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First survey of the hard tick (Acari: Ixodidae) fauna of Nakai District, Khammouane Province, Laos, and an updated checklist of the ticks of Laos

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Abstract

From 2012 to 2014, tick collections for tick and tick-borne pathogen surveillance were carried out in two areas of Nakai District, Khammouane Province, Laos: the Watershed Management and Protection Authority (WMPA) area and Phou Hin Poun National Protected Area (PHP NPA). Throughout Laos, ticks and tick-associated pathogens are poorly known. Fifteen thousand and seventy-three ticks representing larval (60.72%), nymphal (37.86%) and adult (1.42%) life stages were collected. Five genera comprising at least 11 species, including three suspected species that could not be readily determined, were identified from 215 adult specimens: Amblyomma testudinarium Koch (10; 4.65%), Dermacentor auratus Supino (17; 7.91%), D. steini (Schulze) (7; 3.26%), Haemaphysalis colasbelcouri (Santos Dias) (1; 0.47%), H. hystricis Supino (59; 27.44%), H. sp. near aborensis Warburton (91; 42.33%), H. sp. near darjeeling Hoogstraal and Dhanda (5; 2.33%), H. sp. near lagrangei Larrousse (3; 1.4%), H. spp. (16; 7.45%), Rhipicephalus haemaphysaloides (Supino) (5; 2.33%), and R. (Boophilus) microplus (Canestrini) (1; 0.47%). These collections, together with the literature to date, provide evidence for the occurrence of at least 22 ixodid tick species, representing six genera, in Laos. Here we present new records for at least four tick species from WMPA area, Nakai District, Khammouane Province, where tick-borne pathogens may circulate. These preliminary results should serve as a framework for further molecular investigations of putative tick vectors and their pathogens in Laos.

Key words: Laos, Ticks, Ixodidae, Fauna, Distribution

Introduction

Ticks are hematophagous ectoparasites that are known pests and vectors of a wide range of diseases of humans, livestock, pets, and wild animals. In Southeast Asia, taxonomically accurate information on tick species is limited, and the tick-borne diseases of this region remain poorly characterized. Historically, about 104 species of ticks, representing 12 genera, have been known to occur in Southeast Asia (Petney et al., 2007), with the recent addition of two new species of Dermacentor (Apanaskevich et al., 2015a, Apanaskevich et al., 2015b). The relationship between ticks and tick-borne pathogens in the region is largely unknown, even though the presence of these pathogens has been recognized for many years and the number of new pathogens discovered in ticks has increased markedly (Yu et al., 2011, Kernif et al., 2012, Kho et al., 2015).
Laos is a landlocked country, located in Southeast Asia and bordered by China, Vietnam, Cambodia, Thailand and Myanmar. It covers a land area of approximately 236,800 km². Interestingly, Laos is a country where deforestation for agriculture and farming has greatly increased in recent years. In 2012, forests accounted for 67.2% of the total land area, a marked decrease from a 2000 survey showing 71.6% forest cover (World Bank, 2015, http://wdi.worldbank.org/table/3.1). Deforestation activities may alter ecosystem dynamics, changing or creating new breeding habitats for disease vectors as well as enhancing pathogen-host-vector interactions. Evidence shows tick-related diseases are circulating but have been largely neglected (Phongmany et al., 2006, Rattanavong et al., 2014). Advances have recently been made in Laotian infectious disease research, but vector-borne diseases are still misdiagnosed and underestimated because of inadequate clinical training and limited surveillance and laboratory capacity. Moreover, most vector-borne disease research in Laos has focused on mosquitoes and mosquito-borne diseases, with the result that very little attention has been paid to acarology, especially ticks and tick-borne diseases.

No single reference to Laotian tick species is currently available to researchers, in part because the tick fauna of Laos is still largely unknown. Tick species that occur in Laos have been discussed by (i) Toumanoff in 1944 (Toumanoff, 1944), (ii) Hoogstraal et al. and Wilson between the 1960s and 1980s (Hoogstraal et al., 1965, Wilson, 1970, Hoogstraal et al., 1971, Hoogstraal et al., 1973b, Hoogstraal et al., 1985b), (iii) Petney and Keirans in the mid-1990s (Petney et al., 1994, Petney et al., 1995, Petney et al., 1996a, Petney et al., 1996b), (iv) Robbins et al. in 1996 (Robbins et al., 1996), and (v) Kernif et al. in 2012 (Kernif et al., 2012), the most recent work.

