AVIAN DISEASES vol. 18 no. 3

Isolation of Dactylaria gallopava from Broiler-House Litter

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Received 2 April 1974

SUMMARY

The first outbreak of dactylariosis in chickens from Georgia is described. The mortality rate among 60,000 birds comprising six separate flocks of broilers was 3–5%. Diagnosis in each flock was confirmed by histopathology of brain tissue and isolation of Dactylaria gallopava from birds. When a dilution technique and an incubation temperature of 42 °C were used, D. gallopava was isolated from 2 of 75 litter samples from five broiler houses, the first recorded isolation of D. gallopava from chicken litter. It is suggested that the wood chips and sawdust in litter may introduce the fungus into broiler houses.

INTRODUCTION

Dactylaria gallopava (Cooke) Bhatt & Kendrick, 1968, a thermophilic dematiaceous hyphomycete, was originally described as the causal agent of encephalitis in turkey poults (5). The second outbreak of dactylariosis and the first to involve chickens occurred

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Table 1. Thermophilic fungi isolated from 75 chicken-litter samples collected in broiler houses in Gainesville, Georgia.

<table>
<thead>
<tr>
<th>Fungus species</th>
<th>Total no. of positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus fumigatus</td>
<td>1</td>
</tr>
<tr>
<td>Dactyliaria gallopava</td>
<td>2</td>
</tr>
<tr>
<td>Mucor pusillus</td>
<td>13</td>
</tr>
<tr>
<td>Paecilomyces varioti</td>
<td>45</td>
</tr>
<tr>
<td>thermoascus aurantiacus</td>
<td>42</td>
</tr>
</tbody>
</table>

In a flock of 1600 Australorp chickens maintained near Brisbane, Australia (2). In that outbreak 550 five-week-old chicks died. Outbreaks of dactylariosis have since been reported among turkey poults in South Carolina (1) and Maryland (7), and among 2-week-old chickens in southern Indiana (6).

In nature, *D. gallopava* has been found to occur in high-temperature environments such as thermal soils, self-heated coal-waste tips (8) and piles (9), and hot-spring effluents where soil and water temperatures range from 38.5 to 61.5 C and pH ranges from 2.8 to 4.2 (9). Even though dactylariosis is now a well recognized disease, its epidemiology is only partially understood. Blalock et al. (1) did not isolate the fungus from turkey house

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Fig. 1. Lesions of dactylariosis in chicken brain.
litter, but stated that litter could be the probable source of the disease agent.

During the summer of 1973 and extending through January 1974, the first Georgia outbreak of dactylariosis was recorded in Gainesville, one of the state's most concentrated broiler-raising areas. In six separate flocks of approximately 10,000 chickens each, dactylariosis caused a mortality of 3–5%. During the study of this outbreak, 75 litter samples were collected from five of the involved broiler houses and screened for *D. gallopava*. This report presents our observations on this outbreak of dactylariosis and the isolation of *D. gallopava* from the chicken litter.

**MATERIALS AND METHODS**

The affected birds from six separate flocks of broilers from three different producing companies were examined over a period of 10 weeks. The birds were studied for gross symptomatology. The affected brain tissues were examined by histopathology. A portion of the brain tissue from some of the birds was cultured on Sabouraud’s dextrose agar containing chloramphenicol and cycloheximide. The causal organism in each case was identified by colonial and micromorphology.

Seventy-five litter samples were collected from five chicken broiler houses. Each sample, consisting of 20 to 25 g of litter, was scooped up with a plastic spoon at depths of 3 to 5 in. In each broiler house, at least 15 samples were collected covering the entire area of the house. The samples were stored in sterile plastic bags.

One-to-two-g portions of each sample were suspended in test tubes containing 10 ml of sterile distilled water with 0.1% Tween 80. The tubes were thoroughly shaken to homogenize the suspension, and were allowed to stand for 30 minutes. One ml of the supernatant was withdrawn and diluted with 9 ml of sterile distilled water. A third dilution (1 in 10) was similarly prepared.

