Review article

Anaplasma phagocytophilum: An emerging but unrecognized tick-borne pathogen

S. EL HAMIANI KHATAT[1], H. SAHIBI[1]

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Abstract

Several vector-borne pathogens are considered to be emerging or re-emerging. Among these agents, *Anaplasma phagocytophilum* is a tick-borne rickettsiale bacterium of worldwide distribution. This bacterium is increasingly detected in several parts of the world in both ticks and hosts, including animals and humans. *A. phagocytophilum* is the causal agent of a zoonotic disease called 'granulocytic anaplasmosis' and described in several domestic animals and humans. Currently, human granulocytic anaplasmosis is considered to be the third most important vector-borne disease in both the USA and Europe and is also increasingly diagnosed in some Asian countries. However, in many parts of the world, this disease is unknown and no data are available on its epidemiology.

Key words: *Anaplasma phagocytophilum*, Granulocytic anaplasmosis, Epidemiology, Vector-borne disease, Zoonosis.

Résumé

De nombreux agents vectoriels sont actuellement considérés comme émergents ou ré-émergents. Parmi ces organismes, *Anaplasma phagcytophilum* est une bactérie transmise par les tiques de distribution mondiale. Cette bactérie est de plus en plus détectées dans différents pays aussi bien chez le vecteur, la tique, et les hôtes constitués par les animaux et l'homme. *A. phagocytophilum* est responsable d'une maladie zoonotique, décrite chez différents animaux domestiques et chez l'homme, appelée 'anaplasmose granulocytaire'. Actuellement, cette maladie est considérée comme la troisième plus importante maladie vectorielle en Europe et aux USA et est également de plus en plus rapportée en Asie. Toutefois, elle reste méconnue dans une large majorité de pays et aucunes données épidémiologiques n'y sont disponibles.

Key words: *Anaplasma phagocytophilum*, Anaplasmose granulocytaire, Épidémiologie, Maladie vectorielle, Zoonose.

INTRODUCTION

Anaplasma phagocytophilum is an obligate intracellular gram-negative tick-borne rickettsiale bacterium of worldwide distribution (Diniz and Breitschwerdt, 2012; Dumler *et al.*, 2005; Woldehiwet, 2010). The bacterium is usually transmitted through the bites of ticks of *Ixodes* genera (Stuen *et al.*, 2013; Swanson *et al.*, 2006). This organism develops within intracytoplasmic inclusions in granulocytic cells mainly neutrophils (Diniz and Breitschwerdt, 2012; Dumler *et al.*, 2005; Woldehiwet, 2010). *A. phagocytophilum* infects a wide variety of wild and domestic animals and causes an emerging zoonotic tick-borne disease called granulocytic anaplasmosis (Beugnet and Marié, 2009; Dumler *et al.*, 2005; Keesing *et al.*, 2014).

Tick-borne pathogens, a rising hazard in veterinary and human medicine

Vector-borne diseases (VBDs) are caused by various infectious agents including parasites, bacteria and viruses that are transmitted to a host through the bite of hematophagous arthropods (Baneth, 2014; Beugnet and Marié, 2009; Heyman *et al.*, 2010). A wide variety of VBDs affect both human and animals (Heyman *et al.*, 2010). Ticks are considered to transmit the widest number of pathogens when compared to other arthropod vectors. Several of these pathogens are of veterinary and medical importance and cause various diseases including anaplasmosis, babesiosis, Lyme borreliosis, ehrlichiosis and rickettsiosis (Baneth, 2014; Heyman *et al.*, 2010; Nijhof *et al.*, 2007). In geographic areas where mosquitos are not prevalent, TBDs are the primary causes of VBDs (Baneth, 2014; Michelet *et al.*, 2014). VBDs and more specifically tick-borne diseases (TBDs) are a growing economic problem causing serious depression in livestock production worldwide (Heyman *et al.*, 2010). Furthermore, canine VBDs have been of increased focus interest because they constitute an important threat to both canine and human health and are thus of major zoonotic relevance (Krämer *et al.*, 2014; Rizzoli *et al.*, 2014; Schreiber *et al.*, 2014). Human is mostly not considered the main host target of tick-borne pathogens contrary to other vector-borne pathogens such as malaria or leishmaniasis, but an accidental host contaminated during the circulation between the vector and the wildlife host (Baneth, 2014; Michelet *et al.*, 2014).

Several VBDs are considered to be emerging in new regions or re-emerging and changes in their epidemiological features are described including extension of geographical distribution, changes in pathogenicity and increased prevalence (Beugnet and Marié, 2009; Rizzoli *et al.*, 2014; Swanson *et al.*, 2006). Indeed, several TBDs have been

[1] Institut Agronomique et Vétérinaire Hassan II, Maroc

recently reported in previously not affected areas such as babesiosis in northern Germany, Belgium, Poland and the Netherlands, *Candidatus* Neoehrlichia mikurensis in central and northern Europe and Africa, canine monocytic ehrlichiosis is reported to extend from southern Europe to northern areas of France and described recently in Poland, Belgium and the Netherlands, tick-borne encephalitis virus is also extending to northern parts of Europe and granulocytic anaplasmosis in northern Europe (Beugnet and Marié, 2009; Day, 2011; Heyman *et al.*, 2010; Krämer *et al.*, 2014; Schreiber *et al.*, 2014).

Multiple factors are supposed to play a crucial role in ticks expansion, mainly increased animal travelling, climatic changes with global warming, landscape changes, rehabilitation and management with increased urbanization, development of large suburban areas with private gardens, creation of artificial lakes, deforestation and reforestation, increased open-air activities, changes in wild fauna, loss of biodiversity and decreased host population densities (Baneth, 2014; Day, 2011; Rymaszewska and Adamska, 2011; Yancey *et al.*, 2014). All these conditions affect the ecology and epidemiology of infectious diseases, enable the circulation, multiplication and spread of both vectors and pathogens into formerly unaffected areas, promote the creation of niches for vectors and the capacity to vector newly acquired pathogens, and also increase the risk for the host to enter in contact with vectors (Baneth, 2014; Day, 2011; Krämer *et al.*, 2014; Yancey *et al.*, 2014).

The combination of urbanization, climate changes and landscape modification in urban and peri-urban areas create favorable conditions to further increase ticks populations, extend areas of tick exposure for humans, promote the transmission of tick-borne pathogens and increases the number of human patients (Beugnet and Marié, 2009; Nicholson, 2010; Rizzoli *et al.*, 2014). Beside these environmental changes leading to increased hazard exposure to well-known vector-borne pathogens, increased clinician awareness, new diagnostic tools, improved surveillance and increased reporting and communication of tick-borne diseases in several countries can also explain the increased incidence of some tick-borne diseases (Baneth, 2014; Doudier *et al.*, 2010; Folkema *et al.,* 2012; Heyman *et al.*, 2010).

Finally, advances in molecular biology allow the discovery of new species, strains or genetic variants and extend the list of microorganisms able to infect either animals or humans or both (Baneth, 2014; Doudier *et al.*, 2010; Rizzoli *et al.*, 2014). Therefore, TBDs are increasingly recognized as rising hazard for public health (Rizzoli *et al.*, 2014). These observations are particularly obvious for *A. phagocytophilum*. Indeed, the environment suitability of *Ixodes scapularis* and *I. pacificus*, the main vectors for *B. burgdorferi* and *A. phagocytophilum*, seems to increase in Canada (Villeneuve *et al.*, 2011). Furthermore, high prevalences of *A. phagocytophilum* in ticks have been recorded several in European countries (Rizzoli *et al.*, 2014) and its prevalence is increasing in both animals and humans (CDC, 2008; Folkema *et al.*, 2012; Heikkilä *et al.,* 2010). In the USA, both canine and human exposure has progressively increased from 2008 to 2010 and from 2009 to 2010 respectively. The number of reported human cases has increased by 53% during this period (Qurollo *et al.*, 2014).