Herein we present preliminary results of a faunal study of tick species collected during surveys of ticks and tick-borne pathogens in Nakai District, Khammouane Province, and we provide an updated checklist of tick records from Laos. An investigation of Laotian ticks and tick-borne pathogens was launched in late 2012 and continued into 2014 through collaboration between the U.S. Naval Medical Research Center-Asia (NMRC-A) and the Institut Pasteur du Laos (IP-Laos). Its goals were to determine the geographical distribution of putative tick vectors of bacterial and arboviral diseases in the Nakai Nam Theun National Protected Area (NNT NPA), known as the Watershed Management and Protection Authority area (WMPA), located in Nakai District, Khammouane Province, Laos. About 6,900 people live in NNT NPA, clustered in 31 villages, with a density of about 1.95 persons/km². Villagers in the area rely mainly on a number of forestry/agriculture practices, including hunting and gathering of forest and stream products. Livestock and pets are important for their livelihood and these valuable animals are often maintained close to human dwellings (NT2 WMPA 2015, [http://www.nt2wmpa.gov.la/en/people-and-nature]). Water buffalo and cattle are reared in this area by releasing them into the forest and by periodically moving them close to or under village houses. Such practices may facilitate interactions between ticks and their hosts, including the exchange of ticks between wild and domestic animals. They may also lead to dispersal of the tick population, thereby potentially increasing the risk of tick-borne disease transmission. Laotian foresters and scientists working under such conditions have been diagnosed and treated for rickettsial infection (Nakai District hospital, personal communication). Throughout Southeast Asia, mites and ticks are important vectors of these pathogens and potentially other newly emerging infectious agents.

Methods

Study area

Tick collections were conducted in two areas of Nakai District, Khammouane Province: the WMPA area and Phou Hin Poun National Protected Area (PHP NPA) (Figure 1). The WMPA area,
bordering Vietnam and covering a total area of 4,230 square km is the largest protected forest area in Laos and is considered a biodiversity hotspot in Southeast Asia (http://www.nt2wmpa.gov.la/en/ecology/). In order to maximize the diversity of tick species surveyed in this area, ticks were sampled at as many sites as possible in mountainous primary forest (locality range: Lat. 17.74395°N–18.06337°N, Long.105.3337°E–105.4685°E, Alt. 542-690 m). The PHP NPA, located northwest of Nakai District, was selected for tick collection in order to increase the scope and accuracy of our tick sampling. The PHP NPA is located in an area whose ecology contrasts sharply with the WMPA area. This region is one of only two NPAs in Laos that includes portions of the Central Indochina limestone mountains. Tick collection sites in this area were in secondary forests within valleys enclosed by karstic limestone formations and forested mountains. The collection sites were water buffalo grazing habitats near village tobacco and rice fields (locality range: Lat. 17.96798°N–17.98188°N, Long. 104.8198°E–104.8291°E, Alt. 178–203 m). Tick collection was conducted during the dry season in December 2012, February 2013, and February, March and April 2014 (see Table 1 for exact dates and locations). During the course of collecting in March 2014, temperatures for the Nam Noy field-site (forested area of the WMPA) ranged from 18.5 to 39°C, with a relative humidity (RH) range of 49.5 to 89.5%. In April 2014, Korbong village field-site (a village area of the WMPA) experienced a temperature range of 21 to 33.5°C, with a RH range of 51.5 to 88%. This is within the normal seasonal averages for Khammouane Province in April 2011 (25.5°C, range 20.5–30.5°C; 67%, range 51-83%) (Department of Natural Resources and Environment Laos, 2011).

**FIGURE 1**: Map of field tick collection sites in Khammouane Province, Lao PDR. Red stars are tick collection sites, black circles are villages in WMPA area. VT = Vientiane capital. KM = Khammouane Province. Blue line = Mekong River.

**Tick collections and identification**

Ticks were collected by dragging the forest floor and vegetation. White heavy cotton sheets were cut into many sizes: 50 cm, 70 cm, and 100 cm widths x 100 cm long. These makeshift drags were swept along the forest floor/vegetation at about 1-10 minute intervals near animal trails before being visually examined for ticks. Specimens were removed from the sheets using forceps, placed in 1.5 ml cryovials labeled with date and site, and then transported live to the IP-Laos Nakai Field Laboratory. Additionally, ticks were collected from domestic animals by direct hand removal with
forceps during our survey in Korbong village and stored in 80% ethanol. In the Nakai lab, live ticks were killed by freezing at -20°C for 10–30 minutes. Adults, nymphs, and larval ticks were then separated, counted, and subsequently stored at -20°C for transportation on dry ice to IP-Laos in Vientiane for further analysis (species identification, pathogen detection, and discovery).

Adult ticks were preserved in 80% ethanol as Laotian reference tick samples and sent to co-investigator Robbins to confirm morphological identification. The remaining ticks were identified and grouped microscopically on an ice pack at IP-Laos, using the reference determinations from Dr. Robbins, together with related keys and original species descriptions (Robinson, 1926, Yamaguti et al., 1971, Wassef et al., 1984, Wassef et al., 1986, Tanskul et al., 1989, Voltzit et al., 2002). Because there are no morphological identification keys available for preimagines, larval and nymphal stages were grouped by genus. Reference tick samples were deposited in the collection room of the IP-Laos Medical Entomology Laboratory located in Vientiane, the capital of Laos.

TABLE 1: Field site collection coordinates and collection dates.

