

REVIEW

TICK-BORNE PATHOGENS AND DISEASES OF ANIMALS AND HUMANS IN THAILAND

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Abstract. Tick-borne pathogens in Thailand can cause diseases that result in productivity and economic losses in the livestock sector as well as cause debilitating illnesses in humans and their companion animals. With the advent of molecular techniques, accurate identification of tick-borne pathogens and precise diagnosis of disease is now available. This literature review summarizes the various tick-borne pathogens that have been isolated from ticks and their vertebrate hosts in Thailand, covering those protozoa, rickettsiae, bacteria and viruses most responsible for human and veterinary disease with particular emphasis on those that have been characterized molecularly.

INTRODUCTION

Ticks are surpassed only by mosquitoes as arthropod vectors of disease (Dennis and Piesman, 2005). A wide variety of pathogens that can be propagated and transmitted by ticks commonly infect both humans and animals found in close association with man (*eg*, cattle, dogs, cats, rodents and fowl). Tick-borne pathogens in Thailand involve protozoa, rickettsiae, bacteria and *Flavivirus* species and cause diseases such as anaplasmosis, babesiosis, ehrlichiosis and rickettsiosis. Small-scale livestock industry is expanding in this country because of a demand from national markets and an export-based economy. Tick-borne diseases will assume an increasingly greater importance and necessity for control as the demand for meat and other animal products continues to increase with the improvement of Thailand's economy (Chansiri,

1997). High standards of quality control for farm animals and meat products, including disease preventions, are essential to sustain this expanding industry. The increasing popularity of pet ownership together with the existence of large populations of stray dogs and cats in Southeast Asian countries including Thailand provide an ideal environment for tick-borne disease transmission to humans and companion animals (Irwin and Jefferies, 2004).

The accurate detection of tick-borne pathogens is an important aspect of livestock quality control as well as disease diagnosis. Assays based on microscopic or serological examination are often not sensitive enough to detect pathogens, especially when they occur at low infection levels. In addition, the lack of microscopic distinguishing features among pathogen species or the always-present problem of cross-reactivity in serological tests makes accurate identification at the species level difficult. With the advent of molecular diagnostic techniques in the 1990's, sensitive and accurate determinations of pathogen species can now be performed.

This review summarizes the various

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tick-borne pathogens that have been isolated from ticks and their vertebrate hosts in Thailand. In particular, we concentrate on those pathogens that have been detected and characterized using molecular techniques (Tables 1 and 2). This review presents an update of those pathogens borne by ticks that have been detected both in ticks and various vertebrate species, including humans, in Thailand. Future needs for determining the extent of the tick-borne pathogen problem in this country are discussed.

PROTOZOAL PATHOGENS

Babesia

Babesiosis is an emerging tick-transmitted, zoonotic disease of worldwide economic, medical and veterinary importance. It is caused by intra-erythrocytic parasites of the genus *Babesia* (Homer *et al*, 2000). *Babesia* spp infect many types of mammals, especially in the order Rodentia, as well as some species of birds. The manifestations of the disease are broad, ranging from silent infestation to a fulminant malaria-like disease resulting in severe hemolysis and death. In Thailand, *Babesia* parasites have been found in cattle, dogs and a species of rodent (*Bandicota indica*) (Table 1) as well as in cats (Jittapalapong and Jansawan, 1993) and a species of bird (*Dendrocitta vagabunda*; Peirce, 2000).

Bovine babesiosis in particular threatens the cattle industry in tropical and sub-tropical regions, and production of cattle and buffaloes in many Asian countries, including Thailand, has been impaired by this lethal disease (Brockelman, 1989, cited in Tan-Ariya *et al*, 1992). The important causative agents of bovine babesiosis in Asia are *B. bovis* and *B. bigemina* (Bock *et al*, 2004). However, the serological techniques used to diagnose bovine babesiosis do not consistently detect carrier animals and do not specifically eliminate cross-

reactions between *B. bigemina* and *B. bovis* (Wright *et al*, 1987; Figueroa *et al*, 1992). Molecular techniques have often been developed to overcome the problem of serological cross-reactivity among closely related pathogens. In Thailand, a molecular technique for specifically detecting *B. bovis* in cattle was developed in 1992 (Petchpoo *et al*, 1992) and other techniques have been reported more recently (Thammasirirak *et al*, 2003; Patarapadungkit *et al*, 2004). Nevertheless, the extent to which these methods have been used, *eg*, in determining the prevalences of the two bovine *Babesia* species, is unknown because of the lack of published reports that use them.

The prevalence of *Babesia* infections in Thai cattle is highly variable (Nishikawa *et al*, 1990; Phrikanahok *et al*, 2000) but in some areas seropositives as high as 96.7% have been reported (Nishikawa *et al*, 1990). Imported cattle and their hybrid offspring appear to be more susceptible to *Babesia* infection than native breeds (Tan-Ariya *et al*, 1992). However, even native breeds may be susceptible if raised in a babesiosis-free area and then exposed to cattle from a babesiosis-endemic area (Pemayodhin *et al*, 1991). *Rhipicephalus* (formerly *Boophilus*) *microplus* is the recognized tick vector of both *B. bovis* and *B. bigemina* throughout the world (Bock *et al*, 2004). This tick species is widespread in Thailand (Tanskul *et al*, 1983) and a recent study confirmed infection by the parasite, *B. bigemina*, in 45.6% of tick individuals collected from cattle in an enzootic area of Thailand (Jittapalapong *et al*, 2004).

