Main Pet Arthropod-Borne Diseases in Asia

Tokyo 6th-9th November 2012
Many viral, bacterial, and parasitic pathogens have been associated with transmission by arthropods, including several recently identified pathogens in both humans and domestic animals, especially dogs. Ticks, mosquitoes and fleas are the main vectors of pathogen transmission to carnivores and humans.

The important spreading of pathogens by a greater mobility of human populations and their companion animals combined with changes in the ecosystems favorable to survival of ectoparasites have led to the recognition of vector borne diseases in new areas. Vector-borne diseases are also recognized as an emerging infectious threat not only to humans but also to dogs and cats thanks to better detection tools, largely based on molecular technique. Among vectors, ticks are the most important in veterinary medicine regarding the variety of transmitted pathogens.

Studying vector-borne diseases need a multi-disciplinary approach... The majority of vector-borne diseases have zoonotic impact requiring collaborations between physicians and veterinarians.

The objective the 9th Merial Parasitology & Arthropod Borne Diseases Symposium is to provide an update of ticks and Tick Borne Diseases of Pets and their control, focusing on Asia region.

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Introduction

Ixodidae, known as ‘hard ticks’, are giant mites. They have adapted to living in all habitats and to feed on any kind of hosts, from reptiles to mammals. In industrialized countries, they are also known for their major impact on animal health, including pets or sports animals (dogs, horses) and livestock animals (cattle). They are the vector of many pathogenic agents: viruses, bacteria, protozoa or helminthes; some of them being common among humans and animals. The important genera of tick found on pets worldwide are *Ixodes*, *Rhipicephalus*, *Dermacentor*, *Amblyomma* and *Haemaphysalis*.

The tick infestation of pets is really common. It can be seasonal in temperate areas, or year round in warmer places.

The distribution and the density of ticks infesting pets and humans is subject to change, as well as the transmitted diseases. The multifactorial reasons for these changes are related to climate change (less cold winter in temperate zones), wildlife increase (population of wildboars, foxes, deers, rodents), human activities (creation of parks, riversides, walking areas in forests, forest management, increase of sub-urban areas with many gardens and green zones), travelling of pets all around the world...

It appears that the tick threat is now an increasing concern in many places of the world.

**ECOLOGICAL CHARACTERISTICS OF IXODIDAE TICKS**

Hard ticks are cosmopolitan in distribution but each species is restricted to a particular biotope and climate: ecosystem/species called ‘tick areas’. Populations may be subject to strong seasonal changes in each habitat.

Some ticks are adapted to desert areas, especially *Hyalomma* ticks (*H. dromedarii*). Some are adapted to humid tropical climates, especially *Amblyomma* ticks like *Amblyomma variegatum* and *Amblyomma maculatum*. 
Others do like hot climate, either tropical or Mediterranean, especially *Rhipicephalus* ticks for example *Rhipicephalus sanguineus*. Some species are more adapted to cold temperate (*Dermacentor reticulatus, Ixodes ricinus, Ixodes pacificus*) and/or cold continental climates (*Ixodes ricinus, Ixodes scapularis*).

There are biotopes suitable for free-living stages (larvae, nymphs and adults waiting for a host), that may vary for the same species. For example *Ixodes hexagonus* (hedgehog tick) or *Dermacentor reticulatus* (marsh tick), larvae and nymphs may be found in the burrows of rodents or rabbits while adults are found in grass. Regarding *Ixodes scapularis* and *I. ricinus* (forest ticks), all life stages are found in grass, preferentially under the forest cover, but they don’t have the same tropism for hosts.

Some tropisms correspond to each parasitic life stage. Larvae and nymphs may have a tropism for some hosts, such as micromammals (field mice, voles, hedgehogs) or birds, while adults look for herbivores (cattle, deer, horse) or canids. That’s the case for *Ixodes scapularis* and *Ixodes ricinus*. The preference can be strict or not. For example, *Rhipicephalus Boophilus microplus*, the tropical cattle tick, is really restricted to cattle and almost never infest humans or pets; when many other ticks have a non-restricted tropism, for example *Ixodes* ticks which can bite all mammals in the absence of their preferred hosts.

### Ticks Biology

Ticks are temporary ectoparasites and are not intermittent feeders like mosquitoes or permanent parasites like cat & dog fleas or lice. An obligate alternation occurs between free-living and parasitic stages.

#### Living environment variations

Ticks having endophilic stages must be distinguished from those having exophilic stages:

- **Endophilic domestic ticks**: *Rhipicephalus sanguineus* (all stages are present in the dog’s environment: kennels, on the floor, on walls...).
- **Exophilic ticks**: *Ixodes ricinus, Dermacentor reticulatus*. Stages are present in forests, in the woods, along the edge of a field, in vacant lots, public gardens and parks, riversides... There are some exophilic ticks that are ‘troglodytes’: present in the environment but not on the surface, preferably in burrows, which is the case with *Ixodes hexagonus* (the hedgehog tick).

This situation, endophilic versus exophilic, may vary within one species depending on the stages.
Climatic variations

In addition to the biotope, climatic conditions of temperature and humidity play a key role in explaining the presence of one species or another.

- Hygrophilic ticks: they require humidity; do not tolerate excessive heat and desiccation.

It is the case with many *Ixodes* like *I. ricinus* in Europe, *I. scapularis* in the USA, *I. persulcatus* and *I. ovatus* in Asia and Japan, and *Dermacentor* ticks like *D. reticulatus* and *D. variabilis*.

- Xerophilic ticks: they live in warm areas, tolerate desiccation but not frost.

It is the case with *Rhipicephalus sanguineus*. They may prefer warm and humid conditions and be susceptible to desiccation like *Haemaphysalis longicornis* in Asia.

When the conditions are not favorable for activity, ticks undergo diapause: winter and dry-season diapause for ticks under temperate and continental climates. Activity resumption often depends on the season (i.e. spring and fall peak in Western Europe), but this does not exclude activity during winter when temperatures are milder, even temporarily. This can explain canine babesiosis cases all over the year when *Dermacentor* ticks ‘wake up’ based on climate changes.

Choice of host

Ticks can be differentiated by their host tropism. Euryxenous ticks (polytropic) are not host-specific and feed on a wide range of animal species. Stenoxenous ticks (monotropic) have a particular host tropism. This affinity may also vary among life stages.

- *Ixodes ricinus* and *Ixodes scapularis*: larvae and nymphs usually feed on micromammals whereas adults are on the lookout for ungulates (domestic or wild ruminants, wild boars). Larvae and nymphs can nevertheless bite birds or just about any mammal that they encounter (human, dog, cat…), which is why Lyme disease can be passed on to humans or dogs.

- *Ixodes holocylus* (Australian Paralysis Tick): larvae, nymphs and adults usually feed on marsupials (small and large) but they can nevertheless bite any other mammal that they encounter (human, dog, cat…), which explains the cases of tick paralysis (neurotoxin, holocyclotoxin, secreted with the saliva) seen in dogs and in children in Northern and Eastern Australia (mainly Queensland).
• *Haemaphysalis longicornis*: larvae and nymphs usually feed on micromammals whereas adults are on the lookout for ungulates (domestic or wild ruminants). All stages can also bite any mammal that they encounter (human, dog, cat...), which is why this is the vector of canine babesiosis in Japan.

• *Rhipicephalus sanguineus*: All stages prefer to parasitize canids but cats may also be infested.

### Fixation on the host

The ticks will find their hosts by detecting the heat, vibration, shadow, breath \((\text{CO}_2)\) and odors. They especially use their Haller organs located on the Tarsa I to located the host and distance to it.

Ticks can be separated in two groups:
- The questing ticks: they are immobile and wait for their host, usually in the herbs. They go down onto the ground and humus at night or when the conditions are not favorable, and they climb on the vegetation during the day, usually at the hours corresponding to the activity of their host (early hours of the day and at dusk). *Ixodes, Dermacentor, Haemaphysalis, Boophilus* ticks are questing ticks.
- The hunting ticks: they are really mobile and they will walk in the direction of their hosts. They can ‘run’ quite fast. This is the case of some *Amblyomma* and *Hyalomma*, but also for *Rhipicephalus sanguineus* in a certain extent.

The ticks will infest the hosts quickly, in a few seconds. They usually don’t fall on the hosts, they are not on the trees but more on the ground and in the herbs. They will catch the host with their sticky organ located at the end of the tarsa. Then they will use their legs and claws to crawl in the fur, on the skin, and to search a place to attach.

### Choice of attachment site

Once on the host, ticks crawl in the fur along the skin until they find a good spot to start feeding. They usually favor areas of thin skin: ears, undersides of the limbs, scrotum, udder... In order to locate their host, ticks rely on features such as heat, smell, sight and touch.

*Rhipicephalus* ticks prefer to be around or in the dog’s ears while *Dermacentor* and *Ixodes* have less preference.

After having infested their host, the time to find their attachment site takes in average between 4 to 6 hours. During that time, ticks are eventually already in contact with topical acaricides.

### Fixation - Attachment

• Ticks attach to host using their chelicerae and by inserting the hypostome. Firstly they use their pedipalps which are tactile to find the place for attachment. The ticks attach then first with their chelicerae which look like harpoons with 2 terminal hooks. They penetrate the skin and they are retracted by the tick through muscular contraction. It allows the oblique penetration of the hypostome under the skin.

• Upon attachment, the tick secretes a cement for 10-30 minutes (primary and secondary cement produced by types II and III salivary-gland acini). The cement is made up of glycoproteins that polymerize on exposure to air and skin.

• As soon as the attachment occurs, some pathogens may be transmitted. The first pathogen agents to be inoculated must be present in the saliva and immediately infectives. That’s the case of virus, which could be inoculated within 15 minutes after the fixation starts.

### Nutrition – Blood meal

All stages feed: larvae, nymphs and adults (females and males). The males
feed less in volumes and they in fact can bite several times.
The meal lasts between 3 to 7 days on average. The larvae and nymphs feed for shorter periods (3 to 5 days) than the females (5 to 7 days).

The meal is no really a blood meal like for mosquitoes. It contains not only blood but also digested tissue and many leucocytes. The food intake occurs in two phases:

- The first one which can be named a preparation phase involves intense secretory activity, during which the tick produces enzymes and peptides, inducing immunomodulatory, anticoagulant and proteolytic effects. This phase enables the creation of a liquid necrotizing hemorrhagic zone through the digestion of subcutaneous tissue and attraction of many leucocytes (monocytes, phagocytes, granulocytes). This preparation phase takes at least 3 days during which the tick exchanges fluids with the host. The volume of the female tick doesn’t increase too much during that phase (from 2 to 30 mg).

- The second phase is the rapid ingestion phase. The tick ingests the fluids and cells and the female volume increase dramatically [from 30 to 250 mg]. In order to avoid osmotic shock, the female concentrates the ingested meal and excretes the excess fluid.

- Each phase involves the activity of different salivary-gland acini. Besides the enzymes, the saliva contains peptides acting as cytokines with an immunomodulation role. It avoids protective immunity which is uncommon in mammals, except in guinea pigs which develops an basophile hypersensitivity response to tick bite. This immunomodulation allow the attraction of many white blood cells which will be part of the meal and ‘help’ the local necrosis to occur. This is also favorable for the transmission of pathogens.

- During the meal, pathogen agents can be transmitted. The bacteria that are present in the saliva and directly infective are inoculated quite quickly, in average from 8 to 24h after the attachment. It concerns *Ehrlichia, Anaplasma, Rickettsia...* Some other pathogens undergo multiplication and antigenic variations to become infective. That’s the case for *Borrelia burdorteri sensu lato*. Therefore the transmission occurs later, usually between 36 to 48 hours or more. Regarding *Babesia*, the sporozoites have to become infective and migrate to the saliva. They are usually transmitted between 48 to 96 hours after attachment.

- At the end of the blood meal, the females will actively detach and fall off the host on the ground.

**Reproduction and Egg-laying**

The females and males mate on the host. The mating occurs before or during the female meal. Depending the genus, ticks secretes pheromones to attract...
other ticks. The co-feeding is a way to exchange pathogens from ticks to ticks through the same feeding places. The in utero genesis of the eggs starts during the blood meal of the females. When the females fall off their host on the ground, they will search for a crevice to hide in and are prepared to lay their eggs within 48 to 72 hours. The females bend their anterior extremity which splits dorsally on the capitulum (camerostomal groove). The egg laying phase will take 48 to 72 hours and the female will dye at the end. The eggs are protected and clustered together by a yellow lipid wax. In average 3 000 to 10 000 eggs are laid at once.

Life cycles

Ticks molt in the environment, which corresponds to the original pattern, but also on the host when it comes to genera or species that have evolved.

- Classic life cycle: triphasic, occurring among all genera: Each stage requires one meal, then the engorged stage drops off to the ground and molts. The new stage, fasting, awaits a new host to complete the cycle. All important ticks encountered in Europe, North America, and Asia undergo that cycle. Thus, every tick generation requires three hosts.

- Biphasic cycle: The larva molts into a nymph on the first host, meaning that there are two hosts instead of three per tick generation. It is the case for some *Hyalomma* or *Rhipicephalus* ticks.

- Monophasic cycle: The 2 molts occur on the host. This pattern of life cycle is observed in all *Boophilus* (infesting ruminants) and *Margaropus* (infesting Giraffe, Zebra or horses) ticks, as well as in two *Dermacentor* species.

Duration of life cycle: It varies widely and depends on both climate conditions and host behavior. Interruption of the life cycle may occur: egg diapause (exceptional); behavioral diapause of larvae, nymphs or adults, awaiting favorable conditions; diapause in fasting stages up to a year, waiting for a host.
VECTOR ROLE OF TICKS

In veterinary medicine, ticks are the most important vectors in regards to the number of transmitted diseases, their economical importance in production animals and the zoonotic impact, which is not the case in human medicine, where mosquitoes are the ones that play a predominant role.

In order for ticks to be vectors, required conditions include always transstadial transmission. The infected stage is never a vector of transmission.

The transmitted agents and diseases belong to many groups:

- **Virus (>99)**: Tick-borne encephalitis [classic TBE virus, Powassan fever, Kyasanur forest disease, Omsk hemorrhagic fever and Langat virus, ovine encephalomyelitis or louping-ill, Colorado tick fever...].
- **Rickettsiae** [in a wide sense]: ehrlichiosis, anaplasmosis, coxiellosis [Q fever], cowdriosis, Rocky Mountain spotted fever, Mediterranean spotted fever, African spotted fever, Australian spotted fever, Queensland tick typhus, Siberian tick typhus...
- **Protozoa**:
  - *Babesia*: Inevitable and exclusive biological vector, the tick is the definitive host;
  - *Theileria*: Inevitable and exclusive biological vector, the tick is the definitive host;
  - *Hepatozoon canis* [transmission by ingestion of the tick].
- **Helminths**: filarial parasites (*Dipterotoman*).
Canine Babesiosis in Asia

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He started his carrier as a veterinary officer of the Ministry of Agriculture, Forestry and Fisheries in 1986 where he started the study of ticks and tick-borne diseases of animals. In 1994 he started the study of ticks and tick-borne diseases of companion animals in Laboratory of Internal Medicine, Yamaguchi University, where canine babesiosis is endemic. Since 2005 he has been a professor of veterinary internal medicine at Obihiro University of Agriculture and Veterinary Medicine where tick-borne diseases of farm animals are endemic. He is now in charge of the department and the education of internal medicine. His works focused on ticks and tick-borne diseases, including Babesia, Theileria, Rickettsia, Ehrlichia, Anaplasma and Borrelia in both small and large animals.

Introduction

The vast Asian continent includes a wide variety of climates that range from cold to tropical and from humid to arid. Disease-bearing ticks of companion animals in Asia also comprise many species, and one would expect plenty of tick-borne diseases (TBD) in dogs and cats of the continent. However, limited epidemiological information is available on TBD of companion animals in Asian countries. As canine babesiosis is one of the most important TBD in dogs in Asian countries, here the epidemiological aspect of the disease in Asia, more specifically, in Japan, will be reviewed.

BABESIA CANIS (Figure 1)

Babesiosis is a tick-borne disease caused by a protozoan parasite. Both Babesia canis and Babesia gibsoni cause canine babesiosis in Asian countries (Irwin 2005). These parasites infect the red blood cells of dogs and typically cause hemolytic anemia. Three different subspecies of B. canis occur worldwide. Sequence analysis of the 18S rRNA gene of B. canis in free-roaming dogs in Okinawa, Japan, suggested...
that the infecting species there was *B. canis vogeli* [Inokuma et al., 2004]. Most *B. canis* subspecies found in Asia are thought to be *B. canis vogeli*, which causes mild to moderate pathogenesis in dogs [Farwell et al., 1982, Irwin 2005]. *B. canis* is believed to be widely distributed in the world, which is consistent with the global distribution of its vector tick, *R. sanguineus* [Irwin 2005].

**BABESIA GIBSONI** (Figure 2)

*Babesia gibsoni* is more important *Babesia* pathogen in Asia, because infection with *B. gibsoni* generally results in more severe clinical manifestations than does infection with *B. canis*, and may cause multiple organ dysfunctions [Farwell et al., 1982, Inokuma et al., 2005]. *B. gibsoni* is also widely distributed throughout Asia because of the wide ranges of its hosts, which include *H. longicornis* and *R. sanguineus* [Irwin 2005]. Evidence of *B. gibsoni* and *B. canis* infection in dogs has been reported in Israel [Baneth et al., 1998], India [Megat Abd Rani et al., 2011], Thailand [Suksawat et al., 2001], Malaysia [Rajamanickam et al., 1985], South Korea [Lee et al., 2009], Taiwan [Lee et al., 2010], China [Wang et al., 2010] and Japan [Inokuma et al., 2005].

In Japan, *B. gibsoni* is distributed primarily in the western part of the country [Miyama et al., 2005]; few studies have reported canine *B. gibsoni* infection in eastern Japan. Most confirmed cases of *B. gibsoni* infection in eastern Japan have been found in Tosa dogs, a fighting breed [Miyama et al., 2005]. Interestingly, transmission of *B. gibsoni* among these dogs was thought to occur via bite wounds rather than ticks [Miyama et al., 2005]. In a recent epidemiological survey of 115 dogs with suspected *B. gibsoni* infection in eastern Japan, 35 dogs (30.4%) tested positive by polymerase chain reaction and/or enzyme-linked immunosorbent assay for the immunodominant antigen P50 of *B. gibsoni*. These dogs included 28 Tosa dogs, 4 American Pit Bull Terriers and 3 mongrels [Miyama et al., 2005].

More recently, nationwide questionnaire surveillance was conducted regarding the prevalence of *Babesia gibsoni* infection in dogs in 2009 and 2010 [Inokumka et al., 2012]. A total of 6,746 answers were recovered among 9,513 animal hospitals in all 47 prefectures in Japan. Among these, 859 animal hospitals (12.7%) have diagnosed *B. gibsoni* infection in dogs. The numbers of patients were 3,802 and 3,625 in 2009 and 2010, respectively. In Eastern Japan, a total of 89 animal hospitals diagnosed 87 and 75 patients infected with *B. gibsoni* in 2009 and 2010, respectively. Nine clinical cases of *B. gibsoni* infection were confirmed in non-fighting dogs without travel history to Western Japan. It is suggested that natural infection with *B. gibsoni* would have
occurred in Eastern Japan. In Western Japan, a total of 769 animal hospitals diagnosed 3,715 and 3,550 patients infected with *B. gibsoni* in 2009 and 2010, respectively. Especially, many confirmed clinical cases more than 200 per year were reported from some Prefectures (Fig.3).

**TICK VECTORS OF BABESIA IN ASIA**

Many tick species exist in Asian countries. In cold northern regions, *Ixodes persulcatus*, or the taiga tick, is the dominant species, with a range stretching from Eastern Europe and Russia to China and Japan. *Rhipicephalus sanguineus* (Fig.4) is the most common tick species infesting dogs in southern tropical regions of Asia, as well as most of the rest of the world. One study of ectoparasites of domestic animals in Thailand reported that *R. sanguineus* was the only tick species collected from dogs (Changbunjong et al., 2009). This tick species is usually established in kennels which is very unique compared with other species. Thus canine hosts usually continuously attacked by *R. sanguineus*, and it is very difficult to control this tick species completely.

