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Does a Feline Leukemia Virus Infection Pave the Way for *Bartonella henselae* Infection in Cats?▽

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Domestic cats serve as the reservoir hosts of *Bartonella henselae* and may develop mild clinical symptoms or none after experimental infection. In humans, *B. henselae* infection can result in self-limiting cat scratch disease. However, immunocompromised patients may suffer from more-severe courses of infection or may even develop the potentially lethal disease bacillary angiomatosis. It was reasoned that cats with immunocompromising viral infections may react similarly to *B. henselae* infection. The aim of our study was to investigate the influence of the most important viruses known to cause immunosuppression in cats—*Feline leukemia virus* (FeLV), *Feline immunodeficiency virus* (FIV), and *Feline panleukopenia virus* (FPV)—on natural *B. henselae* infection in cats. Accordingly, 142 cats from animal shelters were necropsied and tested for *B. henselae* and concurrent infections with FeLV, FIV, or FPV by PCR and immunohistochemistry. A significant association was found between *B. henselae* and FeLV infections ($P = 0.00028$), but not between *B. henselae* and FIV ($P = 1.0$) or FPV ($P = 0.756$) infection, age ($P = 0.392$), or gender ($P = 0.126$). The results suggest that susceptibility to *B. henselae* infection is higher in cats with concurrent FeLV infections, regardless of whether the infection is latent or progressive. Histopathology and immunohistochemistry for *B. henselae* failed to identify lesions that could be attributed specifically to *B. henselae* infection. We conclude that the course of natural *B. henselae* infection in cats does not seem to be influenced by immunosuppressive viral infections in general but that latent FeLV infection may predispose cats to *B. henselae* infection or persistence.

The outcome of *Bartonella henselae* infection for human patients depends mostly on their immune status. In immunocompetent patients, *B. henselae* causes the self-limiting cat scratch disease, characterized by local granulomatous to abscedating lymphadenitis (10). Immunocompromised hosts, in contrast, are prone to suffer from disseminated cat scratch disease with bacteremia and may develop angioproliferative lesions, including peliosis hepatis or the potentially lethal disease bacillary angiomatosis (38, 39). Domestic cats are one well-known reservoir host of *B. henselae* and transmit the agent to humans through scratch marks or bite wounds. The prevalence of feline infection is high in animal shelters and populations of stray cats, since fleas transmit the agent among cats (6). A recent epidemiologic study revealed a *B. henselae* bacteremia prevalence of 18.7% among cats from animal shelters, whereas only 1% of pet cats in the same area were found to be bacteremic (4). The clinical course and pathology of feline bartonellosis differ from those for human infections. In domestic cats, natural infection has been associated with gingivitis, stomatitis, lymphadenopathy, uveitis, and urinary tract diseases (13, 21, 42). Experimental infection, on the other hand, leads to prolonged and relapsing intraerythrocytic bacteremia with either no clinical symptoms or mild unspecific symptoms (1, 6, 19, 35). Histologically, specific-pathogen-free cats with acute *B. henselae* infections had marked generalized lymphatic hyperplasia (15, 20). In chronic experimental infections, a variety of additional unspecific histological lesions, including lymphocytic interstitial nephritis, cholangitis, hepatitis, and lymphoplasmacytic myocarditis, were observed (20).

In contrast to the availability of data on bartonellosis in otherwise healthy experimentally infected cats, no systematic investigations of the significance and lesions of natural *B. henselae* infection in cats with immunosuppressive diseases have been conducted.

The aim of the present study was to investigate systematically the influence of immunosuppressive infectious diseases on natural *B. henselae* infection in cats. We hypothesized that, as with humans, immunosuppressive diseases in cats may alter both susceptibility to *B. henselae* and the course of infection. Accordingly, we focused on the three most important agents known to cause immunosuppression in cats: feline leukemia virus (FeLV), feline immunodeficiency virus (FIV), and feline panleukopenia virus (FPV).

