EXPERIMENTAL STAPHYLOCOCCAL INFECTION IN BUDGERIGAR
(Melopsittacus undulatus) – DIAGNOSTICS AND THERAPY

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ABSTRACT

Staphylococcal infection in the budgerigar is one of the most common diseases in this species of animal. Studies have been carried out using various methods of experimentally infecting budgerigars with Staphylococcus aureus. The course of the disease, the clinical manifestation of staphylococcosis, as well as the pathological-anatomical changes are followed. Studies have been conducted on the effect of antibiotic therapy, depending on the stage of the infection.

Key words: budgerigar, infection, Staphylococcus aureus, diagnostics, treatment.

Introduction

Bacteria of the genus Staphylococcus are the microorganisms that most commonly cause bacterial parrot infections. Reasons for this are the widespread prevalence of these bacteria in the external environment, on the skin, the mucous membranes of the birds, and their high resistance to the influence of external factors. Many staphylococci are found to be normal microflora for parrots and become pathogenic in stress associated with immune failure and secondary bacteraemia.

The genus Staphylococcus belongs to the family Micrococcaceae. It includes 26 species, 14 of which are isolated from birds. S. aureus is considered to be obligate pathogenic. The remaining species have different pathogenicity depending on the bird species or the immune status of the macro-organism. Of the parrots, S. intermedius, S. saprophyticus, S. haemolyticus, S. hyicus and S. gallinarum are often isolated (Kozlitin, 2017). The major pathogenicity factors, particularly well-exhibited in S. aureus, are exotoxins and enzymes. Toxins are alpha, beta, gamma and delta hemolysins, leucocidin, dermolitic toxin, enterotoxins and others. Exoenzymes are coagulase, fibrinolysin, hyaluronidase, penicillinases and others. Pathogenic strains of staphylococci are usually coagulase positive. Coagulase negative strains are rarely pathogenic or have reduced pathogenicity. Very important for pathogenicity is the factor of thrombo formation. Staphylococci are most resistant among all cocci. They die in humid heat of 80° C at exposure for at least 30 minutes. Many of the staphylococci isolated from birds are resistant to penicillin, streptomycin, and amphenicols (Devrieze et al., 1994; Obreshkov et al., 1978, Popova, 2016; Kaprelyan et al., 1986).

In birds, infections caused by staphylococci are exogenous and endogenous. Exogenous occur when the bacteria invade an open skin or mucous membrane wound, or through the umbilical opening in the chickens. Endogenous most often occur as complications of mold or viral diseases after colonization of the respiratory tract and subsequent septicemia. The disease can be artificially re-
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produced by venous injection of staphylococcal suspension (Obreshkov et al., 1978). The pathological anatomy finding is dependent on the course of the disease (Forbes and Migallon Guzman, 2017; Ritchie et al., 1994).

Staphylococcal infections in parrots are poorly studied. That's why, the aim of the present study was to investigate the clinical and pathological changes in budgerigars after experimental infection with *Staphylococcus aureus*, to conduct microbiological studies and to track the effect of therapy with some modern antibacterial agents.

**Materials and methods**

*Experimental animals.* In the experiments, ten budgerigars (*Melopsittacus undulatus*) at 9 months of age (Fig. 1) were used, of which five were male and five female. The animals were divided by the color into two groups of five green and five blue in cages. Their body mass ranged from 44 to 46 grams. All birds were obtained from a parrot farm in Sofia with registration number 1554-0157. The parrots were fed with "Parrot prestige" from Versele laga twice a day with drinking water at will and under equal zoo-hygienic conditions on the territory of the clinic.

*Clinical tests.* After a seven-day adaptation period, the two groups of birds were subjected to a thorough clinical examination to establish their health status at the time of initiation of the trial. One bird from the green group was not well accepted by the others, constantly chased and driven away from the food. The examination (Fig. 1) included inspection, auscultation, thermometry, microscopic studies by Gram of faecal swabs, ultrasound of abdominal organs, blood samples (from vena jugularis) for whole blood tests (WBT) and biochemistry, deworming. All experimental birds were dewormed externally with Pulmomectine (Pantex, Holland) containing 0.12 mg Ivermectine in 1 ml of the solution.