Description of the pathogen *Anaplasma phagocytophilum*

Anaplasma phagocytophilum is an obligate intracellular gram-negative tick-borne rickettsiale bacterium of worldwide distribution (Berzina *et al.*, 2013; Diniz and Breitschwerdt, 2012; Dumler *et al.*, 2005; Keesing *et al.*, 2014). The name of *A. phagocytophilum* has been given in 2001 to merge three previously distinct agents, the agent of equine granulocytic anaplasmosis or previously ehrlichiosis (*E. equi*), the agent of tick borne fever or pasture fever in sheep and cattle (*E. phagocytophila*) and the agent of human granulocytic anaplasmosis (previously human ehrlichiosis) (Dumler *et al.*, 2001).

This bacterium belongs to the family of *Anaplasmataceae* in the order of *Rickettsiales* (Alleman and Wamsley, 2008; Woldehiwet, 2010). The family *Anaplasmataceae* includes obligate intracellular arthropod-borne α-proteobacteria responsible of endemic and emerging diseases of major relevance in both veterinary and human medicine with important economic and public health outcomes (Pruneau *et al.*, 2014). More specifically, *Anaplasma* and *Ehrlichia* genera include tick-borne pathogens that mostly infect peripheral blood cells (Dumler *et al.*, 2005). Indeed, *A. phagocytophilum* infects myeloid cells of bone marrow, mainly neutrophils but also occasionnaly eosinophils. This organism develops within intracytoplasmic inclusions of varying size (from 1.5 to 6 mm in diameter) and derived from the host cell membrane called "morula" (from Latin morum "mulberry") (Figure 1) (Diniz and Breitschwerdt, 2012; Dumler *et al.*, 2005; Woldehiwet, 2010).

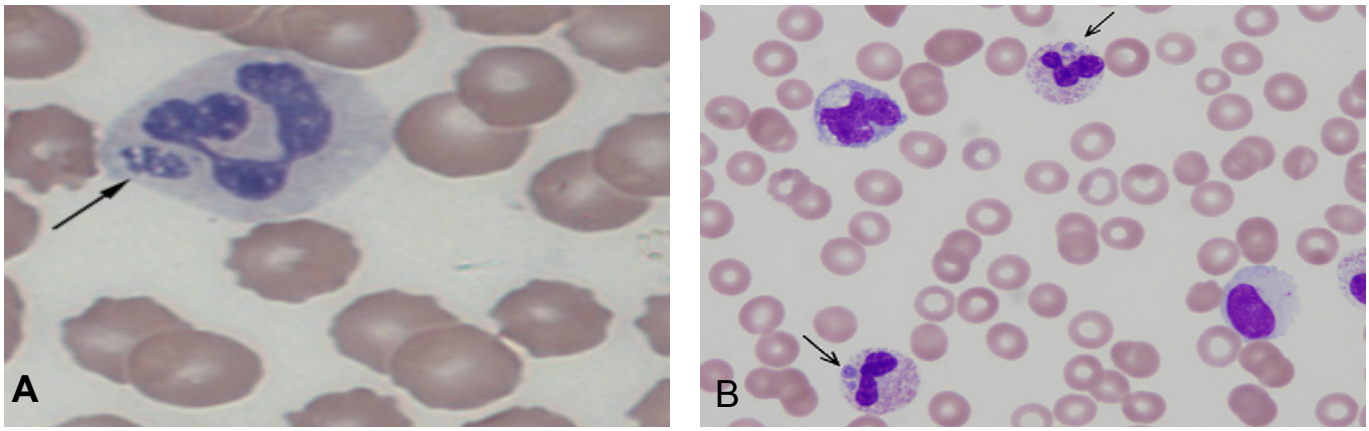

Figure 1: Morulae of A. phagocytophilum morula (black arrows) within a canine neutrophil, Wright's stain, ×100 (A) (Carrade et al., 2009) and in neutrophil of a 65-year-old man with a 2-day history of fever and diarrhea, Wright stain, ×100 (B) (Weil et al., 2012).

A. phagocytophilum causes a zoonotic disease called granulocytic anaplasmosis (Beugnet and Marié, 2009; Dumler *et al.*, 2005; Keesing *et al.*, 2014). Before the mid 1990s and the discovery of the first cases of human granulocytic anaplasmosis (formerly human granulocytic ehrlichiosis) in Wisconsin, *A. phagocytophilum* was thought to infect only domestic animals and free-living reservoirs (Cochez *et al.*, 2011; Dumler *et al.*, 2005; Kybicová, 2010).

Epidemiological features of *A. phagocytophilum*

Geographic distribution

A. phagocytophilum infection in both people and domestic animals has a worldwide geographic distribution that commonly follows the distribution of its vector, *Ixodes spp* ticks (Diniz and Breitschwerdt, 2012; Dumler *et al.*, 2007). Although *A. phagocytophilum* has been identified in several countries, the endemic areas include the upper Midwest, New England, western coast especially northern California, eastern and northeastern regions of the USA, British Colombia and some central and northern European countries (Sainz *et al.*, 2015; Swanson *et al.*, 2006; Keesing *et al.*, 2014). The organism has also been reported in Scandinavia, Baltic countries (Latvia, Lithuania, and Estonia), Finland, Asia, South America and North Africa (Berzina *et al.*, 2013). However, data are lacking in large areas including parts of Asia, Africa, Latin America and Oceania (Swanson *et al.*, 2006). In North Africa, only few data have been recently published in Tunisia, Algeria and Egypt (Azzag *et al.*, 2015; Ben Said *et al.*, 2014; Ghafar and Amer, 2012; M'ghirbi *et al.*, 2009; M'ghirbi *et al.*, 2012).

Vector

A. phagocytophilum is defined as a tick-borne disease, contamination of people and domestic animal occurs mostly after tick bites especially when they come in contact with the vector in host reservoir habitat (Diniz and Breitschwerdt, 2012; Leiby *et al.*, 2002). The bacterium is transmitted most frequently by ticks of *Ixodes persulcatus* complex (Stuen *et al.*, 2013; Swanson *et al.*, 2006). Ticks included in this complex are involved in the transmission of the majority of *Ixodes*-vectored human diseases and the most important species are *I. scapularis*, *I. pacificus*, *I. ricinus* and *I. persulcatus* (Swanson *et al.*, 2006). These ticks have a worldwide distribution but are mainly encountered in the northern hemisphere (Figure 2) (Swanson *et al.*, 2006; Woldehiwet, 2010).

Ixodes species involved in the transmission of the bacterium varies according to the geographic area (Figure 2). In the United States, the main vectors are *I. scapularis* (blacklegged or deer tick) and *I. pacificus* (western-blacklegged tick) (Figure 3) (Doudier *et al.*, 2010; Jin *et al.*, 2012). In Europe, especially in Northwestern areas, the most common vector is *I. ricinus* (sheep or castor bean tick) (Figure 3) (Jin *et al.*, 2012; Swanson *et al.*, 2006). Some authors considered that this tick is the only vector of *A. phagocytophilum* in Europe (Heyman *et al.*, 2010; Sainz *et al.*, 2015). *I. persulcatus* tick (Figure 3) is also present in some European regions especially eastern parts, and can share some areas with *I. ricinus* (Figure 2). This tick species has a geographic area that extends into Japan and is considered the primary vector in Asia (Sainz *et al.*, 2015; Swanson *et al.*, 2006). In eastern Mediterranean areas and North Africa, ticks of *Rhipicephalus, Hyalomma*, and *Haemaphysalis* genera are also involved in the transmission of the bacterium (Ghafar and Amer, 2012; Qablan *et al.*, 2012; M'ghirbi *et al.*, 2012; Sarih *et al.*, 2005). A wide variety of other tick species were found to be positive to *A. phagocytophilum* in the USA, Europe and Asia including other *Ixodes spp., Amblyomma americanum, Dermacentor spp., (*Doudier *et al.*, 2010; Jin *et al.*, 2012; Stuen *et al.*, 2013).

