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Besnoitia Species (Protozoa, Sporozoa, Toxoplasmatidae): Recognition of Cyclic Transmission by Cats

Abstract. *Isosporan oocysts, measuring 13 by 16 micrometers, from a cat in Hawaii produced* Besnoitia *cysts in tissues of mice and rats. Feeding these cysts to cats led to oocyst shedding after 11 to 13 days, continuing for a mean of 11 days. This indicates a two-host cycle for* Besnoitia, *adding an intestinal phase and oocyst production by a carnivore to the already known tissue stages. Thus a representative of* Besnoitia, *similar to other species in cattle, horses, reindeer, impala, other mammals, and reptiles, has been shown to be a coccidian of cats, capable of being spread by fecal contamination.* Besnoitia *is the fourth mammalian tissue parasite, together with* Toxoplasma, Hammondia, *and* Sarcocystis, *found to produce isosporan-type oocysts.*

During studies on coccidia of cats (*1*), typical *Besnoitia* cysts (Fig. 1, C and E) were observed in the omentum and mesentery and on the serosal surfaces of viscera of mice treated with cortisone acetate and inoculated with an unidentified isosporan-type oocyst (Fig. 1, A and B) from the feces of a stray cat in Hawaii. These observations are of interest because the life cycle of *Besnoitia*, a sporozoan parasite of veterinary and biologic importance, has been obscure.

Of 23 cats fed carcasses of mice infected with the oocyst, 17 developed patent infections. Times to oocyst shedding ranged between 11 and 13 days (average, 12 days) and the patent periods ranged between 5 and 12 days (average, 11 days). Several *Besnoitia* cysts isolated from the omentum and mesentery of infected mice were fed intact to three kittens 3 days old, resulting in oocyst shedding after 16 days in one kitten; two kittens died earlier from a viral infection. Single isolated cysts were fed to four cats, two of which developed a 16-fold increase in antibody, although no oocysts were detected.

Schizonts were found both in intestinal epithelial cells and in the lamina propria of the ileum (Fig. 1F). Macrogametes (Fig. 1G), measuring 10 to 13 μm, were larger than those of *Toxoplasma gondii*, *Hammondia hammondi* (*2*), and *Sarcocystis muris* (*3*). Macrogametocytes were found only in goblet cells of the small intestine. Three out of five cats shed oocysts after a second meal of cyst-infected mice and one cat that received a third meal also shed oocysts. Prepatent periods remained between 12 and 13 days, but oocyst shedding lasted only 3 to 7 days. Likewise, a cat previously infected with *T. gondii* and another infected with *S. muris* shed *Besnoitia* oocysts after consuming *Besnoitia* cysts. The skeletal muscles of two cats were fed to other cats and to mice, but no infections resulted.

The *Besnoitia* oocyst is shed unsporulated (Fig. 1A) and contains a finely granular, light brown sporont, which initially fills the oocyst almost completely and later shrinks in size; there is no micropyle or polar granule. Twenty unsporulated oocysts averaged 12 by 17 μm, ranging from 10 to 13 by 16 to 19 μm. When suspended in 1 or 2 percent sulfuric acid at room temperature (24° ± 1°C) and exposed to air, sporulation is complete between 48 and 96 hours. Each oocyst contains two ellipitical sporocysts, without oocyst residuum. Each sporocyst contains four sporozoites and a diffuse granular residuum; no Stieda body was observed (Fig. 1B). The wall of sporulated oocysts is smooth, about 0.5 μm thick, and appears to consist of two layers; the inner layer frequently collapses inward. The average measurement of 100 sporulated oocysts was 13 by 16 μm, ranging from 12 to 15 by 15 to 18 μm. Fifty sporocysts averaged 8 by 11 μm, with ranges from 7 to 8 by 10 to 11 μm. Sporozoites mea-

