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Article

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 km^2. 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/km^2. 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^2, 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).

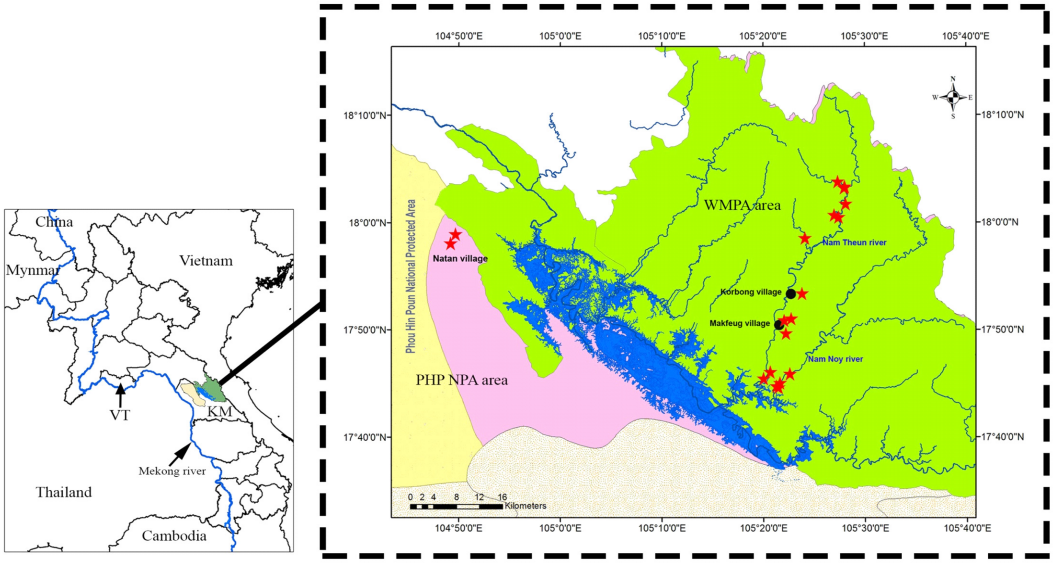

FIGURE 1: Map of field tick collection sites in Khammouane Province, Lao PDR. Red stars are tick collection sites, black circles are villages in WMPA area. VT = Vientiane capital. KM = Khammouane Province. Blue line = Mekong River.

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.

Field site names	Collection dates	Site characteristics*	Latitude	Longitude	Altitude (meters)
WMPA area	11 December 2012 5–7 February 2013 19–23 March 2013 9–16 February 2014 2–3 March 2014	MPF	17.75084	105.3604	542
	12 December 2012	MPF	17.74395	105.3579	579
	12 December 2012	MPF	17.74556	105.3547	564
WMPA area	13 December 2012	MPF	17.76768	105.3445	625
	13 December 2012	MPF	17.75742	105.3337	564
	18 December 2012 16 February 2013	MPF	17.97563	105.4017	626
	16 February 2013	MPF	18.00798	105.4570	579
	18 December 2012	MPF	18.01137	105.4497	621
	20 December 2012	MPF	18.02897	105.4685	598
	12 February 2013	MPF	18.05362	105.4664	651
	13 February 2013	MPF	18.06337	105.4557	690
	14 February 2013	MPF	18.05567	105.4674	656
PHP NP area	5-7 March 2014	VSF	17.967977	104.819818	178
	5-7 March 2014	VSF	17.981883	104.829076	203
WMPA area	30 March 2014	VSF	17.847732	105.367612	597
	1 April 2014	VSF	17.850140	105.378981	576
	31 March 2014	MPF	17.827494	105.370127	676
WMPA area	3–5 April 2014	MPF	17.889201	105.396963	687

*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.

Localities/Hosts	Adults				Nymphs		Larvae		Total	
Genus/Species	Male		Female							
	No	%	No	%	No	%	No	%	No	%
WMPA area										
Vegetation										
Amblyomma sp.	0	-	0	-	1,106	7.34	1,507	10	2,613	17.34
Amblyomma testudinarium	4	0.03	2	0.01	5	0.03	37	0.25	48	0.32
Dermacentor auratus	11	0.07	6	0.04	0	-	0	-	17	0.11
Dermacentor spp.	0	-	0	-	101	0.67	277	1.84	378	2.51
Dermacentor steini	2	0.01	5	0.03	0	-	0	-	7	0.05
Haemaphysalis colasbelcouri	0	-	1	0.01	0	-	0	-	1	0.01
Haemaphysalis hystricis	25	0.17	31	0.21	0	-	0	-	56	0.37
Haemaphysalis sp., ** aborensis*	24	0.16	67	0.44	0	-	0	-	91	0.6
Haemaphysalis sp., **darjeeling*	1	0.01	4	0.03	0	-	0	-	5	0.03
Haemaphysalis sp., **lagrangei*	0	-	3	0.02	0	-	0	-	3	0.02
Haemaphysalis spp.	7	0.05	9	0.06	3,273	21.71	7,193	47.72	10,482	69.54
Ixodes spp.	0	-	0	-	7	0.05	6	0.04	13	0.09
Total	74	0.49	128	0.85	4,492	29.8	9,020	59.84	13,714	90.98
Cows										
Amblyomma testudinarium	3	0.02	1	0.01	1	0.01	0	-	5	0.03
Haemaphysalis hystricis	0	-	2	0.01	0	-	0	-	2	0.01
Rhipicephalus haemaphysaloides	2	0.01	2	0.01	0	-	0	-	4	0.03
Rhipicephalus (*Boophilus*) *microplus*	0	-	1	0.01	0	-	0	-	1	0.01
Total	5	0.03	6	0.04	1	0.01	0	-	12	0.08
Dogs										
Amblyomma testudinarium	0	-	0	-	1	0.01	0	-	1	0.01
Haemaphysalis hystricis	0	-	1	0.01	0	-	0	-	1	0.01
Rhipicephalus haemaphysaloides	0	-	1	0.01	0	-	0	-	1	0.01
Total	0	-	2	0.01	1	0.01	0	-	3	0.02
Total	79	0.52	136	0.9	4,494	29.81	9,020	59.84	13,729	91.08
PHP NP area										
Vegetation										
Amblyomma spp.	0	-	0	-	891	5.91	0	-	891	5.91
Dermacentor spp.	0	-	0	-	4	0.03	99	0.66	103	0.68
Haemaphysalis spp.	0	-	0	-	317	2.1	33	0.22	350	2.32
Total	0	-	0	-	1,212	8.04	132	0.88	1,344	8.92
Total	79	0.52	136	0.9	5,706	37.86	9,152	60.72	15,073	100

