NOTE  Bacteriology

Experimental Infection of Cats with Chlamyphila felis

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ABSTRACT. Cats experimentally infected with a British isolate of Chlamyphila felis (C. felis), B166 strain, by droplet into the eye and nose developed conjunctivitis, mild rhinitis and fever. The chlamyphila were first isolated from conjunctiva, nictitating membrane and then from lung, tonsil, liver, spleen, kidney, nasal and vaginal swabs and blood. These results indicate that C. felis B166 strain first infected and replicated in the conjunctiva and nictitating membrane in cats with symptoms which were mostly limited to conjunctivitis, and then pervaded the whole body by bacteremia.

KEY WORDS: bacteremia, Chlamyphila felis, experimental infection.


The disease caused by Chlamyphila felis (C. felis) is clinically characterized by sneezing and coughing, accompanied by mucopurulent ocular and nasal discharges in cats [1]. Nowadays, C. felis is primarily considered an ocular pathogen with or without rhinitis in cats rather than a pulmonary pathogen [3, 9, 16].

There have been a few reports of seroepidemiological investigation of feline chlamydiosis in Japan [4, 7, 10, 18]. Mochizuki et al. reported that C. felis was found in 26.9% of diseased cats with conjunctivitis and rhinitis [7]. Yan et al. reported that the rate of prevalence of C. felis antibodies was 45.5% in stray cats and 12.3% in pet cats [18]. These reports suggest widespread chlamyphila infected in cats in Japan.

Cats experimentally infected with C. felis developed conjunctivitis, ocular discharges and fever, and this organism has been isolated from some tissues of experimentally infected cats [5, 6, 13, 15]. However, the time course of the disease and of the spread of the organism in the bodies of infected cats are not known clearly.

In this report, we describe the characteristics of clinical disease produced by C. felis after ocular and intranasal exposure. The distribution of C. felis in the bodies of cats was also examined by isolation and immunohistological and pathological examinations.

C. felis B166 strain used in this experiment was isolated from a cat with conjunctivitis in the United Kingdom in 1984 [14].

Twenty-six specific pathogen free (SPF) cats 2 to 6 months old were obtained from a colony maintained on an SPF animal farm. The SPF status of the cats was verified by the culturing of conjunctival swabs in embryonated hens' eggs for chlamydia, CRFK cells for virus, mycoplasma liquid media for mycoplasma, and thioglycolate media for bacteria, and the samples were all negative for these agents. The cats were kept in individual cages, fed commercial dry cat food and supplied water properly. The cats were randomly divided into 11 groups of two or four cats each. Two control cats were kept in a separate room. The experiments were performed according to our institutional guidelines for animal experimentation.

Twenty-five microliters of suspension containing 105.0 ELD50 (50% embryo lethal dose) of virulent, egg-grown feline C. felis B166 strain were inoculated by droplet into the eye and nose of a cat. The ELD50 of the inoculum was determined by yolk sac inoculation of 6-day-old embryonated chicken eggs. Control cats were given phosphate-buffered saline.

Clinical signs, which included conjunctivitis and respiratory diseases, were observed and rectal temperature was recorded daily for all cats. Because ocular signs were the most prominent signs in the preliminary experiment, conjunctivitis was scored by conjunctival hyperemia, serous and mucopurulent ocular discharges, and swelling of the eyelid. Each sign was given a score of between 0 (normal) and 3 (very severe) with scoring intervals of 0.5 for each cat. Left and right eyes were scored separately.

Two inoculated cats were euthanatized and necropsied on each of postinoculation days (PID) 1, 3, 5, 7, 10, 14, 17, 21, 24 and 28. The cats were administered ketamine hydrochloride to reduce pain, and were then exsanguinated by cutting the axillary artery. On PID 10 and 14, four cats each were examined. The two control cats were necropsied on PID 28.

At necropsy, selected tissues (conjunctiva, nictitating membrane, lung, tonsil, liver, spleen and kidney) were collected and analyzed by chlamydial culturing. The tissues were homogenized as a 20% suspension in chlamydial transport medium [12] and stored at –80°C. Each homogenate was inoculated into the yolk sacs of five 6-day-old embryonated chicken eggs. Eggs were observed daily for 10 days after the inoculation. Embryos that died by PID 3 were discarded, and from those that died after PID 3 the yolk sac was harvested and examined for chlamydia using a monoclonal antibody (MAb) against major outer membrane protein (MOMP) of C. psittaci GCP-1 strain [17]. This MAb recognizes C. felis MOMP.