A wider variety of tick species occur in the Asian temperate zone, which includes China, South Korea and Japan (Yamaguchi et al., 1970). Tick species in this region belong to the genera *Haemaphysalis*, *Amblyomma*, *Dermacentor* and *Hyalomma*. The tick species of canine hosts show more variation in Japan than they do in European countries. A systematic survey of canine tick species in Japan revealed that *Haemaphysalis longicornis* (Fig.5) was the most common species found, occurring in 492 of 1221 dogs (40.3%), followed by *Haemaphysalis flava* (196, 16.1%), *R. sanguineus* (59, 4.8%) and *Ixodes ovatus* (50, 4.1%) (Shimada et al., 2003) (Fig. 6). Small numbers of *Haemaphysalis hystricis*, *Haemaphysalis campanulata*, *Haemaphysalis japonica*, *Haemaphysalis ias*, *Ixodes persulcatus*, *Ixodes nipponensis* and *Amblyomma testudinarium* were also recovered.
Seasonal occurrence of *B. gibsoni* infection in dogs is known in the temperate zone. Spring and autumn are the dangerous periods for the infection in dogs in Japan [date not shown]. This can be explained by the seasonal occurrence of *H. longicornis*, the major tick vector of *B. gibsoni* (Fig. 7). In the spring, infected adults and nymphs that passed the winter look for host animals to feed blood. This is the first peak of infection observed in spring from March to May. Then adult females lay eggs and nymphs molt to adults. New larvae would appear from summer to autumn. The peak of larval infestation is found in September when the second peak of *B. gibsoni* infection is occurred. Larvae that successfully infested animals can molt to nymphal stage to survive the cold winter. Tick control is the most important strategy to prevent *B. gibsoni* infection in dogs.

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Prevalence of Canine Tick-Borne Diseases in Malaysia

The common tick-borne diseases of dogs in Malaysia are Ehrlichiosis, Babesiosis, Anaplasmosis, and Hepatozoonosis. Despite the climatic conditions that are conducive for the transmission of tick-borne diseases, there is little published data on their prevalence in dogs in Malaysia and until just recently, only conventional methods were available for the diagnosis of these diseases. Most studies have focused on Selangor state and Kuala Lumpur with a few exceptions that have included Perak and Sarawak (Figure 1), therefore there is a paucity of information about the distribution and prevalence of tick-borne diseases in Malaysia.
Ehrlichiosis

*Ehrlichia canis*, a ubiquitous Rickettsial pathogen of dogs, is the causative agent of canine monocytic ehrlichiosis, the most common and one of the most clinically significant tick-borne diseases of dogs in Malaysia. The earliest published study on *E. canis* in Peninsular Malaysia conducted in the early eighties, revealed a prevalence of 0.2% in owned dogs (Rajamanickam, C., et. al., 1985). In a survey conducted between 1986 and 1995, on average, 58 cases per year were reported at one private clinic in Selangor (Yeoh, E.C., et. al., 1996). There has only been one other published study that determined a serological prevalence rate of 15% in dogs in Perak state using the IFAT (Rahman, W.A, et. al., 2010). In the recent past, numerous unpublished studies have revealed prevalence rates from as low as 0% (Yap, B.K., 2004) to 3.3% (Toh, P.Y., 2002; Yeoh, W.H., 2009) in owned dogs (Figure 2) and 0% (Lim, C.W., 2007) to 1.8% (Yeoh, W.H., 2009) in stray dogs (Figure 3). Molecular diagnostic methods, namely PCR, were recently established for the detection of *E. canis* in Malaysia. The most recent molecular prevalence study revealed 1.2% of owned dogs and 3.4% stray dogs infected with *E. canis* (Nazari, M. et. al., 2011). However, interestingly, efforts to isolate the organism from ticks recovered from dogs have thus far been unsuccessful (Anurrdin, S. H., 2010).

Babesiosis

Babesiosis is also a common tick-borne disease of dogs in Malaysia causing severe clinical disease, often culminating in death. Previous studies have reported *Babesia canis* prevalence rates ranging from 0% (Lim, C.W., 2007) to 4.29% (Yap, B.K., 2004) in owned dogs (Figure 2) and 1.82% (Yeoh, W. H., 2009) to 10.0% (Lim, C.W., 2007) in stray dogs (Figure 3). As these studies identified the *Babesia* spp. based solely on their morphological appearance in erythrocytes it is uncertain whether it was indeed *B. canis* and not some other large *Babesia* spp. that were detected. However, a recent unpublished study using PCR-based detection methods, revealed 2.85% of stray dogs infected with the large *Babesia* and upon analysis of the 18SrRNA gene sequence, it was found to be most similar to *Babesia vogeli* (Zulkifli, R., 2011). *B. gibsoni* prevalence rates seemed to have declined over the years in both stray and owned dogs from as high as 17.7% in stray dogs in 1985 (Rajamanickam, C., et. al., 1985) to 2.85% in the most recent study in 2011 (Zulkifli, R., 2011). Despite this, just the fact that *B. gibsoni* is still present within the canine population is a source of concern due to the challenges associated with treating infected dogs effectively.
Anaplasmosis and Hepatozoonosis

In Malaysia, the observation of *Anaplasma platys* and *Hepatozoon canis* in canine blood has been mostly incidental and both these tick-borne pathogens have as yet not been reported to cause serious disease in dogs. *A. platys* prevalence rates have been as high as 18.3% in stray dogs (Toh, P.Y., 2002) [Figure 3] and 2.5% in owned dogs (Yap, B.K., 2004; Lim, C.W., 2007) [Figure 2]. A recent study revealed a molecular prevalence rate of 1.2% in owned dogs and 10.73% in stray dogs (Nazari, M. et al., 2011). The relatively high prevalence rate but low number of clinical cases, makes one wonder about the possible role *Anaplasma platys* may be playing in co-infections.

*Hepatozoon* prevalence rates have been surprisingly high whereby 15% of stray dogs were found to be infected in a study conducted in 2007 (Lim, C.W., 2007). However, as with the other tick-borne diseases prevalence rates have fortunately decreased over the years.

Conscientious efforts must be made to investigate vector-borne diseases as they are endemic in Malaysia. This is also essential in light of the growing number of emerging and re-emerging vector-borne diseases reported worldwide. With increased urbanization, there is also greater contact between wildlife reservoir hosts, companion animals and humans leading to the risk of zoonotic disease transmission. Furthermore, the increased popularity and mobility of pets increases the risk of disease transmission from companion animals to humans and also the risk of introduction of new diseases in areas where they have previously not been reported. Thus, it is hoped that in the future more energy and resources will be geared towards detecting and monitoring these diseases to provide a more accurate picture of the current situation of canine tick-borne diseases in Malaysia.

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Epidemiology of Ticks and Tick-borne Diseases in Carnivores and Humans in Thailand

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Abstract

Ticks and tick-borne infections are global medical and veterinary public health concerns. Thailand has economically and environmentally changed markedly over the past several decades. The magnitude of free roaming carnivores such as dogs and cats is still represents a considerable problem. Stray animals serve not only as reservoirs for infectious diseases to all pet but also as carriers for zoonotic diseases to humans since they are sharing their environment. Ticks play a significant role in the bridge between reservoir hosts including companion animals and humans. Zoonotic tick-borne diseases are the result of complex and variable interactions, subject to biogeographically rules and human and animal activity. Therefore, the combination of a sizeable population of stray dogs and cats and favorable climatic conditions promote the transmission of arthropod-borne organisms in animals. Tick-transmitted infections associated with protozoa, Rickettsiae, and bacteria are prevalent in Thailand. Babesia canis, Bartonella spp., Ehrlichia spp., Hepatozoon canis, HaemoBartonella spp., and Mycoplasma spp. Ehrlichia infections including E. chaffeensis, E. canis, and Anaplasma phagocytophila, also infect humans and dogs likely contribute to the enzootic cycle and human infection. Whereas many of the tick-borne diseases considered involve dogs as the major host or potential reservoir, the cat play a crucial role in the epidemiology of bartonellosis, especially cat-scratch disease with a variety of clinical syndromes in humans. The large populations of stray cats provide an excellent environment for the dissemination of infections between cats and from cats to humans because cats serve as reservoirs of Bartonella infection. Moreover, feline haemoplasmosis is also found in stray cats and might be predisposing caused of anemia in cats. As long as the intensification of the relationship between humans and companion animals, and the increase of tick population, the risk of zoonotic tick-borne diseases emerging becomes greater.
Introduction

Many of the most serious infectious diseases in the world are transmitted by blood-sucking arthropod vectors including flies and ticks. Arthropod-borne infections affected dogs and cats can cause serious diseases in humans, notably babesiosis, ehrlichiosis, bartonellosis and filariasis. The increasing prevalence of arthropod-borne diseases of pets and their owners has been associated with increased accessibility of contaminated environments and increase the reservoir population such as stray animals\(^1\).

Thailand is located in Southeast Asia and lying within the tropical zones thus boasting annual rainfalls of more than 1,500 mm and annual minimum temperatures between 25\(\text{C}\) to 35\(\text{C}\) with average humidity more than 50% throughout the year. As a result, Thailand provides an excellent habitats and microclimates which favor the survival and multiplication of blood-feeding arthropods such as ticks, mites, lice, fleas, and flies. Therefore, Thailand is afflicted by some tropical zoonotic infections including parasitic infections and many of them are arthropod transmitted. The brown dog tick, \textit{Rhipicephalus sanguineus} is the most common widely distributed tick, found on dogs\(^2\) and cats in Thailand, the main vector of parasitic diseases, and also parasitizing humans\(^3\).

The modernized socio-economic status has resulted in significant urbanization and industrialization that has influenced in pet ownership and ethic regarding pet care. The increasing of pet population are also correlated with the population of stray animals due to the economic recession. Stray animals are left without proper care and leading to the ectoparasites' reproduction so that this will place humans at a greater risk for exposure to pet's parasites and pathogens. Children are at a higher risk than adults, due to their closer physical contact with pets and their own habits. Although dogs and cats can be kept as social companions and pets, the relationship between humans and these pets is complicated and varies enormously according to the specific culture, status, social interests, religious conviction and affluence of individuals.

Tick control is conventionally performed by chemical acaricides that have many unpleasant effects such as toxicity to nearby animals, humans, and environment and an emerging resistant tick generation. An alternative control such as anti-tick vaccine and medicinal plant is required and urgently needed. A few candidate proteins are identified and proved to be potential for vaccine development in Thailand\(^4\)-\(^6\).

**TICK-BORNE DISEASES OF COMPANION ANIMALS IN THAILAND**

Mosquitoes are responsible for more diseases than any other arthropods in human tropical diseases and ticks are recognized as the second most for pathogens transmission. Ticks are notorious vectors of a variety of agents that cause zoonotic infections, including viruses, bacteria, and protozoans. Ticks are the most important vectors of diseases and most prevalent ectoparasites found in dogs and cats\(^7\)-\(^8\). \textit{R. sanguineus} are responsible for transmission of the canine and feline \textit{Babesia}, \textit{Ehrlichia}, \textit{Rickettsiae}, and other bacterial diseases\(^9\)-\(^12\). The life cycle of tick-borne is greatly influenced by environmental temperature and relative humidity. Tick-borne diseases are the result of complex and variable interactions, subject to biogeographical rules, and animal and human activity. As might be predicted, the combination
of a sizeable population of stray dogs and cats, and favorable climatic conditions promote the transmission of vector-borne organisms in companion animals in Thailand. As the ectoparasite population increases, the associated risk of infestation of non-host species become greater and human members of the household may be targeted.

Babesiosis

Babesiosis in companion animals is caused by *Babesia canis* or *B. gibsoni*, tick-transmitted parasites, frequently occurred in dogs and cats. Canine babesiosis is one of the most important arthropod-borne, protozoan haemoparasitic diseases and considered endemic throughout Thailand. Babesiosis of dogs is routinely found with the acute sign of fever, anemia, and the record of tick infestations. In Thailand, the prevalence of *Babesia* infection in pet dogs was 3.8% around Bangkok areas by using staining and light microscopic examination (ME) in 1991. However, ME probably might yield the false negative results in some condition due to the low parasitaemia.

Molecular diagnosis have been used to detect *Babesia* infections as screening test since this technique has the better sensitivity and specificity than of ME. The polymerase chain reaction amplification (PCR) of 18S rRNA gene is performed with modification and optimization of selected PCR parameters. Molecular genotyping have allowed to specify speciation of *Babesia* spp. that is not possible to differentiate by morphological characteristics or ME. From 2002 to 2003, *B. canis* infections were identified in 300 stray dogs by PCR in 17 districts of Bangkok metropolitan areas (Fig. 1b) and sequences of *B. canis* isolation in Thailand was 99% identical to reference *B. canis*.

However, *B. gibsoni* was not detected in these stray dogs.

Three subspecies of *B. canis* has been identified so far. *B. canis vogeli* is transmitted by *Rhipicephalus sanguineus* in tropical and subtropical countries, *B. canis canis* is transmitted

![Figure 1 - Distribution of zoonotic arthropod-borne diseases of dogs and cats in Bangkok, Thailand](image)
by Dermacentor reticulatus in Europe and B. canis rossi is transmitted by Haemaphysalis leachi in Africa. There is a difference of pathogenicity and severity between three groups of B. canis. Different infections of B. canis affects protective immunity because there was no cross protection between subspecies.

In 2010, blood samples were collected from 1,490 stray cats residing in 140 monasteries of 50 metropolitan districts of Bangkok (Fig.1a), and assayed with light microscopy and PCR for evidence of Babesia spp. Pear-shaped merozoites were observed microscopically from two (0.13%) of these cats, while a nested 18S rDNA-based PCR assay detected B. canis vogeli infections in 21 (1.4%) of the cats tested. The prevalence of infection was found in 30% (15/50) of the Bangkok areas (Fig.2).14

Ehrlichiosis

Ehrlichiosis is one of the most clinically significant diseases of pet dogs in Thailand and caused by intracellular Rickettsial organisms, Ehrlichia spp. Ehrlichia organisms are members of the family Rickettsiaceae and are Gram negative, intracellular, pleomorphic bacilli. The geographical distribution of ehrlichiosis has expanded with the distribution of the brown dog tick, R. sanguineus. Ehrlichia spp. that infects dogs, including E. chaffeensis, E. canis, E. ewingii, and Anaplasma phagocytophila, also infect humans and dogs likely contribute to the enzootic cycle and human infections.

Detection of Ehrlichia’s morulae by ME currently represents the gold standard for the diagnosis of ehrlichiosis; however, this approach is presumably insensitive and fails to define the infecting Ehrlichia spp., which may have important clinical and zoonotic implications. The advent of DNA-based molecular technology has greatly facilitated more advance research on ehrlichiosis. Three genogroups of Ehrlichia have been identified by phylogenetic analysis using 16S rRNA gene.

Canine Monocytic Ehrlichiosis is caused by E. canis has been reported throughout the world, but is particularly
prevalent in tropical regions. The infection is mainly transmitted by *R. sanguineus*. In Thailand, canine ehrlichiosis is mainly caused by *E. canis* and is responsible for widespread in pet and stray dogs as well as cats\textsuperscript{15-19}. The other *Ehrlichia* spp. such as *E. chaffeensis* causes human monocytic ehrlichiosis and human granulocytic or granulocytotropic ehrlichiosis is caused by *A. phagocytophila*. The prevalence of ehrlichiosis in pet dogs in Bangkok areas was 14.1% by ME. There was also a report of Ehrlichiosis in cats in Bangkok in 1993\textsuperscript{8}. In 2003, positive rates against *E. canis*, *E. chaffeensis* and *A. phagocytophila* were 22, 22 and 11%, respectively by indirect fluorescence antibody assay (IFA) [Fig. 1d,f,h]\textsuperscript{16}. Most seropositive dogs showed higher titers against *E. canis* than the other two agents and appeared to be the most prevalent species that infects dogs in Bangkok. However, some dogs had higher titers against *E. chaffeensis* than that of *E. canis*, indicating that this important agent of human ehrlichiosis is enzootic or that a pathogen related to *E. chaffeensis* may also infect stray dogs in Thailand. However, the role of dogs in acting as a zoonotic reservoir for human infection with *E. chaffeensis* is still questionable.

Canine Granulocytic Ehrlichiosis has been detected recently in Thailand and is caused by *Anaplasma phagocytophila* (former *E. phagocytophila*). In 2003, feline sera were taken from 287 stray cats in monastery around Bangkok, Thailand. Positive rates against *E. canis*, *E. chaffeensis* and *A. phagocytophila* were 4.9, 5.2 and 2.4%, respectively [Fig. 1c,e,g]\textsuperscript{15}. The antibody titers against *A. phagocytophila* were relatively lower than those of *Ehrlichia* species. Important agent of human ehrlichiosis is enzootic or that a pathogen related to *E. chaffeensis* may also infect stray cats in Thailand since *E. chaffeensis* appears to be the most prevalent agent that infects cats in Bangkok.

**Bartonellosis**

Whereas many of the arthropod-transmitted diseases considered involve dogs as the major host or potential reservoir, cats play a crucial role in the epidemiology of bartonellosis with a variety of clinical syndromes in humans, the best known of which is cat-scratch disease. *Bartonella henselae* are emerging pathogens in human, causing severe diseases in immunocompromised individuals. The warm climate and large populations of stray cats in Thailand provide an excellent environment for the dissemination of arthropod-transmitted infections between cats and from cats to humans because stray cats serve as reservoirs of *Bartonella* infection for transmission to household pet cats via fleas. *B. vinsonii* subsp. *berkhoffii* was isolated from dogs and clinically recognized by endocarditis, granulomatous lymphadenitis and peliosis hepatitis in humans.

In Thailand, the prevalence of *B. henselae* antibodies in 34 domestic cats that come to the Kasetsart Veterinary teaching hospital was 32.3% by using IFA test \textsuperscript{20} and the seroprevalence of *B. henselae* in human blood donors was 5.5\textsuperscript{21}. The prevalence of *Bartonella* infection was investigated in 275 cat samples collected in 1997 and 1998 from 8 areas of Thailand, overall, *Bartonella* species were isolated for 27.6% of cats examined. Most positive cats were found to be stray animals. *B. henselae* and *B. clarridgeiae* were isolated from 82.9% and 11.8% of *Bartonella*-positive cats, respectively\textsuperscript{22}. Coinfection with two species was also found in 5.3% of the bacteremic cats. These data suggest that stray cats represent an important reservoir of *Bartonella* infection for humans in
Thailand\textsuperscript{23–24}. In 2004, \textit{B. vinsonii} subsp. \textit{berkhoffii} was not isolated from all of 350 dog samples collected from Bangkok areas\textsuperscript{22} (Fig. 1i &j).

The presence of \textit{Bartonella} spp. infection in rodents has been reported in Southeast Asia including Thailand\textsuperscript{25}. The highest infected rodent species was found in \textit{R. tanezumi}, \textit{Mus cookii} and \textit{Rattus phylogenetic} in Thailand. Among 1,341 blood samples from 20 different animal species randomly collected, 143 \textit{Bartonella} species isolates by culture that were confirmed by real-time PCR technique targeting the 16S-23S rRNA intergenic spacer region. All 143 \textit{Bartonella} isolates were identified by PCR amplification and sequencing of partial \textit{gltA} gene, \textit{rpoB} gene and ITS region. Phylogenetic analysis of the isolates indicated the presence of several different known species including \textit{B. elizabethae} (7 isolates), \textit{B. cooperplainsensis} (6 isolates), \textit{B. phoceensis} (1 isolate), \textit{B. queenslandensis} (33 isolates), \textit{B. rattimassiliensis} (42 isolates), \textit{B. tribocorum} (11 isolates) and three new putative \textit{Bartonella} species. The overall, \textit{Bartonella} infections were 11\% and found in 14 different rodent species, one rodent genus and one shrew species\textsuperscript{25}.

