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Vector-borne diseases in colony stray cats of Milan city

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CHAPTER 1

Foreword

1. FOREWORD

Vector-borne diseases are caused by a wide range of infectious agents including viruses, bacteria, and parasites (protozoa and helminths), which are transmitted by a variety of arthropod vectors such as ticks, Diptera (mosquitoes, phlebotomine sand flies, muscid flies), lice and fleas.

Vector-borne pathogens or diseases are important because:

• they may be highly pathogenic in dogs and cats
• their transmission is often unpredictable
• their diagnosis and control are difficult
• variable clinical signs can develop after long incubation periods and these are rarely pathognomonic
• animals may have persistent infections and thus act as reservoirs
• several are important zoonoses, such as leishmaniosis, borreliosis, rickettsiosis, bartonellosis and dirofilariosis (ESCCAP).

A wide range of factors may affect the occurrence and spread of VBDs (Otranto et al.2009), : climatic and ecological changes, national regulations on the management of stray dogs and cats together with the relatively unimpeded movement of companion animals to and from countries with a high prevalence of arthropod-transmitted diseases has introduced and promoted the diffusion both the arthropods and the diseases they carry in previously non-endemic areas (ESCCAP, Shaw et al., 2001).

Another important feature of these diseases is their increasing occurrence in wildlife, which act as reservoirs. (ESCCP) Free-roaming dogs and cats are often present in urban and rural areas representing a public health concern (Otrantoet al. 2010) in many countries of the world: these animals may be at high risk of acquiring vector-borne pathogens, mainly because they are often untreated against ectoparasites, thus, representing an easy feeding source for them. In addition, the general conditions of these animals (e.g., poor nutrition) may contribute to susceptibility to some VBDs; likewise, when infected, free-roaming cats and dogs are often neither monitored nor treated against vector-borne pathogens (Otranto et al. 2010) so they can be an easy source of infection for domestic cats who have free access to the external environment. Effective control of vector-borne diseases requires a thorough knowledge of the infectious agents, their vectors and major hosts (ESCCAP).

1.1. FELINE VECTOR-BORNE DISEASES

The arthropod parasites most commonly recognised as vectors of feline diseases are fleas of the family Pulicidae and ticks of the family Ixodidae. The potential of fly, mite or lice species for disease transmission in cats has not been determined (Tabar et al., 2008). However, there are some reported cases of feline leishmaniosis in the veterinary literature, which probably means that sandflies are

also important vectors of disease in cats (Hervas et al., 1999; Leiva et al. 2005). Ctenocephalides felis, the cat flea, is the predominant species of flea found on cats throughout the world and is recognised as a transmission vector for Bartonella henselae infection, yersiniosis, coxiellosis and several rickettsial diseases. Ct felis has also been anecdotally incriminated in the transmission of Mycoplasma haemofelis (Haemobartonella felis) although arthropod transmission has never been confirmed. Adult Ct felis are obligate, haemophagous ectoparasites that spend several months feeding in the stable environment on the skin surface of a single cat; the length of time being dependent on host grooming (Rust 1994). A percentage (2–15%) of Ct felis, particularly males, may move from one cat to another. The intermediate stages of Ct felis do not develop on their host, but rather in close proximity to its resting place (Kern et al 1992). During these stages, Ct felis is more tolerant of temperature and humidity extremes than other flea species. With their serrated mouth parts, adult Ct felis produce cutaneous micro-wounds from which they feed. Disease transmission occurs through contamination of micro-wounds with flea saliva and/or feces, the latter being produced in voluminous quantities at the time of feeding (Silverman et al., 1994). The domestic habitats in which most pet cats live provide optimal environmental requirements for Ct felis development. The free-roaming, hunting behavior of cats encourages exchange between domestic and potentially infected sylvatic flea populations, an important factor in the ecology of several emerging or re-emerging zoonotic diseases (Azad et al., 1997;, Shaws et al., 2001). There is limited information on the species of ticks capable of attaching to and transmitting infectious diseases to cats. The cats seem to be less predisposed to tick-transmitted diseases than dogs. They are likely to have innate resistance or adaptation to infection, limiting the disease development or impairing the tick-to-cat transmission of the infective agents (Shaw et al., 2001). In the cool temperate areas of the northern Europe, the most common species found attached to cats are Ixodes hexagonus (the hedgehog tick), I. ricinus, I. canisuga and I. ventalloi (Hillyard, 1996, Ogden et al., 2000). I hexagonus was found to be the most common tick infesting cats in some surveys in the UK and France (Pichot et al., 1997, Ogden et al., 2000). In warmer regions, cats have been found infested with Dermacentor spp, Rhipicephalus sanguineus and Haemaphysalis spp. (Shaw et al., 2001).

Arthropod-transmitted pathogens are often associated with the induction of immunological abnormalities. Animals that are unable to mount a protective immune response may develop persistent sub-clinical infection which recrudesces with stress or concurrent disease. Chronic antigen exposure may result in exuberant non-protective antibody responses and the induction of polysystemic immune-mediated disease. The role of arthropod-transmitted diseases in chronic, relapsing syndromes with fever and signs of immune-

mediated disease in cats deserves further investigation (Shaw et al. 2001). Even though the infectious organisms discussed here are dealt with as individual causative agents of arthropod-borne disease, there is potential for co-infection to occur. Multiple infections can occur when several arthropod species and a susceptible host share the same biohabitat. Similarly, multiple species and multiple genotypes of the same pathogenic species can occur in an individual animal (Shaw et al., 2001). The same arthropod species may be a vector for several pathogens and co-infection of individual ticks can occur (Schouls et al., 1999). Co-infection may partially explain variations in classic clinical presentation, pathogenicity and response to therapy, and the role of active co-infection rather than sequential exposure, can now be clarified by polymerase chain reaction (PCR) analysis (Kordick et al., 1999b).

1.2. DIAGNOSIS OF VECTOR-BORNE DISEASES

Although history and clinical signs may suggest one of the arthropod-transmitted infectious diseases, diagnosis should be supported by laboratory techniques which include combinations of direct microscopic visualisation or immunodetection of infective organisms in blood or infected tissue, microbial culture, serological testing, immunoblotting, and PCR (Shaw et al., 2001). In endemic areas and in acute disease, direct visualisation (using the light microscope) of pathogen agent in Giemsa stained blood smears and in lymph node, bone marrow aspirates or skin biopsies is valuable. However, in chronic disease, low-grade exposure or subclinical infection, levels of bacteraemia/parasitaemia may be low and direct visualisation does not allow intra-species differentiation (Shaw et al., 2001). The use of direct immunohistochemical or immunocytochemical methods on biopsy samples and cytological preparations from infected animals may increase both sensitivity and specificity but these techniques are currently only available from research laboratories (Shaw et al., 2001). Although culture is still considered the definitive test for the presence of infection, there are considerable challenges associated with culture of arthropod-borne organisms which limit its availability as a diagnostic method in practice (Shaw et al., 2001). As a group, these organisms are often fastidious and may require long periods of culture, special media (Bartonella, Borrelia, Francisella) or eukaryotic cell co-culture systems (Rickettsia, Ehrlichia, Leishmania, Babesia). In addition, the collection and transport of material for culture may represent a considerable zoonotic hazard (Shaw et al., 2001). Microbiological laboratories should be consulted prior to sample submission and sample package and transport should fulfil all biosafety requirements. Serological testing is the most practical and commonly employed diagnostic methodology for arthropod-borne infectious diseases, and the indirect fluorescent antibody (IFA) and enzyme-linked immunosorbent assay (ELISA)

tests are most widely used. However, serological testing in cats is severely limited by poor availability of feline-specific test kits, in addition to the more general disadvantages of reduced ability to identify acute infection, difficulty in differentiating infection from prior exposure or vaccinal titres, and antigenic cross-reactivity between organisms. A rise in immunoglobulin G titre is required to confirm active infection and there is considerable inter-laboratory variation in interpretation of significant titres. Serological testing can be supported by western immunoblotting to characterise and distinguish the different species involved in disease, but immunoblotting has limited availability in practice. Many of the problems of serological diagnosis are circumvented by the use of the PCR, and methods employing PCR have been reported for most of the arthropod-borne infections affecting cats. Advantages of PCR include its sensitivity and specificity, particularly in the early phases of disease, and its versatility. It can be applied to blood, bone marrow, splenic and lymph node aspirates, body fluids such as joint and cerebrospinal fluid and formalin fixed tissue biopsy samples. Small diagnostic samples can be screened for several pathogens simultaneously and PCR can be used to monitor the progress of therapy. However, the sensitivity of PCR produces its own problems in interpretation. The presence of microbial DNA although indicative of current infection, is not diagnostic for disease unless it is present with compatible clinical signs. The use of quantitative PCR may well help to solve this problem in the future (Shaw et al., 2001).

1.3. BARTONELLOSIS

Bartonellosis is caused by fastidious, Gram- negative, intraerythrocytic, arthropod-transmitted bacteria of the genus, Bartonella. Several species have been identified in wild and domestic cats: Bartonella henselae, B koehlerae, B clarridgeiae and 'B weissii' although B henselae is themost prevalent and geographically widespread (Breitschwerdt et al., 2000). Asymptomatic infection with B henselae or B clarridgeiae is common in cats, which are therefore considered to be a major reservoir for human infection. In humans, B henselae and B clarridgeiae have been shown to be the agents of the common, but usually self-limiting cat scratch disease (CSD) (Kordick et al., 1997, 1999a). B henselae has been associated with more profound human syndromes such as the vasculoproliferative disorders bacillary angiomatosis and peliosis hepatis, as well as endocarditis, prolonged bacteraemia and various ocular disorders including Perinaud oculoglandular syndrome, neuroretinitis and chorioretinitis (Cunningham et al., 2000). Bartonella are haemotrophic bacteria which are facultative intracellular parasites of red blood cells and endothelial cells. They can be detected in samples of cat blood as well as in specimens from claws and in saliva (ESCCAP). The major vector of B henselae in cats is the flea, Ct felis (Chomel et al., 1996). B henselae can be visualised in and cultured from Ct felis

for up to 9 days after an infected blood meal (Higgins et al., 1996a); however the role of the cat flea as a biological vector has not been fully determined (Shaw et al., 2001). Crucial for an infection is both the contact with fleas and their faeces. The agent can survive and remain infectious for up to nine days in the faeces of infected fleas (ESCCAP). Cat-to-cat transmission of B henselae occurs via intradermal inoculation of infected faeces from Ct felis (Flexman et al., 1995, Foil et al., 1998). However, the role of the cat flea in the transmission of B henselae from cats to humans has not been proven (Shaw et al., 2001). For the infection of humans, scratches and bites from cats play a crucial role. It is assumed that the oral cavity and the claws of infected cats are contaminated with bacteria-containing flea faeces during grooming and that the agent is transmitted to humans through skin wounds. Another possibility is the iatrogenic transmission in blood transfusions (ESCCAP). The agent B. henselae as well as the primary vector Ctenocephalides felis are distributed worldwide. The highest probability of becoming infected with Bartonella has been associated with cats less than two years of age, cats with access to outdoor areas, stray cats and animals in multi-cat households (ESCCAP). There is no breed or gender predisposition (Kordick et al., 1999c). Although geographic environments with warm temperatures and high humidity are reportedly associated with the highest exposure rates (Breitschwerdt et al., & Kordick, 2000), the prevalence in cool temperate climates is also relatively high; probably because in colder countries, animals are kept in heated domestic or confined environments, facilitating the maintenance of the flea life cycle (Shaw). Disease association with naturally occurring Bartonella infection is difficult to determine because of its high prevalence in apparently asymptomatic cats. Clinical disease is characterized by fever, lethargy, transient anaemia, lymphadenomegaly, neurological dysfunction or reproductive failure has been reported following experimental infections with B henselae and B clarridgeiae (Shaw et al., 2001). However there are limited reports of clinical disease in naturally infected cats. There is a statistical correlation between cats naturally infected with B henselae infection, stomatitis and urinary tract disease (Glaus et al., 1997). Uveitis associated with intraocular Bartonella DNA and ocular IgG production has also been reported in cats (Lappin et al., 1999, Lappin et al., 2000). Uveitis associated with intraocular Bartonella DNA and ocular IgG production has also been reported in cats (Lappin et al, 1999, Lappin et al., 2000). Pathological abnormalities documented in experimentally infected cats have included lymphocytic-plasmacytic myocarditis, lymphocytic cholangiohepatitis, lymphocytic interstitial nephritis and lymph node and splenic hyperplasia (Guptill et al., 1997). The effect of co-infection with FIV and B henselae has been investigated and there is no direct association between FIV and B henselae seropositivity. However, in cats which have serological evidence of both infections, there is an increased risk of

lymphadenopathy and gingivitis (Ueno et al., 1996). The diagnosis of Bartonella infection is best made by culture and/or PCR because of the high prevalence of seropositivity in normal cats. The association of infection and clinical disease is difficult to confirm and requires identification of Bartonella organisms within tissues such as lymph node showing compatible pathology (Shaw et al., 2001). The following diagnostic procedure is recommended:

1. The presence of clinical signs that may be associated with bartonellosis.
2. Exclusion of other causes that might explain the clinical picture.
3. Laboratory tests:

a. The gold standard for the diagnosis of bartonellosis is blood culture. It is also possible to detect Bartonella DNA in samples of blood, tissue, cerebrospinal fluid or aqueous humor by PCR.

b. Antibodies can be detected serologically from approx. 10 to 14 days after infection. A positive serological finding only shows that the cat or dog has already had contact with Bartonella spp. For the diagnosis of clinical bartonellosis repeated testing of serum samples should show a rising antibody titre.

4. Response to treatment with an antibiotic effective against Bartonella spp. However, this may be complicated by the fact that the drugs effective against Bartonella spp. are broad spectrum antibiotics which are also effective against other possible infections which may have been included in the differential diagnosis. Despite following this procedure, a definitive diagnosis of bartonellosis is not always possible (ESCCAP).

Treatment of bartonellosis and elimination of bacteraemia is problematic. Doxycycline, amoxicillin, amoxicillin/clavulanate used at higher than recommended dose rates have been successful in suppressing bacteraemia in experimental infections. Rifampicin and enrofloxacin are also reportedly effective (Regnery et al., 1996, Greene et al., 1996, Kordick et al., 1997). However, total elimination of infection may be impossible despite the use of combination therapy such as rifampicin and doxycycline, and prolonged duration (4–6 weeks) of therapy. In addition, the risk of re-exposure is high (Shaw et al., 2001). Even though the mechanism of transmission of Bartonella infection from cats to humans has not been determined, the prevalence of Bartonella bacteraemia in cats and the risk of Bartonella associated disease in pet owners, is decreased by a vigorous integrated flea control program. When uninfected cats are housed with B henselae bacteraemic SPF cats in an ectoparasite-free environment, there is no evidence of Bartonella transmission between cats (Chomel et al., 1996). Furthermore, when continuous preventative treatment for fleas and ticks was given to two cats and one dog persistently infected with Bartonella species in a single household, the in-contact humans remained serologically negative (Kordick et al., 1998).