In Thai dogs, one species of *Babesia*, namely *B. canis*, has been associated with canine babesiosis (Table 1). Although antigens to another species, *B. gibsonii*, have been detected, infection by the latter cannot be confirmed by molecular methods (Suksawat *et al*, 2001a). *B. canis* is separated into three subspecies based on differences in vector specificity, cross-immunity and pathogenicity

among isolates (Uilenberg, 2006) and these along with molecular differences have resulted in proposals to call them separate species (Zahler *et al*, 1998). However, there has not yet been any consensus among taxonomists regarding the taxonomy of the *B. canis* group. The *B. canis* that occurs in Southeast Asia, sometimes referred to as subspecies *vogelli* (Irwin and Jefferies, 2004), is transmitted by the brown dog tick *Rhipicephalus sanguineus* (Shaw *et al*, 2001a), a tick that is commonly found on Thai dogs (Tanskul *et al*, 1983). Among pet dogs of Samut Prakan Province near Bangkok, 3.75% were infected with *Babesia* based on hematological characteristics (Jittapalapong and Tipsawake, 1991). A similar rate (3.07%) was determined for canine blood samples submitted to a Kamphaeng Saen animal hospital northwest of Bangkok (Salakij *et al*, 1999). A much higher *Babesia* infection rate is expected in populations of stray dogs throughout Thailand because of their greater exposure to ticks.

Babesia infection has also been detected in equine animals in Thailand with 1 and 62 out of 400 tested horses, donkeys and mules being seropositive for *B. caballi* and *B. equi*, respectively (Tantaswasdi *et al*, 1998). Despite the high prevalence of parasites, there have been no clinical reports of the disease equine babesiosis in this country (Tantaswasdi *et al*, 1998).

Recently, a new type of rodent babesia was detected in Thai bandicoots (*Bandicota indica*) using DNA sequence analysis (Dantrakool *et al*, 2004). This protozoon seems to be phylogenetically most closely related to *B. canis*, the dog parasite, although morphologically it looks more like *B. microti*, a parasite of rodents in North America. Because *Babesia* in Thai bandicoot rats is morphologically pleomorphic, it has been suggested there is more than one species. However, the possibility of multiple infections by different species seems quite low because there were

no ambiguities resulting from direct sequencing of the PCR products (Dantrakool *et al*, 2004). More than half (17/33) of the rodents were found to have *Babesia* in their blood. *Haemaphysalis doenitzi* ticks were identified exclusively on *Babesia*-positive *Bandicota indica*, so this tick species may prove to be the vector.

In other parts of the world, babesiosis in humans has been diagnosed initially as malaria (Dantrakool *et al*, 2004). Thus, it is probable that cases of human babesiosis in countries like Thailand, in which malaria is endemic, have been overlooked or misdiagnosed as malaria. Human *Babesia* is considered to come from rodents alongside humans (Dantrakool *et al*, 2004). The fact that *Babesia* has been found in a Thai rodent, that *Babesia* tick vectors like *Rhipicephalus microplus* and *Rhipicephalus sanguineus* occur in Thailand and sometimes infest people (Estrada-Pena and Jongejan 1999) and that first detections of human babesiosis have recently been reported from other Asian countries, including Taiwan, Japan and Korea (Shih *et al*, 1997; Saito-Ito *et al*, 2000; Kim *et al*, 2007) emphasizes the importance of continual surveillance for human babesiosis in Thailand.

Theileria

Protozoa of the genus *Theileria* cause a variety of disease syndromes in domestic and wild ruminants and other mammals (Bishop *et al*, 2004; Jefferies *et al*, 2007). Globally, the two most important species that cause theileriosis in domestic cattle are *T. parva* and *T. annulata* (Bishop *et al*, 2004). Neither of these two parasites has been reported in Thailand. Nevertheless, benign *Theileria* that belong to the *Theileria buffeli/orientalis/sergenti* group, a group of parasites that causes mild disease in cattle (Sarathaphan *et al*, 1999), and other yet unplaced *Theileria* parasites (Table 1), occur in this country. Currently, substantial research is being conducted regarding the taxonomy of benign *Theileria* parasites of

ruminants (Gubbels *et al*, 2000, 2002; Chansiri and Sarataphan, 2002; Kim *et al*, 2004; Allsopp and Allsopp, 2006), but there is as yet no generally accepted system of classifying or naming these parasites. Because of this lack of agreement, we report in Table 1 the *Theileria* species found in Thailand by the names of the strains and types given them by the researchers.

Two genes, the small subunit ribosomal RNA or *ssrRNA* gene and the major piroplasm surface protein or *MPSP* gene, have been used to investigate genetic relations among *Theileria* isolates in Thailand (Table 1). A third gene, the thymidylate synthase or *TS* gene, has also been used by Chansiri and Sarataphan (2002) to compare the Thai isolate *Theileria* sp Thung Song with other *Theileria* types. However, this gene has not been used for other studies of Thai *Theileria* parasites and so we excluded it from Table 1. Three genetically distinct types of *Theileria* have been recorded to occur in Thailand using the *MPSP* gene, *ie*, types B1, C and Thai (Table 1). The number of *Theileria* isolates that are present in Thailand based on the *ssrRNA* gene is not clear, because the two isolates, Thung Song and Thai recorded in Table 1, have not yet been compared phylogenetically within the same analysis.

Theileria parasites have been shown to be highly prevalent among Thai domestic ruminants. Kaewthamasorn and Wongsamee (2006) found that 50% of beef cattle ($n=162$ animals tested) in Nan Province, northern Thailand, were infected with *Theileria* parasites based on blood characteristics. There were no serious observable clinical signs of disease in any of the positive animals, which suggests that *Theileria* infections of beef cattle in this province are normally not life threatening.

