Master thesis

Prevalence of infections with selected pathogens in client-owned cats from the Netherlands

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Table of contents

Abstract

Multiple pathogens of cats are directly associated with clinical abnormalities or have primary importance as zoonotic agents impacting human health. However, limited or no prevalence data is available for many of these pathogens in cats from the Netherlands. The aims of this study were therefore to estimate the prevalence of selected bloodborne and zoonotic pathogens in cats from the Netherlands and to assess for associated risk factors and co-infections. Anti-coagulated blood, sera, and or plasma were obtained from 167 client-owned cats in the Netherlands and transported to Colorado for assay. Previously validated plate based ELISA assays were used to detect antibodies against *Bartonella* species and *Toxoplasma gondii* in plasma or serum. A commercially available kit was used to detect antibodies against feline immunodeficiency virus (FIV) and antigens of feline leukemia virus (FeLV) and *Dirofilaria immitis* (SNAP® feline Triple; IDEXX) in sera or plasma. Total DNA and RNA were extracted from the blood and previously validated molecular assays that amplify the nucleic acids of *Anaplasma* species, *Bartonella* species, *Ehrlichia* species, *Felis catus* gammaherpesvirus 1 (FcaGHV1), feline foamy virus (FFV) and the hemoplasmas were performed. Genetic sequencing was performed to determine the species of some agents and to validate the final results. The estimated seroprevalence rates for *Bartonella* spp. IgG (21 of 163 cats; 12.9%; 95% CI: 8.51-19.02), *T. gondii* IgG (26 of 163 cats; 16.0%; 95% CI: 11.05-22.48), FIV antibodies (6 of 121 cats; 5.0%; 95% CI: 2.21-10.71) and FeLV (4 of 121 cats; 3.3%; 95% CI: 1.23-8.59) were calculated. *Bartonella* spp. DNA (2 of 163 cats; 1.2%; 95% CI: 0.30-4.83), overall hemoplasma DNA (17 of 163 cats; 10.4%; 95% CI 6.55-16.21), FFV DNA (66 of 150 cats; 44.0%; 95% CI: 36.19-52.12) and FcaGHV1 DNA (21 of 162 cats; 13.0%; 95% CI: 8.57-19.14) were amplified from some samples. Risk factors included increasing age for FFV infection and co-occurrences of infection were found for several pathogens. *D. immitis* antigen as well as *Anaplasma* spp. and *Ehrlichia* spp. DNA were not detected in any of the 121 and 163 samples tested respectively.

Introduction

Multiple pathogens have been described in cats which are directly associated with clinical abnormalities or have their primary importance as zoonotic agents impacting human health. Retroviruses, including feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) infection may lead to immunosuppression and virus-induced lymphomas (Hosie et al., 2009; Lutz et al., 2009). Transmission occurs via direct contact between cats during cat fighting (FIV) and via viremic cats, which spread the virus via saliva, nasal secretions, feces and milk (FeLV). Cats may remain persistently infected throughout life (Hosie et al., 2009; Lutz et al., 2009).

Feline foamy virus (FFV), another retrovirus, causes persistent infections as well. Experimental infections of cats with this pathogen have led to histologic abnormalities in lung and kidney tissue but no clear association with disease has been found thus far and is therefore considered apathogenic (Bleiholder et al., 2011; German et al., 2008; Romen et al., 2006).

Little is known about *Felis catus* gammaherpesvirus 1 (FcaGHV1), a gammaherpesvirus which has recently been discovered in the domestic cat (Troyer et al., 2014). However, associations have been found with FIV infections and they are suspected of playing a causative role in FIV associated lymphomas as has similarly been described in HIV positive humans with gammaherpesvirus infection (Beatty et al., 2014; Ertl et al., 2015; Troyer et al., 2014).

Hemoplasmas are the causative agents of feline infectious anemia and have been associated with FIV and gammaherpesvirus infection which supports the role of cat fighting as a route of transmission (Bergmann et al., 2017b; Dean et al., 2008; McLuckie et al., 2016). However the role of fleas in transmission cannot fully be excluded (Woods et al., 2005). The 3 causative agents of hemoplasmosis, differ in their pathogenicity, with *Mycoplasma haemofelis* (Mhf) being the most pathogenic and '*Candidatus* Mycoplasma haemominutum' (Mhm) and '*Candidatus* M turicencis' (Mt) being less pathogenic, which usually do not lead to significant anemia (Jensen et al., 2001; Tasker et al., 2016).
Different tick species that serve as vectors for bloodborne pathogens have been found in the Netherlands. *Ixodes* spp., the vector of *Anaplasma phagocytophilum*, are by far the most predominant species, whereas *Rhipicephalus sanguineus* is less common and mostly introduced by dogs/cats with a travel history (Lappin et al., 2015; Nijhof et al., 2007). *Rhipicephalus sanguineus* is the vector of *Ehrlichia* spp. and most likely for *A. platys* as well, since co-infections with *Ehrlichia* spp. are commonly found (Pennisi et al., 2017a). Infection with the above mentioned pathogens in cats seems less common compared to dogs and there is only limited data available of natural infection in cats. Clinical impact may vary and signs are mostly nonspecific consisting of fever, general illness, anemia, thrombocytopenia and lameness (Breitschwerdt et al., 2002; Pennisi et al., 2017a).
The vector-borne nematode *Dirofilaria immitis* is transmitted by mosquitoes, mostly of the genera *Culex* and *Aedes* (Genchi et al., 2011). Infection may lead to severe pulmonary thromboembolism and eosinophilic inflammatory response in the lungs, but more often disease manifestation is characterized by a less severe chronic course of infection with mild to moderate respiratory signs (Pennisi et al., 2017b). The filarial nematode is endemic in some Mediterranean countries in Europe, but a further spread to more northern and eastern countries has been recognized (Genchi et al., 2014; Pennisi et al., 2017b). Moreover, *D. immitis* also poses an increasing public health risk since human pulmonary dirofilariasis has been described as well (Kronefeld et al., 2014).
Bartonella henselae infection may lead to cat scratch disease in humans, as well as fatal disorders such as bacillary angiomatosis, bacillary peliosis and endocarditis, especially in immunocompromised people. *B. henselae* is the most common species in cats, but others have also been described such as *B. clarridgeia*, *B. koehlerae* and to a lesser extent *B. quintana*. Transmission between cats occurs via the vector *Ctenocephalides felis*. Most cats with *Bartonella* spp. infection are healthy, but fever, lymphadenopathy, anemia, endocarditis, uveitis and neurological dysfunction have been described (Bergmann et al., 2017a; Breitschwerdt et al., 2010; Lappin et al., 2009; Pennisi et al., 2013).
Cats are also the definitive host for *Toxoplasma gondii*, which may lead to congenital toxoplasmosis in humans (Paquet et al., 2013). Transmission in cats occurs via ingestion of oocysts from the environment or ingestion of intermediate hosts infected with tissue cysts (Hartmann et al., 2013). Most cats have subclinical infections while clinical abnormalities such as fever, uveitis, myositis, neurological disease and interstitial pneumonia have also been described, most commonly in immunosuppressed cats as occurs with end stage FIV and FeLV infection or with use of immunosuppressive drugs at high levels (Barrs et al., 2006).
Prevalence data of these pathogens have been described in cats from surrounding European countries but limited recent or no prevalence data is available for many of these pathogens in cats from the Netherlands. The aims of this study were therefore to estimate the prevalence of the above mentioned pathogens and to assess for associated risk factors and co-infections in client-owned cats from the Netherlands

Materials and methods

Samples

A total of 143 remnant EDTA blood samples from client owned cats were obtained from the University Veterinary Diagnostic Laboratory in Utrecht, the Netherlands. Beforehand, these blood samples were stored at 4°C for at least a week. An additional 24 remnant EDTA blood samples and serum samples were obtained from LABOKLIN Laboratory for Clinical Diagnostics GmbH & Co. KG, in Hoensbroek, the Netherlands. All blood samples originated from veterinary clinics in the Netherlands. If known, additional information about breed, age, sex and castration status were provided. All blood samples were evaluated for reasons independent to the study and collected via informed consent.
After collection, 250 µl of whole blood was separated from the EDTA blood samples and the remaining plasma was collected after centrifugation. Whole blood, plasma and serum samples were stored at -80°C before being shipped on dry ice to Colorado State University. Once arrived, samples were stored again at -80°C until being thawed to room temperature prior to assay.

