

ARTHROPOD SPECIES COMPOSITION OF COMMONLY DISEASE TRANSMISSION WHICH PARASITISED ON RAISING ANIMALS IN THE SOUTH REGION, VIETNAM IN 2016

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Summary

The study was conducted at 8 sites which were representative of geographics, habitats and socio - economics of the South region - Lamdong province from 2015 - 2017 by surveys, sample collection in the field and tests in laboratory. The aim of the study was: to determine the species composition and distribution of ticks parasitized on raising animal (dogs, cats, birds, cows, pigs) and to detect ticks of *rickettsia* infection which parasitized on raising animal. The results showed that: Collected 5,293 ticks individuals including 4 species, 3 genus, 1 family. Among them, Rhipicephalus was 2 species of *Rhipicephalus (Rh.) haemaphysaloides* and *Rhipicephalus (Rh.) sanguineus*, Boophilus was 1 species of *Boophilus microplus* and Ixodes was 1 species of *Ixodes (I.) granulatus*. A total of 293 ticks samples (1,460 individual) were tested by PCR. The overall, common *Rickettsia* spp. infection rate was 1.02% (3/293 samples). All these 3 tick samples are parasitized on domesticated dogs (*Canis familiaris*). In particular, the *Rickettsia* spp. infection rate in tick species of *Rh (Rh.) sanguineus* was 1.71% (3/175 samples). Don't detected pathogen of *Orientia tsutsugamushi* in ticks. Tinhbien district (Angiang 2015) and Longthanh district (Dongnai 2015, 2016) Where have tick of *Rickettsia* spp. infection.

Key word: Ticks, raising animals, the South region - Lamdong province.

INTRODUCTION

Medical arthropods also known as vectors borne disease, they are found in almost every continents in the world. Depending on the geographical location, there are different infectious pathogens that have different vectors borne disease. Medical arthropods can live and grow (parasitized) on different host species. Of which, arthropod species parasitized on raising animals which are particularly important role in disease transmission from animal-to-human transmission. In our country, up to now,

investigations have shown that ticks, chigger mites, gamasid mites and fleas which are relatively common arthropods in many regions^[1]. In which, the fleas transmitted plague disease which had been studied extensively, from 2003 to now the plague disease case don't reported.

Ticks (Ixodoidea) is one of the common arthropods which parasitized on raising animals such as dogs, cows, buffaloes, cats, birds, goats, sheep... In humans, ticks can cause many serious harms such as paralysis, poisoning, irritation and allergies. Besides they transmit some infectious diseases that are considered to be a major problem in public health. Ticks are recognized as the main vectors and reservoirs of spotted fever group rickettsiae. Tick - borne rickettsioses are caused by obligate intracellular bacteria belonging to the spotted fever group of the genus *Rickettsia*. These zoonoses are among the oldest known

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vector-borne diseases, and include Mediterranean spotted fever, which is caused by *Rickettsia conorii* and transmitted by the brown dog tick *Rhipicephalus sanguineus*. Additionally they include most of the emerging tick - borne diseases such as the infection caused by *R. slovaca* which is transmitted by *Dermacentor* spp.^[2]. In Vietnam, the study of ticks was also of interest, but there is no study to determination of pathogens in ticks.

Research by the Institute of Malariology - Parasitology - Entomology in Ho Chi Minh City 2015 to 2017 for the purpose of assessing the species composition and distribution of ticks on raising animals in the Southern region - Lamdong province, determining the presence of pathogens for human which are *Oriental tsutsugamushi*; *Rickettsia* spp. on tick parasitized on raising animals.

SUBJECTS AND METHODS

Methods. Time study: From 2015 to 2017 (Collected ticks in rainy season 2015 and dry season 2016). Location of study: In the field of Ninhkieu district (Cantho City), Condao island (Baria - Vungtau province), Lanbiang (Lamdong province), Longthanh district (Dongmai province), Phuquoc island (Kiengiang province), Thanhphu district (Bentre province), Thudaumot town (Binhduong province), Tinhbien district (Angiang province). Labo: Department of Ectoparasitology and Department of Culture - Immunology (Institute of Malariology - Parasitology - Entomology - HCMC).