FeLV infection. FeLV, a gammaretrovirus, causes immunosuppression in as many as 50% of cats with progressive infections, and a replication-defective strain named FeLV-FAIDS causes the fatal feline AIDS (FAIDS) in 100% of viremic cats within the first year of infection (17, 27, 32). According to a recent study by Torres and colleagues (41), four possible courses of FeLV infection can be distinguished by determination of proviral and antigen loads in blood or lymphatic tissues. Besides self-limited (abortive) and regressive courses, latent and progressive infections occur. Latent FeLV infections can be distinguished from progressive infections by the absence of detectable antigen (41). Aside from the risk of infecting other cats following reactivation, the clinical significance of latent FeLV infection remains unclear (28, 30). Recent studies have stated that the prevalence of progressive FeLV infection in

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TABLE 1. Primers and annealing temperatures for the detection of *B. henselae* by PCR

Target gene and assay (segment amplified)	Primer name	Primer sequence (5′–3′)	Annealing temp (°C)	Amplicon size (bp)	Reference(s)
htrA					
Assay I (nt 1209–1372)	3+ (outer forward)	GTG CGT TAA TTA CCG ATC CA	58.0	163	3, 5
	4− (outer reverse)	CCA AAC TCC TAA GGT TAC TGT TTC	58.0		5
	1+ (inner forward)	GCT GGT ATC AAG GCA GGT G	58.0	82	5
	2− (inner reverse)	GCA ATA CGC TTT GCT AGA TCAC	58.0		5
Assay II (nt 1250–1332)	6+ (outer forward)	CCA GAT CAA CAT CCC TTG AAA	58.0	182	5
	5− (outer reverse)	GCG TAA CTT GTG CCA TCA GA	58.0		5
	7+ (inner forward)	TTT GCA ACG TTC GCA TAG ACT	58.0	93	5
	8− (inner reverse)	CAG AAA TCA CAT GAT TAT TGG TCAC	58.0		5
gltA (nt 504–1178)	f1 (outer forward)	GGT CCC AAC TCT TGC CGC TAT G	65.0	675	34
	r1 (outer reverse)	CAG CCG ACA CTG CGT GCT AAT G	65.0		34
	f2 (inner forward)	ATG CCT AAA AAT GTT ACA AGA	58.0	354	34
	r2 (inner reverse)	CGT GCT AAT GCA AAA AGA AC	58.0		34

Germany is between 2.9% (14) and 9% (40), whereas the percentage of latent infections can be as high as 50% (40). Interestingly, in one of these studies, latent infections were significantly more prevalent among cats from animal shelters (40).

FIV infection. FIV, a feline lentivirus, is associated with an AIDS-like syndrome in the terminal phase of infection (29). Its prevalence is highest among free-roaming male cats over the age of 4 years (7). Initial infection is characterized by relatively mild clinical symptoms, including transient fever, anorexia, and generalized lymphadenopathy, which can last as long as several months (31). A second, asymptomatic phase follows and lasts as long as 6 years (16). The onset of feline AIDS is defined by secondary and opportunistic infections due to immunosuppression. The overall prevalence of FIV infection in Germany is thought to range from 2.5% (14) to 8.4% (12).

FPV infection. FPV causes feline panleukopenia, characterized by enteritis, severe lymphatic depletion, and bone marrow necrosis, primarily in young cats. The resulting panleukopenia leads to severe immunosuppression.

Coinfections. Only two previous studies have investigated a possible link between *B. henselae* and FeLV or FIV infection in cats, with conflicting results. A 1997 study observed that the prevalence or clinical signs of *B. henselae* infection in cats were not influenced by FeLV or FIV infection (13). In contrast, a different study found that cats coinfected with FIV and *B. henselae* had a significantly higher frequency of lymphadenopathy than cats infected with FIV alone. However, the prevalence of *B. henselae* infection was not affected by FeLV or FIV infection, as determined by comparison to controls (42). Possible associations of *B. henselae* with FPV infections have not been investigated previously.

This study provides a systematic investigation of natural infections with FeLV, FIV, and FPV, the three most important viral pathogens known to induce immunosuppression in felines, and their possible association with *B. henselae* in naturally infected cats.