True infection rates may be underestimated by hematological analysis. Sarataphan *et al* (2003) collected a total of 247 blood samples from 214 cattle and 33 water buffa-

loes in geographical locations including southern, northern and northeastern Thailand. Microscopic examination of Giemsa-stained blood smears revealed 27.5% of samples were positive for *Theileria* whereas allele-specific PCR amplification of the *MPSP* gene determined a much higher infection rate of 82.6%. Among positive cases, the Thai-type of *MPSP* gene was the most common, comprising 67.6% of samples, whereas the C-type comprised only 8.3% of infections. Mixed infections were also common with 24% of cases containing both *MPSP* gene types (Sarataphan *et al*, 2003).

Ixodid ticks of the genera *Rhipicephalus*, *Amblyomma*, *Hyalomma* and *Haemaphysalis* transmit *Theileria* parasites (Bishop *et al*, 2004). No tick species has been identified as a *Theileria* vector in Thailand, however, three of the four vector genera, namely *Rhipicephalus*, *Amblyomma* and *Haemaphysalis*, are represented by more than 30 species in Thailand (Tanskul *et al*, 1983); the other genus, *Hyalomma*, has not been recorded in this country. *Haemaphysalis anomala*, *Haemaphysalis cornigera*, *Haemaphysalis lagrangei*, *Haemaphysalis nadchatrami*, *Haemaphysalis bandicota*, *Haemaphysalis heinrichi* and *Rhipicephalus haemaphysaloides* are all recorded from Thai domestic cattle (Tanskul *et al*, 1983) and warrant testing for the presence of *Theileria* parasites.

Hepatozoon

Hepatozoon parasites cause hepatozoonosis in vertebrates and are transmitted when a vertebrate ingests an invertebrate infected with these parasites (Baneth *et al*, 2003). Transmission has been shown to be direct, the vertebrate predator directly eats the *Hepatozoon*-infected invertebrate prey (Smith, 1996). However, for vertebrates like snakes for which invertebrates may not be the normal prey, an intermediate vertebrate host has been postulated (Smith, 1996). More than 300 species of *Hepatozoon* species are known (Smith, 1996).

Table 1
Molecular detection of tick-borne pathogens in vertebrate hosts in Thailand.

Pathogens	Vertebrate hosts	References
Protozoa^a		
<i>Babesia bigemina</i>	Cattle	Jittapalapong <i>et al</i> , 2004
<i>B. canis</i>	Dogs	Suksawat <i>et al</i> , 2001a,b
<i>Babesia</i> sp	<i>Bandicota indica</i> (rodent)	Dantrakool <i>et al</i> , 2004
<i>Theileria</i> spp classification based on the <i>ssrRNA</i> gene		
<i>Theileria</i> sp type Thai	Cattle	Kakuda <i>et al</i> , 1998
<i>Theileria</i> sp type Thung Song	Cattle	Chansiri <i>et al</i> , 1999
<i>Theileria</i> spp classification based on the <i>MSPS</i> gene		
<i>Theileria</i> sp type B1	Cattle	Sarathaphan <i>et al</i> , 2003
<i>Theileria</i> sp type C	Cattle, water buffalo	Sarathaphan <i>et al</i> , 1998, 2003
<i>Theileria</i> sp type Thai	Cattle, water buffalo	Kakuda <i>et al</i> , 1998; Sarathaphan <i>et al</i> , 1998, 2003
<i>Hepatozoon canis</i>	Dogs, cats	Jittapalapong <i>et al</i> , 2006; Criado-Fornelio <i>et al</i> , 2007b
Rickettsiales^b		
<i>Anaplasma platys</i>	Dogs	Suksawat <i>et al</i> , 2001a,b; Pinyoowong <i>et al</i> , 2008
<i>Ehrlichia canis</i>	Dogs	Suksawat <i>et al</i> , 2001a,b; Ariyawutthiphan <i>et al</i> , 2008; Pinyoowong <i>et al</i> , 2008
<i>Rickettsia honei</i> strain TT-118	Humans	Jiang <i>et al</i> , 2005
<i>Rickettsia</i> sp strain PMK94	Humans	Gaywee <i>et al</i> , 2007
Other bacteria^b		
<i>Bartonella henselae</i>	Cats	Maruyama <i>et al</i> , 2001

^aProtozoa nomenclature: *Babesia* spp. - Uilenberg (2006); *Theileria* spp – standard system of classification not available yet in the literature (see text).

^bNomenclature for Rickettsiales and other bacteria from Kracht (2004).

Like other blood parasites, *Hepatozoon* infections can be detected by microscopic examination of blood smears. *Hepatozoon* infections have been found using this method in several types of vertebrates in Thailand including stray and pet dogs (Pukkavesa *et al*, 1980; Jittapalapong and Tipsawake 1991; Jittapalapong *et al*, 2006; Niwetpathomwat *et al*, 2006), stray cats (Jittapalapong *et al*, 2006), a species of wild cat (the flat-headed cat, *Prionailurus planiceps*; Salakij *et al*, 2008) and in more than ten snake species (Salakij *et al*, 2001, 2002).