Research subjects: Ticks (Ixodoidea). Hosts of ticks (Ixodoidea) which are raising animal at survey sites. Rickettsiaceae bacteria.

Selection of sample and sample size

The sample size for ticks species composition and distribution survey which were randomly conducted in the field, collecting all ticks samples on raising animals at the survey sites.

The sample size for determining the prevalence of *rickettsia* infection in ticks which were made randomly on all ticks that collected from the field. Tick individuals of the same species, parasitized on the same host or the same host group which were put on a sample, each sample has 1 - 5 tick individuals.

Techniques used in research: Ticks were collected from Raising animal (dogs, cows, cats, chickens, birds,

pigs), Examine carefully the thin skin areas such as the groin, leg, anus, ear, nose, eyelid, crest of the raising animals for take ticks. Collected ticks were placed into plastic vials containing absolute ethanol and taken to the laboratory, where they were identified based on morphologic criteria following taxonomic keys (Phan Trong Cung, 2001)^[3].

For the molecular detection and analysis of *Rickettsia* species, We pooled 1 - 5 ticks of the same species collected from one host raising animal or one host raising group for DNA extraction, DNA was extracted by the ISO-LATE II Genomic DNA Kit (Bioline, South Korea) according to the manufacturer's instructions. Multiplex PCR reactions were performed with total reaction volume was 50µl: 25µl 2x MyTaq HS Mix 2x (Bioline); 2.0µl Multiplex Primer 25 nmol, to detect *Rickettsia* spp. and *Oriental tsutsugamushi*; 20µl distilled water; 3µl of mold DNA. PCR cycle: 94°C for 5 minutes, followed by 45 cycles: 94°C for 30 seconds, 56°C for 30 seconds and 72°C for 45 seconds and ended at 72°C for 7 minutes (Applied Biosystem 2720 Thermocycle, America).

Table 1. Primers for groEL gene of Rickettsiaceae

No	Primer	Sequencing primers	Size	Rickettsiaceae gene segments: groEL
1	SF1 SR2	GATAGAAGAAAAGCAATGATG CAGCTATTTGAGATTTAATTTG	229bp	<i>Rickettsia</i> spp.
2	TF1 TR2	ATATATCACAGTACTTTGCAAC GTTCCCTAACTTAGATGTATCAT	366bp	<i>Oriental tsutsugamushi</i>

PCR products were detected by DNA electrophoresis with 2% agarose in TBE containing ethidium bromide for 30 minutes at 120 volts and bacteria are identified based on PCR product size on.

Research morality: The study was conducted on host species of ticks that did not cause any harms to humans. In addition, the study also discovered host species containing the vector-borne disease for humans and pathogens presenting in these ticks based on which recommendations could be made for local preventive centers.

RESULTS

Species and individuals of raising animals collected at survey sites in the southern region - Lamdong province 2015 - 2016

The total species and individuals of raising animals at surveys were tested for which collected tick samples at 8

survey sites that were 1,013 individuals of 6 species groups. There were 491 dog individuals, 195 cow individuals, 34 cat individuals, 182 chicken individuals, 75 bird individuals and 36 pig animals (Table 2).

Table 2. Individuals of raising animal collected at survey site (2015 - 2016)

Survey sites	Individuals of raising animals						Total
	Dog	Cow	Cat	Chicken	Bird	Pig	
Langbiang	62	26	3	31	3	5	130
Thudaumot	65	0	5	13	5	0	88
Longthanh	51	35	4	11	8	2	111
Ninhkieu	86	0	6	27	34	8	161
Thanhphu	78	52	10	33	8	5	186
Tinhbien	62	41	6	19	19	6	134
Condao	53	25	0	24	15	5	122
Phuquoc	34	16	0	24	2	5	81
Total	491	195	34	182	75	36	1.013

At the survey sites, the individuals of raising animals collected in Thanh Phu district which was the highest (186 individuals), following, Ninh Kieu district with 161 individuals, Tinh Bien district with 134 individuals, Lanbiang with 130 individuals, Con Dao island with 122 individuals, Long Thanh district with 111 individuals, Thu Dau Mot town with 88 individuals, Phu Quoc island was the lowest (only 81 individuals). Of 1,013 raising animals collected in the study area, dog was the highest individuals (491 individuals) and cat, pig were the lowest (34 individuals cat and 36 individuals pig).

