Induced Systemic Candidiasis in Young Broiler Chickens*

R. D. Wyatt, D. G. Simmons, and P. B. Hamilton
Department of Poultry Science and Department of Microbiology,
Raleigh, North Carolina 27607

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SUMMARY
Systemic candidiasis was induced in broiler chickens 14 days old by intravenous injection of a suspension of viable Candida albicans cells. Injection resulted in decreased body weight, moderate mortality, swollen and reddened livers and kidneys, pancreatitis, and disturbances of the nervous system. Three types of neural disturbances were observed: 1) extreme opisthotonus with spasmodic tremors; 2) extreme torticollis with cranial rotation of 270 degrees; and 3) extreme torticollis which resulted in the head being drawn in a medial-ventral direction. None to 50% of the inoculated birds exhibited these neural disturbances, depending on the isolant of C. albicans used. Microbiological examination of internal organs and blood revealed that C. albicans localized in the meninges of the brain. There was also a significant isolant-related effect of C. albicans on the growth rate of the inoculated birds. These easily quantitated differential effects of various isolants of C. albicans offer the prospect of correlating biochemical characteristics with virulence and obtaining information about the mechanism of pathogenicity of this microorganism.

INTRODUCTION
Candidiasis is a disease of man, poultry, wild birds, and a variety of other animals. It occurs in a mucocutaneous form as localized lesions of the mucocutaneous membranes and in a systemic form in which the infection is disseminated throughout the body. In man, candidiasis may involve the mouth, skin, vagina, nails, intestine, and lungs as well as a septicemia (1,3,16,21,23). In chickens, this disease generally manifests itself as a localized infection of the mucous membranes, particularly the crop (3), and thus is

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Table 1. Effect of intravenous injection of Candida albicans on body weight and mortality of young broiler chickens.

<table>
<thead>
<tr>
<th>Isolant no.</th>
<th>Weekly body weight (g)</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Control</td>
<td>286 ± 9</td>
<td>545 ± 11a</td>
</tr>
<tr>
<td>1</td>
<td>255 ± 5</td>
<td>329 ± 11a</td>
</tr>
<tr>
<td>2</td>
<td>264 ± 8</td>
<td>416 ± 13b</td>
</tr>
<tr>
<td>3</td>
<td>281 ± 5</td>
<td>385 ± 11c</td>
</tr>
<tr>
<td>4</td>
<td>263 ± 7</td>
<td>348 ± 11c</td>
</tr>
<tr>
<td>5</td>
<td>271 ± 9</td>
<td>412 ± 22b</td>
</tr>
</tbody>
</table>

A Birds were two weeks old at inoculation. Each value represents the mean body weight ± SEM for all birds in a group. Values in a column followed by different letters differ significantly (P < 0.05).

B Mortality is expressed as number of deaths per number of birds inoculated.

the mucocutaneous form of the disease. Overcrowding, inadequate nutrition, antibiotic therapy, mycotoxicosis, and debilitation appear to aid the establishment of Candida albicans in the crop (4,7,9,21,24,25). In a number of avian species, the infection results in a characteristic “turkish towel” appearance of the crop (3,16). A lesser degree of infection may result in a slight thickening of the crop, with only moderate yeast colonization or no visual yeast growth (16). In addition to crop infections, C. albicans was reported by Beemer et al. (2) to be the etiological agent responsible for an epidemic venereal disease in geese. Blaxland and Fincham (5) observed pronounced neural disturbances in turkeys experiencing chronic crop mycosis. These neural disturbances were characterized by a staggering gait and twitching of the head. Birds dying from crop mycosis were observed to have an S-shaped flexure of the neck, with their legs stretched backward. It was postulated that these symptoms were the result of a systemic candidiasis, though no experimental proof was offered.

Because no information was available about chickens experiencing systemic candidiasis, this investigation was undertaken to develop a method of inducing systemic candidiasis in young broiler chickens and to provide a description of the systemic form of the disease.

MATERIALS AND METHODS

Animal husbandry. Day-old broiler chicks were obtained from a commercial source and maintained in electrically heated batteries with feed and water available ad libitum. The feed was a commercial broiler-starter ration from which all medications had been omitted. At 2 weeks of age 60 birds were selected for this experi-
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on the basis of uniformity of body weight and overall appearance.

