrombosis, abscess formation, arthritis, synovitis and marked heterophilic infiltration in affected organs. Macroscopic and microscopic lesions in the various tissues of the chukar partridge chicks following intravenous injection of *Staphylococcus aureus* are summarized in Tables 2 and 3. Macroscopic lesions in brain tissues following the injection of partridges were mostly inapparent, but the histopathological examination of the brain tissue revealed congestion and thrombosis. Experimentally induced staphylococcosis as well as naturally occurring disease have been reported in chickens (Mutalib et al 1983a, McNamee et al 2000, Jiang et al 2015), turkeys (Linares et al 2001, Corrand et al 2012), pet birds (Briscoe et al 2008, Huynh et al 2014, Iverson et al 2015), birds of prey (Poorbaghi et al 2012, Bezjian 2014), waterfowl (Degernes et al 2011, Mondal et al 2014), and other avian species (Le-
lesions observed during induced systemic staphylococcosis may be the result of the mechanical influence of *Staphylococcus aureus* in the affected organs since, cultures of affected tissues yielded various levels of *Staphylococcus aureus*.

Another hypothesis to account for the injuries of the affected organs would be the toxin production. It is shown that *Staphylococcus aureus* contains the protein-A, which is a cell-wall component, implicated in the immunogenicity and may be a virulence factor (Mutalib et al 1983b, Pauli et al 2014). Furthermore, there are other toxins and enzymes produced by *Staphylococcus aureus*, which seem to correlate with the pathogenesis. These chemicals including, protease, hemolysins, leukocidin, lipase, hyaluronidase, deoxyribonuclease, dermonecrotic toxin, fibrinolysin, exfoliative toxins, and enterotoxins (Andreasen et al 1993b, Wilson et al 2011, Gould et al 2012, Du Toit 2014). In addition to the so-called toxins and enzymes, certain inherent properties of *Staphylococcus aureus* have been postulated to be associated with its effects. For example, Haden et al. (2007) showed that *Staphylococcus aureus* sepsis induces mitochondrial biogenesis in some tissues. Histologically, the heterophilic infiltration was seen in affected organs following systemic staphylococcosis in partridge chicks. Andreasen et al. (1993a) demonstrated that supernatants of pathogenic *Staphylococcus aureus* resulted in increased chemotaxis of heterophils. This finding can be correlated with the heterophilic infiltration. Now, new efforts are required to find new biochemicals and mechanisms that might be of great relevance in pathogenesis of *Staphylococcus aureus* in partridge.

**Conclusion**

The diversity of clinical signs and lesions that were observed suggests the susceptibility of partridge chicks to intravenous inoculation of *Staphylococcus aureus*. The alterations may be due to different factors including mechanical influence, toxin or toxic by-product and mechanisms, which contribute to the overall virulence of the organism.

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