![Figure 1: Common flock (a), echography (b) and thermometry of the birds (c).](image)

*Microbiological studies.*

*Nutrient media.* Blood agar, *S. aureus* agar, agar of Mueller Hinton, Mac Conkey and Sabouraud with chloramphenicol (BUL BIO NCIPD Ltd. - Bulgaria), as well as Colorex orientation agar (HiMeida Laboratories Pvt. Ltd. Mumbai India, acquired by Ridadom-Sofia) were used.
**Taxonomic identification** of the isolated bacteria was performed by microscopic examination of Gram stained preparations, taking into account the cultural features and biochemical parameters by means of Staphy test 24 (Erbalachema) and microtests (HiMaida Laboratories Pvt. Ltd. Mumbai India, acquired by Ridadom-Sofia). The isolation and identification of the bacteria was performed in accordance with the Bergey’s Manual of Determinative Bacteriology (Holt et al., 1994).

**Receiving of a pathogenic strain of Staphylococcus aureus.** For the purpose of the experiment, the acquisition and multiplication of a field pathogenic strain of *S. aureus* was necessary. The strain was isolated from a sick parrot *P. erithacus* with severe necrotic foot pyoderma. The bird was brought with a worsened general condition and a high degree of self-injury in the area of the joints, feet and tibiotarsus. After taking a sample of the affected areas with a sterile swab, a seeding on blood agar, Mac Conkey, Colorex Orientation agar, and Sabouraud agar with chloramphenicol was made. The Petri dishes with seedings were placed in a thermostat for incubation at 37° C for 48 hours. The preparation of the bacterial strain to infect the recipients included also suspending it in saline solution. Dilution was done with the Mac Ferland 2 standard, which corresponds to a concentration of 6x10⁸ cells/ml.

**Determination of the sensitivity** of isolated bacteria to antimicrobial agents was performed using the classic agar-gel diffusion method of Bauer et al. (1966). Standard discs for antibioticograms (Ridacom-Sofia) were used after inoculation of a bacterial suspension in an exponential growth phase at a concentration of 2.10⁶ cells/ml on Muller-Hinton agar. Cultivation was performed at 37° C for 24 hours. The results are interpreted by the three-step system of Bauer et al. (1966) after measuring the diameters of the inhibition zones in millimeters.

**Oral infection of the birds.** To nine budgerigars were given by 1.0 ml of the staphylococcus suspension with the help of a hand-feeding parrot probe (Fig. 2). One of the blue birds was inoculated directly from the agar culture without dilution. The stress of the birds was minimal and there were no incidents.

![Image of oral infestation with a probe](image-url)

**Parenteral infection of the birds.** Ten days after regaining the normal general condition some of the surviving birds were subjected to parenteral infection with *S. aureus*. Two of the blue and two
of the green parrots were inoculated intravenous. For this purpose, the animals were anesthetized with Isoflurane (by inhalation anesthesia apparatus, “Dreger Sula”). After the right vein was found, therein was injected by 0.1 ml of the suspension prepared of *S. aureus* diluted in saline by Mac Ferland standard 2 (6x10^8 cells/ml). The same dose was also given intramuscularly to two birds and subcutaneously to two more birds, of which two blue and two green. The idea of subcutaneous infection was to simulate wound infection, one of the common causes of staphylococcal diseases in parrots.

**Histopathological tests.** After surgical excision tissue samples of liver, heart and intestines from parrots with staphylococcal infection were routinely fixed in 10% buffered formalin, rinsed in water and after dehydration in graded ethanol and xylene clearing, materials were embedded in paraffin. Tissue sections (3–5 µm thick) were stained in H&E and examined by a light microscope (Leica DM 5000B, Wetzlar, Germany). The sections were examined standardly for the presence of characteristic features for toxic organ lesions and inflammation and the microscopic observations were evaluated.