<table>
<thead>
<tr>
<th>Field site names</th>
<th>Collection dates</th>
<th>Site characteristics*</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Altitude (meters)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WMPA area</td>
<td>11 December 2012</td>
<td>MPF</td>
<td>17.75084</td>
<td>105.3604</td>
<td>542</td>
</tr>
<tr>
<td></td>
<td>5–7 February 2013</td>
<td>MPF</td>
<td>17.74395</td>
<td>105.3579</td>
<td>579</td>
</tr>
<tr>
<td></td>
<td>12 December 2012</td>
<td>MPF</td>
<td>17.74556</td>
<td>105.3547</td>
<td>564</td>
</tr>
<tr>
<td>WMPA area</td>
<td>13 December 2012</td>
<td>MPF</td>
<td>17.76768</td>
<td>105.3445</td>
<td>625</td>
</tr>
<tr>
<td></td>
<td>13 December 2012</td>
<td>MPF</td>
<td>17.75742</td>
<td>105.3337</td>
<td>564</td>
</tr>
<tr>
<td></td>
<td>18 December 2012</td>
<td>MPF</td>
<td>17.97563</td>
<td>105.4017</td>
<td>626</td>
</tr>
<tr>
<td>PHP NP area</td>
<td>16 February 2013</td>
<td>MPF</td>
<td>18.00798</td>
<td>105.4570</td>
<td>579</td>
</tr>
<tr>
<td>WMPA area</td>
<td>18 December 2012</td>
<td>MPF</td>
<td>18.01137</td>
<td>105.4497</td>
<td>621</td>
</tr>
<tr>
<td>PHP NP area</td>
<td>20 December 2012</td>
<td>MPF</td>
<td>18.02897</td>
<td>105.4685</td>
<td>598</td>
</tr>
<tr>
<td>PHP NP area</td>
<td>12 February 2013</td>
<td>MPF</td>
<td>18.05362</td>
<td>105.4664</td>
<td>651</td>
</tr>
<tr>
<td>PHP NP area</td>
<td>13 February 2013</td>
<td>MPF</td>
<td>18.06337</td>
<td>105.4557</td>
<td>690</td>
</tr>
<tr>
<td>PHP NP area</td>
<td>14 February 2013</td>
<td>MPF</td>
<td>18.05567</td>
<td>105.4674</td>
<td>656</td>
</tr>
<tr>
<td>WMPA area</td>
<td>5-7 March 2014</td>
<td>VSF</td>
<td>17.967977</td>
<td>104.819818</td>
<td>178</td>
</tr>
<tr>
<td>WMPA area</td>
<td>5-7 March 2014</td>
<td>VSF</td>
<td>17.981883</td>
<td>104.829076</td>
<td>203</td>
</tr>
<tr>
<td>PHP NP area</td>
<td>30 March 2014</td>
<td>VSF</td>
<td>17.847732</td>
<td>105.367612</td>
<td>597</td>
</tr>
<tr>
<td>WMPA area</td>
<td>1 April 2014</td>
<td>VSF</td>
<td>17.850140</td>
<td>105.378981</td>
<td>576</td>
</tr>
<tr>
<td>PHP NP area</td>
<td>31 March 2014</td>
<td>MPF</td>
<td>17.827494</td>
<td>105.370127</td>
<td>676</td>
</tr>
<tr>
<td>WMPA area</td>
<td>3–5 April 2014</td>
<td>MPF</td>
<td>17.889201</td>
<td>105.396963</td>
<td>687</td>
</tr>
</tbody>
</table>

*MPF = Mountainous primary forest, VSF = Valley secondary forest.

Results

Tick species composition and distribution in Nakai District

A total of 15,073 ticks were collected during our survey, comprising larval (60.72%), nymphal (37.86%), and adult (1.42%) life stages. Five tick genera were identified: *Haemaphysalis* Koch, 1844
(72.92%), Amblyomma Koch, 1844 (23.61%), Dermacentor Koch, 1844 (3.35%), Ixodes Latreille, 1795 (0.09%), and Rhipicephalus Koch, 1844 (including Boophilus Curtice, 1891) (0.04%). The majority of our tick samples were collected by dragging trails and vegetation in the WMPA (91.08%) and PHP NPA (8.92%) areas. Only 0.1% of our specimens were collected from domestic animal hosts in the WMPA area. No adult ticks were collected in the PHP NPA area (Table 2).

**TABLE 2:** Number of ticks collected by species and percentage of larvae, nymphs, adult males and females collected from two study localities by hosts.