Source and identification of isolants The isolants of *C. albicans* were obtained from the crops of birds being processed in a commercial poultry-processing plant. One isolate was found in five flocks. Crop swabs were streaked on Pagano-Levin agar (Difco, Detroit, Mich.) and incubated at 37°C for 48 hr. Typical pink colonies were subcultured on Sabouraud's dextrose agar (Difco). Identification was based on chlamydospore formation, germ-tube formation, and the fermentation and assimilation patterns of carbohydrates as outlined by Lodder (14).

Preparation of *C. albicans* suspension. The inoculum was prepared by incubating each isolate in Sabouraud's dextrose broth (Difco) for 18 hr. at 42°C. The broth cultures were centrifuged, and the resulting pellet was washed three times with sterile 0.85% saline in 0.05M phosphate buffer (pH 7.4). After the third washing the cell suspension was adjusted by optical density to 10 million viable cells/ml based upon enumeration of colonies by the pour-plate method.

Experimental design. There were 6 groups of 10 birds each. One group served as the control, and each of the other five groups was inoculated with one of the isolates of *C. albicans*. The experimental birds were injected intravenously with 0.5 ml of a suspension of the appropriate isolate of *C. albicans*, while the control birds received 0.5 ml of sterile phosphate-buffered saline (pH 7.4). Mortality was recorded daily, and the birds were weighed weekly. The birds were examined for abnormal behavior and gross symptoms at the weekly weighing. While the results of a single trial are reported, the experiment was repeated for a total of three trials with similar results.

Necropsy. Three weeks after inoculation, the birds were killed by a lethal dose of sodium pentobarbital. Gross pathological findings were recorded. Specimens of neural tissue, liver, kidney, spleen, and pancreas were removed and fixed in 10% neutral buffered formalin. Tissues were prepared for histological examination and stained with hematoxylin and eosin by standard methods.

Microbiological examination. Prior to killing, a sample of blood was removed aseptically from the brachial vein and plated on Sabouraud's dextrose agar, Pagano-Levin agar, and blood agar. After killing, samples of neural tissue, liver, spleen, kidney, pancreas, and bursa were taken by searing the exposed surface with a
hot spatula and inserting a sterile microbiological loop in the seared area. The tissue removed was streaked on Saboraud's dextrose agar, Pagano-Levin agar, and blood agar. All plates were incubated for 48 hours at 37 C and examined for microbial growth.

**Statistical analysis.** Statistical analysis of the body weights was by an analysis of variance in which an F-ratio was determined. If the F-ratio indicated significance, the least-significant difference was calculated (6).

## RESULTS

**Weight gain.** The initial effect observed as the result of intravenous injection of *C. albicans* was a marked depression in the growth rate of the inoculated birds. Table 1 shows the weekly body weights of the six groups. A statistical analysis revealed that all isolants slowed the growth of the infected birds throughout the 3-week experimental period. There was also a statistically significant (P < 0.05) differential body-weight effect by the various

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**Fig. 1.** Photograph of a broiler exhibiting extreme opisthotonus and spasmodic tremors of the head.
isolants used. For example, isolants 1 and 4 caused a severe depression in growth, whereas isolants 2, 3, and 5 had a lesser effect throughout the experiment.

**Mortality.** Table 1 also shows the cumulative mortality over the 3-week experimental period. Mortality was only moderate (0-20%) during the study. While the differences in mortality were slight, they did appear to be related to the severity of the growth depression.

**Clinical signs.** None to 10% of the inoculated birds exhibited neural symptoms by the second week postinoculation. The early neural symptoms manifested themselves as moderate opisthotonus and tremors of the head. By the third week after inoculation, 10-50% of the inoculated birds exhibited some form of neural abnormality and three distinct manifestations of neural disturbances were observed. The first was characterized by extreme opisthotonus, with spasmodic tremors of the head. Such an affected bird is shown in Fig. 1. Affected birds would assume a squatting position and remain motionless except for the tremors of the head.