Authors	Year	Journal	Number of tested cats	Characteristics of feline population	Methods	Location	Prevalence
EUROPE							
SPAIN							
Laia Solano-Gallego et al	2006	Veterinary Microbiology 118 (2006) 274–277	168		IFA+ PCR	northeastern Spain	IFAT: 71.4%; PCR: 7/168 (4,2%)
M D Tabar et al	2008	Veterinary Parasitology 151 (2008) 332–336	100	cast	PCR	Barcelona area; Spain	B. clarridgeiae 1%
Tania Ayllo´n et al	2012	VECTOR-BORNE AND ZOONOTIC DISEASES Volume 12, Number 2, 2012	680	680 (539 (79.3% of total) client-owned cats ; 141 (20.7% of total) stray cats were enrolled at five humane societies in the Madrid area)	IFA (1: 64)+ PCR	Madrid, Spain.	IFAT: B henselae stray cats 24.1% (n = 31); client-owned cats 23.7% (n = 128). All cats in 162/680 23.8%. PCR: Bartonella spp. DNA was amplified and sequenced from 2 (0.3%): one from a client-owned cat and the other from a stray cat. B. henselae DNA from one cat (0.15%), and B. clarridgeiae DNA from another cat (0.15%)
PORTUGAL							
A. S. Alves et al	2009	Journal Compilation 2009 European Society of Clinical Microbiology	51	Fifty-one cats (domestic, shelter and stray) from Lisbon and E´vora	PCR	PORTUGAL	35/51 (67.7%); The prevalence of Bartonella bacteraemia is higher in shelter (76.9%) than in domestic cats (68.2%) and all stray cats tested (n = 2) were

		and Infectious Diseases, CMI, 15 (Suppl. 2), 1–3					positive.
IRLANDA							
F Juvet et al	2010	Journal of Feline Medicine and Surgery (2010) 12, 476e482	116 whole blood; 83 serum samples	41 client-owned and 75 stray cats	PCR+ELISA	IRLANDA	PCR: B henselae alone 3 (2.6%), 3 (4%); B clarridgeiae alone 1 (0.9%) 1 (1.3%); B henselae and B clarridgeiae 1(0.9%) 1 (1.3%) ; ELISA: Bartonella species 22 (26.5%) 20 (32.8%) 2 (9.1%)
UNITED KINGDOM							
AD Bennett et al	2011	Journal of Feline Medicine and Surgery (2011) 13, 553e557	78	78 cats were entered into the study. Of the cats, 61 were client-owned and 17 were from rescue centres; 40 were clinically ill and 38 were considered healthy	PCR on blood semples	Scotland	B henselae 5.8%
USA							
B J. Luria et al	2004	Journal of Feline Medicine and Surgery (2004) 6, 287e296	553	feral casts	IFA >1:64	Northern Florida	B. henselae 33.6 (186/553)

J B Case et al	2006	Journal of Feline Medicine and Surgery (2006) 8, 111e117	170	domestic cats living in private homes, feral cat colonies, and animal shelters from California and Wisconsin	IFA	USA	14.7% for Bartonella henselae
A M Ishak et al	2007	Journal of Feline Medicine and Surgery (2007) 9, 1e7	89 cats with anemia; 87 healthy cats	the blood of cats with anemia and a control group of healthy cats	PCR	USA	Bartonella species was amplified from five of 89 cats with anemia (5.6%) and seven of 87 healthy cats (8.0%)"
CANADA							
A Kamrani et al	2008	The Canadian Journal of Veterinary Research 2008;72:411–419	646 healthy pet cats; 45 feral cats; 50 fleas	healthy pet cats in Ontario	PCR	Canada	Healthy cats: Bartonella spp. by PCR and by culture combined was 4.3% (28/646) [3.7% (24/646) Bartonella henselae, 0.6% (4/646) Bartonella clarridgeiae]; feral cats: The prevalence of Bartonella henselae (58%); fleas: B. henselae 90% (45/50); B. clarridgeiae 12% (6/50)
AFRICA							
R Lobetti and M R Lappin	2012	Journal of Feline Medicine and Surgery 14 (2012) 857–862	102		PCR+ELISA	South Africa	PCR: cats [Bartonella henselae: five cats (4.9%); Bartonella clarridgeieae: three cats (2.9%). ELISA: cats, 24 (23.5%) wereseropositive for Bartonella IgG
AUSTRALIA							

VR Barrs et al	2010	Australian Veterinary Journal Volume 88, No 5, May 2010	111 cats		PCR+ELISA	easthern Australia	PCR: 11/42 (26.2%) were positive for Bartonella spp. DNA in their blood. ELISA: Bartonella spp. IgG was detected in 42 cats (37.8%), of which
ASIA							
A S Mokhtar and S Tay	2011	Am. J. Trop. Med. Hyg., 85(5), 2011, pp. 931–933	209 fleas;	209 fleas (Ctenocephalides felis);	PCR	Malaysia	B. henselae 11.5%; B. clarridgeiae 19.1%

1.4 EHRLICHIOSIS

Ehrlichia are vector-borne, Gram-negative, obligate intracellular bacteria. In Europe, Ehrlichia canis is the aetiological agent of canine monocytic ehrlichiosis (CME). This pathogen infects mainly lymphocytes and monocytes wherein the typical, microscopically visible microcolonies (morulae) develop. The main host of E. canis is the dog (other canids can serve as reservoirs of infection); the vector is the tick Rhipicephalus sanguineus. All stages (larvae, nymphs, adults) of R. sanguineus preferentially feed on canids and may acquire E. canis from bacteraemic animals. The pathogen may overwinter in infected ticks. Trans-stadial (from larva to nymph to adult), but probably no transovarial, transmission occurs (ESCCAP). Ehrlichia canis or a closely related species has been described in cats (ESCCAP). For many years, Ehrlichia-like inclusions have been detected in monocytes, lymphocytes and granulocytes of cats with febrile illness and thrombocytopenia in many countries (Buoro et al., 1989, Bouloy et al., 1994, Beaufils et al., 1999, Bjoersdorff et al., 1999), as has serological evidence of Ehrlichia infection (Peavy et al., 1997, Matthewman et al., 1996, Beaufilset al., 1999). Molecular techniques confirmed the presence of E. canis in cats with clinical signs compatible with monocytic ehrlichiosis in North America (Breitschwerdt et al., 2002).

As in other species, ehrlichiosis in cats is presumed to be tick-transmitted, but definitive evidence for the route of transmission is lacking (Shaw et al., 2001). Risk factors associated with positive serology for E canis and/or E risticii have been studied (Stubbs et al., 2000) and cats with outdoor exposure are more likely to have positive E canis and/or E risticii serology; while there is no association with breed or age. Clinical signs reported in cats with ehrlichiosis are extremely varied. For the monocytic ehrlichiosis, the signs are: intermittent fever, anorexia, weight loss, vomiting and diarrhoea. Clinical-pathological discoveries include anaemia, thrombocytopenia, leukopenia and hyperglobulinaemia. It is still unknown whether cats become persistently infected or develop immunity as a result of chronic infection, similar to what happens with dogs (Shaw et al., 2001). No association with FIV or FeLV has been reported in literature (Shaw et al., 2001). Definite diagnosis of feline ehrlichiosis is made by demonstration of intra-leucocytic inclusions in, or PCR of, a peripheral blood sample. However, the presence of inclusions in infected cats is variable (Stubbs et al., 2000, Bjoersdorff et al., 1999). Serological (IFA) testing is available for both E risticii and E canis infections in cats (Stubbs et al., 2000) but considerable cross-reaction occurs between ehrlichial species and a rising titre is required to confirm active infection. Although controlled therapeutic trials are lacking, administration of doxycycline or tetracycline is the treatment of choice for feline ehrlichiosis and clinical response is reported to be excellent (Peavy et al., 1997, Bjoersdorff et

al., 1999, Beaufils et al., 1999). Antibiotic therapy should be administered for a minimum period of 21–28 days. The primary measure for the prevention of Ehrlichia infections is an effective protection against tick infestation (ESCCAP). E. canis is not considered a zoonotic agent (ESCCAP).

Authors	Year	Journal	Number of tested cats	Characteristics of feline population	Methods	Location	Prevalence	Notes
EUROPE								
ITALIA								
S. Vita et al	2005	Veterinary Research Communications, 29(Suppl. 2) (2005) 319–321	203		IFI assays and all samples were initially tested at a dilution of 1:40	central Italy (Abruzzo)	1% E.canis	a mother and a son, who had been mainly living indoors and who had antibody titers of 1: 160 and 1:2560, respectively
SPAIN								
E AGUIRRE et al	2004	Ann. N.Y. Acad. Sci. 1026: 103–105 (2004)	122	cats	IFAT+PCR	central Spain	IFAT: E. canis 10% PCR: 0%	
A. Ortuno	2005	J. Vet. Med. B 52, 246–248 (2005)	235	cats	IFA	Northeastern Spain	17.9%.	Most positive sera (83.3%) showed low antibody titres (<1:80)
Laia Solano-Gallego et al	2006	Veterinary Microbiology 118 (2006) 274–277	168		IFAT+PCR	northeastern Spain	IFAT: 11.3% PCR:0%	
M D Tabar et	2008	Veterinary	100	cats	PCR	Barcelona area;	1%	

al		Parasitology 151 (2008) 332–336				Spain		
T. Ayllo´n et al	2009	Journal Compilation 15 (2009) (Suppl. 2), 4–5	52	client-owned cats	PCR	central Spain	3.8%	
Tania Ayllo´n et al	2012	VECTOR-BORNE AND ZOONOTIC DISEASES Volume 12, Number 2, 2012	680	680 (539 (79.3% of total) client-owned cats ; 141 (20.7% of total) stray cats were enrolled at five humane societies in the Madrid area)	IFA (Cut-off titers were established at 1:40)+ PCR	Madrid, Spain.	stray cats 6.4% (n = 9); client-owned cats 10.8% (n = 58); All cats in 67/680 9.9%, PCR: 0%	
IRLANDA								
F Juvet et al	2010	Journal of Feline Medicine and Surgery (2010) 12, 476e482	116 whole blood	41 client-owned and 75 stray cats	PCR	IRLANDA	0%	
UNITED KINGDOM								
AD Bennett et al	2011	Journal of Feline Medicine and Surgery (2011) 13, 553e557	78	78 cats were entered into the study. Of the cats, 61 were client-owned and 17 were from rescue centres; 40 were clinically ill and 38 were considered healthy	PCR	Scotland	0%	

USA								
J M Eberhardt et al	2006	Journal of Feline Medicine and Surgery (2006) 8, 164e168	112 cats	feral and relinquished cats in Phoenix and Nogales, Arizona	PCR	USA	0%	
M R Lappin et al	2006	Journal of Feline Medicine and Surgery (2006) 8, 85e90	92 whole blood semples	cats brought to Veterinarians in Alabama (BG and AR), Maryland (Brunt), and Texas (Burney)	PCR	USA	0%	
AFRICA								
R Lobetti and M R Lappin	2012	Journal of Feline Medicine and Surgery 14 (2012) 857–862	102		PCR	South Africa	0%	
AUSTRALIA								
VR Barrs et al	2010	Australian Veterinary Journal Volume 88, No 5, May 2010	111 cats		PCR	easthern Australia	0%	
ASIA								
A S Mokhtar and S Tay	2011	Am. J. Trop. Med. Hyg., 85(5), 2011, pp. 931–933	209	fleas (Ctenocephalides felis)	PCR	Malaysia	R. felis 2.9%	

1.5 RICKETTSIAL INFECTIONS

These arthropod-transmitted, intracellular infections are rarely reported to cause clinical disease in cats. However, as domestic cats are susceptible to infection with a number of rickettsial species and are hosts for their arthropod vectors, they may play an increasingly important role in the epidemiology of these diseases in humans (Shaw et al., 2001). The spotted fever group of rickettsiosis are significant problems for man and dogs. In the Americas, Rocky Mountain spotted fever caused by Rickettsia rickettsia (transmitted by Dermacentor andersoni, D. variablis, R. sanguineus and Amblyomma cajennese) is the most important of this group, while in Europe, Asia and Africa, Mediterranean spotted fever caused by R. conorii (transmitted by R. sanguineus) is of greatest concern. The role of dog in these human infections is in transporting infected ticks into the domestic environment and the risk to owners of removing infected ticks from their animals (Days, 2011). Seropositivity indicates cats are also susceptible to infection with these tick-transmitted rickettsial species (Greene & Breitschwerdt 1998; Matthewman et al., 1997b). Clinical disease associated with infection has not been identified in cats. Whether cats play a role in the epidemiology of these diseases through interaction with the wild rodent reservoir of infection and ticks is unknown (Shaw et al., 2001).

In these group of infections there are also two causes of flea-transmitted human typhus, Rickettsia typhi transmitted by rodent fleas with a world-wide distribution and the recently characterised and closely related R. felis in south-western USA (Schriefer et al., 1994a, 1994b, Higgins et al., 1996b). The reservoir potential for domestic pets for R. typhi is not proven, but R. felis is found in dogs, cats and cat fleas (Ct. felis) (McElroy KM et al., 2010; Kenny MJ et al., 2003). R. felis may be transmitted transovarially and trans-stadially in successive generations of Ct. felis and infection can be maintained in cats fleas for up 12 generations without the fleas having fed on an infected hosts. Infection has also been transmitted among cats by infected fleas (C.E. Greene et al., 2013). Thus the domestic pet may introduce infected fleas into the home environment, which then become the means for human infection. So Ct felis is now a recognised vector for typhus and will support infections with both rickettsial species (Noden et al., 1998). This vector has a catholic host range and is particularly well adapted to feed on opossums and cats. In endemic areas of the USA, peri-urban opossums are major reservoir hosts for R felis (Schriefer et al., 1994b) but the reservoir potential of cats has not been determined. Experimental infection of cats with R felis has been demonstrated (Wedincamp et al., 2000) as has seropositivity to R typhi (Sorvillo et al., 1993, Matthewman et al., 1997b). The pathogenic potential of either rickettsial species for cats is unknown. What is in no doubt, is that cats will transport Ct felis into domestic surroundings and as

transovarial and trans-stadial transmission of R felis has been shown (Azad et al., 1992), a domestic focus of infection for humans could be established**.**

Authors	Year	Journal	Number of tested cats	Characteristics of feline population	Methods	Location	Prevalence	Notes
EUROPE								
ITALIA								
G Capelli et al	2009	Parasites & Vectors 2009, 2(Suppl 1):S8	320 fleas (257 C. felis and 63 C. canis)	257 C. felis and 63 C. canis from 117 animals	PCR	north-eastern Italy	11.9%	38 (11.9%) C. felis fleas, 13 from cats (17.6%) and 25 from dogs (10.2%) werepositive for R. felis
SPAIN								
E AGUIRRE et al	2004	Ann. N.Y. Acad. Sci. 1026: 103–105 (2004)	122	cats	IFA	central Spain	N. risticii 2.4%	
Laia Solano-Gallego et al	2006	Veterinary Microbiology 118 (2006) 274–277	168	cats	IFA	northeastern Spain	R. conorii 44%	
M D Tabar et al	2008	Veterinary Parasitology 151 (2008) 332–336	100	cats	PCR	Barcelona area; Spain	R spp. 1%	
Tania Ayllo´n et al	2012	VECTOR-BORNE AND ZOONOTIC DISEASES Volume 12, Number 2,	680	680 (539 (79.3% of total) client-owned cats ; 141 (20.7% of total) stray cats were enrolled at five humane societies in the Madrid	IFA (Cut-off titers were established at 1:40)+ PCR	Madrid, Spain.	IFAT:stray cats 2.1% (n = 3) for N. risticii; client-owned cats 0.7% (n =	

		2012		area)			4) for N. risticii; all cats 7/680 1%. PCR: 0%	
PORTUGAL								
A. S. Alves et al	2009	Journal Compilation 2009 European Society of Clinical Microbiology and Infectious Diseases, CMI, 15 (Suppl. 2), 1–3	32 fleas from Fifty-one cats (domestic, shelter and stray)	Fifty-one cats (domestic, shelter and stray) from Lisbon and E´vora	PCR	PORTUGAL	29 of which (90.6%) were C. felis, one (3.1%)was C. canis and two (6.3%) were unidentifiable. Only C. felis fleas were infected, six (40.0%) with B. clarridgeiae and six (40.0%) with R. felis	
UNITED KINGDOM								
AD Bennett et al	2011	Journal of Feline Medicine and Surgery (2011) 13, 553e557	78	78 cats were entered into the study. Of the cats, 61 were client-owned and 17 were from rescue centres; 40 were clinically ill and 38 were considered healthy	PCR	Scotland	0%	

USA								
J B Case et al	2006	Journal of Feline Medicine and Surgery (2006) 8, 111e117	170	domestic cats living in private homes, feral cat colonies, and animal shelters from California and Wisconsin	IFA	USA	17.2% of cats for Rickettsia rickettsii 14.9% for R akari, 4.9% for R typhi, 11.1% for R felis	
M R Lappin et al	2006	Journal of Feline Medicine and Surgery (2006) 8, 85e90	92 whole blood semples; 92 fleas from the same cats	cats brought to Veterinarians in Alabama (BG and AR), Maryland (Brunt), and Texas (Burney)	PCR	USA	0%	
J M Eberhardt et al	2006	Journal of Feline Medicine and Surgery (2006) 8, 164e168	112 cats	feral and relinquished cats in Phoenix and Nogales, Arizona	PCR	USA	Neorickettsia risticii 0%	0%
D B Bayliss et al	2009	Journal of Feline Medicine and Surgery (2009) 11, 266e270	90 cats with and without fever	cats with and without fever	IFA	USA	R felis: cats with fever 5.6%; cats without fever 2.2%; R rickettsii: cats with fever 6.6%: cats without fever 2,2%	

CANADA								
A Kamrani et al	2008	The Canadian Journal of Veterinary Research 2008;72:411–419	45 fera cats; 50 fleas	feral cats and fleas	PCR	Canada	Cats: R. felis 0%; fleas: R. felis 18% (9/50)	
AUSTRALIA								
VR Barrs et al	2010	Australian Veterinary Journal Volume 88, No 5, May 2010	111 cats		PCR	easthern Australia	0%	
ASIA								
A S Mokhtar and S Tay	2011	Am. J. Trop. Med. Hyg., 85(5), 2011, pp. 931–933	209	fleas (Ctenocephalides felis)	PCR	Malaysia	R. felis 2.9%	

1.6 COXIELLOSIS

Coxiella burnetii is an extracellular, arthropod-transmitted, spore-forming bacterium which in cats produces subclinical infection but in humans causes Q fever, a disease associated with fever, arthralgia, myalgia, hepatitis and respiratory symptoms. A wide range of wild and domesticated animals including cats are considered In wildlife reservoir cycles, C burnetii is commonly transmitted by arthropod vectors, including ticks. In addition, the sporulated form of C burnetii is highly resistant to environmental extremes and can be spread between hosts by ingestion or aerosol dissemination of infected fluids such as milk, urine and vaginal/uterine secretions, or by ingestion of infected tissues such as placental material (Shaw et al. 2001; Nagaoka et al., 1998). Cross-reactivity between C burnetii and B henselae has been reported in human studies (LaScola et al., 1996) and although this has not been investigated in cats, it may inflate seroprevalence figures for Coxiella (Shaw et al., 2001.

