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Vet Pathol 1988 25: 369
DOI: 10.1177/030098588802500506

The online version of this article can be found at:
http://vet.sagepub.com/content/25/5/369
Acute Fibrinopurulent Blepharitis and Conjunctivitis
Associated with Staphylococcus hyicus, Escherichia coli, and Streptococcus sp. in Chickens and Turkeys

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Abstract. Multiple outbreaks of acute severe fibrinopurulent lesions of the eyelids occurred in chickens and turkeys. Lesions began as tiny foci of epidermal necrosis and ulceration and spread to involve the entire eyelid. Scabs overlying the epidermis contained large gram-positive cocci; lesser numbers of small cocci and gram-negative bacilli were in more superficial areas. Staphylococcus hyicus was isolated from birds in all stages of the disease. Escherichia coli and Streptococcus sp. were isolated only during severe stages; no anaerobic bacterial pathogens were isolated. Vasculitis and perivascular lymphocytic infiltrates in deep layers of the dermis suggested that a staphylococcal toxin may have been involved. The disease was not reproduced by scarifying S. hyicus onto the eyelids or by intravenous inoculation of retrovirus-infected chickens.

Avian staphylococcosis occurs in several syndromes: omphalitis in day-old chicks, severe dermatitis of adult birds (diffuse or multifocal purulent lesions limited to the dermis), and septicemia associated with chronic, disseminated staphylococcosis, i.e., localization of staphylococci in tissues with major inflammatory lesions in joints (synovitis), tendon sheaths (tendonitis), heart valves (endocarditis), and bone marrow (osteomyelitis). Staphylococci are commensal organisms in birds and are residents of skin, nasal passages, conjunctiva, and feathers. Cutaneous staphylococcosis is usually associated with previous injury, e.g., bruises, thorn injuries, insect bites. Superinfection of lesions with Clostridium perfringens type A or other bacteria is common and occurs in young adult birds following adverse environmental conditions such as excessive cold or heat.

Staphylococcus aureus and S. epidermidis have been isolated from poultry. In general, S. aureus is considered pathogenic for poultry, while S. epidermidis, which is coagulase-negative and a poor protein A producer, is not. Some pathogenic avian staphylococci cannot be typed by current methods. Here we report clinical and pathologic characteristics of a new form of cutaneous staphylococcal infection of poultry.

Materials and Methods

Multiple outbreaks of blepharitis in the production unit of the laboratory occurred over a 5-year period, 1980 to 1986. During this time, we examined five separate groups of adult chickens and one flock of turkeys. The disease had occurred repeatedly but sporadically in different poultry houses which were adjacent to a swine production facility and were served by a common service unit.

Lesions were sampled, and necropsies were done on five chickens from each affected flock. Tissues were placed in 10% buffered formalin, processed by standard methods, and stained with hematoxylin and eosin (HE), Brown-Brenn gram stain, and alcian blue periodic acid-Schiff stain. In four groups of chickens, standard aerobic and anaerobic cultures for bacteria were done; in the fifth, titrations of bacteria in conjunctivae and exudate were done. Staphylococci from natural disease were identified according to the characteristics described by Kloos and Jorgensen.

Reproduction of the disease was attempted. National Animal Disease Center (NADC) strain New Hampshire Red chickens were housed in isolated rooms and given feed and water ad libitum. Culture fluids containing S. hyicus, E. coli, or Streptococcus sp. were scarified into the junction of the epidermis and mucosa of the eyelid of six chickens. Saline was scarified into the eyelid of six control chickens.

Attempts to produce systemic disease were done by intravenous inoculation of ten normal chickens and ten chickens given an avian retrovirus. Rates of clearance of bacteria from the bloodstream were determined by injecting 12, 10-week-old chickens intravenously with 0.1 ml containing $1.0 \times 10^6$ Staphylococcus hyicus. Two infected and one control bird were killed at 0, 2, 6, 24, 96, and 144 hr after inoculation. Tissues were examined by standard cultural, histologic, and electron microscopic methods.

To enhance susceptibility to bacteria, 10-day-old white Leghorn strain 9 chickens (Iowa State University, Ames, IA) were given an avian retrovirus. Myeloblastosis-associated virus, subgroup B, MAV-2 (0) (provided by Dr. Ralph Smith, Colorado State University) at a dose of 10^7 PFU/chick, was given intravenously via the jugular vein. 10 days after hatch.
Anemia, reflecting bone marrow stem cell suppression, was monitored by hematocrit; values dropped on day 12, were lowest at day 17, and had recovered at day 21 of age. Suppressed chicks were given *S. hyicus* intravenously (as above) at 20 days of age.

