AN OUTBREAK OF STAPHYLOCOCCOSIS IN YOUNG CHICKENS

Author(s)
SATO, Gihei; MIURA, Shiro; MIYAMAE, Takeo

Citation
Japanese Journal of Veterinary Research, 6(3): 167-180

Issue Date
1958-11-15

DOI
10.14943/jjvr.6.3.167

Doc URL
http://hdl.handle.net/2115/1736

Type
bulletin

File Information
KJ00002373188.pdf
AN OUTBREAK OF STAPHYLOCOCCOSIS IN YOUNG CHICKENS

Gihei SATO, Shiro MIURA and Takeo MIYAME

Department of Epizootiology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan

(Received for publication, July 14, 1958)

INTRODUCTION

There are a number of publications concerning staphylococcosis in poultry. Occurrences of chicken staphylococcosis such as arthritis, vesicular dermatitis, or omphalitis have been reported in several countries. Extensive studies on properties of pathogenic staphylococci of chicken origin were carried out by Jungherr and Plastridge, and by Smith11,12.

In 1938, Kawanishi and Nakamura reported a chicken staphylococcosis which had previously never been described either in Japan or in other countries. The most characteristic lesion of the diseased birds was edema on the wings, which was due to serous inflammatory exudate containing blood. Affected birds always died from septicemia 2~4 days after the onset of the symptoms. In general, 4~9-week-old chickens suffered from the disease. Kawanishi and Nakamura isolated coagulase positive and hemolytic or non-hemolytic staphylococcus from the lesions. They also could reproduce the same conditions in chickens by inoculation with the isolated strains of Staphylococcus citreus. Recently, in Italy, Rossi observed some cases of gangrene on the wings of chickens. The disease was characterized by the most frequent occurrence of edema and gangrene on the wings. He isolated Staphylococcus aureus and Clostridium perfringens Type D from the lesions. At the same time, he detected pure staphylococci from a crusty dermatitis and ocular or articular lesions. In addition, Mondini and Quaglio described a widespread generalized infection of fowls showing gangrene on the wing-tip or in other portions of the body. The disease was caused by the infection of Staphylococcus aureus or occasionally of Pseudomonas pyocyanea.

The above-described chicken staphylococcosis appears to be characteristic, in respect of the presence of marked edema or gangrene in infected birds. Moreover, cutaneous staphylococcosis in chickens reported by Fielder in Australia may be added to the group of the diseases described by Kawanishi and Nakamura, Rossi, and Mondini and Quaglio.

In October, 1956, the present authors observed an outbreak of chicken
staphylococcosis almost similar to that described by Kawashima and Nakamura, although some differences were recognized. The present paper gives detailed descriptions of the outbreak and of various properties of isolated staphylococcus.

Experiment

1. Symptoms

The disease occurred in two flocks of young male White Leghorn chicken at a small breeding farm in Sapporo. This farm has produced broilers by rearing male chicks purchased from a hatchery in other prefecture. The first flock of 1,000 day-old chicks which hatched on August 8th was purchased from a hatchery in Toyohashi city. After brooding for about 3 weeks, chickens were removed to battery. Chickens began to die at the age of about 40 days. Within the period from 40 to 80 days after hatching, more than 90% chickens of the flock died. According to the breeder’s anamnesis, gangrene of a dark red color was found on the wings or in other portions of the body. Initial symptoms of the disease was lacrimation. Widespread lesions were found for a few days before death. Diseased birds died within about one week following the manifestation of the initial symptoms.

The second flock of 1,000 male day-old chicks which hatched on September 1st was introduced from the same hatchery. Losses of chickens in battery started from about 40 days after hatching. On October 25th, three diseased chickens (Nos. 1, 2, and 3) of the flock were examined soon after death by the present authors. Outward appearances of

**FIG. 1. Appearance of the Affected Chickens (Nos. 1, 2 and 3)**

**FIG. 2. Characteristic Lesions on the Wings of the Chickens**
Lesions characterized by marked serous exudation and gangrene were found all over one or both wings and on the neck, breast and abdominal region. In one case of the three illustrated (No. 2), muscular tissues were separated from the wing bones due to a marked subcutaneous edema and gangrene. Feathers on the lesions easily fell out. By October 26th, about 20% chickens of the flock were lost.

