



# Identification of a new type of babesia species in wild rats (*bandicota indica*) in Chiang Mai province, Thailand

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【 84 】

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【 学位論文題目 】

Identification of a New Type of Babesia Species in  
Wild Rats ( *Bandicota indica* ) in Chiang Mai  
Province, Thailand  
(タイ、チェンマイプロビンスの野ネズミに確認された  
新しいタイプのバベシア)

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## INTRODUCTION

Babesiosis is a typical zoonotic disease that is caused by *Babesia* spp., tick-borne intraerythrocytic protozoan parasites. Only a few of the more than 100 *Babesia* spp. have been known to infect human. In Europe, a small number of human babesiosis cases have been attributed to *B. divergens*, the cattle babesia, while hundreds of human babesiosis cases in the northeast and midwest United States have been caused by *B. microti*, the rodent babesia. Newly emerging species, referred to as WA1 and CA1, have been found to cause human infection in the western United States. Recently sporadic cases of human babesiosis by *B. microti* have been consecutively reported in Taiwan and Japan, where *B. microti*-like parasites had earlier been identified in wild rodents, *Bandicota indica* and *Rattus coxinga* in Taiwan, and *Apodemus speciosus* and *Apodemus argenteus* in Japan.

Human babesiosis has sometimes been diagnosed initially as malaria because of the similarity between the two diseases or the two parasites. Thus, it is likely that cases of human babesiosis in countries to which malaria is endemic have been overlooked or misdiagnosed as malaria. Apart from Taiwan and Japan, little is known about rodent babesias in Asian countries, although *Babesia* infection is indeed common in other animals. Accordingly, this study examined babesial infection in wild rats in Thai areas where malaria is endemic in order to evaluate the possibility of the emergence or latent existence of human babesiosis.

## MATERIALS AND METHODS

Rats were trapped from three areas of Chiang Mai Province, i.e., Muang District, San Sai District and Doi Lo Sub-district, and from one area of Mae Hong Son Province, i.e., Mae Sariang District during April 2000 to May 2002. The species of captured rats and of ticks infesting them were identified according to the key characteristics described by Lekagul and Jeffrey (1988) and by Tanskul and Inlao (1989), respectively. Intraerythrocytic parasites were microscopically examined for approximately 100 oil power fields on Giemsa-stained thin blood smears. A number of intraerythrocytic parasite-positive rats were killed and their heparinized heart blood was collected for DNA preparation.

The small subunit ribosomal RNA gene (SSUrDNA) fragments of the parasites, which were amplified from the blood by PCR with a primer set of AnI and

BnI based on the highly conserved regions of SSUrDNA sequences of eukaryotes, were ligated into a plasmid and sequenced. The sequences were confirmed not to contain any sequence errors due to *Taq* polymerase by directly sequencing the PCR products with two pairs of primers: AnI-CR1 and CF1-BnI. The CR1 and CF1 primers were designed to match the regions of SSUrDNA conserved in various *Babesia* spp. but not in rodent SSUrDNA sequences. Almost full length of SSUrDNA sequence was obtained and the phylogenetic analysis based on the sequences was performed by the neighbor-joining and maximum-likelihood methods.

## RESULTS

A total of 47 wild rats comprising 30 *Bandicota indica* and 17 *Rattus exulans* were trapped in this study. By microscopic observation, *Babesia* sp. was identified in 17 of 30 *Bandicota indica* (parasitemia 0.03-11.02%), but not in any of 17 *R. exulans*. *Babesia*-positive *Bandicota indica* were captured at all of three areas in Chiang Mai but not in Mae Hong Son Province. Twelve of 17 *Babesia*-positive *Bandicota indica* were infested with *Haemaphysalis doenitzii* ticks. No other species of ticks were identified on any of captured wild rats.

On Giemsa-stained thin blood smears, Thai babesial parasites appeared pleomorphic. Ring-shaped trophozoites resembling ringforms of *Plasmodium falciparum*, pyriform-shaped trophozoites, annular trophozoites and irregularly large trophozoites were often observed. Four trophozoites, so-called "Maltese cross", were occasionally but not typically seen. Double infection and multiple infection were frequently observed on the smear of higher parasitemia. In general, the Thai babesial parasites appeared morphologically similar to *B. microti*.