![Figure 2](image_url)

**Fig. 2.** Photograph of a broiler exhibiting a sigmoid flexure of the neck with cranial rotations of 270 degrees.
Table 2. Effect of intravenous injection of *Candida albicans* on the incidence and type of neural disturbance in young broiler chickens.

<table>
<thead>
<tr>
<th>Isolant no.</th>
<th>Opisthotonus with tremors</th>
<th>Torticollis with 270° rotation of head</th>
<th>Torticollis in medial-ventral direction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>1</td>
<td>0/8</td>
<td>1/8</td>
<td>0/8</td>
</tr>
<tr>
<td>2</td>
<td>0/10</td>
<td>1/9</td>
<td>0/9</td>
</tr>
<tr>
<td>3</td>
<td>1/9</td>
<td>2/9</td>
<td>1/9</td>
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<tr>
<td>4</td>
<td>1/9</td>
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<td>4/10</td>
</tr>
<tr>
<td>5</td>
<td>1/10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Incidence is expressed as number exhibiting the particular disturbance per number of survivors.*

The second type of disturbance was characterized by torticollis, with the neck in a sigmoid flexure resulting in cranial rotation of 270 degrees, though no tremors were observed. An affected bird with this type of disturbance is shown in Fig. 2. These birds were able to stand and move about, though they frequently walked in circles when frightened. The direction of circular movement was consistent for a given bird, though 75% of the birds that circled did so in a counterclockwise direction. The third category of disturbance was an extreme torticollis in a medial-ventral direction, resulting in the beak resting on the abdominal wall, with the head in continuous tremors. An affected bird with this type of disturbance is shown in Fig. 3.

Table 2 shows the percentage of the survivors experiencing neural disturbances 3 weeks after inoculation with the different isolants of *C. albicans*. Only one bird from each of the groups inoculated with isolants 1 and 2 exhibited neural involvement, and only two birds exhibited neural disturbances in the group inoculated with isolant 3. The group inoculated with isolant 4 was the only one to exhibit all three types of neural disturbances. The group inoculated with isolant 5 had the highest number of birds with neural symptoms. Isolant 5 may possess a more specific neurotropism since 40% of the inoculated birds exhibited extreme torticollis in a medial-ventral direction. There appeared to be no strict correlation between growth rate, mortality, and severity of neural involvement, although isolant 4 was ranked first for its growth inhibition and neurotropism and second for its lethality.

No paresis or convulsions were observed in any of the birds. The sensory organs appeared to be unaffected, although it was extremely difficult to elicit an auditory response. Affected birds did possess voluntary motor function of the head and neck region,
as evidenced by their ability to eat and drink. A normal posture could be maintained for 30–60 seconds following manual extension of the neck.

**Gross lesions.** Upon necropsy, all birds that had exhibited an abnormal positioning of the head were found to have a slight sigmoid flexure of the keel bone, with no evidence of traumatic breast blisters. This presumably was the result of prolonged squatting with the body resting on the keel bone. In every case of the abnormal positioning of the head and neck, the neck was straight at death. The flexure involved the cervical vertebra, with no single vertebra or section involved consistently. No muscular atrophy was evident, and the bone structure appeared normal. In all birds inoculated with *C. albicans*, the kidneys were swollen and dark, the liver exhibited a dark mahogany discoloration, the bursa was swollen and vascular, and a mild pancreatitis with diffuse petechia was noted. In approximately 20% of the inoculated birds, the meninges were slightly hemorrhagic but the brain tissue appeared normal.

![Fig. 3. Photograph of a broiler experiencing extreme torticollis with the head drawn in a medial-ventral direction.](image)
Microbiological examination. Cultures of liver, spleen, kidneys, bursa, pancreas, and blood were negative for microbial contamination. Cultures of meninges were positive for C. albicans in all birds experiencing neural involvement. The apparent severity of infection was somewhat variable, however, as evidenced by the number of colonies on agar streak plates. Brain, spinal cord, sciatic nerve, and inner-ear cultures were negative for microbial contamination.

