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Inoculation with *Bartonella henselae* followed by feline herpesvirus 1 fails to activate ocular toxoplasmosis in chronically infected cats

CC Powell[1], DL Kordick[2], MR Lappin[1]

[1]*Colorado State University, , Department of Clinical Sciences, 300 W. Drake, Fort Collins, Colorado 80523-1620;* [2]*Blue Ridge Pharmaceuticals, 4249-105 Piedmont Parkway, Greensboro, NC 27410 USA*

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Infection by *Toxoplasma gondii* is very common in cats although most remain disease free. The factors that trigger development of uveitis in some cats infected with *T gondii* have not been elucidated, but infection by more than one organism may be contributory. In this study, cats chronically infected with *T gondii* were inoculated with *Bartonella henselae* followed by FHV-1 to test the hypothesis that immune stimulation by multiple infections will reactivate ocular toxoplasmosis. Anterior uveitis and chorioretinitis were not detected in the cats with chronic *T gondii* infection thus allowing rejection of the hypothesis using this experimental design.

Chronic and recurrent uveitis is common in cats and has the potential to cause severe ocular damage, resulting in visual impairment or blindness. The cause of uveitis is often difficult to determine with certainty however, up to 74% of cats with uveitis have serological evidence of infection by *Toxoplasma gondii* (Chavkin et al 1992). Many healthy cats are also seropositive for *T gondii* (Lappin et al 1992a) and the organism or intraocular production of *T gondii* specific antibodies can be detected in the aqueous humour even though there is no evidence of existing or previous ocular disease (Lappin et al 1992b, Burney et al 1998). Why some cats infected with *T gondii* develop uveitis and others do not is unknown. In humans, ocular toxoplasmosis is most common following transplacental infection (Dubey & Beattie 1998); this may be true in cats as well. Recently, kittens were shown to develop transient chorioretinitis and anterior uveitis as the result of either transplacental infection or infection in the early neonatal period, without developing other clinical signs of toxoplasmosis (Powell & Lappin 2001). Since non-specific immune stimulation in cats previously infected with *T gondii* is known to increase production of *T gondii* specific IgG in both aqueous humoir and CSF (Lappin et al 1996a) and the seroprevalence of other infectious agents, such as *Bartonella henselae* (up to 80%) (Chomel et al 1995) and feline herpesvirus-1 (up to 90%) (Maggs et al 1999a), is very high in cats, mixed infection or immune mediated reactions may also contribute to disease development. In addition, both *B henselae* (Lappin et al 2000, Lappin & Black 1999) and feline herpesvirus-1 (Maggs et al 1999b) have been linked to uveitis in some cats.

The present study was undertaken to test the hypothesis that immune-stimulation from coinfection with *B henselae* or feline herpesvirus-1 (FHV-1) will reactivate uveitis in kittens infected with *T gondii* in the neonatal period. Four kittens from a separate study, infected in the neonatal period with *T gondii* (Powell & Lappin 2001), comprised the experimental group. Eight kittens, either purchased as specific pathogen free, or born in-house to specific pathogen free queens, were used in the control groups. *Toxoplasma* infected kittens (Group A) were *T gondii* seropositive (Lappin et al 1989) and had previously developed chorioretinitis lesions consistent with ocular toxoplasmosis (Davidson et al 1993). Ocular lesions were resolved for a minimum of 14 weeks prior to the start of this study. Group A kittens were sequentially inoculated with *B henselae* and FHV-1, with 12 weeks between infections. The four control kittens in Group B

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were sequentially infected with *B henselae* and FHV-1 with the same inoculum and time line as for Group A. The four control kittens in Group C were infected only with FHV-1 with the same inoculum and time line as Groups A and B. *Bartonella henselae* was initially isolated from a woman with uveitis (Breitschwerdt & Kordick 1995) and maintained frozen. The organism was subcultured and then passaged in two kittens before inoculation into the kittens described here; infection was confirmed by blood culture. Kittens from Groups A and B were inoculated with *B henselae* by infusing 1 ml of blood from the donor cat. The USDA vaccine challenge strain of FHV-1 was obtained from the Center for Veterinary Biologics Laboratory and maintained frozen at −70°C until used.* All cats in each group were administered 20 mg ketamine (Vetalar; Park Davis) and 0.05 mg acepromazine (Vedco) IV and administered the vaccine challenge dose of FHV-1 by use of an atomiser (half volume into the oropharynx and quarter volume into each nostril). Inoculation with FHV-1 was done 12 weeks after infection with *B hensleae*. A general physical examination (temperature, pulse, respiration rate, and lung auscultation) was done daily for 2 weeks after inoculation with each organism. Ocular examinations were done prior to inoculation with *Bartonella* or FHV-1 and then weekly for 10 weeks after inoculation with each organism. Ocular examinations included slit lamp biomicroscopy and indirect ophthalmoscopy, after pupil dilation with 1% tropicamide (Tropicacyl 1%; Akorn). Blood was collected from Group A and Group B cats weekly for 6 weeks post inoculation for performance of *Bartonella* culture.

Bartonella henselae was cultured from the blood of all Group A and Group B cats, confirming infection. Systemic illness was not detected in cats from Groups A or B after infection with *B henselae*. All of the 12 cats developed mild upper respiratory disease and conjunctivitis within 10 days of inoculation with FHV-1 suggesting that infection by the organism occurred. Reactivation of *T gondii* chorioretinitis was not detected in Group A cats. Group B cats were ophthalmoscopically normal for the duration of the study. Two cats in Group C developed mild anterior uveitis 11 and 25 days post-infection that resolved within 1 week of appearance. Posterior segment lesions were not detected in Group C cats. A summary of the experimental protocol and results can be found in Table 1.