\textbf{Mycoplasma (\textit{Haemobartonella SPP.}) Infections}

\textit{Mycoplasma haemofelis} (Mhf) and \textit{‘Candidatus Mycoplasma haemominutum’} (Mhm), commonly referred to feline hemoplasmas, are organisms formerly known as \textit{HaemoBartonella felis}. They are pleomorphic Gram-negative bacteria that parasitize on the surface of feline erythrocytes. Feline hemoplasmas infections cause hemolytic anemia, thrombocytopenia, fever and potentially fatal hemolytic crisis. These organisms are uncultivated bacteria pathogen and usually diagnosed through the microscopic examination of blood smears. A total of 1,488 blood samples were randomly collected from stray cats that lived in monasteries in 50 districts of Bangkok. Primers targeting on 16S rRNA gene that had been designed, were used for feline hemoplasma investigation. These primers were amplified for 170 bp and 193 bp of \textit{M. haemofelis} and \textit{‘Candidatus M. haemominutum’}, respectively. A total of 16.9\% (252/1,488) and 25.9\% (386/1,488) were positive for Mhf and Mhm, respectively and 1.6\% (24/1,488) for both species. Normally, cats is unable to eliminate the organism after they have been infected, increasing of ages will be increased the chance of infection for \textit{M. haemofelis} and \textit{‘Candidatus M. haemominutum’}. Bangkok was an endemic area for infection of \textit{‘Candidatus M. haemominutum’} and \textit{M. haemofelis}, because the high proportion of Bangkok district (98 - 100\%) was found positive detection\textsuperscript{26–27}.

\textbf{Hepatozoonosis}

Canine and feline hepatozoonosis is a protozoan disease of pet animals in tropical, sub-tropical and temperate regions all over the world. Blood samples were collected from 308 stray dogs and 300 stray cats residing in 30 metropolitan districts of Bangkok, and assayed with light microscopy and PCR for evidence of \textit{Hepatozoon canis} infection. Gamonts were observed in blood smears for 2.6\% of dogs and 0.7\% of cats tested with microscopy. Conversely, the PCR assay detected \textit{Hepatozoon} in blood from 11.4\% of dogs and 32.3\% of cats tested. The prevalence of infection was the same between male and female dogs or cats, and PCR-positive dogs and cats were found in 36.7\% and 46.7\% of the districts surveyed, respectively\textsuperscript{28}. 
FACTOR ASSOCIATED WITH TRANSMISSION OF COMPANION ZOONOTIC TICK-BORNE DISEASES IN THAILAND

The increased in incidence of canine and feline zoonotic tick-borne diseases might be mostly associated with the changes in human behaviors such as recreation and travelling (Fig. 3), the changes in ecology such as increased wildlife abundance (rodents), and the climatic changes such as more raining and flooding increased the tick survival rate. In Thailand, a number of stray animals is climbing due to no law enforcement to the irresponsibility owners. Thailand has the culture and Buddhist religion that contradict to the law and there are very difficult for authority to strictly perform. Eventually, stray animals might become causing more threatening in the near future since they will be the habitat or reservoir of ectoparasites including ticks.

The role of ticks in the epidemiology of zoonotic tick-borne diseases such as babesiosis and ehrlichiosis is well understood but, for diseases such as bartonellosis, and mycoplasmosis, their roles are still unclear.

Stray cats in Thailand are quite unique for the living styles and their roles as reservoirs. Most stray cats are found outdoor in monasteries and lived with the less care by monks or caretakers so that these cats might serve as reservoirs of Bartonellosis, Hepato-

zoonosis, Ehrlichiosis and Brugian filariasis for transmission to household pet cats via arthropod vectors. There are questions regarding the mechanism of transmission and what are the missing animals during transmission. In Asia, Bartonella infection have been investigated in different rodents from several countries. Therefore, rodents might play an important role in the transmission of pathogens to other animals or humans via arthropod vectors since they are reside or share the environment.

Many pathogens are difficult to detect or confirmed at present time without sophisticated tools such as molecular technique and some diseases are existed in a subclinical state, presenting new challenges associated with diagnosis to veterinarians. Despite the modernizations of veterinary practice and changing disease prevalence, it is crucial that veterinarians retain their professionals to the prevention and control of zoonoses in companion animals. Veterinarians should inform or educate animal owners for some zoonotic details caused by their own pets. To get more attention, Veterinarians have to repeat this message and consider this mission more seriously for not only a better health of animals but also for owners.
REFERENCES


Introduction
Vector-borne diseases in humans such as malaria, Japanese encephalitis and scrub typhus have been well known in Korea while vector-borne diseases in companion animals and their public health significance have been relatively under-recognized in this country. Especially, limited reports are available on the status of vector-borne disease transmission among dogs and cats. Recent reports suggest that arthropod-transmitted infections of dogs associated with protozoa, filaria, Rickettsia and bacteria are prevalent in the country. As global warming is affecting climate conditions of Korea, sub-tropical parasitic diseases such as malaria and leishmaniasis that have not been prevalent in South Korea are now emerging.

Among canine vector-borne diseases, the heartworm disease, caused by *Dirofilaria immitis*, is by far the most important and wide-spread internal disease of dogs in Korea. *Thelazia callipaeda* which causes ocular lacrimation in dogs and humans has been found in the country. Among protozoan parasites, *Babesia gibsoni* is a common pathogen which is transmitted to dogs by ticks and possibly by being bitten by infected dogs. Although the occurrence is rare, *Anaplasma* and *Ehrlichia* have also been reported in Korea. The etiologic agent of the Lyme disease, *Borrelia burgdorferi* has been identified from both humans and ticks. The development of clinical Lyme disease was reported in humans. Other pathogens that have not been found in Korea include *Babesia canis*, *B. vogeli*, *B. canis rossi*, *Bartonella vinsonii*, and *Hepatozoon canis*.

**TICKS IN KOREA**
As vectors for a number of viral and Rickettsial diseases of man and animals worldwide, ixodid ticks are noxious insects in Korea, too. They are involved in the transmission of *Theileria*, *Anaplasma*, *Borrelia*, *Rickettsia* and *Babesia* in humans and animals. A total of 32 species in 8 genera has so
far been found in Korea (Table 1).
A majority of ticks distributed in Korea is *Haemaphysalis longicornis* (Fig. 1 & 2).

**Ticks collected from dogs**

A total of 15 species in 6 genera has been collected from the body of dogs (Table 2). From a survey from April 2008 through May 2009, ticks were collected from domestic, stray, and military working dogs when they were examined at any of the four US Army veterinary treatment facilities within the Republic of Korea. Approximately 2,500 dogs were examined during this period, and a total of 411 were collected from 18 dogs. The collected species were *H. longicornis*, *H. flava*, *Ixodes nipponensis*, and *Rhipicephalus sanguineus*. *H. flava* and *I. tanuki* were also collected from a

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**Table 1: Ticks of Korea and their hosts**

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Argas</em></td>
<td><em>A. boueti</em> Roubaud &amp; Colas-Belcour, 1933</td>
<td>bat</td>
</tr>
<tr>
<td></td>
<td><em>A. japonicus</em> Yamaguti, Clifford &amp; Tipton, 1968</td>
<td>swallow</td>
</tr>
<tr>
<td></td>
<td><em>A. vespertilionis</em> (Latrella, 1802)</td>
<td>bat</td>
</tr>
<tr>
<td><em>Otobius</em></td>
<td><em>O. megnini</em> (Duges, 1884)</td>
<td>horse</td>
</tr>
<tr>
<td><em>Amblyomma</em></td>
<td><em>A. testudinarium</em> Koch, 1844</td>
<td>dog, cattle, horse, pig, deer</td>
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<tr>
<td><em>Boophilus</em></td>
<td><em>B. annulatus</em> Say (Say, 1821)</td>
<td>dog, cattle, horse, deer, chicken</td>
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<tr>
<td></td>
<td><em>B. microplus</em> (Canestrini, 1887)</td>
<td>dog, cattle, horse, deer</td>
</tr>
<tr>
<td><em>Dermacentor</em></td>
<td><em>D. coreus</em> Keegan &amp; Toshioka 1957</td>
<td>horse</td>
</tr>
<tr>
<td></td>
<td><em>D. marginatus</em> (Schulze, 1776)</td>
<td>cattle, sheep, goat, horse</td>
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<td></td>
<td><em>D. reticulatus</em> (Fabricius, 1794)</td>
<td>dog, cattle, horse, rabbit</td>
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<td></td>
<td><em>D. silvarum</em> (Olenov, 1903)</td>
<td>dog, cattle, horse, rabbit, mouse</td>
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<td><em>Haemaphasalis</em></td>
<td><em>H. campanulata</em> Warburton, 1908</td>
<td>dog</td>
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<td></td>
<td><em>H. concinna</em> Koch, 1844</td>
<td>cattle</td>
</tr>
<tr>
<td></td>
<td><em>H. cornigera</em> Neumann, 1897</td>
<td>cattle, field mouse</td>
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<td></td>
<td><em>H. flava</em> Neumann, 1897</td>
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<td></td>
<td><em>H. japonensis</em> Warburton, 1908</td>
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<td></td>
<td><em>H. japonica</em> douglasiei Nutt &amp; Warburton, 1915</td>
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<td></td>
<td><em>H. kutchensis</em> Hoogstral &amp; Trapdo, 1915</td>
<td>pheasant</td>
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<td></td>
<td><em>H. longicornis</em> Neumann, 1901</td>
<td>cattle, human, horse, rabbit</td>
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<td><em>Ixodes</em></td>
<td><em>I. cavi palp</em> Nuttal &amp; Warburton, 1908</td>
<td>cattle, goat, sheep</td>
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<td></td>
<td><em>I. granulatus</em> Supino, 1897</td>
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<td></td>
<td><em>I. japonensis</em> Neuman, 1904</td>
<td>hedgehog, rabbit</td>
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<td></td>
<td><em>I. monosinuos</em> Saito, 1967</td>
<td>human, goat</td>
</tr>
<tr>
<td></td>
<td><em>I. nipponensis</em> Kitaoka &amp; Saito, 1967</td>
<td>dog, cattle, human, deer</td>
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<td><em>I. persulcatus</em> Schulze, 1930</td>
<td>dog, cattle, human, horse</td>
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<td></td>
<td><em>I. ovatus</em> Neumann, 1899</td>
<td>dog, cattle, human, deer</td>
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<td></td>
<td><em>I. ricinus</em> (Linne, 1758)</td>
<td>dog, sheep, mouse</td>
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<tr>
<td></td>
<td><em>I. signatus</em> Birula, 1895</td>
<td>sea gull</td>
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<tr>
<td></td>
<td><em>I. tanuki</em> Satio, 1964</td>
<td>Korean raccoon dog</td>
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<tr>
<td></td>
<td><em>I. turdus</em> Naktsugi, 1942</td>
<td>field mouse, birds</td>
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<td></td>
<td><em>I. vespertilionis</em> Koch, 1844</td>
<td>bat</td>
</tr>
<tr>
<td><em>Rhipicephalus</em></td>
<td><em>R. sanguineus</em> (Latrielle, 1806)</td>
<td>dog</td>
</tr>
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</table>

Cho and Lee, 2004 [1]
Korean raccoon dog (Nyctereutes procyonoides koreensis) caught in Korea.

Ticks collected from humans
Depending on the geographic areas, the incidence and causative species of ticks are different. *I. nipponensis* was the most common causative species of ticks responsible for tick bites. Other common tick species of human bites in Korea include *I. ovatus, I. persulcatus, I. monospinosus, H. flava* (Fig. 3 & 4) and *H. longicornis*. Among these, *Borrelia burgdorferi*, the causative agent of the Lyme disease, was isolated from *I. persulcatus* collected from Chungju, Korea.

Table 2: Ticks collected from dogs in Korea

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
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<tbody>
<tr>
<td>Amblyomma</td>
<td><em>A. testudunarium</em></td>
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<tr>
<td>Boophilus</td>
<td><em>B. annulatus</em></td>
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<td><em>B. microplus</em></td>
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<td>Dermacentor</td>
<td><em>D. reticulatus</em></td>
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<td><em>D. silvarum</em></td>
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<td>Haemaphysalis</td>
<td><em>H. campanulata</em></td>
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<td><em>H. flava</em></td>
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<td><em>H. japonica</em></td>
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<td></td>
<td><em>H. longicornis</em></td>
</tr>
<tr>
<td>Ixodes</td>
<td><em>I. nipponensis</em></td>
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<td><em>I. persulcatus</em></td>
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<td><em>I. ovatus</em></td>
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<tr>
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<td><em>I. ricinus</em></td>
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<tr>
<td>Rhipicephalus</td>
<td><em>R. sanguineus</em></td>
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</table>

Excerpted from Yamaguti et al., 1971 [19]
Tick-borne pathogens identified from dogs

So far, only one dog has been diagnosed to have *Anaplasma* sp. in Korea. A 4-year-old female Shunauzer dog referred to the Veterinary Teaching Hospital of Chungbuk National University due to anorexia and depression showed PCR-positive to *A. platys*. Tetracycline and doxycycline medication for 3 months returned the condition of the dog to normal. The presence of ticks or other arthropods from the infected dog was not mentioned by the authors. However, epidemiologic studies indicate that dogs can be exposed to many tick-borne pathogens in Korea. In a serological survey for canine vector-borne diseases, *Dirofilaria immitis*, *Anaplasma phagocytophilum*, *Ehrlichia canis*, and *Borrelia burgdorferi* infections in rural hunting and urban shelter dogs mainly from southwestern regions of the Republic of Korea (South Korea) were compared. From a total of 229 wild boar or pheasant hunting dogs, the number of serologically positive dogs for any of the four pathogens was 93 (40.6%). The highest prevalence observed was *D. immitis* (22.3%), followed by *A. phagocytophilum* (18.8%), *E. canis* (6.1%) and the lowest prevalence was *B. burgdorferi* (2.2%). In contrast, stray dogs found within the city limits of Gwangju showed seropositivity only to *D. immitis* (14.6%), and none of the 692 dogs responded positive for *A. phagocytophilum*, *E. canis* or *B. burgdorferi* antibodies. This study indicates that the risk of exposure to vector-borne diseases in rural hunting dogs can be quite high in Korea, while the urban environment may not be suitable for tick infestation on dogs, as evidenced by the low infection status of tick-borne pathogens in stray dogs.

Canine babesiosis in Asia, North America, northern and eastern Africa and Europe is usually attributed to *Babesia gibsoni*, a tick-borne, intraerythrocytic parasite (Fig. 5). First reported in 1962, it is also the main causative agent of canine babesiosis in South Korea. Autochthonous canine babesiosis other than by *B. gibsoni* has not been reported in Korea. It is quite common to observe manifestation of clinical babesiosis following surgery or during immunosuppressive therapy that the disease may recur in dogs with latent infection with *B. gibsoni*. The most common dog breed recognized in Korea to harbor the organism is American Pit Bull Terriers. There is a strong association between having recently been bitten by another dog and infection with *B. gibsoni*. Seo et al. (1996) investigated the prevalence rate of *B. gibsoni* antibody in mixed-breed dogs, American Pit bull terriers, and military dogs by using IFA. The results indicated that 60 dogs (7.8%) in 774 hybrid dogs, 78 dogs (81.3%) in 96 Pit bull terriers, and 15 dogs (15.6%) in 96 military dogs (German Shepherd) were exposed to *B. gibsoni*.

**Tick vector for *B. gibsoni***

The tick vector for *B. gibsoni* was thoroughly studied by Higuchi group of Japan who extensively investigated the developmental stages of *B. gibsoni* in different organs of the vector tick, *H. longicornis*. Although no reports...
are available on the vector ticks for *B. gibsoni* in Korea, it is assumed that *H. longicornis* which is commonly found ticks throughout the Korean peninsula may well serve as the vector.

**Tick-borne pathogens identified from ticks**

Vector-borne pathogens such as *Ehrlichia chaffeensis*, *Anaplasma phagocytophilum*, and *A. bovis* were identified from *H. longicornis* and *I. persulcatus* ticks collected from nine provinces of Korea by TaqMan real-time PCR\(^2\). Amplification of a 16S rRNA gene fragment of *Ehrlichia* and *Anaplasma* species was observed in 364 tick DNAs (45.3% of the total). Of the 364 positive ticks, species-specific PCRs confirmed that 35 *H. longicornis* and one *I. persulcatus* were positive for *A. phagocytophilum* and one *I. persulcatus* was positive in *E. chaffeensis*.

In another study, five species of ticks in two genera (*Haemaphysalis* spp. and *Ixodes* spp.) collected from small wild-caught mammals or by dragging/flagging in Korea contained species-specific fragments of *A. phagocytophilum*, *A. platys*, *E. chaffeensis*, *E. ewingii*, *E. canis*, and *Rickettsia* sp. as evidenced by the PCR assay\(^3\). The presence of *Bartonella*-specific DNA was also demonstrated in *H. longicornis*, *H. flava*, *I. turdus*, *I. persulcatus* and *I. nipponensis* ticks which indicates that these ticks may be involved in the transmission of *Bartonella* sp. in Korea\(^4\).

Lyme disease spirochete, *Borrelia burgdorferi sensu lato*, was identified and characterized from the midgut suspensions of three *Ixodes* ticks and heart tissue from one mouse, *Apodemus agrarius*, collected from Chungbuk and Kangwon provinces\(^5\).

**Tick-borne pathogens identified from animals**

To assess the potential public health threat to U.S. Forces Korea, DNA extracted from spleen tissues of rodents that were collected at selected U.S. military installations and training sites in Korea was assayed by PCR for *Ehrlichia* and *Anaplasma* species\(^5\). *Ehrlichia*- and *Anaplasma*-specific DNAs were identified from the spleen tissues of *Apodemus agrarius* (striped field mouse, 157/196), *Mustela sibirica* (weasel, 1/1), and *Cricetulus riton nestor* (Korean greater long-tailed hamster, 1/1). Species-specific DNA fragments of *E. canis*, *E. ewingii*, *A. phagocytophila*, and *A. platys* were amplified by PCR techniques. The striped field mouse appears to play a major role as a reservoir for latent infections of various *Ehrlichia* or *Anaplasma* species in Korea. In another study, 424 spleen samples from small captured mammals (389 rodents, 33 insectivores, and 2 weasels) were screened for selected zoonotic pathogens from which species-specific DNA fragments of *A. phagocytophilum*, *A. platys*, *E. chaffeensis*, *E. ewingii*, *E. canis*, and *Rickettsia* sp. were amplified by PCR assay\(^3\). *Bartonella* infections in ticks, mites and small mammals collected from various military installations and training sites in Korea were also documented\(^4\). *Bartonella* spp. was identified in ticks (5.2% of 1,305 ticks), in mesostigmatid mites (19.1% of 21 mites) and in small mammals (13.7% of 424). Possible occurrence of tick-borne encephalitis in Korea was suggested by Kim et al (2008) by demonstrating the tick-borne encephalitis virus (TBEV) DNA in *H. longicornis* and *I. nipponensis* by RT-nested PCR\(^6\).

**Tick-borne pathogens identified from humans**

The presence of *A. phagocytophila* and *E. chaffeensis* was first indicated by Heo et al. (2002) who reported that 0.4% and 1.8% of sera from 491
Korean patients with acute febrile diseases serologically reacted with *E. chaffeensis* and *A. phagocytophilum* tests by IFA, Western blotting, and TaqMan realtime PCR, respectively. Also, serum samples from 271 Korean patients with symptoms of high fever were tested to detect antibodies against *A. phagocytophilum* and *E. chaffeensis* by IFA and Western blot assay. The presence of these pathogens was evidenced, because 24 (8.9%) and 29 (10.7%) of the sera reacted with *A. phagocytophilum* and *E. chaffeensis* antigens by the Western blot assays, respectively.

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Canine babesiosis in Taiwan

Introduction
Taiwan lies on the western edge of the Pacific Ocean and belongs to subtropical area. It enjoys warm weather all year round. Ticks and flea are the major observed ectoparasites. *Rhipicephalus sanguineus* is the most observed tick species in dogs. Tick-borne diseases, such as Ehrlichiosis and Babesiosis, are increasing in Taiwan. Canine babesiosis, which is caused by hemoprotozoan organisms of the genus *Babesia* that include both *Babesia canis* and *Babesia gibsoni*, is characterized by hemolytic anemia, fever, and splenomegaly, and mostly is a life-threatening disease in Taiwan. The prevalence of *B. canis* and *B. gibsoni* were 2.36% and 14.31%, respectively, from an investigation of stray dogs in northern Taiwan (Young’s master thesis from National Pingtung University of Science and Technology, 2007). In our teaching hospital, *Babesia gibsoni* infection is the mostly observed infectious disease, which results in anemia.