<table>
<thead>
<tr>
<th>Localities/Hosts</th>
<th>Adults</th>
<th>Nymphs</th>
<th>Larvae</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genus/Species</td>
<td>Male No</td>
<td>Female No</td>
<td>Male No</td>
<td>Female No</td>
</tr>
<tr>
<td>WMPA area</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amblyomma sp.</td>
<td>0</td>
<td>0</td>
<td>1,106</td>
<td>7.34</td>
</tr>
<tr>
<td>Amblyomma testudinarium</td>
<td>4</td>
<td>0.03</td>
<td>2</td>
<td>0.01</td>
</tr>
<tr>
<td>Dermacentor auratus</td>
<td>11</td>
<td>0.07</td>
<td>6</td>
<td>0.04</td>
</tr>
<tr>
<td>Dermacentor spp.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Dermacentor steini</td>
<td>2</td>
<td>0.01</td>
<td>5</td>
<td>0.03</td>
</tr>
<tr>
<td>Haemaphysalis colasbelcouri</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.01</td>
</tr>
<tr>
<td>Haemaphysalis hystricis</td>
<td>25</td>
<td>0.17</td>
<td>31</td>
<td>0.21</td>
</tr>
<tr>
<td>Haemaphysalis sp., *aborensis</td>
<td>24</td>
<td>0.16</td>
<td>67</td>
<td>0.44</td>
</tr>
<tr>
<td>Haemaphysalis sp., *darjeeling</td>
<td>1</td>
<td>0.01</td>
<td>4</td>
<td>0.03</td>
</tr>
<tr>
<td>Haemaphysalis sp., *lagrangei</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0.02</td>
</tr>
<tr>
<td>Haemaphysalis spp.</td>
<td>7</td>
<td>0.05</td>
<td>9</td>
<td>0.06</td>
</tr>
<tr>
<td>Ixodes spp.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
<td>0.49</td>
<td>128</td>
<td>0.85</td>
</tr>
<tr>
<td>Cows</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amblyomma testudinarium</td>
<td>3</td>
<td>0.02</td>
<td>1</td>
<td>0.01</td>
</tr>
<tr>
<td>Haemaphysalis hystricis</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0.01</td>
</tr>
<tr>
<td>Rhipicephalus haemaphysaloides</td>
<td>2</td>
<td>0.01</td>
<td>2</td>
<td>0.01</td>
</tr>
<tr>
<td>Rhipicephalus (Boophilus) microplus</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.01</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>0.03</td>
<td>6</td>
<td>0.04</td>
</tr>
<tr>
<td>Dogs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amblyomma testudinarium</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Haemaphysalis hystricis</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.01</td>
</tr>
<tr>
<td>Rhipicephalus haemaphysaloides</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.01</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0.01</td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>0.52</td>
<td>136</td>
<td>0.9</td>
</tr>
<tr>
<td>PHP NP area</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amblyomma spp.</td>
<td>0</td>
<td>0</td>
<td>891</td>
<td>5.91</td>
</tr>
<tr>
<td>Dermacentor spp.</td>
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<td>0</td>
<td>4</td>
<td>0.03</td>
</tr>
<tr>
<td>Haemaphysalis spp.</td>
<td>0</td>
<td>0</td>
<td>317</td>
<td>2.1</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>0</td>
<td>1,212</td>
<td>8.04</td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>0.52</td>
<td>136</td>
<td>0.9</td>
</tr>
</tbody>
</table>

* Determination tentative.
Of 215 adult ticks, 27.44% (34 females, 25 males) were identified as *Haemaphysalis hystricis* Supino; 7.91% (6 females, 11 males) as *Dermacentor auratus* Supino; 4.65% (3 females, 7 males) as *Amblyomma testudinarium* Koch; 3.26% (5 females, 2 males) as *Dermacentor steini* (Schulze); 2.33% (3 females, 2 males) as *Rhipicephalus haemaphysaloides* (Supino); 0.47% (1 female) as *Haemaphysalis colosbelcourii* (Santos Dias); and 0.47% (1 female) as *Rhipicephalus (Boophilus) microplus* (Canestrini). The remaining indeterminate identifications include 42.33% (67 females, 24 males) as *H.* sp. near *aborensis* Warburton; 7.45% (9 females, 7 males) as *H.* spp.; 2.33% (4 females, 1 male) as *H.* sp., near *darjeeling* Hoogstraal and Dhanda; and 1.40% (3 females) as *H.* sp. near *lagrangei* Larrousse. No adult ticks of the genus *Ixodes* were collected during our study. Tick species diversity was found to be higher in WMPA area than in PHP NPA, especially in the case of the genus *Haemaphysalis*. As shown in Fig. 2, 5 genera and at least 10 species were collected in WMPA area, whereas only 3 genera were collected in PHP NPA. In WMPA area, *A. testudinarium* and *H. hystricis* were collected from both ground/vegetation and animals (cows and dogs), while *R. haemaphysaloides* was collected from cows and dogs and *R. (B.) microplus* was collected only from a cow (Table 2).

**FIGURE 2:** Species composition of ticks collected from WMPA area (left hand) and PHP NP area (right hand).

Updated checklist of hard ticks known to occur in Laos, based on literature and the results of this study

On the basis of our study and the 12 papers published since 1944 that discuss Laotian ticks, the following 6 genera and 22 species, at least 4 of them new, are now known to occur in Laos (*indicates species previously recorded in Laos and found in this study; **new record for Laos):**

**Genus Amblyomma Koch, 1844** (including *Aponomma* Neumann, 1899)

*Amblyomma (Aponomma) crassipes* (Neumann, 1901)

Reference: Petney *et al.*, 1996b

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*Amblyomma helvolum* Koch, 1844
Reference: Petney *et al.*, 1995
Known hosts and localities in Laos: not indicated. The authors refer to accession number RML 37800 (ex *Varanus* sp. (reptile): 1 ♂, forest, Saigon Road, South Laos, 27 July 1960, M. Nadchatram).

*Amblyomma (Aponomma) paxtoni* (Neumann, 1910)
Reference: Petney *et al.*, 1996b
Known hosts and localities in Laos: not indicated. The authors cite Teng & Jiang, 1991.