Infected cats are considered important reservoirs for human coxiellosis (Shaw et al., 2001). C burnetii appears to be frequently carried in the vagina of healthy cats in endemic areas (Nagoaka et al., 1998) and contact with infected parturient cats is a risk factor for human infection (Pinsky et al., 1991, Morita et al., 1994). Diagnosis of active infection with Coxiella is made by demonstration of a rising antibody titre, PCR or immunohistochemical techniques. Treatment of cats may be required in households where there is increased risk of human infection. Coxiella infections are variably susceptible to single agent therapy with macrolides (erythromycin, azithromycin), potentiated sulphonamides and fluoroquinolones for 2–4 weeks duration. Combination therapy of doxycycline and fluoroquinolones with rifampicin may be more effective (Shaw et al., 2001, Levy et al., 1991).

1.7 YERSINIOSIS

Yersinia pestis is non-spore-forming bacterium which causes Plague. Infection is maintained in reservoir rodent populations by flea transmission. Localised foci of disease occur in temperate, semi-arid areas throughout the world and with semi-urban development now extending into endemic areas of plague, there is increasing risk of domestic cats being infected by bites from rodent fleas acquired during hunting, or morecommonly by ingestion of infected small mammals. Ct felis is a relatively ineffective vector for plague transmission. Bacteraemic cats are a source for human infection either directly through aerosol spread, bites or scratches or indirectly by transporting infected fleas into the domestic environment (Shaw et al., 2001, Macy, 1998, Gage et al., 2000). Both domesticated and wild cats are more susceptible to clinical yersiniosis than dogs. In experimentally infected cats, most develop mild to moderately severe clinical disease with subsequent recovery, although some develop an acute fulminating

and fatal syndrome (Gasper et al., 1993). In naturally infected cats are described two main clinical syndromes:Bubonic plague associated with fever, dehydration, weight loss and lymphadenopathy with abscessation and draining tracts affecting the cervical, retropharyngeal and submandibular lymph nodes, which may be followed by recovery or by haematogenous spread with progression to the often fatal, septicaemic syndrome. In cats, pneumonic involvement during this stage is common and dissemination by aerosol may occur to in-contact humans.
Less commonly reported gastrointestinal syndrome with vomiting, diarrhoea, tonsillar and lingual lymph node enlargement and necrotic stomatitis (Eidson et al., 1991, Carlson, 1996).
Fluorescent antibody testing and rising serological titres provide a presumptive diagnosis. Confirmation of diagnosis is made by demonstration of bacteria in affected tonsillar tissue or lymph node aspirates with appropriate Gram and Giemsa staining characteristics, followed by culture. Stringent biosafety procedures should be carried out in collection and transport of specimens and culture requires a containment laboratory. The decision to treat cats with plague should always take into consideration the zoonotic risk. In particular, the potential for aerosol spread from pulmonary lesions in infected cats should be evaluated by thoracic radiology. Appropriate protective clothing and gloves should be worn and all contaminated material or surfaces should be disinfected. Yersinia pestis is sensitive to routine disinfectants and a variety of antibiotics including aminoglycosides, doxycycline, chloramphenicol and fluoroquinolones .
Fluorescent antibody testing and rising serological titres provide a presumptive diagnosis. Confirmation of diagnosis is made by demonstration of bacteria in affected tonsillar tissue or lymph node aspirates with appropriate Gram and Giemsa staining characteristics, followed by culture. Stringent biosafety procedures should be carried out in collection and transport of specimens and culture requires a containment laboratory. The decision to treat cats with plague should always take into consideration the zoonotic risk. In particular, the potential for aerosol spread from pulmonary lesions in infected cats should be evaluated by thoracic radiology. Appropriate protective clothing and gloves should be worn and all contaminated material or surfaces should be disinfected. Yersinia pestis is sensitive to routine disinfectants and a variety of antibiotics including aminoglycosides, doxycycline, chloramphenicol and fluoroquinolones (Shaw et al., 2001, Bonacorsi et al., 1994). Therapy should be continued for a minimum of 21 days. Doxycycline is most commonly used in the bubonic syndrome and can be used for prophylaxis in exposed, subclinical cats (Shaw et al., 2001, Macy 1998).

1.8 TULARAEMIA

The aetiological agent of tularaemia in mammals and birds is the tick-transmitted bacterium, Francisella tularensis. These organisms are distributed throughout the temperate and sub-Arctic areas of the northern hemisphere and there is geographic variation in strain, the species of tick vector and reservoir hosts involved. Two biovars are recognised and cats are susceptible to both (Baldwin et al., 1991). F tularensis tularensis is distributed throughout North America and is associated with a tick-rabbit cycle. F tularensis palearctica has a broad distribution throughout the northern hemisphere and has a more complex epidemiology involving a hare/rabbit reservoir-tick/mosquito cycle. Tick vectors include species of Dermacentor, Ambylomma, Ixodes and Haemaphysalis. Cats may also be infected by ingesting infected rodent or lagomorph prey. Infected domestic cats may transmit tularaemia to humans by bites and scratches (Capellan et al., 1993, Rodon et al., 1998, Arav-Boger 2000). Cats appear more susceptible to naturally occurring clinical tularaemia than dogs and younger cats appear more predisposed. Clinical signs include fever, marked lethargy, anorexia, regional or generalised lymphadenopathy with abscessation, splenomegaly and/or hepatomegaly with abscessation, oral ulceration, leucopenia, icterus and in some cases death (Baldwin et al., 1991, Gliatto et al., 1994, Woods et al., 1998). Presumptive diagnosis may be made serologically but confirmation requires culture of infected tissue or body fluids using rigorous biosafety procedures to prevent human infection. Therapeutic regimes for feline tularaemia have not been thoroughly investigated and are adapted from human cases. Aminoglycoside, tetracycline, chloramphenicol and fluoroquinolone antibiotics have been advocated for 2–4 weeks duration (Enderlin et al., 1994).

1.9 BORRELIOSIS

There are currently 11 known species/genotypes of the Borrelia burgdorferi complex (=sensu lato) which are spirochaetes that infect many mammals and birds and are transmitted by ticks (Ixodes ricinus, I. hexagonus, and I. persulcatus) (ESCCAP). Human infections are of major public health importance and although infections have been demonstrated in dogs, they are not of major clinical importance. Humans as well as dogs acquire Borrelia infection when exposed to infected ticks but there is no interdependency between dogs and humans in terms of transmission. Positive serology in cats has also been reported, but disease in cats, if it occurs at all, is poorly understood and there is therefore little data concerning the prevalence of infection, clinical appearance and treatment options for cats (ESCCAP). Currently, ticks of the family Ixodidae and mostly of the genus Ixodes are recognized vectors of B. burgdorferi. Larval, nymph and adult female tick vectors can acquire Borrelia when feeding on an infected “reservoir host”, which is an animal harbouring the

pathogen as a long-term infection. It is also the case that ticks can become infected with the spirochaetes when feeding next to infected ticks (co-feeding transmission). Several animal species have been identified as reservoirs of Borrelia in Europe, including many mammals and birds. Borrelia in ticks disseminate to the salivary glands and are transmitted trans-stadially but there is no transovarial transmission. The tick must be attached for at least 16-24 hours before pathogen transmission to a new host occurs. Borrelia remains in the skin of a host before travelling to other tissues. In some cases, it can take up to 4 weeks before a systemic infection develops (ESCCAP). Much of the published borreliosis research in dogs and cats relates to the Genogroup I species, B. burgdorferi sensu stricto, which is the main cause of borreliosis in humans and dogs in the USA. However, B garinii and B afzelii are the major pathogenic species for humans in Europe and in Japan, B japonica causes human disease (Shaw et al., 2001, Dressler et al., 1994, Wilske et al., 1996, Hubalek, et al.,1997). The results of experimental infection of cats with US strains of B burgdorferi are contradictory with respect to inducing clinical signs. Burgess (1992) reported no apparent disease following infection while others (Gibson et al., 1993, Omran et al., 1997) reported some cats with non-specific signs including fever, lethargy, stiffness and arthritis. Clinical and pathological signs referable to hepatic, gastrointestinal, neurological and cardiac disease are also reported (Omran et al., 1997). There is some evidence that persistent infection without disease may occur (Shaw et al., 2001). Clinical manifestations in naturally-infected cats are uncommon (ESCCAP). Clinical borreliosis has not been fully characterized but techniques used to indicate infection in cats include PCR and serology. Cyclical increases in both B burgdorferi-specific IgM and IgG levels with associated neutropenia have been reported in experimentally infected cats (Omran et al 1997). Detection of Borrelia by culture, cytology or PCR may be difficult, timeconsuming and expensive. The organism is rarely found in blood, urine, joint fluid or CSF, but can be detected in skin and synoviae. Therapeutic protocols have not been specifically described for cats. However, doxycycline, amoxicillin, azithromycin, penicillin, ceftriaxone and cefotaxime are used in human and canine cases and duration of therapy should extend to 30 days (Shaw et al., 2001).

1.10 ANAPLASMOSIS

Anaplasma spp. are vector-transmitted, Gram-negative, obligate intracellular bacteria. In Europe, A. phagocytophilum (formerly Ehrlichia phagocytophila) and A. platys (formerly E. platys) have been reported from domestic dogs. They infect predominantly neutrophil and rarely eosinophil granulocytes (A. phagocytophilum) or platelets (A. platys), respectively, and develop into typical microcolonies (morulae) that are observable by light microscopy (ESCCAP).

Anaplasma phagocytophilum is the agent of Canine granulocytic anaplasmosis (CGA); it can infected dogs, cats, humans, horses, sheep, roe deer, red deer, small rodents, goats, cattle, llamas which are recognized as reservoir and while Ixodes ricinus is recognized as its vector tick in nonfeline hosts (ESCCAP) (Greene). Trans-stadial but not transovarial transmission of A. phagocytophilum occurs in the Ixodes vector. Usually, tick feeding for 24-48 hours is required for the transmission of this agent to susceptible dogs. The incubation period in the mammalian host is 1 to 2 weeks. After endocytosis, A. phagocytophilum develops by binary fission into morulae in the phagosomes predominantly of neutrophils and rarely eosinophils. Cells infected with A. phagocytophilum are found in the circulating blood as well as in the tissues of the mononuclear phagocytic system, such as the spleen, liver and bone marrow (ESCCAP). The geographical distribution of infections with A. phagocytophilum generally correspond to the distribution of their respective (or supposed) tick vectors. With increasing travel of dogs with their owners, infections must also be expected to occur in previously non-endemic areas (ESCCAP). Reports of Anaplasma spp. infections in cats are rare. Of the naturally infected cats with A. phagocytophilum DNA in the blood, all lived in an I. scapularis, I. pacificus or I. ricinus endemic region, all were presumed to be allowed to roam outdoors, and four were known to be infested by Ixodes spp. At the time of inizial examination. Therefore, this genus of ticks can also likely serve as the vector transmitting A. phagocytophilum to cats. C. felis taken from cats in United States, Portugal and Australia had negative test results for Anaplasma spp. DNA. Rodents are commonly infected with A. phagocytophilum; however it is unknown whether ingestion of or direct contact with rodents plays a role in A. phagocytophilum infection in cats (M.R.Lappin and E.B. Breitschewerdt, Greene 4th ed.). Cats with A. phagocytophilum infections suffer from lethargy, anorexia, fever, lymphadenopathy, anaemia and thrombocytopaenia (ESSCAP; Bjoersdorff et al 1999, Shaw 2001, Lappin et al 2001). The diagnosis of Anaplasma spp. Infections is generally based on serology and/or PCR. Antibodies may be detected by indirect immunofluorescent assay (IFA) using A. phagocytophilum antigens. Seroconversion may occur 1-4 weeks after exposure and, thus, cats with acute infections can be serologically negative. Additionally, in endemic areas, positive IFA results may result from a previous infection and may not necessarily be indicative of an acute infection. Generally, two serological tests at an interval of 2-3 weeks need to be carried out to monitor if seroconversion has occurred. A positive result of a single serological test combined with clinical signs is not sufficient evidence for a diagnosis of anaplasmosis. Specific assays for the detection of A. phagocytophilum and A. platys are performed by specialized laboratories. A PCR-positive result generally confirms an infection. A PCR-negative result does not exclude the possibility of an infection. A definitive

diagnosis is made when morulae can be observed in neutrophil (and more rarely eosinophil) granulocytes (A. phagocytophilum) on microscopical examination of blood smears. To increase the diagnostic sensitivity, buffy coat smears should be examined. Positive results should be confirmed by PCR analysis. The treatment of anaplasmosis consists of the administration of antirickettsial agents and symptomatic treatment. Tetracyclines are the most commonly used compounds, with doxycycline at a dosage of 10 mg/kg/day over 3-4 weeks being the most commonly used treatment scheme. With correct treatment, the prognosis of A. phagocytophilum infections is fairly good. The primary measure for the prevention of Anaplasma infections is an effective protection against tick infestations (ESCCAP). Thus, exposure of cats to potential arthropod vectors should be avoided or tick control maintained with acaricidal products that are approved for use on cats (Lappin et al., 2013). A. phagocytophilum can likely be transmitted by blood; therefore, cats used as blood donors should be screened for infection by using serum antibody tests or PCR assays, and positive cats should be excluded as donors. Although A. phagocytophilum is known to infect both people and cats, no association has ever been exhibited between human infection and cat contact. If cats are housed indoor and arthropod control is maintained, the risk to people should be minimal (Lappin eta al., 2013).