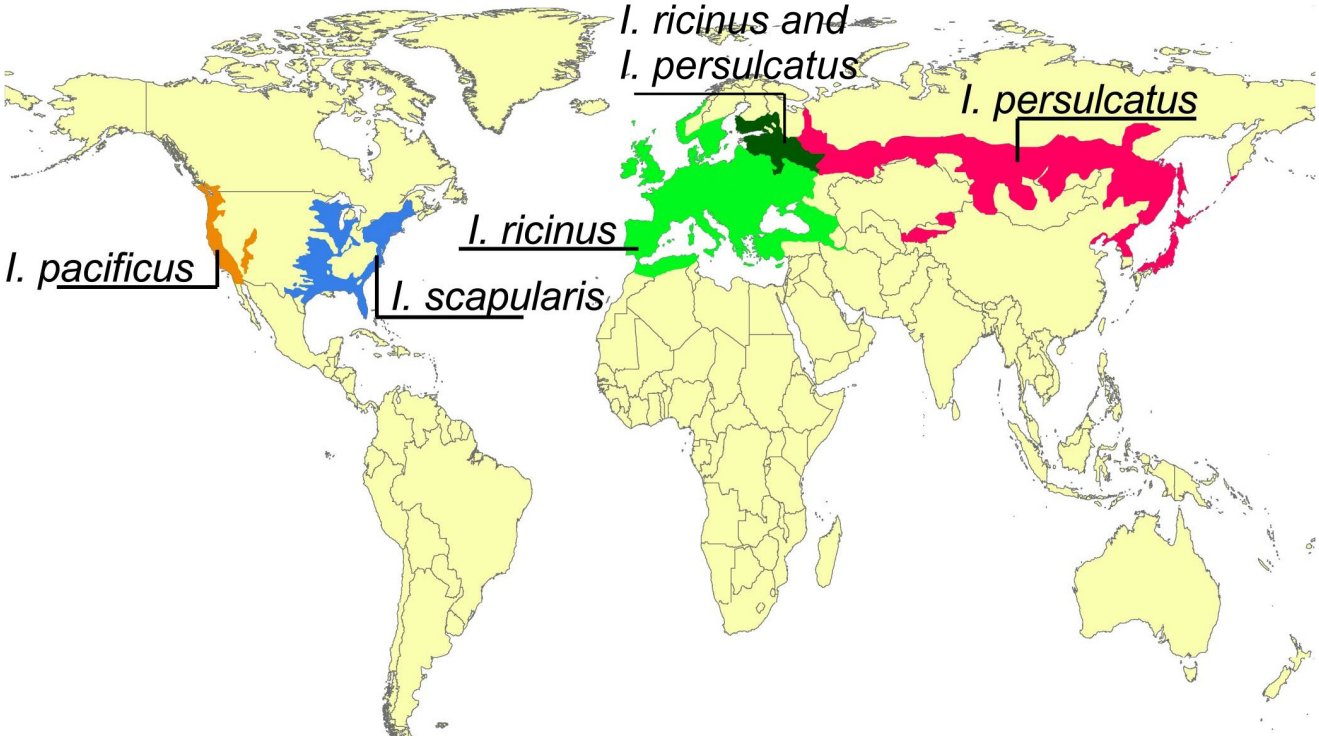

Figure 2: Worldwide geographic distributions of Ixodes tick species, vectors of A. phagocytophilum (Adapted from Swanson et al. 2006)

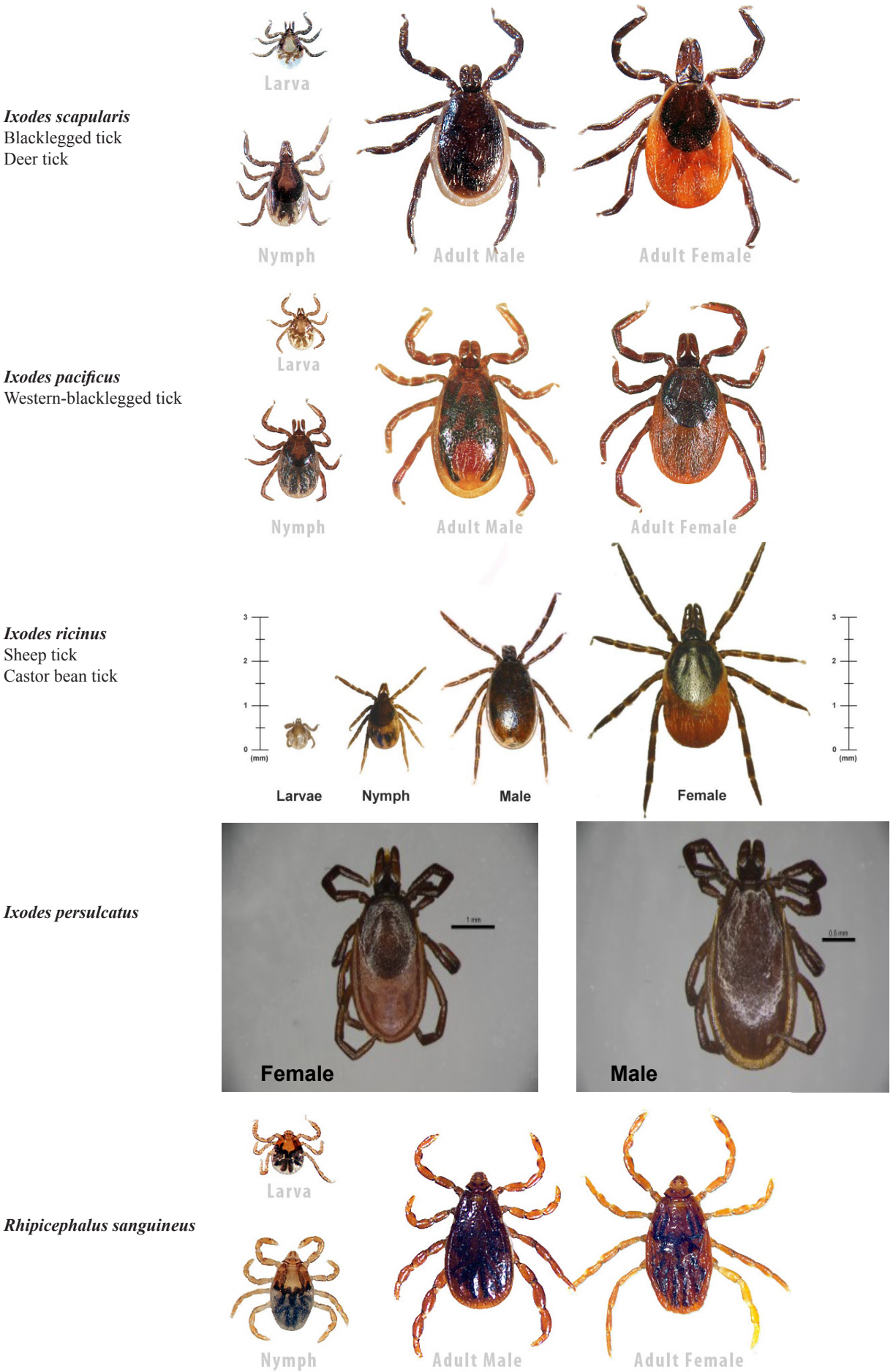

Figure 3: Ticks involved in the transmission of A. phagocytophilum in the USA, Europe, Asia and the Mediterranean region. I. scapularis I. pacificus, R. sanguineus (TickEncounter Resource Center of the University of Rhode Island), I. ricinus (Stanek et al. 2012), tick developmental stages and I. persulcatus (Online photographic guide to ticks, Bristol University tick ID).