*Amblyomma testudinarium* Koch, 1844
Reference: Petney *et al.*, 1995
Known hosts and localities in Laos: not indicated. The authors refer to accession number RML 37803 (ex *Mydaus multiceps*: 1 ♂, 16 km south of Thateng, Bolovens Plateau, 3,400 ft altitude, 24 July 1960, M. Nadchatram).

In this study: vegetation, cows and dogs in WMPA area, Nakai District, Khammouane Province.

*Amblyomma (Aponomma) varanense* (Supino, 1897)
Reference: Petney *et al.*, 1996b
Known hosts and localities in Laos: southern Laos. The authors cite accession number RML 37799 (ex *Varanus* sp. (reptile): 4 ♂, forest, Saigon Road, South Laos, 27 July 1960, M. Nadchatram).

Genus *Dermacentor* Koch, 1844

*Derma centor auratus* Supino, 1897
Reference: Hoogstraal *et al.*, 1985b
Known hosts and localities in Laos: domestic pig, Xieng Khouang Province.
In this study: vegetation, WMPA area, Nakai District, Khammouane Province.

*Derma centor steini* (Schulze, 1933)
Reference: first record in Laos.
Known hosts and localities in Laos: None.
In this study: vegetation, WMPA area, Nakai District, Khammouane Province.

Genus *Haemaphysalis* Koch, 1844

*Haemaphysalis sp., possibly aborensis* Warburton, 1913
Reference: Hoogstraal *et al.*, 1971
In this study: vegetation, WMPA area, Nakai District, Khammouane Province.

*Haemaphysalis asiatica* (Supino, 1897)
Reference: Robbins *et al.*, 1996
Known hosts and localities in Laos: ex *Catopuma temminckii*, Lak Sao (18.10N, 104.55E), Khammouane Province.

*Haemaphysalis colasbelcouri* (Santos Dias, 1958)
Reference: first record in Laos.
Known hosts and localities in Laos: None.
In this study: vegetation, WMPA area, Nakai District, Khammouane Province.

*Haemaphysalis sp., near darjeeling* Hoogstraal and Dhanda, 1970
Reference: first record in Laos.
Known hosts and localities in Laos: None.
In this study: vegetation, WMPA area, Nakai District, Khammouane Province.

*Haemaphysalis doenitzi* Warburton and Nuttall, 1909
Reference: Hoogstraal *et al.*, 1973b

**Haemaphysalis heinrichi** Schulze, 1939
Reference: Wilson, 1970
Known hosts and localities in Laos: 2 ♂. Vientiane Province, vicinity of Ban Van Heua, 800 m, ex *Melogale personata* (Geoffroy) (BBM-La 41668), early 1968, N. K. Chinyavong.

*Haemaphysalis hystricis* Supino, 1897
Reference: Hoogstraal *et al.*, 1965
Known hosts and localities in Laos: ex human (feeding on arm) 1 ♂, Phong Saly, 30 April 1929, R. Wheeler (MCZ); ex *Mydax multiiceps*: 1 ♂, 2 ♀, 16 km south of Thateng, Bolovens Plateau, 3,400 ft altitude, 24 July 1960, M. Nadchatram (RML 37803).
In this study: vegetation, dog and cow in WMPA area, Nakai District, Khammouane Province.

**Haemaphysalis sp., near lagrangei** Larrousse, 1925
Reference: first record in Laos.
Known hosts and localities in Laos: None.
In this study: Vegetation, WMPA area, Nakai District, Khammouane Province.

**Haemaphysalis ornithophila** Hoogstraal & Kohls, 1959
Reference: Wilson, 1970
Known hosts and localities in Laos: all records from Vientiane Province, Ban Van Heua, 800 m, 12 April 1965, J. L. Gressitt. 2 NN, ex bird, (LA 1007); 5 NN, host not listed, probably bird, (LA 1010).

Genus *Ixodes* Latreille, 1795

**Ixodes ovatus** Neumann, 1899
Reference: Robbins *et al.*, 1996
Known hosts and localities in Laos: ex *Chrotogale owstoni*, Lak Sao (18.10N, 104.55E), Khammouane Province.

**Ixodes spinicoxalis** Neumann, 1899
Reference: Wilson, 1970
Known hosts and localities in Laos: 1 N, Vientiane Province, vicinity of Ban Van Heua, 800 m, ex *Callosciurus flavimanus* (Geoffroy)? or *Callosciurus finlaysoni* (Horsfield)? (BBM-La 41669), early 1968, N. K. Chinyavong.

Genus *Nosomma* Schulze, 1919

**Nosomma monstrosum** (Nuttall and Warburton, 1908)
Reference: Toumanoff, 1944
Known hosts and localities in Laos: water buffalo from Vientiane.

Genus *Rhipicephalus* Koch, 1844 (including *Boophilus* Curtice, 1891)

*Rhipicephalus haemaphysaloides* Supino, 1897
In this study: cow and dog in Korbong village, WMPA area, Nakai District, Khammouane Province.