Species composition and distribution of ticks on raising animals at survey sites in the Southern region - Lamdong province 2015 - 2016

Species composition and individuals of ticks on raising animals

Collected 5,293 ticks individuals of 4 species, 3 genus, 1 families. In which Rhipicephalus was 2 species: *Rhipicephalus (Rhipicephalus) haemaphysaloides*, *Rhipicephalus (Rh.) sanguineus*; Boophilus was 1 species: *Boophilus microplus* and Ixodes was 1 species: *Ixodes (Ixodes) granulatus*

Table 3. Species composition and individuals of tick (Ixodoidea) collected

No	Tick species	Individuals
	Ixodidae Muray, 1877	
1	<i>Boophilus microplus</i> Canestrini, 1887	1.288
2	<i>Ixodes (Ixodes) granulatus</i> Supino, 1897	1
3	<i>Rhipicephalus (Rhipicephalus) haemaphysaloides</i> Supino, 1897	511
4	<i>Rhipicephalus (Rh.) sanguineus</i> Latreille, 1804	3.493
Totals		5.293

The total of tick individuals collected at 8 survey sites which were 5,293. In which, *Rhipicephalus (Rh.) sanguineus* was the highest (3,493 individuals), following *Boophilus microplus* was 1,288 individuals, *Rhipicephalus (Rh.) haemaphysaloides* was 511 individuals and was the lowest (only 1 individuals) (Table 2).

Distribution of ticks on raising animals at the survey sites

Investigated 6 groups of raising animals at 8 sites in the Southern region - Lamdong province. The results showed that, ticks parasitized on groups of raising animals the following:

Table 4. Distribution of ticks on raising animals at southern region - Lamdong province

No	Species of tick	Species of raising animals
1	<i>B. microplus</i>	Cow (<i>Bos</i> sp.)
2	<i>I. (I.) granulatus</i>	Dog (<i>Canis familiaris</i>)
3	<i>Rh. (Rh.) haemaphysaloides</i>	Dog (<i>Canis familiaris</i>)
4	<i>Rh. (Rh.) sanguineus</i>	Dog (<i>Canis familiaris</i>)

Boophilus microplus parasitized on cow (*Bos* sp.). *Rhipicephalus (Rh.) haemaphysaloides*, *Rhipicephalus (Rh.) sanguineus*, and *Ixodes (Ixodes) granulatus* parasitized on dogs (*Canis familiaris*) (Table 4).

In the two survey times on the rainy season (2015) and the dry season (2016) in 8 sites in the Southern region - Lamdong province, we determined the distribution of ticks on raising animals in the survey sites as Fig 1.

Two tick species parasitized on dogs were *Rh. (Rh.) haemaphysaloides* and *Rh. (Rh.) sanguineus* which were distributed in all survey sites; *B. microplus* parasite mainly cows which was distributed in 7 survey sites and *I. (I.) granulatus* was distributed only in one survey site (Phuquoc island). Phuquoc island had the highest tick

species composition (4 species), following, Tinhbien district (3 species), Condao island (3 species), Langbiang (3 species), Longthanh (3 species), Thanhphu (3 species), Ninhkieu district (2 species) and Thudaumot town (2 species).