Molecular techniques are necessary for distinguishing and identifying *Hepatozoon* species. Sequences of the *18S rDNA* gene are most often used for genetically comparing and identifying *Hepatozoon* isolates (Baneth *et al*, 2000; Criado-Fornelio *et al*, 2007a; Allen *et al*, 2008). In Thailand, *Hepatozoon canis* has been identified in stray dogs, pet dogs and stray cats based on the *18S rDNA* gene (Jittapalapong *et al*, 2006; Criado-Fornelio *et al*, 2007b) whereas a yet unnamed species different to *H. canis* was found in the flat-headed cat (Salakij *et al*, 2008).

Table 2
Molecular detection of tick-borne pathogens in ticks in Thailand.

Pathogen	Tick species ^c	Source	References
Protozoa^a			
<i>Babesia bigemina</i>	<i>Rhipicephalus (Boophilus) microplus</i>	Cattle	Jittapalapong <i>et al</i> , 2004
Rickettsiales^b			
<i>Anaplasma platys</i>	<i>Dermacentor auratus</i>	Dogs	Parola <i>et al</i> , 2003a
<i>Anaplasma</i> sp strain AnAj360	<i>Amblyomma javanense</i>	Pangolin	Parola <i>et al</i> , 2003a
<i>Anaplasma</i> sp strain AnHl446	<i>Haemaphysalis lagrangei</i>	Bear	Parola <i>et al</i> , 2003a
<i>Ehrlichia</i> sp strain EBm52	<i>Rhipicephalus (Boophilus) microplus</i>	Cattle	Parola <i>et al</i> , 2003a
<i>Rickettsia honei</i> strain TT-118	<i>Ixodes granulatus</i>	<i>Rattus rattus</i>	Kollars <i>et al</i> , 2001
<i>Rickettsia</i> sp strain SP2464	<i>Ixodes granulatus</i>	<i>Rattus rattus</i>	Kollars <i>et al</i> , 2001
<i>Rickettsia</i> sp strain RDa420	<i>Dermacentor auratus</i>	Bear	Parola <i>et al</i> , 2003a
<i>Rickettsia</i> sp strain RDla440	<i>Dermacentor</i> sp	Pig nest	Parola <i>et al</i> , 2003a
<i>Rickettsia</i> sp strain ATT	<i>Amblyomma testudinarium</i>	Vegetation	Hirunkanokpun <i>et al</i> , 2003
<i>Rickettsia</i> sp strain HOT1	<i>Hemaphysalis ornithophila</i>	Vegetation	Hirunkanokpun <i>et al</i> , 2003
<i>Rickettsia</i> sp strain HOT2	<i>Hemaphysalis ornithophila</i>	Vegetation	Hirunkanokpun <i>et al</i> , 2003

^a*Babesia* nomenclature from Uilenberg (2006).

^bNomenclature for Rickettsiales and other bacteria from Kracht (2004).

^cTick nomenclature from Barker and Murrell (2004).

Definitive hosts for *Hepatozoon* species consist of a variety of hematophagous invertebrates including ticks, mites, sand flies, tsetse flies, mosquitoes, fleas, lice, reduviid bugs, and leeches (Smith, 1996). Ticks have been shown to be vectors for *Hepatozoon* parasites in mammals, birds and lizards (Smith, 1996). In Southeast Asia, *H. canis* is the major *Hepatozoon* species known to infect dogs. Dogs have been shown to become infected with *H. canis* after ingesting the brown dog tick, *Rhipicephalus sanguineus* (Baneth *et al*, 2001), a species commonly found on dogs in many parts of the world, including Thailand (Tanskul *et al*, 1983). Hepatozoonosis is a commonly reported illness of Thai dogs (Pukkavesa *et al*, 1980; Jittapalapong *et al*, 2006; Criado-Fornelio *et al*, 2007b). In Southeast Asia, gamonts are only occasionally found in the peripheral blood of dogs (as inclusions in neutrophils and occasionally monocytes),

suggesting that *Hepatozoon* infections in this part of the world are generally subclinical (Irwin and Jefferies, 2004). This contrasts with other locations where hepatozoonosis can cause serious illness (Baneth, 2001).

Based on hematological examination, estimates of the prevalence of canine *Hepatozoon* infections in stray and pet dogs in Thailand are low at 2.6 and 2.2%, respectively (Jittapalapong and Tipsawake, 1991; Jittapalapong *et al*, 2006). Hepatozoon infections have also been detected in stray cats but at very low rates (0.7%, Jittapalapong *et al*, 2006). However, estimates based on microscopic examination of blood smears may underestimate infection rates. Jittapalapong *et al* (2006) also determined prevalences based on PCR detection of *Hepatozoon canis* and determined infection rates of 11.4 and 32.3%, respectively, in the same samples from stray dogs and cats that were examined

hematologically.

The unexpected high prevalence of *H. canis* parasites among stray cats found by Jittapalapong *et al* (2006) indicates that their role in the epizootiology of hepatozoonosis should be investigated. Both stray dogs and cats have potential roles as reservoirs or sources of pet infections with *H. canis* in Thailand.

RICKETTSIALES

The Order Rickettsiales is a diverse assemblage of bacterial species, many of which are associated with ticks. With the advent of molecular techniques, genetic relationships among microbes have become easier to characterize. This is especially so for the Order Rickettsiales, which has undergone extensive taxonomic revision since genes suitable for taxonomic comparison were identified. The currently accepted classification of Rickettsiales and the one used in this review is based on Dumler *et al* (2001). This order comprises two families, the Rickettsiaceae and the Anaplasmataceae, both of which contain tick-borne pathogens. The Rickettsiaceae contain two genera, *Rickettsia* and *Orientia*, of which only the *Rickettsia* include tick-borne species. The Anaplasmataceae are comprised of four genera, three of which, *Ehrlichia*, *Anaplasma* and *Neorickettsia*, include species carried by ticks. The fourth genus, *Wolbachia*, is widespread in insects and has been found in several other types of invertebrates but has not been recorded in ticks.