**Results**

**Clinical examination** of experimental birds prior to infection. The inspection revealed a normal general condition, expressed as movement everywhere in the cells, vocalization, food search, active protection when captured. The feathering, the skin, the visible mucous membranes were in the normal parameters. Auscultation did not detect any noises in the lungs and air sacs. Internal body temperature ranged from 39.9–42.4° C for all individuals. The microscopic studies of the Gram stained fecal slides from each parrot showed a normal flora of Gram-positive lactobacilli, a lack of Gram-negative colli forms and megabacteria (*Macrorhabdus ornitogaster*). No abnormal changes in liver, spleen, muscle stomach, and genital organs have been detected at the abdominal echography. For this purpose, a sonography device, Chison ECO 1, was used.


**Evaluation of a pathogenic strain of S. aureus.** At reading the result, tipical for *S. aureus* smooth and round S-colonies with golden-yellow color were observed on blood agar and Colorex orientation agar, having strong catalase activity. Growth on Mac Conkey and Sabouraud agar was not detected. Colonies of the growth were taken and placed on *S. aureus* agar and incubated again for 48 hours in a thermostat. The characteristic growth of this bacterium on this agar, with a specific pale violet color, was readed (Fig. 3 a). In Gram-stained smears were observed coccus-shaped, violet-colored bacteria in large clusters formations (Fig. 3 b). Identification of the species was also carried out with a “Staphytest 24”, in which the bacterium was identified as *Staphylococcus aureus subs. aureus* (Fig. 3 c).
Figure 3: Typical growth of *Staphylococcus aureus* on *S. aureus* agar (a), microscopic preparation by Gram (b) and species identification with *Staphyltest 24* (c).

**Determination of the sensitivity** of the isolated bacteria to antimicrobials. The results of the antibioticogram are presented in Table 1.

**Table 1. Sensitivity of the isolated bacteria to antimicrobial means *in vitro***

<table>
<thead>
<tr>
<th>Antimicrobial mean</th>
<th>Contents of the disc (μg)</th>
<th>Sensitivity of the strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>10 u</td>
<td>R</td>
</tr>
<tr>
<td>Amoxicilin+Clavulanic acid</td>
<td>10</td>
<td>I</td>
</tr>
<tr>
<td>Linco-spectin</td>
<td>15+200</td>
<td>S</td>
</tr>
<tr>
<td>Tylosin</td>
<td>15</td>
<td>R</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>75</td>
<td>R</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>5</td>
<td>S</td>
</tr>
<tr>
<td>Marbofloxacin</td>
<td>5</td>
<td>I</td>
</tr>
<tr>
<td>Pradofloxacin</td>
<td>5</td>
<td>S</td>
</tr>
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*S* – sensitive; *I* – intermediate; *R* – resistant

**Oral infection of the birds.** During the two days following the infection, a change in the general condition of the experimental animals was not observed. On the third day, however, there was some change in the behavior of the birds. All were quieter, more drowsy, slightly bristling, with a strong lousing. Some of the parrots had mild diarrhea and in Gram stained faecal smears, deviations from the normal microflora were observed - increase in the total number of microorganisms in a field of vision, as well as a relative increase in Gram-negative bacteria. Most of the birds swiftly scraped the area of the throat. Taking food and water from everyone was normal. The internal body temperature was within the normal range for the species. Two more days later, most of the parrots regained their usual behavior, with the exception of one, the "green" bird, which was constantly harassed from the others. It was bristling, refusing food and water. At catching with a hand it did not resist. His weight was 25 grams, and the internal body temperature was 37.4°C. The next day it was dead. The following changes were noted in the autopsy: the external view revealed a strong cachexia. The
sternum stood out strongly, with the amount of pectoral muscles being minimal. The feathers in the anus area were heavily polluted with fecal masses, and were easily plucked off. After opening the abdomen, there was a strong hyperemia of all internal organs and numerous hemorrhages on the liver, intestine, pancreas (Fig. 4 a). There were purulent foci on air sacs and lung edema. Catarrhal enteritis with hemorrhages was found in the lining of the intestine. Numerous petechial hemorrhages were also observed in the lining of the trachea and the endocardium. A heart and lung culture was made on blood agar and \textit{S. aureus} agar. After 24 hours there was a strong growth of the bacteria on the nutrient media, which undoubtedly proved that it was the cause of the bird's death.