Serological assays

Plasma and serum were not thawed more than twice prior to assay and all serological assays were performed within 1 day after thawing. Assays performed per sample was dependent on plasma or serum volume availability per cat. Previously described plate-based enzyme-linked immunosorbent assays (ELISA) were used to detect *T. gondii* and *Bartonella* spp. specific antibodies in plasma or serum of all 167 samples at Colorado State University, Fort Collins, CO, USA (Lappin et al., 2009; Vollaire et al., 2005). Because the *T. gondii* and *Bartonella* spp. ELISA were only described for use with serum, plasma and serum samples available of the same cat (n=24) were ran on the same ELISA plates in order to compare antibody titer responses and to adjust plasma titer cutoff points if needed. *Dirofilaria immitis* antigen, FeLV antigen and FIV antibody were detected in plasma or serum of 123 samples using a commercially available kit (SNAP® feline Triple; IDEXX laboratories, Westbrook, ME, USA). All assays were performed according to the manufacturer's user manual.

Molecular assays

Total DNA of all 167 samples was extracted from 200 µl of whole blood with QIAcube® HT automated purification instrument and QIAamp® 96 DNA QIAcube® HT kit. DNA of *Ehrlichia* spp., *Anaplasma* spp. (Lappin et al., 2004), hemoplasmas (Jensen et al., 2001), *Bartonella* spp. (Jensen et al., 2000), and FcaGHV1 (Troyer et al., 2014) were amplified by previously described conventional polymerase chain reaction (PCR) assays. DNA PCR products were visualized by agarose gel electrophoresis with EZVision® One DNA Dye (Amresco®; Solon, OH, USA). Because Mhf and Mt PCR products are within the same band size, further sequencing was performed to validate the final results (Macromolecular Resources, Colorado State University, Fort Collins, Colorado, USA). FFV DNA was amplified and measured using a previously described real-time PCR assay (Lee et al., 2017). Appropriate positive and negative controls were evaluated in each assay.

Statistical analysis

Statistical analysis was performed with use of the statistical software Stata version 14.2 (StataCorp. 2016, College station, TX, USA). Prevalence and 95% confidence intervals were estimated for each pathogen. Differences in each pathogen prevalence according to sex, castration status and age group were tested for statistical significance using univariate and multivariate logistic regression analysis. The same method was also used to test the significance of coinfections by pair. Significance level was defined as $P< 0.05$.

Results

Animals

After adjustments were made for samples that originated from the same cat, a total of 163 cats were included in this study. Sex, castration status and age were reported in 138 (76 males, 62 females), 139 (125 castrated, 14 non-castrated) and 156 cats respectively. The age ranged from 1,5 months to 19 years with a mean age of 9.5 years. Breed was reported for 159 cats (95 European Shorthairs, 25 undefined, 10 Maine Coons, 4 British Shorthairs, 4 Siamese, 4 crossbreeds, 3 Birmans, 2 Scottish Folds, 2 Persians, 2 Sphynx cats, 2 Oriental Shorthairs, 2 Angoras, 1 Abyssinian, 1 Norwegian Forest cat, 1 Ragdoll and 1 Chartreux).

Serological assays results

Toxoplasma gondii and *Bartonella* spp. ELISA antibody titers of both plasma and serum samples originating from 24 cats were evaluated. Similar titers were found for plasma and serum in the *T. gondii* ELISA but the *Bartonella* spp., plate predominantly gave higher titers in plasma compared with serum (4 plasma samples were positive at 1:64 while they were negative in serum, 1 plasma sample that was positive at 1:128 was positive in serum as well at 1:256). Based on these findings antibody titers of 1:128 and higher were considered positive in plasma for the *Bartonella* spp. ELISA, while titers of 1:64 and higher remained the cutoff point in plasma for the *T. gondii* ELISA.
Based on this, 21 of 163 cats (12.9%; 95% confidence interval: 8.51-19.02) were *Bartonella* spp. IgG positive, and 26 of 163 cats (16.0%; 95% CI: 11.05-22.48) were *T. gondii* IgG positive. Further, 6 of 121 cats (5.0%; 95% CI: 2.21-10.71) were FIV antibodies positive, and 4 of 121 cats (3.3%; 95% CI: 1.23-8.59) were FeLV antigen positive. *Dirofilaria immitis* antigen was not detected in any of the 121 samples tested.

Molecular assay results

Total *Bartonella* spp. DNA was amplified from 2 of 163 cats (1.2%; 95% CI: 0.30-4.83), with band sizes corresponding to *Bartonella henselae* (1/163) and *Bartonella clarridgeia* (1/163). Both *Bartonella* spp. PCR positive cats were seropositive as well. DNA of the hemoplasmas was amplified from 17 of 163 cats (10.4%; 95% CI: 6.55-16.21). A total of 15 of these cats were positive for Mhm alone, one cat was positive for Mhf alone, and 1 cat was coinfected with both Mhf and Mhm. A total of 150 and 162 cats were assayed for FFV and FcaGHV1 DNA respectively. FFV DNA was amplified from 66 of 150 cats (44.0%; 95% CI: 36.19-52.12) whereas FcaGHV1 DNA was amplified from 21 of 162 cats (13.0%; 95% CI: 8.57-19.14). *Anaplasma* spp. and *Ehrlichia* spp. DNA were not amplified from any of the 163 samples tested. An overview of both serological and molecular assay results can be found in Table 1.

Table 1. Prevalence of infection with selected pathogens in client-owned cats from the Netherlands.

Pathogen	n	Positive n (%)	95% confidence interval (%)
Serological assays:			
***Bartonella* species**	163	21 (12.9%)	8.51-19.02 %
Toxoplasma gondii	163	26 (16.0%)	11.05-22.48 %
FIV	121	6 (5.0%)	2.21-10.71 %
FeLV	121	4 (3.3%)	1.23-8.59 %
Dirofilaria immitis	121	0 (0%)	0-3.00 % *
Molecular assays:			
***Bartonella* species**	163	2 (1.2%)	0.30-4.83 %
Bartonella henselae		1 (0.6%)	
Bartonella clarridgeia		1 (0.6%)	
***Ehrlichia* / *Anaplasma* species**	163	0 (0%)	0-2.24 % *
Hemoplasmas	163	17 (10.4%)	6.55-16.21 %
Mhm alone		15 (9.2%)	
Mhf alone		1 (0.6%)	
Mhf and Mhm		1 (0.6%)	
FFV	150	66 (44.0%)	36.19-52.12 %
FcaGHV1	162	21 (13.0%)	8.57-19.14 %

FIV= feline immunodeficiency virus, FeLV= feline leukemia virus, Mhm= '*Candidatus* Mycoplasma haemominutum', Mhf= *Mycoplasma haemofelis,* FFV= feline foamy virus, FcaGHV1= *felis catus* gammaherpesvirus 1.
* calculated with a one-sided binominal exact test.