Rickettsiaceae

Rickettsial infections or rickettsiosis (also called typhus) are a major cause of febrile illness in people throughout the world. Of the two genera comprising the Rickettsiaceae, the *Rickettsia* include species that are tick-borne within a group called the Spotted Fever Group or SFG.

The first Thai member of the SFG to be

discovered, called the Thai tick typhus isolate, TT-118, was obtained from a mixed pool of *Ixodes* spp and *Rhipicephalus* spp larval ticks collected from *Rattus rattus* trapped in Chiang Mai Province in 1962 (Robertson and Wisseman, 1973). TT-118 was subsequently determined to be a strain of *Rickettsia honei*, the etiologic agent of Flinders Island spotted fever in Australia (Stenos *et al*, 1998). Several documented cases of human patients exhibiting signs and symptoms of SFG rickettsiosis have been reported in Thailand and in many cases their sera were reactive to SFG rickettsial antigens. Three patients at a Chiang Mai hospital showed serological reactions to several SFG rickettsias but the tests could not confirm which rickettsial species were involved (Sirisanthana *et al*, 1994). SFG rickettsias were also serologically detected in 20 of 46 patients presenting with rickettsiosis symptoms at a hospital on the central Thai-Myanmar border in western Thailand (Ellis *et al*, 2006). Further serological testing of 8 of these patients showed antibodies to the SFG rickettsias, *R. conorii* India, *R. helvetica* and *R. felis* (Parola *et al*, 2003b). Another three patients from northeastern Thailand were seropositive for *R. helvetica* (Fournier *et al*, 2004). Eight (0.9%) of 854 febrile patients had antibodies to *R. helvetica* in a survey that included a total of five hospitals from northeastern, Central and southern Thailand (Suttinont *et al*, 2006). In the latter survey, *R. helvetica* antibodies were detected in patients from both northeastern and southern but not Central Thailand.

Serological surveys in two parts of Thailand have shown the presence of antibodies to TT-118 to be widespread in communities. A survey of 122 farmers in Chiang Rai, northern Thailand, determined that spotted fever antibodies were markedly prevalent (9.0 to 21.3%) with specific reactivity to the TT-118 strain occurring in 8.2% of people (Takada *et al*, 1993). In a similar survey in suburban Bangkok, 4% of 215 people who donated

blood were positive for TT-118 infection (Strickman *et al*, 1994). These survey results along with evidence of infections in hospital patients suggest that SFG rickettsias are widely distributed in various parts of Thailand.

The first molecular confirmation of a SFG rickettsial infection in a Thai person was published by Jiang *et al* (2005) who used five different genes to show the presence of *R. honei* infection in a patient. Later, Gaywee *et al* (2007) confirmed molecularly the presence of DNA of another SFG rickettsia that was very similar to *R. japonica*, the causative agent of Japanese spotted fever in Japan (Mahara, 2006) in a patient at a Bangkok hospital. *Rickettsia* have also been determined molecularly in ticks in Thailand (Table 2). *Rickettsia honei* and another undescribed *Rickettsia* species were detected in the tick, *Ixodes granulatus*, whereas two unnamed *Rickettsia* isolates were found in *Dermacentor auratus* and unidentified *Dermacentor* larvae. In addition, three strains of SFG *Rickettsia* were detected in *Amblyomma testudinarium* and *Hemaphysalis ornithophila* (Hirunkanokpun *et al*, 2003). Infection rates in these two tick species were high with 30% of *Amblyomma testudinarium* and 16.8% of *Hemaphysalis ornithophila* testing positive for *Rickettsia* DNA. Three of the four infected tick species, *Ixodes granulatus*, *Dermacentor auratus* and *Amblyomma testudinarium*, have been recorded infesting humans (Tanskul *et al*, 1983). So, these ticks are potential sources of human rickettsial infections in Thailand.

Humans are not the only vertebrates to be infected with *Rickettsia* in Thailand. In a study by Suksawat *et al* (2001a) antigens to three *Rickettsia* species were detected in Thai dogs. The highest prevalence was recorded for *R. prowazekii* (24%), the flea-borne agent of epidemic typhus. However, the prevalence in dogs of antigens to the tick-borne SFG human pathogen *R. rickettsii* (12%) was also high. Antigens to another tick-borne *Rickett-*

sia, *R. canadensis*, were also found but at a comparatively lower prevalence (4%) than the other two. The role of the latter *Rickettsia* in human infection has not been definitely established (Parola *et al*, 2005).

Anaplasmataceae

Ehrlichiosis is an acute disease that triggers flu-like symptoms in both humans and animals and is caused by infection of parasites that were all previously placed in the genus *Ehrlichia*. However, evidence from molecular phylogenetic analyses using the *16S rRNA* gene and *groESL* operon nucleic acid data and from serological cross-reactions suggests that the genus *Ehrlichia* should be split into four genera, which are now designated as *Ehrlichia*, *Anaplasma*, *Neorickettsia* and *Wolbachia* (Dumler *et al*, 2001).

