BRIEF NOTE

Ocular Lesions in Experimental Canine Brucellosis

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Besides characteristic affection of the genital organs, several investigators have noticed involvement of the central nervous system [3, 4], vertebrae [5] or ocular system [7] in chronic canine brucellosis caused by Brucella canis (B. canis). In the course of experiments on B. canis infection in dogs, we observed incidentally corneal opacification with recurrence in two of three beagles having chronic infection. This brief report deals with ocular lesions of these cases.

The cases were male beagles 15 months of age having had no agglutinin against B. canis at the beginning of the experiment. A strain of B. canis, ZD-14, an isolate from a spontaneous case in this laboratory [8], was used for inoculation. The organism, which was grown aerobically on tryptosoy agar (TSA) (Eiken, Tokyo) slant at 37°C for two days, was suspended in phosphate-buffered saline pH 7.4, (PBS), and 2x10^8 colony forming units (CFU) were inoculated into the cephalic vein. For collecting aqueous fluid, dogs were anesthetized with Ketalar® (Park, Davis & Sankyo, Tokyo), and the surface of the eyes was washed by flushing sterilized PBS with a syringe. The aqueous fluid was withdrawn from the anterior chamber of the eyes with a 27 gauge needle inserted at the lateral limbal margin of cornea. Usually 0.2 to 0.5 ml of the fluid was obtained. Immediately after collection, 0.1 ml of the fluid was inoculated directly on a TSA plate for detection of B. canis. The rest of samples was subjected to agglutination test according to a modification of Carmichael’s method [9]. At the same time, about 3 ml of blood was sampled aseptically from the cephalic vein and then 0.1 ml each of the blood was inoculated on two TSA plates for examination of bacteremia. Serum was obtained from the rest of blood samples for agglutination test. The inoculated plates were incubated aerobically at 37°C for 7 days. Suspected colonies developed on plates were examined for morphology after Gram’s stain and agglutination using anti-B. canis rabbit serum. At autopsy, the both eyes were removed, fixed in 10% neutral buffered formalin, and were embedded in paraffin. Sections 5 μm thick were prepared and stained with hematoxylin and eosin.

The clinical histories of the two cases (Cases A and B) are summarized in Fig. 1,
Table 1. Recovery of *B. canis* and agglutinin titer in serum and eyes

<table>
<thead>
<tr>
<th>Case</th>
<th>Days after inoculation</th>
<th>Recovery of <em>B. canis</em></th>
<th>Agglutinin titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Blood</td>
<td>Aqueous fluid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R  L</td>
<td>R  L</td>
</tr>
<tr>
<td>A</td>
<td>244</td>
<td>— —</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>301</td>
<td>— +</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>382</td>
<td>— —</td>
<td>ND</td>
</tr>
<tr>
<td>B</td>
<td>244</td>
<td>+ ND</td>
<td>1:1280</td>
</tr>
<tr>
<td></td>
<td>301</td>
<td>+ —</td>
<td>1:640 ≥ 1:20 &gt; 1:640</td>
</tr>
<tr>
<td></td>
<td>385</td>
<td>— —</td>
<td>1:640 ≥ 1:20 &gt; 1:2560</td>
</tr>
</tbody>
</table>

*Not done or not determined.
R: Right eye. L: Left eye.

Fig. 1. History of corneal opacification

<table>
<thead>
<tr>
<th>Case</th>
<th>Eye</th>
<th>Days after inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200</td>
<td>250 300 350 400</td>
</tr>
<tr>
<td>A</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L</td>
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<td>B</td>
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<td>L</td>
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</tr>
</tbody>
</table>

Remarks.
Height shows the severity of ophthalmitis.

and the results of bacteriological and serological examinations are presented in Table 1.

**Case A:** Bilateral corneal opacity, more severe in the right eye, was first recognized on day 238 postinoculation. The opacity abated after a duration of 3 weeks. Corneal opacification recurred bilaterally on day 280, also with more severe affection in the right eye. At the next week, however, changes in the left eye became much more intense and later, both eyes showed changes of similar intensity. Bacteriological examination revealed only 1 CFU of *B. canis* from the right eye at day 301. Corneal opacity in the both eyes disappeared 5 weeks after the second onset, but another recurrence was observed bilaterally on day 350 with a duration of one week. Furthermore, unilateral severe opacity in the right eye came again on day 376 (Fig. 2). The dog was autopsied on day 382 when the opacity in the right eye still remained. Turbid aqueous fluid from the anterior chamber of the right eye was clotted immediately after collection, whereas that from the left eye remained clear without clotting within a few hours.