That *T gondii* causes both anterior and posterior uveitis in cats with generalised toxoplasmosis is undisputed (Dubey & Beattie 1993) since the organism is readily identified on histopathology. However, in seropositive cats with uveitis and no other clinical evidence of disease, the organism is rarely found (Peiffer & Wilcock 1991). This has caused some to question the role of *T gondii* in development of ocular disease in cats (Davidson & English 1998). Although evidence exists to support its involvement (Lappin et al 1992a, Lappin et al 1992b, Lappin et al 1993, Lappin et al 1996b), the mechanisms are elusive and likely are dependent on other factors that favour development of uveitis in some cats but not others. Potentially, the organism is present in the eye and causing direct tissue damage but due to low numbers, is difficult to detect. Another possibility is the organism somehow initiates either autoimmunity or ocular hypersensitivity. In a non-human primate model of ocular toxoplasmosis, intraocular injection of *T gondii* antigen in previously infected animals resulted in anterior uveitis, vitreous inflammation, and retinal vasculitis suggesting development of hypersensitivity (Newman et al 1982). We hypothesised that interactive effects of immune stimulation with infection by other agents could potentially trigger uveitis in eyes previously sensitised to *T gondii.* However, activation of chronic *T gondii* chorioretinitis or anterior uveitis was not documented in Group A cats of this study following coinfection by *B henselae* and FHV-1. These results allow for rejection of the hypothesis using this experimental design. Whether other forms of immune stimulation or other coinfections will activate toxoplasmic uveitis is unknown.

Bartonella henselae associated uveitis has been documented frequently in humans (Ormerod & Dailey 1999) and has been suggested in 2 veterinary publications in cats (Lappin et al 2000, Lappin & Black 1999), however the majority of infected cats remain disease free. How bartonella and other infectious agents are involved with triggering uveitis is complex and ill defined. Experimental evidence strongly supports immune-mediated mechanisms for uveitis in humans (Gery & Nussenblat 1996) and a relationship between *B henselae* associated uveitis and the presence of HLA-B27 has been proposed (Kerkhoff & Rothora 2000). Genetics may

*USDA, Animal and Plant Health Inspection Service, Veterinary Services, Center for Veterinary Biologics-Laboratory, Ames, IA, USA.

Table 1. Summary of experimental protocol and results

Group/ kitten #	Date of birth	Infection date			Ocular signs		
		T gondii	*Bartonella henselae*	FHV-1	Initial detection date	No longer detectable	Location*
A–1	10/11/98	TPN†	31/7/99	22/10/99	4/2/99	22/3/99	CR
A–2	10/11/98	TPN	31/7/99	22/10/99	14/1/99	5/3/99	CR
A–3	10/11/98	TPN	31/7/99	22/10/99	14/1/99	5/3/99	CR
A–4	17/11/98	TPN	31/7/99	22/10/99	4/2/99	26/3/99	AU & CR
B–1	22/10/98	—	31/7/99	22/10/99	—	—	—
B–2	22/10/98	—	31/7/99	22/10/99	—	—	—
B–3	22/10/98	—	31/7/99	22/10/99	—	—	—
B–4	22/10/98	—	31/7/99	22/10/99	—	—	—
C–1	18/6/99	—	—	22/10/99	2/11/99	9/11/99	AU
C–2	18/6/99	—	—	22/10/99	16/11/99	23/11/99	AU
C–3	18/6/99	—	—	22/10/99	—	—	—
C–4	18/6/99	—	—	22/10/99	—	—	—

*AU, Anterior uveitis; CR, chorioretinitis.
†TPN, Transplacental or neonatal.

also play a role in cats, which could explain why none of the cats in this study developed ocular disease after inoculation with *B henselae*. It is also possible that strain differences are involved and this strain of *B henselae*, although pathogenic in humans, is not pathogenic in cats. Additionally, the isolate used in this study had been frozen and subcultured which may have changed the virulence of the isolate. However, in an attempt to stimulate antigenic expression that might have been altered by in vitro culture, the strain was passaged twice through kittens before being used as an inoculum.

FHV-1 is commonly associated with ophthalmic disease. Principle manifestations include conjunctivitis, dendritic ulcers, and possibly, corneal sequestra. It was previously believed that if FHV-1 was associated with intraocular inflammation it was via extension from a diseased cornea. Unlike herpes simplex virus infection in humans, FHV-1 infection has not been considered a cause of endogenous uveitis in cats. Local production of FHV-1 antibody and FHV-1 DNA was detected in some cats with 'idiopathic' uveitis (Maggs et al 1999b). These results led to the conclusion that FHV-1 infection could potentially result in uveal tract inflammation. This possibility is supported by the transient uveitis seen in two kittens in this study from Group C. Since these cats were initially specific pathogen free, were infected only with FHV-1, and were kept in a controlled environment, uveitis caused by another infectious agent is very unlikely. Although uveitis could have occurred due to trauma, physical and ocular examination did not support trauma as a cause. To our knowledge, this is the first report of anterior uveitis in cats experimentally inoculated with FHV-1 with no evidence of corneal disease. Our findings support the hypothesis that FHV-1 may be associated with uveitis in cats.

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