*Rhipicephalus (Boophilus) microplus* (Canestrini, 1888)
Reference: Petney *et al.*, 1996a
Known hosts and localities in Laos: not indicated. The authors refer to accession number RML 37810 (ex dog: 1 ♂ Ban Theuong, 18 km. N.W., Xieng Khouang, Laos, 3,450ft altitude, 9 August 1960, M. Nadchatram).
In this study: Cow in Korbong village, WMPA area, Nakai District, Khammouane Province.
**Rhipicephalus sanguineus** (Latreille, 1806)

References: Wilson, 1970, Kernif et al., 2012

Known hosts and localities in Laos: Wilson, 1970 recorded 95♂♂, 119♀♀, 16 NN, 56 LL, Vientiane Province, Vientiane 170m, on old wall (of building?), 15 December 1968, N. K. Chinyavong. Kernif et al., 2012 recorded from dogs, Luang Namtha Province (20°55′N, 101°07′E), northwest Laos.

**Discussion**

This is the first study in Laos to address the hard tick fauna of Nakai District, Khammouane Province. Our field work yielded five ixodid genera and at least 10 species. Taken together with a review of the literature to date, 6 genera comprising 22 species (including suspected species from this study) are now known to occur in Laos. In our study, number and species diversity of ticks were higher in the WMPA area than in the PHP NPA area. These results reflect differences in the habitats and hosts at our two collection sites. In the WMPA area, most tick collections were made by dragging in primary forest where wild animals were present. In this area, livestock, such as village water buffalo and cattle, are allowed to roam freely, and this practice may increase the risk of tick-borne pathogens being transmitted between wild and domestic animals (L’Hostis et al., 2002) as well as humans.

In this study, *Haemaphysalis*, the most species-rich tick genus in Southeast Asia (52 *Haemaphysalis* species are known from this region, over 30% of the world haemaphysalid fauna) (Petney et al., 2007), was the dominant genus found in the forests of the WMPA area. Among tick genera, *Haemaphysalis* is cited as an example of tick-host coevolution through the preservation of primitive (*Aponomma*-like) structures in some species, their manifestation of structural differences in each developmental stage, as well as structural differences between the sexes of individual species (Hoogstraal et al., 1985a). Very little is known about these ticks in Southeast Asia and particularly in Laos. Therefore, further studies of *Haemaphysalis* taxonomy, both morphological and molecular, are urgently needed. Species names reported herein are those of species that have been found in countries adjoining Laos. *Haemaphysalis* species determinations were largely based on the work of Tanskul and colleagues (Tanskul et al., 1983, Tanskul et al., 1989). However, several adult specimens in our collections remain undetermined and are awaiting further studies of tick-host relationships, as well as additional morphological and molecular genetic analyses. We further expect that future studies of Laotian *Haemaphysalis* will result in a number of nomenclatural changes.

*Haemaphysalis aborensis*, a suspected species in this study, was the most frequently collected adult tick. This species, belonging to subgenus *Aborphysalis*, was redescribed in all stages by Hoogstraal et al. (1971). Subgenus *Aborphysalis* contains 5 species: *H. kyasanurensis* Trapido, Hoogstraal, and Rajagopalan; *H. aborensis*; *H. formosensis* Neumann; *H. atherurus* Hoogstraal, Trapido, and Kohls; and *H. capricornis* Hoogstraal. *Haemaphysalis aborensis* is known to occur in India, Nepal, Myanmar, Laos, Vietnam and China (Hoogstraal et al., 1971, Chen et al., 2010). It is found on a wide range of hosts, including Bovidae, Suidae, Moschidae, Hystricidae, Cervidae, Felidae, Phasianidae, Tupaiidae and Paridae, but there are no records from humans (Guglielmone et al., 2014). The medical and veterinary significance of this species is unknown.

*Haemaphysalis hystricis* was the second most frequently collected adult tick. This species is a member of subgenus *Kaiseriana*, which contains at least 9 species in Thailand (Tanskul et al., 1983). *Haemaphysalis hystricis* has often been misidentified as *H. bispinosa* Neumann, *H. birmaniae* Supino, *H. semermis* Neumann, and *H. papuana nadchatrami* Hoogstraal, Trapido, and Kohls. For this reason, all stages of *H. hystricis* were redescribed by Hoogstraal et al. (1965), who included a summary of hosts and distribution (Hoogstraal et al., 1965). *Haemaphysalis hystricis* occurs
throughout the Australasian, Oriental, and Palearctic zoogeographic regions, where it parasitizes a wide range of hosts, including humans and several orders of Mammalia. Records also exist for Cuculidae and several families of Passeriformes, although Aves are considered exceptional hosts (Guglielmone et al., 2014). In our study, this tick species was collected from vegetation, cows and dogs. *Haemaphysalis hystricis* is a putative vector of pathogens in the genera *Rickettsia*, *Coxiella*, *Ehrlichia* and *Trypanosoma* (Parola et al., 2003, Thekisoe et al., 2007, Ando et al., 2010, Arthan et al., 2015).