On October 26th and 27th, the flock was observed by the present authors. Diseased birds showed eye symptoms such as lacrimation, inflammation, swelling, or adhesion of eyelids. Abrasion was found occasionally on the eyelids. Also focal hemorrhages were observed on the wings or on the skin of the body. Ruffled feathers, drooping wings and recumbency were recognized, but no lameness. Characteristic lesions on the wings and body were found in some cases which died on October 27th. Localized necrosis, scab formation and fowl-pox-like nodules were observed in almost all birds examined.

For the period from October 27th to November 1st, soluble aureomycin was given in drinking water to all chickens of the flock.

On November 1st, characteristic lesions were observed no longer in the diseased birds. Eight sick chickens were separated from the flock for clinical observation. Eye symptoms such as lacrimation, swelling or adhesion of eyelids and also respiratory disturbances were found among them. Almost all birds were recumbent and some of them extended their necks in respiration. Scab formation was observed on the face or on the wings. Fowl-pox-like nodules were present on the face, especially around the eyes. Next day, one of the above 8 birds was killed for pathological examination. A soybean-sized node on the face was proved histologically to be fowl pox. Two of the remaining birds died from respiratory disturbances. Before November 6th, four out of remaining 5 birds died.

On November 7th, symptoms of diseased birds of the flock were like those observed on November 1st. Only one bird showed a slight swelling of left hock joint. That bird died next day.

On November 21st, survivors of the flock appeared to be normal. During the outbreak, about 70% of 1,000 chickens of the flock died.

2. Pathology

A total of 31 cases including 27 died and 4 killed chickens were pathologically-anatomically examined. Marked lesions showing subcutaneous edema and gangrene were found on the wings and other parts of the body. These lesions were dark red in appearance. However the nasty smell described by Kawashima and Nakamura was not experienced from the lesions. In some cases, the necrosis was confined to small areas. In the later outbreak, scab formation was occasional. Nodules of fowl pox were present on the face, mainly on eyelids. Cheese-like exudates were found in eyes or in mouth of some cases. Slight swellings of hock joint were found in only one case. Gross appearance of pathological changes in the visceral organs was not marked.

Five cases consisting of 3 died and 2 killed chickens which were obtained in the initial outbreak were examined histopathologically. The principal changes consist of, as a rule, circulatory disturbances, such as hyperemia and histiocytic cell hyperplasia as reticulo-
endothelial activities were observed in the visceral organs except muscular lesions. No fowl-pox-like findings were noted among them. In the muscles of the wings, characteristic changes such as severe hemorrhages, hyperemia, edema, thrombosis, cell infiltration and masses of bacteria were found. In one case other than the above-described, some nodules on the face were histologically proved to be fowl pox by the presence of inclusion bodies (BOLLINGER bodies).

3. Bacteriology

1) Bacterial cultivation

A total of 22 carcasses of chickens, 18 died and 4 killed, were examined bacteriologically. In smears of the characteristic lesions small clumps of Gram-positive cocci were found. Materials such as visceral organs, eye discharges, and muscular tissues of lesions were cultivated on 5% sheep blood agar. In order to search for enterobacteriaceae, selenite broth and MACCONKEY agar were employed for cultivation of visceral organs and intestinal contents. Materials of the characteristic lesions of the first 3 cases were inoculated into thioglycollate media for examination of anaerobic organisms.

Pure culture of staphylococcus was obtained from gangrenous lesion, confined necrosis and scab. Several birds manifested septicemia. Infection of Salmonella potsdam or of Salmonella gallinarum-pullorum was observed in some cases. Gram-negative small rods or Gram-positive cocci other than Staphylococcus aureus were detected chiefly from the throat or trachea of some cases. However, no anaerobic organisms were detected from the materials. The results of bacteriological examinations are shown in table 1.

It will be seen from table 1 that eye discharges yielded growth of staphylococcus with consistency. A loopful of discharges gave a pure and rich growth of staphylococcus, though sometimes mixed infections with other organisms were found. At the same time, a rich growth of staphylococcus was obtained from nostril discharges in almost all cases. Eye discharges of an attacked chicken, which survived, were examined bacteriologically for over 2 months. Pure culture of staphylococcus was obtained from the discharges at least 50 days.