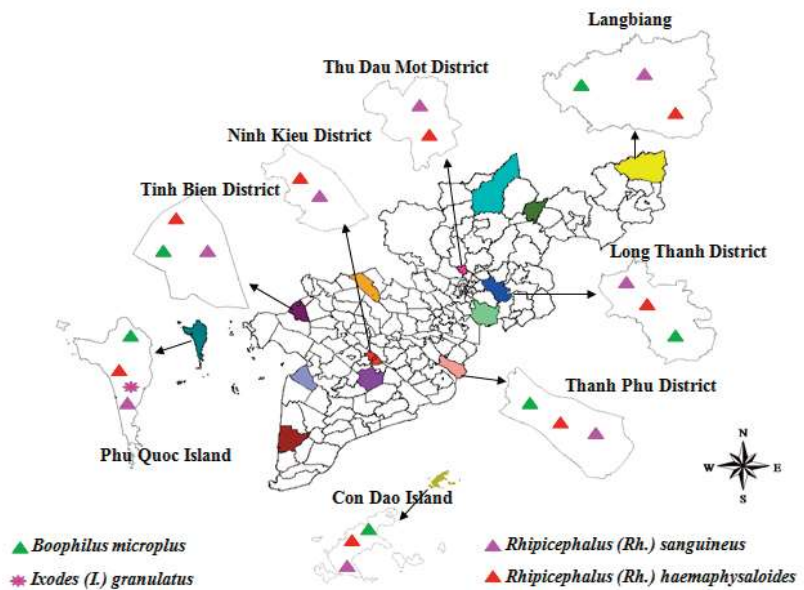


Fig 1. Distribution diagram of ticks parasitized on raising animals at 8 survey sites in southern region - Lamdong province (2015 - 2016)

Pathogen present on ticks parasitized on raising animals in the southern region - Lamdong provice 2015 - 2016

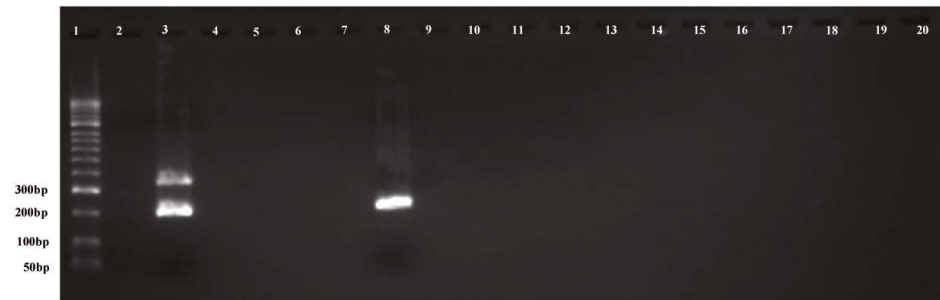


Fig 2. The results of the PCR detected DNA of *Rickettsia* spp.

Note: Well 1: DNA bands (50 bp); well 2: negative; well 3: positive of *Rickettsia* spp. (299 bp) and *O. tsutsugamushi* (366 bp); Wells 4 - 20: tick samples; Well 8: tick sample was positive with DNA of *Rickettsia* spp. (299 bp).

Table 5. Analysis results of rickettsiaceae on ticks by PCR technique

No	Species of tick	Samples (individuals)	Sample and rate (%) (+) with <i>Rickettsia</i> spp.	Sample and rate (%) (+) with <i>O. tsutsugamushi</i>
1	<i>B. microplus</i>	78 (390)	0	0
2	<i>I. (I.) granulatus</i>	1 (1)	0	0
3	<i>Rh. (Rh.) haemaphysaloides</i>	39 (191)	0	0
4	<i>Rh. (Rh.) sanguineus</i>	175 (878)	3 (1,71)	0
Totals		293 (1.460)	3 (1,02)	0

Analysis of 293 ticks sample (each sample contains 1 - 5 individuals) to determine the presence of rickettsiaceae, resulted in the following (table 5): ticks collected on raising animals in the southern region, Lamdong province which was infection rate of *Rickettsia* spp. 1.02 %. Of which only 3/175 samples of *Rh. (Rh.) sanguineus* infected *Rickettsia* spp. with infection rate 1.71%. there was no positive sample for *Orientia tsutsugamushi*.