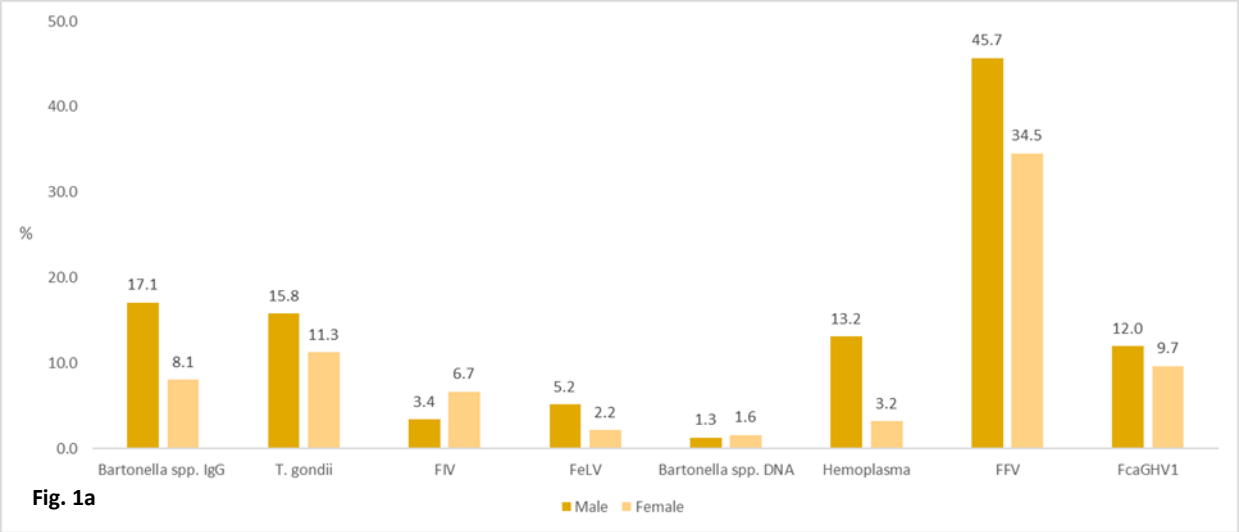

Fig. 1a

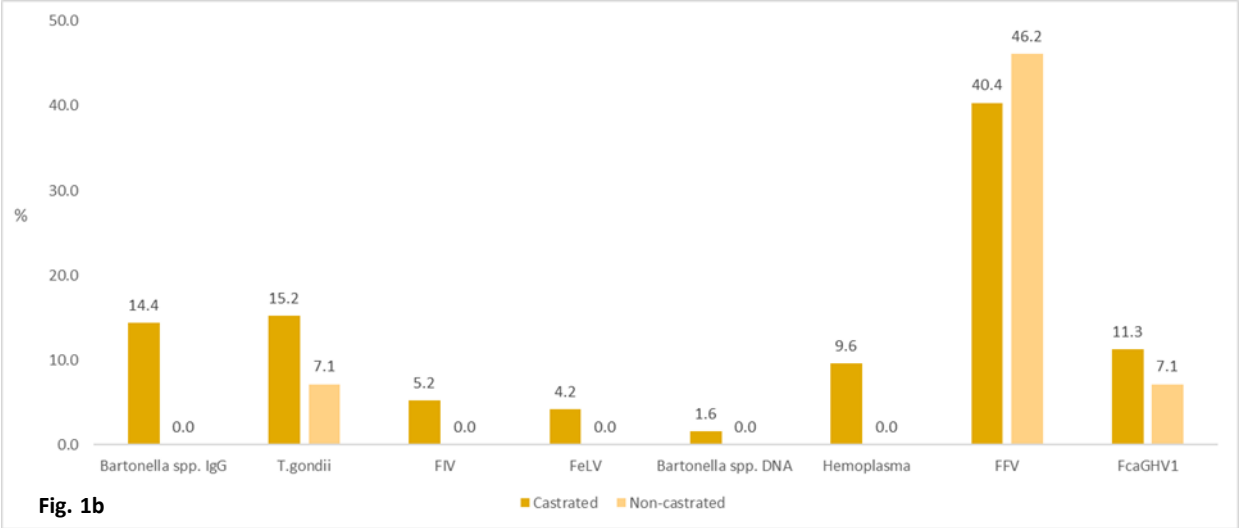

Fig. 1b

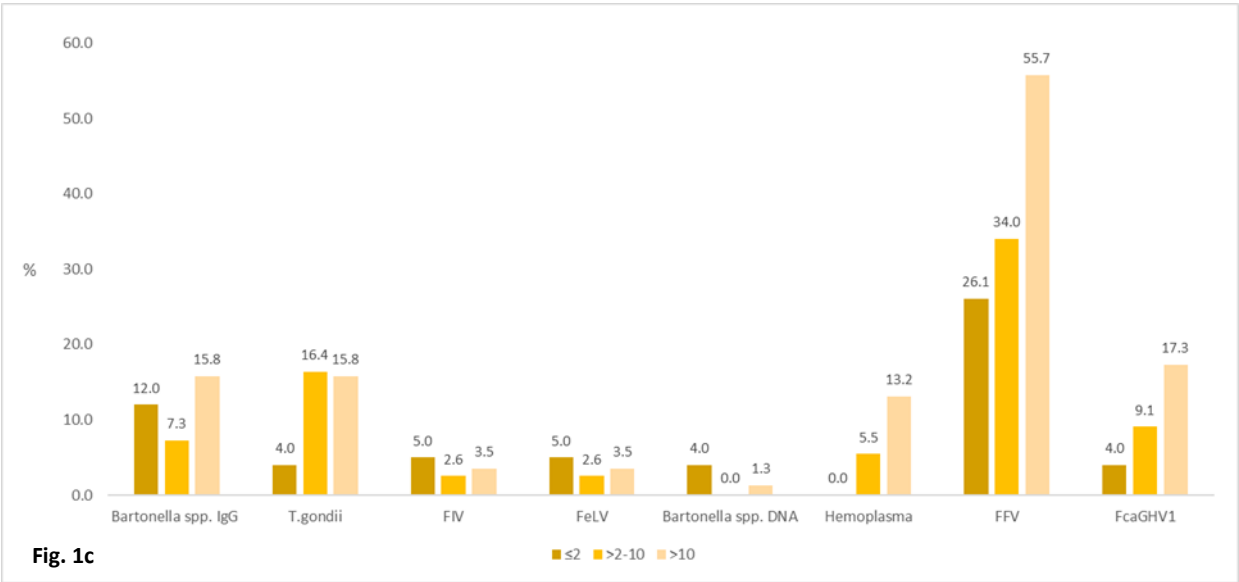

Fig. 1c

Figure 1. Seroprevalence of *Bartonella* species., *Toxoplasma gondii*, feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV), and molecular prevalence of *Bartonella* spp., hemoplasmas, feline foamy virus (FFV) and *Felis catus* gammaherpesvirus 1 (FcaGHV1) according to sex (Fig. 1a), castration status (Fig. 1b) and age groups (in years) (Fig. 1c).

Table 2. Univariable and multivariable logistic regression analysis to identify risk factors for selected pathogens in client-owned cats from the Netherlands.