![Image of parrot autopsy](image)

**Figure 4:** Autopsy of the first dead parrot (a), strong cachexia (b); hyperemia of the internal organs and catarrhal enteritis (c) on the third day after parenteral infection with \textit{S. aureus}.

The result of oral infection showed high resistance of parrots to oral \textit{S. aureus} infection despite the strong pathogenicity of the strain and the massive amount of the given infectious agent. The birds themselves overcome the disease, developing only short-term septicemia. Only the parrot that was in poor condition because of the harassment of other birds, probably with reduced resistance and weakened immunity, developed severe disease.

**Parenteral infection.** At the twentieth hour of the inoculation of the pathogen, the intravenously infected birds showed signs of septicemia. And the fours were bristling, wings downed low down, lack of vocalisation, they had very little desire for food. They were sitting next to the food bowls, but they did not eat. They had polyuria. One of the green parrots had diarrhea, and one of the blue - fast breathing. The Gram faecal smear showed increased microbial content, with Gram negative bacteria predominance. The internal body temperature was from 39.0° C to 39.2° C. In the blood tests for WBT, only a white blood cell reaction was found, with leukocytes reaching 60 000/ml, as well as strong monocytosis.

**The treatment** of the blue birds was started by oral administration of suspension of Veraflox (Pradofloxacin 25 mg/ml), 0.05 ml once daily. The nutrition was with a mash for manual feeding Roudybush 2 ml per os, three times a day. They were placed in a controlled environment (in cuvious at 28° C and 70% humidity). Green parrots were treated under the same conditions but without
giving them the antibiotic. 24 hours after the onset of treatment in the blue birds there was a visible improvement, with increased movement in the cage, more frequent feeding, vocalization, and body temperature increase to 40.5° C. The green birds were getting worse, standing at the bottom of the cage motionless, their body temperature had fallen to 38° C. On the morning of the third day, both were dead.

Pathoanatomically, a highly cachexic carcass was observed (Fig. 4 b, c), the body mass was 26 grams. The air sacs and internal organs were covered with hemorrhages, such were observed and under the epicardium. A strong catarrhal enteritis was found in the intestine. The liver and spleen were highly hyperemic. As a result of the cultures of blood agar, S. aureus agar and Colorex orientation agar, a pure culture of S. aureus was isolated (Fig. 5 a, b) and the species was biochemically confirmed.

![Figure 5: Colonies of Staphylococcus aureus on blood agar in cardiac culture (a) and in air sacs culture (b); treatment with a Veraflo suspension of a bird from the group of the treated (c).](image)

The parrots of which the bacterium was inoculated subcutaneously and intramuscularly within 20 days showed no clinical signs, there was no deviation in their physiological parameters. From the taken throat secretions for control cultures, there was no growth of staphylococci on the agars, id est there was no development of even subclinical infection.

Histopathology. The results from the histological analyses are shown on figures 6, 7 and 8. In the liver morphological features of acute liver injury were found: apoptosis and necrosis of hepatocytes and the presence of congestion of the central veins and hepatic sinusoids and detectable dilatation of the last. Histopathological findings in small intestines were mainly focused in columnar mucosal epithelium and lamina propria in the proximal region of villi intestinales. Also apoptotic cells with pycnotic nuclei were observed in the covering epithelium of the villi. Heart muscle did not show any observable changes.
Figure 6: Liver of a parrot infected with *S. aureus*. Notable centilobular parenchymal lesions (4-point star) (A); intranuclear vacuolations (v) and intracytoplasmic vacuolations (vv) (B, C, D); congestion of the central vein and hepatic sinusoids and dilatation (5-point star) (A, B); margination of the chromatin (blue arrow) (E), ballooning degeneration of hepatocytes (d) (B, C); group cell necrosis (n) (B); karyocytomegaly (m) (B, C, E); cell fusions (f) (B, C, D); apoptotic bodies (a) (B, E). H&E
Figure 7: Duodenum of a parrot infected with *S. aureus*. Congestion of mucosal blood vessels (4-point star) (A), edema in proximal region of villi (blue arrow) (B, D); detachment of the epithelium and its basement membrane from the underlying tissues, increasing spaces to *lamina propria mucosae* (red arrow) (A, B, C, D); apoptotic cells (black arrow) (B, C). H&E.