Reservoir hosts

The absence of transovarian transmission in the vector makes it unable to act as a reservoir of *A. phagocytophilum.* Although a wide range of animal species can be infected by the bacterium, hosts might fulfill several characteristics to be considered as competent reservoir. Indeed, a host reservoir must be fed on by an infected vector tick at least occasionally, take up a critical number of the infectious agent during the bite by an infected tick, allow the pathogen to multiply and survive for a period in at least some parts of his body and might allow the pathogen to find its way into other feeding ticks (Rizzoli *et al.*, 2014; Stuen *et al.*, 2013). Several mammalian species are considered as host reservoir and thus, enable the persistence of the bacterium between seasons of tick activity and its spread through their movement in case of migratory animals (Stuen *et al.*, 2013). Among them, rodents and wild cervids are considered to be the principal host reservoir of the bacterium (Alleman and Wamsley, 2008; Beugnet and Marié, 2009; Nicholson *et al.*, 2010).

In Europe, even though *A. phagocytophilum* has been detected in a wide range of wild animal species, the reservoir host for the human pathogenic strain is still unknown (Heyman *et al.*, 2010; Michalik *et al.*, 2012; Strasek Smrdel *et al.*, 2015). Indeed, the reservoir competence of rodents is not established and cervids are reported to disseminate mainly variants that have not been isolated in humans with possible exception for red deer (Michalik *et al.*, 2012). Similarly, no information is available on the reservoir hots competence of wild animals for *A. phagocytophilum* in Asia (Cao *et al.*, 2006). Only few studies have been carried and showed relatively high prevalence rates of infection by *A. phagocytophilum* in wild ruminants and small mammals (Stuen *et al.*, 2013).

A. phagocytophilum has been also detected in several other wild vertebrates including boar, fox, bear, European bison, donkey, moose, hare, Eurasian lynx, birds and reptiles (Kybicovà, 2010; Stuen *et al.*, 2013). However, their role in the bacterium life cycle is not assessed (Stuen *et al.*, 2013). In some geographical areas, several bird species are thought to be either competent host reservoirs of the bacterium or to contribute to the circulation and spread of infected ticks (Diniz and Breitschwerdt, 2012; Little, 2012; Woldehiwet, 2010).

Life cycle of *A. phagocytophilum* transmission by Ixodes tick species

A. phagocytophilum is considered to be naturally maintained in a tick-rodent cycle (Figure 4) (Cao *et al.*, 2006). The interaction between reservoir hosts and tick-borne pathogens is variable and has a direct impact on transmission dynamics. In the case of bacterial tick-borne infections that often lead to an immune system response in the host reservoir or to its death limiting the bacteriaemic phase, ticks represent a critical feature for the maintenance of the enzootic cycle in nature. The perpetuation of cycles can be ensured either by the transmission of the pathogens between different tick development stages (transstadial transmission), or between generation (transovarian transmission) or between ticks during cofeeding (Swanson *et al.*, 2006).

The life cycle of *Ixodes* ticks lasts for almost 2 years (Littman *et al.*, 2006; Swanson *et al.*, 2006) and its duration depends on climatic conditions varying from less than a year in tropical regions to three or more years in temperate regions (Heyman *et al.*, 2010). This life cycle comprises four distinct developmental stages: egg, larva, nymph and adult. *Ixodes* ticks activity varies according to the life stage and they mostly quest on vegetation in prime suburban real estate (Littman *et al.*, 2006; Swanson *et al.*, 2006). The feeding behavior at each life stage has a directly effect on the risk of tick-borne pathogens transmission (Swanson *et al.*, 2006). All *Ixodes* species of public health relevance need to feed on a new host at each life stage after hatching and the blood meal is completed in three to five days (Heyman *et al.*, 2010; Swanson *et al.*, 2006). Ticks belonging to *I. persulcatus* complex are exophilic, anthropophilic and nonspecific feeding ticks. Consequently, they can have their blood meal on both various host reservoirs and on humans (Stuen *et al.*, 2013; Swanson *et al.*, 2006).

A. phagocytophilum is transmitted to the host during the bite of a nymphal or adult tick infected during previous stages (larval or nymphal) (Diniz and Breitschwerdt, 2012; Doudier *et al.*, 2010; Nicholson *et al.*, 2010) (Figure 4). Transmission of *A. phagocytophilum* to the host during tick feeding occurs usually within 24 to 48 h. Consequently, at least daily removal of ticks should be recommended in association with ectoparasites preventive treatment in endemic areas to avoid the transmission of the bacterium (Cockwill *et al.*, 2009). As nymphs have very small size (approximately 1 mm), they are often able to feed much longer and are at increased risk to transmit tick-borne pathogens such as *A. phagocytophilum* (Heyman *et al.*, 2010; Swanson *et al.*, 2006). In contrast, adult ticks are bigger making them more quickly detectable and removed before disease transmission (Swanson *et al.*, 2006). Due to the transstadial transmission, nymphs and adult ticks contaminated in a previous stage last infected after molting and are able to contanimate susceptible hosts during the following blood meals.

Adult female ticks require an additional feeding to devolop their egg (Heyman *et al.*, 2010; Swanson *et al.*, 2006). This additional blood meal could explain the higher prevalence of *A. phagocytophilum* in adult ticks reported in some studies (Stuen *et al.*, 2013). As *A. phagocytophilum* is not transmitted transovarially among *Ixodes* ticks (Dumler *et al.*, 2005; Stuen *et al.*, 2013; Swanson *et al.*, 2006), larvae are mostly considered free from infection until hatching and having their first blood meal (Doudier *et al.*, 2010; Swanson *et al.*, 2006). Another consequence of the absence of transovarial transmission is that when adult femal tick laid their eggs, the bacterial cycle is interrupted (Bakken and Dumler, 2008; Stuen *et al.*, 2013).

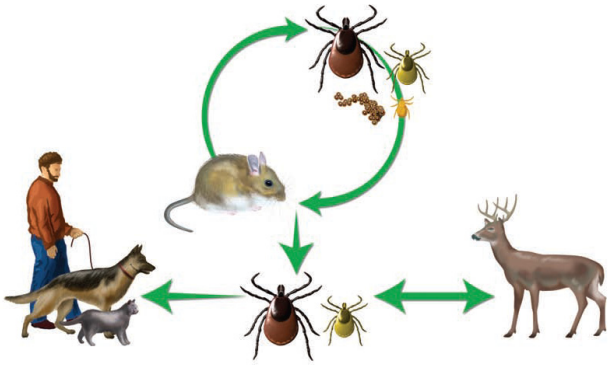

Figure 4. Life cycle of A. phagocytophilum (Diniz and Breitschwerdt, 2012). The perpetuation of the bacterium in nature is ensured through the transmission from competent reservoir hosts (small mammals and/or wild cervids) to the tick vector. Incidental transmission to other hosts including domestic carnivores and humans is also possible after tick bite.

A. phagocytophilum a zoonotic pathogen of veterinary and public health importance

A large variety of mammals are receptive to *A. phagocytophilum*. However, the disease has been described only in dogs, horses, cattle, sheep, goats, llamas, cats and humans (Eberts *et al.*, 2011; Gorna *et al.*, 2013; Mazepa *et al.*, 2010). People and domestic animals are considered incidental hosts (Poitout *et al.*, 2005).

In domestic ruminants

A. phagocytophilum was first described in domestic ruminants including sheep, goat, deer and cattle (Carrade *et al.*, 2009; Jin *et al.*, 2012; Woldehiwet, 2010). The disease was named tick-borne fever (TBF) in ruminants and was first described in sheep in Scotland in 1932 (Cochez *et al.*, 2011; Jin *et al.*, 2012). Later in the 1950s, the bacterium was identified as the causative agent of pasture fever in cattle in England (Beugnet and Marié, 2009). Then, *A. phagocytophilum* has been reported in sheep and cattle with TBF in several countries of Europe including Ireland, Scandinavia, Netherlands, Austria, Switzerland and Spain (Beugnet and Marié, 2009; Woldehiwet, 2010). Recently, the organism has also been identified in camelids (Ben Said *et al.*, 2014; Carrade *et al.*, 2009; Mentaberre *et al.*, 2013).