			Univariable logistic regression analysis		Multivariable logistic regression analysis	
Pathogens	**Variables**	**Categories**	**Odds-ratio (95% CI)**	**P value**	**Odds-ratio (95% CI)**	**P value**
Serological assays:						
***Bartonella* species**	Sex	Male vs. female	2.35 (0.79-7.01)	0.125	2.55 (0.84-7.78)	0.098
	castration	Castrated vs. non-castrated	n.c.	n.c.	n.c.	n.c.
	Age	>2-10 vs. ≤2	0.58 (0.12-2.79)	0.492	0.45 (0.09-2.30)	0.335
		>10 vs. ≤2	1.38 (0.35-5.33)	0.645	0.89 (0.21-3.77)	0.881
		>10 vs. >2-10	2.39 (0.73-7.86)	0.151	2.01 (0.58-6.97)	0.273
Toxoplasma gondii	Sex	Male vs. female	1.47 (0.54-4.00)	0.447	1.41 (0.50-3.98)	0.519
	castration	Castrated vs. non-castrated	2.33 (0.29-18.87)	0.428	n.c.	n.c.
	Age	>2-10 vs. ≤2	4.70 (0.56-39.28)	0.154	3.72 (0.43-32.39)	0.234
		>10 vs. ≤2	4.50 (0.55-36.50)	0.159	2.49 (0.29-21.43)	0.407
		>10 vs. >2-10	0.95 (0.37-2.46)	0.930	0.67 (0.23-1.95)	0.461
FIV	Sex	Male vs. female	0.50 (0.08-3.13)	0.459	0.47 (0.07-3.28)	0.450
	castration	Castrated vs. non-castrated	n.c.	n.c.	n.c.	n.c.
	Age	>2-10 vs. ≤2	0.50 (0.03-8.44)	0.631	0.51 (0.03-8.87)	0.641
		>10 vs. ≤2	0.69 (0.06-8.06)	0.768	0.66 (0.06-7.92)	0.743
		>10 vs. >2-10	1.38 (0.12-15.79)	0.795	1.31 (0.11-15.24)	0.832
FeLV	Sex	Male vs. female	2.4 (0.24-23.88)	0.455	2.77 (0.27-28.18)	0.389
	castration	Castrated vs. non-castrated	n.c.	n.c.	n.c.	n.c.
	Age	>2-10 vs. ≤2	0.50 (0.03-8.44)	0.631	0.39 (0.02-6.86)	0.518
		>10 vs. ≤2	0.69 (0.06-8.06)	0.768	0.60 (0.05-7.26)	0.688
		>10 vs. >2-10	1.38 (0.12-15.79)	0.795	1.55 (0.13-18.11)	0.727
Molecular assays:						
***Bartonella* species**	Sex	Male vs. female	0.81 (0.05-13.27)	0.885	1.03 (0.06-17.58)	0.983
	castration	Castrated vs. non-castrated	n.c.	n.c.	n.c.	n.c.
	Age	>2-10 vs. ≤2	n.c.	n.c.	n.c.	n.c.
		>10 vs. ≤2	0.32 (0.02-5.31)	0.427	0.28 (0.02-4.82)	0.383
		>10 vs. >2-10	n.c.	n.c.	n.c.	n.c.
Hemoplasmas	Sex	Male vs. female	4.55 (0.96-21.59)	0.057	4.96 (0.94-26.16)	0.059
	castration	Castrated vs. non-castrated	n.c.	n.c.	n.c.	n.c.
	Age	>2-10 vs. ≤2	n.c.	n.c.	n.c.	n.c.
		>10 vs. ≤2	n.c.	n.c.	n.c.	n.c.
		>10 vs. >2-10	2.62 (0.69-10.03)	0.158	1.86 (0.44-7.82)	0.398
FFV	Sex	Male vs. female	1.64 (0.79-3.39)	0.182	1.65 (0.78-3.52)	0.192
	castration	Castrated vs. non-castrated	0.79 (0.29-2.55)	0.780	0.59 (0.16-2.15)	0.426
	Age	>2-10 vs. ≤2	1.46 (0.49-4.38)	0.500	1.06 (0.33-3.41)	0.923
		>10 vs. ≤2	3.56 (1.26-10.12)	**0.017***	2.90 (0.92-9.09)	0.069
		>10 vs. >2-10	2.44 (1.15-5.18)	**0.020***	2.73 (1.17-6.39)	**0.020***
FcaGHV1	Sex	Male vs. female	1.27 (0.43-3.80)	0.665	1.25 (0.41-3.81)	0.697
	castration	Castrated vs. non-castrated	1.65 (0.20-13.63)	0.640	0.92 (0.10-8.55)	0.940
	Age	>2-10 vs. ≤2	2.40 (0.27-21.70)	0.436	1.85 (0.19-17.82)	0.595
		>10 vs. ≤2	5.03 (0.62-40.60)	0.129	3.80 (0.43-33.69)	0.230
		>10 vs. >2-10	2.09 (0.70-6.28)	0.186	2.06 (0.57-7.45)	0.271

FIV= feline immunodeficiency virus, FeLV= feline leukemia virus, FFV= feline foamy virus, FcaGHV1= *felis catus* gammaherpesvirus 1.
n.c.= non calculable, because one of either categories contained zero positives.
* significant association.

Risk factors and co-infections

Associations of each pathogen with sex, castration status, age group and co-occurrence with other pathogens were assessed. Because of low numbers of Mhf, all hemoplasmas were assessed together for risk factor analysis. The same applied for the cats positive for *Bartonella* spp. DNA.

The seroprevalence of *T.gondii* antibodies was higher in the older age groups. Cats in the age group ≤ 2 years had a seroprevalence of 4.0% (1/25 cats), while cats in the age groups >2-10 years and >10 years had a seroprevalence of 16.4% (9/55 cats) and 15.8% (12/76 cats) respectively. However, the difference was not significant.

Hemoplasma infection was more common in male cats (10/76 cats; 13.2%) than in female cats (2/62 cats; 3.2%) with an odds ratio of 4.96 (95% CI: 0.94-26.16), which approached statistical significance (P=0.059).

The prevalence of FcaGHV1 DNA also increased with age, but the difference was not significant. Cats ≤ 2 years had a prevalence of 4.0% (1/25), cats >2-10 years had a prevalence of 9.1% (5/55) and cats > 10 years had a prevalence of 17.3% (13/75).

Univariate logistic regression analysis demonstrated a significant association between age groups and FFV infection. Cats >10 years were more likely to have a FFV infection compared with cats ≤ 2 years (OR: 3.56; 95% CI: 1.26-10.12; P=0.017) and cats >2-10 years (OR: 2.44; 95% CI: 1.15-5.18; P=0.020). However, after adjustments were made for potential confounders (sex and castration status) multivariate logistic regression analysis only demonstrated a significant difference between cats >10 years and cats >2-10 years (OR: 2.73; 96% CI: 1.17-6.39; P=0.020). For an overview of the risk factor analysis see Table 2.

Regarding co-infections, *T. gondii* and *Bartonella* spp. IgG were significantly associated with each other (OR: 3.94; 95% CI: 1.14-13.57; P=0.03), which was also true for hemoplasma with FFV (OR: 6.76; 95% CI: 1.24-36.94; P=0.027), hemoplasma with FcaGHV1 infection (OR: 66.66; 95% CI: 9.96-445.99; P=<0.001) and FFV with FcaGHV1 infection (OR: 3.62; 95% CI 1.02-12.92; P=0.047). See Table 3.

Table 3. Significant co-occurrences of selected pathogens in client-owned cats from the Netherlands, calculated with multivariable logistic regression analysis.

