ISOLATION OF ORNITHOSIS BEDSONIAE FROM MITES COLLECTED IN TURKEY QUARTERS AND FROM CHICKEN LICE

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In a preliminary report, Meyer and Eddie (1960) noted the isolation of a bedsonia* from arthropods taken in association with poultry. The implications of this report warranted further analysis.

The first isolation from arthropods was made from a group of unidentified ectoparasites taken from White Rock roosters raised in a midwestern hatchery. For nearly 10 years the Hooper Foundation had purchased roosters from this hatchery in order to produce immune serum. Serum from each rooster was first tested in the indirect complement fixation (ICF) test to ascertain the absence of any reaction against the ornithosis group. In the spring of 1959 a group of roosters heavily infested with ectoparasites revealed reactors to the ICF test. Ectoparasites collected from a rooster with an ICF titer of 1:64 were pooled, ground, emulsified with broth in a mortar, cultured, and injected into mice, 0.5 ml intraperitoneally and 0.3 ml intranasally. At autopsy of mice sacrificed on the 21st day, the spleens were moderately enlarged and the lungs were spotted with grey pinpoint lesions. These organs were emulsified and inoculated into a new set of mice. Typical greyish pulmonary lesions developed, in which elementary bodies identical to those of the ornithosis bedsonia were seen. On additional mouse passage the virulence increased slightly, and some mice succumbed to the infection (figure 1). In similar tests of ectoparasites taken from a rooster with an ICF titer of 1:16, a bedsonia was again isolated.

The possibility that the source of the virus was the mice rather than the arthropods was considered. Cowen stock mice have been used in the Hooper Foundation psittacosis work since 1932 and are routinely tested for members of this group every 2 or 3 months. There has never been any evidence of the infection in this stock.

After the incidental, provocative observation, further isolations were sought from the same flock. The shaft louse, Menopon gallinae (Linnaeus)† was collected from 10 hens and a serum sample was taken from each bird. In tests similar to those in the first instance, a bedsonia was isolated from 1 collection of lice. The host had an ICF titer of 1:4.

In additional tests, these 3 isolates proved to be pathogenic for parakeets and guinea pigs. They produced an in-

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Most of the field-collected material for this study was obtained through the cooperation of Dr. R. A. Tjalma, Department of Hygiene and Preventive Medicine, State University of Iowa, and Dr. M. A. Holmes, Public Health Veterinarian, Oregon State Department of Public Health.

* A member of the psittacosis-lymphogranuloma venereum group of viruses.

† The mallophagan Menopon gallinae normally spends its entire life on the body or feathers of the host bird. It is a chewing insect which ingests particulate matter such as barbs of feathers, scales, and organic debris on the hosts.
fection fatal to mice on intraperitoneal injection or intranasal instillation of a 10⁻⁴ dilution. A toxin for mice was demonstrated by intravenous injection of a 10⁻⁴ dilution. A CF antigen prepared from lungs of mice infected with one of these isolates fixed known positive psittacosis serum.

Further attempts to isolate the virus from arthropods varied from the preceding tests in that the collections were made from the environment previously occupied by infected birds rather than from the birds themselves. The collection sites were in and around 2 barns on a turkey ranch that had been under observation for the past 4 years because of the unexplained recurrence of ornithosis. On August 31, 1959, 20 plastic bags of nest litter and debris were taken from 11 different sites (table 1). All turkeys had been removed from these places June 9, 1959.

The material in plastic bags was held at room temperature in the laboratory for 15 to 108 days. As time permitted, arthropods were separated from the collected material by the Berlese funnel technique (Baker and Wharton, 1952). Most of the collected organisms were Acarina, the majority of which were alive. After preliminary segregation of the specimens into easily recognizable groups with the aid of a dissecting microscope, batches of not more than 200 each were transferred into clean collection vials containing sterile physiologic saline solution. Samples were mounted in polyvinyl alcohol for later definitive identification. The mites in saline were held in the refrigerator at 0±4°C until they could be tested for virus.

Tests for virus were performed on 120 pools of material, of which 117 were of arthropods (table 2). Bedsoniae were isolated from 7 of these pools (table 3). One pool had been held in the laboratory from September until January when the tests could be undertaken—about 7 months after any possible access to live turkeys and 5 months after collection of debris on the premises. Another mite pool from which bedsoniae had been isolated in late September was held at 4°C and retested in January at which time it was still positive.