Two recent studies that investigated the prevalence of *A. phagocytophilum* in Tunisia and Canaries Islands found a prevalence of 29.2 and 3% respectively (Ben Said *et al.*, 2014; Mentaberre *et al.*, 2013). The disease, also called bovine ehrlichiosis, is mostly described in spring and autumn in animals grazing in favorable biotopes for ticks (Guyot *et al.*, 2011). Clinical signs associated with TBF and pasture fever include a sudden onset of high fever, anorexia, dullness, arthritis, oedema of legs and weight loss. Less frequently, cutaneous and mucous membrane hemorrhage were also described (Ben Said *et al.*, 2014; Guyot *et al.*, 2011; Stuen *et al.*, 2013).

A. phagocytophilum infection can also induce immune deficiency in ruminants (Ben Said *et al.*, 2013; Guyot *et al.*, 2011) leading to secondary opportunistic infections such as *Staphylococcus aureus*, *Pasteurella spp.* and *Listeria monocytogenes* infections (Carrade *et al.*, 2009). The severity of fever varies according to the age of the animal, the variant involved, the host species and the immunological status of the individual affected. Young animals and tick-free individuals placed on infested pasture seem to be the most sensitive to *A. phagocytophilum* infection (Stuen *et al.*, 2013). In addition, the disease is considered to be a cause of important productivity loss in dairy cattle including drop in milk yield, reduced weight gain in infected bullocks and lambs, reduced fertility and abortions (Ben Said *et al.*, 2014; Beugnet and Marié, 2009; Stuen *et al.*, 2013).

In horses

The disease was initially known as equine granulocytic ehrlichiosis (EGE) and was first reported in California in the late 1960s (M'ghirbi *et al.*, 2012; Pusterla and Madigan, 2013). Other cases were then described in several parts of Europe and America including Scandinavia, Switzerland, the United Kingdom, France, Brazil, Canada, Florida, Washington, Oregon, New York, Colorado, Illinois, Minnesota, Indiana, Wisconsin, New Jersey and Connecticut (Beugnet and Marié, 2009; Pusterla and Madigan, 2013; Woldehiwet, 2010). In Europe, the seroreactivity to *A. phagocytophilum* in horses has been assessed in several countries and varies from 6.52 to 22.3% (M'ghirbi *et al.*, 2012).

A recent study carried out on 60 horses living in regions favorable to *I. ricinus* tick in Tunisia revealed a prevalence of 67 and 13% by serology and PCR respectively. The prevalence by PCR is similar to reported rates in Italy (M'ghirbi *et al.*, 2012). Equine granulocytic anaplasmosis is described as an acute disease with an estimated incubation period of less than two weeks. The disease in mainly characterized by fever, lethargy, inappetence, staggering or ataxia, distal limb edema, reluctance to move and icterus (Franzen *et al.*, 2009; M'ghirbi *et al.*, 2012; Pusterla and Madigan, 2013). Occasionally, cardiac arrhythmia is reported including ventricular tachycardia and premature ventricular contractions and are possibly due to myocardial vasculitis (Pusterla and Madigan, 2013). Clinical presentation is unspecific like in other animal species and mimics other infectious diseases including Lyme disease, leptospirosis, babesiosis, theileriosis, equine herpes virus and equine infectious anemia virus (M'ghirbi *et al.*, 2012). These clinical signs can also be associated with hematological modifications including thrombocytopenia, leucopenia, neutropenia, lymphopenia and mild anemia. The evolution is mostly favorable, even without treatment, with recovery several weeks after the onset of symptoms (Franzen *et al.*, 2009; M'ghirbi *et al.*, 2012; Pusterla and Madigan, 2013). Indeed, subclinical infections are estimated to reach up to 50% of equine cases and thus the disease seems largely underestimated in most endemic European countries (M'ghirbi *et al.*, 2012). However, fatalities exist and can be due to opportunistic infections or traumatic injuries secondary to the lack of coordination (Pusterla and Madigan, 2013).

The severity of the diseases varies according to the age with horses less than 1 year old displaying limited clinical signs, those younger than 4 years old developing mild clinical signs and those older than 4 years old develop progressive characteristic disease (Pusterla and Madigan,

2013). The duration of the disease varies from 3 to 16 days. During natural infection, the peak of antibody titer occurs 19 to 81 days after the onset of clinical signs. Immunity seems to be protective and lasts for at least 2 years (M'ghirbi *et al.*, 2012; Pusterla and Madigan, 2013). In experimentally infected horses, antibody titer starts to progressively decline 300 days post-infection unless reinfection occurs (M'ghirbi *et al.*, 2012).

In companion animals (cats and dogs)

A. phagocytophilum infection was recognized for the first time in dogs in 1982 in California (Little, 2012; Woldehiwet, 2010). The disease has been described in several states of the USA but also in Europe as soon as the late 1980s (Beugnet and Marié, 2009; Carrade *et al.*, 2009). Most dogs infected by *A. phagocytophilum* remain apparently healthy. This is suggested by the discrepancy between the high seroprevalence and the relative low number of sick dogs in endemic areas (Carrade *et al.*, 2009; Diniz and Breitschwerdt, 2012; Ravnick *et al.*, 2014). Therefore, most immuno-competent dogs seem to control this infection and thus develop a subclinical or mild and self-limiting disease (Diniz and Breitschwerdt, 2012). However, some dogs infected by *A. phagocytophilum* develop an unspecific illness mostly characterized by an acute onset of fever, lethargy, depression, decreased appetite or anorexia, weight loss and musculoskeletal pain or discomfort (Beugnet and Marié, 2009; Carrade *et al.*, 2009; Diniz and Breitschwerdt, 2012). Lymphadenopathy, splenomegaly and hepatomegaly are also frequently described (Diniz and Breitschwerdt, 2012; Little, 2012). Less frequent clinical signs include digestive signs, polyuria, polydipsia, respiratory signs, pale mucous membranes, hemorrhagic diathesis, collapse, uveitis, scleral congestion, endocarditis, polymyositis and neurological signs (Carrade *et al.*, 2009; Cockwill *et al.*, 2009; Diniz and Breitschwerdt, 2012; Mazepa *et al.*, 2010; Ravnik *et al.*, 2011; Silaghi *et al.*, 2011).

Most important hematological and serum biochemistry profile modifications associated with canine granulocytic anaplasmosis include thrombocytopenia, mild to moderate nonregenerative normocytic normochromic and increased liver enzymes activity (Carrade *et al.*, 2009; Diniz and Breitschwerdt, 2012; Kohn *et al.*, 2008; Silaghi *et al.*, 2011). The severity of the disease is variable, from a subclinical infection to an acute form with severe clinical presentation (Poitout *et al.*, 2005; Ravnik *et al.*, 2011). The variability of the severity of clinical signs can be due either to the presence of co-infections, the immune response of the host or the virulence of strains/variants (Carrade *et al.*, 2009; Diniz and Breitschwerdt, 2012; Mazepa *et al.*, 2010). The prognosis of the disease in dogs is usually favorable with a rapid remission after doxycycline therapy (Mazepa *et al.*, 2010; Ravnick *et al.*, 2014). However, few fatality cases are reported (Bexfield *et al.*, 2005; Kohn *et al.*, 2008; Mazepa *et al.*, 2010).

More recent experimental studies have shown that cats are also susceptible to the infection by *A. phagocytophilum*. The first case in feline species was reported in the late 1990s (Beugnet and Marié, 2009; Lappin *et al.*, 2012; Little, 2012). Then only few reports described feline cases of granulocytic anaplasmosis in European countries including Sweden, Switzerland, Finland and Italy (Gorna *et al.*, 2013). Even though granulocytic anaplasmosis is increasingly diagnosed all over the world, the disease seems to be rarely identified among cats. This can be due to their particular behavior that can remove ticks during grooming or to differences in the pathogenesis of the disease in this species (Gorna *et al.* 2013; Heikkilä *et al.*, 2010).