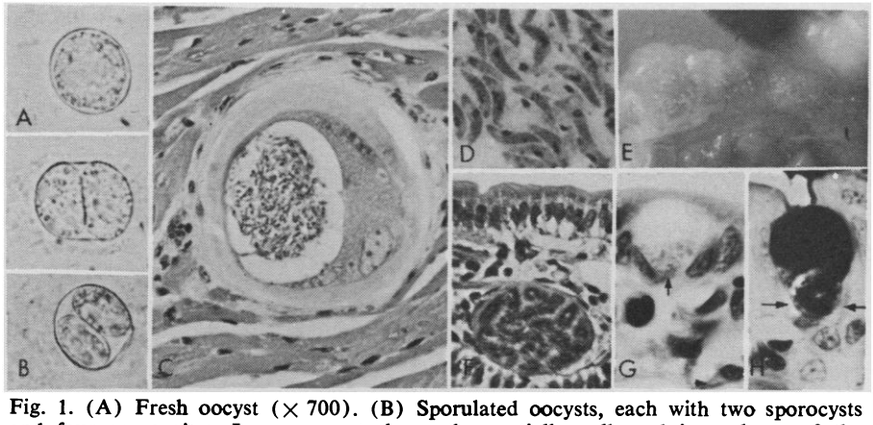

Fig. 1. (A) Fresh oocyst (× 700). (B) Sporulated oocysts, each with two sporocysts and four sporozoites. Lower oocyst shows the partially collapsed inner layer of the oocyst wall (right lower center) (× 700). (C) Section of cyst, in the myocardium of a mouse, with pale-staining cyst wall surrounding host cell, several nuclei of which are in the cytoplasm at the right, with the microorganisms in the cytoplasmic vacuole on the left [hematoxylin and eosin stain (H and E); × 280]. (D) Bradyzoites from cyst in impression film (Giemsa stain; × 700. (E) Cysts developing in intestinal wall of a mouse 48 days after the oral administration of oocysts (× 2). (F) Macroschizont from intestinal lamina propria of cat infected for 11 days (H and E stain; × 280). (G and H) Macrogametocytes in goblet cells of the small intestine of a cat, 16 days after infection. Each parasite (arrows) is at the base of the cells. The mucin remained unstained with H and E (G) but stained deeply with periodic acid—Schiff and hematoxylin (H), which renders the parasite (between arrows) as a series of granules (× 700).

sured about 2 by 10 μm within the sporocyst, and 2.5 by 8 μm outside of the sporocyst when stained with Giemsa.

Oocysts in saline (10^3 to 10^5, 1 week old) were infectious to mice after oral and intraperitoneal administration. However, this was irregular and made numerical comparisons of oocyst numbers and infectious doses impossible. Apparently the oocyst wall resists digestion within the mouse gut. Infectivity for mice was markedly increased when oocysts were ruptured before they were administered, or were exposed first to saline equilibrated with CO_2 for 30 minutes at 37°C, then to 0.5 to 1 percent trypsin (1 : 300) for 2 hours with either 1 to 5 percent cat bile or taurocholic acid. Mice treated with cortisone (2.5 mg injected subcutaneously twice a week) were more heavily infected than nontreated mice. After 2 to 3 months many of the cysts had ruptured and were replaced by granulomas which were about five times the diameter of the cyst (*4*). However, laboratory rats showed similar infections with intact or digested oocysts, and cysts persisted longer than in mice (*4*). After oocysts and sporozoites were inoculated intraperitoneally into mice and rats, cysts were found between 30 and 60 days concentrated in the omentum and mesentery. After peroral administration, cysts were found in the wall of the ileum and cecum (Fig. 1E). Hamsters were not susceptible.

Oocysts were not infectious to six cats to which they were fed, since no oocyst shedding occurred over a period of 42 to 56 days. However, when four of these cats were fed cysts, oocyst shedding followed.

Cysts of *Besnoitia* (Fig. 1C) become visible to the naked eye 4 to 6 weeks after infection of mice or rats (Fig. 1E). They are spherical and measure between 90 and 210 μm in diameter. The cyst wall is 1 to 30 μm thick and encloses the entire host cell, probably a fibroblast. The zoite of *Besnoitia* (Fig. 1D) develops in large numbers within a cytoplasmic vacuole, gradually occupying almost the entire cell, which becomes multinucleate (Fig. 1C). Individual zoites are crescentic and measure 2 to 3 by 10 to 13 μm. With minor variations the new *Besnoitia* cyst resembles those of *B. jellisoni* of mice and *B. besnoiti* in cattle, and of the species in reindeer, horses, and lizards (*4*).

Serologic studies included indirect fluorescent antibody (IFA) tests (*1*), in which zoites from *Besnoitia* cysts were used, and sectioned cysts from heavily infected tissues fixed in 5 percent glacial acetic acid in 100 percent ethanol. Eleven weeks after oral inoculation with oocysts, six mice had developed antibody titers of 1 : 1000 to 1 : 8000 with the homologous antigen. Two of these mice fed to cats proved to be infectious.