Agglutinin titers of the aqueous fluid taken during the onset and at autopsy were almost equal to or higher than those of serum sampled at corresponding times (Table 1).

Histological changes were non-granulomatous iridocyclitis (Fig. 3) and exudative retinitis. Diffuse infiltration of plasma cells and circumscribed lymphoid nodules near the encirculating artery were observed in the iris. There were remarkable congestion, slight hemorrhage and diffuse infiltration of plasma cells sometimes having Russell’s bodies in the ciliary body. Plasma cell infiltration was seen into not only the vessel layer but also the ciliary processes (Fig. 4) and lymphocytes were seen between fibrinous meshwork in the posterior chamber, trabecular channel and substantia propria covering the Descemet’s membrane near the scleral border. In the latter area, there was also some hemorrhage. The cor-
neal endothelium was shown to have vacuolated cytoplasm and detached from the Descemet's membrane, where moderate infiltration of plasma cells and neutrophils was seen. Serous exudate and some leukocytes were seen in the anterior chamber as well as in the vitreous body, and the latter was recognized as a white mass when the formalin-fixed eye globes were opened. There was diffuse infiltration of plasma cells between the inner plexiform layer and the nerve-fiber layer of retina. Some serous exudate and a few lymphocytes were seen between the choroid and detached retina (Fig. 5). Infiltration of plasma cells and lymphocytes was seen in the choroid near ora serrata of the left eye, although no pathological changes were found in the choroid of the right eye. In addition, some lymphocytes were accumulated around the anterior ciliary vein in the scleral border of both eyes.

**Case B:** Ophthalmic changes were seen only on the left eye throughout the observation. Corneal opacification was first detected on day 217 postinoculation, persisted for 8 weeks, and then abated. The recurrence occurred on day 280 persisting for 10 weeks. Opacity was not so remarkable for 5 weeks after the onset and then it became again severe with a transient abatement at 7th week. In the left eye hyphemia was observed without corneal opacity on day 384. This dog was autopsied on day 385 when some erythrocytes were seen at the bottom of the anterior chamber of the left eye without clotting nor hemolysis. Aqueous fluid of the affected left eye was clotted with slight hemolysis immediately after collection. Aqueous fluid of the affected eye showed agglutinin titer of 1:640 at day 301 and 1:2560 at autopsy, whereas that of non-affected eye was negative. Serum titer remained at 1:640. Only 2 CFU of *B. canis* was detected from the fluid of left eye at day 301.

Histopathology of the affected eye was essentially similar to that of Case A. Though degree of iridocyclitis was less severe in Case B, more marked retinal changes and hemorrhages than those of Case A were found in Case B as shown in Figs. 6 and 7.

The ocular changes described above were considered to be caused by *B. canis* infection, since agglutinin titers of aqueous fluid of affected eyes were usually higher than serum titers. Local formation of antibodies might be suggested by histological findings such as remarkable infiltration of plasma cells in the iris and ciliary body. Serofibrinous exudate in the eye globes might be due to increase of vascular permeability. Retinal detachment may have been produced secondarily by the exudate in the vitreous body.

The findings mentioned above resemble clinically as well as pathologically those observed in "blue eye" in canine viral hepatitis [2] or equine periodic ophthalmia which is suspected of leptospiral infection [1, 6]. The recurrence nature of ophthalmic disorders in the present cases might be more analogous to the latter. However, higher agglutinin titer in the aqueous fluid than in serum was not reported in equine periodic ophthalmia [1] nor in "blue eye" [2]. Further studies must be done to make clear pathogenesis of ocular lesions in canine brucellosis.

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References


Explanation of Figures

Fig. 2. Corneal opacification of the right eye of Case A 382 days after intravenous inoculation.

Fig. 3. Iridocyclitis in the right eye of Case A. Marked inflammatory cells in trabecular channel, iris and posterior chamber. Note detached epithelium of ciliary processes. ×120.

Fig. 4. Plasma cell infiltration in ciliary process and detached epithelium of Case A. ×600.

Fig. 5. Retina of Case A. Perivascular infiltration of lymphocytes in retina and serous exudate between choroid and slightly detached retina. ×600.

Fig. 6. Detached retina and optic papilla of Case B. ×120.

Fig. 7. Remarkable disorganization of detached retina and hemorrhage in vitreous body of Case B. ×300.