*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 colasbelcouri* (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).

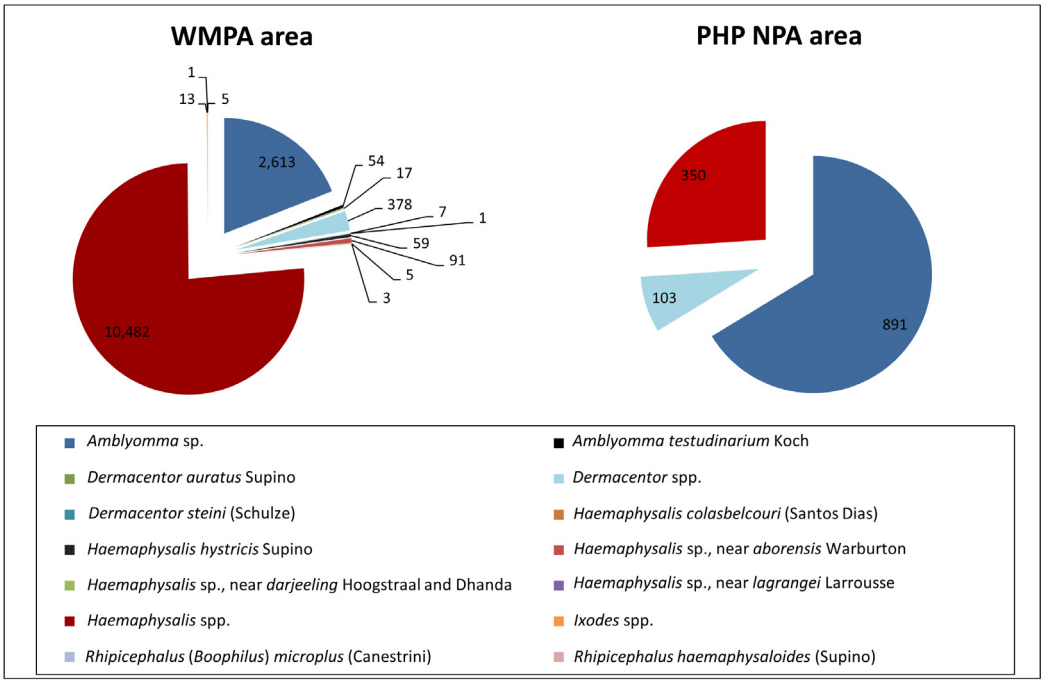

FIGURE 2: Species composition of ticks collected from WMPA area (left hand) and PHP NP area (right hand).

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

Known hosts and localities in Laos: not indicated. The above authors cite a collection of 3 ♂ and 1 ♀ ex *Bungarus fasciatus*, Xieng Khouang, Laos, listed in the Ph.D. dissertation of T.S. Kaufman, 1972.

***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*) *pattoni* (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

****Dermacentor 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.

*****Dermacentor 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

Known hosts and localities in Laos: Phongsaly. Ex *H. hodgsoni subcristata*, Phu Mon, 30 January 1929, Hendee: 19 ♂, 5♀ (HH 41,610).

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

Known hosts and localities in Laos: ex *Centropus bengalensis*. Ban Ton Phung, 26 February 1953, H. G. Deignan; 1 ♂ (HH 44,004) (RML32709).

***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 *Mydaus multiceps*: 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: l 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**

References: Petney *et al.*, 1996a, Robbins *et al.*, 1996

Known hosts and localities in Laos: not indicated. Petney & Keirans, 1996 refer to accession number (RML 37807). Robbins *et al.* ex *Catopuma temminckii*, Lak Sao (18.10N, 104.55E).

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, Tupaiidea 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 darjeeling, 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 *Borrelia* 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 (Hoogstraal *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 spinicoxalis* 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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