Serums from 13 mice with titers equal to or greater than 1 : 1000 with the homologous *Besnoitia* were negative at 1 : 32 when tested against *B. jellisoni* tachyzoites in the dye test. Cross-reactions of 1 : 128 were found between *B. jellisoni* and the new *Besnoitia* sp. in the IFA test. Such serums were also tested against *Toxoplasma* and *Hammondia* antigens by the IFA test. They were usually negative at 1 : 16, but an occasional titer of 1 : 32 was found to one or the other antigen. Cats that were seronegative, or had titers of 1 : 4 to the new *Besnoitia* by IFA before exposure, developed titers of 1 : 64 or higher after oocyst shedding.

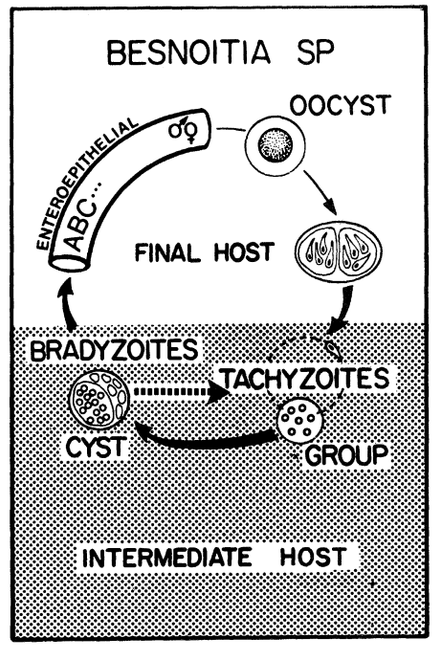

Fig. 2. Postulated life cycle of *Besnoitia* sp. The oocyst (right) is shed unsporulated by cats, the final host. With sporulation, two sporocysts and eight sporozoites are formed. In mice and rats, experimental intermediate hosts, tachyzoites (rapidly multiplying stages) appear in groups, which are more numerous in *B. jellisoni* than in the species presented here. Subsequently bradyzoites (slowly multiplying organisms) develop in cysts which persist during chronic infection. Bradyzoites of *B. jellisoni* and *B. besnoiti* are infectious to other intermediate hosts (interrupted arrow); but this transfer has not been achieved with the species under study. When cysts are ingested by cats, a multiplicative cycle with an unknown number of generations (A, B, C . . .) takes place in the intestinal epithelium, after which male and female gametocytes and, later, oocysts are formed.

The diagnostic feature of *Besnoitia* has been its characteristic cyst composed of a fibrillar wall enclosing hypertrophied and hyperplastic host cell nuclei and a cytoplasmic vacuole containing many bradyzoites (*4*). The relation of the cyst to the oocyst (13 by 16 μm) is based on four cat-mouse-cat passages in which the oocyst-cyst sequence was observed in the absence of other oocysts or sporocysts, or other tissue cysts. *Isospora felis* and *I. rivolta* were observed in some fecal specimens of experimental cats, but they give rise to distinctly different cysts (*5*). The serologic findings and the biologic studies support the hypothesis that the new oocyst (13 by 16 μm) results from the sexual phase of the *Besnoitia* cycle. During the prepatent period nine samples of cat feces were not infectious to mice; the appearance of infectivity of three samples for mice preceded microscopic recognition of oocysts by 1 day. Five inocula containing unsporulated oocysts were noninfectious to mice; six inocula of oocysts from the same lot became infectious to mice between 59 and 64 hours, during which time sporulation increased from 16 to to 60 percent. This hypothesis was also supported by tests designed to separate oocysts and infectivity. Filtration of oocyst suspensions through a bed of glass beads (*6*) was carried out. This showed a close correlation between a pore size of more than 15 to 17 μm, which allowed passage of oocysts and infectivity, and a smaller pore retaining both oocysts and infectivity.

Features of the life cycle of *Besnoitia* are summarized in Fig. 2. Apart from the well-known genera, it will be compared to *Hammondia hammondi*, an obligatory muscle parasite which unlike *Sarcocystis* shows slender bradyzoites and enteroepithelial multiplicative stages and yields unsporulated oocysts much like *Toxoplasma* from which it differs by the obligatory host change (*1*, *2*, *7*). Unlike *Isospora* and