MATERIALS AND METHODS

Patients. From October 2006 to November 2008, blood and tissue specimens were collected during necropsy from 70 male and 72 female cats from animal shelters in the Berlin, Germany, area. Cats were divided into two age groups: 0 to 12 months and >1 year. At necropsy, tissue samples of mandibular, retropharyngeal, and popliteal lymph nodes, tonsils, spleen, liver, and peripheral blood were collected. All specimens were split in half; one half was frozen at −20°C and the other half was fixed in 10% formalin and embedded in paraffin through graded alcohols. In addition, histopathology was performed on all major organ systems using 4-μm-thick hematoxylin-and-eosin-stained sections from formalin-fixed, paraffin-embedded tissue specimens.

PCR testing for *B. henselae*. DNA was extracted from all frozen tissue samples by using a commercial kit (QiaAmp DNA Mini kit; Qiagen, Hilden, Germany). *B. henselae* DNA was amplified using two separate nested PCR assays targeting different segments of the *htrA* gene (GenBank accession no. L20127), as described previously (5). Primer sequences are given in Table 1. Samples that tested positive in either of the two *htrA* assays were confirmed by replication of both *htrA* assays and by a second species-specific nested PCR targeting the citrate synthase (*gltA*) gene of *B. henselae* (GenBank accession no. L38987) as described by Regnath et al. (34). PCR was performed in the same way as for the *htrA* assays, except for an annealing temperature of 65°C in the first amplification round and a primer concentration of 25 pmol with 1 mM $MgCl_2$. For all nested PCR assays, the detection limit was determined to be approximately 1 CFU by serial dilution of culture-grown, heat-deactivated *B. henselae*. Bacterial DNA from culture-grown, hydrolyzed *B. henselae* strain Berlin-1 (kindly provided by Mardjan Arvand, Rostock University, Rostock, Germany) was used as a positive control. Negative controls with no template DNA were included in all amplifications. PCR products were visualized by ethidium bromide staining after agarose gel electrophoresis.

Immunohistochemical detection of *B. henselae*. Tissue samples from all PCR-positive cats underwent immunohistochemical analysis (IHC) using *Bartonella*-specific, affinity-purified rabbit immunoglobulin G (IgG) as a primary antibody, as described previously (5). Vero cells infected with *B. henselae* were used as positive controls, whereas uninfected Vero cells served as negative controls.

Detection of FeLV, FIV, and FPV infections. The primers and annealing temperatures for all amplifications are summarized in Table 2. Otherwise, PCR conditions were identical to those for *B. henselae*.

FeLV infection. Bone marrow was tested for the FeLV genome by seminested PCR (18, 40) targeting the U3 long terminal repeat (LTR) region of the FeLV provirus (GenBank accession no. L25632). To distinguish between progressive and latent FeLV infections, IHC of bone marrow, spleen, and mandibular lymph nodes was performed for all cats with detectable proviral DNA in the bone marrow. Tissue from a feline case of FeLV gp70 antigen-positive malignant lymphoma served as a positive control. Antigen was retrieved by microwave heating (560 W) for 12 min in 10 mM citric acid (pH 6.0) supplemented with 0.05% Triton X-100. As a primary antibody, mouse monoclonal IgG directed against the envelope protein gp70 of feline leukemia virus, diluted 1:200 (catalog no. MCA1897; AbD Serotec, Duesseldorf, Germany), was used. Negative controls, consisting of purified mouse IgG from preimmune sera diluted 1:200 (Mouse Super Sensitive Negative Control HK119; BioGenex Laboratories, San Ramon, CA), were included for all slides. Biotinylated goat anti-mouse IgG, diluted 1:200 (Vector Laboratories Inc., Burlingame, CA), was used as a secondary antibody. Staining was carried out using a streptavidin-biotin-peroxidase

TABLE 2. Primers and annealing temperatures for the detection of FIV, FeLV, and FPV by PCR