The isolates produced nonfatal infections in mice on primary intraperitoneal, subcutaneous, or intranasal inoculation. On the second or third intranasal and intraperitoneal passage there was some enlargement of the spleen, and focal pulmonary areas of consolidation contained typical elementary bodies. In further passage the pulmonary infections were fatal (figure 2). Some isolates killed all mice on intraperitoneal injection, some only a few. The 2 isolates most thoroughly studied are antigenuically related to the psittacosis group. Virus was recovered from some parasites that survived the infection. The guinea pig susceptibility has been somewhat irregular. All have had antibody titers, but not all have succumbed.

The identification of representative samples of arthropods collected on the turkey ranch are given in tables 2 and 3. These include both parasitic and free-living mites, numerous bark lice or psocids, and a few coleopterans or beetles. Three of the infected pools consisted of free-living glycyphagid mites, mounted samples of which were Glycyphagus cadaverum (Schrank). These are free-living mites commonly found infesting old skins and feathers, dried stored food products, and organic debris. One infected pool was a mixture of Haemogamasus, Haemolaelaps, Orni-
Table 3.—Orithornis virus isolations from field-collected nest arthropods 6 months after removal of turkeys.

<table>
<thead>
<tr>
<th>Arthropods</th>
<th>Pools tested</th>
<th>Number of arthropods</th>
<th>Pools</th>
<th>Arthropods per pool</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycyphagidae</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>Glycyphagus coleoptratus</em></td>
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<td>1356</td>
<td>1</td>
<td>54</td>
</tr>
<tr>
<td><em>Cheyletidae</em></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Acaropsis coarctata</em> (Berlese)</td>
<td>1 (f)</td>
<td>27</td>
<td>1</td>
<td>15</td>
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<td><em>Mesostigmata</em></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Chalcopterus attenuatus</em></td>
<td>2</td>
<td>236</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td><em>Dermestidae</em></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Oriolomysicus candidus</em> (Can. et Foz.)</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

![Image of chicken on a turkey farm.](image)

**Figure 2.** Isolation of orithornis virus from arthropods from an Oregon turkey ranch.

Thornys and Cheyletus species. The first 2 of these genera contain species demonstrated to take vertebrate blood when available but to feed on other small arthropods when driven by hunger. Orithornis is a genus of strictly parasitic mites, depending upon vertebrate blood for food. Cheyletes eruditus (Berlese), the species in this pool, is considered a free-living predator, capturing and feeding on other small arthropods. Cheyletid mites were the sole forms in 2 other positive isolations of the virus. A preserved sample of 1 of these was *Acaropsis coarctata* (Berlese), a cosmopolitan, presumably predacious species; the other was an apparently new genus of mite resembling Chalcopterus. The 7th isolation of virus was made from a pool of 3 mites identified as an undescribed species of Araneus of the family Neoparasiidae. Members of this mesostigmatic family are free-living mites, generally regarded as predacious.

The data bring up the question of poultry ectoparasites (mites and lice, or other arthropods) infesting the environment of poultry, participating in some way in the spread or maintenance of the orithornis bedsonia, and, if so, how. This virus is able to survive without a host for some time, and it is possible that its presence in or on arthropods for the periods demonstrated here is merely a reflection of its durability on an inanimate substrate. Further work is needed to clarify this point. The fact remains that bedsoniae have definitively been isolated from ectoparasitic biting lice of poultry and from 4 different kinds of free-living mites, some of which retained active virus for at least 6 months after the date of collection.

It is simple to visualize how arthropods such as these could become either externally or internally contaminated with a virus passed in feces or nasal droplets of infected birds. Once contaminated, the arthropods could spread the infection (a) by being ingested in the poultry feed or water, (b) by being ingested as the birds pick at their feathers, (c) by crawling into nasal and mouth cavities, or (d) by being mixed in airborne dust and inhaled. Presumably, blood sucking parasites, such as *Orithornis syrvarum*, could pick up the virus by feeding during a period of viremia of the avian host.

Additional arthropods from a variety of sources are being examined, and the first steps are being taken to learn some of the possibilities of transmission. Early in 1960 some miscellaneous nesting and other material was collected from a turkey ranch in California where there had been no clinical evidence of orithornis. The removed mites were separated according to species, pooled in groups not exceeding 100, and subjected to mouse passage. We found 1 pool of *Glycyphagus codarius* (70), 2 of unidentified members of the family Acaridae (200), 1 of *Cheyletes eruditus* (1), and 1 of *Haemapelops castis* (3), totaling 5 pools and 274 mites. Routine mouse passage through 3 sets of mice revealed no evidence of bedsonia.