PRELIMINARY RESULTS OF A RETROSPECTIVE STUDY IN NATIONAL TAIWAN UNIVERSITY VETERINARY HOSPITAL (NTUVH)
1564 anemic dogs were presented at National Taiwan University Veterinary Hospital between February 2010 and August 2011, infectious diseases accounted for 10.36% (162/1564) with different pathogens, including *Babesia gibsoni, Dirofilaria immitis, Ehrlichia canis, Babesia canis, and Anaplasma platys*. The prevalence of *Babesia canis*, *B. gibsoni* and *Ehrlichia canis* were 1.8%, 51.2% and 14.8%, respectively. *B. gibsoni* revealed the predominated pathogen. The prevalence of *B. gibsoni* in...
male was higher than in female and overpresenting between 3 to 4 years old dogs. 38.6% of infected dogs have not received regular or none ectoparasite prevention. Only 25% of them have received regular ectoparasite prevention. In dogs infected by *B. gibsoni*, anorexia (86.3%), lethargy (81.54%), abnormal urine color (62.96%) and pale mucous membrane (59.3%) were the most common clinical signs. Fever presented in only 34.3% of the infected dogs. Splenomegaly (65.79%) and hepatomegaly (63.16%) were noted in image examination. The major hematological abnormalities included thrombocytopenia (65.52%) and macrocytic hypochromic anemia (23.86%). The distributions of mild (PCV≥25, <37), moderate (PCV≥15, <25) and severe (PCV<15) anemia were 28.2%, 45.9% and 25.9%, respectively.

**DIAGNOSIS**

Most of the infected dogs present mild to severe anemia. The confirmed diagnostic methods of *Babesia* spp. includes direct blood smear examination and PCR test. We used multiplex-nested PCR to detect *B. spp* infection and further differentiate between the different genotypes (*B. gibsoni* Asian genotype, North American genotype and *B. canis*). More than 100 cases that were genotyped for *Babesia* in the NTUVH lab between 2005 and 2010 were identified as the *B. gibsoni* Asian genotype, suggesting that the *B. gibsoni* Asian genotype is the major pathogen responsible for canine babesiosis in Taiwan.

**TREATMENT**

Various combination strategies for treating *Babesia gibsoni* have been described. However, relapses after administering some combinations of anti-*Babesia* drugs and the presence of drug-resistant *B. gibsoni* still pose significant challenges to veterinarians, especially Asian genotype. Most studies have showed that no single drug is sufficient for the treatment of this disease. Therefore, drug combinations appear to be a better choice for treating *B. gibsoni* infections. We have published a prospective study to compare the efficacy of a combination of clindamycin, diminazene, and imidocarb [CDI] to that of a combination of atovaquone and azithromycin [AA] for the treatment of *B. gibsoni*. The dogs in the CDI group exhibited higher recovery rates and lower relapse rates during treatment for *B. gibsoni*. In the same study, the M121I mutation in the *B. gibsoni* CYTb gene was detected in all AA-relapse and AA-nonremission dogs but not in CDI-relapse dogs, which demonstrates that the M121I mutation is associated with atovaquone resistance.
No vaccine for *Babesia gibsoni* is available. Prevention for ectoparasites exhibits the most effective methods at present in Taiwan. Owners of infected dogs were inquired by an unofficial questionnaire regarding on the time period that they observed the ectoparasites again, and the results was about 20 to 28 days in summer after the commercialized ectoparasital agent was applied. Therefore, ectoparasital agent is suggested to apply every 3 weeks.

**PREVENTION**

No vaccine for *Babesia gibsoni* is available. Prevention for ectoparasites exhibits the most effective methods at present in Taiwan. Owners of infected dogs were inquired by an unofficial questionnaire regarding on the time period that they observed the ectoparasites again, and the results was about 20 to 28 days in summer after the commercialized ectoparasital agent was applied. Therefore, ectoparasital agent is suggested to apply every 3 weeks.

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**Table 1: Efficacy of different drug combinations in treatment of *Babesia gibsoni***

<table>
<thead>
<tr>
<th>Drug combinations</th>
<th>Recovery rate</th>
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<td>DCM</td>
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CDI: Diminazine 3.5mg/kg IM, a single dose on the day of presentation, Imizol 6mg/kg, a single dose on the day after the diminazine was administered, Clindamycin 30mg/kg BID - C: Clindamycin 30mg/kg BID - AA: Atovaquone 13.3mg/kg q8h, Azithromycin 10mg/kg q24h - DC: Diminazine 3.5mg/kg IM, a single dose on the day of presentation, Clindamycin 30mg/kg BID - DCM: Diminazine 3.5mg/kg IM, QW for 2 weeks, Clindamycine15mg/kg BID, Metronidazole 5 mg/kg BID.
Dynamics of pathogen transmission by ticks with particular reference to *Ehrlichia canis*

**Frans Jongejan**

Director of the Utrecht Centre for Tick-borne Diseases (UCTD) at the Faculty of Veterinary Medicine in the Netherlands and holds an extra-ordinary professorship at the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria in South Africa.

His main research interests concern tick transmission studies with particular reference to tick-borne pathogens of dogs. In these studies, he uses *in vitro* feeding models, wherein ticks attach and successfully feed on artificial membranes.

He is a consultant to the pharmaceutical industry and has published 199 papers on ticks and tick-borne diseases.

**Introduction**

A broad range of protozoan and bacterial pathogens is transmitted through bites of infected vector ticks feeding on dogs (Chomel, 2011). Pre-eminent tick-borne diseases of dogs are in particular, canine monocytic ehrlichiosis (*Ehrlichia canis* infection), babesiosis (*Babesia* spp.), granulocytic anaplasmosis (*Anaplasma phagocytophilum* infection) and *Borrelia burgdorferi* infection (Shaw et al., 2001; Jongejan and Uilenberg, 2004).

**CANINE MONOCYTIC EHRLICHIOSIS**

The Rickettsial pathogen *Ehrlichia canis* has a worldwide distribution coinciding with the vector tick, *Rhipicephalus sanguineus*. The vast majority of cases of canine monocytic ehrlichiosis (CME) are therefore seen in (sub-)tropical regions which are hospitable to *R. sanguineus* ticks (Fig.1). *Ehrlichia canis* is transstadially transmitted (from larvae to nymphs...
or from nymphs to adults) but also intrastadially transmitted through male *Rhipicephalus sanguineus* ticks (Bremer et al., 2005). Due to the strict host specificity of the tick, dogs serve both as a reservoir and a domestic animal host for *E. canis* (Stich et al., 2008). In dogs, *Ehrlichia canis* develops in monocytes and macrophages, whereas in ticks the infection can be localized in midgut and salivary glands (Fig. 2). Pathology of CME is characterized by thrombocytopenia, leukopenia, fever, depression and bleeding tendencies (Harrus et al., 1999; de Castro et al., 2004).

**DYNAMICS OF TICK TRANSMISSION**

Information on the transmission dynamics of pathogens by ticks is rather limited being surprisingly scanty documented for canine tick-borne pathogens, including *E. canis*. In general, the duration of attachment that is required for ticks to transmit vary according to the bacterial, protozoan, or viral nature of the pathogen concerned. Some information is available on the transmission dynamics of *Borrelia* spirochetes which appears to occur only rarely within the first 24 hours after tick attachment, whereas in the same period for instance most *Anaplasma phagocytophilum* bacteria have already been transmitted. Furthermore, most viral pathogens can be transmitted very fast even within minutes after attachment of the infected vector tick. Finally, protozoan parasites require additional time, usually several days, for their sporoblasts to mature into sporozoites in the tick salivary glands before they can be secreted into the saliva and transmitted to the mammalian host. It has been reported for example that *Babesia microti* is transmitted by *Ixodes scapularis* ticks between 36 and 48 hours after tick attachment, while the attachment duration required for *Rhipicephalus appendiculatus* ticks to transmit *Theileria parva* is at least 72 hours.

**PREVENTION OF PATHOGEN TRANSMISSION**

Relatively little research has been carried out to determine the ability of tick control products to prevent transmission of tick-borne diseases to dogs. Some studies suggest that topically applied tick control compounds can aid in the prevention of transmission of specific tick-borne pathogens. Most published studies have however aimed to demonstrate the acaricidal efficacy of a particular compound against a range of ixodid tick species found on dogs. This is also reflected in the current guidelines for evaluation of efficacy of ectoparasiticides which focus on treatment, prevention and control of ticks only (Marchiondo et al., 2007).
As far as the prevention of CME concerned, field studies conducted in West Africa revealed that fipronil on itself could reduce the transmission of *E. canis* to susceptible dogs (Davoust et al., 2003). Furthermore, application of a combination of 10% imidacloprid/ 50% permethrin reduced *E. canis* exposure in dogs under field conditions in southern Italy (Otranto et al., 2008). In addition, the ability of fipronil to prevent transmission of *Borrelia burgdorferi* by field-collected *Ixodes scapularis* ticks, was already reported several years ago (Jacobson et al., 2004).

**MODELS FOR BLOCKING OF PATHOGEN TRANSMISSION**

Recently, the ability of CERTIFECT® (Merial, GA, USA), a novel combination of fipronil, amitraz and (S)-methoprene in a spot-on formulation was shown to protect dogs from *Borrelia burgdorferi* and *Anaplasma phagocytophilum* infections transmitted by field-collected *I. scapularis* ticks (McCall et al, 2011). Importantly, a transmission blocking model has recently been developed wherein experimental batches of infected ticks were generated under laboratory conditions. This model concerned the transmission blocking of *Babesia canis* by infected *Dermacentor reticulatus* ticks. It was shown that CERTIFECT® applied up to 28 days prior to infestation with *Babesia canis* infected *Dermacentor reticulatus* ticks successfully prevented the development of canine babesiosis (Jongejan et al, 2011). Subsequently, a similar approach has been used to demonstrate prevention of transmission of *E. canis* by infected *R. sanguineus* ticks. This model includes, in addition to a standardized infection and challenge load with *Ehrlichia canis*-infected ticks, one or more treatment-groups to test the duration of preventive activity. Both *B. canis* as well as *E. canis* transmission blocking models can further be employed to test various, novel combinations of tick control compounds. Significantly, adaptation of these studies to the blocking of transmission of other tick-borne pathogens, such as *Babesia vogeli* transmitted by *R. sanguineus* ticks, or *Anaplasma phagocytophilum* transmitted by *Ixodes* ticks, has now become feasible.

**IN VITRO MODEL**

The dynamics of pathogen transmission *in vivo* can be compared to *in vitro* feeding assays, initially developed for ixodid ticks by Kröber and Guerin (2007). The methods originally developed for *in vitro* feeding of adult *I. ricinus* ticks, have now been successfully adapted in the Utrecht Centre for Tick-borne Diseases to meet the requirements of a range of other ixodid tick species, such as *Ixodes hexagonus*, *Dermacentor reticulatus*, and also *R. sanguineus* (Fig. 3). Surprisingly, for instance, when infected *R. sanguineus* ticks (Fig. 4) were evaluated in the *in vitro* feeding model, transmission of *E. canis* was detected already within 8 hours after tick attachment.

Fig. 3. Adult *Rhipicephalus sanguineus* ticks attached and feeding through a silicone membrane can either acquire *E. canis* from an infected blood meal or transmit *E. canis* to non-infected blood. There is a clear tendency of ticks to cluster (photograph: Utrecht Centre for Tick-borne Diseases)
CONCLUSION

The early events after tick attachment which lead to the actual transmission of pathogens are crucial and need to be studied in further detail in order to optimize the blocking of pathogen transmission. It will only be a matter of time before guidelines for evaluation of efficacy of ectoparasiticides will include specific recommendations regarding the use of models wherein blocking of pathogen transmission by specific acaricides can be demonstrated. Finally, in vitro feeding models will be of great value in understanding the precise kinetics of pathogen transmission, which may already be initiated within hours after attachment of a tick.

Acknowledgements

Research on the dynamics of *Ehrlichia canis* transmission using *in vitro* feeding of ticks is conducted at the Utrecht Centre for Tick-borne Diseases (UCTD) at Utrecht University supported by Merial SAS, France. Special thanks are due to Pr. Frédéric Beugnet for his continued and stimulating interest in these studies.

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Clinical Aspects of the main canine *Rhipicephalus*-borne diseases: Monocytic Ehrlichiosis, Thrombocytic Anaplasmosis and Hepatozoonosis

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His research interests focus on the pathogenesis, diagnosis and treatment of veterinary and zoonotic vector-borne infectious diseases including leishmaniosis, canine ehrlichiosis, babesiosis, hepatozoonosis, trypanosomiasis and dirofilariasis. Prof. Baneth is involved in the study of zoonotic and veterinary diseases in the Mediterranean Basin, Uzbekistan, Ethiopia and South America funded by the European Union and other international research agencies. He is the author of more than 140 scientific publications and book chapters.
Introduction
Pathogens transmitted by blood-sucking arthropods such as ticks, fleas, sand flies and mosquitoes cause some of the most widespread and severe diseases of animals and humans. Dogs are affected by a wide spectrum of virus, bacteria, protozoa and helminth pathogens transmitted by arthropod vectors. Transmission of pathogens is often mediated via the vector’s saliva during the course of a blood meal, however, other modes of vector transmission can occur. When referring to transmission of pathogens by ticks such as *Rhipicephalus sanguineus*, ingestion of the vector by the canine host as in hepatozoonosis, or transovarial transmission via the tick’s egg as in some species of *Babesia*, may occur. STUDY 1: Tick efficacy of the fipronil + amitraz combination at different concentrations of amitraz.

**EHLRICHA CANIS**

Etiology and epidemiology
*Ehrlichia canis*, the etiologic agent of canine monocytic ehrlichiosis, has been recognized worldwide as an important canine infectious agent (Harrus and Waner 2011). It has been reported from Africa, Asia, America, and Europe. Autochthonous (non-imported) cases of *E. canis* in Europe have been reported mostly from Spain, Portugal, Southern France, Italy, the Balkans, Turkey and Greece. Serologic evidence of canine *E. canis* infection has been reported from Japan since the 1990’s (Inokuma et al., 1998) with the first PCR confirmation of infection in a dog in 2001 (Suto et al., 2001).

*Ehrlichia canis* morulae found in monocytes and macrophages are a «microcolony» of bacteria surrounded by a membranous vacuole. Morulae may contain 100 or more *Ehrlichiae* organisms. *Ehrlichia canis* is transmitted by the three-host tick *Rhipicephalus sanguineus*. The pathogenesis of the disease involves an incubation period of 8-20 days, followed by 3 consecutive phases: an acute phase which lasts 1-4 weeks, a subclinical phase which may last from months to years, and a chronic phase. Not all infected dogs develop the chronic severe form of the disease and the conditions that lead to the development of this stage are unknown (Cohn 2003; Harrus and Waner 2011). *Ehrlichia canis* can also be transmitted by blood transfusion and it is recommended to screen for its presence in the blood of donor dogs.

Clinical findings
The clinical presentation of the disease caused by *E. canis* may vary (Harrus et al., 1997; Frank and Breitschwerdt 1999; Cohn 2003; Gaunt et al., 2010; Harrus and Waner 2011), and the clinical signs most frequently reported are lethargy, anorexia, fever, lymphadenomegaly, splenomegaly and hemorrhages, mainly petechiae, ecchymoses and epistaxis. Ocular manifestations of canine ehrlichiosis include anterior uveitis, keratoconjuctivitis, hyphema, glaucoma, chorioretinitis and retinal detachment. Polyarthritis and polymyositis have also been described in *E. canis* infection. The neurological abnormalities found in canine ehrlichiosis are associated with vasculitis, meningoencephalitis, and lymphocytic infiltration of the central and peripheral nervous system or hemorrhages. *Ehrlichia canis* infection has been termed by some clinicians as the «silent killer». It is often not apparent during the early and subclinical stages of infection, and when the disease is diagnosed in the chronic stage, it may be too late to save the canine patient, as treatment may not be helpful in reversing the severe
pancytopenia caused by bone marrow suppression associated with this disease.

Laboratory abnormalities in canine monocytic ehrlichiosis include hematologic and serum biochemistry changes [Harrus et al., 1997; Frank and Breitschwerdt 1999; Cohn 2003; Harrus and Waner 2011]. Thrombocytopenia is the most frequent hematological abnormality occurring in more than 90% of cases. Anemia, usually non-regenerative normocytic and normochromic, is another common finding in this disease. In addition, mild to severe leucopenia is a frequent abnormality. Hyperglobulinemia, hypoalbuminemia and mild elevation of alkaline phosphatase (ALP) and alanine aminotransferase (ALT) activities are frequently reported in ehrlichiosis. Dogs in the chronic severe stage of the disease may develop severe pancytopenia as their bone marrow becomes hypocellular. The prognosis of these chronically ill dogs is grave.

Immune-mediated responses play a major role in the pathogenesis of *E. canis* infection. Anti-platelets antibodies have been demonstrated less than a week after experimental *E. canis* infection of dogs. Platelet aggregation abnormalities, anti-nuclear antibodies, RBC autoagglutination with positive Coombs’ test, and circulating immune-complexes have been shown in infected dogs and are associated with the disease process [Harrus and Waner 2011].

The decrease in platelets during canine ehrlichiosis is a result of several mechanisms. These mechanisms include increased consumption with vascular endothelial changes, platelet sequestration and pooling in the spleen, thrombophagocytosis with immunological destruction, a decrease in the half life time of circulating platelets possibly due to opsonization with antibodies, and production impairment due to bone marrow destruction and hypocellularity. In addition to the decrease in circulating platelet number, platelets dysfunction (thrombocytopathy) has also been implicated as an additional factor contributing to lack of platelet functionality in canine monocytic ehrlichiosis.

**Diagnosis**

The laboratory diagnosis of *E. canis* infection includes evaluation of the hemogram and serum biochemistry panel [Cohn 2003; Harrus and Waner 2011]. The detection of morulae in monocytes in stained blood smears is rare and can not serve as a main diagnostic option (Figure 1). Detection of the presence of *E. canis* DNA by PCR is highly sensitive and specific and has become the most useful diagnostic test for the confirmation of canine ehrlichiosis. Several conventional and real-time PCR protocols have been described for *E. canis* and the assay can be performed on blood or tissue including the spleen and bone marrow [Baneth et al., 2009; Gaunt et al., 2010; Harrus and Waner 2011].

Anti-*E. canis* antibodies persist long after recovery from the disease. Serum antibodies are thought not to be protective or play an important role in eliminating this intracellular infection. Serology is indicative of exposure to *E. canis* and may often

![Figure 1 - Ehrlichia canis morula in the cytoplasm of a monocyte from the blood of a naturally-infected dog (x100 magnification)](image-url)
be helpful in ruling out progressive infection. Antibodies may not be detectable during the early stage of infection. However, seropositive dogs with previous exposure to the pathogen may also present due to other urgent disease conditions.

**Treatment**

Ehrlichia canis is susceptible to doxycycline which is highly efficient in clearing rickettsemia in acute cases of *E. canis* infection (Cohn 2003). Clinical recovery is noticed within 48-72 hours, yet treatment should be continued for 3 weeks, as some dogs may remain carriers when shorter treatments are applied. Treatment with the injectable drug imidocarb dipropionate has been shown to be ineffective in totally eliminating *E. canis*. However, it is often used in combination with doxycycline when *Babesia* co-infection is suspected.

**Prevention**

The control of tick infestation by topical treatment with acaricidals and environmental eradication of ticks is recommended for the prevention of *E. canis* infection. No commercial vaccine against *E. canis* infection is currently available.

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**ANAPLASMA PLATYS**

**Etiology and epidemiology**

*Anaplasma platys* (previously known as *Ehrlichia platys*) was first identified in 1978 in Florida (Harvey et al., 1978). It is a Gram-negative, obligate intracellular bacterium belonging to the family *Anaplasma taceae* and closely related to *Anaplasma phagocytophilum* and *Anaplasma marginale*. *Anaplasma platys* infects canine platelets and causes a disease...
commonly recognized as infectious canine cyclic thrombocytopenia. The presumed natural vector of *A. platys* is the tick *R. sanguineus* and DNA of this bacterium has been reported from this tick species in several countries, however, experimental infection studies have not demonstrated transmission by *R. sanguineus* conclusively (Simpson et al., 1991; Gaunt et al., 2010). Like *E. canis*, *A. platys* may also be accidentally transmitted by blood transfusion (Simpson et al., 1991).