Assay/target gene (segment amplified)	Primer name	Primer sequence (5′–3′)	Annealing temp (°C)	Amplicon size (bp)	Source or reference(s)
FIV provirus/*gag* p26 (nt 1057–1639)	E 1+ (forward)	CCA CAA TAT GTA GCA CTT GAC C	56.0		11
	E 2− (outer reverse)	GGG TAC TTT CTG GCT TAA GGT G	56.0	583	11
	N 4− (inner reverse)	TAA TGG TCT GGG AGC ATC AG	56.0	249	This report
FeLV provirus/U3 LTR (nt 2107–2290)	f1 (outer forward)	TTA CTC AAG TAT GTT CCC ATG	55.0	184	18
	r1 (reverse)	AGG TCG AAC TCT GGT CAA CT	55.0		18, 40
	f2 (inner forward)	CTT GAG GCC AAG AAC AGT TA	52.7	104	18, 40
FPV/VP2 gene (nt 851–1022)	1A+ (forward)	CAT TGG GCT TAC CAC CAT TT	55.0	172	This report
	2− (reverse)	GGT GCA CTA TAA CCA ACC TCA GC	55.0		37

system (Vectastain Elite ABC kit; Vector Laboratories Inc.) with 3,3′-diaminobenzidine tetrahydrochloride as the chromogen (DAB buffer tablets; Merck, Darmstadt, Germany).

FIV infection. Peripheral blood was tested for FIV infection by seminested PCR targeting the proviral *gag* p26 region (GenBank accession no. NC_001482) as described by English et al. (11).

FPV infection. FPV infection was detected by histopathology and standard PCR of bone marrow and peripheral lymph nodes of all cats, using primers targeting the VP2 gene (GenBank accession no. GQ169552) of FPV (37).

Statistical evaluation. To test for associations between *B. henselae* infection and FeLV, FIV, or FPV infection, sex, or age, two-tailed Fisher exact tests were performed using SPSS (version 16.0) statistical software with a specified significance level (P) of <0.05.

RESULTS

The median age of the cats was 12 months, with a range of 0.5 months to 18 years and a mean age of 48 months. Fifty percent were 0 to 12 months old, and 47.2% were older than 1 year. The ages of four cats (2.8%) were unknown. All cats either had died spontaneously or were euthanized due to the lesions summarized in Table 3.

The overall prevalence of *B. henselae* infection was found to be 7.7%. In 11 of 142 cats, *B. henselae* DNA was detected by PCR in blood and/or peripheral lymph nodes, spleen, tonsils, and bone marrow, with the highest rate of detection in peripheral lymph nodes and blood (Table 4). In 3 of the 11 cats, *B. henselae* was also identified immunohistochemically in lymphatic tissues. The bacteria were characterized by a strong cytoplasmic signal in individual histiocytic cells, similar to the pattern seen in human cat scratch disease (22). However, histopathology and immunohistochemical analysis failed to identify any organ lesions that could be attributed to *B. henselae*. None of the lesions previously linked with experimental or natural infection were observed at higher frequencies in cats with *B. henselae* infection than in controls in this study.

TABLE 3. Lesions resulting in death or euthanasia for the cats included in this study[a]

Lesion	No. of cats affected
Inflammatory lesions	
Gastroenteritis due to feline panleukopenia virus	62
Gastroenteritis due to other causes	9
Respiratory infections	15
Urogenital diseases	4
Feline infectious peritonitis	5
Other	5
Hypertrophic cardiomyopathy	14
Neoplasia of various organs	9
Degenerative diseases	8
Trauma	2
Miscellaneous	9

[a] A total of 142 cats were studied.

Table 5 summarizes the prevalence of *B. henselae* infection in relation to infections with FeLV, FIV, or FPV. Six of 142 cats (4.2%) were infected with FeLV; 4 of these were coinfected with *B. henselae.* Interestingly, latent FeLV infection (indicated by positivity for provirus and negativity for gp70) was found in three of the four cats with concurrent FeLV and *B. henselae* infections, whereas only one of two cats with progressive FeLV infections (provirus positive, gp70 positive) was coinfected with *B. henselae*. Blood from four cats (2.8%) tested positive for FIV proviral DNA, but none of these cats had a *B. henselae* infection. One of the four cats had typical lesions consistent with the lymphadenopathy stage of FIV infection. The other three cats had no histological changes, suggesting that they were in the asymptomatic stage. FPV infection was detected in 62 of 142 cats (43.7%) by characteristic histopathological changes of lymphatic depletion, bone marrow necrosis, and intestinal crypt epithelial necroses. All cases were confirmed by PCR for FPV. Of the 62 cats with FPV infection, only 4 were coinfected with *B. henselae*.

