FRANCISELLA-LIKE ENDOSYMBIONT IN A TICK COLLECTED FROM A CHICKEN IN SOUTHERN THAILAND

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Abstract. *Francisella* is a genus of bacterial pathogens potentially lethal to humans. We report here for the first time a novel *Francisella*-like endosymbiont discovered in a hard-tick (*Rhipicephalus sanguineus* s.l.) obtained from a chicken (*Gallus domesticus*) in Thailand. The phylogenetic results indicate the 16S rDNA sequences of this *Francisella* bacterium form a unique clade with the *Francisella*-like endosymbiont of the tick species, *Amblyomma varanense* and *Amblyomma helvolum*, that have previously been found on snakes in Thailand. This species of *Francisella* is in a different group from the other *Francisella*-like endosymbionts previously reported from other countries. No *Francisella* was detected in *Haemaphysalis wellingtoni* ticks obtained from chickens in this study.

Keywords: ticks, *Francisella*-like endosymbiont, chicken, Thailand

INTRODUCTION

Ticks are important vectors of zoonoses that affect human health worldwide and can transmit viruses, bacteria and protozoa. A variety of avian species have been reported to be hosts for ticks: *Amblyomma americanum* and *Ixodes brunneus* have been found on wild turkeys (*Meleagris gallopavo*) (Scott *et al*, 2010), *Ixodes turdus* and *Haemaphysalis flava* have been found mainly on passerine birds (Yamauchi, 2001) and *Ixodes ricinus* has been found on red grouse chicks (Kirby *et al*, 2004) .

Francisella is a genus of bacterial pathogens that can cause potentially fatal disease. *Francisella tularensis* causes tularemia, also known as rabbit fever and deer-fly fever (Francis, 1921). The disease can be transmitted by ticks, biting flies, water, food and aerosols (Tärnvik, 2007). *Francisella novicida* had been isolated from a Thai patient from southern Thailand (Leelaporn *et al*, 2008). In Thailand, *Francisella*-like endosymbionts (FLEs) have been reported from snake ticks (Sumrandee *et al*, 2014).

In southern Thailand, indigenous chickens live in close contact with people.

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The chickens walk around freely and forage on the ground for their natural diet, which consists of worms, insects, seeds, and plants. Pathogen-carrying ticks on chickens can cause health problems for local people. Little is known about the presence of *Francisella* spp in indigenous chickens in southern Thailand. Here, we report a novel *Francisella*-like endosymbiont discovered in *Rhipicephalus sanguineus* s.l. obtained from a chicken (*Gallus domesticus*) in Thailand for the first time.

MATERIALS AND METHODS

Tick collection

Ticks were randomly collected (June 2014) from two indigenous chickens (*Gallus domesticus*) from one suburban house in Surat Thani Province, southern Thailand (9°07′51.2″ N latitude, 99°21′44.0″ E longitude). The ticks were removed from neck and cockscomb of the chickens and kept in 70% ethanol at 4°C before being sent to the Faculty of Science of Mahidol University in Bangkok, Thailand for species identification (Kohls, 1957). Ticks were also collected from a cat body (*Felis catus*) in the same household where the ticks were collected from chickens. No ticks were found in chickens and cats from the other nearby houses.

DNA extraction and *Francisella* detection

The ticks were cleaned before DNA extraction. DNA extraction was performed individually for adult ticks while the nymphs (developmental stage) were pooled, using the QIAamp DNA Extraction Kit for Tissue (Qiagen, Hilden, Germany) following the manufacturer's protocol. The quality of the extracted tick DNA was determined by amplification of the tick mitochondrial (mt) 16S rDNA gene using the primers 16S + 1 and 16S - 1 as previously described (Williams *et al*, 1992). For the detection of *Francisella* 16S rDNA in tick samples, each PCR reaction was carried out using primers and conditions as described previously (Williams *et al*, 1992). The PCR fragment of the positive sample was purified and sequenced.

Phylogenetic analysis

Nucleotide sequences of *Francisella* 16S rDNA (1,054bp) were aligned by the MegAlign tool using DNASTAR® Lasergene software. Neighbour-joining (NJ) was used to generate phylogenetic relationships using PAUP 4.0b10 software. Bootstrap values >50% were indicated above branches (1,000 replicates). *Francisella halioticida* was selected as an outgroup.

RESULTS

Five ticks (adults: three ticks from one chicken and another two ticks from another chicken) and two nymphs (from a cat) were identified to the species or genus levels (only adult tick can be identified to species, while larva and nymph can be identified to the genus level by a tick taxonomist): *Rhipicephalus sanguineus* s.l. (two ticks, both female), *Haemaphysalis wellingtoni* (three ticks, all male) and *Haemaphysalis* spp (two nymphs). Both *R. sanguineus* s.l. specimens and one *H. wellingtoni* were obtained from one chicken. Another two *H. wellingtoni* ticks were collected from a second chicken. Two *Haemaphysalis* nymphs were collected from a cat from the same household, as well. All ticks were positive for the primers 16S + 1 and 16S - 1.

For *Francisella* detection, DNA samples were checked for the presence of *Francisella* 16S rDNA sequences. Positive results were obtained in one out of the five ticks collected from the two chickens; the positive result was obtained for a *R.*

sanguineus s.l. female. The other ticks (*H. wellingtoni*) collected from the same chicken were negative for *Francisella* 16S rDNA. The *Haemaphysalis* nymphs collected from the cat (*Felis catus*) in the same household were also negative for *Francisella*.