Figure 8: Myocardium of a parrot infected with *S. aureus*. Normal morphology (A, B). H&E

The treatment with Veraflox lasted for twenty days (Fig. 5 c). The sick birds have completely recovered. Their control cultures from throat samples on the environments used were completely sterile. The dose of the preparation (15 mg/kg body weight) and the frequency of its administration have been successfully determined - at 24 h interval, at which an antimicrobial effect occurs without adverse side effects for the patient.
Discussion

According to a number of authors (Harrison and Lidhtfood, 2006; Tully and Dorrestein, 2009; Doneley, 2016), birds are relatively resistant to staphylococcal infections. A significant immunosuppression is required to develop a disease. External environmental factors such as bad zoo-hygienic conditions, stress from change in husbandry conditions, owner change, avitaminosis, and poor nutrition can become a prerequisite for disease development. It starts in a sharp form. If the bird is not treated but is survived, it usually goes into chronic infection for years.

Our results confirm those of some authors (Kozlitin, 2017; Sakas, 2002), according to which staphylococcal infections always start after immunosuppression. Data from current studies indicate that intravenous injection of a staphylococcus suspension necessarily leads to infection but other methods of infection - only in the presence of predisposing factors. This is in line with the outcomes of Obreshkov et al. (1978). As is known, the immunity at passing the disease is perishable. The bird may suffer a second time in a relatively short time (Kozlitin, 2017; Samour, 2016). The data from our research confirms this. We have found that it is not difficult to reproduce a staph. infection in birds subjected to such an infection shortly before.

The results from the histological analyses exhibited morphological features of acute liver injury: apoptosis and necrosis of hepatocytes and the presence of congestion of the central veins and hepatic sinusoids and detectable dilatation of the last. Perhaps toxins induced hemodynamic changes led to moderate hepatic congestion and by mechanical clamping made bile ducts invisible. This obstruction of the duct system especially affecting the intrabiliary system - Hering duct system could has concomitant impact on the dual blood supply of the affected liver. The hallmarks of the acute hepatic injury found were the centilobular parenchymal lesions-hepatocellular apoptoses and focal necroses. Common findings concerning the parenchymal functions and deterioration were the observed intracytoplasmic and intranuclear vacuolations, degeneration of the nuclei or margination of the chromatin, ballooning degeneration of hepatocytes - clear cytoplasm without nuclear displacement and apoptotic bodies all confirming the apoptotic changes. In some areas signs for necrosis were found - karyorehxis and spotty necrosis affecting single cells or confluent group cell necrosis. The cytoplasmic vacuolations observed could be caused by the oxidative damages of the membrane lipids and other cellular component of cells during the focal dissolution of hepatic cords. The lipid peroxidation is crucial in the pathogenesis of tissue injury. The degeneration of hepatocytes attributed to Reactive Oxygen Species which is a cellular response to the action of xenobiotics and bacterial toxins released. The appearance of apoptotic hepatocytes indicated very recent hepatocyte damage as kupffer’s cells reaction was not obvious in the region. Absence of fibrotic changes was evidential for very rapid processes too. One of the main findings was the presence of pleomorphism of the hepatic cells with karyocytomegaly and some signs of cell fusions-like processes. Cell fusion is supposed to be a road to polyploidization in the liver, which process has not been extensively investigated, and its contribution to a variety of conditions, such as infections, carcinogenesis and aging still remains unclear (Lizier et al., 2018). The acute hepatocellular injury resulted in rare cell proliferations reacting in direction to regenerate- biunucleated hepatocytes proliferating the bile ductules, which is common symptom of a toxic shock syndrome. Necroinflammatory activity with plasma cells and neutrophils was not observed, along with low kupffer’s cells content, perhaps of the early stage of liver injury and fast lethal end. The acute hepatic failure affecting more of the parenchyma often complicates with metabolic pathways injury and hepatic encephalopathy and death.
All the lesions in small intestines were perhaps connected to haemodynamic changes by strong congestion and dilatation of blood vessels. This led to congestive phenomena and mild edema of the mucosa of villi, detachment of the epithelium and basement membrane and increased spaces to the underlying structures of lamina propria mucosae. Structural damage observed here in the intestinal mucosa of proximal villi could have crucial role affecting mucosal barrier functions and absorption of nutrients.