Statistical evaluation revealed a significant association between *B. henselae* and FeLV infections ($P = 0.00028$), but not between *B. henselae* infection and FIV ($P = 1.0$) or FPV ($P = 0.348$) infection, age ($P = 0.524$), or sex ($P = 0.126$).

DISCUSSION

In the present study, 142 cats from animal shelters were tested for *B. henselae* infection and concurrent viral infections known to cause immunosuppression in cats. Interestingly, the prevalence of *B. henselae* infection was found to be significantly correlated only with FeLV infection, regardless of whether this was latent or progressive. There was no correlation with FIV or FPV infection or with other allegedly immunosuppressive conditions, including cachexia, very old age, or very young age. At the same time, there was no evidence that the course and pathology of *B. henselae* infection are influenced either by FeLV infection or by other conditions known to cause immunosuppression in cats.

There are several possible explanations for the observed

TABLE 4. Details for 11 cats that tested positive for *B. henselae* infection, including data on coinfections with FeLV, FIV, or FPV[a]

Cat no.	Sex, age (mo)	Detection of *B. henselae*		FeLV infection[b]	Other viral infections	Cause of death
		By PCR	By IHC			
1	Male, 96	Popliteal ln	Popliteal ln	Latent	None	Hypertrophic cardiomyopathy
2	Male, 108	Blood	Negative	Latent	None	Enteric leiomyoma
3	Male, 3	Popliteal ln, tonsils, spleen, blood	Tonsils, spleen	No infection	None	Urolithiasis, hydronephrosis, emaciation
4	Male, NA	Tonsils	Negative	No infection	FPV	Necrotizing enteritis
5	Female, 3	Spleen, bone marrow	Spleen	No infection	FPV	Necrotizing rhinitis, bone marrow necroses
6	Male, 4	Blood	Negative	Latent	FPV	Necrotizing enteritis and rhinitis
7	Male, adult	Tonsils, blood; mandibular, retropharyngeal, and popliteal ln	Negative	Progressive	None	Chronic myocarditis, hypertrophic cardiomyopathy, lymphadenopathy
8	Male, 144	Blood	Negative	No infection	None	Bilateral interstitial nephritis, uremia
9	Female, 36	Blood	Negative	No infection	None	Epileptic seizures
10	Female, 5	Blood	Negative	No infection	FPV	Necrotizing enteritis
11	Male, 96	Blood	Negative	No infection	None	Suppurative and necrotizing bronchopneumonia

[a] IHC, immunohistochemical analysis; FeLV, feline leukemia virus; FIV, feline immunodeficiency virus; FPV, feline panleukopenia virus; ln, lymph nodes; NA, not available.
[b] Cats with latent FeLV infections were positive for provirus and negative for gp70; those with progressive FeLV infections were positive both for provirus and for gp70.

association of FeLV and *B. henselae* infection. On the one hand, both *B. henselae* and FeLV are transmitted by fleas, which may account for the high coinfection rate (43). On the other hand, both agents have a tropism for hematopoietic progenitor cells. FeLV subgroups A and C (FeLV-A and FeLV-C) have been shown to infect bone marrow cells of all three lineages (9). In addition, FeLV-C inhibits erythroid progenitor cells at the level of CFU, resulting in aplastic anemia (2, 33). Maendle et al. (24) have recently demonstrated invasion and persistence of *B. henselae* in human $CD34^+$ hematopoietic progenitor cells, which resulted in reduced proliferation but did not affect the cells' erythroid differentiation. As a consequence, infection was sustained even through the cells' maturation, accounting for the presence of *B. henselae* inside human erythrocytes. In contrast, the agent was shown to be unable to invade human erythrocytes directly. Whether the same mechanism applies to the cat is not known. However, infection of hematopoietic progenitor cells may be an explanation for the high number of intracellular *B. henselae* bacteria previously detected in feline red blood cells by immunofluorescent techniques (36). Initially, this seemed to conflict with the finding that direct invasion of feline erythrocytes by *B. henselae* is not very efficient (25). Based on these results, we hypothesize that infection of hematopoietic progenitor cells by FeLV prepares the ground for *B. henselae*, making the cells more susceptible either to infection by *B. henselae* or to bacterial persistence. The mechanisms and functional aspects of this putative interaction will require further investigation.