It is an evident fact that pure staphylococcal cultures were obtained from the apparently normal subcutaneous tissues of the face, chiefly around the eyes or the cheeks, of several birds. These birds suffered from the disease, but they were proved bacteriologically not to have septicemia. A pure staphylococcal growth was obtained from the swellings of the hock joint of one bird which died from septicemia.

Salmonella potsdam or Salmonella gallinarum-pullorum was detected from several birds which died or were killed. These salmonella types were isolated mainly from the unabsorbed yolks. Isolation of Salmonella potsdam from the chickens with exception of chick embryos appears to have been reported neither in Japan nor in other countries.

Although, in this outbreak, a variety of pathological findings or mixed infections were recognized in the birds, a remarkable consistency was noted in the isolation of staphylococcus and in the character of all the strains of the organism isolated, indicating strongly that the staphylococcus so isolated had some degree of specificity.
<table>
<thead>
<tr>
<th>ORGANISMS DETECTED</th>
<th>TERMINATION</th>
<th>NO. OF CHICKENS EXAMINED</th>
<th>EYE DISCHARGES</th>
<th>SKIN LESIONS</th>
<th>THROAT OR TRACHEA</th>
<th>LUNGS</th>
<th>HEART</th>
<th>SPLEEN</th>
<th>LIVER</th>
<th>KIDNEY</th>
<th>UNABSORBED YOLK</th>
<th>FECES</th>
<th>NO. OF POSITIVE CHICKENS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>Died</td>
<td>18*</td>
<td>17</td>
<td>15</td>
<td>12</td>
<td>14</td>
<td>6</td>
<td>7</td>
<td>10</td>
<td>1</td>
<td>.</td>
<td></td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Killed</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>.</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Salmonella potsdam</td>
<td>Died</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Killed</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Salmonella gallinarum-pullorum</td>
<td>Died</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Killed</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Others</td>
<td>Died</td>
<td>18</td>
<td>8</td>
<td>1</td>
<td>13</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>.</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Killed</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>.</td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>

* In one of these 18 cases, eye discharges were not cultivated.
2) Characters of the isolated staphylococcus

The isolated staphylococcus was identified with *Staphylococcus aureus* from the following characters.

**Morphology**  In the tissues or in broth culture, Gram-positive and non-motile cocci were arranged frequently singly, in pairs, in short chains, or sometimes in small clusters. Size of the coccus was relatively small.

**Pigment production**  The staphyloccocal culture produced yellowish orange pigment on skimmilk agar (10%).

**Growth on agar containing 7.5% NaCl**  Positive.

**Biochemical**  Seventeen strains examined produced acid from glucose, galactose, saccharose, maltose, lactose, trehalose, and glycerine without exception. Some of them did so from salicin, raffinose, xylose and arabinose. However, the strains did not attack inulin, inositol, dulcitol and sorbitol. All 139 strains examined fermented mannitol and liquefied gelatin. They reduced nitrates. Litmus milk showed acid with clot and was decolorized. Hydrogen sulfide and indol were not produced. VOGES-PROSKAUER reaction and methylred test were positive. Methylene blue was reduced weakly. Solid horse serum was liquefied by all 25 strains examined.

**Hemolysis**  Agar plates containing 5% whole blood of sheep, horse, rabbit or chicken were not affected by the isolated staphylococcus after leaving for 24 hours at room-temperature following incubation for 48 hours at 37°C.

**Coagulase production**  The technique described by WILSON and MILES was employed. The isolated strains coagulated rabbit plasmas usually later in comparison with well-known pathogenic strains. Chicken plasmas were coagulated with a few exceptions. It is an interesting fact that agar culture seemed unsuitable for detection of coagulase of the isolated strains, because diluted rabbit plasma inoculated with fresh agar culture of the staphylococcus was frequently not coagulated. The controlled pathogenic strains, of course, were positive consistently when either broth or agar culture was used.

**Toxin production**  Preparation of toxin was made referring to the descriptions by KOJIMA. Overnight broth culture of the 4 isolated strains and a controlled (209 P) were inoculated respectively into semisolid horse meat agar containing 2% polypeptone, 0.3% NaCl and 0.3% agar. The inoculated media were incubated for 5 days in air containing about 20% carbon dioxide. Then the media were filtered through filter paper. The filtrates were centrifuged at 3,000 r.p.m. for one hour and the supernatants were sterilized by Seitz filter. These filtrates were stored in ice-box for 3 days before the application.