In cats, the bacterium causes clinical signs similar to those described in dogs including fever, lameness, enlarged lymph nodes, lethargy, tachypnoea, anorexia and weight loss (Heikkilä *et al.*, 2010; Lappin *et al.*, 2012; Little, 2012). Periodontal disease, gingivitis, vomiting, abdominal pain, pharyngitis, polydipsia, hematuria, muscle and joint pain, conjunctivitis and neurologic signs including hyperesthesia, tremors, incoordination and shyness have also been reported (Gorna *et al.*, 2013; Heikkilä *et al.*, 2010; Little, 2012). However, whether these symptoms are attributed to anaplasmosis or to opportunistic infections secondary to an immune deficiency associated with *A. phagocytophilum* infection is unknown (Heikkilä *et al.*, 2010). Like in dogs, these clinical signs are often accompanied by hematological and biochemical abnormalities including thrombocytopenia, lymphopenia, neutrophilia with a left shift, anemia, monoclonal gammopathy, and increased liver enzymes activity (Gorna *et al.*, 2013; Heikkilä *et al.*, 2010; Lappin *et al.*, 2012). Thrombocytopenia is the most common hematological abnormality like described in other species and is mild to moderate in cats (Heikkilä *et al.*, 2010).

In humans

Currently, human granulocytic anaplasmosis (HGA) is considered to be the third most important vector-borne disease in both the USA and Europe and is also increasingly diagnosed in some Asian countries (Dumler, 2012; Li *et al.*, 2011; Zhang *et al.*, 2008). In the USA, anaplasmosis is a nationally notifiable disease (Bakken and Dumler, 2008; CDC, 2008) and the number of cases has critically and rapidly increased (Figure 5). Between 2000 and 2010, incidence has increased from 1.4 to 6.1 cases per million inhabitants (Dahlgren *et al.*, 2011; Rizzoli *et al.*, 2014).

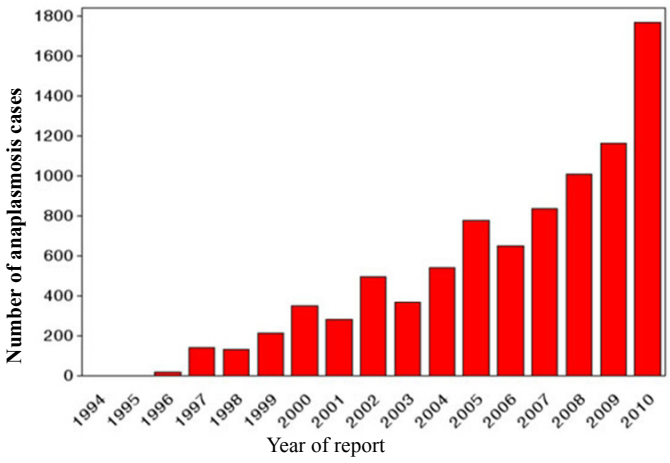

Figure 5: Number of annual human granulocytic anaplasmosis cases in the USA from 1994 to 2010 (http://www.cdc.gov/anaplasmosis/stats/)

HGA is an unspecific flu-like illness mostly characterized by fever, headache, chills, myalgia and malaise (Dumler, 2012; Folkema *et al.*, 2012; Rymaszewska and Adamska, 2011). Symptoms usually appear ten days to three weeks after tick bite (Heyman *et al.*, 2010; Stuen *et al.*, 2013). In most cases, clinical signs are mild and self-limited, with favorable evolution even without treament (Cochez *et al.*, 2011; Jin *et al.*, 2012). However, some patients can develop severe life-threatening complications (Cochez *et al.*, 2011; Dumler, 2012; Jin *et al.*, 2012). Due to the potential serious outcomes associated with the disease, the Infectious diseases Society of America recommends to give antimicrobial therapy to every person suspected to have HGA on the basis of clinical presentation although mild or self-limiting pending the laboratory results and to do not delay the treatment (Heyman *et al.*, 2010).

CONCLUSION

Although *A. phagocytophilum* is known as a veterinary pathogen causing disease in ruminants since more than 70 years, its zoonotic potential is more recently recognized. The ability of causing disease in humans with potential severe outcomes, the worldwide distribution and the rising focus on ticks and tick-borne diseases increased physician interest and awareness on this emerging pathogen. The difficulty of diagnosis either in human or in veterinary medicine and the importance of giving early treatment to avoid severe complications highlight the importance of improved knowledge on this pathogen especially epidemiological information. Epidemiological data are also crucial to perform effective preventive strategies. Although the number of publications on *A. phagocytophilum* and granulocytic anaplasmosis is important and increasing, several data are still missing including epidemiological informations in huge parts of Asia, Latin America and Africa and implications of strain variability in the pathogenicity of the disease. Several works on this pathogen are ongoing in Morocco in ruminants, dogs, ticks and humans in order to elucidate mechanisms of transmission and epidemiological context on this infection.

REFERENCES

Alleman A.R. and Wamsley H.L. (2008). An update on anaplasmosis in dogs. *Vet. Med.* 103: 212-220.

Azzag N., Petit E., Gandoin C., Bouillin C., Ghalmi F., Haddad N. and Bouloui A.J. (2015). Prevalence of select vector-borne pathogens in stray and client-owned dogs from Algiers. *Comp. Immunol. Microbiol. Infect. Dis.* 38: 1-7.

Bakken J.S. and Dumler S. (2008). Human granulocytic anaplasmosis. *Infect. Dis. Clin. North Am.* 22: 433-448.

Baneth G. (2014). Tick-born infections of animals and humans: a common ground. *Int. J. Parasitol.* 44: 591-596.

Ben Said M., Belkahia H., Sayahi L., Aloui M., Jemli M.H., Hadj Mohamed B., Sassi L., Darghouth M.A., Djaïem A.A., Bayoudh M. and Messadi L. (2014). First serological study of the prevalence of *Anaplasma phagocytophilum* in dromedary (*Camelus dromedarius*) in Tunisia. *Bull. Soc. Pathol. Exot.* 107:1-6.

Berzina I., Capligina V., Bormane A., Pavulina A., Baumanis V., Ranka R., Granta R. and Matise I. (2013). Association between *Anaplasma phagocytophilum* seroprevalence in dogs and distribution of *Ixodes ricinus* and *Ixodes persulcatus* ticks in Latvia. *Ticks Tick Borne Dis.* 4: 83-88.

Beugnet F. and Marié J.L. (2009). Emerging arthropod-borne diseases of companion animals in Europe. *Vet. Parasitol.* 163: 298-305.

Bexfield N.H., Villiers E.J. and Herrtage M.E. (2005). Immune-mediated haemolytic anaemia and thrombocytopenia associated with *Anaplasma phagocytophilum* in a dog. *J. Small Anim. Pract.* 46: 543-548.

Cao W.C., Zhan L., He J., Foley J.E., De Vlas S.J., Wu X.M., Yang H., Richardus J.H. and Habbema J.D. (2006). Natural *Anaplasma phagocytophilum* infection of ticks and rodents from a forest area of Jilin Province, China. *Am. J. Trop. Med. Hyg.* 75: 664-668.

Carrade D.D., Foley J.E., Borjesson D.L. and Sykes J.E. (2009). Canine granulocytic anaplasmosis: a review. *J. Vet. Int. Med.* 23: 1129-1141.

Centers for Disease Control and Prevention (CDC). (2008). *Anaplasma phagocytophilum* transmitted through blood transfusion: Minnesota, 2007. *MMWR Morb. Mortal Wkly. Rep.* 57: 1145-1148.

Cochez C., Ducoffre G., Vandenvelde C., Luyasu V. and Heyman P. (2011). Human anaplasmosis in Belgium: a 10-year seroepidemiological study. *Ticks Tick Borne Dis.* 2: 156-159.