TABLE 5. Prevalence of *B. henselae* infection in relation to viral infections associated with immunosuppression in cats

Viral coinfection	No. of cats[a]	
	B. henselae positive (n = 11)	*B. henselae* negative (n = 131)
FeLV		
Latent infection	3	1
Progressive infection	1	1
FIV positivity	0	4
FPV positivity	4	58
None of the above	3	67

[a] A total of 142 cats were studied.

There was no apparent association between *B. henselae* infection and FIV infection in the present study. This is consistent with the findings of previous studies in cats (13, 42). The lack of correlation between the prevalence of FIV and *B. henselae* infection may be due to the fact that FIV, like HIV and unlike *B. henselae* and FeLV, is not transmitted by fleas. Similarly, HIV is not arthropod borne (8). In contrast to *B. henselae* and FeLV, FIV does not affect hematopoietic progenitor cells (23). Similarly, although HIV may induce severe bone marrow depression, infection of hematopoietic progenitor cells *in vivo* is extremely rare (26).

To our knowledge, a possible link between FPV and *B. henselae* infection has not been investigated before, although FPV infection is a common infection of young cats, leading to abrupt systemic lymphatic depletion and bone marrow necrosis. In the present study, FPV and *B. henselae* infections were not associated. This may have been due to the young age of the cats tested here and the generally acute and rapid course of feline panleukopenia, which killed cats before they had a chance to be coinfected with *B. henselae*.

We found the prevalence of *B. henselae* bacteremia among cats from animal shelters to be markedly lower (7.7% versus 18.7%) than that observed in a previous study in the same geographical area (4). This difference may be due to biological variance, or it may be linked to better flea control in the contributing animal shelters. A seasonal influence on prevalence can largely be excluded, since the sampling spanned 26 months. Interestingly, in 3 of the 11 infected cats (27%), the agent was detected in various organs but there was no bacteremia. This may suggest that *B. henselae* is sustained in internal organs of the reservoir host even after bacteremia has been cleared. However, we collected blood samples only once during necropsy, so it is possible that several of the cats were

in a nonbacteremic phase of *B. henselae* infection, which could have been identified by two or more subsequent blood tests for living animals. Histopathological and immunohistochemical analyses disclosed no specific organ changes for *B. henselae* infection. Immunohistochemistry revealed a cytoplasmic signal in just a few histiocytes within lymphatic tissues, a pattern similar to that observed in human cat scratch disease (22). None of the lesions previously described in experimental or natural feline bartonellosis, including stomatitis, lymphadenopathy, uveitis, urinary tract diseases, cholangitis, hepatitis, and myocarditis, were observed at a higher frequency in *B. henselae*-infected cats than in uninfected controls, and in this respect there was no difference between cats with diseases known to cause immunosuppression and controls.

In conclusion, our results suggest that susceptibility to *B. henselae* infection may be increased in cats with FeLV infection, whether latent or progressive. This could be due to interference at the level of the agents' target cell, possibly the hematopoietic progenitor cell. In contrast, the course of *B. henselae* infection seems not to be influenced by concurrent infection with agents known to cause immunosuppression, including FeLV and FPV. However, due to the low overall prevalences of FeLV and FIV infections in this study, controlled coinfection studies will be necessary in order to confirm and further characterize the effects of coinfections with these immunosuppressive viral agents on *B. henselae* infection in cats.

In addition, these findings may help to elucidate the significance of latent FeLV infection in cats.

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