Authors	Year	Journal	Number of tested cats	Characteristics of feline population	Methods	Location	Prevalence
ITALY							
W. Tarello	2005	Veterinary Record (2005)156, 772-774	250	cats from Italy with Ehrlichia-like inclusion bodies in their neutrophils	cytological demostration of ehrlichial morulae in neutrophils; characteristic signs of disease; absence of concurret conditions; responce to treatment to doxycyclina	central Italy	15/250 (6%)
EUROPE							
SPAIN							
E AGUIRRE et al	2004	Ann. N.Y. Acad. Sci. 1026: 103–105 (2004)	122	cats	IFA + PCR	central Spain	4.9% IFAT; 0% PCR
Laia Solano-Gallego et al	2006	Veterinary Microbiology 118 (2006) 274–277	168	cats	IFAT+PCR	northeastern Spain	11,30% IFAT; 0% PCR
M D Tabar	2008	Veterinary Parasitology 151 (2008) 332–336	100	cast	PCR	Barcelona area; Spain	1%
T. Ayllo´n et al	2009	Journal	52	client-owned cats	PCR	central Spain	7.7%

		Compilation 15 (2009) (Suppl. 2), 4–5					
Tania Ayllo´n et al	2012	VECTOR-BORNE AND ZOONOTIC DISEASES Volume 12, Number 2, 2012	680	client-owned and stray cats from	IFAT+PCR	Madrid, Spain.	IFAT stray cats: 9.1% (n = 8); client-owned cats 5.7% (n = 49); all cats 57/680 8.4%. PCR: 0%
PORTUGAL							
A. S. Alves et al	2009	Journal Compilation 2009 European Society of Clinical Microbiology and Infectious Diseases, CMI, 15 (Suppl. 2), 1–3	18 fleas from Fifty-one cats (domestic, shelter and stray)	Fifty-one cats (domestic, shelter and stray) from Lisbon and E´vora	PCR	PORTUGAL	0%
IRLANDA							
F Juvet et al	2010	Journal of Feline Medicine and Surgery (2010) 12, 476e482	116 whole blood	41 client-owned and 75 stray cats	PCR	IRLANDA	0%
UNITED KINGDOM							
S. E. Shaw et al	2005	Veterinary Record (2005)157, 645-648	60 cats	ill owned cats	PCR	UNITED KINGDOM	1.6%

AD Bennett et al	2011	Journal of Feline Medicine and Surgery (2011) 13, 553e557	78	78 cats were entered into the study. Of the cats, 61 were client-owned and 17 were from rescue centres; 40 were clinically ill and 38 were considered healthy	PCR	Scotland	0%
AFRICA							
R Lobetti and M R Lappin	2012	Journal of Feline Medicine and Surgery 14 (2012) 857–862	102		PCR	South Africa	0%
USA							
J M Eberhardt et al	2006	Journal of Feline Medicine and Surgery (2006) 8, 164e168	112 cats	feral and relinquished cats in Phoenix and Nogales, Arizona	PCR	USA	0%
M R Lappin et al	2006	Journal of Feline Medicine and Surgery (2006) 8, 85e90	92 whole blood semples	cats brought to Veterinarians in Alabama (BG and AR), Maryland (Brunt), and Texas (Burney)	PCR	USA	0%
Sarah A. Billeter et al	2007	Veterinary Parasitology 147 (2007) 194–198	460	domestic felines	IFA at a 1:50; PCR	United States	IFAT 4.3%; PCR 0%
A M Ishak et al	2007	Journal of Feline Medicine and Surgery (2007) 9, 1e7	89 cats with anemia; 87 healthy cats	the blood of cats with anemia and a control group of healthy cats	PCR	USA	0%

AUSTRALIA							
VR Barrs et al	2010	Australian Veterinary Journal Volume 88, No 5, May 2010	111 cats		PCR	easthern Australia	0%

1.11 HAEMOTROPIC MICOPLASMAS INFECTIONS

The haemotropic mycoplasmas ('haemoplasmas') are mycoplasmal bacteria that infect erythrocytes, attaching to the red blood cell surface (S. Tasker). These species, including Haemobartonella felis, renamed Mycoplasma haemofelis and two new feline species: 'Candidatus Mycoplasma haemominutum' and 'CandidatusMycoplasma turicensis'. One recent study from the USA suggested that cats could be further infected with an organism similar to one of the canine haemoplasmas called 'Candidatus Mycoplasma haematoparvum' (Sykes et al., 2007). The feline haemoplasma species that are currently recognised vary in pathogenicity, with some isolates consistently inducing haemolytic anaemia whereas others result in few noticeable clinical signs. However, it is not only the infecting species or isolate that influences clinical signs — duration of infection and host factors, such as the presence of concurrent disease or retrovirus status, can also play a part. Age, too, may be important in that it has been suggested that younger cats are more susceptible to clinical haemoplasmosis (Sykes et al., 2008; Tasker S et al., 2009). Other factors such as infecting organism dose or route of infection may also impact on outcome. Studies evaluating risk factors for haemoplasma infection have generally found that older male cats, with outdoor access, are more likely to be infected. The increased prevalence in male cats, together with reports that cat bite abscesses and outdoor roaming are risk factors, have led to the suggestion that horizontal transmission may occur via fighting. In line with this both 'Candidatus M haemominutum' and 'Candidatus M turicensis' DNA has been found in the saliva of infected cats (Willi et al., 2006;.Dean et al., 2008). However, recent studies failed to show the successful transmission of 'Candidatus M turicensis' infection between cats by the oral or subcutaneous inoculation of 'Candidatus M turicensis'-infected saliva, even though the same dose of 'CandidatusM turicensis' in blood led to successful transmission when inoculated subcutaneously (Museux et al., 2009). More work in this field is required but it seems probable that haemoplasma transmission via social contact is unlikely but that aggressive interaction (eg, cat bites) may result in transmission if the recipient cat is exposed to infectious blood rather than just saliva. The cat flea Ctenocephalides felis has been incriminated in the transmission of haemoplasmosis between cats although only transient transmission of M haemofelis to a cat via the haematophagous activity of Ct felis was demonstrated (Woods 2005). Studies have found evidence of feline haemoplasma infection in fleas collected from cats (Kamrani et al., 2008; ,Shaw et al., 2004; Lappin et al., 2006; Hornok et al., 20109and in some ticks (Willi et al., 2007; Tauroura et al., 2005).. Additionally, in one US study (Hackett et al., 2006), community-sourced cats that were housed indoors without a history of ticks or fleas were less likely to have haemoplasma infections than those that had access outdoors or had a history of ticks or fleas. However , direct evidence for

the role of vectors in natural/field haemoplasma transmission between cats is lacking. It has been certainly seen haemoplasma transmission between cats in the absence of vectors so, even if fleas or ticks have the ability to act as vectors for haemoplasmosis in cats, other means of haemoplasma transmission must exist (Tasker et al.,2010).

Transmission via contaminated blood transfusions has been reported (Willi et al., 2006),4 and the use of freshly collected blood from a haemoplasma-infected blood donor for transfusion would very likely result in transmission of infection to the recipient cat. The risk of haemoplasma transmission when using stored blood for transfusions depends on the viability of haemoplasmas in stored blood. Other possible modes of haemoplasma transmission include vertical transmission from queen to kittens during pregnancy, at birth or via lactation. Although not reported, transmission may also be possible via the use of multi-use vials between cats, or the inappropriate use of the same equipment (eg, surgical instruments) on different cats without adequate cleaning/sterilisation, particularly if blood contamination was significant and only a short time elapsed between consecutive procedures (Tasker et al., 2010). Most of the haemolysis associated with haemoplasma infection is believed to be extravascular in nature, occurring in the spleen and liver especially, but also in the lungs and bone marrow. Intravascular haemolysis has also been reported, as has increased osmotic fragility of haemoplasma-infected red blood cells (Willi et al., 2005) Positive Coombs' tests and autoagglutination, indicating the presence of erythrocyte-bound antibodies, have been demonstrated in anaemic M haemofelis-infected cats (Tasker et al., 2009) . Such erythrocyte-bound antibodies could be responsible for immune-mediated destruction of red blood cells. in most cats the erythrocyte-bound antibodies appeared only after anaemia had started to develop. The absence of erythrocyte-bound antibodies at the onset of development of anaemia could reflect a problem with the sensitivity of detection of erythrocyte-bound antibodies; however, an alternative explanation is that erythrocyte-bound antibodies appear as a result of haemoplasma-induced haemolysis, rather than initiating the haemolysis. Indeed it has been documented disappearance of these antibodies with antibiotic and supportive treatment alone, without the need for specific glucocorticoid treatment, questioning the key role of such antibodies in haemoplasma-induced anaemia. the clinical signs of haemoplasma infection depend on factors such as the haemoplasma species involved, stage of infection and whether concurrent diseases or infections are present. Common signs exhibited by acutely ill cats include pallor, lethargy, anorexia, weight loss, depression and dehydration. Intermittent pyrexia is often seen, particularly in the acute stage disease, as is splenomegaly, which may reflect extramedullary haematopoiesis. Jaundice is uncommon unless severe acute haemolysis occurs. Cats that recover from haemoplasma infection may remain

chronically infected (Tasker et al., 2010). Long-term carrier status appears to be especially common following 'Candidatus M haemominutum' infection, although suspected clearance of this infection has been reported with and without antibiotic treatment (Willi et al., 2006). M. haemofelis-infected cats may spontaneously clear infection from peripheral blood after infection without antibiotic treatment, and such clearance has also been reported with 'Candidatus M. turicensis' infection. In any haemoplasma-infected carrier cat the potential for reactivation of infection exists, which can result in clinical disease (Foley et al., 1998), although this seems to be quite rare once haemoplasma infection has become established beyond acute infection (Tasker et al., 2010). When anaemia is induced by haemoplasma infection, it is typically regenerative (or pre-regenerative if sampling occurs very soon after the onset of anaemia), macrocytic, and normo- or hypochromic. However, significant reticulocytosis is not always present, even when pre-regenerative cases are excluded, possibly due to concurrent retroviral infections or other disease processes. Release of sequestered erythrocytes from the spleen may also result in a marked rise in red blood cell count without an accompanying reticulocytosis (Tasker et al., 2010). Serum biochemistry may reveal hyperproteinaemia due to dehydration or an acute phase response, and increased liver enzyme levels may arise from hepatic hypoxic damage. Hyperbilirubinaemia can result from the haemolysis (Tasker et al., 2009). Diagnosis of haemoplasma infection used to rely on cytological examination of a Romanowsky-stained blood smear, with organisms appearing on the surface of erythrocytes as rounded bodies singly, in pairs, or occasionally in chains. Diff-Quik or filtered Giemsa stains can be used. However, cytological diagnosis has poor sensitivity and specificity. many cases diagnosed as being haemoplasma-infected on the basis of blood smear interpretation in practice have been false positives, with stain precipitate, Howell-Jolly bodies and artefacts due to slow blood smear drying being the most common reasons for error. Additionally cytology cannot differentiate between haemoplasma species (Tasker et al., 2010). Polymerase chain reaction is known to be more sensitive than cytology for haemoplasma detection. (S. Tasker et al., 2010)Quantitative PCR assays exist that amplify and differentiate all three feline haemoplasma species. Only a small amount of blood is required for PCR. Most diagnostic laboratories request 1 ml of anti-coagulated blood, although in reality PCR is usually performed using DNA extracted from smaller volumes of blood (0.1 or 0.2 ml). EDTA-anticoagulated blood is usually required since heparin inhibits PCR. DNA is quite stable within blood samples so rapid or cold shipping of samples is not required. Blood sampling for PCR should preferably be undertaken before a cat undergoes any antibiotic treatment (Tasker et al., 2010). Following recovery from acute clinical disease, cats can remain chronically infected. In some cases this chronic carrier status is lifelong, whereas in others anti biotic therapy or

spontaneous elimination of the organism results in apparent clearance of infection. Cats usually remain PCR positive during chronic carrier status. Up to 10% of healthy cats at any time are infected with feline haemoplasmas (Kamrani et al., 2008; Tasker et al., 2003), emphasising that a positive PCR result does not always correlate with the presence of clinical haemoplasmal disease. This must be considered when interpreting haemoplasma PCR results. If haemoplasma infection is diagnosed in a cat showing clinical signs then treatment should be instigated, ideally to clear infection but certainly to induce a clinical response. Treatment is also indicated in cases with concurrent infections or diseases that could exacerbate the potential for disease due to haemoplasma infection (eg, in an immunosuppressed FIV-infected cat). In cases in which treatment is instigated, monitoring can be performed so that treatment efficacy can be assessed. Tetracycline and fluoroquinolone antibiotics are effective at reducing haemoplasma organism numbers in the blood, although consistent predictable elimination of haemoplasma infection has not been shown in any study.(Tasker et al., 2010) However, antibiotics usually do improve clinical signs and haematological abnormalities associated with haemoplasma infection, even if elimination of haemoplasma infection does not always result. Cats clinically ill with haemoplasmosis are often profoundly dehydrated, so correction of this with intravenous fluid therapy is important. The degree of anaemia in haemoplasmosis cases may be masked by the dehydration causing haemoconcentration, so haematological parameters must be monitored after correction of dehydration. Encouraging food intake (eg, offering tempting warmed food, hand feeding) is important in inappetent cats. Although cats are often able to tolerate anaemia quite well, if the anaemia is severe (PCV <12%) and/or has developed acutely and is accompanied by significant associated clinical signs, a blood transfusion. Tetracyclines, such as oxytetracycline at a dosage of 22 mg/kg q8h PO (Tasker et al., 2002) , are usually effective in the treatment of haemoplasmosis. Doxycycline (10 mg/kg q24h PO) is usually the preferred tetracycline for the treatment of feline hemoplasmosis, with prolonged treatment courses (up to 8 weeks) recommended to increase the chance of eliminating infection (Tasker et al., 2004). Evidence exists for doxycycline efficacy against all three feline haemoplasma species, although controlled studies have only been performed for M haemofelis (Dowers et al., 2002; Tasker et al., 2004; Dowers et al., 2009). Fluoroquinolones are also often an effective treatment for haemoplasmosis. Enrofloxacin (5 mg/kg q24h PO) has been successfully used to treat M haemofelis infection in controlled studies. Marbofloxacin has also been effective at reducing M haemofelis and 'Candidatus M haemominutum' organism numbers in the blood of treated cats in controlled studies, using the recommended UK dosage of 2 mg/kg q24h PO, although the fall in 'Candidatus M haemominutum' organism numbers was less pronounced

than that of M haemofelis Tasker et al., 2006).The efficacy of 14 days of pradofloxacin treatment of experimental M haemofelis infection has recently been reported (Dowers et al., 2009), where it was found to be effective (at the standard 5 mg/kg q24h PO dose, as well as a higher dose of 10 mg/kg q24h PO) at improving clinical signs and haematological values, and lowering M haemofelis copy numbers in the blood. It has been reported the anaemia induced by haemoplasma infection is in part immune-mediated and so corticosteroids have been recommended as adjunct therapy (VanSteenhouse et al., 1993).However, the value of corticosteroids in the treatment of haemoplasmosis has not been proven and, as mentioned earlier, anaemic Coombs'-positive cats infected with M haemofelis respond to antibiotic treatment alone (Tasker et al., 2009), which calls into question the need for immunosuppressive treatment. Indeed in some cases concurrent corticosteroid therapy has been associated with a delay in obtaining apparent haemoplasma clearance from the blood. It has been shown that a 'Candidatus M turicensis' infected cat that received methylprednisolone acetate prior to infection developed a more severe anaemia than an immunocompetent cat (Willi et al., 2005), so corticosteroids have the potential to exacerbate disease too. Corticosteroid treatment is recommended only considering corticosteroid treatment in cases that are deteriorating despite administration of an appropriate antibiotic and in which immunemediated haemolytic anaemia (IMHA) is thought to be a major component of the disease, or in cases in which haemoplasma infection is not strongly believed to be the underlying cause of disease in cases that are deteriorating despite administration of an appropriate antibiotic and in which immunemediated haemolytic anaemia (IMHA) is thought to be a major component of the disease, or in cases in which haemoplasma infection is not strongly believed to be the underlying cause of disease.

Recently, PCR has been used to show evidence of haemoplasma infection in humans. PCR has documented the presence of an organism very similar to M suis in people in China, although clinical haemoplasmosis was not common. (Yuan et al., 2007; Yuan et al., 2009). In another study, a M haemofelis-like organism was detected in an immunosuppressed HIV-positive patient who had concurrent bartonellosis and a history of cat scratches (Santos et al., 200). However, the contribution of the M haemofelis-like infection to the man's clinical signs was not discussed. These reports certainly suggest the existence of zoonotic haemoplasma infections, which will need to be further investigated to clearly define the epidemiology of human haemoplasmosis.