Co-occurrence		Odds ratio (95% CI)	P value
Bartonella species IgG	*Toxoplasma gondii* IgG	3.94 (1.14-13.57)	0.030
Hemoplasmas DNA	FFV DNA	6.76 (1.24-36.94)	0.027
Hemoplasmas DNA	FcaGHV1 DNA	66.66 (9.96-445.99)	<0.001
FFV DNA	FcaGHV1 DNA	3.62 (1.02-12.92)	0.047

FFV= feline foamy virus, FcaGHV1= felic catus gammaherpesvirus 1

Discussion

The aims of this study were to estimate the prevalence of selected bloodborne and zoonotic pathogens in cats from the Netherlands and to assess for associated risk factors and co-infections.
A *Bartonella* spp. seroprevalence of 12.9% was found in our study (21 of 163 cats), which was lower than the 52% found in a previous study in the Netherlands (Bergmans et al., 1997), but comparable with results from other European countries, with seroprevalences ranging from 8-53% (Pennisi et al., 2013). Bergmans et al. also found an almost tenfold higher prevalence of bacteremia (22%) compared with the 2.1% (2/163) found in the present study. However, more recent studies performed in Germany showed comparable results with our study, with evidence of bacteremia in 2.5% and 0% of cats (Bergmann et al., 2017a; Morgenthal et al., 2012). This difference in prevalence might be explained by the fact that the present study solely and the German studies predominantly consisted of client-owned cats, which probably have been less in contact with fleas or were treated with flea control products. Most cats in the study of Bergmans et al. were shelter cats which probably have had higher flea infestation. Flea infestation, outdoor lifestyle, shelter cats and living in a multicat environment are all previously described risk factors (Bergmann et al., 2017a; Pennisi et al., 2013). In the present study no associations were found for sex, castration status or age. Previous studies have shown that young age is a risk factor for bacteremia which might be explained by the fact that the immune system of young cats may have more difficulties to suppress a primary infection in comparison with older cats (Bergmann et al., 2017a; Pennisi et al., 2013). In the present study, 1 cat was infected with *B. henselae* and 1 cat with *B. clarridgeia*, which are the most common *Bartonella* spp. in cats (Bergmann et al., 2017a).
Toxoplasma gondii seroprevalence was 16.0% (26 of 163 cats) in the study described here, which was comparable with the seroprevalence of 18.2% from a previous study conducted in the Netherlands (Opsteegh et al., 2012). In the previous study *T. gondii* seroprevalence was strongly associated with age with higher seroprevalence in older cats and peak seroprevalence rates around 20-30% in cats >4 years of age. Our study also showed higher seroprevalence rates in cats >2 years of age, even though this association was not significant and seroprevalence rates in these age groups were lower (16.4% for the age group >2-10 years and 15.8% for the age group >10 years) compared with the previous study. Since other described risk factors such as being a stray cat, hunting behavior and eating raw meat might have been less present in our study, this could be an explanation for our lower peak prevalence rates (Opsteegh et al., 2012). Worldwide seroprevalence rates for *T. gondii* antibodies have been described to reach up to 50%, especially in stray cats. However, seropositive cats are unlikely to shed oocysts, as antibodies need two to three weeks to develop after infection and at that time cats usually do no longer shed (Hartman et al., 2013). The prevalence of oocysts in feces is therefore much lower, which was also confirmed by Schares et al. (2008), who found an oocyst prevalence of 0.11% in cat feces from Germany and other European countries.
In our study plasma instead of serum was used to determine antibody titers for *T. gondii* and *Bartonella* spp.. The ELISAs used in this study have predominantly been described for use with serum, but a study in which plasma was used has also been described (Levy et al., 2008). There has however never been any study in which antibody titers from simultaneously obtained plasma and serum samples have been compared for these specific assays. Even though one would expect that antibody titers would be the same for both sample types, since serum only differs from plasma in that it does not contain fibrinogen and clotting factors, one must realize that not only differences in sample composition but also differences in blood sample preparation and storage condition could influence the results. Ilies et al. (2017) found that sample collection methods did not influence IgG concentration, but in our study *Bartonella* spp. IgG titers did differ in plasma compared to serum. All plasma samples with a titer of 1:64 (n=4) were negative in serum and one plasma sample with a titer of 1:128 was positive in serum

at 1:256. Based on these finding we adjusted the titer cutoff point for plasma in which titers of 1:128 and higher were considered positive. However, more research is needed in which greater numbers of both plasma and serum samples are used to compare antibody titers for this specific assay, and to be able to make more reliable conclusions about the antibody titer outcomes for its use with plasma as well.

Six of 121 cats (5.0%) appeared to be FIV infected in our study, which was higher than a recent prevalence (1.5%) found in Germany by Bergmann et al. (2015). Worldwide the seroprevalence is highly variable between regions and countries and has been estimated at 1-14% in healthy cats and up to 44% in sick cats (Hosie et al., 2009). Other, less recent studies performed in the Netherlands found seroprevalence rates of 0-1% in healthy cats and 3-7% in diseased cats (Egberink et al., 1993; Lutz et al., 1988; Weijer et al., 1988). In the present study we had no information about the clinical background of the cats and what the proportion of sick cats in our sample was. However, because a large proportion of the samples originated from cats that were referred to the Department of Clinical Sciences Companion Animals of the Utrecht Veterinary Faculty, which is a specialized clinic, we should take into account that our sample might have had a higher proportion of sick cats and thus this would explain the higher prevalence in our study. Male cats, free roaming cats, non-castrated cats, increasing age and sick cats have al previously been described as risk factors for FIV infection (Gates et al., 2017; Hosie et al., 2009). Because of the low number of FIV positive cats in the present study, no associations were found for sex, castration status, age or co-infections.

In a multi-cat household without control of FeLV infection, it has been described that the course of infection develops as follows: 30-40% persistent viremic cats (p27 antigen and provirus positive), 30-40% latent viremic cats (p27 negative and provirus positive), 20-30% abortive infections (p27 and provirus negative, but antibody positive) and 5% of cats develop an atypical course of infection being p27 positive and provirus negative (Lutz et al., 2009). In our study the p27 antigen was demonstrated by use of a commercial ELISA (SNAP® feline triple; IDEXX laboratories), which is an indicator of infection but not always of viremia, since soluble p27 could also be detected with the absence of provirus. Therefore, in case of a clinical indication, active infection is usually confirmed by use of a PCR, which is also able to detect latently infected cats (Lutz et al., 2009). The prevalence of FeLV in Europe has been estimated to be less than 1% in individually kept cats and up to 20% in multi-cat households. However, thanks to effective eradication programs and vaccinations FeLV infection in Europe has greatly decreased (Lutz et al., 2009). A recent study in Germany confirms this decrease, with FeLV infection prevalence rates consisting of 1.9% persistent infections, 1.5% latent infections and 4.5% abortive infections (Bergmann et al., 2015). Our study estimated a FeLV antigenemia prevalence of 3.3% (4 of 121 cats) which is higher than expected. However, for the same reason as the higher estimated FIV infection in our study, the higher prevalence of FeLV antigen positive cats may be explained by the fact that cats in our study mainly originated from a specialized clinic, which includes an oncology center. Especially cats with lymphoma or leukemia are at higher risk of being FeLV infected compared with cats without lymphoma or leukemia. This was confirmed by Stützer et al. (2011) who found a 20.8% prevalence of FeLV antigenemia in cats with lymphoma or leukemia in Germany. Thus, a higher proportion of sick cats, possibly with a contribution of cats with lymphoma or leukemia may explain the estimated prevalence of FeLV found in the present study. Further, no risk factors were found in the present study. Risk factors previously found in other studies include multi-cat households, young age, high population density and poor hygiene (Lutz et al., 2009).

None of the cats in our study were *D. immitis* antigen positive. This was not surprising, since this parasite is not endemic in this country. However, a study performed in Germany reported *D. immitis* infected mosquitoes (Kronefeld et al., 2014) and infection has been reported in dogs in the Netherlands as well (Genchi et al., 2014). *D. immitis* infection rates in cats appear to be lower than in dogs (about 9-18% of the rate in unprotected dogs) and the detection of infection is also more difficult

in cats compared to dogs, making false negative results more likely. The parasitic life cycle in the cat differs in that from the dog by the fact that only a low number of L3 larvae develop into adults, production of microfilariae rarely occurs, or only at low loads, and microfilaremia lasts for a shorter period. In our study a commercial ELISA was used which detects circulating antigens of adult *D. immitis* females but has a low sensitivity in cats. Detection of anti-*D. immitis* antibodies would have given us more information about parasitic exposure, irrespective of the parasitic load. Because of global warming as well as increasing numbers of travelling dogs, *D. immitis* will continue to spread to more northern and eastern located countries, which will increase its relevance throughout Europe (Pennisi et al., 2017b).