In the 1970s, substantial epizootic losses of military dogs occurred in Singapore, Thailand, Malaysia and Vietnam (Irwin and Jefferies, 2004). The causative agent was determined to be *Ehrlichia canis*, an obligate intracellular parasite that infects the host's monocytes after transmission by *Rhipicephalus sanguineus*. Many affected dogs developed a rapidly fatal hemorrhagic disease characterized by lethargy, fever, severe epistaxis, and petechial or ecchymotic hemorrhages. The development of terminal bone marrow suppression, to which German shepherd dogs seemed uniquely sensitive, gave rise to the original term "tropical canine pancytopenia" for this disease (Irwin and Jefferies, 2004). In Thailand, 161 cases of ehrlichiosis were identified serologically in a population of 316 military working dogs, of which 54 dogs exhibited clinical or hematological signs of ehrlichiosis (Davidson *et al*, 1975). Clinical and serologic recognition of ehrlichiosis among pet dogs in widely separated regions of Thailand suggests the disease has been endemic in Thailand for an extended time. Under such circumstances it is possible that pet and stray dogs serve as a source of infection for the epizootic that

occurs in Thai military working dogs (Davidson *et al*, 1975).

The first molecular evidence (Table 1) that Anaplasmataceae species infect dogs in Thailand was provided by Suksawat *et al* (2001b) who confirmed infections by *E. canis* and *A. platys*. The detection of a supposed third species, *A. phagocytophilum* (formerly *E. equi*), by Suksawat *et al* (2001b) was later determined to be a PCR artifact (Suksawat *et al*, 2002) and so currently there is no molecular evidence for *A. phagocytophilum* infection in Thai dogs. Nevertheless, antibodies to *A. phagocytophilum* as well as to *E. canis*, *E. chaffeensis* and *N. risticii* have been detected in dogs that presented to a Bangkok veterinary teaching hospital with clinical signs of ehrlichiosis (Suksawat *et al*, 2001a). The results of this study indicate that dogs in Thailand have substantial exposure to vector-borne diseases (Suksawat *et al*, 2001a).

Several other domestic animals have also been reported to be infected with Anaplasmataceae pathogens. In a survey of seven provinces throughout Thailand, up to 74.2% of calves were determined to be seropositive for *A. marginale* (Phrikanahok *et al*, 2000). This bacterium is the most prevalent tick-borne pathogen of animals worldwide and is responsible for severe morbidity and mortality in temperate, subtropical, and tropical regions (Palmer *et al*, 2000). The severity of *Anaplasma* infection in Thailand was demonstrated in a case study in Samut Prakan Province, Central Thailand, in which anaplasmosis was determined to be the cause of death of five of 30 non-immune cattle (Pemayodhin *et al*, 1991). *Anaplasma* DNA was also found to be common in goats in Satun Province, southern Thailand with 16.9% and 6.7% of meat and dairy goats being infected, respectively (Jittapalapong *et al*, 2005). Cats are normally considered not to be infected with *Ehrlichia* pathogens. Nevertheless, *Ehrlichia*-like inclusions have been detected in the peripheral

blood of naturally infected cats in Thailand (Jittapalapong and Jansawan, 1993).

Human infections with *Ehrlichia* were first reported in Thailand by Heppner *et al* (1997) who determined about 40% of volunteers in a malaria prophylaxis study from Sangkhla Buri District, Kanchanaburi Province on the Thai-Myanmar border had antibodies to *E. chaffeensis*, the agent of human monocytic ehrlichiosis in the USA. The exact identity of the ehrlichial agent reported by Heppner *et al* (1997) as *E. chaffeensis* cannot be determined because of the existence of cross-reactivity among closely-related *Ehrlichia* species. For example, cross-reactivity has been reported among *E. canis* and *E. chaffeensis* antigens in America (Perez *et al*, 1996; Kordick *et al*, 1999). To date, isolates of *E. chaffeensis* or molecular evidence of infection is limited to the USA (Suksawat *et al*, 2001a).

A survey of ticks conducted in the same part of Thailand where human *Ehrlichia* infection was discovered using PCR to detect bacteria of the order *Rickettsiales*, including those in the family Anaplasmataceae (Parola *et al*, 2003b). Ticks were collected from peridomestic and wild animals, from people, or by flagging the vegetation (Table 2). Three *Anaplasma* spp were found, including *A. platys*, which was obtained from *Dermacentor auratus* ticks collected from dogs, and two unnamed *Anaplasma* species, one from *Amblyomma javanense* ticks collected on a pangolin and the other from *Haemaphysalis lagrangei* ticks collected from a bear. One unnamed *Ehrlichia* species was determined in *Rhipicephalus microplus* ticks collected from cattle. Two of these tick species, *Dermacentor auratus* and *Haemaphysalis lagrangei*, have been collected from humans (Tanskul *et al*, 1983).

In summary, tick-borne parasites of the family Anaplasmataceae confirmed to be present in Thailand include *A. platys* and *E. canis* (Table 1), which can cause severe canine monocytic ehrlichiosis and mild to mod-

erate canine infectious cyclic thrombocytopenia in dogs (Irwin and Jefferies, 2004). Of these, genetic and serological evidence for *E. canis* infection in humans exists (Perez *et al*, 1996), although not in Thailand. In addition, *A. platys* and another unnamed *Anaplasma* spp were detected in ticks that sometimes feed on humans. The potential presence in Thai dogs of *E. chaffeensis* supported by serological evidence is of concern. *E. chaffeensis* infection has mild effects on dogs (Irwin and Jefferies, 2004). However, in humans in the USA, this parasite can cause severe and even fatal human monocytic ehrlichiosis. The question concerning whether *E. chaffeensis* occurs in Thailand or not has not been answered. However, *Ehrlichia* strains very closely related to American *E. chaffeensis* have been found in ticks in other countries of the region (South-east China-Cao *et al*, 2000; Japan-Shibata *et al*, 2000; Vietnam-Parola, *et al*, 2003b). These findings highlight the potential for novel anaplasmoses and ehrlichioses to develop in Thailand as well as the need for accurate diagnostic tools for determining the identities of tick-borne pathogens in this country.