### Results

**Clinical findings**

Small erosions at the junction of the conjunctiva and skin of the eyelid were typical of early stages of disease.
A small amount of fibrinopurulent exudate covered the erosions and, as lesions progressed, ulcers developed and expanded peripherally to involve the entire skin of the eyelid (Fig. 1). Chickens of both sexes were affected similarly and both had bilateral involvement. As purulent exudate covered the eyelids, the skin of the upper and lower eyelids was adhered and chickens could not see. When eyelids were forcibly retracted, corneas were generally clear of inflammation.

**Histopathology**

Early in the course of disease, small foci of necrosis and ulceration contained inflammatory exudates, plant material, and large cocci (Figs. 2, 3). At the most severe stage of disease, eyelids had diffuse necrosis with loss of epithelium, massive accumulation of fibrinopurulent exudate, and marked inflammation of underlying connective tissues. The scab was separated from underlying tissue by a narrow rim of heterophils, and this, in turn, by a zone of macrophages in a matrix of fibrin.

Scabs that covered the lesions were composed of inspissated fibrin, pyknotic heterophils, and bacteria. Large gram-positive cocci were present both as free bacterial cells throughout the scab and as microcolonies, which were more superficial in the scab (Fig. 4). Smaller gram-negative rods were also present but were not as common and were generally limited to outer parts of the scab.

Interstitial connective tissue contained much fibrin and albuminous granular material adjacent to eroded epithelium. Capillaries were markedly dilated. Deeper in the dermis, blood vessels were dilated, mast cells were decreased in numbers, and vasculitis was severe. Venules and smaller veins were dilated and surrounded by inflammatory cells and fibrin deposits (Fig. 5). Arterioles were only rarely degenerate and were less severely affected by inflammation. In advanced lesions, especially in late stages, blood vessel groups were surrounded by plasmacytes and lymphocytes (Fig. 6). Lacrimal glands of affected eyes were enlarged and contained large accumulations of lymphocytes and plasmacytes, which were especially prominent around the ducts.

Lacrimal glands of recovered birds had foci of lymphocytes, often with germinal centers. In late stages of
Table 1. Bacterial titers in eyelid tissues of four chickens with conjunctivitis.

<table>
<thead>
<tr>
<th>Chicken</th>
<th>Stage of Disease</th>
<th>Staphylococcus hyicus</th>
<th>E. coli</th>
<th>Streptococcus sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Early</td>
<td>$2.8 \times 10^4$</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>2</td>
<td>Peak</td>
<td>$5.8 \times 10^6$</td>
<td>$9.4 \times 10^4$</td>
<td>$3.8 \times 10^6$</td>
</tr>
<tr>
<td>3</td>
<td>Peak</td>
<td>$9.0 \times 10^6$</td>
<td>$7.4 \times 10^5$</td>
<td>$2.8 \times 10^6$</td>
</tr>
<tr>
<td>4</td>
<td>Recovery</td>
<td>&lt;100</td>
<td>0</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

Bacteriology

Gram-stained smears of exudates contained irregular clusters of gram-positive cocci which were approximately 0.5-1.5 μm in diameter. Staphylococci were cultured from all affected eyes (Table 1). On blood agar, colonies were less than 5 mm diameter, white, and round. Aerobically, the organisms produced acid in sucrose, trehalose, fructose, ribose, glucose, and lactose. Bacteria were susceptible to lysostaphin, but not lysozyme. Coagulase was not produced. The bacterium was thus identified as *Staphylococcus hyicus*. Identification was confirmed by Dr. P. B. Smith, Nosocomial Infections Laboratory, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia. On in vitro tests, all isolates of *S. hyicus* were sensitive to the following antibiotics: amikacin, ampicillin, chloramphenicol, chlorotetracycline, erythromycin, gentamicin, penicillin, streptomycin, and tetracycline.

Ultrastructurally, bacterial cells grown in broth cultures were round or coccoid, had thick cell walls, and separation membranes. They did not have a capsule, flagellum, or pili.

Experimental infection

Intradermal scarification of *S. hyicus* into the epithelium of the eyelid did not cause acute fibrinopurulent blepharitis (Table 2). A transitory area of erythema and tiny scabs developed but did not expand beyond the point of scarification and healed by 7 days after inoculation.