*Haemaphysalis darjeeing*, another suspected species in this study, is known to occur in the Oriental Region, including India, Nepal, Myanmar, Thailand, and Malaysia. It has been characterized as a parasite of hill and mountain forest-dwelling game animals and occurs from the eastern Himalayas of India to the Chiang Mai area of northwestern Thailand (Hoogstraal et al., 1970). This species has never been recorded from Vietnam (Kolonin, 1995, Kolonin, 2003), so there is a high probability that the species name used here is incorrect. However, Hoogstraal and Dhanda (1970) described this species as a member of the *H. birmaniae* group, subgenus *Haemaphysalis*, placing it close to *H. birmaniae* and *H. atherurus* (Hoogstraal et al., 1970). Known hosts include humans, Bovidae, Cervidae, Suidae, and Mustelidae (Guglielmone et al., 2014). The medical and veterinary significance of this species is unknown.

*Haemaphysalis lagrangei*, a third suspected species in this study, was redescribed and described in all stages by Hoogstraal et al., 1973, who classified it as a member of the *H. bispinosa* group, subgenus *Kaiseriana*, which also includes *H. longicornis* Neumann, *H. ramachandrai* Dhanda, Hoogstraal and Bhat, *H. bispinosa*, and *H. renschi* Schulze. Among these species, *H. lagrangei* most closely resembles *H. longicornis* (Hoogstraal et al., 1973a). Within the *H. bispinosa* group, only *H. lagrangei* is known to occur in Vietnam (Kolonin, 1995, Kolonin, 2003) and two species, *H. lagrangei* and *H. bispinosa*, occur in Thailand (Tanskul et al., 1983). *Haemaphysalis lagrangei* has also been recorded from Malaysia, China and Cambodia (Hoogstraal et al., 1973a, Chen et al., 2010). This tick is known to parasitize a wide range of hosts, including humans, several orders of Mammalia, Phasianidae, Falconidae, Laniidae, Muscicapidae, Varanidae, Cervidae, Mustelidae and Viveridae (Guglielmone et al., 2014). Hoogstraal et al. noted that this species experiences structural variations and differences in size related to its hosts (Hoogstraal et al., 1973a), so species determinations reported herein may be incorrect. Because reports of the veterinary and medical importance of this tick have increased in Southeast Asia over the past decade, particularly with regard to its role as a vector of *Anaplasma* spp., *Coxiella* spp., *Theileria* spp., and other *Rickettsia* spp. (Parola et al., 2003, Arthan et al., 2015, Sumrandee et al., 2015), further studies of the taxonomy, vector ecology, and host and pathogen relationships of *H. lagrangei* in Laos are warranted.

*Haemaphysalis colasbelcouri* was originally described as *Aponomma colasbelcouri*. Hoogstraal and Wilson (1966) described this species as *H. vietnamensis* (Hoogstraal et al., 1966) and a member of subgenus *Alloceraea*. As discussed by Guglielmone et al. (2009), *H. colasbelcouri* is the current valid name for this tick. Within subgenus *Alloceraea*, *H. colasbelcouri* is thought to be a structurally primitive prototype of today’s haemaphysalids by virtue of its *Aponomma*-like characters and its occurrence in tropical-temperate forest habitats (1200-1450 m altitude) of Southeast Asia, which are hypothesized to be the evolutionary point of origin of genus *Haemaphysalis* (Hoogstraal et al., 1966, Hoogstraal et al., 1985a). In this study, one female tick was collected from vegetation in mountainous forest of the WMPA area (locality: 18.06337°N, 105.4557°E, at 690 m altitude), indicating that the environment in this area is favorable for primitive haemaphysalids and likely also is responsible for the high diversity of structurally advanced *Haemaphysalis* species that are found in this area. Bovidae and Cervidae have been reported as hosts of *H. colasbelcouri*, and human infestations have also been recorded (Guglielmone et al., 2014), but the medical and veterinary
significance of this species remains unknown. Further studies of the biology of Laotian *Haemaphysalis* are needed to document host specificity and disease transmission in this area.

Twenty-one species of *Amblyomma* (including 9 formerly assigned to genus *Aponomma*) are known to occur in Southeast Asia, and 5 of these have been reported from Laos (Petney et al., 1995, Petney et al., 1996b). During this study, adults of *A. testudinarium* were collected from vegetation, cows and dogs in the WMPA area. Because the immature stages of Southeast Asian *Amblyomma* remain poorly known, larvae and nymphs were identified by association with adults. Only adults of *A. testudinarium* were collected from the WMPA area; therefore, all immature *Amblyomma* collected from this area were considered to represent *A. testudinarium*. However, immatures that were collected from the PHP NPA area were classified as *Amblyomma* sp. because no adults were collected there. *Amblyomma testudinarium* occurs throughout the Australasian, Oriental and Palearctic Zoogeographic Regions. Its host range is broad and includes humans (Guglielmone et al., 2014). This species has been associated with a rash-like illness in Japan (Natsuaki et al., 2014) and is reported to be a putative vector of severe fever with thrombocytopenia syndrome virus (SFTSV) as well as spotted fever group rickettsiae (Yun et al., 2014, Sun et al., 2015). It has also been found to harbor *Borreliia* spp. (Hou et al., 2015).