**Hemotoxin**  Tube test was carried out by the method of SMITH. The Seitz filtrates of four chicken strains did not affect either sheep or rabbit erythrocytes even at the dilutions of 1 : 10. On the contrary, the Seitz filtrate of the controlled strain lysed rabbit erythrocytes at 1 : 160 and sheep erythrocytes at 1 : 20 after leaving for 19 hours in room temperature following incubation at 37°C for two hours.

**Lethal toxin**  The Seitz filtrates of the isolated strains were intravenously injected in a dose of 0.4 ml into two or three mice and into one rabbit in a dose of 4 ml. The filtrate of one of the 4 strains isolated was injected intravenously into a chicken in a dose of 4 ml. The above described inoculation doses were determined according to the descriptions
Staphylococcosis in Young Chickens

of SMITH\(^{10}\). Observation period was 48 hours. None of the animals injected were affected, while mice injected with the Seitz filtrate prepared from the controlled pathogenic strain died almost immediately after injection.

Dermonecrotoxin: A dose of 0.2 ml of the Seitz filtrates was injected intradermally into a rabbit as described by SMITH\(^{11}\) and a dose of 0.1 ml into a guinea pig in a similar manner. Observation was carried out for 7 days. All the strains isolated yielded negative results. On the contrary, the controlled strain was strongly positive.

**Sensitivity to antibiotics or sulpha'iso.'Ca.'zol** Sensitivity disks (Eiken Co., Tokyo) were employed for examination of sensitivity of the 3 isolated strains. These strains were judged as sensitive against penicillin, dihydrostreptomycin, oxytetracyclin, chloramphenicol and chlortetracyclin. They were resistant against sulpha'iso.'zol.

4. Experimental Inoculation

1) Small animals

Broth or agar cultures of two strains, one of which was isolated from gangrene on the wing (No. 1) and the other from eye discharges of a survived chicken (No. 19), were inoculated into adult rabbit, guinea pigs and mice by various routes. The animals were kept under observation for 4 weeks after injections and they were examined bacteriologically. The results obtained are indicated in table 2.

Each of 4 rabbits which survived showed bacteriemia at 24 hours following injection. In one of them, which had been injected with a dose of 0.5 ml of broth culture, bacteriemia were found also at the period from 8 to 10 days following injection. No pathological changes were observed in post mortem examination. Guinea pigs subcutaneously injected showed small induration and scab formation at the site of injection, but not severe lesions. Mice inoculated subcutaneously died from septicemia within a few days after injection and some of them showed widespread edema and gangrene in the abdominal region where injection was made.

2) Chickens

A dose of 0.25 ml of horse meat infusion broth culture of the isolated strain No. 1 was injected intravenously into 2 White Leghorn chickens (about 6-month-old) as indicated in table 2. They survived, though bacteriemia were recognized during several days after injection.

Further experiments were carried out using young New Hampshire chickens (45-day-old). Inocula were prepared from overnight agar culture of the isolated strains (Nos. 1 and 19). Chickens injected were killed after a period from 4 to 6 weeks after injection. The results obtained are shown in table 3.

Severe infections similar to those observed in natural cases were found in the cases of subcutaneous or wound infections. Widespread edema and gangrene were found all over the wing. Chickens inoculated intravenously were affected severely as described by SMITH\(^{12}\). They were recumbent and showed drooping wings. In survivors, localized lesions at the site of injection healed within about one week after injection.

In order to ascertain the possibility of wound infection in natural outbreak, some
### Table 2. Pathogenicity of the Isolated Staphylococcus against Small Animals