**Summary**

Bedsoniae were isolated by mouse passage from 2 different collections of *Menopon gallinae* (Linnæus) from chickens in which there had been serologic, but not clinical, evidence of this infection. They were also isolated from 7 of 117 pools of arthropods collected from a turkey ranch where orithornis had been enzootic or epizootic for 4 years. Three pools were of *Glycyphagidae*, 2 of *Cheyletidae*, 1 of Neoparasiidae, and 1 of a mixture of parasitic mesostigmatic and free-living cheyletid mites. Whether and, if so, how these arthropods participate either in per-
petuating or spreading this infection among poultry or other birds remains an open question.

**ADDENDUM**

The finding that bedsonia survive in litter material and may be isolated from mites living in it has been followed up by exploratory experiments. Straw from horse stables near San Francisco contained many of the mite species found in the tests described in this paper.

Major groups of mites were separated alive from stable straw under the stereoscopic microscope. The mites thus collected did not show a bedsonia virus contamination when control-tested by mouse passage. Sample polyvinyl alcohol preparations of each of the mite groups were identified as follows: *Glycyphagus cadaverum* (Schräck), 1 pool of 100 mites; *Oenoglyphus plumiger* (Koch), 1 pool of 26 mites; *Chelotes eruditus* (Schräck), 1 pool of 130 mites; *Homogamasus pontiger* (Berlese), 1 pool of 38 mites; 2 mixed pools of 100 and of 54 mites, containing Glycyphagus micractis Goedenma, Tydeidae, and other unidentified mites.

After the straw and mites were found to be negative for bedsonia, the straw was placed to a depth of about an inch in the bottom of a specially constructed metal box. Two parakeets were intranasally given 0.5 ml of a 10% mouse lung suspension of a virulent bedsonia (Texas turkey isolate) and then placed in the metal box with the litter containing the arthropods. Both birds died on the 5th day. The organs and intestinal content contained bedsonia. One mite of Glycyphagus species was found on each of the dead birds, but 3 mouse passages revealed no bedsonia.

A sample of arthropods was removed every week for 167 days at which time the experiment was terminated. The arthropods were separated into pools by gross characteristics visible with the stereoscopic microscope and later tested by mouse passage. Bedsonia were isolated from samples taken on the 15th, 29th, 36th, 45th, 56th, 106th and 167th day.

On the 56th day of the experiment, 2 Java sparrows were placed in the metal box with the remaining arthropods. The box had not been cleaned after removal of the dead parakeets. The sparrows did not show any symptoms and after 2 months they were bled out. No collections of mites were made during this period. The external surfaces of the birds were examined for arthropods, but none were found. The interiors of the ears and nose were not examined. Organs from these sparrows were used for mouse passage and bedsonia were isolated from both birds. Arthropods were again collected and tested (159th, 159th and 167th days). Bedsonia were isolated from the last 2 samples. The results are summarized in table 4.

In interpreting these results it is recognized that gross separation of arthropods with the stereoscopic microscope does not assure that all were of the same species as those represented in samples mounted for definitive identification. However, there is reasonable assurance that the pool used for viral isolations were of the major categories listed in the table.

Two auxiliary observations were made in the course of other work: (1) It was noted that experimentally infected mice were infested with *Polyplax serrata*. Three pools of 200 each were tested and bedsonia were isolated from 1 pool. (2) Some infected roosters were found to be infested with *Oriahenopsys syzygus*, and bedsonia were isolated from 1 of 2 pools of 100 each.

Two more confirmatory tests were made: (1) The isolates from *Glycyphagus, Chelotes*, *Oriahenopsys syzygus*, *Polyplax serrata* and *Aenogenus gallinae* were injected into parakeets, 2 per test, and the survivors were sacrificed and tested about 5 weeks later. Virus was isolated from all birds, 4 of which died. (2) A yolk sac antigen was prepared with several isolates (3 from *Chelotes* 1 from *Polyplax serrata*) and known positive serum was used with these antigens in the CF test. The titers were 1:256 to 1:1024.

**REFERENCES**


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