At PID 1, 3, 5, 7, 10, 14, 17, 21, 24, and 28, conjunctival, nasal and vaginal swabs were taken from all live cats. The
swabs were placed in chlamydia transport medium [12] and stored at –80°C. They were tested for chlamydophila by egg inoculation as described above.

At necropsy, blood samples were collected from the cats for chlamydial culturing. They were put into glass containers which contained glass beads, shaken hard several times, and then stored at room temperature for a few min. The top clear layer was collected and stored at –80°C until it was tested for chlamydophila by egg inoculation as described above.

At necropsy, selected tissues (conjunctiva, nictitating membrane, lung, tonsil, liver, spleen and kidney) were collected for immunohistological examination. They were fixed in 10% formalin and embedded in paraffin using standard methods. Sections were tested immunohistologically using a Histofine SAB-PO(M) kit (Nichirei, Inc. Tokyo, Japan) and a MAb against MOMP of C. psittaci GCP-1 strain according to the manufacturer’s instructions.

Selected tissues (conjunctiva, nictitating membrane, lung, tonsil, liver, spleen and kidney) were collected at necropsy, fixed and embedded as described above. Sections were stained with hematoxylin and eosin.

Sera were collected at necropsy for testing for the antibodies against chlamydophila using an indirect microimmunofluorescence (MIF) test according to the method of Pudjiatmoko et al. [10]. Antigen was prepared from C. felis Fe/Cello strain [2] propagated in yolk sac. It was purified in the form of elementary bodies by sucrose density gradient ultracentrifugation.

The most prominent eye sign was conjunctivitis with severe swelling of the eyelid. Conjunctival hyperemia and serous or mucopurulent ocular discharge reached a peak on PID 7 to 9 and then gradually subsided. The average daily clinical eye scores after C. felis inoculation are shown in Table 1. Most cats exhibited bilateral conjunctivitis, with the exception of two cats, which exhibited unilateral conjunctivitis at first and exhibited bilateral conjunctivitis a week later. In addition, some cats also developed mild respiratory signs, which included slight nasal discharge and occasional sneezing. Fever developed after the clinical conjunctivitis. Pyrexia higher than 40.0°C was observed in most cats necropsied on PID 10, 14, 17 and 21 (Table 1). No clinical signs were observed in the control cats throughout the observation period.

Chlamydophila was first isolated from the conjunctiva and the nictitating membrane on PID 3, then from the lung and tonsil on PID 10, and finally from the liver, spleen and kidney on PID 14 (Table 2).

Chlamydophila was isolated from conjunctival swabs of all cats on and after PID 3, from nasal swabs of all cats on and after PID 5, and from vaginal swabs of some cats on and after PID 14 (Table 3).

Chlamydophila was isolated from the blood of some cats on PID 14, 17 and 21 (Table 2).

Chlamydophila was detected in the conjunctiva (Fig. 1) and nictitating membrane on and after PID 3 in all cats, and occasionally in the lung, tonsil, liver, spleen and kidney after PID 14 (Table 2).

Palpebral conjunctiva consisted of moderate focally extensive conjunctivitis on PID 3 to 10, and granuloma appeared from PID 14 to 28. Mild inflammation of the nictitating membranes appeared from PID 3 to 24. There were hardly any notable pathological changes in lung, tonsil, liver, spleen or kidney (data not shown).

MIF serum antibody with a titer higher than 8 was first detected on PID 21 and persisted until PID 28 (Table 1).

Experimental inoculation with C. felis produces conjunctivitis, mild rhinitis and fever of relatively late onset in cats [5, 6, 13]. In the present study, conjunctivitis first appeared on PID 5 to 7 in most cats, reached a peak from PID 7 to 10, and then tended to recover, with some exceptions in which it persisted until PID 28. This accords with the typical course of experimental feline C. felis infection reported by several authors [5, 8, 13, 15]. Most naturally infected cats which suffer from chlamydial conjunctivitis exhibit unilateral conjunctivitis [3, 9, 15]; however, the cats used in this