1.12 CYTAUXZOONOSIS

Cytauxzoonosis is a tick-transmitted protozoal disease caused by Cytauxzoon felis affecting wild and domestic felids (Meinkoth and Kocan, 2005). It was

described in several south central, south eastern (Meinkoth and Kocan, 2005), and mid-Atlantic states of the USA (Birkenheuer et al., 2006). The presumed main reservoir host of this infection appears to be the wild felid bobcat (Lynx rufus) (Kocan et al., 1985). Ticks are considered the vector and transmission of C. felis by Dermacentor variabilis (Blouin et al., 1984) and Amblyomma americanum (Reichard et al., 2010) has been demonstrated experimentally. C felis has developmental stages in both macrophages and erythrocytes of infected cats. In experimentally infected cats, proliferation of organisms in mononuclear phagocytic cells particularly in the lungs, results in marked vascular damage and occlusion, release of inflammatory mediators and DIC. The intra-erythrocytic stage induces both intravascular and extravascular haemolysis (Hoover et al., 1994). This produces a rapidly progressive syndrome in most cats characterized by acute fever, haemolytic anaemia, lymphadenomegaly, anorexia, dyspnoea, and in severe cases, poor peripheral perfusion followed by hypothermia, coma and death (Shaw et al., 2001). The most common clinicopathological findings are anemia, depression, anorexia, vomiting, icterus, splenomegaly, hepatomegaly and high fever (Birkenheuer et al., 2006). Hypothermia typically develops just prior to the death (Greene et al., 2006). Historically, it was thought that the disease was always fatal in domestic cats. However, survival after infection (Walker and Cowell, 1995) and persistent blood parasitemia without clinical illness (Brown et al., 2008, 2010) have been documented in a few cases. In contrast, wild felids rarely manifest clinical illness and generally develop a subclinical erythroparasitemia (Meinkoth and Kocan, 2005). Nevertheless, occasional cases of fatal cytauxzoonosis, with clinical signs and large schizont-filled macrophageswithin blood vessels, have been reported also in wild felids (Carli et al., 2012; Garner et al., 1996; Nietfeld and Pollock, 2002; Peixoto et al., 2007). Interestingly, in the last years, other species of Cytauxzoon infecting wild and domestic felids have sporadically been described. A new species of Cytauxzoon named Cytauxzoon manul was molecularly characterized from a Pallas' cat imported into Oklahoma from Mongolia (Ketz-Riley et al., 2003; Reichard et al., 2005). Moreover, molecular recognition of a Cytauxzoon-like parasite was documented in a domestic cat (Criado-Fornelio et al., 2004) and Iberian Lynx from Spain (Luaces et al., 2005; Millán et al., 2007, 2009). Recently, Hepatozoon canis and Cytauxzoon sp. co-infection has been described in a cat from France (Criado-Fornelio et al., 2009) and a clinical cytauxzoonosis has been described in three cats from Trieste (Italy) (Carli et all 2012). Unfortunately, there is paucity of information about epidemiological and clinic-pathological aspects of infection by species of Cytauxzoon other than C. felis. Diagnosis is made by demonstration of intra-erythrocytic organisms with appropriate morphology in blood, lymph node, bone marrow or splenic aspirates in combination with compatible clinical signs (Shaws et al. 2001) and by PCR on the same samples

(Carli et all 2012). Anti-protozoal therapy using imidocarb diproprionate or diminazene aceturate is recommended. A single injection provides sufficient residual activity in the majority of cases. Supportive fluid therapy is essential and DIC should be managed using heparin. Broad spectrum antibiotic therapy with enrofloxacin or cephalosporins is also recommended (Shaw et al., 2001).

1.13 BABESIOSIS

Babesia species are tick-borne apicomplexan parasites of erythrocytes that infect a variety of domestic and wild animals, and humans. Babesiosis in domestic cats is a more rare clinical infection in comparison with its canine counterpart. Clinical domestic feline babesiosis has mostly been reported from South Africa where infection is mainly due to Babesia felis, a small Babesia that causes anemia and icterus (Penzhorn et al., 2004; Schoeman et al., 2001). B. felis also infects African wild felids including lions, cheetahs and servals (Bosman et al., 2007). Other reports of domestic feline babesiosis have mostly been sporadic. Babesia cati was reported from a cat in India (Mudaliar et al., 1950) and a few cases of infection in domestic cats by unnamed Babesia parasites were reported in France, Germany, Thailand and Zimbabwe (Bourdeau, 1996; Jittapalapong and Jansawan, 1993; Moik and Gothe, 1997; Stewart et al., 1980). A large form Babesia, B. canis presentii, was described in cats from Israel (Baneth et al., 2004). Interestingly, the presence of Babesia species typical to dogs in domestic cats is detected sporadically by molecular techniques often without compelling evidence of clinical infection. Molecular evidence for infection by B. canis and B. microti-like piroplasmid in cats was provided in a study from Spain and Portugal (Criado-Fornelio et al., 2003b). In addition, B. vogeli has been identified by blood smear examination and PCR in stray cats from metropolitan Bangkok, Thailand (Simking et al., 2010). The naturaltransmission of babesiae to vertebrate hosts occurs through the bite of a vector tick. Although information on some of the tick vectors for canine babesial species is available, very litle is known regarding the tick transmission of feline babesial species (Solano-Gallego, et al.,). Some species of Babesia including B. canis have been shown to be infective to animals only 2–3 days after tick attachment (Schein et al., 1979). Babesia spp. are generally highly host-specific with regard to both the transmitting tick species and the mammalian host. After being ingested with a blood meal, Babesia stages penetrate the gut epithelium of the tick, multiply and migrate to different organs including the tick ovary and salivary glands. Transovarial transmission from infected adult female ticks to their progeny occurs with large Babesia spp. And thus their larvae (“seed ticks”) can be an important source of infection. Female Dermacentor spp. generally require a period of initial feeding before Babesia sporozoites are available for transmission within their saliva to the dog; in male ticks, transmission may be more rapid as they repeatedly feed taking only small

amounts of blood, they perform co-feeding with females and possibly feed from several different hosts. Sporozoites infect erythrocytes where they differentiate into merozoites and divide by binary fission eventually causing cell lysis (ESCCAP). The pathology that Babesia inflicts in the host varies considerably with the different species and sub-species involved, and also with the host's individual immune status, age, concurrent infections or illness and response to infection (Irwin, 2009). Haemolytic anemia and systemic inflammatory response syndrome leading to multipleorgan dysfunction syndrome account for most of the clinical signs observed in canine and feline babesiosis (Taboada and Lobetti, 2006). In general, Babesia species cause a haemolytic anemia which is multifactorial and is the predominant clinical manifestation inducing a number of immune responses that may have a devastating influence (Ayoob et al., 2010a). Haemolytic anemia can occur due to direct red blood cell lysis by replicating intracellular parasites which cause a combination of intravascular and extravascular haemolysis. A number of mechanisms are involved with red blood cell destruction. These include the binding of antibodies to cell surface and complement activation (, production of serum haemolytic factors, erythrocyte oxidative damage and increased red blood cell phagocytosis, creation of spherocytes, and a decrease in the osmotic fragility of red blood cells (Carli et al., 2011). Intense haemolysis results in haemoglobinemia, haemoglobinuria, bilirubinemia and bilirubinuria. Thrombocytopenia alone is observed in many cases of babesiosis andmay relate to immune, splenic sequestration or coagulatory consumption of platelets from haemolytic or vascular injury. 006). Tissue hypoxia is an important contributor to many of the clinical signs caused by the most Babesia spp.(Solano-Gallego et al., 2011). Babesia infection in cats is associated with anorexia, lethargy, anemia and icterus. Information on the clinical manifestations of domestic feline babesiosis is limited mostly to publications on B. felis infection in South Africa (Ayoob et al., 2010b; Penzhorn et al., 2004; Schoeman et al., 2001). In a study on B. felis that included 56 cats (Schoeman et al., 2001), 80% were less than 3 years old and there was no specific breed or gender predilection. Most cats were anorectic and lethargic. Macrocytic hypochromic regenerative anemia was present in the majority of infected cats. Hyperbilirubinemia was present in 86% of the cats and alanine aminotransferase activity was elevated in 89%. Thirty two percent of the cats were concurrently infected with feline leukemia virus (FeLV) and 14% with feline immunodeficiency virus (FIV). Babesia canis presentii infection in a cat from Israel co-infected with FIV and Candidatus Mycoplasma haemominutum was accompanied by fever, icterus, moderate anemia and thrombocytopenia which resolved following anti-babesial therapy (Baneth et al., 2004). The only systemic abnormality recordedina catwith B. cati infection in India was fever (Mudaliar et al., 1950). Diagnosis is made by demonstration of intraerythrocytic protozoal

organisms with appropriate morphology in blood, lymph node, bone marrow or splenic aspirates in combination with compatible clinical signs. Sensitivity is limited by low-level parasitaemia in chronic cases or where prevalence is low (Shaw et al., 2001). The polymerase chain reaction (PCR) is a sensitive and specific diagnostic techniquewhich is frequently employed for the diagnosis of babesiosis. It is particularly useful for detection of infection in animals with a low parasitaemia levels and for speciation of parasites (Solano-Gallego et al., 2011. No serological tests are routinely available for cats (Shaw). The drug most commonly recommended for treatment of babesiosis in cats caused by B. felis is the antimalarial, primiquine phosphate at the dosage of 0.5 mg/kg PO sid for 3 days (more than 1 mg/kg is lethal in cast) ; the response to the treatment is usually good but there can be occasional to frequent relapses (Ayoob et al. 2010b, Penzhorn et al. 2004); feline babesiosis caused by B. canis presentii can be treated with Imidocarb dipropionate (2.5–3mg/kg IM) with a good response (Baneth et al. 2004). Blood transfusions are rarely required (Shaw et al., 2001).

1.14 LEISHMANIOSIS

Leishmaniosis is caused by an intracellular protozoan parasite of the genus Leishmania and is transmitted by sandflies. L. infantum infection is common in dogs in endemic areas of Europe, the Middle East and many tropical and subtropical areas of the world and causes serious systemic and cutaneous disease in susceptible animals (Shaw et al., 2001). In contrast to dogs, natural infection and clinical disease in domestic cats caused by Leishmania species appear to be rare. Clinical disease and subclinical infections have been reported sporadically in the following regions and countries where canine leishmaniosis is endemic: southern Europe; North Africa, Iraq; Iran; central and South America (Solano-Gallego and G. Baneth 2013). Whether the low prevalence of infection/disease in endemic areas is due to under-reporting or to the fact that cats have a high degree of natural resistance is unknown. However, cases of feline systemic clinical disease and asymptomatic infection due to L infantum and other species are reported (Passos et al., 1996, Ozon et al., 1998, Hervas et al., 1999) and wild cats have been incriminated as reservoirs for leishmaniosis in endemic Mediterranean countries (Shaw et al., 2011). Clinical leishmaniosis in domestic cats has been reported to involve L. infantum in Europe and Brazil; L. Mexicana in Texas; L. venezuelensis in Venezuela, and L. braziliensis and L. amazonensis in Braszil (Solano-Gallego and G. Baneth, 2013). Animals living in an endemic area can develop specific antibodies against leishmania and they can be evidentiated by means of IFAT. The antibody titers appear to be lower in affected cats than in dogs, even if the number of clinical cases is very scanty (Mancianti F 2004, Barnes et al 1993). Serologic and PCR surveys of cat

population in canine leishmaniosis-endemic regions in southern Europe and Middle East indicate that feline infection is more widespread than clinical disease. Epidemiologic studies have described seroprevalence rates ranging from 0,9% to 30%. The true role of cat as reservoir of L. infantum infaction remains unclear, even if transmission of L. infantum to sandflies (P. perniciosus) from chronically infected cat under experimental condition has been reported (Solano-Gallego and G. Baneth, et al., 2013). Cutaneous leishmaniasis is the more frequent form in cats and it was reported from several countries. Typical signs include nodular to ulcer or crusty lesions on the nose, lips, ears, eyelids, diffuse areas of alopecia and granulomatous dermatitis: clinical signs of cutaneous FL are unspecific and in endemic area this infection must be taken into account (Mancianti F 2004, Barnes et al 1993) . Visceral leishmaniasis with involvement of spleen, liver, bone marrow, lymph node, uvea, and kidney have been less commonly described and are thought to be associated with immunosuppression caused by leukemia virus and immunodeficiency virus infections or other concomitant diseases (Solano-Gallego et al.,2013): liver and spleen are interested, with lymph nodes and kidney (Mancianti F.2004) in association with jaundice, vomiting, hepatomegaly, splenomegaly, lymphadenomegaly, membranous glomerulonephritis and granulomatous gastroenteritis (Ozon et al 1998).As in dogs, hyperproteineimia with hypergammaglobulinemia is a common finding in feline leishmaniosis. (L. Solano-Gallego et al.,2013) Untreated affected cats can frequently die. Some of the affected cats were FIV and/or FeLV positive and these viroses such as stress may induce an impaired cellular immune response. However the resistance of the cat to leishmania infection probably depends on genetic factors, not strictly related to cell mediated immunity (Mancianti F. 2004). Presumptive diagnosis is most commonly made by the demonstration of protozoal organisms in tissue biopsy specimens using both light and electron microscopic examination or by the demonstration of specific antibodies against leishmania using IHAT, Western blot or IFAT (Mancianti F 2004, Shaw et al. 2001). PCR on blood samples or better on bone marrow or lynph node aspirates offers a sensitive and specific diagnostic tool for this disease (Mancianti F 2004, Shaw et al, 2001,. Solano-Gallego et al., 2013).

Very little published information is available on drug therapy for feline lieshmaniasis. One cat in Spain with primarily cutaneous lesions was treated with 5 mg/kg of meglumine antimoniate subcutaneously combined with 10 mg/kg ketoconazole orally. A 4-week course of combine therapy was repeated three times, with 10 days without therapy between each course, and resulted in the resolution of cutaneous lesions. Successful treatment of cats with clinical improvement was achieved in the majority of cases with 5 to 10 mg/kg allopurinol, given twice daily. Cats with disseminated of infection required longer allopurinol treatment of at least 6 months in comparison to the shorter course

reported beneficial for cats with only cutaneous lesions. Topical treatment of cutaneous L. Mexicana infection in a cat with clotrimazole and subsequently with paromomycin did not improve the dermal lesions (Solano-Gallegoet al. 2013).

Authors	Year	Journal	Number of tested cats	Characteristics of feline population	Methods	Location	Prevalence	Notes
ITALY								
Poli et al	2002	Veterinary Parasitology 106 (2002) 181–191	110	Owned apparently healthy cats submitted to practitioners for vaccination program	IFAT	Italy (Liguria and Tuscany)	1/110 (0.9%)	Titre 1:160
Vita et al	2005	Veterinary Research Communications, 29(Suppl. 2) (2005) 319–321	203		IFAT	central Italy (Abruzzo)	33 of 203 cats tested (16.3%);	all samples were initially tested at a dilution of 1:40; 20 of these 33 1 a 40; ten cats a titer of 1:80, two cats a titer of 1:160, and one a titer of 1:320;
Pennisi	2004	Veterinary Research Communications, 28 (2004) 363–366	4	Four domestic cats with dermatological signs	microscopic (smears from cutaneous ulcers and cysts, and from lymph node needle-aspiration) and serological investigations (IFAT)	Italy (Messina district)	4/4; 100%	
Pennisi et al	1998	Proceeding SiSVet	93	57 FIV+ stray and owned cats and 36 FIV- stray cats	IFAT	Sicily	55/93 (59%) 40/57 FIV+ (70%) and 14/36 FIV-	Statistical association between FIV+ and FeL + and FIV+ and value of L. infatum IFAT (higher)

Pennisi et al	2000	Proceeding Sisvet	89	Owned cats	IFAT + PCR on blood	Sicily	54/89 PCR+ (61%), IFAT+ 68%	
Boari et al		Proceeding Terrasini	288	Mainly stray cats	IFAT	Abruzzo	36/288 (12.5%)	
EUROPE								
SPAIN								
Solano-Gallego et al	2007	Am. J. Trop. Med. Hyg., 76(4), 2007, pp. 676–680	455	445 cats living in ecoregions around the Northwestern Mediterranean basin; Barcelona (N 390), Tarragona (N 12), and the island of Mallorca (N 43). Clinical veterinarians collected all samples. Samples from Barcelona had two different origins: 255 stray cats from the animal pound of Barcelona and 135 cats examined at the Veterinary Teaching Hospital of the Universitat Autònoma de Barcelona.	ELISA+WB	Spain (Northeastern Iberian Peninsula and Balearic Islands: Barcelona, Tarragona, Mallorca)	5.3-6.3% Seroprevalence by ELISA-prot A was 6.29%, and that by ELISA-IgG was 5.25%. There were also 13.03% and 6.56% of uncertain results for ELISAprot A and ELISA-IgG, respectively	