Our results show that it is essential for an accurate diagnosis to confirm it microbiologically by taking material for seeding from affected internal organs, yolk bladder, skin. It is necessary to distinguish pathogenic staphylococci from non-pathogenic by the coagulation test of rabbit plasma. Staphylococcosis is required to differentiate from escherichiosis, streptococcosis, klebsielliosis, acute parasitic and viral infections.

The treatment of staph. infections starts with antibiotics after a mandatory antibioticgram. Our results show that pradofloxacin is a reliable preparation for the treatment of staph infections in parrots. Also important is the use of immunostimulants, probiotics and painkillers. At skin lesions is applied topically iodine preparations, such with chlorhexidine, dyes. If there is a lot of necrotic tissue, surgical removal is required.

The results of our observations in the course of current studies confirm the view of most authors (Doneley, 2016; Forbes and Gusman, 2017; Kozlitin, 2017) that stressful conditions in parrots necessarily lead to a weakening of immunity. According to our observations, feeding on birds and the family environment are perhaps the most important factors in maintaining good bird health. Parrots successfully conceal their clinical signs, and good looks are not a guarantee of good health. Therefore, with a view to successful overall prevention, it is important to provide to parrots quality food, preferably by leading manufacturers in the industry. It is necessary to provide optimal zoo hygienic conditions for cultivation, with emphasis on noise, light, cleanliness and rodent control. It is essential to comply with the ecological requirements for each species of parrot. We also recommend conducting a prophylactic examination and basic tests, at least once a year.

For prophylaxis of staphylococcal infections, the most important is the proper diet and observance of impeccable hygiene in avian premises and those for incubation and breeding chickens (Harrison and Lightfoot, 2006; Mitchell and Tully, 2016). Usually feeding mainly with cereal mixtures leads to avitaminosis A, liver lipomatosis, stress, immune compromise and staph. infections. According to our observations, particularly susceptible are the grey parrot (Psittacus erithacus) and the cockatiels (Nymphicus hollandicus) that most often develop frontal sinusitis with eye inflammation and formation of nasal stones. Giving formulated blends does not always solve the problem. In many of these foods, vitamins are also missing, and have experienced various negative reactions due to improper storage or poor quality. It is advisable to give the supplements regardless of the type of food. Our experience shows that very good in this regard are the products of the company Nekton, combining all the necessary vitamins, minerals and amino acids of good quality. The training of the personnel in the breeding grounds also is important for the prevention. All should wear sterile protective clothing and manipulate parrots with gloves and mask. The air temperature is required to be 30° C, humidity 70%, and there should be no air current, which corresponds to data of Harrison and Lightfoot (2006) and Mitchell and Tully (2016).
Conclusion

1. *S. aureus* does not belong to the normal parrots microflora and it is a strong pathogen for them. The most sensitive to this bacterial species are birds with stress-weakened immunity.

2. Oral infection with *S. aureus* results in the development of a disease only in birds with an immune compromise, regardless of the dose of the pathogen. Venous infection is the surest way to reproduce the infection, all animals become sick without exception. Intramuscle and subcutaneous inoculation of the bacteria in dose 0.1 ml of the suspension of $6 \times 10^8$ cells/ml does not cause disease.

3. Veralox (Pradofloxacin) given orally at a dose of 15 mg / kg g. once every 24 hours is an excellent way to eliminate staphylococcal infections in parrots.

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References