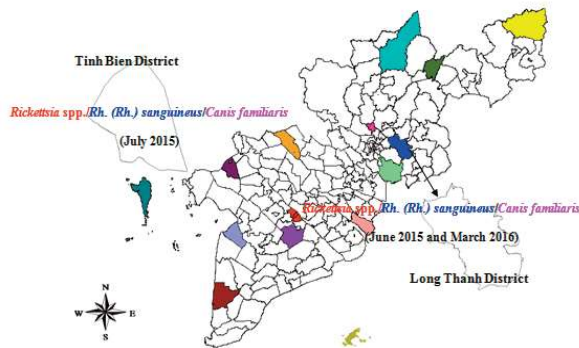


Fig 3. Distribution diagrams of *Rickettsia* spp. on ticks parasitized on raising animals in the southern region, Lamdong province

At 8 survey sites (Figure 3), three positive ticks sample with DNA of *Rickettsia* spp. Which were *Rh. (Rh.) sanguineus*. They were found at two sites: Tinh Bien district, An Giang province in July 2015 and Long Thanh district, Dong Nai province in June 2015 and March 2016. These 3 positive ticks sample which are parasitized on domesticated dogs (*Canis familiaris*).

DISCUSSION

We have collected 1,013 individuals of raising animal. Of which, there were 491 dog individuals, 195 cow individuals, 34 cat individuals, 185 bird individuals, 75 bird individuals and 36 pig individuals. And collected 5,329 tick individuals (Ixodoidea), analyzed 304 tick samples (1,496 individuals) by PCR. From the results of the above analysis, we have determined the species composition, distribution of ticks parasitized on raising animals as well as the infection status of rickettsiaceae on ticks in the Southern region, Lamdong province.

The tick species composition parasitized on raising animals in the Southern region, Lamdong province 2015 - 2016

Tick species composition were collected from raising

animals in 2015 - 2016 in southern region, Lamdong province which are higher than tick species composition in other areas such as Quangbinh and Hatinh (Nguyen Van Chau, 2005) collected 3 ticks species^[4]. Meanwhile, Quangnam and Kontum region (Nguyen Van Chau, 2004) collected 4 tick species^[5]. Cattien National Park (Ho Dinh Trung, 2006) collected 4 ticks species^[6].

However, ticks species composition in the southern region, Lamdong province which are less than that of the study of Khamsing Vongphayloth *et al.*, (2016), First survey of the hard tick (Acari: Ixodidae) fauna of Nakai District, Khammouane Province, Laos and an updated checklist of the ticks of Laos. As result, 11 species, 5 genera: *Amblyomma testudinarium*, *Dermacentor auratus*, *D. steini*, *Haemaphysalis colasbelcourti*, *H. hystricis*, *Haemaphysalis* sp. (Similar to *H. aborensis*), *Haemaphysalis* sp. (Similar to *H. darjeeling*), *Haemaphysalis* sp. (Similar to *H. lagrangei*), *Haemaphysalis* spp., *Rhipicephalus haemaphysaloides*, *R. (Boophilus) microplus* were collected^[7]; Telleasha L. Gray and *et al.*, (2016), A survey of ticks (Acari: Ixodidae) of companion animals in Australia. A total of 4,765 individual of 11 tick species: *Haemaphysalis triglycerides*, *Haemaphysalis bancrofti*, *Haemaphysalis longicornis*, *Ixodes cornuatus*, *Ixodes myrmecobii*, *Ixodes hirsti*, *Ixodes holocyclus*, *Ixodes tasmani*, *Rhipicephalus australis*, *Rhipicephalus sanguineus*, *Bothriocroton* sp.^[8].

Distribution of ticks parasitized on raising animals in the survey sites in the Southern - Lam Dong province 2015 - 2016

Dog was the main host of *Rh. (Rh.) sanguineus* and *Rh. (Rh.) haemaphysaloides*; Cow was the main host of *B. microplus*. Distribution of ticks (Ixodoidea) parasitized on raising animals at the survey sites: *Rh. (Rh.) haemaphysaloides* and *Rh. (Rh.) sanguineus* parasitized on dogs which distributed in all survey sites; *B. microplus* parasitized on cows which distributed at 6 sites, because the main host of this species is cattle but in Ninhkieu district and Thudaomot town are an urban habitat that does not have buffaloes so it is not possible to find them. *I. (I.) granulatus* was found only in Phuquoc island district (Figure 1).