Histopathological examination. Histopathologic examination of liver, kidney, spleen, pancreas, brain, spinal cord, sciatic nerve, and inner ear revealed no evidence of C. albicans infection. In several birds, sections of the meninges exhibited mycelial-like structures identical to those observed in deep mycoses caused by C. albicans (3).

DISCUSSION

These data indicate that systemic candidiasis with deleterious effects can be induced by intravenous inoculation of broiler chickens. The first indicator of systemic candidiasis was a slowing of growth. After 1 week postinoculation an analysis of variance of body weights revealed that isolant 1 caused the most severe growth depression, isolants 3 and 4 caused intermediate growth depression and isolants 2 and 5 caused the least depression. After the second and third weeks postinoculation an analysis of variance revealed that isolants 1 and 4 give the greatest growth depression, and isolants 2, 3, and 5 the least. The differential effect of C. albicans on body weight implies differences in virulence among the isolants studied. Certain inherent properties of C. albicans have been postulated to be associated with virulence (19). For example, Seelig (21) and Kobayashi et al. (13) have found organisms of the genus Candida to contain an endotoxin-like material. Mankowski (15) isolated a glycoprotein from culture filtrates of C. albicans which retarded the growth of newborn mice upon repeated subcutaneous injections. Iwata et al. (10,12) reported the isolation of highly toxic material from C. albicans. This material, named canditoxin, was later shown to be an alkaline phosphatase (11). Chattaway et al. (8) failed to demonstrate toxin production by C. albicans; however, they did demonstrate the presence of an extracellular peptidase and an acid phosphatase in mycelial extracts of C. albicans. Now, biochemical investigations of Candida can perhaps be correlated with some of its easily quantitated in vivo effects, yielding information about its mechanism of pathogenicity.
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The relatively low mortality observed indicates that chickens are somewhat resistant to the effects of intravenous injection of viable *C. albicans* cells. Morelli and Rosenberg (18) found that an intravenous injection of $1.4 \times 10^6$ cells/animal resulted in 100% mortality in both complement-positive and complement-negative mice. Albano and Schmitt (1) observed similar results in random bred mice injected with $5.5 \times 10^6$ cells/animal. These levels of inoculum compare with $5.0 \times 10^6$ cells/bird used in this study, but the mortality did not exceed 20% with any isolant.

Natural involvement during candidiasis has been reported (5). It is not known, however, whether these disturbances were caused solely by systemic candidiasis or other interacting factors. Neural symptoms similar to those caused by induced systemic candidiasis are associated with other diseases. For example, opisthotonos, also known as “star-gazing,” can be inherited in quail (20,22), and similar disorders are related to infectious diseases (4) and nutritional deficiencies in chickens (7,25). The neural disorders observed during induced systemic candidiasis appear to differ from “star-gazing.” For example, Savage and Collins (20) observed that the backward movement of the head of quail occurred only when the birds were excited or when an opaque object was placed above the bird. All the neural symptoms observed during systemic candidiasis were spontaneous and required no stimulus. Circular movement, frequent squatting, and voluntary movement of the head are common to both inherited “star-gazing” and systemic candidiasis.

The neural disturbances observed during induced systemic candidiasis may be the result of a mechanical influence of *C. albicans* in the neural tissue since cultures of meninges yielded high numbers of *C. albicans*. Another hypothesis to account for the involvement of the neural system would be that of toxin production. The internal organs exhibited evidence of cellular damage as determined by gross pathology and histopathology; however, cultures of those organs were negative for *C. albicans*. This suggests the participation of a toxic material in this disease.

The diversity of the neural symptoms observed suggests differences in the specificity of *C. albicans* or a toxic by-product(s) for the nervous system. The relatively low frequency of neural disturbances caused by isolants 1, 2, and 3 and the high incidence caused by isolants 4 and 5 also suggests strain differences in virulence. No strict correlation was apparent between body-weight
depression and incidence of neural symptoms. This could be interpreted to indicate that the effects observed during systemic candidiasis are caused by many factors which contribute to the overall virulence of the organism. Furthermore, quantitative variation of one or more factors could have a pronounced influence on the virulence of a particular strain.

REFERENCES


ACKNOWLEDGMENTS

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