The *Francisella* 16S rDNA sequences obtained from *R. sanguineus* s.l. (SRT 95) were submitted to GenBank (accession number KP659194). This sequence showed the highest sequence identity (1052/1056, 99.62%) with a *Francisella* endosymbiont, *Amblyomma varanense* clone APOH2 16S ribosomal RNA gene, partial sequence (accession number KF268342), from a snake in Thailand. Using Blast search results, this *Francisella* 16S rDNA sequence was also 98.77% (1043/1056) identical to *Dermacentor auratus* clone SSPG3 16S ribosomal RNA gene, partial sequence (JQ764629) and 98.68% (1043/1057) identical to *Ornithodoros moubata* symbiote B gene for a 16S rRNA partial sequence (AB001522).

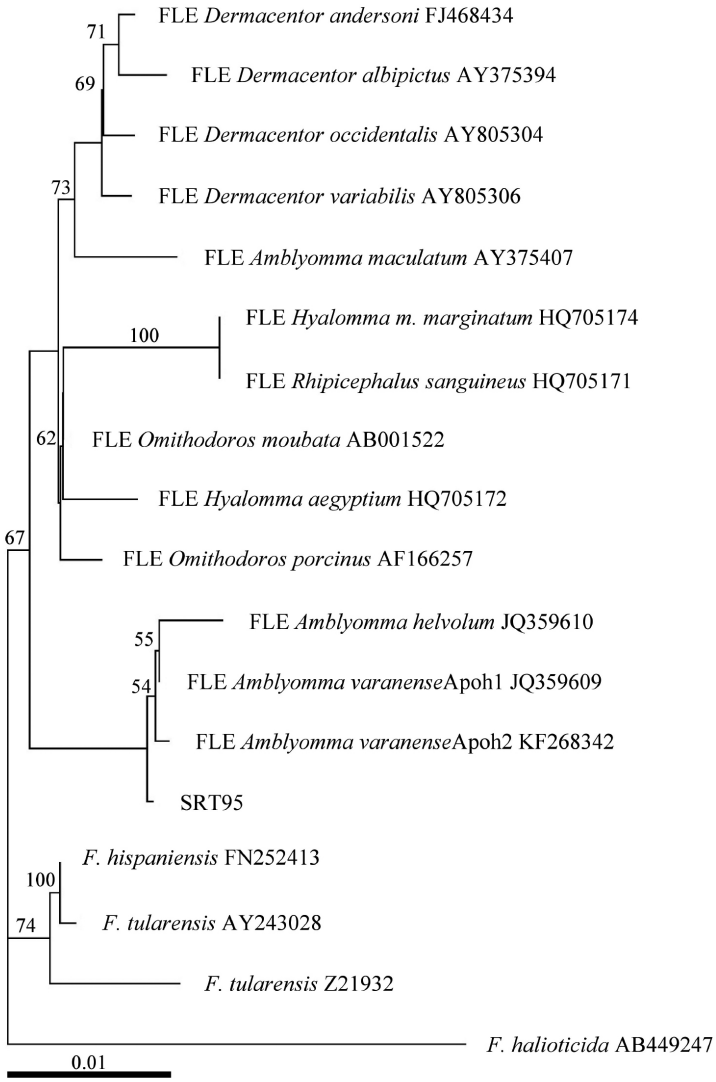

Fig 1–Neighbour-joining phylogenetic analysis of a *Francisella* 16S rDNA sequence obtained from a *Rhipicephalus sanguineus* s.l. specimens obtained from a chicken. *Francisella halioticida* was selected as an outgroup.

The phylogenetic analysis results indicate the 16S rDNA sequences from the *Francisella* infecting the *R. sanguineus* s.l. taken from *G. domesticus* represent a unique clade within the *Francisella* endosymbiont of *A. varanense* and the *Francisella* endosymbiont of *A. helvolum* and are evolutionarily closely related to this group (Fig 1). This type of *Francisella* forms a different clade than *F. tularensis* strains (pathogenic bacteria).

DISCUSSION

FLE emerged from an infective ancestral organism (Scoles, 2004). FLE have

a worldwide distribution in both hard and soft ticks (Szigeti *et al*, 2014), namely, in the genera *Ixodes*, *Amblyomma*, *Dermacentor*, and *Ornithodoros* (Scoles, 2004). Ivanov *et al* (2011) reported detecting FLE in *R. sanguineus* s.l., *R. bursa, R. turanicus*, *Dermacentor marginatus, D. reticulatus*, *I. ricinus*, *Hyalomma marginatum,* and *H. aegyptium* ticks. The effect of FLE on vector competency and for the transmission of *F. tularensis* by ticks remains unclear. Characterization of these organisms has largely been limited to PCR-based methods because these bacteria are not readily culturable on microbiological agar (Tärnvik, 1989). In this study, a novel FLE closely related to those found in *A. varanense* and *A. helvolum* collected from snakes was discovered in a hard-tick (*R. sanguineus* s.l.) from a chicken (*G. domesticus*) in Thailand. A previous phylogenetic study found several FLE form a monophyletic clade closely related to pathogenic *Francisella* species transmitted by ticks (Scoles, 2004). However, in this study, we found a FLE in *R. sanguineus* s.l. which was distinct from other FLE previously reported. This species of *Francisella* is in a different group of FLE from other tick genera and forms a different clade of FLE found in *R. sanguineus* s.l. HQ705171 reported previously (Ivanov *et al*, 2011). Our findings suggest we found a novel strain of FLE infecting *R. sanguineus* s.l. in southern Thailand reported here for the first time. The pathogenicity to mammals of this FLE detected is unclear; futher studies are needed to determine this.

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