Martin-Sanchez et al	2007	Veterinary Parasitology 145 (2007) 267–273	183 (+28 control cats from non endemic area)	Owned cats; 183 cats from endemic area Southern Spain; 28 cats from the North of France (Strasbourg area)	IFAT+PCR on blood. In PCR positive cats in which it was possible to obtain another sample of blood, bone marrow and/or lymph node aspirate in a period of 15–20 days, this was cultured and Giemsa stained	Southern Spain	47/183 (25.7%) PCR IFAT: 60.0% Ab titers > 10; 28.3% with ab titer ≥40; 6.1% of cats presented an Ab titers > o = 160: 8 cats had an antibody titer = 160, 1 with Ab = 640, 1 with Ab = 1280, and 1with Ab = 5120	Seropositive with Ab titre ≥10. The highest proportion of positive PCRs (31.6%) was observed in cats with an Ab titer=20. By the contrast, the population with a high Ab titer (≥160) is the one that present the smallest proportion of positive PCRs (18.2%); 7 cats with a positive PCR were tested by culture and microscopic examination of Giemsa-stained samples. In 3 of them, amastigotes were observed in the stains. No culture was positive.
Ayllon et al	2012	VECTOR-BORNE AND ZOONOTIC DISEASES Volume 12, Number 2, 2012	680	680 (539 (79.3% of total) client-owned cats ; 141 (20.7% of total) stray cats from Madrid area)	IFAT+PCR on blood	Central Spain (Madrid)	IFAT: stray cats 6.4% (n = 9); client-owned cats 3%(n = 16); all cats 25/680 3.7%. PCR: 0.6%; 4 /680; 3 were client-owned cats, and 1 was a stray cat	IFA (1:50)
T Ayllon et al	2008	Animal Biodiversity and Emerging	233	cats attended at the Veterinary Teaching Hospital in Madrid	IFAT+PCR	Central Spain (Madrid)	1.29% 3/233 with a titer > 1:100; 2 of	IFAT (1:100 cut-of titer)

		Diseases: Ann. N.Y. Acad. Sci. 1149: 361–364 (2008)		for different medical or surgical reasons			3 seropositive cats had an antibody titer = 1:400, 1 had an antibody titer = 1:100. 7 cats had a titer = 1:50. Considering all the positive cats the prevalence was 4.29%. PCR: 1/233 (0.43%)	
M D Tabar et al	2008	Veterinary Parasitology 151 (2008) 332–336	100	cats	PCR	Barcelona area; Spain	3%	
K Sherry et al	2011	VECTOR-BORNE AND ZOONOTIC DISEASES Volume 11, Number 3, 2011	105	cats living outdoors in two shelters	ELISA	Spain (Ibiza)	13.2%	Sera were tested for IgG antibodies against L. infantum, Toxoplasma gondii, and feline immunodeficiency virus (FIV) and for the detection of feline leukemia virus (FeLV) p27 antigen by enzymelinked immunosorbent assay (ELISA)
PORTUGAL								
Duarte et al	2010	Journal of Feline	180	Stray cats	IFAT	Lisbon	0.6%	Cut-off 1/40

		Medicine and Surgery (2010) 12, 441-446						
Maia et al	2008	VECTOR-BORNE AND ZOONOTIC DISEASES Volume 8, Number 4, 2008; 555-559	23	Stray cats	PCR on blood (IFAT on 20 cats)	Lisbon	7/23 (30.4%) PCR 4/20 IFAT. 3 cats presented an IFAT titer of 1:2 and 1 cat presented a titer of 1:16. In 3 of these 4 cats, PCR and real-time PCR were positive.	The serum obtained from each animal was diluted from 1/2 to 1/64.
Cardoso et al	2010	Veterinary Parasitology 174 (2010) 37–42	316	Domestic cats	DAT+ELISA	Northern Portugal	6 positive to DAT; 9 positive to ELISA; The overall Seroprevalence was 2.8%	
Maia et al	2010	Veterinary Parasitology 174 (2010) 336–340	142	130 client-owned cats+12 stray cats	IFAT+PCR on blood	the Lisbon metropolitan area, Portugal	20.3% PCR (28/138) 1/76 IFAT	The serum obtained from each animal was diluted from 1/2 to 1/256
FRANCE								
Bez	1992	PHD tesis Ecole Vet	174		IFAT		0.6%	

		Lyon						
Marechal	1993	PHD tesis Ecole Vet Lyon	110		IFAT, WB, ELISA		2.7%	
GREECE								
Diakou et al	2009	Journal of Feline Medicine and Surgery (2009) 11, 728e730	284	stray adult cats, living in the major area of Thessaloniki (Northern Greece)	IgG ELISA	the major area of Thessaloniki, Northern Greece	11 (3.87%)	
AFRICA								
Michael et al	1982	J Egypt Soc Parasitol. 1982 Jun; 12(1):283-6.	80	Stray cats	IHA	Egypt	3.75%	
Morsy et al	1988	J Egyptian Soc Parasitol	28	Stray cats	IHA	Egypt	3.6%	
Morsy and El Seoud	1994	J Egypt Soc Parasitol. 1994 Apr; 24(1):199-204.	60	pet and stray cats	IHA	Egypt	3.3%	Microscopic confirmation
SOUTH AMERICA								
Simoes-Mattos et al	2001	Ciencia Animal	84	Stray cats	ELISA	Brazil	10.7%	
Vides et al	2011	Veterinary Parasitology 178 (2011) 22–28	55	55 cats with dermatologic lesions from two major animal shelters of Aracatuba, Southeastern Brazil	Citology, ELISA, IFAT, PCR; immunohisto chemistry (IHC) in skin lesions of all cats	Aracatuba, Southeastern Brazil	VL was diagnosed in 27/55 (49.1%) cats with dermatological problems. 10/27 (37.0%) cats (citology of lymphoid); 14/27 (51.9%) ELISA;	

							6/27 (22.2%) IFAT; 5/27 (18.5%) both IFAT and ELISA; IHC positive 9/27 (33.3%) cats; 5/27 (18.5%) were positive only for IHC; 3/52 (5.76%) positive both IHC and PCR on tissue	
Longoni et al	2012	Comparative immunol microbiol infect diseases	95	Stray cats	ELISA+WB	Mexico		
Dourado-Coehlo et al	2011	Parasitol Res (2011) 109:1009–1013	70	Cats form urban and rural area	ELISA+IFAT	Andradina Municipality, São Paulo State, Brazil	4.2% ELISA 0% IFAT	*Leishmania chagasi*
Costa et al	2010	Brazilian Journal of Veterinary Research and Animal Science **Volume:** 47 **Issue:** 3 **Pages:** 213-	200	cats	Citology+ELISA	Brazil	14.5% (31/200) of the feline population studied, with 4% (8/200) of positivity by parasitological diagnosis and 11.5% (23/200) by serology	*L. infantum chagasi*

		217						
Coehlo et al	2011	Veterinary Parasitology 176 (2011) 281–282	52	cats sent by their owners to the Center for Zoonosis Control at Andradina Municipality, São Paulo State, Brazil, for euthanasia	Citology on imprints of poplited lymph node, bone marrow and spleen. The samples positive at citology were subjected to PCR	Andradina Municipality, São Paulo State, Brazil	5.76% (3/52)	*L. infantum chagasi*
USA								
R Wells et al	2012	Veterinary Record 2012 171: 654	1	PCR	imported from South Africa three weeks beforehand	USA	100%	

1.15 HEPATOZOONOSIS

Hepatozoonosis is a tick-transmitted, protozoal disease caused by species of the intraleucocytic parasite, Hepatozoon. Hepatozoonosis of domestic cats has been reported from several countries including: India, South Africa, Nigeria, the USA, Brazil, Israel, Spain and France (Baneth, 2011; Patton, 1908, Leeflang and Ilemobade, 1977, Van Amstel, 1979, Ewing, 1977, Perez et al., 2004, Klopfer et al., 1973, Tabar et al., 2008 and Beaufils et al., 1998). It has mostly been reported from regions where canine infection is also present. The species of Hepatozoon that infect cats has not been definitely identified and it is currently unknown if only a single species is found in cats and whether it is similar to species described in other animals. Some studies have referred toHepatozoon felis as the species of Hepatozoon infecting felids (Tabar et al., 2008) whereas other authors have determined that H. canis is responsible for feline hepatozoonosis (Wenyon, 1926 and Jittapalapong et al., 2006). Unlike other ticktransmitted diseases, infection with Hepatozoon in dogs occurs by ingestion of infected Rhipicephalus sanguineus ticks rather than by tick bites, and cats are presumed to be infected by the same route, even if the vectors of feline hepatozoonosis are not known. As in dogs with H canis infection, a characteristic clinical syndrome in cats associated with hepatozoonosis is difficult to define. Feline hepatozoonosis is associated with infection of muscle tissues. Hepatozoon meronts have been identified in the myocardium and skeletal muscles of domestic cats and wild felids with hepatozoonosis (Klopfer et al., 1973, Beaufils et al., 1998 and Kubo et al., 2006), and elevated activities of the muscle enzyme creatinine kinase were found in the majority of cats with hepatozoonosis in a retrospective study of this disease (Baneth et al., 1998). The level of parasitaemia is usually low in cats with less than 1% of the neutrophils containing gamonts. Co-infection with FIV, FeLV and Haemobartonella felis has been reported with feline hepatozoonosis (Baneth et al 1998). Diagnosis as in the other arthropod-borne protozoal infections, is by demonstration of intra-leucocytic organisms with characteristic morphology in peripheral blood or bone marrow smears. Response to therapy with doxycycline for 14–21 days has been recommended (Baneth et al 1995a).

1.16 STRATEGY OF CONTROL

Vector control

The importance of prophylactic flea control in the management of bartonellosis has been demonstrated (Chomel et al 1996, Kordick & Breitschwerdt 1998; Shaw 2001)). This emerging zoonosis is an important reason to maintain routine. prophylactic flea control measures in cats and safe, effective, residual insecticides

are now available for use (Insecticides/Acaricides: Fipronil, Selamectina, Nitenpyram, Imidocloprid. Insect growth regulators: Fipronil/Methoprene, Lufenuron, Pyriproxifen). Control measures should combine both adulticide and insect development inhibitors that are licensed for use in cats. Tick control in cats is also recommended in endemic areas. However, there is a marked deficit of literature concerning the efficacy of acaricides for tick infestation in cats. This may reflect the technical difficulties involved in performing such studies. One obvious problem lies in establishing large enough patent tick infestations in both control and treated groups to ensure reliable estimates of efficacy. A further problem lies in toxicity of pyrethroids in cats which limit their use as acaricides in this species. Fipronil is the only acaricide which has been extensively investigated as an acaricide for use in
cats (Wiedemann 2000; Shaw 2001).

It is different for feral cats: free-roaming cats are often untreated against ectoparasites. Because feral cats should only be handled after they are anesthetized, treatment of parasites at the time of sterilizazion is limited to topical and injectable products. Products selection should be based on safety, spectrum, cost and ease of treatment. The advantage of a single treatment for parasites at the time of surgery is uncertain. However cats with heavy flea infestations can be sprayed with cat-safe flea spray before surgical preparation for immediate control of fleas. Another option is rectal administration of nitempyram. There are also numerous topical products available for flea control with longer duration activity. Many topical products have activity against intestinal parasites and ear mites. Products with efficacy against fleas, roundworms, and hookworms are likely to provide the greatest health benefit during feral cat-TNR programs. Avermectina drugs should be avoided in young debilitated kittens, because neurologic side effects have been reported in this group. Parasiticides can be mixed in food for ongoing treatment, but this is usually not practical such as roundworms and hookworms cannot be controlled, similar to the case for wildlife species (Levy,).

Vaccination

The development of vaccination programmes for control of arthropod-borne infections in cats would benefit both feline and human health. However, there are intrinsic problems with producing vaccines against a group of organisms that can readily evade and manipulate the host immunity Moreover, vaccine development requires detailed knowledge of the nature of the target species immune response to immunodominant epitopes, and such studies are lacking for these feline micro-parasites. Of the diseases reviewed here, feline vaccination has been proposed only for the control of human CSD (Shaws 2001).

Demographic control of feral cats

Free-roaming animals constitute a major reservoir for infectious diseases in dogs and cats in general population. Trapping, neutering and returning animals to their environment has been done for free-roaming cats in many areas (Greene et al., 2013). Same programs are quite elaborated, including extensive veterinary care, surveillance for infectious diseases, colony registration, monitoring and adoption of tame cats; others focus solely on sterilization. Whereas most programs are run by small volunteer groups dependent on donations for operating costs, a growing number are operated with public funds by municipal animal control agencies with the belief that sterilization is ultimately more efficient and cost-effective than ongoing extermination (Levy 2013).

CHAPTER 2

Colony stray cats of Milan

2. COLONY STRAY CATS OF MILAN

Worldwide domestic and feral cat (Felis catus) numbers have increased. Concerns regarding high populations of feral cats in urban areas include wildlife predation, public nuisance and disease (Jones AL et al., 2011). In fact feral cats can serve as a direct or indirect source of infection for outdoor pet cats (Stojanovic V et al., 2011) and for their owners. Thus, feline urban populations are recognized as public health hazards (Mendes-de Almeida et al., 2011).

Debate about the actual impact of free-roaming cats on the environment, on nonfelid and felid wildlife, on domestic feline health, ad as a reservoir of both feline and zoonotic diseases is going. This debate is often emotional, fueled largely by lack of sound scientific data on which to form credible conclusions. Separating the impacts of owned cats that roam outdoors from those of unowned ones is also difficult. Increasing social awareness towards unowned free-roaming cats has resulted in a new concept of "community" or "village" cats. This terminology describes how cats are regarded in much of the world. In addition to cat that clearly have an identified owner and residence, most communities are also populated by colonies of free roaming cats occupying a wide spectrum of socialization level and interactions with human society. This cats are "owned" by the community and the community is their "home" (Levy, 2013).

In the territory of the city of Milan there are a lot of these "community cats" living in small colonies in close association with human activities such as schools, restaurants and neighborhoods, considered as an integral component of the overall cat population. They are managed by volunteers who take care of their nutrition, their census and their capture during TNR (trap-neuter-return) programs implemented by local health authority (ASL).

In Milan there are more than 450 cats colonies, but is difficult to estimate the real number of feral cats. These colony stray cats are protected by national, regional and municipal laws and regulations: usually they can live free-roaming and cannot be moved from the places they are established. Their capture is allowed only for sterilization and health care, but they have to return them to their colonies.

The feral cats can carry the same diseases as pet cats, but the risk of infection transmission is affected by factors such as the cats proximity and relationship to humans and other animals (particularly owned cats), route of transmission, pathogen persistence in environment, and predation. Currently, there are sparse data available to quantify the risks contributed by feral cats compared to that of pet cats. Cetrain conditions, such as rabies, bite wounds, bartonellosis, zoonotic and non zoonotic enteropathogens, arthropods and arthropod-borne diseases and feline infections such as feline immunodeficiency virus (FIV) and feline

leukemia virus (FeLV) are of interest to public and/or feline health. For these reasons we have concuted studies on the prevalence of vector-borne diseases in colony stray cats of Milan city" (J K Levy, Chapter 98, Greene 4TH Ed) .

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CHAPTER 3

Serological and molecular evaluation of Leishmania infantum in stray cats in nonendemic area in Northern Italy

SCIVAC INTERNATIONAL CONGRESS 2013
Canine Leishmaniasis and other vector-borne diseases: current state of knowledge
MARCH 8th- 10th 2013; PISA – ITALY

3. SEROLOGICAL AND MOLECULAR EVALUATION OF LEISHMANIA INFANTUM INFECTION IN STRAY CATS IN A NON-ENDEMIC AREA IN NORTHERN ITALY

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Tipologia: Ricerca Originale
Area di interesse: Medicina interna

Purpose of the work.

To assess the prevalence of leishmaniosis in a large representative sample of stray cats from this non-endemic area, and to analyze
the results according to clinical, laboratory and infectious data.

Materials and used methods.

Blood samples were collected from 233 European shorthair stray cats from urban colonies in Milan, northern Italy, during a trap-neuter-release (TNR) program. The following data were recorded: sex (n=233), age (n=233), body condition score (n=215), area of colony of provenance i.e. one of the seven municipalities of Milan (n=233), health status based on physical examination (n=233), and dermatological evaluation (n=121), complete blood cell count (n=127), antibodies to FIV relative to the gp40 and p24 FIV antigens, the FeLV p27 antigen (n=137), and Toxoplasma gondii IgG antibodies (n=79). The presence of anti-L. infantum antibodies was measured by an indirect immunofluorescence antibody test (IFAT) performed according to the recommendations of OIE using MHOM/IT/80/IPT1 as a whole-parasite antigen fixed on multispot slides and fluorescently-labeled anti-feline gamma as conjugate. L. infantum DNA was amplified from 200 μl of whole blood by real-time PCR. The target for amplification was a 116-bp fragment in the constant region of the kDNA minicircle.
The primers used were QLK2-UP 5'-GGCGTTCTGCGAAAACCG-3' and QLK2-DOWN 5'-AAAATGGCATTTTCGGGCC-3'; the TaqMan probes were Q Leish Probe 2 and 5'-FAM TGGGTGCAGAAATCCCGTTCA-3'- Black Hole. Factors associated with the L. infantum positivity were considered statistically significant when $P<0.05$.
Outcomes.