Infection of either one or two of the hemoplasmas was detected in 17 of 163 cats (10.4%), which was comparable with prevalence rates of 9.4% and 7.2% previously found in Germany by Bergmann et al. (2017b) and Morgenthal et al. (2012) respectively. In our study Mhm was by far the most predominant species which was similar to the aforementioned studies. Mhf, which is the most pathogenic hemoplasma spp., was only found in 2 cats (either alone or as co-infection with Mhm) and Mt was found in none of the cats. Bergmann et al. also did not detect Mt in their study while Morgenthal et al. did. In our study, hemoplasma DNA was more common in male cats than in female cats, which almost reached significance (P=0.059), and is in agreement with previous studies (Bergmann et al., 2017b). This finding supports the presumable main route of transmission via cat fighting. Associations found in other studies such as outdoor access, shelter cats, multi-cat households and FIV infection also support this transmission route for hemoplasmas (Bennett et al., 2011; Bergmann et al., 2017b).

None of the cats in our study were found to be infected with *Anaplasma* or *Ehrlichia* spp. No previous prevalence studies for these pathogens have been conducted in the Netherlands so far, but in Germany prevalence rates of 0.1% and 0.4% were found for *A. phagocytophilum,* while *Ehrlichia* spp. were not detected (Bergmann et al., 2015; Morgenthal et al., 2012; Hamel et al. 2012). Since *Ixodes* spp., which serve as vector for *A. phagocytophilum*, are the most predominant tick species in the Netherlands, and *A. phagocytophilum* infected ticks have been found in this country, one would expect to find infected cats here as well (Nijhof et al., 2007). Future serological studies could provide us with more information about *Anaplasma* spp. exposure in cats from the Netherlands rather than recent infections alone. *Ehrlichia* spp. and *A. platys* infections, on the other hand, are less likely for cats in the Netherlands, since their vector *R. sanguineus* is less common in this country and mostly introduced by dogs and cats with a travel history (Nijhof et al., 2007; Pennisi et al., 2017a). However, their importance may increase with climate changes or with increasing numbers of imported animals.

In the study described here, evidence of FFV infection was detected in 44.0% (66/150) of cats. To our knowledge no FFV prevalence studies based on molecular assays have been performed thus far, but previously performed seroepidemiological studies estimated a prevalence of 39% and 36% in cats from Germany (Bleiholder et al., 2011) and Switzerland (Romen et al., 2006) respectively, which was comparable with our results, but much higher seroprevalences have been described outside of Europe as well (Winkler et al., 1999). FFV prevalence rates based on molecular assays or serological assays might be comparable because FFV transmission results in persistent infections. Winkler et al. (1999) demonstrated that in 30 randomly selected cats all FFV PCR positive cats were FFV antibody positive as well and that only one FFV antibody positive cats was PCR negative. This PCR negative result might have been because of a low proviral load which could not be detected by PCR. If the real-time PCR assay in our study was not able to detect low viral loads as well, one would expect that the true prevalence of infected cats would be higher. Future studies in which both serological assays as well as the recently used real-time PCR assay would be used together, could possibly provide us with more information about this. In our study FFV infection increased with age in which cats >10 years were significantly more likely to be FFV infected than cats >2-10 years. Other studies also demonstrated that increasing age was associated with a higher prevalence (Bleiholder et al., 2011; Winkler et al., 1999).

The major transmission route is believed to be via saliva, mainly by social contact between cats such as with grooming and sharing food (Pinto-Santini et al., 2017). The exact role of FFV virus infection in cats is not clear yet. FFV infection has a high prevalence in both healthy and diseased cats and since no association with clinical disease has been discovered thus far it is considered apathogenic. A previous study in which 8 cats were experimentally infected, however, did reveal histologic evidence of mild glomerulonephritis and a moderate interstitial pneumonia of all cats (German et al., 2008). Moreover, the role of FFV in exacerbating other viral infections has not been studied yet but simian immunodeficiency virus (SIV) infected macaques in a research setting were sicker and died sooner if they were also naturally infected with simian foamy virus (SFV) (Pinto-Santini et al., 2017). It would thus be interesting to find out if a FFV infection influences disease progression in FIV infected cats as well. Other than for SFV, there is currently no evidence of zoonotic transmission for FFV (Pinto-Santini et al., 2017).

Finally, our study found a prevalence of 13.0% (21/162 cats) for FcaGHV1 infection, which was in agreement with previous found prevalence rates of 9.6-19.1% in Germany, Austria, Australia, Singapore, the UK and the USA (Beatty et al., 2014; Ertl et al., 2015; McLuckie et al., 2016). Previous studies showed that FcaGHV1 infection increased with age, which was also found in our study (not significant) and supports horizontal rather than vertical transmission. In our study we also found a clear association between FcaGHV1 and hemoplasma infection which was in agreement with Beatty et al. and McLuckie et al.. This in combination with previously found associations with male sex, non-castrated cats and FIV supports the role of aggressive contact as the mode of transmission (Beatty et al., 2014; Ertl et al., 2015; McLuckie et al., 2016; Stutzman-Rodriguez et al., 2016). The pathogenic role of FcaGHV1 infection in cats still remains unknown and further research is needed to determine its relevance. Especially because human gammaherpesvirus infections have shown to play a causative role in HIV associated lymphomas, and a similar association of FcaGHC1 with FIV infection might exist as well (Troyer et al., 2014).

Further, co-occurrences of FcaGHV1 with FFV, FFV with hemoplasma and *T. gondii* with *Bartonella* spp. IgG were found. These co-occurrences have presumably been found because infections with these pathogens increased with age and thus were more frequently found as co-infections in older cats.

Conclusions

Results of this study confirm that *Bartonella* spp., *T. gondii*, FIV, FeLV, hemoplasma, FFV and FcaGHV1 infection occurs in client-owned cats from the Netherlands. Further research is needed to understand the exact pathogenic role of FFV and FcaGHV1 infection in cats. The main limitation of our study is the fact that our sample mainly consisted of cats that were referred to a specialized clinic. Our results may therefore not represent the average Dutch cat population, but rather a cat population with a higher proportion of diseased cats. Nonetheless, our study still gives a good insight in the occurrence of relevant infectious agents in cats from the Netherlands and these pathogens should thus be on differential lists of cats with appropriate clinical signs.

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Conflict of interest

The authors declare that there was no conflict of interest.

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References

BARRS, V.R., MARTIN, P. and BEATTY, J.A., 2006. Antemortem diagnosis and treatment of toxoplasmosis in two cats on cyclosporin therapy. Australian Veterinary Journal, 84(1-2), pp. 30-35.

BEATTY, J.A., TROYER, R.M., CARVER, S., BARRS, V.R., ESPINASSE, F., CONRADI, O., STUTZMAN-RODRIGUEZ, K., CHAN, C.C., TASKER, S., LAPPIN, M.R. and VANDEWOUDE, S., 2014. Felis catus gammaherpesvirus 1; a widely endemic potential pathogen of domestic cats. Virology, 460-461(1), pp. 100-107.

BENNETT, A.D., GUNN-MOORE, D.A., BREWER, M. and LAPPIN, M.R., 2011. Prevalence of Bartonella species, haemoplasmas and Toxoplasma gondii in cats in Scotland. Journal of Feline Medicine and Surgery, 13(8), pp. 553-557.

BERGMANN, M., ENGLERT, T., STUETZER, B., HAWLEY, J.R., LAPPIN, M.R. and HARTMANN, K., 2017a. Prevalence of Bartonella species infections in cats in Southern Germany. Veterinary Record, 180(13), pp. 325.

BERGMANN, M., ENGLERT, T., STUETZER, B., HAWLEY, J.R., LAPPIN, M.R. and HARTMANN, K., 2017b. Risk factors of different hemoplasma species infections in cats. BMC Veterinary Research, 13(1),.