In Southeast Asia, 7 species (including 2 new species) belonging to genus *Dermacentor* are known to occur (Petney et al., 1996a, Apanaskevich et al., 2015a, Apanaskevich et al., 2015b). During our study, 2 species were collected: (i) *Dermacentor auratus* is widespread in the Oriental Zoogeographic Region, having been recorded from Sri Lanka, India, Nepal, Bangladesh, China, Myanmar, Thailand, Vietnam, Laos, Malaysia and Indonesia (Hooogastraal et al., 1985b, Chen et al., 2010); this species parasitizes a broad range of mammalian hosts, including Suidae, Cervidae, Sciuridae, Felidae, Mustelidae, Ursidae, Rhinocerotidae, Canidae, Boidae, Tragulidae, Cercopithecidae, Hystricidae, Muridae, and humans, as well as members of the avian family Phasianidae (Guglielmone et al., 2014). Many rickettsiae, protozoa and viruses have been isolated from *D. auratus*, such as *Anaplasma* sp. and *Rickettsia* sp. (Parola et al., 2003), *Hepatozoon* species similar to *Hepatozoon felis* (Sumrandee et al., 2015), Lanjan virus (Tan et al., 1967) and Kyasanur Forest virus (Sreenivasan et al., 1979). (ii) *Dermacentor steini*. We report the first collection of this species from Laos, although *D. steini* occurs widely in the Australasian and Oriental Zoogeographic Regions. Its known hosts include Suidae, Muridae, and Boidae, but also Elapidae and Varanidae (Guglielmone et al., 2014). The medical and veterinary significance of this species has not been adequately investigated, but *D. steini* feeds on humans and many of the same hosts as *D. auratus* (Wassef et al., 1988), so there is a high probability that co-infection may occur among these ticks, and *D. steini* may vector diseases similar to those transmitted by *D. auratus*.

*Ixodes*, the most species-rich tick genus in the world, currently comprises about 244 described species (Guglielmone et al., 2014). In Southeast Asia, 14 species are known, 6 of which parasitize humans (Petney et al., 1994, Guglielmone et al., 2014). Two *Ixodes* species have been recorded from Laos, *Ixodes ovatus* Neumann and *Ixodes spinicohalis* Neumann, from Viverridae (*Chrotogale owstoni*) and Sciuridae (*Callosciurus* spp.), respectively (Wilson, 1970, Robbins et al., 1996). Robbins et al. 1996 recorded *I. ovatus* in Lak Sao (18.10N, 104.55E), located near our study area. There is a high probability that this tick’s host chiefly inhabits forested areas, such as those where our tick collections were made. In our study, only nymphs of *Ixodes* were collected from ground vegetation. Further studies of tick hosts should provide valuable data on the distribution of Laotian *Ixodes* species and their relationship to disease.

Only 3 species of *Rhipicephalus* (including *Boophilus*) occur in Southeast Asia, and all have been reported from Laos (Wilson, 1970, Petney et al., 1996a, Robbins et al., 1996, Kernif et al., 2012). In the present study, *Rhipicephalus haemaphysaloides* was collected from cows and dogs in Korbong village. This species was also recorded in Lak Sao, near our study area (Robbins et al., 1996).
Rhipicephalus haemaphysaloides occurs in the Australasian, Oriental and Palearctic zoogeographic regions. Human infestations have been reported, and known hosts include mammals (Canidae, Muridae, Bovidae, Herpestidae, Soricidae) and birds (Cuculidae, Timaliidae, Phasianidae) (Guglielmone et al., 2014). In laboratory studies, R. haemaphysaloides has been shown to be a putative vector of Kyasanur Forest virus and Rickettsia spp. (Bhat et al., 1978, Hsu et al., 2011).

Rhipicephalus (Boophilus) microplus. Only one adult of this species was collected during our survey, from a cow in Korbong village. This one-host tick occurs in tropical regions around the world, where its hosts include Bovidae, several orders of Mammalia and Aves, Bufonidae, Chamaeleonidae and Elapidae (Guglielmone et al., 2014). Many pathogens are associated with this tick, including Babesia spp., Borrelia spp., Coxiella spp., Rickettsia spp., and Theileria spp. (Kernif et al., 2012, Hou et al., 2015, Sumrandee et al., 2015).

Our study of Laotian ticks has yielded important new records for at least four hard tick species from the WMPA area, Nakai District, Khammouane Province, where tick-borne pathogens may circulate among wild/domestic animals and people. This work represents a first step toward elucidating the tick fauna of Laos and will be followed by molecular investigations to characterize putative tick vectors and their pathogens. In this regard, it should be noted that errors in tick identification, especially in the case of the difficult genus Haemaphysalis, can lead to the erroneous reporting of tick species as disease vectors. Also, tick collection by dragging can have the effect of excluding immature stages when these live in burrows or other sequestered host breeding sites. Further studies on tick-host relationships and the ecology of each developmental stage should provide useful data on tick taxonomy and population dynamics. We have no doubt that additional studies of these and other hematophagous arthropods in Laos will yield considerable new information on the identity, ecology, and distribution of pathogens in Southeast Asia.

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