*Anaplasma platys* has been reported from Europe, Asia, Australia and the Americas. DNA of *A. platys* has been reported in blood of dogs from or in ticks from a large number of countries including Brazil, Chile, Argentina, Greece, Israel, France, Spain, Portugal, Taiwan, China, Japan, Thailand, Venezuela, Australia, Congo and the United States. Dog infection in the United States was reported from several states including: Florida, Louisiana, Mississippi, Texas, Arkansas, North Carolina, Pennsylvania, Illinois, Idaho, and California. In Japan, *A. platys* infection has been reported from both dogs and ticks (Inokuma et al., 2003; Unver et al., 2003).

**Clinical findings**

*A. platys* causes a cyclic thrombocytopenia that could result in bleeding, including petechiae and ecchymoses, although most infected dogs are probably able to control infection without demonstrating clinical signs. Bacteremia and thrombocytopenia occur in cycles of approximately 10 to 14 days. The clinical findings associated with infection according to published studies include: anorexia, lethargy, fever, weight loss, lymphadenomegaly, petechiae and ecchymoses, thrombocytopenia and anemia (Kontos et al., 1991; Harrus et al., 1997). Other studies have described asymptomatic natural infection associated with *A. platys*. Infected dogs are frequently co-infected with *E. canis*, and an experimental infection of dogs with both agents has shown that dogs with simultaneous infections had more severe clinical manifestations than those infected only with one agent (Gaunt et al., 2010).

**Diagnosis**

Detection of *A. platys* morulae in canine platelets can be made upon examination of Giemsa-stained blood smear by microscopy (Figure 2), however, confirmation of infection should be made by specific PCR.

![Figure 2 - Anaplasma platys morula in a thrombocyte from the blood of a naturally-infected dog (x100 magnification).](image)

**Treatment**

*Anaplasma platys* is susceptible to doxycycline with at the same dose and duration used for *E. canis* treatment (Gaunt et al., 2010).

**Prevention**

Due to the high likelihood that *A. platys* is transmitted by *R. sanguineus*, the control of tick infestation by topical treatment with acaricides and environmental eradication of ticks is also recommended for the prevention of this infection.
Etiology and epidemiology

Canine hepatozoonosis is a tick-borne disease caused by apicomplexan protozoa. In contrast to most tick-borne pathogens that are transmitted via the tick saliva, *Hepatozoon* infects dogs by ingestion of ticks containing infective sporozoites in their hemocoel (Baneth et al., 2003). Two different species of *Hepatozoon* infect dogs, *Hepatozoon canis* in the Old World and South America, and *Hepatozoon americanum* in the southern USA (Allen et al., 2011; Baneth 2011). Canine hepatozoonosis with *H. canis* is also prevalent in Japan and the first case of this infection was described in 1991 (Murata et al., 1991).

Clinically, *H. canis* infection varies between being asymptomatic in dogs with a low parasitaemia, to a severe disease with anemia, profound lethargy and cachexia in dogs with a large number of circulating parasites (Baneth et al., 1995; Baneth and Weigler, 1997). *Hepatozoon americanum* infection is manifested mainly by gait abnormalities and musculoskeletal pain due to myositis and periosteal bone lesions. *Hepatozoon americanum* infection is an emerging disease that is spreading north and east. It was originally detected in Texas in 1978 and has since been reported also from a large number of other American states. Recently, *H. canis* was also reported in the southeastern USA and some *H. canis - H. americanum* co-infections have been described (Allen et al., 2011).

The main vector of *H. canis* is the brown dog tick *Rhipicephalus sanguineus* which is found in warm and temperate regions all over the world, making the potential distribution of *H. canis* wide (Baneth et al., 2001). In addition, *H. canis* is transmitted by the tick *Amblyomma maculatum* in Brazil. The Gulf Coast tick *Amblyomma maculatum* is the vector of *H. americanum* in North America. Both of the *Hepatozoon* species that infect

**REFERENCES**

dogs are transmitted transstadially in their tick vectors. *Hepatozoon canis* has also been shown to be transmitted transplacentally from the dam to its pups (Murata et al., 1993), whereas recent evidence supports that transmission of *H. americanum* also by predation and ingestion of tissue forms from mammalian host’s tissues (Johnson et al., 2009).

**Clinical findings**

*Hepatozoon canis* infection can vary from being sub-clinical to severe and life-threatening in dogs with extreme lethargy, cachexia, and anemia. A sub-clinical infection to mild disease is the most common presentation of *H. canis* infection and it is usually found in dogs with a low level of parasitemia (1-5%), while severe illness can be found in dogs with a high parasitemia sometimes approaching 100% of the blood neutrophils. High parasitemia rates are sometimes accompanied by extreme neutrophilia reaching as high as 150,000 leukocytes/µl blood (Baneth and Weigler, 1997).

So far, there is no evidence of age, gender, or breed predisposition for hepatozoonosis. Dogs with hepatozoonosis are more likely to be from a rural community as compared with an urban setting, probably due to a higher exposure rate to ticks. Conditions that weaken the immune system such as co-infection with other pathogens increase the susceptibility to new infection with *H. canis* or allow the reactivation of existing infection.

**Diagnosis**

*Hepatozoon canis* mainly infects the hemolymphatic tissues and blood-forming organs including the bone marrow, lymph nodes, and spleen, and detection of infection can be carried out by blood smear microscopy (Figure 3) or blood PCR. *Hepatozoon americanum* primarily infects skeletal and cardiac muscular tissues and causes myositis and severe lameness, and the diagnosis of this infection is achieved mostly by tissue histopathology or blood PCR (Kargánchez et al., 2006; Allen et al., 2011).

**Treatment**

The current treatment protocol for *H. canis* infection is the administration of imidocarb dipropionate at 5–6 mg/kg every 14 days until gamonts are no longer detectable in blood smears. The prognosis of treated dogs with a low *H. canis* parasitemia is generally good even if decrease of parasitemia is slow and requires several repeated imidocarb treatments. The prognosis for dogs with a high parasitemia is good to guarded and it is sometimes associated with the outcome of a concurrent illness.

*Hepatozoon americanum* infection is treated with a combination oral therapy of trimethoprim-sulfadiazine (15 mg/kg every 12 hours), pyrimethamine (0.25 mg/kg every 24 hours), and clindamycin (10 mg/kg every 8 hours) for 14 days. After initial relief from clinical disease signs is obtained, remission can be prolonged with the oral administration of the coccidiostat decoquinate at 15 mg/kg mixed in food every 12 hours for two years. Relapse of clinical disease is
common following the discontinuation of treatment (Baneth 2011).

**Prevention**

Prevention of *H. canis* and *H. americanum* infection consists of the use of topical acaricides and environmental parasiticides, prevention of oral ingestion of ticks, and of predation on infected mammal hosts. No commercial vaccines are available for canine hepatozoonosis.

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Clinical aspects of the main canine *Ixodes*-borne diseases: Lyme disease and Granulocytic Anaplasmosis

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Luc Chabanne is a Professor at VetAgro Sup - Campus Vétérinaire in Lyon, France, where he is the head of the Small Animals Department since 2007. His teaching domain is veterinary internal medicine, with special interest in Haematology and Immunopathology. Pr. Chabanne graduated from the Ecoles Vétérinaires Francaises in 1985.

His research is centred on canine immunopathology. After studying the canine model of Systemic Lupus Erythematosus until 2005, he focuses now on vector-borne diseases in dogs and cats, particularly in comparative pathology (zoonotic pathogens and/or valuable disease models): *Babesia*, *Ehrlichia* and *Anaplasma*.

**Introduction**

Members of the *Ixodes ricinus* complex including *I. ricinus* in Europe, *I. persulcatus* in Asia, *I. scapularis* in eastern North America, and *I. pacificus* in western North America, are the most important vectors of protozoa, bacteria, and viruses that cause severe, debilitating disease to humans, domestic animals, and wildlife (Sonenshine 1991). Both Lyme borreliosis and granulocytic anaplasmosis are *Ixodes* spp.-transmitted bacterial diseases that may clinically affect dogs, causing fever and lameness.

**INSIGHTS ABOUT THE PATHOGENS**

Lyme borreliosis is a disease caused by infection with spirochetes belonging to the *Borrelia burgdorferi sensu lato* complex, which is a genetically diverse group. At least six genospecies have been found in humans and dogs. *Borrelia burgdorferi sensu stricto* predominates in the United States. In Europe, *Borrelia burgdorferi sensu stricto* also is found (in about 10% of isolates), but *B. afzelii* and *B. garinii* are more common (Rauter 2005). In Asia, *I. persulcatus* can transmit different *Borrelia* species. A recent epidemiological survey of *Borrelia* in Asia revealed that *B. garinii*, *B. afzelii*, *B. japonica*, *B. valaisiana* and *B. sinica* are the major pathogens of this region. *B. japonica*, *B. tanukii* and *B. turdi* have also been isolated in Japan from *Ixodes ovatus*, *I. tanuki* and
I. turdus, respectively (Kawabata 1993; Fukunuga 1996). More recently, a new Borrelia species closely related to B. valaisiana has been isolated from ticks and rodents in China, Taiwan, Korea and Okinawa prefecture (Japan) (Takada 2001; Masuzawa 2004). Lyme disease is the most frequent tick-borne disease among people in the northern hemisphere and human infections are of major public health importance. This multisystemic disorder is a well-recognized disease in humans but as yet, it is not clearly defined in dogs (Krupka 2010; Littman 2006), and the pathogenesis of Borrelia species other than B. burgdorferi sensu stricto remains to be studied.

Granulocytic anaplasmosis is caused by Anaplasma phagocytophilum (formerly Ehrlichia phagocytophila, E. equi or Human Granulocytic Ehrlichiosis agent), a Gram-negative, obligate intracellular bacteria of the order Rickettsiales, family Anaplasmataceae, affecting neutrophil granulocytes. It was initially known as the causative agent of tick-borne fever or pasture fever in sheep and cattle in Europe. E. equi granulocytic anaplasmosis was then documented in 1968 in California, and was later found in other parts of United-States and Europe. During the early 1990s, A. phagocytophilum has emerged as a cause of human disease in the United States and Europe. Canine granulocytic anaplasmosis due to A. phagocytophilum was first recognized in California in 1982 (Madewell 1982), before its description in Europe. Recently, the number of dogs infected with A. phagocytophilum has risen (Nicholson 2010), and A. phagocytophilum has been detected from naturally infected cats (Bjøeersdorff 1999; Lappin 2004; Tarello 2005; Heikkila 2010). Although the organism occurs worldwide, the disease is infrequently reported in Asian countries. DNA fragments of A. phagocytophilum were first detected in Ixodes persulcatus in China (Cao 2003) and Haemaphysalis longicornis in South Korea (Kim 2003). Human patients infected with A. phagocytophilum have also been reported from South Korea (Heo 2002) and China (Zhang 2011). DNA fragments of the agent were recently detected in I. ovatus, I. persulcatus and H. megaspinosa (Ohashi 2005; Kawahara 2006; Yoshimoto 2010), as well as in Japanese sika deer (Kawahara 2006) and cattle (Ooshiro 2008; Jilintai 2009). There are no case reports to date of domestic Asian dogs infected with A. phagocytophilum, while serology (Zhang 2011, 2012) and PCR analysis (Zhang 2012) attest dog exposure and infection.

**CLINICAL ASPECTS**

Predominant clinical signs reported in dogs with Lyme borreliosis or granulocytic anaplasmosis are nonspecific. Clinical suspicion usually begins with clinical evaluation of a febrile, lameness or painful patient with reluctance to move.

**Lyme borreliosis**

Besides asymptomatic seroconversion, clinical features of Lyme borreliosis in humans have included dermatitis, arthritis, meningoencephalitis, and myocarditis. Some of these manifestations have also been observed in dogs (Krupka 2010; Littman 2006).

Clinical illness in experimentally infected dogs occurred 2 to 6 months after exposure, and the severity and propensity to develop signs of illness seem to vary inversely with the animal’s age and immune status. Acute signs of fever (39.5°C to 40.5°C), shifting leg lameness, articular swelling, lymphadenomegaly, anorexia, and general malaise are described. The skin is seldom visibly affected and does not show the easily recognisable
erythema migrans seen in human infections.

Lameness in a particular limb often lasts for a few days and then may shift to another limb or disappear. The first limb affected is usually closest to the site of tick attachment. Despite the transient nature of the arthritis, inflammatory changes can persist in the joint as evidenced by abnormal synovial fluid analysis with neutrophilic synovitis (suppurative polyarthritis). Later on, after intermittent episodes of lameness a subclinical chronic periartthritis develops.

Although a later manifestation of Lyme borreliosis in people is often neurologic signs, neuropathologic findings were described only in experimentally infected dogs as an asymptomatic encephalitis, mild perineuritis, or meningitis. In dogs presumed to be naturally infected based on positive serologic results, clinical signs of neurologic dysfunction were not correlated with positive serologic reactivity to *B. burgdorferi* (Jäderlund, 2007). Nucleic acids of *B. burgdorferi* were not found in the brain tissue or central nervous systems of dogs with a variety of natural CNS diseases (Barber 2010; Jäderlund, 2009).

Cardiac arrhythmias secondary to myocarditis and myocarditis have also been uncommonly reported in canine Lyme disease.

Renal disease (Lyme-associated protein-losing nephropathy) in conjunction with suspected Lyme borreliosis was described in certain breeds: Labrador and Golden retrievers mainly in the US (Littman 2006), and Bernese mountain dogs in Europe (Gerber 2007, 2009), but there is a little existing proof of cause and effect.

No specific haematological or biochemical changes are associated with canine Lyme disease.

### Granulocytic anaplasmosis

The clinical manifestations in dogs range from a mild self-limited illness (Canelas Domingos 2011; Carrade 2009; Kohn 2008, 2011; Eberts 2011), to a life-threatening infection. However, as in human, most canine infections probably result in minimal or no clinical manifestations. The incubation period is of 1 or 2 weeks. In dogs infected with *A. phagocytophilum* that develop illness, the common clinical signs are nonspecific: fever, lethargy and anorexia.

In canine cases, reluctance to move, weakness, stiffness, lameness, or soreness from undetermined origin have been commonly reported. Overt joint pain and inflammatory arthritis have been documented in some cases.

Less commonly, soft and nonproductive coughing, labored breathing, polydipsia, gastrointestinal signs including vomiting and diarrhea, seizures and proprioceptive deficits, hemorrhage, manifested as mucosal petechiae, melena, or epistaxis, have been reported. Results of physical examinations are often unremarkable; dogs may exhibit mild lymphadenopathy, splenomegaly or slight hepatomegaly.

The most consistent laboratory abnormality is mild to severe thrombocytopenia, which occurs in 80 to 90% of dogs. Morulae are frequently observed in peripheral granulocytes on blood smears, lymphopenia and a mild to moderate nonregenerative normochromic normocytic anemia are not uncommon. Both neutrophilia or neutropenia have been reported, whereas monocytes and reactive lymphocytosis are occasionally observed in dogs. When evaluated, the bone marrow of infected dogs showed normal or increased cellularity, and increased absolute numbers of megakaryocytes were observed in correlation with thrombocytopenia, indicating an
active thrombopoiesis. Abnormalities most commonly observed on serum biochemistry profile are a mild increase in hepatic enzyme activities (especially alkaline phosphatase), and also mild hypoaalbuminemia, hyperglobulinemia, hypophosphatemia, and hyperamylasemia.

**Co-infection with **Borrelia and Anaplasma**

Because *B. burgdorferi* is maintained largely in a vector-reservoir-host system similar to *A. phagocytophilum*’s, co-infection with *B. burgdorferi* and *A. phagocytophilum* is frequently detected (Nieto 2009). The clinical consequences of dual infection should be considered. The two organisms may enhance one another’s pathogenicity, and simultaneous or sequential infections with *B. burgdorferi* and *A. phagocytophilum* are more likely to induce disease than single infections (Beall 2008; Bowman 2009).

**CONCLUSION**

In addition to being multisystemic disorders caused by tick-borne bacteria, Lyme borreliosis and granulocytic anaplasmosis in dogs share common features, which generate controversies in the diagnosis of such diseases. Actually, many dogs appear to tolerate infection without developing overt clinical disease, so much so that non-clinical or occult infection should be the rule and disease development the exception.

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Heartworms: Not as simple as once thought

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Doug Carithers graduated from the Iowa State University College of Veterinary Medicine, and achieved certificate degree in the Executive Veterinary Program at the University of Illinois. He practiced veterinary medicine and surgery in Iowa for 10 years, prior to being recruited for a position in industry. At Merial for 19 years, Dr. Carithers has been the Director of Applied Research and Publications for the past ten years. In that role, he has developed and conducted over 60 clinical and field studies, including large-scale field trials for over 1000 dogs on PREVICOX and over 400 horses treated with EQUIOXX. He conducted the 2001, 2004, 2007 and 2010 American Heartworm Society heartworm incidence surveys. In addition to coordinating trials and surveys, he has authored or co-authored over 30 scientific articles and is currently authoring a parasitology atlas for educating pet owners. He has lectured nationally and internationally at veterinary and parasitology meetings and conferences, and participated in national and international round table discussions on pain medication and parasitology. He is a member of the Iowa VMA, AVMA, AAVP, WAAVP and AHS. He is past Vice President of the American Heartworm Society and is currently serving on the AHS board as Editor and also serves on committees for the AAVP and AHS. Previously, Dr. Carithers served on the boards of the National Commission on Veterinary Economic Issues (NCVEII) for 10 years and the Companion Animal Parasite Council (CAPC), serving on committees for these organizations as well as the North American Veterinary Medical Education Consortium (NAVMEC) for the American Association of Veterinary Medical Colleges (AAVMC).

Historically, as medical professionals, we have tended to view the activity of pharmaceutical drugs, including antiparasitics, as a dose-dependent phenomenon. The proper or accepted dose in an infected animal would be expected to kill the target (parasite or bacteria) with direct activity against the target organism. The drug might affect the activity of the invader’s metabolism, reproductive capability, or ability to feed and move, thus directly affecting viability and killing the target. Logically, a dramatically higher dose would be expected to increase the killing activity, speed or efficacy of a drug. While progressively lower doses, might reduce the killing speed, activity and/or efficacy, essentially reaching a dose where the drug only affect the most susceptible organisms.
Much of our understanding of the action of antiparasitic drugs was based on studies performed against *Caenorhabditis elegans*, a free living nematode with a direct life cycle that is easy to maintain, therefore it offers a great nematode-model for bench-studies. *C. elegans* did play a huge role in screening for macrocyclic lactone antibiotics, which possessed ivermectin-like anthelmintic activity. It also seemed logical to assume that the same direct neuromuscular activity of the avermectins, resulting in motor paralysis and resultant death seen in invertebrates and *C. elegans* would be the same in all nematode species, including the filarial worm, *Dirofilaria immitis*. Studies on with ivermectin and *C. elegans* (and eventually, studies on other nematodes and related macrocyclic lactones) demonstrated that ivermectin had a high affinity for nematode glutamate-gated chloride ion channels (GluCls), causing neuromotor paralysis. In invertebrates and nematodes, the paralytic action affects digestion and/or motility, directly resulting in death of the worm.

Paralysis, directly resulting in death, would also seem to be the case with heartworms, at least on the surface. In-vivo, low doses of ivermectin (~3nM peak plasma concentrations) and other macrocyclic lactones (MLs) are known to rapidly clear microfilarial infections in humans and animals. While for in-vitro studies, it was long ago established that extremely high concentrations of MLs were necessary to cause any apparent paralysis of microfilariae. Studies continue to performed and in many cases, conclusions related to ML efficacy are being touted, based upon these in-vitro results. Even doing so without considering or understanding a dose disparity exists between in-vivo and in-vitro activity.