BERGMANN, M., ENGLERT, T., STUETZER, B., HAWLEY, J.R., LAPPIN, M.R. and HARTMANN, K., 2015. Prevalence of selected rickettsial infections in cats in Southern Germany. Comparative immunology, microbiology and infectious diseases, 42, pp. 33-36.

BERGMANS, A.M.C., DE JONG, C.M.A., VAN AMERONGEN, G., SCHOT, C.S. and SCHOULS, L.M., 1997. Prevalence of Bartonella species in domestic cats in The Netherlands. Journal of clinical microbiology, 35(9), pp. 2256-2261.

BLEIHOLDER, A., MÜHLE, M., HECHLER, T., BEVINS, S., VANDEWOUDE, S., DENNER, J. and LÖCHELT, M., 2011. Pattern of seroreactivity against feline foamy virus proteins in domestic cats from Germany. Veterinary immunology and immunopathology, 143(3-4), pp. 292-300.

BREITSCHWERDT, E.B., ABRAMS-OGG, A.C.G., LAPPIN, M.R., BIENZLE, D., HANCOCK, S.I., COWAN, S.M., CLOOTEN, J.K., HEGARTY, B.C. and HAWKINS, E.C., 2002. Molecular evidence supporting Ehrlichia canis-like infection in cats. Journal of Veterinary Internal Medicine, 16(6), pp. 642-649.

BREITSCHWERDT, E.B., MAGGI, R.G., CHOMEL, B.B. and LAPPIN, M.R., 2010. Bartonellosis: An emerging infectious disease of zoonotic importance to animals and human beings. Journal of Veterinary Emergency and Critical Care, 20(1), pp. 8-30.

DEAN, R.S., HELPS, C.R., GRUFFYDD JONES, T.J. and TASKER, S., 2008. Use of real-time PCR to detect Mycoplasma haemofelis and 'Candidatus Mycoplasma haemominutum' in the saliva and salivary glands of haemoplasma-infected cats. Journal of Feline Medicine and Surgery, 10(4), pp. 413-417.

EGBERINK, H.F., HENDRIKS, P., SLAPPENDEL, R.J. and HORZINEK, M.C., 1993. The diagnosis and epidemiology of feline immunodeficiency virus (FIV) infection in The Netherlands. Tijdschrift voor diergeneeskunde, 118 Suppl 1, pp. 43S-44S.

ERTL, R., KORB, M., LANGBEIN-DETSCH, I. and KLEIN, D., 2015. Prevalence and risk factors of gammaherpesvirus infection in domestic cats in Central Europe Herpes viruses. Virology Journal, 12(1),.

GATES, M.C., VIGEANT, S. and DALE, A., 2017. Prevalence and risk factors for cats testing positive for feline immunodeficiency virus and feline leukaemia virus infection in cats entering an animal shelter in New Zealand. New Zealand veterinary journal, 65(6), pp. 285-291.

GENCHI, C., KRAMER, L.H. and RIVASI, F., 2011. Dirofilarial infections in Europe. Vector-Borne and Zoonotic Diseases, 11(10), pp. 1307-1317.

GERMAN, A.C., HARBOUR, D.A., HELPS, C.R. and GRUFFYDD-JONES, T.J., 2008. Is feline foamy virus really apathogenic? Veterinary immunology and immunopathology, 123(1-2), pp. 114-118.

HAMEL, D., BONDARENKO, A., SILAGHI, C., NOLTE, I. and PFISTER, K., 2012. Seroprevalence and bacteremia [corrected] of Anaplasma phagocytophilum in cats from Bavaria and Lower Saxony (Germany). Berliner und Münchener tierärztliche Wochenschrift, 125(3-4), pp. 163-167.

HARTMANN, K., ADDIE, D., BELÁK, S., BOUCRAUT-BARALON, C., EGBERINK, H., FRYMUS, T., GRUFFYDD-JONES, T., HOSIE, M.J., LLORET, A., LUTZ, H., MARSILIO, F., MÖSTL, K., PENNISI, M.G., RADFORD, A.D., THIRY, E., TRUYEN, U. and HORZINEK, M.C., 2013. Toxoplasma Gondii Infection in Cats: ABCD guidelines on prevention and management. Journal of Feline Medicine and Surgery, 15(7), pp. 631-637.

HOSIE, M.J., ADDIE, D., BELÁK, S., BOUCRAUT-BARALON, C., EGBERINK, H., FRYMUS, T., GRUFFYDD-JONES, T., HARTMANN, K., LLORET, A., LUTZ, H., MARSILIO, F., PENNISI, M.G., RADFORD, A.D., THIRY, E., TRUYEN, U. and HORZINEK, M.C., 2009. Feline immunodeficiency ABCD guidelines on prevention and management. Journal of Feline Medicine and Surgery, 11(7), pp. 575-584.

ILIES, M., IUGA, C.A., LOGHIN, F., DHOPLE, V.M., THIELE, T., VÖLKER, U. and HAMMER, E., 2017. Impact of blood sample collection methods on blood protein profiling studies. Clinica Chimica Acta, 471, pp. 128-134.

JENSEN, W.A., FALL, M.Z., ROONEY, J., KORDICK, D.L. and BREITSCHWERDT, E.B., 2000. Rapid identification and differentiation of Bartonella species using a single-step PCR assay. Journal of clinical microbiology, 38(5), pp. 1717-1722.

JENSEN, W.A., LAPPIN, M.R., KAMKAR, S. and REAGAN, W.J., 2001. Use of a polymerase chain reaction assay to detect and differentiate two strains of Haemobartonella felis in naturally infected cats. American Journal of Veterinary Research, 62(4), pp. 604-608.

KRONEFELD, M., KAMPEN, H., SASSNAU, R. and WERNER, D., 2014. Molecular detection of Dirofilaria immitis, Dirofilaria repens and Setaria tundra in mosquitoes from Germany. Parasites and Vectors, 7(1),.

LAPPIN, M.R., BREITSCHWERDT, E., BREWER, M., HAWLEY, J., HEGARTY, B. and RADECKI, S., 2009. Prevalence of Bartonella species antibodies and Bartonella species DNA in the blood of cats with and without fever. Journal of Feline Medicine and Surgery, 11(2), pp. 141-148.

LAPPIN, M.R., BREITSCHWERDT, E.B., JENSEN, W.A., DUNNIGAN, B., RHA, J.-., WILLIAMS, C.R., BREWER, M. and FALL, M., 2004. Molecular and serologic evidence of Anaplasma phagocytophilum infection in cats in North America. Journal of the American Veterinary Medical Association, 225(6), pp. 893-896+879.

LAPPIN, M.R., CHANDRASHEKAR, R., STILLMAN, B., LIU, J. and MATHER, T.N., 2015. Evidence of Anaplasma phagocytophilum and Borrelia burgdorferi infection in cats after exposure to wild-caught adult Ixodes scapularis. Journal of Veterinary Diagnostic Investigation, 27(4), pp. 522-525.

LEE, J.S., MACKIE, R.S., HARRISON, T., SHARIAT, B., KIND, T., KEHL, T., LÖCHELT, M., BOUCHER, C. and VANDEWOUDE, S., 2017. Targeted enrichment for pathogen detection and characterization in three felid species. Journal of clinical microbiology, 55(6), pp. 1658-1670.

LEVY, J.K., CRAWFORD, P.C., LAPPIN, M.R., DUBOVI, E.J., LEVY, M.G., ALLEMAN, R., TUCKER, S.J. and CLIFFORD, E.L., 20 08. Infectious diseases of dogs and cats on Isabela Island, Galapagos. Journal of Veterinary Internal Medicine, 22(1), pp. 60-65.