Cockwill K.R., Taylor S.M., Snead E.C.R., Dickinson R., Cosford K., Malek S., Lindsay L.R. and Diniz P.P. (2009). Granulocytic anaplasmosis in three dogs from Saskatoon, Saskatchewan. *Can. Vet. J.* 50: 835-840.

Dahlgren F.S., Mandel E.J., Krebs J.W., Massung R.F. and McQuiston J.H. (2011). Increasing incidence of *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* in the United States, 2000–2007. *Am. J. Trop. Med. Hyg.* 85: 124-131.

Day M.J. (2011). One health: the importance of companion animal vector-borne diseases. *Parasit. Vectors.* 4: 49-54.

Diniz P.P. and Breitschwerdt E.B. (2012). *Anaplasma phagocytophilum* infection (canine granulocytic anaplasmosis) in *Infectious Diseases of the Dog and Cat*, Fourth Edition, edited by Greene CE., Elsevier, 244-254.

Doudier B., Olano J., Parola P. and Brouqui P. (2010). Factors contributing to emergence of *Ehrlichia* and *Anaplasma spp.* as human pathogens. *Vet. Parasitol.* 167: 149-154.

Dumler J.S., Barbet A.F., Bekker C.P., Dasch G.A., Palmer G.H., Ray S.H., Rikihisa Y. and Rurangirwa F.R. (2001). Reorganization of genera in the families *Rickettsiaceae* and *Anaplasmataceae* in the order *Rickettsiales*: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia*

with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and - HGE agent' as subjective synonyms of *Ehrlichia phagocytophila. Int. J. Syst. Evol. Microbiol.* 51: 2145-2165.

Dumler J.S., Choi K.S., Garcia-Garcia J.C., Barat N.S., Scorpio D.G., Garyu J.W., Grab D.J. and Bakken J.S. (2005). Human granulocytic anaplasmosis and *Anaplasma phagocytophilum. Emerg. Infect. Dis.* 11: 1828-1834.

Dumler J.S., Madigan J.E., Pusterla N. and Bakken J.S. (2007). Ehrlichioses in humans: epidemiology, clinical presentation, diagnosis, and treatment. *Clin. Infect. Dis.* 45 (Suppl 1): S45-S51.

Dumler J.S. (2012). The biological basis of severe outcomes in *Anaplasma phagocytophilum* infection. *Immunol. Med. Microbiol.* 64: 13-20.

Eberts M.D., Diniz P.P., Beall M.J., Stillman B.A., Chandrashekar R. and Breitschwerdt E.B. (2011). Typical and atypical manifestations of *Anaplasma phagocytophilum* infection in dogs. *J. Am. Anim. Hosp. Assoc.* 47 (6): e88-e94.

Folkema A.M., Holman R.C., McQuiston J.H. and Cheek J.E (2012). Trends in Clinical Diagnoses of Rocky Mountain Spotted Fever among American Indians, 2001–2008. *Am. J.Trop. Med. Hyg.* 86: 152-158.

Franzen P., Aspan A., Egenvall A., Gunnarsson A., Karlstam E. and Pringle J. (2009). Molecular evidence for persistence of *Anaplasma phagocytophilum* from acute experimental infection. *J. Vet. Intern. Med. 23*: 636-642.

Ghafar M.W. and Amer S.A. (2012). Prevalence and first molecular characterization of *Anaplasma phagocytophilum*, the agent of human granulocytic anaplasmosis, in *Rhipicephalus sanguineus* ticks attached to dogs from Egypt. *J. Adv. Res.* 3: 189-194.

Gorna M., Adaszek L., Policht K., Skrzypczak M. and Winiarczyk S. (2013). Detection of *Anaplasma phagocytophilum* in a cat. *Vet. Med-Czech.* 58: 3 9-43.

Guyot H., Ramery E, O'Grady L., Sandersen C. and Rollin F. (2011). Emergence of bovine ehrlichiosis in Belgian cattle herds. *Ticks Tick Borne Dis.* 2: 116-118.

Heikkilä H.M., Bondarenko A., Mihalkov A., Pfister K. and Spillmann T. (2010). *Anaplasma phagocytophilum* infection in a domestic cat in Finland: case report. *Acta Vet. Scand.* 52: 62-67.

Heyman P., Cochez C., Hofhuis A., van der Giessen J., Sprong H., Porter S.R., Losson B., Saegerman C., Donoso-Mantke O., Niedrig M. and Papa A. (2010). A clear and present danger: tick-borne diseases in Europe. *Expert Rev. Anti. Infect. Ther.* 8: 33-50.

Jin H., Wei F., Liu Q. and Qian J. (2012). Epidemiology and control of human granulocytic anaplasmosis: a systematic review. *Vector Borne Zoonotic Dis.* 12: 269-274.

Keesing F., McHenry D.J., Hersh M., Tibbetts M., Brunner J.L., Killilea M., LoGiudice K., Schmidt K.A. and Ostfeld R.S. (2014). Prevalence of human-active and variant 1 strains of the tick-borne pathogen *Anaplasma phagocytophilum* in hosts and forests of Eastern North America. *Am. J. Trop. Med. Hyg.* 91: 302-309.

Kohn B., Galke D., Beelitz P. and Pfister K. (2008). Clinical features of canine granulocytic anaplasmosis in 18 naturally infected dogs. *J. Vet. Intern. Med.* 22: 1289-1295.

Krämer K., Schaper R., Schunack B., Połozowski A., Piekarska J., Szwedko A., Jodies R., Kowalska D., Schüpbach D. and Pantchev N. (2014). Serological detection of *Anaplasma phagocytophilum*, *Borrelia burgdorferi* sensu lato and *Ehrlichia canis* antibodies and *Dirofilaria immitis* antigen in a countrywide survey in dogs in Poland. *Parasitol. Res.* 113: 3229-3239.

kybicova, K (2010). *Borrelia burgdorferi sensu lato* and *Anaplasma phagocytophilum* in the Czech Republic, PhD Thesis, Charles University in Prague.

Lappin M.R. and Breitschwerdt E.B. (2012). *Anaplasma phagocytophilum* infection (feline granulocytic anaplasmosis) in Infectious Diseases of the Dog and Cat, Fourth Edition, Chapter 26, *Ehrlichia* and *Anaplasma* Infections p254-256.

Leiby D.A., Chung A.P.S., Cable R.G., Trouern-Trend J., McCullough J., Homer M.J., Reynolds L.D., Houghton R.L., Lodes M.J. and Persing D.H. (2002). Relationship between tick bites and the seroprevalence of *Babesia microti* and *Anaplasma phagocytophila* (previously *Ehrlichia* sp.) in blood donors. *Transfusion* 42: 1585-1591.

Li H., Zhou Y., Wang W., Guo D., Huang S. and Jie S. (2011). The clinical characteristics and outcomes of patients with human granulocytic anaplasmosis in China. *Int. J. Infect. Dis.* 15: 859-866.

Little SE. (2012). Ehrlichiosis and anaplasmosis in dogs and cats. *Vet. Clin. North. Am. Small Anim. Pract.* 40: 1121-1140.

Littman M.P., Goldstein R.E., Labato M.A., Lappin M.R. and Moore G.E. (2006). ACVIM small animal consensus statement on Lyme disease in dogs: diagnosis, treatment, and prevention. *J. Vet. Intern. Med.* 20: 422-434.

Mazepa A.W., Kidd L.B., Young K.M. and Trepanier L. (2010). Clinical presentation of 26 *Anaplasma phagocytophilum*-seropositive dogs residing in an endemic area. *J. Am. Anim. Hospl. Assoc.* 46: 405-412.

Mentaberre G., Gutiérrez C., Rodríguez N.F., Joseph S., González-Barrio D., Cabezón O., de la Fuente J., Gortazar C. and Boadella M. (2013). A transversal study on antibodies against selected pathogens in dromedary camels in the Canary Islands, Spain. *Vet. Microbiol.* 167: 468-473.