<table>
<thead>
<tr>
<th>ANIMALS</th>
<th>KIND OF CULTURE</th>
<th>DOSE</th>
<th>ROUTE</th>
<th>Died/Injected</th>
<th>Days to Death (Post Mortem Cultivation)</th>
<th>Died/Injected</th>
<th>Days to Death (Post Mortem Cultivation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td>Broth</td>
<td>0.5 ml</td>
<td>i. v.</td>
<td>0/1</td>
<td>Survived (-)</td>
<td>0/1</td>
<td>Survived (-)</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>0.05</td>
<td>&quot;</td>
<td>0/1</td>
<td>&quot; (-)</td>
<td>0/1</td>
<td>&quot; (-)</td>
</tr>
<tr>
<td>Chicken</td>
<td>Broth</td>
<td>0.25</td>
<td>&quot;</td>
<td>0/2</td>
<td>(Not Done)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Broth</td>
<td>0.2</td>
<td>i. p.</td>
<td>0/4</td>
<td>Survived (-)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>0.5</td>
<td>&quot;</td>
<td>0/4</td>
<td>(Not Done)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mice</td>
<td>Agar</td>
<td>2 mg</td>
<td>&quot;</td>
<td>1/4</td>
<td>2 (Septicemia)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>&quot;</td>
<td>s. c.</td>
<td>4/5*</td>
<td>2~3 (Septicemia)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guinea pig</td>
<td>Agar</td>
<td>10</td>
<td>&quot;</td>
<td>0/1</td>
<td>Survived (-)</td>
<td>0/1</td>
<td>Survived (-)</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>1 i. p.</td>
<td></td>
<td>1/1</td>
<td>1 (Septicemia)</td>
<td>1/1</td>
<td>2 (Septicemia)</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>2 i. c.***</td>
<td></td>
<td>0/1</td>
<td>Survived (-)</td>
<td>1/1</td>
<td>2 (Septicemia)</td>
</tr>
</tbody>
</table>

*Staphyloccoci were detected from the kidney of a survivor.

**i. c.: intracardial.
### TABLE 3. Experimental Inoculation of the Isolated Staphylococcus in Young Chickens

<table>
<thead>
<tr>
<th>DOSE (mg)</th>
<th>ROUTE</th>
<th>STRAIN NO. 1 Died/Injected</th>
<th>Days to Death</th>
<th>STRAIN NO. 1 Died/Injected</th>
<th>Days to Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>s. c.</td>
<td>1/1</td>
<td>1 (Septicemia)</td>
<td>0/1</td>
<td>(Eye Discharges)</td>
</tr>
<tr>
<td></td>
<td>i. m.</td>
<td>0/1</td>
<td>(Injection Site)</td>
<td>0/1</td>
<td>(-)</td>
</tr>
<tr>
<td>1</td>
<td>i. v.</td>
<td>2/2</td>
<td>3 and 5 (Septicemia)</td>
<td>0/1</td>
<td>(-)</td>
</tr>
<tr>
<td>5</td>
<td>i. p.</td>
<td>0/1</td>
<td>(Nostril Discharges)</td>
<td>0/1</td>
<td>(-)</td>
</tr>
<tr>
<td>20</td>
<td>Wound</td>
<td>1/1</td>
<td>2 (Septicemia)</td>
<td>0/1</td>
<td>(-)</td>
</tr>
<tr>
<td>1</td>
<td>i. o.</td>
<td>0/1</td>
<td>(-)</td>
<td>0/1</td>
<td>(-)</td>
</tr>
<tr>
<td></td>
<td>i. n.</td>
<td>0/1</td>
<td>(Trachea)</td>
<td>0/1</td>
<td>(-)</td>
</tr>
</tbody>
</table>

*Heavy bacterial suspension was rubbed into cut on the skin of under part of the wing.

i. m.: intramuscular.

i. o.: intracocular.

i. n.: intranasal.

Experiments were carried out. Fourteen 40-day-old New Hampshire chickens were employed for inoculation. Skin of under part of the right wing of each bird was rubbed circularly within a diameter of one cm by ampoule cutter. Thus an adequate abrasion was obtained. One drop of bacterial suspension, which contained different amounts of 24 hours agar culture of strain No. 1, was rubbed into each abrasion. Observation was made for 2 weeks. In 2 cases (Nos. 3 and 12), cut rather than abrasion was prepared. The results of the experiment are shown in table 4.

Localized lesions as abscesses at the site of injection recovered within about one week. No staphylococcus was detected from the site of injection in post mortem examination. A bird inoculated with a dose of 0.01 mg died from septicemia, but no alteration were found at the site of injection.

From the above results, it may be supposed that the isolated staphylococcus was low virulent for experimental animals and chickens. Moreover, under the experimental conditions, wound infections appear to occur rarely.