OTHER BACTERIAL PATHOGENS

Several types of bacterial pathogens other than rickettsial bacteria are often carried by ticks. They include *Bartonella* spp which cause bartonellosis, a disease associated with a variety of clinical syndromes in humans, the best known of which is cat scratch disease (Irwin and Jefferies, 2004) and which may also be responsible for several conditions in cats and dogs (Breitschwerdt *et al*, 1999; Shaw *et al*, 2001b), *Borrelia* spp, which cause lyme disease and relapsing fever in humans (Parola and Raoult, 2001; Rebaudet and Parola, 2006), *Coxiella burnetii*, which causes Q fever throughout the world and infects arthropods, birds, pets, domestic and wild mammals, as well as humans

(Rodolakis, 2006), and *Francisella tularensis*, the cause of tularemia in humans and other vertebrates (Cunha and Johnson, 2001).

Bartonella species are gram-negative bacteria that infect erythrocytes, endothelial cells and macrophages, often leading to persistent blood-borne infections (Billeter *et al*, 2008) in mammals including humans (Dehio, 2004). Several *Bartonella* species have been detected in ticks and this evidence along with clinical studies in which *Bartonella* infection of animals or humans likely followed tick exposure have led to suggestions that ticks may be vectors of these pathogens (Billeter *et al*, 2008). Nevertheless, no reports have shown ticks to be *Bartonella* vectors, whereas several other blood-feeding arthropods, including a sandfly (*Lutzomyia verrucarum*), a louse (*Pediculus humanus humanus*) and two flea species (*Ctenocephalides felis*, *Ctenophthalmus nobilis nobilis*), have been confirmed as vectors (Billeter *et al*, 2008). Of the *Bartonella* species with serological or molecular evidence of occurrence in mammals in Thailand (see below), only *B. henselae* and *B. vinsonii* ssp *berkhofii* are known from ticks in other parts of the world (Billeter *et al*, 2008) but not Thailand.

Evidence of *Bartonella* presence in Thailand has been provided by several studies that have shown infection in various mammal species. Blood samples of cats and dogs that presented to an animal teaching hospital in Bangkok were found to have antibodies to *B. henselae* and *B. vinsonii* ssp *berkhofii*, respectively (Boonmar *et al*, 1997; Suksawat *et al*, 2001a). Molecular techniques have detected several *Bartonella* strains in three species of rodents (*Bandicota indica*, *Rattus losea* and *Rattus rattus*) in Chiang Rai Province, northern Thailand (Castle *et al*, 2004) as well as determined *B. clarridgeiae* and *B. henselae* to be present in 27.6% of cats throughout Thailand (Maruyama *et al*, 2001). A *Bartonella* strain detected in the blood of three human

patients from Khon Kaen Province in north-eastern Thailand has been proposed as a new *Bartonella* species, *B. tamiae* (Kosoy *et al*, 2008).

Borrelia species causing lyme disease and relapsing fever are not known to be in Thailand or elsewhere in Southeast Asia (Reboudet and Parola, 2006). *Borrelia* species that cause lyme disease are transmitted by *Ixodes* ticks and those that cause relapsing fever are transmitted by *Ornithodoros* ticks. Both tick genera are represented by several species in Thailand (Tanskul *et al*, 1983). However, none of the tick species recorded as *Borrelia* vectors in other parts of the world are known in Thailand (Tanskul *et al*, 1983; Parola and Raoult, 2001). Hirunkanokpun *et al* (2003) examined ticks for several human pathogens, including *Borrelia*. They were unable to detect *Borrelia* DNA in 334 ticks representing 14 species tested. However, none of the species tested by Hirunkanokpun *et al* (2003) were in the genera *Ixodes* or *Ornithodoros*.

Tularemia in humans and animals is caused by *F. tularensis*, a small gram-negative intracellular bacterium (Parola and Raoult, 2001). It seems to be a disease of temperate zones of the Northern Hemisphere (Cunha and Johnson, 2001). The pathogen is transmitted to humans by bites from infected arthropod vectors with the primary vectors being ticks and deer flies, as well as by various other routes, including the direct handling of infectious carcasses, the ingestion of contaminated food, vegetation or water, and the inhalation of infectious dust, soil or aerosols (Ellis *et al*, 2002). Domestic and wild animal population movements may be important in the spread of diseases like tularemia. In a recent case, potentially *Francisella*-infected prairie dogs were distributed to wholesalers, retailers, and persons worldwide, including Thailand (Avashia *et al*, 2004). Despite the potential for spread of *Francisella* in Thailand through importation of infected animals, there

have been no published reports of tularemia or *Francisella* infections in Thailand. However, a warning of tularemia has been posted recently by the Thailand's Department of Disease Control (MCOT enews, 2008). Hirunkanokpun *et al* (2003) tested 14 tick species for several pathogens including *Francisella* but the results were negative for *Francisella* DNA.