Toxoplasma, but similar to *Hammondia* and *Sarcocystis* (*7*), the oocysts of the new *Besnoitia* sp. are not infectious to the final host. Although a host change appears obligatory between mouse and cat as in *Sarcocystis* and *Hammondia* (*1*, *2*), a facultative transmission among efficient intermediary hosts may be possible as observed with *B. jellisoni*, *B. besnoiti*, and *B. darlingi*, all of which can be subinoculated from one to another intermediate host (*4*, *7*, *8*). It should be pointed out that an oocyst stage of *B. jellisoni* has not been recovered in eleven cats, six bobcats, and one cougar, or in other carnivores examined so far [three foxes, three dogs, one coyote, eight procyonids, four skunks, one owl, one hawk, and one boid, one colubrid, and three viperid snakes (J.K.F.)] *Besnoitia jellisoni* persists in mice for over a year, but mice are relatively poor hosts for the *Besnoitia* under study. In laboratory rats, cysts are more stable. The native intermediary host in Hawaii has not been identified; however, another cat shedding oocysts, 13 by 16 μm, was observed in Hawaii 7 days after it arrived in the laboratory. Since oocysts were found before the known prepatent period, it seemed likely that the cat had been infected outside the laboratory. Mice to which some of these oocysts were fed developed high (> 1 : 1000) IFA titers to *Besnoitia bradyzoites*.

The identification of a carnivore as a final host of *Besnoitia* brings this organism into a closer relation with *Toxoplasma*, *Hammondia*, and *Sarcocystis*, the cycles of which were recently described (*2*, *9*, *10*). These findings appear to confirm the recently reported life cycle of *B. besnoiti* from cattle (*11*) and may help to uncover life cycles of *B. tarandi* from reindeer, *B. bennetti* from horses, and others which are of economic and veterinary importance. It may also be possible to devise control measures, once the host supporting the sexual cycle has been identified.

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Luminous and Chromatic Flickering Patterns Have Opposite Effects

Abstract. *When stimulated in phase by a sinusoidally flickering, uniform field, the red and green cone systems tend to inhibit each other. This inhibition is minimized by (i) counterphase (luminance) patterns or (ii) red/green (chromaticity) flicker. However, when (i) and (ii) are combined, the usual flickering-pattern effect is reversed: instead of enhancing chromatic flicker, counterphase patterns tend to suppress it.*

At frequencies below 10 hertz, the visual sensitivity to sinusoidally flickering stimuli depends on the spatial pattern of the stimulus. This low-frequency sensitivity is least when the flickering stimulus is a patternless, uniform field with no surround, but is greatly increased by so-called counterphase patterns, in which different parts of the visual field flicker in opposite phases (*1*). The effectiveness of such stimuli increases with the number of edges (that is, phase reversals) in the pattern; the optimum stimulus is a rectilinear, counterphase grating with stripes subtending about 10 minutes of visual angle. However, even a single edge (for example, a split field with right and left halves flickering in opposite phases) is sufficient to greatly increase the flicker sensitivity, as shown by the open symbols in Fig. 1.

Sensitivity to luminous flicker characteristically reaches a maximum at about 10 hertz, decreasing at higher and lower frequencies. The low-frequency flank of this passband can be explained in terms of lateral inhibition in the retina (*2*). When the entire field is flickering uniformly, inhibiting pathways provide a form of automatic gain control, which tends to counteract the variation of the stimulus if it is varying slowly enough. Therefore, the uniform-field sensitivity decreases at low frequencies.

The presence of a counterphase edge interferes with this gain control, because the increasing stimulus on one side of the edge and the decreasing stimulus on the other side have opposite effects on the local gain, so that it tends to remain constant in the vicinity of the edge. Therefore, the stimulus amplitude is not attenuated as much near a counterphase edge as it is in a uniform field; this increases the flicker sensitivity as shown by the open squares in Fig. 1. The split-field sensitivity still shows a maximum at 8 hertz, decreasing for lower frequencies, probably because lateral inhibition is still effective at some distance from the counterphase edge (*3*).

However, quite different results are obtained when the hue of the stimulus is varied while its luminosity is held constant. Chromatic flicker sensitivity is typically a low-pass (rather than a bandpass) function of frequency; it decreases monotonically over the same frequency range where the luminous flicker sensitivity is increasing, as shown by the filled symbols in Fig. 1. In these experiments, the chromaticity was varied sinusoidally about the yellow point indicated by the CIE diagram inset in Fig. 1 (*4*). At full modulation, the extreme chromaticities were a saturated red and green (also shown in the inset), very similar to van der Horst's chromatic flicker primaries (*5*); their luminance ratio was adjusted to minimize low-frequency luminous flicker. However, none of these choices is critical,