The serology test for L. infantum showed that 25.3% (59/233) of the cats had L. infantum seroreactivity, 38 (16.3%) had antibody titers of 1:40, 15 (6.4%) had titers of 1:80 and 6 (2.6%) had antibody titers of 1:160.
All blood samples tested using real time PCR were negative for the presence of L. infantum DNA. Only FIV seropositive status was statistically associated with seroreactivity to L. infantum (multivariate logistic regression: P=0.0098 and OR = 7.34 (95%CI=1.96 to 27.59).
Conclusions.
This study is the first epidemiological investigation of feline Leishmania infection in the metropolitan area of Milan, which is a non-endemicarea for leishmaniosis. Our results were surprising, since no autochthonous canine cases of leishmaniosis have ever been reported in this region in northern Italy. In countries in southern Europe where leishmaniasis is endemic, serological investigations performed in feline populations using different techniques have revealed prevalence rates that range from less than 1% to more than 60%.1 As here, previous epidemiological studies have used IFAT to detect antibodies to Leishmania spp. in cats.1,2,3,4
None of the peripheral blood samples we examined using real-time PCR were positive for parasite DNA. PCR has been used previously by others, either alone or in combination with serology, as in our study, to assess the prevalence of feline Leishmania infection. Blood is not the best specimen for PCR diagnosis of leishmaniasis. Specifically, PCR performed on canine blood has lower sensitivity, specificity and positive and negative predictive values compared to PCR performed on canine lymph node aspirates, 5 and this may be true for samples from cats as well. However, blood sampling is less invasive and is easy to perform, particularly for epidemiological studies involving numerous subjects, as in our survey. In addition, in many previous studies, L. infantum DNA was found in whole blood samples from cats.1,2,3,4
Our survey may be an overestimation due to the possibility of IFAT crossreactivity between L. infantum and other pathogens, possible on some serologic tests, especially those that use a whole-parasite antigen, as we did here. This has been demonstrated in dogs in that IFAT cross-reactivity has been reported for L. infantum and Trypanosoma cruzi, Leishmania braziliensis and Ehrlichia canis infection.6
There was no significant correlation between T. gondii-positivity and L. infantum- positivity in our study. This may suggest a lack of cross-reactivity with Toxoplasma parasites.
Additional studies that include parasite isolation are needed to clarify our findings on feline leishmaniasis in this geographic area.

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CHAPTER 4

3. Molecular study on vector-borne infections in urban feral colony cats in northern Italy

Original research

3. Molecular study on vector-borne infections in urban feral colony cats in northern Italy

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Abstract

Feline vector-borne diseases are caused by a wide range of pathogens, which are transmitted by arthropods. Many of these infections have zoonotic implications and feral cats may potentially act as sentinels of human and pet health. The present study investigated the prevalence of vector-borne infections in feral colony cats in the city of Milan in northern Italy. Blood samples from 260 feral cats were evaluated, with conventional PCR, for the presence of DNA associated with hemoplasmas (Mycoplasma haemofelis and Mycoplasma haemominutum), Rickettsia spp., Anaplasma phagocytophilum, Ehrlichia spp. and Babesia microti. Odd ratios (OR) were calculated to identify risk factors for infection with vector-borne pathogens. Positive PCR was found in 156 out of 260 subjects (60%), with a prevalence of 33.1% for hemoplasmas, 31.9% for Rickettsia spp., 17.7% for A. phagocytophilum , 6.7% for Ehrlichia spp. (out of 30 samples), and 1.2% for B. microti spp (out of 168 samples). Statistical analysis revealed a correlation between infections with Rickettsia spp. and hemoplasmas (OR=1.95, P=0.02). Additionally, Rickettsia spp. infection was associated with ocular infection (OR=2.21, P=0.02). We conclude that vector-borne infections, including zoonotic diseases, are present in feral cats of Milan. Thus, domestic

cats exposed to the outdoors should be routinely monitored and treated for ectoparasites to minimize disease onset and potential transmission of zoonotic agents to humans. Moreover, as these vector-borne infections are transmitted through blood, feline blood donors from this area should be screened by PCR.

Keywords: feral cats, northern Italy, PCR, vector-borne infection, Mycoplasma haemofelis, Mycoplasma haemominutum, haemoplasmas, Rickettsia spp., Anaplasma phagocytophilum, Ehrlichia spp., Babesia microti

Introduction

Feline vector-borne diseases (FVBDs) are an emerging problem worldwide due to their frequency and morbidity. Furthermore, most cases of FVBDs are zoonotic and feral cats may potentially serve as sentinels for human infection (Kile et al, 2005). FVBDs, such as rickettsiosis, anaplasmosis, ehrlichiosis, and babesiosis, are caused by diverse pathogens transmitted by arthropod vectors, particularly fleas and ticks (Birkenheuer 2012; Greene, Kidd and Breitschwerdt 2012; Lappin and Breitschwerdt 2012; Lappin and Breithschwerdt 2012; Messick and Harvey 2012).

Free-roaming feral cats are a public health concern in urban and rural areas of Italy (Slater et al, 2008). However, the environmental and medical impact of free-roaming cats is not fully appreciated since scientific data on these populations are lacking. Feral cats are predisposed to FVBDs because they live outdoors, have constant exposure to ticks and fleas, and prey on urban wildlife (such as small rodents) that harbor pathogens (Bown et al, 2003; Beck 2010). Additionally, free-roaming cats are often neither monitored nor treated for vector-borne pathogens. Although several studies worldwide have reported on the epidemiology and emergence of FVBDs (Ebani and Andreani 2002; Aguirre 2004; Vita 2005; Solano-Gallego 2006; Lappin 2006; Hackett 2006; Billeter 2007; Tabar 2008; Gentilini 2009; Barrs 2010; Roura 2010; Bennett 2011; Ayllon 2012), only a few studies have focused on feral colony cats (Luria 2004; Ehberhardt 2006; Kamrani 2008; Juvet 2010; Simking 2010) and, in particular, no studies exist on the feral cats of northern Italy.

Identification and epidemiological surveillance of the various zoonotic diseases in cats may advance the development and implementation of protective measures by national and regional agencies. Notably, some etiological agents of FVBDs can directly be transmitted through blood (Gary et al, 2006). With advances in therapeutics, administration of whole blood or blood components is becoming more common in feline medicine. Our knowledge on the prevalence of blood transmitted pathogens in cats would provide useful information for evaluating the risks associated with transmission of blood-borne infections when cats are potential blood donors. Therefore, an understanding of the prevalence

of FVBDs would used to limit the spread of zoonotic diseases in feline populations. The present study evaluated the prevalence of selected vector-borne agents in feral colony cats of Milan and identified possible risk factors for these infections.

Materials and methods

Sample population and data collection

During a 2-year collection period (January 2008 to January 2010), blood samples were taken from 260 feral cats from urban colonies in Milan (northern Italy), under a trap-neuter-release (TNR) program approved by the local authority of the city council. The program was conducted as previously described (Spada et al 2012, 2013).

The cats (n=260) were collected from eight zones of Milan including 3 from zone 1 (1.2%), 11 from zone 2 (4.2%), 108 from zone 4 (41.5%), 12 from zone 5 (4.6%), 27 from zone 6 (10.4%), 55 from zone 7 (21.2%), 22 from zone 8 (8.5%), and 22 from zone 9 (8.5%). All the cats were of Domestic Shorthair breed. Their age distribution, estimated by dentition, included 118 (45.4%) kittens (≤ 6 months) and 142 (54.6%) adult cats (>6 months). The group consisted of 90 males (34.6%) and 170 females (65.4%). The Body Condition Score (BCS) was recorded for 243 cats (LaFlamme et al, 1994) with a median score of 4.3 ± 0.73 (range 2-8 out of 9). Moreover, 225 cats (92.6%) had a BCS score range of 4-6, indicating normal weight. Only 18 (7.4%) cats were underweight (BCS = 1-3).

Health evaluation, based on clinical examinations, of the study group revealed 72 (27.7%) healthy cats and 188 (72.3%) unhealthy cats. In unhealthy cats, clinical abnormalities included enlarged lymph nodes (n=133, 51.2%), pale mucous membranes (n=14, 5.4%), stomatitis (n=101, 38.8%), signs of respiratory tract infection (n=22, 8.5%), and signs of ocular infection (n=40, 15.4%). The cats were not systematically examined for the presence of ticks or fleas and so the rates of ectoparasitism were not recorded.

Hematological and serological analyses

While the cats were anesthetized, blood samples were collected aseptically from the jugular vein and placed in EDTA-treated tubes and in serum separator tubes. Within 24 h of sample collection, EDTA-anticoagulated blood was evaluated for complete blood count (CBC, n=150) using the ADVIA 120 System (Siemens Healthcare Diagnostics, Milan, Italy). Median hematocrit was 25.2% (range 13-38), the median white cell count was 10.770 x 109/l (range 1.516-23.240) and the median thrombocyte count was 374 x109/l (range 90-800). We detected anemia (Ht <24%) in 69/150 (46.0%) cats, leukopenia (WBC

<10,570/μL) in 14/150 (9.3%) cats, leukocytosis (WBC >14,390/μL) in 5/150 (3.3%) cats, and thrombocytopenia (PLT <200,670/μL) in 10/150 (6.7%) cats (Moritz et al, 2004). Surplus blood was stored at -20°C and used for PCR assay. Sera separated from whole blood were tested for antibodies to FIV (gp40 and p24 FIV antigens; n=166) and FeLV p27 antigen (n=166) using a commercial ELISA kit (Snap FeLV/FIV Combo Plus Test, Idexx Laboratories, Hoofddorp, Netherlands). Thirteen (7.8%) cats tested positive for FIV and 6 (3.6%) were positive for FeLV. The presence of IgG antibodies against Toxoplasma gondii was evaluated using a commercial indirect fluorescent antibody test (IFAT) kit (Fuller Laboratories, Fullerton, CA, USA). An antibody titer ≥1:64 was considered indicative of T. gondii exposure (Dubey and Lappin 2012) and was detected in 31/113 (27.4%) cases.

PCR assay

Conventional PCR was performed on blood samples to amplify pathogen-associated DNA associated with hemoplasmas (M. haemofelis - Mhf and M. haemominutum – Mhm), Rickettsia spp, A. phagocytophilum, Ehrlichia spp. and B. microti. The reaction mixture included 2 μl of template DNA, 0.25 mM dNTPs, 0.4 mM of each primer, 1X reaction buffer and 2.5 U Tap DNA polymerase (GoTaq® DNA Polymerase, Promega, Madison, WI USA). The volume of this mixture was adjusted to 25 μl with sterile water. Primer names, sequences and amplicon size (bp) are summarized in Table 1 and PCR conditions have been previously reported (Tzianabos et al 1989; Persing et al 1992; Munderloh et al 1996; Jensen et al 2001; de la Fuente et al 2005). PCR reactions were performed using an automated thermocycler. PCR products were resolved using 1% agarose gels and fragment size was estimated using a DNA molecular weight marker (1Kb DNA Ladder; Promega Madison, WI USA). Control reactions were done in the absence of template DNA to rule out contaminations during PCR.

Statistical analysis

The prevalence of each pathogen was calculated and reported as the percent of cats with a positive test result. Univariate analysis of categorical data was performed using the chi-square test (cell frequencies of >5) or Fisher's exact test (cell frequencies of ≤5). Any parameters statistically linked to positive PCR results were used in a logistic regression model to test for independent risk factors associated with infection. Associations were considered statistically significant when $P<0.05$. Both the P value and odds ratio (OR) with 95% confidence interval (CI) are reported. Data were analyzed using MedCalc Software (version 12.3.0; Mariakerke, Belgium).

Results

Overall, DNA of one or more pathogen was detected in 60% (156/260) of blood samples tested. Of the positive samples, 102/156 cases were infected with only one pathogen and 54/156 (34.6%) contained multiple pathogens (Table 2). Specifically, 46/156 cases (29.5%) were positive for two pathogens and 8/156 (5.2%) samples contained three pathogens.

Statistical association between pathogen-positive samples and risk factors are shown in Table 3. There were no correlations between colony location, age, gender, BCS, CBC abnormalities, FIV, FeLV, T. gondii status and vector-borne infections. Variables linked to PCR-positive results were co-infection between hemoplasmas and Rickettsia spp (OD=1.95, P=0.02, 95%CI=1.13-3.35). Moreover, the presence of ocular infections is associated with Rickettsia spp. infection (OR=2.21, P=0.02, 95%CI=1.11-4.38).

The prevalence of Babesia spp. and Ehrlichia spp. was low (1.2% and 6.7%, respectively), thus statistical investigation of these pathogens was not performed. Data from these pathogens was available in only 168 and 30 samples respectively because the limited blood was not enough for completion of the analysis.

Discussion

We present the first study investigating the prevalence of FVBDs in urban feral colony cats from the city of Milan. It is difficult to assess the overall prevalence of feline infection by different pathogens due to the limited amount of available data, difficulties in comparing information from studies using different diagnostic tools (i.e. molecular and serological) and confounding factors associated with distinct study populations (i.e. feral and domestic cats).

To our knowledge there is only one study on the prevalence of hemoplasmas in domestic cats (Gentilini et al, 2009). This study was performed at a veterinary teaching hospital in northern Italy and reported positive PCRs (nested) in 18.9% of domestic cats. The authors found that hemoplasma PCR-positive status was not significantly associated with anemia, which is consistent with our results here. This discrepancy could be explained by the stage of infection or the higher prevalence of M. haemominutum (Mhm), a less pathogenic species than M. haemofelis (Mhf). In the study of Gentilini et al., the prevalence of Mhm and Mhf was 17.3% and 5.9%, respectively. However, in our study Mhm was not differentiated from Mhf.

The prevalence of hemoplasmas in feral cats was higher in our study (33.1%) compared to other studies worldwide: 20.5% in Florida (Luria et al, 2004), 12.5% in Arizona (Eberhardt et al, 2006), and 13.3% in Ireland (Juvet et al, 2010). A study from Ontario reported a 47% prevalence of Mhf, although that was determined from a small sampling (n=45) of stray cats (Kamrani et al, 2008).

High incidence of hemoplasmas in stray cats is not surprising as outdoor access is a recognized risk factor for infection. For example, in a study of 191 domestic cats from Barcelona (Spain), hemoplasmas were detected in 12% of cases and outdoor access was found to be a risk factor for infection (OR=3.8) (Roura et al, 2010). Additionally, in a study involving feline blood donors from the USA, the prevalence of hemoplasmas was 19.7% in domestic cats with outdoor access, and only 3.6% in domestic cats not allowed outdoors (Hackett et al, 2006). The higher incidence of hemoplasmas in domestic cats that are allowed outdoors and in cats with a pre-existing vector-borne infections, as observed in our study with Rickettsia spp. (OD=1.95, P=0.02), support the suggestion that ticks and fleas could be responsible for transmitting these diseases, as previously reported (Willi et al 2007).

With the exception of Rickettsia acari and Rickesstia felis, which are transmitted by mites and fleas respectively, ticks are the main vectors for Rickettia spp. (Greene, Kidd and Breitschwerd 2012). Rickettsia spp. were detected in 13-15.7% of ticks collected from public parks in northern Italy (Corrain et al, 2012; Capelli et al, 2012). Recent studies have revealed high levels of R. felis (responsible for flea-borne spotted fever in humans) in fleas (Ctenocephalides felis and Ctenocephalides canis) collected from house and stray cats from north-eastern Italy (Capelli et al 2009). Despite their presence in arthropod vectors, Rickettsia infections in cats are generally low worldwide. We detected an association between PCR positive cats for Rickettsia spp. and the presence of ocular infection (OR=2.2, P=0.02). In dogs, Rickettsia infection can cause ocular abnormalities (Greene, Kidd and Breitschwerd 2012) and, although not documented, we suggest the same could occur in felines.

Human granulocytic anaplasmosis, caused by rickettsial bacteria, occurs in 6% of patients living in northern Italy with a history of tick bites (Beltrame et al, 2006). The prevalence of the bacteria in the vector tick, Ixodes ricinus, is between 1.5% (Capelli et al 2012) to 9.2% in northern Italy (Aureli et al, 2012).