One-ml portions from the third dilution tube were pipetted into a flask containing 100 ml of yeast glucose (YG) agar [yeast extract, 5.0 g; glucose, 10.0 g; agar, 20.0 g; water, 1000 ml (3)]. This medium had been sterilized at 120 C for 15 minutes and cooled to 50 C. In order to prevent bacterial contamination, gentamycin sulfate was added to the cooled medium to give a concentration of 100 µg/ml. The medium was then aseptically poured into five petri plates and allowed to solidify. The plates were incubated at 42 C and were observed periodically over a period of 2 weeks.
RESULTS

The affected birds were weak, incoordinated, down on their sides, and generally unthrifty. Antemortem examination revealed birds with various degrees and manifestations of disturbance of the central nervous system, such as ataxia resulting in loss of equilibrium, head tremors, and torticollis.

Histopathological examination of the brains showed abscesses of various sizes in different parts of the tissues. The gross lesions varied in color from almost white to brown to reddish tan (Fig. 1). No other significant lesions were detected. When stained with periodic acid-Schiff stain, several granulomatous areas showing inflammatory cellular infiltration were seen. Observed in the necrotic areas were giant cells and hyphal elements (1.2 to 3.5 μ in diameter) (Fig. 2).

Table 1 summarizes the results of the litter isolation studies. *D. gallopava* was recovered from two of the 75 litter samples studied. Growth of *D. gallopava* appeared on the third day of incubation at 42 C. This rapidly growing fungus was readily recognized by the production of a distinctive diffusable reddish-brown pig-

![Fig. 2. Dactyilaria gallopava, branched, septate hyphae in chicken brain tissue, Periodic acid-Schiff stain. ×1100.](image-url)
Dactylaria gallopava in broiler-house litter

The two *D. gallopava* isolates developed in pure culture on the primary isolation plates.

Subcultures on Sabouraud's dextrose agar were fast growing, dark brown, velvety, and surrounded by a reddish-brown pigment that diffused into the medium (Fig. 3). Microscopic examination of the colony showed dematiaceous yellowish brown septate branched hyphae measuring 1.5–3.5 μ in diameter. Conidiophores were more or less erect, simple, short, hyaline to lightly pigmented, septate, and denticulate. They bore clavate to cuneiform two-celled conidia that measured 12–16 × 4–5 μ (Fig. 4).

**DISCUSSION**

The present outbreak, which began in the summer of 1973 and persisted into February 1974, represents the first record of dactylariosis for the state of Georgia. It caused a 3–5% mortality in six separate flocks of approximately 10,000 chickens each. The dis-

Fig. 3. Eight-day-old colony of *D. gallopava* on Sabouraud dextrose agar incubated at 25 C.
ease in the flocks appeared to be self-limited since it had a low rate of spread and was confined to birds 1 to 5 weeks old.

The isolation of *D. gallopava* from 2 of the 75 litter samples revealed its presence for the first time in such an environment. The fact that dactylariosis affected only 1-to-5-week-old chicks and that *D. gallopava* was isolated from litter shortly after it had been spread out into the broiler houses makes us suspect that the sawdust piles in the local sawmills are the prime source of the pathogen. Plans have been made to collect sawdust-pile samples in the yards of litter vendors to determine whether they harbor *D. gallopava*.

Other thermophilic fungi were also present in the litter. The species isolated are presented in Table 1. The predominant thermophiles were *Paecilomyces varioti* and *Thermoascus aurantiacus*.

Those fungi, along with *Aspergillus fumigatus* and *Mucor pusillus*, developed on the isolation plates either in pure culture or in various combinations. Dennis and Gee (4) investigated the microbial flora of broiler-house litter in England. They also utilized a dilution technique but incubated their isolation plates

![Image of D. gallopava](https://example.com/image)

**Fig. 4.** *D. gallopava*, 7-day-old slide culture on Sabouraud's dextrose agar showing conidiophores and conidia. ×800.
Dactylaria gallopava in broiler-house litter

at 25 C. A large variety of molds were isolated from fresh and used litter. Among the 41 species identified, only one, Paecilomyces variotii, can be classified as a thermophilic fungus (3). It was the most common species isolated from our Georgia litter. The contrast between the species isolated by Dennis and Gee in England and those isolated in Georgia by us emphasizes the selective value of incubation at 42 C when seeking thermophilic fungi such as D. gallopava in heavily contaminated substrates.

REFERENCES


ACKNOWLEDGMENTS

The authors acknowledge help with the chicken isolations by Dr. Glen Washburn, Georgia Poultry Laboratories, and help with the histopathological studies by Dr. Oscar Fletcher, Poultry Disease Research Center, University of Georgia, Athens.