According to Phan Trong Cung *et al.* (2001)^[3], the distribution of Ixodoidea in Vietnam such as: there were 6 tick genus appearance in all regions (*Amblyomma*, *Aponomma*, *Boophilus*, *Haemaphysalis*, *Ixodes* and *Rhipi-*

cephalus) with varying amounts. Dermacentor genus were not present in delta areas. Hyalomma genus were found only in Central - South and South - West Central. The Argas genus were only found in the northern delta but we discovered *Argas* sp. on the bat at Langsen, Longan province; For Ixodes, there were two species, *Ixodes (I) granulatus* distributed in Quangninh, Thainguyen, Bacgiang, Gialai, Daklak, Binhphuoc, Lamdong and *Ixodes (Af.) pilosus* in Thainguyen^[3]. However, species of *Ixodes (I) granulatus* was found in the Phuquoc Island, Kiengiang province and this tick species parasitized on dogs.

Pathogen present on ticks parasitized on raising animals in the souther region, Lamdong provice 2015 - 2016

A total of 293 tick samples (1,460 individuals) were determined DNA of *Rickettsia* spp. and *O. tsutsugamushi* by PCR. Only three samples were positive for DNA of *Rickettsia* spp. and there wasn't samples which was positive for DNA of *O. tsutsugamushi*. These three tick samples which were *Rhipicephalus sanguineus* species, the remaining tick species were negative. Thus, the ticks samples were positive with *Rickettsia* spp. per tick samples total which were tested in the Southern region, Lamdong province (collected from July - October 2015 and March - April 2016) that was 1.02% and the samples of *Rh. sanguineus* were infection of *Rickettsia* spp. for 1.71%. In three ticks samples for positive with DNA of *Rickettsia* spp., a sample was collected at the Tinhbien in July 2015, two samples at Longthanh district in June 2015 and March 2016. The hosts for the three positive samples with DNA of *Rickettsia* spp. which were dogs (Fig 3). The tick rate of *Rickettsia* spp. infection in this study was much lower than in orther studies. Márquez (2008). The rate of *Rhipicephalus sanguineus* ticks in the South West of Spain which were infected *rickettsia* for 18%^[9]. Fatma Khrouf (2013), Detected *Rickettsia* in *Rh. sanguineus* in south-eastern of Tunisia for 37.4%^[10]. Meng Zhang (2013) Determined the presence of *O. tsutsugamushi*, of which 2/13 tick samples was positive for *O. tsutsugamushi* in Shandong Province, China^[11].

CONCLUSION

In conclusion, the species composition of ticks (Ixodoidea) parasitized on raising animals in the souther region, Lamdong in 2015 - 2016 includes 1 families, 3 genus, 4 species: *Boophilus microplus*, *Ixodes (Ixodes) granulatus*, *Rhipicephalus (Rhipicephalus) haemaphysaloides*, *Rh. (Rh.) sanguineus*. The dominant tick species were *Rh. (Rh.) sanguineus* and *B. microplus*.

Distribution of ticks parasitized on raising animal in southern region, Lamdong province: Ticks were mainly found on dogs and cows. Two ticks species of *Rh. (Rh.) sanguineus*, *Rh. (Rh.) haemaphysaloides* were widely distribution (8/8 survey sites), *B. microplus* were distribution in 6/8 survey sites, *Ixodes (Ixodes) granulatus* was found only in Phuquoc island district.

The samples of ticks infected *Rickettsia* spp. which was 1.02% (3/293 samples), in which the percentage of *Rh. (Rh.) sanguineus* samples infected *Rickettsia* spp. which was 1.71% (3/175 samples). No ticks samples (0/293 samples) were postive with *Orientia tsutsugamushi*.

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