M'ghirbi Y., Ghorbel A., Amouri M., Nebaoui A., Haddad S. and Bouattour A. (2009). Clinical, serological, and molecular evidence of ehrlichiosis and anaplasmosis in dogs in Tunisia. *Parasitol. Res.* 104: 767-774.

M'ghirbi Y., Yaïch H., Ghorbel A. and Bouattour A. (2012). *Anaplasma phagocytophilum* in horses and ticks in Tunisia. *Parasit. Vectors* 5: 180-186.

Michalik J., Stańczak J., Cieniuch S., Racewicz M. and Siko B. (2012).Wild Boars as Hosts of HumanPathogenic Anaplasma phagocytophilum Variants. *Emerging Infectious Diseases* 18: 998-1001.

Michelet L., Delannoy S., Devillers E., Umhang G., Aspan A., Juremalm M., Chirico J., van der Wal F.J., Sprong H., Boye Pihl T.P., Klitgaard K., Bødker R., Fach P. and Moutailler S. (2014). High-throughput screening of tick-borne pathogens in Europe. *Front Cell Infect. Microbiol.* 4: 1-13.

Nicholson W.L., Allen K.E., McQuiston J.H., Breitschwerdt E.B and Little S.E. (2010). The increasing recognition of rickettsial pathogens in dogs and people. *Trends Parasitol.* 26: 205-212.

Nijhof A.M., Bodaan C., Postigo M., Nieuwenhuijs H., Opsteegh M., Franssen L., Jebbink F. and Jongejan F. (2007). Ticks and associated pathogens collected from domestic animals in the Netherlands. *Vector Borne Zoonotic* Dis. 7: 585-596.

Poitout F.M., Shinozaki J.K., Stockwell P.J. Holland C.J. and Shukla S.K. (2005). Genetic variants of *Anaplasma phagocytophilum* infecting dogs in Western Washington State. *J. Clin. Microbiol.* 43: 796-801.

Pruneau L., Moumène A., Meyer D.F., Marcelino I., Lefrançois T. and Vachiéry N. (2014). Understanding *Anaplasmataceae* pathogenesis using "Omics" approaches. *Front Cell Infect. Microbiol.* 4: 86-92.

Pusterla N.and Madigan J.E. (2013). Equine granulocytic anaplasmosis. *J. Equine Vet. Sci.* 33: 493-496.

Qablan M.A., Kubelová M ., Siroký P., Modrý D. and Amr Z.S. (2012). Stray dogs of northern Jordan as reservoirs of ticks and tick-borne hemopathogens. *Parasitol. Res.* 111: 301-307.

Qurollo A.B., Chandrashekar R., Hegarty B.C., Beall M.J., Stillman B.A., Liu J., Thatcher B., Pultorak E., Cerrito B., Walsh M. and Breitschwerdt E.B. (2014). A serological survey of tick-borne pathogens in dogs in North America and the Caribbean as assessed by *Anaplasma phagocytophilum*, *A. platys*, *Ehrlichia canis*, *E. chaffeensis*, *E. ewingii*, and *Borrelia burgdorferi* species-specific peptides. *Infect. Ecol. Epidemiol.*: 10.3402/iee.v4.24699.

Ravnik U, Bajuk B. P., Lusa L. and Tozon N. (2014). Serum protein profiles, circulating immune complexes and proteinuria in dogs naturally infected with *Anaplasma phagocytophilum. Veterinary Microbiology* 173: 160-165.

Ravnik U., Tozon N., Strasek Smrdel K. and Avsic Zupanc T. (2011). Anaplasmosis in dogs: the relation of haematological, biochemical and clinical alterations to antibody titre and PCR confirmed infection. *Vet. Microbiol.* 149: 172-176.

Rizzoli A., Silaghi C., Obiegala A., Rudolf I., Hubálek Z., Földvári G., Plantard O., Vayssier-Taussat M., Bonnet S., Špitalská E. and Kazimírová M. (2014). *Ixodes ricinus* and its transmitted pathogens in urban and peri-urban areas in Europe: new hazards and relevance for public health. *Front Public Health* doi:10.3389/fpubh.2014.00251.

Rymaszewska A. and Adamska M. (2011). Molecular evidence of vector-borne pathogens coinfecting dogs from Poland. *Acta Vet. Hung.* 59: 215-223.

Sainz A., Roura X., Miró G., Estrada-Peña A., Kohn B., Harrus S. and Solano-Gallego L. (2015). Guideline for veterinary practitioners on canine ehrlichiosis and anaplasmosis in Europe. *Parasit. Vectors* 8: 75-94.

Sarih M., M'Ghirbi Y., Bouattour A., Gern L., Baranton G. and Postic D. (2005). Detection and identification of *Ehrlichia* spp. in ticks collected in Tunisia and Morocco. *J. Clin. Microbiol.* 43: 1127-1132.

Schreiber C., Krücken J., Beck S., Maaz D., Pachnicke S., Krieger K., Gross M., Kohn B. and von Samson-Himmelstjerna G. (2014). Pathogens in ticks collected from dogs in Berlin/Brandenburg, Germany. *Parasit. Vectors* 7: 535-344.

Silaghi C., Kohn B., Chirek A., Thiel C., Nolte I., Liebisch G. and Pfister K. (2011). Relationship of molecular and clinical findings on *Anaplasma phagocytophilum* involved in natural infections of dogs. *J. Clin. Microbiol.* 49: 4413-4414.

Stanek G., Wormser G.P., Gray J. and Strle F. (2012). Lyme borreliosis. *Lancet* 379: 461-473.

Strašek Smrdel K., Loewenich F.D, Miroslav Petrovec M. and Avšič Županc T. (2015). Diversity of ankA and msp4 genes of Anaplasma phagocytophilum in Slovenia. *Ticks and Tick-borne Diseases* 6:164–166.

Stuen S., Granquist E.G. and Silaghi C. (2013). *Anaplasma phagocytophilum*—a widespread multi-host pathogen with highly adaptive strategies. *Front Cell Infect. Microbiol.* 3: 1-33.

Swanson S.J., Neitzel D., Reed K.D. and Belongia E.A. (2006). Coinfections acquired from *Ixodes* ticks. *Clin. Microbiol. Rev.* 19: 708-727.

Villeneuve A., Goring J., Marcotte L. and Overvelde S. (2011). Seroprevalence of *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Ehrlichia canis*, and *Dirofilaria immitis* among dogs in Canada. *Can. Vet. J.* 52: 527-530.

Weil A.A., Baron E.L., Brown C.M. and Drapkin M.S. (2012). Clinical findings and diagnosis in human granulocytic anaplasmosis: a case series from massachusetts. *Mayo Clin. Proc.* 87: 233-239.

Woldehiwet Z. (2010). The natural history of *Anaplasma phagocytophilum*. *Vet. Parasitol.* 167: 108-122.

Yancey C.B., Hegarty B.C., Qurollo B.A., Levy M.G., Birkenheuer A.J., Weber D.J., Diniz P.P. and Breitschwerdt E.B. (2014). Regional seroreactivity and vector-borne disease eo-Exposures in dogs in the United States from 2004–2010: utility of canine surveillance. *Vector Borne Zoonotic Dis.* 14: 724-732.

Zhang L., Liu H., Xu B., Lu Q., Li L., Chang L., Zhang X., Fan D., Li D., Jin Y., Cui F., Shi Y., Li W., Xu J. and Yu X.J. (2012). *Anaplasma phagocytophilum* infection in domestic animals in ten provinces/cities of China. *Am. J. Trop. Med. Hyg.* 87: 185-189.

Zhang L, Liu Y, Ni D, Li Q, Yu Y, Yu XJ, et al. (2008). Nosocomial transmission of human granulocytic anaplasmosis in China. *JAMA* 300:2263–70.