<p>| Table 1. Clinical signs and antibody responses of cats inoculated with Chlamydophila felis |
|---------------------------------|---------------------------------|---------------------------------|----------------|</p>
<table>
<thead>
<tr>
<th>Postinoculation day</th>
<th>Conjunctivitis (Average score)</th>
<th>Respiratory disease</th>
<th>Fever* antibody titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 / 24&lt;sup&gt;0&lt;/sup&gt; (0.0)</td>
<td>0 / 24&lt;sup&gt;0&lt;/sup&gt;</td>
<td>0 / 24&lt;sup&gt;0&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>0 / 22&lt;sup&gt;0&lt;/sup&gt; (0.0)</td>
<td>0 / 22</td>
<td>0 / 22</td>
</tr>
<tr>
<td>5</td>
<td>8 / 20&lt;sup&gt;0&lt;/sup&gt; (0.5)</td>
<td>0 / 20</td>
<td>0 / 20</td>
</tr>
<tr>
<td>7</td>
<td>16 / 18&lt;sup&gt;0&lt;/sup&gt; (3.0)</td>
<td>0 / 18</td>
<td>0 / 18</td>
</tr>
<tr>
<td>10</td>
<td>13 / 16&lt;sup&gt;0&lt;/sup&gt; (2.5)</td>
<td>2 / 16</td>
<td>3 / 16</td>
</tr>
<tr>
<td>14</td>
<td>11 / 12&lt;sup&gt;0&lt;/sup&gt; (2.2)</td>
<td>3 / 12</td>
<td>4 / 12</td>
</tr>
<tr>
<td>17</td>
<td>6 / 8&lt;sup&gt;0&lt;/sup&gt; (0.9)</td>
<td>2 / 8</td>
<td>6 / 8</td>
</tr>
<tr>
<td>21</td>
<td>1 / 6&lt;sup&gt;0&lt;/sup&gt; (0.2)</td>
<td>2 / 6</td>
<td>5 / 6</td>
</tr>
<tr>
<td>24</td>
<td>1 / 4&lt;sup&gt;0&lt;/sup&gt; (0.1)</td>
<td>1 / 4</td>
<td>0 / 4</td>
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<tr>
<td>28</td>
<td>1 / 2&lt;sup&gt;0&lt;/sup&gt; (0.3)</td>
<td>0 / 2</td>
<td>0 / 2</td>
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<tr>
<td>28 (control)</td>
<td>0 / 2&lt;sup&gt;0&lt;/sup&gt; (0.0)</td>
<td>0 / 2</td>
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</table>

<sup>a)</sup> Indirect microimmunofluorescence.
<sup>b)</sup> Rectal temperature higher than 40°C.
<sup>c)</sup> No. of positive cats/no. of inoculated cats.
<sup>d)</sup> Geometric mean of 2 or 4 cats whose blood were collected as necropsy.
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study exhibited bilateral conjunctivitis. It has been reported that conjunctivitis of cats infected with a higher dose of *C. felis* was bilateral, while that of cats infected with a lower dose was unilateral [13]. The infective dose of *C. felis* used in this study may have been higher than that in cases of natural infection. In this study, the conjunctivitis was serious, but the respiratory disease was very mild; thus, *C. felis* is considered an ocular pathogen rather than a pulmonary pathogen in cats. Fever was observed from PID 10 to 21. These observations were almost identical to those previously reported for *C. felis* infection in cats [5, 6, 13].

In an isolation experiment, chlamydophila was isolated from conjunctiva, nictitating membrane, lung, tonsil, liver, spleen and kidney. These results were concident with the results of immunohistological examination in which chlamydophila was detected in the same tissues. There have been no previous reports about the detection of chlamydophila in such a large number of tissues. The isolation of chlamydophila from the blood of cats infected with *C. felis* indicated that *C. felis* could be disseminated via infectious blood in the bodies of cats.

Pathological examination revealed no marked change in tissues except in the conjunctiva and nictitating membrane during the experimental period. This suggested that *C. felis* B166 strain caused disease which was limited to conjunctivitis.

In this study, serum antibody titer higher than 8 was first detected by an indirect MIF test on PID 21. It has been reported that complement-fixing antibody plays a small role in protection against chlamydial infection in cats [6, 15]. Therefore, it remains necessary to examine the relationship between the MIF antibody titer and the protection against *C.
In humans, the major disease caused by Chlamydia trachomatis (C. trachomatis) are trachoma and sexually transmitted diseases [11]. Cats inoculated with C. felis develop conjunctivitis with severe swelling of the eyelid. This was associated with isolation of chlamydophila from vaginal swabs. C. felis caused chronic salpingitis followed by persistent infection of the oviduct, which suggests that genital transmission and infertility may occur in the infected cats. It is inferred that the pathognomonic nature of C. trachomatis infection in humans may be very similar to that of C. felis infection in cats.

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REFERENCES