Coxiella burnetii, the agent for Q-fever, can infect a broad spectrum of susceptible hosts including domestic animals (livestock and pets), wildlife and even non-mammalian species including reptiles, fish, birds and ticks (Cutler *et al*, 2007). Human infection is usually via shedding of *C. burnetii* in milk, feces, urine and birth products from infected ruminants. Infection is mainly acquired through inhalation of infectious aerosols, at parturition (including normal births) or at slaughter. In humans, infection is often asymptomatic, and in its mild form can be mistaken for other flu-like illnesses. It can, however, be associated with chronic or even fatal infections, usually endocarditis. Though this organism is endemic in the environment, infection is rarely reported. Ticks can be infected with *C. burnetii*. Their significance for causing human disease has yet to be established (Cutler *et al*, 2007). *Coxiella burnetii* infection in humans has been reported in Thailand. Supattamongkol *et al* (2003) reported *C. burnetii* to be present in 9 of 678 (1.3%) febrile patients in northeastern Thailand based on serological tests. In another serological study, one positive case in 133 was detected in febrile patients in a hospital near the Thai-Myanmar border in Kanchanaburi (Ellis *et al*, 2006). The authors considered the single positive case to be insufficient evidence for the presence of *C. burnetii* in the area. Their conclusion is supported by another survey of febrile patients at the same hospital conducted using molecular markers in which no cases of *C. burnetii* infection were found (Parola *et al*, 2003a).

FLAVIVIRAL PATHOGENS

The mammalian tick-borne flavivirus complex includes Kyasanur forest disease, Langkat, Louping ill, Negishi, Omsk hemorrhagic fever, Powassan, and tick-borne encephalitis viruses (Labuda and Nuttall, 2004). Of these, only Langkat virus has been found in Thailand. The vector-competent hosts of these viruses are ticks belonging to the family *Ixodidae* (hard ticks). The most serious of these viruses is the tick-borne encephalitis virus, which is distributed from northern Europe through Siberia to northern Asia (Labuda and Nuttall, 2004). No cases of tick-borne encephalitis have been reported in Southeast Asia.

Langkat virus (isolate TP21) was originally isolated in the 1950s from a pool of *Ixodes granulatus* ticks from forest rats caught in Malaysia (Smith, 1956). Antibodies to Langkat-TP21 were detected in sera of 6 of 51 forest rats. Some human sera that had antibodies to Langkat-TP21 also had antibodies to Japanese encephalitis virus, but this was considered to indicate cross-reactivity between the two viruses and not conclusive evidence of human infection with Langkat virus (Smith, 1956). Langkat virus is the only representative of the tick-borne flavivirus group that has not been found to cause human disease in natural foci (Il'enko *et al*, 1968). Recently, antibodies to Langkat virus were detected in the sera of one human (114 tested) and one orangutan (71 tested) in Malaysia (Wolfe *et al*, 2001). However, it is difficult to form conclusions on the basis of one positive result for each primate species. In Thailand, a strain of Langkat virus (isolate T-1674), was isolated from a pool of *Haemaphysalis papuana* ticks collected from vegetation in Khao Yai National Park (Bancroft *et al*, 1976). No antibodies were found in any of 190 *Rattus* or other small mammals sampled in the same area.

Both Langkat virus carrier ticks, *Ixodes granulatus* and *Haemaphysalis papuana*, are

widely distributed in Thailand (Tanskul *et al*, 1983) and this suggests that the virus may be present in other areas of the country (Bancroft *et al*, 1976). Both tick species have been recorded on humans (Tanskul *et al*, 1983) and may potentially transmit Langkat virus to humans.

CONCLUSION

Ticks and pathogens borne by ticks are common in urban, rural and forest environments in Thailand. Hematological and serological evidence indicates that such pathogens can infect and cause severe disease in humans and domestic animals. However, these techniques often do not allow the identification of pathogens to species level. In recent years, however, the advent of molecular techniques has resulted in accurate identification of tick-borne pathogens and diagnosis of the diseases they cause. In particular, the use of techniques such as multiplex and real-time quantitative PCR can greatly enhance our understanding of the epidemiology of tick-borne diseases in Thailand.

Large numbers of infected stray cats and dogs roam streets, fresh open markets, and other public places in Thailand's urban areas. These stray cats and dogs may act as sources of tick-borne zoonotic diseases (Jittapalapong *et al*, 2006). Companion animals seem most at risk for contracting tick-borne diseases from these stray animals (Irwin and Jefferies, 2004). Nevertheless, experimental evidence that conclusively demonstrates the roles and relative importances of stray dog and cat populations as well as the feral rodent populations in infecting companion animals is lacking but is needed in Thailand.

In Thailand's rural and forest areas, different ticks and tick-borne diseases exist. Livestock, particularly cattle, are at risk for severe disease and even death caused by tick borne *Babesia* and *Anaplasma* species that

may result in substantial losses to rural livelihoods. Despite these diseases' severity, little is known of the extent of each disease-causing pathogen species throughout Thailand. The risk factors associated with each bovine tick-borne disease have not been characterized. Better management and therefore greater relief for cattle and other livestock owners can only come about from better understanding of these diseases. Finally, livestock, wildlife and animal products imported into Thailand should be carefully monitored for tick-borne and other diseases.

Rickettsioses and perhaps babesioses may be a hazard to humans who work or spend their leisure time in Thailand's rural and forest areas. People such as forest workers and ecotourists who spend time in dense vegetation, especially in the high tick season, are at greatest risk. Very little is known of the pathogens carried by ticks in these areas or of their vertebrate reservoir hosts. Yet, as reforestation programs continue in this country and increasing numbers of tourists visit Thailand's national parks, it can be expected that many more people will contract tick-borne diseases. Knowing what tick-borne pathogens are present and the tick species and vertebrate hosts that carry them in these areas may help in the rapid diagnosis of novel diseases that present in people who visit these areas.

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