An in-vitro bench-study performed on microfilariae of *D. immitis* harvested from dogs across the southern US, noted paralysis in microfilariae exposed to various increasing concentrations of ivermectin and other macrocyclic lactones (MLs). It was also noted that several isolates were not ‘paralyzed’ by the high concentrations of MLs, thus it was surmised that these isolates weren’t susceptible to MLs. Interestingly, microfilariae are not the targeted life-stage of *Dirofilaria immitis*, those targets are the L3 and L4 larvae. Bearing this in mind, a separate study was designed using a L3 larval motility assay rather than a MF motility assay. In this study, mosquitoes were allowed to feed on blood containing MF (including the same non-susceptible field MF isolates cited as not-susceptible in the previous study), and develop to infective L3 larvae. The ability of these L3 larvae to actively migrate in the face of exposure to various concentrations of MLs was measured. Interestingly, the L3 larvae that developed from MF cited as non-susceptible responded identically to the presence of MLs as L3 larvae from known, ML-susceptible isolates. So, this might cause one to rethink the value of paralysis in any in vivo microfilarial assays.

In late 2010, Moreno, et al published their findings on a series of studies on the microfilariae of *Brugia malayi*. One of the studies described an antibody staining technique targeting the AVR-14A sub-unit of the GluCl. The investigators found GluCls, expressing AVR-14A were only found in the musculature surrounding the excretory/secretory (ES) vesicle and nowhere else in the microfilariae. Further, in vitro exposure of these MF to ivermectin [0.1µM] decreased the proteins typically produced by the ES vesicle of these microfilariae. Thus, it is possible that the paralysis of the muscle surrounding the ES vesicle accounts for the mechanism of action of this drug against this stage. These data support a role of ES
proteins in some form of modulation and/or evasion of the host immune response by filarial nematodes\textsuperscript{18}. The potential consequence of this is that production of immunomodulatory proteins produced by the mf would be reduced and that they would potentially be more vulnerable to components of the host’s immune system. These findings actually support theories explaining considerations of heartworm MF clearance proposed over 30 years ago, and since that time\textsuperscript{14-19}.

In fact, there are several studies showing synergistic effects between MLs and cells of the innate immune system against filarial larvae of other species \textit{in vitro}\textsuperscript{20-21-22}. There are also several studies in patients infected with \textit{Onchocerca volvulus} that demonstrate dramatic changes in circulating chemokine levels following ivermectin treatments\textsuperscript{23-24-25}.

Studies recently presented by Dr. A.F. Vatta\textsuperscript{26} and Dr. Adrian J. Wolstenholme\textsuperscript{27} at the American Association of Veterinary Parasitologists meeting in August of this year, examined the potential contributions from the canine immune system to the anti-heartworm actions of ivermectin. It was noted that peripheral blood mononuclear cells (PBMC) from uninfected dogs adhere to \textit{D. immitis} mf in the presence of low concentrations of ivermectin and uninfected dog serum; removal of either the drug or the serum reduces the level of adherence. The PBMC do not have an immediate effect on the viability or movement of the mf, but the investigators hypothesize that the binding may cause the rapid removal from the circulation observed \textit{in vivo}. Ivermectin- and serum-dependent binding of isolated polymorphonuclear cells to \textit{D. immitis} mf was also observed.

Interestingly, nearly 28 years ago, two separate papers reported histologic findings post heartworm microfilarial treatment with ivermectin. From samples collected at 48, 72 and 144 hours microgranulomas involving microfilariae were found (lungs, kidneys, liver, spleen, skeletal and cardiac muscles, diaphragm, lymph nodes, gastrointestinal tract and pancreas, with glial nodules seen in the CNS) and described histologically\textsuperscript{28-29} In one of these studies, samples were collected at 18 hours. At this time, a dense mass of RBC, WBC, and macrophages plus many microfilariae was found in pulmonary alveolar septae\textsuperscript{29}. Similar reactions were seen in liver, kidney, and spleen\textsuperscript{29}. It is logical to deduct that this clustering of cells was simply the result of the agglutinated microfilariae and PBMC and polymorphonuclear cells being filtered in the capillary beds, as they were too large to pass through. Then, at 48-144 hours the localized reactions seen were the result of regular clean-up activity, which in some immunocompetent animals would potentially result in a specific antifilarial immune response.

According to Dr. Wolstenholme, when considering the available data, rather than considering 2 dimensions and jumping to dose-related conclusions, we need to consider the impact of the ‘third dimension’, the host immune system, in order to fully appreciate how IVM affects filarial species.
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**In-vitro demonstration of the synergistic acaricidal effect of a fipronil+amitraz combination on *Rhipicephalus sanguineus***

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During Master of Sciences, PhD and post-doctoral studies, she specialized in Parasitology with a specific interest in Arthropods and Vector Borne Diseases. Her works focused on Tse-Tse fly control in Africa, molecular detection of Tick-Borne pathogens and Chikungunya virus transmission in Indian Ocean. From 2007 to 2010, she was assistant Professor at the Parasitology Department of Maisons-Alfort Veterinary School and Head of the Toxoplasma research group of the National Agency for Food Safety (ANSES). She then joined Merial as Technical Director Pet Parasiticides for Europe.

**Introduction**

The potential synergistic effect of the combination of fipronil plus amitraz was tested against unfed *Rhipicephalus sanguineus* in two *in-vitro* experiments using fipronil and amitraz alone or the combination of the two actives. The first assessed the killing effect by contact, while the second assessed the motility of ticks in presence of the actives.

**CONTACT TESTING**

During the contact experiment, 10 unfed ticks (5 males and 5 females) were placed in vials which walls were coated and containing filter impregnated papers (top and bottom of the vials) either with acetone (control), fipronil alone (serial dilutions from 0.0016 to 25 ppm), amitraz alone at 12.5 ppm, or fipronil (serial dilutions from 0.0016 to 25 ppm) + amitraz at 12.5 ppm and held under constant conditions [24°C, 95% RH]. The objective was to assess if the addition of a small amount of amitraz was able to potentiate the killing effect of fipronil, and especially the speed of kill.

No significant death was observed in the control vials or amitraz alone, which confirmed that amitraz at 12.5 ppm had no lethal effect. The lethal concentrations (LC, at the 50 and 90% level) in the fipronil and fipronil+amitraz groups were compared at 6, 24 and 48 hours (hrs) after placement of ticks into vials. The mortality of the ticks was concentration dependant in the two groups, with a significantly higher mortality in the fipronil+amitraz group at the same dose of fipronil. At 24h the combination reached the LC90 level with neither of the actives alone killing ticks, the added effect to fipronil is thus a truly synergistic action. The synergistic ratios were calculated and the LC50 ratio between the two groups were >7.3, 137 and 97 at
6, 24 and 48 hrs, respectively. A similar response was observed when comparing the LC90 ratios. The synergistic action was maximal at 24 hrs because at 48 hrs ticks had been exposed to fipronil long enough to display its acaricidal activity. This contact test clearly demonstrated a synergistic effect of amitraz combined with fipronil, with a clear increase in the speed of kill.

**MOTILITY TESTING**

A second *in vitro* experiment was conducted to measure the motility of *R. sanguineus* with or without stimulation by CO\(_2\), when exposed to fipronil alone, amitraz alone, or fipronil+amitraz, similar to the previous study. Under natural conditions, CO\(_2\) mimicks the presence of a host and the ticks are stimulated showing increased movements (questing behavior). Contrary, in the absence of a stimulus, the ticks usually remain immobile. Ticks that remain motionless despite stimulation by CO\(_2\) can be considered as dead, and ticks that are moving in the absence of stimulation display an abnormal behavior.

In the present experiment, 10 ticks were placed each in a Petri dish and a specific laser movement analyzer was used (LemnaTec Scanalyzer Imaging System). The motility of ticks was studied at either 4 or 24 hrs after exposure to the active compounds. Petri dishes were treated with solutions of either ethanol (control), fipronil (5 serial dilutions from 0.005 to 1.3 µg/cm\(^2\)), amitraz at 0.32 µg/cm\(^2\) or fipronil+amitraz, based on the same concentrations of fipronil as above with the addition of amitraz at 0.32 µg/cm\(^2\). Five successive experiments (one per each fipronil concentration) comparing 4 Petri dishes (one per group) were conducted. In the absence of stimulation, the observations at 4 hrs showed a significant increase of tick motility in both, amitraz and fipronil+amitraz groups compared to the control or fipronil alone groups. After stimulation with CO\(_2\), ticks in all groups displayed motility at the 4 hrs time point. At 24 hrs, in the absence of stimulation, significantly increased movements were only observed in the amitraz alone group. In all other groups, ticks were motionless. After stimulation with CO\(_2\), ticks in the fipronil+amitraz group showed significantly lower motility or no activity at all when compared to all other groups where ticks were moving. Those results can be interpreted by an acaricidal effect in 2 phases of the combination of fipronil+amitraz compared to the other groups:

- In the first phase, at least during the first 4 hrs, amitraz induces a behavioral change with increased movements even in the absence of stimulation (hyperactivity, restlessness). This was observed in both, the amitraz alone and fipronil+amitraz groups. Such a behavioral change should lead to disruption of attachment and inhibition of blood feeding on the host;

- A second phase, between 4 and 24 hrs, during which the ticks quickly died compared to the amitraz alone [no mortality] or fipronil alone [significant mortality only at the highest dose].

**CONCLUSION**

The two experiments clearly demonstrated the synergistic effect of fipronil with amitraz at an amitraz dose that is not lethal for ticks.

**REFERENCES**

Introduction

Different flea species can infest companion animals: *Ctenocephalides felis* ("the cat flea"), *C. canis* ("the dog flea"), *Pulex irritans* ("the human flea or fox flea"), *Archeopsylla erinacei* ("the hedgehog flea"). In more than 90% of cases, the "cat flea" (*Ctenocephalides felis*) is found on cats and dogs (Guaguère and Beugnet, 2006). Rarely, rodent fleas or flea species of small carnivores/insectivores or birds fleas can be observed (*Spilopsyllus cuniculi*, *Xenopsylla cheopis*, *Ceratophyllus gallinae* and *Leptosylla segnis*). Globally, *Ctenocephalides felis felis* is the dominant sub-species, but in Africa and the middle east, *Ctenocephalides felis strongylus* can be more common. In Asia, *Ctenocephalides felis orientis* is also found. These two sub-species are morphologically really similar to *Ctenocephalides canis*.

*C. felis felis* is not host specific and can take its blood meal on various mammalians (domestic and wild carnivores, opossums, rodents, rabbits, ruminants, humans). The cat and dog owners are frequently bitten and they often get pruritic papules on their legs and ankles.

Fleas are the most common ectoparasite that infests pets both in rural and urban habitats. The cat fleas are adapted to their environment, outdoor as well as indoor, and persist through all seasons even in temperate countries even if clinical manifestations are more prevalent from spring to autumn.

The presence of fleas is often quite well tolerated, particularly in cats. However, a flea infestation can provoke intense pruritus. Some animals will develop flea allergic dermatitis with more clinical signs on the skin.

*C. felis* is recovered from 5 to more than 50% of cats and dogs depending the surveys, with variations linked to the country, the season, the areas, the treatments...

*C. canis* is more specific and rarely found on cats or other mammals. However,
many surveys conducted worldwide show that the spectrum of flea species found on dogs varies largely in the different geographical areas. *C. canis* seems to be more adapted to cold climate, rural environment and dogs living outside, whereas *C. felis* is originated from Africa and is more adapted to warm climate and is found from rural to urban areas and on animals living indoors or outdoors.

**TAXONOMY - MORPHOLOGY**

Fleas belong to the Siphonaptera Order of insects. It is a small, wingless insect with 2 to 4 mm in length, with a yellow/brown color. There are approximately 2500 species of fleas that are divided within 15 families and 200 genera. Most fleas of medical and veterinary importance belong to the Pulicidae family.

The fleas usually have well developed eyes, as well as three-segment antennae that are located in antennal fossae on each side of the head. The mouthparts are well adapted to blood sucking. The two labial palps serve to locate the feeding site. Thereafter, the other mouthparts are used to pierce the skin towards a capillary [they are called stylets]. They then form a feeding canal and a salivary canal.

*Ctenocephalides felis felis* is a wingless insect of the Pulicidae family. It is 2-4 mm long and dark orangy brown in colour. The front of its head is rounded and has two perpendicular combs lined with dark brown teeth. The body is laterally compressed to facilitate movement between hairs.

The third pair of legs is very well developed and adapted to jumping. *Ctenocephalides canis* is smaller than *C. felis*, the head is shorter and the first spine of the genal ctenidium measures half of the second in *C. canis* and the same length in *C. felis*.

The third pair of legs is bigger than the others, adapted to jump in order to facilitated the host infestation. The average jumping distance of *Ctenocephalides felis felis* is 20 cm (2-48 cm); that of *Ctenocephalides canis* is 30 cm (3-50 cm). The height of the jump is about 15 cm with 25 cm being the maximum height attained by *Ctenocephalides felis*.

The flea eggs are small (0.2 – 0.5 mm), ovoid and white to yellow-white.

There are 3 successive worm-like larval stages, eyeless, legless, formed with a head [with mandibules] and 12 posterior segments. They measure from 1.5 to 8 mm in length.

The flea pupae is formed in a sticky cocoon which is often surrounded by many debris which helps to its camouflage.
Adult fleas take their first blood meal within 40 minutes of arriving on the dog and then breed within the following 48 hours. Females and males usually stay on their host. If they accidentally fall off, they will reinfest quickly. The transmission from one host to another is possible but not the major way of infestation. Each female can lay up to 50 eggs at peak, starting in average to lay a few eggs 36 hours after the host infestation. The females will lay eggs during their life span which is usually short (15 to 30 days) with an average of 25 eggs a day. They take an average of 4 blood meals per day.

The eggs are not fixed to the host and fall to the ground as the dog moves around. It has been calculated that they stay in average 2 hours on the pet’s skin before falling. It allows a contact with insecticide and Insect Growth Regulator that may be present on the animal’s skin. When temperature and humidity are optimal, the eggs hatch in 3-7 days on the ground. Usually, the maximal number of eggs will be found where the pets are resting.

The larvae are a few millimetres in length. They are non-parasitic and feed on organic debris, in particular skin debris and adult flea faeces (desiccated blood). They like dark and humid conditions and can move horizontally, about 20 cm, in secluded places (for example, under a sofa, the base of carpet or rug fibres, inside the pet bedding). Larvae 1 and Larvae 2 are really sensitive to UV and desiccation.

Having passed through 3 larval stages over a period of between a week to a month, each L3 spins a cocoon in which it metamorphoses into an flea pupae and a pre-merged flea within about 10 days. If the hosts are present in the environment, adult fleas emerge rapidly. If not, the non-emerged adult fleas can survive for several months (from 6 to 12), protected in their cocoon. Non-emerged fleas are an important, readily available source of fleas. They are also relatively protected from the effects of insecticides. Newly-emerged fleas actively seek out a host (preferably a cat or dog) and can survive for about a week without a blood meal.

Environmental conditions affect the development and timing of flea life cycles. Each stage in the life cycle is susceptible to desiccation. A relative humidity of 85% is optimal. Temperature can accelerate or slow down development. For *C. felis*, the minimum temperature seems to be 22°C with the optimum being 25-26°C. Temperatures above 30°C reduce the adult life-span. In winter, an outside temperature below 0°C is fatal for larvae and pupae. Inside, at 17-19°C, the life cycle slows down considerably but pre-emerged adults survive.
ECOLOGY AND DIFFICULTY OF CONTROL

Most pet owners just wait to see their pets itching and then want to kill fleas on their pets. But the most difficult aspect of flea control is an aspect that the majority of pet owners don’t even know exists, namely controlling the pre-existing environmental infestation related to cat flea ecology.

It is for this reason that the concept of integrated flea control is so important. By the time the pet owner notices fleas on their pets there is already a large biomass of flea life stages, that is flea eggs, larvae and pupae, present in the pet’s environment. The biology of the flea dictates that it will take, on average, 1-2 months for these life stages to complete their development - for the flea eggs to develop into larvae, then into pupae and finally into pre-merged adults ready to emerge from the pupal cocoon and jump onto a passing pet. Hence it is biologically impossible to resolve a flea infestation overnight, regardless of which product we choose to treat the pets with.

It is important to realise that the length of time it will take to resolve any individual flea problem is governed by a number of factors that we just can’t know the answer to at the time that we begin treating the pet.

These questions include:

- Where are the fleas coming from? Indoors/outdoors or a combination of both. The source of fleas can be neighbouring cats and dogs, but also stray cats, wild animals like possums, raccoons...The reservoir of pupae could be located outside of the house or even the garden, in a place where dogs and cats often go.
- How much flea biomass is already present in the environment? In tropical, Mediterranean countries, the life cycle never stops. Under temperate, continental climate, it stops outside, slowdown inside, and explodes in spring.
- How long it will take for this biomass to complete its development? This can vary based on fluctuations at the microclimate level.
- Is there an ongoing source of new flea eggs in the environment?

Waiting for more favourable conditions. Based on this consideration, fleas can live all year round with a sudden explosion of the population in spring.

At suitable humidity, the life cycle of *Ctenocephalides felis* lasts 14 days at 29°C. We may consider that in average, the full life cycle is obtained in 3 to 4 weeks.

The emergence of fleas from their cocoons is influenced by various factors. A shadow, footsteps, vibrations (for example, from a hoover) can all trigger emergence. Typically, a dog catches fleas by passing through an infested environment, either outdoors during the right season, or indoors (e.g. when visiting someone else’s house). Frequently, cats bring fleas into a house. The fleas then breed and become a source of infestation for dogs sharing the environment.
These unknowns explain why the resolution of flea infestations will vary from household to household, from year to year, even from season to season. Remember, the flea lifecycle doesn’t only occur inside the house, there is a large outdoor reservoir of flea populations through the interaction between untreated pets/strays/feral and native animals and the flea.

**VECTOR ROLE OF PET FLEA**

Fleas are competent vectors for numerous microbial pathogens of medical and veterinary importance. Plague and Murine typhus where historically known. But in regards to cat and dog fleas, we may consider other transmitted diseases like: cat-scratch disease [one bartonellosis], feline anemia [formerly “haemobartonellosis”], and flea spotted fever.

**Cat Scratch Disease**

*(Bartonella henselae)*

There is an abundant literature concerning the cat scratch disease and other *Bartonella* infections but there is a few indication that those bacterial infections are emerging or that their epidemiology is changing. The number of human cases of Cat Scratch Disease is not decreasing despite flea control and it seems that bartonellosis is now touching adults when it was considered as a child disease.

**Feline and Dog Anemia**

*due to Mycoplasma*

There are publications worldwide concerning the infection of cats by “HaemoBartonella” now called *Mycoplasma Haemoplasma hae-mominutum* or *M. haemofelis* and of the dogs by *M. haemocanis*. The prevalence of infections are usually high, from 20 to 40% or more. The pathogenicity is still considered as low except in case of co-infections (with FIV-FeLV in cats).

**Flea Spotted Fever to Rickettsia felis**

Recently, *Rickettsia felis* emerged as a new pathogen for humans, responsible for the flea-borne spotted fever, also called cat flea typhus. This bacterium was first detected in the cat flea, *Ctenocephalides felis*, by molecular biology techniques, in 1990. Its distribution is considered to be cosmopolitan as for its main vector, *Ctenocephalides felis*.

In fleas, the prevalence can be very high and varies according to the ecological environment and the season in the year. Although *C. felis* is the main biological vector for *R. felis*, this bacterium has also been detected in *C. canis, Pulex irritans*, the human flea, and *Archeopsylla erinacei*.

Although the disease is probably ubiquitous, up to now only few clinical cases have been reported throughout the world. The disease may be misdiagnosed as a tick-borne rickettsiosis. Classical symptoms reported are fever, maculopapular rash and eschar.

Very few cases of disease in animals due to *R. felis* infections are reported in the literature. Interestingly, in Spain, DNA of *R. felis* was found in the serum of a dog living in a house where two persons showed a flea-borne spotted fever, evidenced by PCR. The dog didn’t express fever but symptoms of fatigue, vomiting and diarrhea were reported. In a similar situation in Germany, one dog from a family in which two persons suffered from flea-borne spotted fever, was found infected by *R. felis* but showed no symptoms.
Teniasis to *Dipylidium caninum*

Even if it is not truly a vector borne disease, the fleas being the natural intermediate host of the cestode. The teniasis to *Dipylidium caninum* should be included in the list of disease related to the presence of fleas. Even if it is exceptional, this cestode is zoonotic in case of accidental flea ingestion by a human.

