SIMILARITY BETWEEN ARTHRITIS VIRUS AND FAHEY-CRAWLEY VIRUS

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SUMMARY
Agar-gel precipitin and virus-neutralization tests suggest that the agent of viral arthritis and Fahey-Crawley virus belong to the same serotype. Both viruses produce similar gross and histologic changes in chickens.

INTRODUCTION
Fahey and Crawley (4) isolated a virus from cases of chronic respiratory disease (CRD) in chickens and demonstrated infectivity on the basis of virus-neutralization tests. Subramanyam and Pomeroy (11) inoculated the Fahey-Crawley virus into chickens that were 1 day, 3 weeks, and 6 weeks old and into 6-week-old turkeys, and demonstrated a mild respiratory disease, low neutralizing titers, and a viremia lasting 2–4 days. The virus was recovered from lung and trachea for 3–10 days postinoculation (PI). Petek et al. (9) examined the physicochemical properties of the Fahey-Crawley agent and determined that it was a reovirus.

Olson et al. (7) isolated a virus from cases of synovitis and differentiated the arthritis virus from Mycoplasma synoviae on the basis of physicochemical properties (6). Pathologic changes in chickens associated with viral arthritis infection were described by Kerr and Olson (5). Spontaneous occurrence of the disease in a broiler flock was reported by Olson and Solomon (8). Studies by Walker (12) and Rossi et al. (10) showed that this same viral arthritis agent was a reovirus.

EXPERIMENTAL
Sexed female White Leghorn chicks were obtained from a commercial hatchery. Fahey-Crawley virus in the 4th passage was obtained from S. B. Hitchner, Cornell Universities, Ithaca, N. Y.
Table 1. Number of chickens that developed swelling of the tendon sheaths or histologic lesions of the metatarsal tendons and tendon sheaths following inoculation with the Fahey-Crawley virus.

<table>
<thead>
<tr>
<th>Route of Inoculation</th>
<th>Infectious embryo fluid</th>
<th>No. chickens</th>
<th>No. with swelling (Days PI)</th>
<th>No. with histologic lesions (40 days PI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Footpad</td>
<td>Yolk</td>
<td>5</td>
<td>5 5 5 5 4 1</td>
<td>5</td>
</tr>
<tr>
<td>Footpad</td>
<td>Allantoic</td>
<td>5</td>
<td>5 5 5 5 5 3</td>
<td>5</td>
</tr>
<tr>
<td>Intranasal</td>
<td>Yolk</td>
<td>3</td>
<td>0 0 0 0 0 0</td>
<td>0</td>
</tr>
<tr>
<td>Intranasal</td>
<td>Allantoic</td>
<td>3</td>
<td>0 0 0 0 0 1</td>
<td>1</td>
</tr>
<tr>
<td>Contact controls</td>
<td></td>
<td>5</td>
<td>0 0 0 0 0 0</td>
<td>0</td>
</tr>
</tbody>
</table>

Fahey-Crawley virus in the 5th passage was harvested from yolk and allantoic fluid. The latter materials were inoculated into the footpad of 5 chicks or intranasally into 3 chicks, and 5 were maintained as contact controls. The design of the experiment is shown in Table 1.

The chicks were held for 43 days and then necropsied. The chickens were observed for swelling in the footpad, metatarsal tendons, and tarsus 3, 9, 20, 28, and 40 days postinoculation (PI). Since many of the birds showed no gross swelling at 40 days PI, tissues were taken from the metatarsal area, fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin, and examined histologically.

Serum was collected from day-old chicks and subsequently at weekly intervals, and the agar-gel precipitin (AGP) test was conducted as described by Crowle (2). The agar was prepared by adding 1.25 g Noble agar and 8 g salt to 100 ml of demineralized water containing 1:10,000 merthiolate in a 0.01M phosphate buffer at pH 7.2. The agar was dissolved by slowly heating the mixture to boiling. Seven to ten ml of agar was poured into 100 × 50-mm plastic petri dishes and allowed to cool. A design was made of one central well and 6 outer wells. The wells were 3 mm in diameter and 3 mm apart.

Antigens were prepared from ground infected chorioallantoic membranes, usually from dead embryos. At the start, care was taken to harvest membranes from live embryos that had been inoculated on the CAM. Later, however, satisfactory antigen was prepared from the CAM of embryos inoculated by the yolk sac or allantoic route. Each CAM was tested for specificity against specific antiserum before use.

The virus-neutralization test was conducted with Fahey-Crawley and arthritis virus antiserum and Fahey-Crawley virus
with the infectivity 50% endpoint determined by plaque reduction or embryo mortality. The constant-serum and virus 10-fold dilution technique on the dropped CAM was used. Attempts to conduct the neutralization test in embryonating eggs by the yolk sac or allantoic route of inoculation met with failure.

The number of plaques on the CAM were counted and given the following numerical classification: 0, 10, 20, 30, 40, or 50, respectively indicating none, 1-9, 10-19, 20-29, 30-39, or 40 plaques or more.