### 5. Treatment

For 5 days after detection of the disease, 0.1% soluble aureomycin (Aureomycin soluble-Lederle) was given in drinking water to the chickens of the flock. Widespread
TABLE 4. Experimental Wound Infection in Young Chickens

<table>
<thead>
<tr>
<th>KIND OF WOUND</th>
<th>DOSE (mg)</th>
<th>NO. OF CHICKEN</th>
<th>TERMINATION (Days to Death)</th>
<th>LOCAL CONDITIONS</th>
<th>POST MORTEM CULTIVATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abrasion</td>
<td>10</td>
<td>1</td>
<td>Survived</td>
<td>Scab</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>&quot;</td>
<td>&quot;</td>
<td>—</td>
</tr>
<tr>
<td>Cut</td>
<td>10</td>
<td>3</td>
<td>&quot;</td>
<td>Not Done</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6</td>
<td>&quot;</td>
<td>Not Done</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>7</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Abrasion</td>
<td>0.5</td>
<td>8</td>
<td>&quot;</td>
<td>Not Done</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>9</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>10</td>
<td>Induration</td>
<td>Not Done</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>11</td>
<td>&quot;</td>
<td>Scab</td>
<td>&quot;</td>
</tr>
<tr>
<td>Cut</td>
<td>0.1</td>
<td>12</td>
<td>Died (10)</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Abrasion</td>
<td>0.01</td>
<td>13</td>
<td>Survived</td>
<td>Normal</td>
<td>Not Done</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>Died (5)</td>
<td>&quot;</td>
<td>Septicemia</td>
</tr>
</tbody>
</table>

Skin lesions were not found after the treatment, but localized skin lesions and diphtheric symptoms developed chiefly. The 3rd flock of 530 chicks which was introduced to the same farm on 20th September, 20 days later than the 2nd flock was also given the same drugs for 5 days from the age of 37 days. No infected birds were found among them even throughout their battery stages. Thus, the drugs appeared to be somewhat effective on the disease, though other influencing factors such as cold weather and some improved hygienic conditions might be considered. At an early period of the outbreak, the owner of the flock failed to cure the diseased birds by injection of penicillin.

DISCUSSION

Regarding the outbreak of chicken staphylococcosis, no detailed report other than that of KAWASHIMA and NAKAMURA has been published in Japan, though recently a few outbreaks of the disease were reported\(^5\). KAWASHIMA and NAKAMURA reported 12 outbreaks of edema disease of chickens which were examined during the period from 1931 to 1936. According to the personal communication of Dr. IWAMORI, professor of Gifu University, cases of chicken staphylococcosis almost similar to that described by KAWASHIMA and NAKAMURA have been occurring frequently in the central district of Honshu in the past several years. The disease occurred only during the battery breeding as reported by KAWASHIMA and NAKAMURA.
In foreign countries, arthritis appears to be a pathological condition which was found most frequently in chicken staphylococcosis as described by Hofstad. Kawashima and Nakamura were the first to report the most characteristic lesions showing edema, chiefly on the wings of affected chickens.

Pathological changes of the diseased chickens reported by the present authors seem to be somewhat different from those described by Kawashima and Nakamura, in respect to absence of offensive odor or severe edema. Moreover, in the present outbreak, ocular infection was observed from the early period and crusty dermatitis mainly at the later period. These conditions seem to resemble those reported by Rossi.

Kojima investigated 5 staphylococcus strains isolated by Kawashima and Nakamura. The strains were coagulase positive. Three of them produced $\alpha$-hemolysin. The remaining 2 strains were $\beta$-hemolysin producer and non-hemolytic respectively. The strains of Staphylococcus aureus isolated by the present authors were non-hemolytic but coagulase positive. Previously Jungherr and Plastridge reported that in the determination of pathogenicity and etiologic significance of avian staphylococci, a positive rabbit plasma coagulase test detects definitely pathogenic strains. Smith stated that non-hemolytic staphylococci were isolated frequently from the various lesions in chickens. Moreover, he stated that no correlation between hemolysis and pathogenicity was recognized and that a coagulase test was most reliable to determine the pathogenicity of staphylococci of chicken origin. Experimental inoculation with broth culture of the strains isolated by the present authors failed to kill adult rabbits and adult chickens. However, intravenous injection of a dose of 1 mg of agar culture killed about 6-week-old chickens. Accordingly, the strains isolated by the present authors are probably pathogenic on the basis of the criteria described by Jungherr and Plastridge or by Smith, though they were low virulent for small animals, especially for rabbits.