**Putative transmission**

Many authors have mentioned the possible role of fleas as vectors of other pathogens. FIV and FeLV have been studied but no proof of a vector role has been published. It is key to remind that finding DNA from pathogens in fleas collected on their hosts through PCR technique does not mean anything else than the fleas has ingested a part of pathogen agents (killed or lived). A recent controversy appeared regarding the role of fleas in the transmission of *Leishmania infantum* to dogs. We may imagine that the distribution of canine leishmaniosis would be really different if the fleas could play a vector role. To demonstrate a vector role, experimental designs but using the natural transmission from and the natural host are needed, from host to vector and from vector to host.
Fleas and Ticks as an emerging threat for transmitted diseases to humans in Asia

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Abstract
Many bacterial, viral and parasitic pathogens have been associated with transmission by fleas and ticks, including several recently identified pathogens, both in humans and domestic animals, especially dogs and cats. The emergence or re-emergence in dogs and cats of these flea and tick-borne diseases has a multifactorial origin. Better animal care, better diagnostic tools, and a broader distribution of the vectors in favorable habitats through population migrations (travel with owned pets, translocation or commercial trade of pets) are some of the factors contributing to emergence and recognition of these new pathogens. The present review will focus on the recent epidemiological studies which support the emergence or re-emergence of zoonotic flea and Tick-Borne pathogens in dogs and cats, especially in Asia and Australia.

Introduction
Many viral, bacterial, and parasitic pathogens have been associated with transmission by fleas and ticks, including several recently identified pathogens in both humans and domestic animals, especially dogs and cats. Being hematophagous, ticks and fleas are second and third to mosquitoes as the source of pathogen transmission to humans [Shaw et al., 2001], but first as the source of pathogen transmission to animals. Across Europe (including the UK), North & Central America, Australasia, parts of Asia (China, Korea and Japan) and sub-Saharan Africa, ticks may be common in wooded or heathland areas, especially in spring and summer. There are an estimated 800 species of ticks worldwide, but fewer than 100 occur in North America with only a dozen or so North American tick species known to parasitize human or dogs with any frequency and to transmit micro-organisms of medical importance (Fritz, 2009; Berrada and Telford, 2009).

In Asia, India and Australia, endemic tick and flea species are also involved in transmission of zoonotic pathogens top humans. For instance, in India, seven genera of hard ticks and three genera of soft ticks have been reported [Singh et al., 2011]. The most important genera are Hyalomma, Haemaphysalis, Rhipicephalus and Argas. Similarly, there are more than 2,200 flea species, fleas feeding on mammals can transmit bacteria to humans, such as murine typhus and bubonic plague, various Rickettsiae, including Rickettsia typhi and Rickettsia felis. Cat fleas (Ctenocephalides felis) are the main vector of Bartonella henselae and also various Mycoplasma spp. (formerly known as Haemoplasma and Hemo Bartonella). Fleas can also transmit parasites, such as the tapeworm Dipylidium caninum. [Eisen and Gage, 2012; McElroy et al., 2010] However, only a few species of fleas can feed on
humans, mainly *Pulex irritans*, *Ctenocephalides felis*, the cat flea, less frequently *Ctenocephalides canis*, the dog flea, and several rodent flea species, including the oriental rat flea, *Xenopsylla cheopis*.

Within the last few decades, newly identified flea and tick-borne diseases or re-emergence of known flea or tick-borne diseases with new geographical patterns or prevalence have been described around the world. The important spreading of pathogens by a greater mobility of human populations and their companion animals, combined with changes in the ecosystems favorable to survival of ectoparasites, have led to the recognition of flea and tick-borne diseases in areas usually considered as free of these infections. For tick-borne zoonoses, it includes babesiosis in northern Germany and the Netherlands, canine monocytic erlichiosis in Mediterranean countries (Beugnet and Marié, 2009), or Rocky Mountain Spotted Fever in Arizona, Mexico and Brazil, associated with transmission by *Rhipicephalus sanguineus* ticks (Nicholson et al., 2010). *Rhipicephalus sanguineus*, the brown dog tick, is a good example of ‘parasite globalization’ to its ubiquitous distribution which has clearly been facilitated by dog movements with their owners or through trade. As more attention is given to the care of our companion animals, especially in developed countries (Berrada and Telford, 2009), and better detection tools, largely based on molecular techniques, become available, allowing for more sensitive and specific detection of tick-borne pathogens (Shaw et al, 2001), tick-borne diseases are recognized as an emerging infectious threat not only to humans but also to dogs. The recognition of fleas as vectors of *Rickettsia felis* and *Mycoplasma* spp. also illustrate that concept. The present review will focus on the recent epidemiological studies which support the emergence or re-emergence of flea- and tick-borne pathogens in dogs and cats around the world, and more specifically in the Asian and Australian regions.

**EMERGENCE OF VIRAL TICK-BORNE PATHOGENS IN DOGS**

Most tick-borne pathogens in dogs are of bacterial or protozoal etiology (Shaw et al., 2001), but a few clinical cases of viral tick-borne encephalitis (TBE), a flavivirus, have been described in dogs in central Europe (Leschnick et al., 2002; Weissenböck and Holzmann. 1997), where *Ixodes ricinus* ticks are the main vector of the virus. Usually dogs are less susceptible than humans, but symptomatic infection (fever, lethargy and neurologic signs) has been described. In Austria, seroconversion rates of 24% were reported in dogs without clinical evidence of TBE. This viral disease is on the rise in human populations in central and northern Europe (Suss, 2008), and therefore, more cases may be diagnosed in dogs in the near future. Migrating birds were found to carry TBE-virus infected *I. ricinus* ticks, and they play a role in the geographic dispersal of TBEV-infected ticks (Waldenström et al., 2007). In Japan, in Oshima, in the southern part of Hokkaido, a tick-borne encephalitis (TBE) patient was identified in 1993; in addition TBE virus was isolated from the blood samples of sentinel dogs, ticks pools, and rodents spleens in 1995 and 1996 (Takashima et al., 2001).

Looping-ill virus, another flavivirus, which was initially reported from sheep in the northern highlands of Scotland and England, has now been detected beyond these limited geographic areas, as loping-ill virus is prevalent in Great Britain, in Ireland, on the Iberian Peninsula, in Turkey and Bulgaria, and possibly in Japan (Dobler, 2010). A few cases have been described in dogs (Dobler, 2010).
Among the bacterial diseases transmitted by ticks to dogs, the Borreliae, Ehrlichiae, and Rickettsiae are the main bacterial families at issue and for which better diagnostic tools have facilitated identification and understanding of their epidemiology (Shaw et al., 2001, Nicholson et al., 2010).

**Borrelia burgdorferi** and other Borrelia genogroups

Lyme disease has certainly been recognized as one of the major emerging tick-borne diseases of humans, both in temperate regions of North America and in Europe (Fritz, 2009). Lyme borreliosis is transmitted by different species of *Ixodes* ticks, mainly *I. scapularis* in the northeastern and upper Midwestern United States, and *I. pacificus* along the Pacific coast in North America (Fritz, 2009), *I. ricinus* in Europe, where the distribution of infected ticks is expanding, even in urban areas (Shaw et al., 2001), and *I. persulcatus* in Asia. In Europe, most cases of Lyme borreliosis occur in the Scandinavian countries and in central Europe (especially in Germany, Austria and Switzerland) (Shapiro, 2008). In the United Kingdom, serological surveys for *B. burgdorferi* infection have indicated a prevalence of 4.5% to 11% in dogs, and *B. burgdorferi sensu lato* was detected in 5 (4%) of 120 sick dogs that had not travelled abroad (Shaw et al., 2005). *Borrelia burgdorferi sensu lato* is present in approximately 5% to 35% of *I. ricinus* in Germany, depending on the region (Nau et al., 2009). *Borrelia burgdorferi* and *B. vogeli* have been detected in *I. ricinus* ticks in central and northern Italy (Otranto and Dantas-Torres, 2010). In France, *Ixodes ricinus* is found throughout the country except in the border areas of the Mediterranean coast and in regions with an altitude above 1500 meters, and the *Borrelia* infestation rate of *Ixodes* is approximately 7%, with wide disparity in incidence between administrative districts (Blanc, 2009). In humans, the French national prevalence of Lyme disease is estimated to be between 9.4 to 16.4 per 100,000 persons (about 5,000 new cases per year) with peak rates of 86 to 200 per 100,000 persons in Alsace, the most endemic region (Blanc, 2009).

The number of known endemic areas is also expanding. Lyme disease endemic areas in Canada are increasing because the range of *I. scapularis* is expanding in the eastern and central provinces. In southeastern Canada, most populations of *I. scapularis* occur in Carolinian forests, and it was predicted that *I. scapularis* populations could establish in more northerly woodland types than those in which they currently exist (Ogden et al., 2006). Since 1997, detection of human cases of Lyme disease and passive surveillance for ticks has led to the identification of infected populations of *I. scapularis* in southern Ontario, Nova Scotia, southeastern Manitoba and New Brunswick (Ogden et al., 2009). Dogs are sensitive indicators of Lyme borreliosis prevalence because they have a high level of exposure to ticks (Stone et al., 2005). In disease-endemic areas, ≥50% of unvaccinated dogs have been reported to be infected, even though only about 5% to 10% of dogs exposed to infected ticks develop clinical borreliosis (Fritz, 2009). The prevalence of Lyme borreliosis in dogs correlates with infection in humans, as well as entomologic indicators of disease transmission (Stone et al., 2005). Testing of more than 900,000 dogs in the USA for the C6 peptide derived from *B. burgdorferi* showed that the greatest prevalences of dog infection were in the Northeast (11.6%) and Midwest (4%) and the lowest in the Southeast (1.0%) and West (1.4%)
(Bowman et al., 2009). However, prevalence was the highest (>40%) in areas where human illness was most common (Bowman et al., 2009; Little et al., 2010).

In Australia, Lyme disease has been reported in humans and cattle but was not detected in 57 dogs from the Brisbane area, Queensland (Ballock et al., 1993). A serosurvey in Brazil showed that 23 (9.7%) of 237 dogs were seropositive by ELISA for *B. burgdorferi* and 20 of these were also confirmed by Western blotting (Joppert et al., 2001).

Lyme disease has been described in dogs in mainland China (Wen et al., 2003; Xia et al., 2012). *Ixodes persulcatus* plays a leading role in the transmission of *Borrelia burgdorferi* to humans in northern part of China (Zhang et al., 1997); whereas *I. granulatus* and *Haemaphysalis bispinosa* might serve as principal vectors of *Borrelia burgdorferi* in the southern region. It is also endemic in Japan, mainly on the northern island of Hokkaido (Azuma et al., 1994), but a new *Borrelia* species was identified in ticks collected on cats from Okinawa in southern Japan. Ticks removed from 1136 dogs and 134 cats all over Japan were examined for *Borrelia* infection by PCR and sequencing (Hiraoka et al., 2007). The 5S-23S rDNA intergenic spacer of *Borrelia* was detected from two *Ixodes persulcatus* ticks from two dogs and two unidentified *Ixodes* spp. from another two dogs in Hokkaido, and two *Ixodes granulatus* ticks from two cats in Okinawa. Additionally, two *I. granulatus* from the same cats were also positive. Sequence analysis of the PCR products revealed that the one from Hokkaido was similar to *B. garinii*, the three from Hokkaido to *B. tanukii*, and the four from Okinawa to a novel *Borrelia* sp. closely related to *B. valaisiana*.

Cats also suffer from tick bites and are hosts for *Ixodes* ticks. However, no case of a cat naturally infected with clinical LB has been described so far (Krupka and Straubinger, 2010).

Nevertheless, experimental infections via spirochete inoculation different from tick exposure resulted in short-lived bacteremia. If the cats were exposed to tick bites, they developed lameness and multilocalized inflammations such as arthritis or meningitis. Cats also developed measurable antibody responses. In northern parts of the United States, where LB is endemic, 13% to 47% of cats were found to be seropositive. It still is unclear why cats do not react as sensitively to a Borrelial infection as dogs, but it is hypothesized that they are not as susceptible to the dissemination of the spirochetes or that their immune response can neutralize the bacteria before clinical illness occurs. In the mid-1970s, Steere et al. (1978) and colleagues noted that a significant number of patients with Lyme disease had cats and had noted ticks on their pets, compared with their unaffected neighbors.

**Ehrlichiosis and Anaplasmosis**

Among the Anaplasmataceae, *Ehrlichia canis* was the first monocytic ehrlichiosis to be identified in dogs by Donatien and Lestoquard in Algeria in 1935 (Donatien and Lestoquard, 1935). The disease is endemic in many warm countries (usually below 45° latitude) (Davoust et al., 2003). In the 1970s the disease gained extensive attention when a large number of American military dogs, mainly German shepherds, died from the disease during the Vietnam War (Fritz, 2009). The infection naturally occurs through the bites of the brown dog tick *Rhipicephalus sanguineus*, which is endophilic and frequently occurs in kennels and wall crevices. *E. canis* transmission is trans-stadial. The tick becomes infected when ingesting blood from a dog that hosts *E. canis*,
in most cases during the acute phase of the disease. In infected ticks, *E. canis* proliferates in hemocytes, salivary cells, and intestinal cells. When the next tick stage bites another dog, a flow of mononuclear cells towards the inflammatory zone favors dissemination of the bacteria to the new host [Davoust et al., 2003]. In a trial to reduce the level of infection in dogs in two endemic areas of Africa (Dakar and Djibouti), the monthly use of a tick preventative (Fipronil) was effective to highly reduce the number of canine monocytic ehrlichiosis [Davoust et al., 2003]. Prevalence of up to 22% in dogs suspected of being infected with tick-borne diseases in southern Portugal was reported recently using a Polymerase chain reaction (PCR) detection method [Alexandre et al., 2009]. In Italy, *R. sanguineus* is the most common tick species infesting dogs and an important vector for *E. canis* in most of the country [Otranto and Dantas-Torres, 2010]. The prevalence of *E. canis* infection in dogs (n=601) using real time PCR was respectively 2.9%, 8%, and 9.7% in northern, central, and southern Italy [Solano-Gallego et al., 2006c]. Due to the geographical extension of *R. sanguineus* throughout continental Europe, canine monocytic ehrlichiosis tends to extend northward in countries such as Belgium and the Netherlands where the first autochthonous cases have recently been identified [Beugnet and Marié, 2009]. In France, a prevalence of less than 1% (0.33%) was reported recently [Pantchev et al., 2009]. However, a survey conducted between June 2006 and July 2007 among veterinary practices revealed that canine ehrlichiosis has been diagnosed in all parts of the country but is enzootic only in southern France, especially Corsica [Bourdeau, 2008]. An extension towards the Southeast and the center of the country also was reported [Bourdeau, 2008]. The global annual prevalence was estimated to range between 0.9 and 3 cases per thousand dogs with an average annual incidence of 2.1 cases per thousand dogs [Bourdeau, 2008]. In Israel, 30% of the 195 dogs tested were seropositive for *E. canis* [Levi et al., 2006]. In the USA, canine ehrlichiosis is a sporadic disease [Keefe et al., 1982]; however, a high prevalence (36%) of active infection was detected recently in dogs infested by *Rhipicephalus sanguineus* in northeastern Arizona [Diniz et al., 2010]. On the other hand, the disease is highly endemic in Southeast Asia, including Vietnam and Thailand. In Thailand, 71% (35/49) of the dogs tested were seropositive for *E. canis* [Suksawat et al., 2001]. In Japan, 15 (10%) of 150 dogs from Yamaguchi prefecture were highly seropositive for *E. canis* [Watanabe et al., 2004]. The agent of human monocytic ehrlichiosis, *E. chaffeensis*, has also been identified in dogs in North America [Kordick et al., 1999]. *E. chaffeensis* is restricted to the southeastern and south-central USA, deer being the likely reservoir host and the lone star tick, *Amblyoma americanum*, the principal tick vector [Fritz, 2009]. Infection of coyotes is common in Oklahoma, and canine infections are usually subclinical or mild [Nicholson et al., 2010]. In other parts of the world, PCR evidence of *E. chaffeensis* has been detected in domestic dogs from Korea, Venezuela [Yabsley, 2010], and Brazil [Oliveira et al., 2009]. Among the granulocytic ehrlichiosis and anaplasmosis, *Ehrlichia ewingii* is a pathogen of both humans and dogs which share a common geographic distribution (South Central and southeastern USA), a common tick vector (*A. americanum*), and seasonality [spring to autumn] with *E. chaffeensis* in the USA [Fritz, 2009]. Infection with this organism usually results in a mild to moderate
febrile disease in both dogs and people (Nicholson et al., 2010). Deer and possibly dogs are considered to be prime candidates to serve as mammalian reservoirs for *E. ewingii* (Nicholson et al., 2010). Outside of the USA, infection of dogs was reported in Cameroon (Ndip et al., 2005) and in Brazil (Oliveira et al., 2009). *Anaplasma phagocytophilum* is the causative organism of human granulocytic anaplasmosis and is transmitted by *Ixodes* spp. ticks (Nicholson et al., 2010). In North America, the tick species involved in the transmission of Lyme borreliosis also are involved in the transmission of *A. phagocytophilum*, mainly *I. scapularis* and *I. pacificus*. Therefore, the geographic distribution of this infection parallels that of Lyme borreliosis. The main reservoir species involve sigmodontine rodents in North America. The highest seroprevalence in dogs was reported from the Midwest (6.7%), the Northeast (5.5%), and California (4.8%) (Bowman et al., 2009). *Ixodes ricinus* is the main vector in Europe and *I. persulcatus* and *Dermacentor silvarum* in Asia and Russia (Carrade et al., 2009). Other *Ixodes* spp. ticks also have been implicated in transmission, including *I. trianguliceps*, *I. hexagonus*, and *I. ventralis* in Europe. In Europe, *A. phagocytophilum* infection in dogs was reported first in Switzerland and Sweden in the late 1980s (Beugnet and Marié, 2009) and now has been recognized in most countries, with its main vector being *I. ricinus*. The prevalence of infection in ticks ranges from 1.2% to 8.7% (Jensen et al., 2007). Evidence of canine infection is on the rise in most European countries, including Germany, where about 42% of healthy dogs tested were seropositive (Jensen et al., 2007), and clinical cases were reported recently in France and Italy (Beugnet and Marié, 2009; Otranto and Dantas-Torres, 2010). In the United Kingdom, infection by *A. phagocytophilum* also has been detected in 1 of 120 sick dogs tested that had not travelled outside of the country using molecular methods (Shaw et al., 2005). In Israel, 9% of 195 dogs tested were seropositive for *A. phagocytophilum* (Levi et al., 2006). In dogs, *A. platys* multiplies inside platelet vacuoles and causes canine infectious cyclic thrombocytopenia, which generally does not cause severe clinical signs. This disease was reported first in dogs in the USA (Shaw et al., 2001), and cases were detected in tick-infested dogs in North Carolina (Kordick et al., 1999). However, this infection is found worldwide with a higher incidence in tropical and subtropical areas such as South America where the brown tick *R. sanguineus* is assumed to be the vector. It is an emerging disease in Europe where *A. platys* has been diagnosed in infected dogs in Spain, Italy (mainly central 23% and southern 11.3% parts of the country), Portugal, and France (Beugnet and Marié, 2009; Cardoso et al., 2010; Otranto and Dantas-Torres, 2010). In the People Republic of China, many tick species have been identified to carry zoonotic erlichiae. In southern China, *E. chaffeensis* was detected in Amblyomma testudinarium ticks from infested cattle, *Haemaphysalis yenii* ticks from hare, and *Ixodes ovatus* ticks from *Muntiacus reevesi* (Wen et al., 2003). *E. canis* was identified in *Rhipicephalus sanguineus* ticks from dogs (Guangdong Province) and *Boophilus microplus* ticks from goats. A new species of the genus *Ehrlichia*, closely related to *E. chaffeensis*, and *Anaplasma marginale* was identified in *B. microplus* ticks from cattle in Tibet. In northern China, *E. chaffeensis* was detected in *Dermacentor silvarum* and *I. persulcatus* ticks; the granulocytic
Ehrlichial agents were detected in *I. persulcatus* ticks from an area where Lyme disease is endemic. Canine ehrlichioses were found in southern China (Guangdong province) and *E. canis* and *E. platys* were identified in dogs; human ehrlichioses were demonstrated by amplifying the 16S rRNA genes of *E. chaffeensis* and granulocytic *Ehrlichia* agents from patients’ blood specimens. In comparison of 16S rRNA gene sequences, the sequences of *E. chaffeensis*, *E. canis*, and *E. platys* in China were found to be different from that in other countries at certain nucleotide positions (Wen et al., 2003). In central Taiwan, it was shown that highly enzootic communities of ehrlichiosis/anaplasmosis in healthy dogs was scattered in mountainous environment at elevations between 100m and 1000m, those of heartworm disease was mainly distributed in urbanized plains (Yuasa et al., 2012). After multiple logistic regression analysis, it further showed that older age group and outdoor housing were associated with higher risk of heartworm infection; being male and having tick infestation were associated with higher risk of *E. canis* infection, whereas being male and free-roaming were associated with higher risk of *Anaplasma* infection.