**RESULTS**

Table 1 shows the number of birds that developed swelling in the pelvic limb following inoculation and the number showing histologic lesions. The birds inoculated into the footpad showed a marked swelling in the pad extending to the metatarsal tendons but generally not involving the tendons above the tarsus. No swelling was observed in the uninoculated leg. Between 28 and 40 days most of the swellings had disappeared, though histologic examination revealed chronic inflammatory lesions similar to those described for viral arthritis (5).

Histologic lesions observed in cross sections of the metatarsal digital flexor tendons of birds 43 days PI were characterized by extensive fibrosis and the presence of numerous lymphoid follicles in the tendon sheaths. There were hypertrophy and hyperplasia of the synovial lining cells and a diffuse infiltration of lymphocytes, plasma cells, macrophages, and a few heterophils. Clumps of heterophils and desquamated synovial cells were occasionally present in the synovial spaces (Figs. 1, 2).

Table 2. Number of chickens from which serum samples gave positive precipitin lines when reacted against arthritis and Fahey-Crawley virus antigens.

<table>
<thead>
<tr>
<th>Route inoculation</th>
<th>Material inoculated</th>
<th>No. chickens</th>
<th>Number showing positive precipitin lines (days postinoculation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>Footpad</td>
<td>Yolk</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Footpad</td>
<td>Allantoic</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>IN</td>
<td>Yolk</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>IN</td>
<td>Allantoic</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Contact controls</td>
<td></td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

Allantoic = allantoic fluid.
C = Fahey-Crawley virus antigen.
V = arthritis virus antigen.
IN = intranasally.
Table 3. Neutralization of the Fahey-Crawley virus by serum from chickens exposed via the footpad to the Fahey-Crawley or arthritis virus causing embryo mortality and reduction of plaques on the CAM.

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Number of plaques$^a$</th>
<th>The log_{10} 50% end point</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plaque forming</td>
</tr>
<tr>
<td>Virus titer</td>
<td>38</td>
<td>4.7</td>
</tr>
<tr>
<td>Negative serum</td>
<td>28</td>
<td>4.3</td>
</tr>
<tr>
<td>Fahey-Crawley</td>
<td>25</td>
<td>2.5</td>
</tr>
<tr>
<td>WVU 2937 A</td>
<td>25</td>
<td>2.9</td>
</tr>
<tr>
<td>WVU 2937 B</td>
<td>16</td>
<td>3.1</td>
</tr>
</tbody>
</table>

$^a$Calculated on basis of 0 to 50 at base titer.
WVU 2937 A and B, viral arthritis antiserum.

Table 2 shows the results of the AGP test comparing the Fahey-Crawley and arthritis virus antigens when reacted against serum from chickens exposed to Fahey-Crawley virus. Essentially equal numbers of serums formed precipitin lines with the Crawley and arthritis virus antigen.

Table 3 shows the results of the virus-neutralization tests. On the basis of plaques and embryo mortality, there was at least one log reduction in titer when positive serum was compared with negative serum. The degree of plaque reduction was marked: respectively $28 \times 10^{4.3}$, $25 \times 10^{2.5}$, and $20 \times 10^{3}$ for negative serum, Fahey-Crawley, and arthritis virus antiserum.

Fig. 1. Chronic inflammation in the metatarsal digital flexor tendon sheaths 43 days postinoculation with the Fahey-Crawley virus by the footpad route. $\times$100.
It is concluded that the Fahey-Crawley virus and the arthritis virus WVU 2937 belong to the same antigenic group.

DISCUSSION

Viral arthritis has been present in poultry flocks since 1954. The first isolation by the author was in a flock from which both viral arthritis and infectious synovitis (*Mycoplasma synoviae*) agents were isolated. The two agents were separated on the basis of susceptibility to antibiotics (7). On the same basis, some isolates obtained by Cover (1) and Thayer (12) may have been similar viruses. The arthritis virus remained a laboratory curiosity until 1967, when severe outbreaks occurred in England and the United States. Other severe outbreaks have occurred in this country but have not been reported. The condition has generally been confused with infectious synovitis.

Many workers have attempted to produce respiratory signs by respiratory exposure of chickens and turkeys with the Fahey-Crawley virus, but with varied results. Work of Subramanyam and Pomeroy (11) indicated slight respiratory distress and a viremia when chickens and turkeys were exposed intranasally. Joint lesions,

Fig. 2. Chronic inflammation in the metatarsal digital flexor tendon sheaths 43 days postinoculation with the Fahey-Crawley virus by the footpad route. ×400.
however, have not been mentioned. This is not surprising since only a small percentage of infected chickens developed arthritis.

In addition to the respiratory tract and synovium, reoviruses have been isolated from the intestinal tract (3). The relationship and pathogenicity of these viruses need further study. The most significant pathologic aspect of reovirus infection in chickens is the effect on the synovial membrane.

REFERENCES


ACKNOWLEDGMENTS

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