Kawashima and Nakamura isolated Bacterium edematitis maligni and Staphylococcus citreus from 2 cases of diseased chickens. However, they reported that mixed infections of anaerobic organism in diseased birds should be considered as accidental. Rossi detected Clostridium perfringens Type D and Staphylococcus aureus from edema and gangrene of chickens. So far as the present authors observed, mixed infections of anaerobic organisms were not found in the diseased birds.

Staphylococci have been recognized as a secondary invader in the chicken diseases such as laryngotracheitis, cold or fowl pox. Jungherr and Plastridge examined many strains derived from fowl pox lesions. These strains were admitted as pathogenic. In the present outbreak, fowl pox infections also seemed to occur.
from the early period. Lacrimation or inflammation of eyes as early symptoms of the diseased chickens would be due to the infection of fowl pox virus. In order to ascertain this point, washings of eyes of the birds which died in the first period of the outbreak were smeared into defeathered follicles or wound on the skin of chicken. The treated chicken showed fowl-pox-like lesions. The lesion materials of this chicken were applied into another chicken in a similar manner. Also the second chicken showed the similar lesions. However, further experiments such as filtering test or histopathological examinations of lesion were not made. Occurrence of fowl pox infection in the present outbreak was finally demonstrated by histopathological examination of nodules on the face of an affected bird or by manifestation of diphtheric symptoms of chickens of the flock. From the above results, it may be affirmed that staphylococcosis mixed with fowl pox infection occurred in the present outbreak, though it was not certain whether or not the isolated strains of *Staphylococcus aureus* were primary invaders.

Eyes of all the birds examined were infected severely with staphylococcus. From this fact, it is supposed that eyes which were perhaps infected with fowl pox virus might be a primary focus of growth of the staphylococcus. Recently Mondini and Quaglio suggested that various skin conditions such as fowl pox, offer a portal of entry for staphylococci. This point should be clarified by further experiment.

The staphylococcosis described by Kawashima and Nakamura is known as so-called “battery disease”, because of intimate connection between the outbreak of the disease and battery breeding of chickens. Kawashima and Nakamura imagined that a wound of chicken body caused during battery breeding may offer a portal of entry for the bacteria. Staphylococcal infections of possibly traumatic origin are seen in so-called “battery blister” described by Jungherr and Plastridge, in bumblefoot and generally in arthritis of chickens. However, Kawashima and Nakamura failed to prove this point by experimental wound infection. Also in the present authors’ experiments the same results were obtained.

Fielder reported that cutaneous staphylococcosis occurred in 3 flocks of chickens which originated from the same hatchery. In the present outbreak, chickens of 2 flocks which were purchased from the same hatchery suffered from the disease. Chicks which hatched together with the above 2 flocks were sold to 9 breeders who were asked by mail about the conditions of chickens concerned. Only one of them replied that chickens of his flock suffered from similar disease not confirmed to be due to staphylococcal infection.

Avian staphylococcosis seems to be essentially of sporadic character. Outbreak of the disease seems to be promoted by various factors such as poor hygienic conditions, coccidiosis, or nutritional conditions. Regarding the last factor, Rossi
supposed that an excessive feeding of protein was the determinant for the explosion of the disease. The present authors consider that, in the present instance, poor hygienic conditions, such as would lead to the occurrence of fowl pox, were responsible for the outbreak.

**SUMMARY**

Acute staphylococcosis characterized by widespread edema and gangrene on the wing, neck, or other parts of the body occurred in about 6~9-week-old White Leghorn chickens which were being kept in battery. Symptoms of the diseased chickens changed into confined skin necrosis, crusty dermatitis or diphtheric conditions associated with fowl pox infection.

About 70% of a flock consisting of 1,000 chickens died during the outbreak.

Many strains of *Staphylococcus aureus* were isolated from the various lesions of birds which died or were killed. Ocular infection of the staphylococcus was found from the initial stage of the disease.

The isolated staphylococcus is coagulase positive, non-hemolytic and does not produce toxin. It is low virulent for rabbits, guinea pigs or mice. Young chickens were killed by intravenous injection with a dose of 1 mg of the staphylococcus culture. Wound infection appears to occur rarely under experimental conditions.

The authors wish to express their gratitude to Assist. Prof. Fujimoto of the Department of Veterinary Pathology for his kind help in pathological study. The present work was supported in part by a Grant in Aid for Co-operative Research from the Ministry of Education, for which the authors would like express their cordial thanks.
REFERENCES