Intravenous inoculation of *S. hyicus* led to transient bacteremia and accumulation of bacteria in the spleen and liver; there were no significant differences between normal and retrovirus-infected chickens (Fig. 7). By 48 hr *S. hyicus* could not be cultured from the blood of infected chickens. In contrast, at 144 hr, large numbers of bacteria remained in the spleen. Although growth was briefly suppressed, the birds did not appear sick, and there was no clinical evidence of localization of staphylococci in joints or other tissues.

Bacteria were trapped in greatest numbers in the spleen. In the first 2 hr after intravenous inoculation, they were localized primarily in reticular sheaths (Fig. 8). In contrast, by 24 hr, significant numbers were in the periarteriolar lymphoid sheath (Table 3). In ultrastructural analysis of the lymphoid sheath, staphylococci were found only within large dendritic cells (Fig. 9). They were not in or associated with lymphocytes or vascular tissue. Inside dendritic cells, staphylococci were within phagosomes or phagolysosomes (Fig. 10).

Discussion

*Staphylococcus hyicus* was the dominant agent in these cases of bacterial blepharitis and conjunctivitis. *Escherichia coli* and streptococci probably enhanced the severity of the disease; they may in some unknown way have been required for disease, even though *E. coli* did not cause conjunctivitis when scarified onto conjunctivae of chickens. *S. hyicus*, subsp. *hyicus*, a coagulase-variable bacterium previously implicated in exudative epidermitis and septic polyarthritis of swine, could have originated from pigs housed near...
Fig. 8.  Staphylococci (arrows) in dendritic cells, periarteriolar lymphoid sheath, spleen, chicken, 24 hr after intravenous inoculation. Silver stain.

Fig. 9.  Periarterial lymphoid sheath, spleen, virus-infected chick, 24 hr after intravenous injection of staphylococci. Lymphocytes (Lc) are adjacent to macrophages (Mac). Mac 1 lacks secondary lysosomes or other residual bodies but partially engulfs small lymphocytes; Mac 2 is less dense, has fewer dendrites, and contains large secondary lysosomes, one of which contains a necrotic lymphocyte. Mac 3 has many dendrites, primary and secondary lysosomes, and staphylococci.

Fig. 10.  Staphylococci in vacuoles in a splenic phagocyte (enlargement, Fig. 9).
the poultry units. The lack of pathogenicity of this isolate of *S. hyicus* for chicks supports swine as the original source of infection.

In the experiment that failed to produce systemic infection, it is unlikely that the inoculum (1.0 × 10^6 CFU) contained too few staphylococci. Avian and mammalian staphylococci have been differentiated by evaluating effects after inoculation of 1-day-old chicks subcutaneously. In chicks, the minimum dose of mammalian strains that caused hemorrhage was 11 × 10^3 bacteria; the lowest lethal dose varied from 11 × 10^3 to 26 × 10^3.

Vascular lesions distant from the site of bacterial colonization suggest that a toxin might have been released into the tissues, even though no systemic lesions attributable to a bacterial toxin were present. Cytoexotoxins produced by coagulase-negative staphylococci, similar to the lecithinase (delta toxin) of *S. aureus*, have been implicated as a cause of necrosis of intestinal mucosa of human infants. Slime production in these chickens is compatible with this type of membrane injury.

Production of protein A is probably not related to the pathogenicity of *S. hyicus*. Although porcine isolates vary in their reactions for protein A, 32 strains of *S. hyicus* from cows and two from poultry were devoid of this cell wall component. Slime production is a virulence factor of coagulase-negative staphylococci that colonize plastic shunts and foreign bodies. Biochemically uncharacterized, slime (determined by alcian blue staining of empty culture tubes) has been equated with thick extracellular granular coats covering bacterial cells. Slime was not seen in isolates from these studies.

It is unlikely that sepsis is a consequence of infection with this strain of *Staphylococcus hyicus*. First, there was no evidence of bacteremia in the natural disease. Second, experimental intravenous infection did not lead to overt tissue lesions. Staphylococci probably enter the bloodstream from conjunctival lesions but are destroyed before producing significant lesions. Others have shown that experimental intravenously injected *S. aureus* are cleared from blood in approximately 6 hr. However, *S. aureus* multiplied and reappeared in circulating blood by 12 hr, reached titers of 1 × 10^9/ml blood, and caused signs of joint infection at 48 to 120 hr.

The plant material seen in lesions histologically probably originated from litter and may have initiated the first lesions. Trauma of skin and mucous membranes is often required to reproduce dermatitis with staphylococci, but the interactions of trauma and infection of three bacterial species could not be established in this study.

### References


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