LUTZ, H., ADDIE, D., BELÁK, S., BOUCRAUT-BARALON, C., EGBERINK, H., FRYMUS, T., GRUFFYDD-JONES, T., HARTMANN, K., HOSIE, M.J., LLORET, A., MARSILIO, F., PENNISI, M.G., RADFORD, A.D., THIRY, E., TRUYEN, U. and HORZINEK, M.C., 2009. Feline leukaemia ABCD guidelines on prevention and management. Journal of Feline Medicine and Surgery, 11(7), pp. 565-574.

LUTZ, H., EGBERINK, H., ARNOLD, P., WINKLER, G., WOLFENSBERGER, C., JARETT, O., PARODI, A., PEDERSEN, N. and HORZINEK, M., 1988. Feline T-lymphotrophic lentivirus (FTLV): Experimental infection and occurrence in some countries of Europe. Kleintierpraxis, .

MCLUCKIE, A., TASKER, S., DHAND, N.K., SPENCER, S. and BEATTY, J.A., 2016. High prevalence of Felis catus gammaherpesvirus 1 infection in haemoplasma-infected cats supports co-transmission. Veterinary Journal, 214, pp. 117-121.

MORGENTHAL, D., HAMEL, D., ARNDT, G., SILAGHI, C., PFISTER, K., KEMPF, V.A.J. and KOHN, B., 2012. Prevalence of haemotropic Mycoplasma spp., Bartonella spp. and anaplasma phagocytophilum in cats in Berlin/Brandenburg (Northeast Germany). Berliner und Munchener tierarztliche Wochenschrift, 125(9-10), pp. 418-427.

NIJHOF, A.M., BODAAN, C., POSTIGO, M., NIEUWENHUIJS, H., OPSTEEGH, M., FRANSSEN, L., JEBBINK, F. and JONGEJAN, F., 2007. Ticks and associated pathogens collected from domestic animals in the Netherlands. Vector-Borne and Zoonotic Diseases, 7(4), pp. 585-595.

OPSTEEGH, M., HAVEMAN, R., SWART, A.N., MENSINK-BEEREPOOT, M.E., HOFHUIS, A., LANGELAAR, M.F.M. and VAN DER GIESSEN, J.W.B., 2012. Seroprevalence and risk factors for Toxoplasma gondii infection in domestic cats in The Netherlands. Preventive veterinary medicine, 104(3-4), pp. 317-326.

PAQUET, C., YUDIN, M.H., ALLEN, V.M., BOUCHARD, C., BOUCHER, M., CADDY, S., CASTILLO, E., MONEY, D.M., MURPHY, K.E., OGILVIE, G., VAN SCHALKWYK, J. and SENIKAS, V., 2013. Toxoplasmosis in Pregnancy: Prevention, Screening, and Treatment. Journal of Obstetrics and Gynaecology Canada, 35(1), pp. 78-79.

PENNISI, M.G., HOFMANN-LEHMANN, R., RADFORD, A.D., TASKER, S., BELÁK, S., ADDIE, D.D., BOUCRAUT-BARALON, C., EGBERINK, H., FRYMUS, T., GRUFFYDD-JONES, T., HARTMANN, K., HORZINEK, M.C., HOSIE, M.J., LLORET, A., LUTZ, H., MARSILIO, F., THIRY, E., TRUYEN, U. and MÖSTL, K., 2017a. Anaplasma, Ehrlichia and Rickettsia species infections in cats: European guidelines from the ABCD on prevention and management. Journal of Feline Medicine and Surgery, 19(5), pp. 542-548.

PENNISI, M.G., MARSILIO, F., HARTMANN, K., LLORET, A., ADDIE, D., BELÁK, S., BOUCRAUT-BARALON, C., EGBERINK, H., FRYMUS, T., GRUFFYDD-JONES, T., HOSIE, M.J., LUTZ, H., MÖSTL, K., RADFORD, A.D., THIRY, E., TRUYEN, U. and HORZINEK, M.C., 2013. Bartonella Species Infection in Cats: ABCD guidelines on prevention and management. Journal of Feline Medicine and Surgery, 15(7), pp. 563-569.

PENNISI, M.G. et al., November, 2017b, 2017-last update, Dirafilariosis in cats. Available: http://www.abcdcatsvets.org/dirofilarioses/ [February, 2018].

PINTO-SANTINI, D.M., STENBAK, C.R. and LINIAL, M.L., 2017. Foamy virus zoonotic infections. Retrovirology, 14(1),.

ROMEN, F., PAWLITA, M., SEHR, P., BACHMANN, S., SCHRÖDER, J., LUTZ, H. and LÖCHELT, M., 2006. Antibodies against Gag are diagnostic markers for feline foamy virus infections while Env and Bet reactivity is undetectable in a substantial fraction of infected cats. Virology, 345(2), pp. 502-508.

SCHARES, G., VRHOVEC, M.G., PANTCHEV, N., HERRMANN, D.C. and CONRATHS, F.J., 2008. Occurrence of Toxoplasma gondii and Hammondia hammondi oocysts in the faeces of cats from Germany and other European countries. Veterinary parasitology, 152(1-2), pp. 34-45.

STÜTZER, B., SIMON, K., LUTZ, H., MAJZOUB, M., HERMANNS, W., HIRSCHBERGER, J., SAUTER-LOUIS, C. and HARTMANN, K., 2011. Incidence of persistent viraemia and latent feline leukaemia virus infection in cats with lymphoma. Journal of Feline Medicine and Surgery, 13(2), pp. 81-87.

STUTZMAN-RODRIGUEZ, K., ROVNAK, J., VANDEWOUDE, S. and TROYER, R.M., 2016. Domestic cats seropositive for Felis catus gammaherpesvirus 1 are often qPCR negative. Virology, 498, pp. 23-30.

TASKER, S., December 2016, 2016-last update, Haemoplasmosis in Cats [Homepage of European Advisory Board on Cat Diseases], [Online]. Available: www.abcdcatsvets.org/haemoplasmosis-in-cats/.

TROYER, R.M., BEATTY, J.A., STUTZMAN-RODRIGUEZ, K.R., CARVER, S., LOZANO, C.C., LEE, J.S., LAPPIN, M.R., RILEY, S.P.D., SERIEYS, L.E.K., LOGAN, K.A., SWEANOR, L.L., BOYCE, W.M., WINSTON VICKERS, T., MCBRIDE, R., CROOKS, K.R., LEWIS,

J.S., CUNNINGHAM, M.W., ROVNAK, J., QUACKENBUSH, S.L. and VANDEWOUDE, S., 2014. Novel gammaherpesviruses in North American domestic cats, bobcats, and pumas: Identification, prevalence, and risk factors. Journal of virology, 88(8), pp. 3914-3924.

VOLLAIRE, M.R., RADECKI, S.V. and LAPPIN, M.R., 2005. Seroprevalence of Toxoplasma gondii antibodies in clinically ill cats in the United States. American Journal of Veterinary Research, 66(5), pp. 874-877.

WEIJER, K., VAN HERWIJNEN, R., SIEBELINK, K., UYTDEHAAG, F. and OSTERHAUS, A., 1988. Prevalence of FTLV (feline T-lymphotropic lentivirus) infections in cats in The Netherlands and in West Germany. Tijdschrift voor diergeneeskunde, 113(19), pp. 1063-1064.

WINKLER, I.G., LÖCHELT, M. and FLOWER, R.L.P., 1999. Epidemiology of feline foamy virus and feline immunodeficiency virus infections in domestic and feral cats: A seroepidemiological study. Journal of clinical microbiology, 37(9), pp. 2848-2851.

WOODS, J.E., BREWER, M.M., HAWLEY, J.R., WISNEWSKI, N. and LAPPIN, M.R., 2005. Evaluation of experimental transmission of Candidatus Mycoplasma haemominutum and Mycoplasma haemofelis by Ctenocephalides felis to cats. American Journal of Veterinary Research, 66(6), pp. 1008-1012.