In the United Kingdom, various rodent species have been shown to harbor A. phagocytophilum (Bown et al, 2003). DNA associated with A. phagocytophilum has been detected in naturally infected domestic cats from Finland (Heikkila et al, 2010), the United Kingdom (Shaw et al, 2005), and Italy (Tarello 2005). In our study, 17.7% of cats were infected with A. phagocytophilum, which is higher than other studies worldwide. These studies were performed in domestic cats from eastern Australia (Barrs et al 2010), domestic cats from the USA (Lappin et al 2006), blood donor cats from the USA (Hackett et al, 2006), and feral cats from northern Florida (Luria et al, 2004), Arizona (Eberhardt et al, 2006) and Ireland (Juvet et al, 2010). In some cases, A. phagocytophilum DNA was not detected by PCR in seropositive cats (mainly

IFAT) from the USA (Billeter et al, 2007), and Spain (Solano-Gallego et al, 2006, Aguirre et al, 2004).

The prevalence of Ehrlichia in our study group (n=30) was 6.7%. Ehrlichia spp. that infect cats have been not fully determined (Lappin and Breitschwerdt 2012). The majority of epidemiological data on Ehrlichia spp. infection in cats are based on serological surveys. To the best of our knowledge, only two studies address Ehrlichia prevalence in Italy. One study serologically detected Ehrlichia in 10.2% of stray cats from shelters in Tuscany (Ebani and Andreani 2002). Another study from the Abruzzo region detected two seropositive cats out of 203, although both were PCR-negative (Vita et al, 2005). A study from Spain found that only one out of 100 cats was positive for Ehrlichia spp. (Tabar et al, 2008). In central and northeastern Spain, despite seroprevalence of 10.6% (Aguirre et al, 2004) and 11.3% (Solano-Gallego 2006) respectively, no Ehrlichia-associated DNA was amplified by PCR. Similarly, the pathogen was not amplified 146 blood donor cats in USA (Hackett et al, 2006).

The first confirmed autochthonous case of human B. microti infection in Europe was recently reported (Hildebrandt et al, 2007) and followed by a study that isolated B. microti in ticks (Ixodes ricinus) removed from dogs and cats (Lempereur at al 2011). Moreover, a recent study reported the presence of zoonotic B. microti in rodents from Croatia (Beck et al, 2010). Information on the prevalence of this pathogen in the feline population worldwide is limited. We detected a low prevalence (1.2%), which is in agreement with the value (1.4%) in stray cats from Thailand (Simking et al, 2010). Babesia species were not detected in domestic cats from Spain (Tabar et al, 2008).

These results are particularity important in cases where blood transfusions are used for treatment of cats. Because FVBDs infections usually precede overt symptoms, donated blood may harbor vector-borne pathogens. Additionally, feline hemoplasmas were demonstrated to be transmitted by blood (Gary et al, 2006) and possibly Ehrlichia spp., A. phagocytophilum and Rickettia spp. (Lappin and Breitschwerd 2012; Lappin and Breitschwerd 2012) can be transmitted through blood. Thus, feline blood donors should be tested for pathogens in regions where these infections are prevalent, as suggested by American College of Veterinary Internal Medicine Consensus Statement (Wardrop et al 2005).

This study had certain limitations considering information on the differentiation of hemoplasmas (Mhf versus Mhm) in our study population was lacking. Each type of hemoplasma has unique prognostic concerns, as their pathogenicity varies from being asympthomatic (e.g. MHM) to hemolytic anemia (e.g. MHF) (Messick and Harvey 2012). Moreover, some pathogens have been investigated only at the genus level (e.g. Rickettsia spp. and Ehrlichia spp.) and we lacked information about the species that infected our population. Ectoparasitism was

not investigated and, consequently, we did not identify potential vectors associated with the pathogens in this study. Regardless of these limitations, we believe that this study provides new and useful information on feline vector-borne infections in Italy.

In conclusion, we found that vector-borne infections were present in the feral feline population of Milan. Indeed, pet cats with outdoor access in this region should be regularly monitored and treated for ectoparasites to minimize health risks to humans and pets. Importantly, feline blood donors in northern Italy should be screened with PCR for these emerging infections.

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Table 1. The names, sequences and amplicon size (bp) of PCR primers used in this study

Organism detected	**Primer name**	**Nucleotide sequence (5'-3')**	**Amplicon size (bp)**	**Reference**
Rickettsia spp	Tz-15-19	TTCTCAATTCGGTAAGGGC	246	Tzianabos et al 1989
	Tz-16-20	ATATTGACCAGTGCTATTTC		
Babesia microti	BAB1	CTTAGTATAAGCTTTTATACAGC	238	Persing et al, 1992
	BAB4	ATAGGTCAGAAACTTGAATGATACA		
Ehrlichia spp	PER1	TTTATCGCTATTAGATGAGCCTATG	451	Munderloh et al, 1996
	PER2	CTCTACACTAGGAATTCCGCTAT		
Hemoplasmas (Mycoplasma heamofelis* and *Mycoplasma haemominutum)	fHf	ACGAAAGTCTGATGGAGCAATA	170-193	Jensen et al, 2001
	rHf	ACGCCCAATAAATCCG(A/G)ATAAT		
Anaplasma phagocytophilum	MSP4AP5	ATGAATTACAGAGAATTGCTTGTAGG	849	de la Fuente et al, 2005
	MSP4AP3	TTAATTGAAAGCAAATCTTGCTCCTATC		

Table 2. The prevalence of vector-borne infections in feral colony cats based on PCR detection of bacterial DNA.

Vector-borne pathogen (number of cats tested for)	**Number of infected cats**	**Observed prevalence (%)**	**95%CI**
Hemoplasmas (n=260)	86	33.1	26.5-40.9
Rickettsia spp. (n=260)	83	31.9	25.4-39.6
Anaplasma phagocytophilum (n=260)	46	17.7	13.0-23.6
Ehrlichia spp. (n=30)	2	6.7	0.8-24.1
Babesia microtii (n=168)	2	1.2	0.1-4.3
Hemoplasmas + *Rickettsia spp*	29	11.1	7.5-16.0
Hemoplasmas + *A. phagocytophilum*	9	3.5	1.6-6.6
A. phagocytophilum + *Rickettsia* spp	6	2.3	0.8-5.0
Rickettsia spp + *Ehrlichia* spp	2	0.8	0.1-2.8
Hemoplasmas + *A. phagocytophilum* + *Rickettsia* spp	7	2.7	1.1-5.5
Hemoplasmas + *A. phagocytophilum* + *B. microtii*	1	0.4	0.01-2.1

Table 3. The prevalence of hemoplasma, Rickettsia spp., and A. phagocytophilum in feral colony cats based on PCR detection of bacterial DNA

Factor	**Category**	**Hemoplasmapositive number (%)**	**P value**	***A. phagocytophilum* positive number (%)**	**P value**	***Rickettsia* spp. positive number (%)**	**P value**
Origin of the cats	zone 1	0/86 (0.0)	P=0.54	0/46 (0.0)	P=0.96	1/83 (1.2)	P=0.57
	zone 2	5/86 (5.8)	P=0.57	3/46 (6.5)	P=0.66	3/83 (3.6)	P=0.99
	zone 4	40/86 (46.5)	P=0.31	24/46 (52.2)	P=0.15	41/83 (49.4)	P=0.10
	zone 5	4/86 (4.7)	P=0.77	0/46 (0.0)	P=0.21	2/83 (2.4)	P=0.40
	zone 6	12/86 (14.0)	P=0.27	0/46 (0.0)	P=0.18	4/83 (4.8)	P=0.07
	zone 7	14/86 (16.3)	P=0.23	15/46 (32.6)	P=0.06	19/83 (22.9)	P=0.76

	zone 8	8/86 (9.3)	P=0.92	0/46 (0.0)	P=0.05	9/83 (10.8)	P=0.48
	zone 9	3/86 (3.5)	P=0.07	4/46 (8.7)	P=0.82	4/83 (4.8)	P=0.23
Age	Juvenile (≤ 6 months)	37/86 (43.0)	P=0.69	21/46 (45.7)	P=0.90	42/83 (50.6)	P=0.31
	Adult (>6 months)	49/86 (57.0)		25/46 (54.3)		41/83 (49.4)	
Gender	Male	60/86 (69.8)	P=0.37	14/46 (30.4)	P=0.63	22/83 (26.5)	P=0.08
	Female	26/86 (30.2)		32/46 (69.6)		61/83 (79.5)	
BCS	Poor (1-3/9)	8/78 (10.3)	P=0.37	1/44 (2.3)	P=0.26	5/83 (6.0)	P=0.74
	Good (4-6/9)	70/78 (89.7)		43/44 (97.7)		78/83 (94.0)	
Health status	Healthy	25/86 (29.1)	P=0.84	18/46 (39.1)	P=0.08	24/83 (28.9)	P=0.88
	Unhealthy	61/86 (70.9)		28/46 (60.9)		59/83 (71.1)	
Clinical abnormalities in unhealthy cats	Lymph node enlargement	46/86 (53.5)	P=0.69	21/46 (45.7)	P=0.51	45/83 (54.2)	P=0.59
	Pale mucous membranes	3/86 (3.5)	P=0.51	4/46 (8.7)	P=0.46	1/83 (1.2)	P=0.08
	Stomatitis	30/86 (34.9)	P=0.43	14/46 (30.4)	P=0.26	25/83 (30.1)	P=0.07
	Signs of respiratory tract infection	10/86 (11.6)	P=0.29	3/46 (6.5)	P=0.82	9/83 (10.8)	P=0.48
	Signs of ocular infection	14/86 (16.3)	P=0.92	7/46 (15.2)	P=0.85	19/83 (22.9)	**OR=2.21 CI=1.11-4.38 P=0.02**
CBC abnormalities	Anaemia	29/59 (49.2)	P=0.69	17/37 (45.9)	P=0.89	35/71 (49.3)	P=0.59
	Leukopenia	4/59 (6.8)	P=0.56	2/37 (5.4)	P=0.53	5/71 (7.0)	P=0.53
	Leukocytosis	2/59 (3.4)	P=0.66	3/37 (8.1)	P=0.18	2/71 (2.8)	P=0.90

	Thrombocytopenia	6/59 (10.2)	P=0.30	3/37 (8.1)	P=0.99	6/71 (8.5)	P=0.63
Hemoplasmas PCR results	Positive	-	-	18/46 (39.1)	P=0.43	36/83 (43.4)	**OR=1.95 CI=1.13-3.35 P=0.02**
	Negative	-	-	28/46 (60.9)		47/83 (56.6)	
A. phagocytophilum PCR results	Positive	18/86 (20.9)	P=0.43	-	-	14/83 (16.9)	
	Negative	68/86 (79.1)		-	-	69/83 (83.1)	
Rickettsia spp. PCR results	Positive	36/86 (41.9)	**OR=1.95 CI= 1.13-3.35 P=0.02**	14/46 (30.4)		-	
	Negative	50/86 (58.1)		32/46 (69.6)	P=0.95	-	-
B. microti PCR results	Positive	1/63 (1.6)		1/42 (2.4)		0/83 (0.0)	
	Negative	62/63 (98.4)	P=0.71	41/42 (97.6)	P=1.00	83/83 (100)	P=0.49
Ehrlichia spp. PCR results	Positive	0/13 (0.0)		0/5 (0.0)		2/12 (16.7)	
	Negative	13/13 (100)	P=0.59	5/5 (100)	P=0.74	10/12 (83.3)	P=0.30
FIV test results	Positive	5/64 (7.8)		5/39 (12.8)		5/78 (6.4)	
	Negative	59/64 (92.2)	P=0.82	34/39 (87.2)	P=0.35	73/78 (93.6)	P=0.68
FeLV test results	Positive	2/64 (3.1)		0/39 (0.0)		2/78 (2.6)	
	Negative	62/64 (96.9)	P=0.90	39/39 (100)	P=0.36	76/78 (97.4)	P=0.75
T. gondii test results	Positive	13/46 (28.3)		6/23 (26.1)		18/65 (27.7)	
	Negative	33/46 (71.7)	P=0.92	17/23 (73.9)	P=0.94	47/65 (72.3)	P=0.83

ATTACHMENT 1

Scientific Publications on Peer Review Internetional Journals with Impact Factor

1. Eva Spada, Daniela Proverbio, **Alessandra Della Pepa**, Roberta Perego, Luciana Baggiani, Giada Bagnagatti DeGiorgi, Giulia Domenichini, Elisabetta Ferro and Fausto Cremonesi, 2012, **Seroprevalence of feline immunodeficiency virus, feline leukaemia virus and Toxoplasma gondii in stray cat colonies in northern Italy and correlation with clinical and laboratory data**, Journal of Feline Medicine and Surgery 14(6) 369–377DOI: 10.1177/1098612X12437352

2. Daniela Proverbio, Eva Spada, Roberta Perego, **Alessandra Della Pepa**, Giada Bagnagatti De Giorgi, Luciana Baggiani, 2012, **Assessment of blood types of Ragdoll cats for transfusion purposes,** Veterinary Clinical Pathology Manuscript ID: VCP-12-1903.R1

3. Daniela Proverbio, Giada Bagnagatti de Giorgi, **Alessandra Della Pepa**, Luciana Baggiani, Eva Spada, Roberta Perego, Carlo Comazzi, Angelo Belloli, 2012, **Preliminary evaluation of total protein concentration and electrophoretic protein fractions in fresh and frozen serum from wild Horned Vipers (Vipera ammodytes ammodytes),** Veterinary Clinical Pathology Vet Clin Pathol 0/0 (2012) 1–5 ©2012 DOI:10.1111/j.1939-165x.2012.00486.x.

4. Eva Spada, Daniela Proverbio, Alessandra Della Pepa, Giulia Domenichini, Giada Bagnagatti De Giorgi, Giorgio Traldi and Elisabetta Ferro, 2013, **Prevalence of faecal-borne parasites in colony stray cats in northern Italy,** Journal: Journal of Feline Medicine and Surgery DOI: 10.1177/1098612X12473467

ATTACHMENT 2

Scientific Publication on National Journals and Proceedings of National Conferences

1. Spada E., Perego R., Domenichini G., **Della Pepa A**., Proverbio D. **TOXOPLASMOSI NEL GATTO; Rassegna di Medicina Felina Anno 14, Numero 2, 2010, pp 7-12.**
2. Spada E., Perego R., Bagnagatti De Giorgi G., **Della Pepa A.**, Baggiani L., Proverbio D, **SINDROME ACQUISITA DA FRAGILITA' CUTANEA FELINA; Rassegna di Medicina Felina Anno 15, Numero 4, 2011, pp 7-13**
3. Spada E., Perego R., Bagnagatti De Giorgi G., **Della Pepa A**., Baggiani L., Proverbio D., **GRUPPI SANGUIGNI NEL GATTO E MEDICINA TREASFUSIONALE FELINA; Rassegna di Medicina Felina, Anno 15, Numero 3, 2011, pp 7-13.**
4. Proverbio D, Spada E, **Della Pepa A**, Baggiani L, Perego R, Bagnagatti De Giorgi G, Ferro E, **COMPARISON OF A RAPID ELISA WITH IFAT FOR DETECTING OF IgG ANTIBODIES SPECIFIC TO TOXOPLASMA GONDII IN FELINE SERUM,** Atti del LXV Convegno Nazionale SISVet 7-10 Settembre 2011, Tropea-Drapia (VV), ITALIA.
5. Proverbio D, Perego R, Spada E, Baggiani L, Bagnagatti De Giorgi G, Della Pepa A, Ferro E **COMPARISON OF A SEMI-AUTOMATED ELECTROPHORETIC SYSTEM AND A QUANTITATIVE BIOCHEMICAL METHOD FOR THE EVALUATION OF ALKALINE PHOSPHATASE ISOENZYMES IN CANINE SERUM;** Atti del LXIV Convegno Nazionale SISVet 7-10 Settembre 2010, Asti (AL), ITALIA.
6. Spada E, Proverbio D, Baggiani L, Perego R, Milici A, Domenichini G, **Della Pepa A**, Bagnagatti De Giorgi G, Ferro E, **DISTRIBUTION OF A, B AND AB BLOOD TYPE IN RAGDOLL CATS** Atti del LXIV Convegno Nazionale SISVet 7-10 Settembre 2010, Asti (AL), ITALIA.