In mainland China, a survey of the occurrence of *Dirofilaria immitis*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *Anaplasma phagocytophilum* in dogs was undertaken between October 2008 and October 2009 (Xia et al., 2012). A total of 600 blood samples were taken from dogs in four cities: 300 in Beijing, 150 in Shenzhen, 30 in Shanghai and 120 in Zhengzhou. All samples were tested for the heartworm antigen and antibodies of canine *B. burgdorferi*, *E. canis* and *A. phagocytophilum* by using the canine [SNAP® 4Dx®] test kit. The occurrence of *D. immitis*, *B. burgdorferi*, *E. canis* and *A. phagocytophilum* was 1.17% (7/600), 0.17% (1/600), 2.17% (13/600) and 0.5% (3/600), respectively. In Shenzhen city, 2% (3/150), 8.67% (13/150) and 2% (3/150) of samples were positive for *D. immitis*, *E. canis* and *A. phagocytophilum*, respectively. The occurrence of heartworm antigen was 0.33% (1/300) in Beijing, 2.00% (3/150) in Shenzhen, 3.33% (1/30) in Shanghai and 1.67% (2/120) in Zhengzhou. *E. canis* and *A. phagocytophilum* were detected only at one site, Shenzhen, while the only occurrence of *B. burgdorferi* was at Beijing. Therefore, the dog population in China is at potential risk for *D. immitis*, *B. burgdorferi*, *E. canis* and *A. phagocytophilum* infection, the risk being especially high in southern China (Xia et al., 2012).

In Thailand, in 49 sick dogs with fever, anemia or thrombocytopenia, seroreactivity (IFA test) was most prevalent to *E. chaffeensis* (74%) and *E. canis* (71%) antigens, followed by *A. phagocytophilum* (58%), *Bartonella vinsonii* subsp. berkhoffii (38%), *E. risticii* (38%), *R. prowazekii* (24%), *Babesia canis* (20%), *R. rickettsii* (12%), *R. canadá* (4%), and *B. gibsoni* (4%) antigens (Suksawat et al., 2001). By PCR amplification, 10 dogs were found to be infected with *E. canis*, 5 with *E. platys*, and 3 with *Babesia canis*. *Ehrlichia canis* and *E. platys* have also been reported in dogs in Japan (Inokuma et al., 2001).

Cats are also susceptible to *Ehrlichia canis* and *Anaplasma phagocytophilum* infection. Natural exposure to these pathogens in the United States has been assessed by serologic and molecular assays (Billeter et al., 2007); 30% to 38% seropositivity to *A. phagocytophilum* was observed from 84 cats from the northeastern United States, and evidence of infection with the agent was reported from several clinically ill cats from Massachusetts.
and Connecticut. 20/460 (4.3%) sero+ in cats from all over the USA. Similarly, seropositive cats for *Ehrlichia* (11%) and *Anaplasma* (2%) were identified in Spain among 168 tested animals (Solano-Gallego et al., 2006a).

**Rickettsiae infections**

Several infections are associated with tick-borne *Rickettsiae* in dogs. Among these, Rocky Mountain Spotted fever (RMSF) and Mediterranean Spotted fever are the more severe and widespread (Nicholson et al., 2010; Shaw et al. 2001).

**Rickettsia rickettsii**

RMSF is a potentially fatal disease of humans and dogs caused by *R. rickettsii*. It is widely distributed in the Americas. In the USA, infection is transmitted by infected *Dermacentor variabilis*, the American dog tick, or *D. andersonii*, the Rocky Mountain wood tick. Recently, *R. sanguineus* has been implicated as a new vector of RMSF in Arizona and Northern Mexico (Nicholson et al., 2010). *Amblyomma americanum* also can occasionally transmit *R. rickettsii* (Fritz, 2009). In Central and South America, *Amblyomma cajennense* (Cayenne tick) and *A. aureolatum* (golden dog tick) have been implicated as the vectors to humans and dogs (Walker et al., 2008). Two cases were reported recently in dogs in Brazil (Labruna et al., 2009). In the USA, RMSF is a seasonal disease and occurs throughout the USA during the months of April through September. Over half of the human cases occur in the south-Atlantic region of the USA (Delaware, Maryland, Washington D.C., Virginia, West Virginia, North Carolina, South Carolina, Georgia, and Florida), and the highest incidence rates have been reported in North Carolina and Oklahoma. RMSF tends to be more common in young (<3 years old) dogs, and more than 80% of clinical cases occur in dogs that are frequently outdoors. Dogs have long been recognized as efficient sentinels for RMSF in human populations, and infections in canines often have been associated with an increased risk for the disease in owners or residents in close proximity (Paddock et al., 2002).

**Rickettsia conorii**

Mediterranean spotted fever (MSF) is caused by infection by *R. conorii* which is transmitted through the bite of *R. sanguineus* ticks or by accidental mucus membrane inoculation of *R. conorii* from crushed ticks. However, only a small proportion of *R. sanguineus* ticks are infected with *R. conorii* as infection rates are generally below 15% (Rovery and Raoult, 2008). The disease is distributed widely across southern Europe, Northern Africa, southern Africa, the Middle East, the Indian subcontinent, and Asia (Nicholson et al., 2010). In Europe, the disease in humans appears to be in its second emergence since its discovery (Rovery and Raoult, 2008). Dogs are considered to be important transport hosts, capable of carrying infected ticks close to their owners (Rovery and Raoult, 2008), and sporadic cases have been associated with the dispersion of *R. sanguineus* in northern France, Belgium, and Germany (Beugnet and Marié, 2009). In areas of southern Europe, a correlation between the percentage of the canine population with antibodies to *R. conorii* and the intensity of human infection has been proven. Seropositivity was higher in dogs from habitats of patients who had MSF (Rovery and Raoult, 2008). *Rickettsia conorii* seroprevalence in dogs is high (26%-60%) in disease-endemic regions (Solano-Gallego et al., 2006b; Otranto and Dantas-Torres, 2010). In dogs, clinical signs of disease have rarely been reported. Illness has been associated with *R. conorii* natural infection in only 2 dogs.
since human MSF was described in 1932, but infection with R. conorii in 3 acutely ill, febrile Yorkshire terrier dogs from Sicily, Italy, was recently confirmed by PCR, DNA sequencing, and seroconversion [Solano-Gallego et al., 2006b].

Other Rickettsiae
R. parkeri has been recognized only recently as a human pathogen in the USA and possibly in Uruguay [Nicholson et al., 2010]. It was detected in a dog in Bolivia [Tomassone et al., 2010], and serological evidence of dog exposure was demonstrated in Brazil [Nicholson et al., 2010]. R. massiliae is another emerging Rickettsial pathogen found in R. sanguineus ticks. R. massiliae has been detected in several countries of southern Europe and of Africa, and in Arizona, USA, but it is still not known if dogs are naturally infected [Eremeeva et al., 2006].

PROTOZOAN TICK-BORNE DISEASES IN DOGS

Babesia and Theileria
Babesiosis, also known as piroplasmosis, is one of the most important tick-borne infections of dogs, and has a worldwide distribution. Piroplasms of domestic animals encompass two main genera, Babesia and Theileria. Although babesiosis is mainly tick-borne, B. gibsoni is an emerging disease with molecular evidence of clonal expansion due to non-vectored transmission by blood exchange during fighting and biting [Irwin, 2009]. Molecular genotyping of canine piroplasms has resulted in the identification of four large and at least four small parasites. Babesia canis was reclassified into three sub-species (B. canis canis, B. canis rossi and B. canis vogeli), and a fourth ‘large’ Babesia species was described in sick dogs in North Carolina [Birkenheuer et al., 2004; Irwin, 2009]. Babesia canis canis mainly infects dogs in Europe and is transmitted by Dermacentor spp. Babesia canis vogeli, transmitted by R. sanguineus, has a wide distribution in tropical, subtropical, and Mediterranean countries and has been reported in continental Europe [Beck et al., 2009]. Finally, B. canis rossi is reported in sub-Saharan Africa and South Africa, being transmitted by Haemaphysalis elliptica [Irwin, 2009].

The three small Babesia species are:
1) Babesia gibsoni, mainly reported in Asia, including Japan and Korea [Lee et al., 2009], where the tick vector is Haemaphysalis longicornis and in southeastern Australia [Jefferies et al., 2003] but has a sporadic distribution worldwide,
2) Babesia conradae, reported in dogs in the western USA [Kjemtrup et al., 2006],
3) a B. microti-like piroplasm [named Theileria annae] found in the Iberian Peninsula and more recently in Croatia [Beck et al., 2009] and thought to be transmitted by I. hexagonus. In addition, three Theileria species have been isolated in a small number of dogs’ blood in Europe [Theileria (Babesia) equi and Theileria annulata] and from dogs in South Africa [Irwin, 2009]. Finally, Theileria equi, a small piroplasm of horses, has been reported in dogs from Spain [Criado-Fornellio et al., 2003].

In Europe, distribution of canine babesiosis is expanding, as autochthonous cases have been reported in the Netherlands [Matjila et al., 2005] and more recently a case was reported from Norway [Oines et al., 2010]. In several non-endemic European countries, including Switzerland, Germany, and Belgium, reported outbreaks of
Babesia canis have been associated with D. reticulatus ticks. In fact, E. canis already has been found in dogs introduced into The Netherlands, either alone or in combination with Babesia canis (Matjila et al., 2005). In Croatia, a prevalence of 3.42% (29 of 848) in asymptomatic dogs was reported, and sequence analysis revealed the presence of Babesia canis canis in 20 dogs (69%), Babesia gibsoni in six dogs (21%), Babesia canis vogeli in two dogs (7%), and Theileria annae in one dog (3%); the first report of this Theileria species outside Spain and Portugal (Beck et al., 2009). In Europe, human babesiosis is caused by B. divergens, B. microti, and EU1 and is thought to be transmitted by Ixodes ricinus (Vannier et al., 2008). EU1 is a species closely related to B. odocoilei and known to infect white-tailed deer. It was first identified in 2003 when two asplenic patients from the Tyrol region of Austria and the Alpine region of Italy developed a severe illness that was found to be caused by this new Babesia species.

In the North America, dogs positive for Babesia DNA were located in 29 US states and 1 Canadian province (Ontario). Babesia gibsoni was the species most commonly detected, as it was present in blood samples from 131 (91%) of 144 dogs (Birkenheuer et al., 2005). In Asia, babesiosis has been reported in Japan (B. microti-like), Korea (K01), Taiwan (TW1), and India (Vannier et al., 2008).

Severe babesiosis may develop in patients with immunodeficiency caused by splenectomy, malignancy, immunosuppressive therapy, or HIV co-infection. Patients older than 50 years or people who experience B. divergens or B. duncani infections also are at risk for severe disease (Vannier et al., 2008).

Hepatozoonosis

Hepatozoon canis has long been recognized to infect and cause disease in dogs worldwide, including Japan, India, Africa, the Middle East, Spain, France, Italy, Greece, Brazil, Thailand, and the Philippines (Potter and MacIntire, 2010). In Brazil, almost 60% of 92 healthy dogs tested in the State of Espirito Santo were PCR positive for Hepatozoon canis (Spolidorio et al., 2009). In 1978, cases of hepatozoonosis were recognized for the first time in the southern USA (Little et al., 2009). Recent reports showed that H. canis is present in some regions of North America (south-central and southeastern USA) (Potter and MacIntire, 2010) and the Caribbean islands (Grenada; detected in 5 (7%) of 73 dogs) (Yabsley et al., 2008), where its vector tick, R. sanguineus, is endemic (Little et al., 2009). While all canine infections were attributed initially to H. canis, in 1997 a novel species, H. americanum was identified in dogs in the southern USA. Contrary to H. canis infection, H. americanum, typically results in a severe debilitating course of illness which, in the absence of treatment, is usually fatal (Potter and MacIntire, 2010). The range of H. americanum is likely expanding along with the range of its vector tick, Amblyomma maculatum, the Gulf coast tick, and co-infections with H. canis and H. americanum also have been identified (Little et al., 2009).

In Europe, of 108 dogs tested in France for hemoprotozoa, one dog was infected by H. canis (0.9%) and another by Babesia canis vogeli (0.9%) (Criado-Fornelo et al., 2009), and in Germany, H. canis was detected in 2.7% of more than 4,600 dogs imported from endemic areas or that had travelled to endemic areas (Menn et al., 2010). In that same study, Babesia canis and E. canis were detected in 23.45 and 10.1% of the dogs.
**BARTONELLA, ANOTHER POTENTIAL TICK-BORNE PATHOGEN**

Recent reports involving humans and canines suggest that ticks should be considered as potential vectors of *Bartonella* spp. (Billeter et al., 2008). However, this concept is still controversial (Telford and Wormser, 2010). The first demonstration of the role of ticks in *Bartonella* infection was made in the 1920s, when *B. bacilliformis* was recovered from tick viscera after feeding on bacteremic monkeys (Chomel et al., 2009). PCR or culture methods have been used to detect *Bartonella* in ticks, either questing or host-attached, throughout the world. Case studies and serological or molecular surveys involving humans and canines provide indirect evidence supporting transmission of *Bartonella* species by ticks (Billeter et al., 2008). Prevalence of specific antibodies or detection of *Bartonella* DNA was observed more frequently in dogs infected by other known tick-borne pathogens (Chomel et al., 2009). However, critically important experimental transmission studies have not been performed for *Bartonella* transmission by many potential arthropod vectors, including ticks (Billeter et al., 2008). A recent publication reported successful experimental transmission of *B. henselae* from *I. ricinus* salivary glands to cats (Cotté et al., 2008) but no proof of natural vector capacity has been published yet for *Bartonella* transmission by ticks (Chomel et al., 2009). Despite numerous molecular surveys to detect *Bartonella* DNA in ticks, there is little evidence that *Bartonella* spp. can replicate within ticks and no definitive evidence of transmission by a tick to a vertebrate host (Angelakis et al., 2010). *B. vinsonii* subsp. *berkhoffii* was isolated from 2 out of 71 dogs from Yanggu County in Shandong Province, indicating the presence of this bacterium in China (Li et al., 2006). On the contrary, it has not been detected in dogs from Taiwan (Tsai et al., 2011).

**Q FEVER (COXIELLA BURNETII)**

Q fever has been associated with exposure to parturient cats and dogs in the USA and Canada and is mainly transmitted by aerosols (McQuiston and Child, 2002). However, it can also be tick-borne. Q fever occurs almost all over the Japan (Porter et al., 2011), with an estimated 7 to 46 cases per year since 1999 when Q fever became a notifiable disease in humans in Japan (127 million inhabitants) (Mahara, 2006). Dogs and cats have been found to be positive for *C. burnetii* by serology and bacteriology throughout the Japanese territory, and cats can be considered as a possible source of human infection, whereas no human cases have so far been related to dog infection (Porter et al., 2011). Stray cats were found to have a higher incidence of infection than domestic cats with seroprevalence ranging from 6.7% in one study to almost 19% in another one (Porter et al., 2011). For dogs, seroprevalence ranging from 10% to 17% was reported (reviewed in Porter et al., 2011).

**EMERGENCE OF FLEA-BORNE PATHOGENS**

*Bartonella* species and cat scratch disease

Cat scratch disease has been diagnosed in many parts of Asia, including China, Taiwan, Japan and many countries in South East Asia. In Japan, most feline strains are *B. henselae* type I, as also reported in cats from the Philippines (Chomel et al., 2006). CSD has also been reported in Australia. In this
country, most human cases seem to be associated with *B. henselae* type I whereas cats are more likely to be infected with *B. henselae* type II (Dillon et al., 2002). Domestic cats represent the main reservoir in these countries. However, the role of dogs varies greatly from lack of detection (Japan, Maruyama, personal communication), to infection by very diverse *Bartonella* species, as in Thailand (Bai et al., 2010).

**Yersinia pestis, plague**

Cats are highly susceptible to plague and have been shown to be the source of human cases in the USA (Gage et al., 2000). Dogs have also been shown to be a potential source of human exposure to plague (Gould et al., 2008). In China, 12 natural plague foci remain, covering more than 291 counties in 19 provinces (Li et al., 2008). The Yunnan Province includes two of these plague foci. After a human outbreak in Yulong County (Yunnan Province) in October 2005, 689 dogs and 151 cats samples were collected in and from around the infected area. Using the result of PHA $\geq 1:40$ as positive criterion, 162 of 689 (23.5%) sera from domestic dogs and 40 of 151 (26.5%) sera from domestic cats were positive for plague (Li et al., 2008). The study showed that the seropositive rates of domestic cats in the villages around a human-infected village were much higher than that in villages further away, and high antibody titers against the F1 antigen were detected in domestic cats (from 1:80 to 1: 20,480), suggesting that domestic cats might play an important role in the spread of plague in the Yunnan Province.

**Murine typhus (R. typhi) and flea-borne spotted fever (R. felis)**

Cat fleas are potentially able to vector Rickettsial diseases including murine typhus (*Rickettsia typhi*) and a closely related zoonotic disease agent, *Rickettsia felis* which are potential human health threats wherever cat, rat or flea populations are dense (Gerhold and Jessup, 2012). Cats are inapparent carriers of *R. typhi*, and outbreaks have been associated with free-roaming cat colonies in Hawaii. Other reported cases of murine typhus in the United States are focused in central and south-central Texas and Los Angeles area. In the Los Angeles *R. typhi* focus, 90% ($n = 9$) of collected cats were seropositive for *R. typhi* antibodies, whereas no seropositive cats ($n = 21$) were found in the control areas where no human infections were reported (Gerhold and Jessup, 2012). Murine typhus is endemic in many parts of Asia, but is mainly related to rats and rat fleas. The role of cats in the epidemiology of the infection has not been investigated in that part of the world.

Flea-borne spotted fever is caused by *R. felis*, and is mainly transmitted by the cat flea and is maintained by transstadial and transovarial transmission in *C. felis* (Hirunkanokpun et al., 2011). More than 70 human cases of flea-borne spotted fever have been reported in various parts of the world, including the USA (Texas, California, Oklahoma), South America (Brazil, Peru, Mexico), Europe (Spain, Germany, France), Asia (Thailand, Laos, South Korea, Taiwan) and Africa (Egypt, Tunisia) (Reif and Macaluso, 2009). The first human cases of flea-borne spotted fever were recently described in Victoria, Australia (Williams et al., 2011). *R. felis* has been detected in cat flea in Western Australia and more recently in 3 (2.3%) of dogs from the northern territories in a remote indigenous community in Maningrida (Hii et al., 2011). *R. felis* has also been detected in humans with a rash in Sri Lanka (Angelakis et al., 2012) and in fleas (6/209; 2.9%) collected on domestic dogs and cats in various locations of Malaysia (Mokhtar and Tay, 2011) and cat fleas collected
Dogs and cats can be a source of several tick and flea-borne zoonoses, especially by bringing these vectors in the human environment. Proper vector control and hygiene measures, including regular immunization, may help reduce the risk of dispersion of these zoonoses.