A 1,704-bp SSUrDNA sequence of a Thai *Babesia* isolate from a positive rat trapped at Doi Lo in 2000 (the BiCM002 isolate) was determined. It was completely identical to that of the other five *Babesia* isolates, regardless of the capture site or year. The sequence of SSUrDNA for *Babesia* sp. from Thai *Bandicota indica* has been submitted to DDBJ under accession no. AB053216.

The best phylogenetic tree by maximum-likelihood analysis for 39 SSUrDNA sequences selected based on the results of a FASTA similarity search was obtained. Four monophyletic groups (A to D) were constructed. Group A contained large babesias (*Babesia sensu stricto*); group B contained *Theileria* spp.; group C contained

piroplasms isolated from humans (WA1 and CA1) and from canines (*Babesia gibsoni*) in the western United States; and group D contained *B. microti* and related small babesias. Within group A, three monophyletic subgroups of A1 (*B. canis* and *Babesia* sp. from an Okinawa dog), A2 (*B. gibsoni* [Asia 1 and 2]), and A3 (several ungulate babesias) were reconstructed. The *Babesia* sp. from Thai *Bandicota indica* was clustered with subgroup A1. The neighbor-joining tree also reconstructed the monophyly of each of the four groups (A to D), positioning the Thai *Babesia* sp. as the sister clade to subgroup A1.

## DISCUSSION

Babesiosis as well as malaria are caused by the intraerythrocytic protozoan parasites of the genus *Babesia* and *Plasmodium*, respectively. There appear to be many similarities between manifestations of human babesiosis and malaria, including the morphological features of the parasites on the blood smear and clinical symptoms such as fever and hemolytic anemia. Trophozoites of *Babesia* spp. always lack the pigment. These morphological features are believed to distinguish *Babesia* spp. from *Plasmodium* spp. However, only ring-shaped trophozoites were often seen on smears of *B. microti*-infected animals especially with low parasitemia. The ring-shaped trophozoites of *B. microti* were quite similar to ring forms of *P. falciparum* that does not show any pigment at the ring form-stage. Thus, babesiosis could be misdiagnosed as falciparum malaria. Moreover, most antimalarial drugs have no effects on babesiosis. Therefore, babesiosis in malaria endemic areas might be misdiagnosed as drug-resistant malaria. In this context, the identification of babesial parasites in wild rats in Chiang Mai, Thailand, where malaria is endemic, seems to deserve attention, considering recent Asian cases of human babesiosis emerged in Taiwan and Japan, where *B. microti*-like parasites had earlier been identified in wild rodents.

*Babesia* is grouped informally into the small babesias (*Babesia* sensu lato) and the large babesias (*Babesia* sensu stricto). This morphological classification is generally consistent with the molecular phylogenetic classification based on SSUrDNA sequences, which discriminates the small and large babesias. Although the Thai babesia in *Bandicota indica* appeared morphologically quite similar to *B. microti*, a small rodent babesia, it was shown to be closest to *Babesia canis*, a large canine babesia, by phylogenetic analysis based on the SSUrDNA sequences. However, the

Thai babesia may be different from *Babesia muris*, a large rodent babesia identified in a white rat, *Rattus norvegicus*, because *Babesia muris* was reported to show typical morphological features of the large babesias. The SSUrDNA sequence of *Babesia muris* has not been studied.

It was interesting that the Thai babesia was identified in *Bandicota indica*, one of the rat species in which *Babesia*-like parasite was previously found in Taiwan, where a case of human babesiosis emerged. The morphological features of the Thai and Taiwanese babesias in *Bandicota indica* were quite similar to each other. Although the babesia in *Rattus coxinga* was suspected to be an etiological parasite in the Taiwanese case of human babesiosis, the relationship between the two Taiwanese rodent babesias in *Bandicota indica* and *R. coxinga* and between Thai and Taiwanese rodent babesias, at present, remain unclear.

Only *H. doenitzi* ticks, which sometimes infest human, were identified on *Babesia*-positive *Bandicota indica*, so this species seemed to be the most probable vector tick of the Thai babesia in *Bandicota indica*. Although at present it is unknown whether the Thai babesia is infectious in humans, a close watch should be kept on human babesiosis in Thailand.

論文審査の結果の要旨			
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論文題目	Identification of a New Type of <i>Babesia</i> Species in Wild Rats ( <i>Bandicota indica</i> ) in Chiang Mai Province, Thailand. タイ、チェンマイプロビンスの野ネズミに確認された新しいタイプのバベシア		
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緒言

バベシア症は、マダニ媒介性赤血球内寄生原虫であるバベシア属原虫による人畜共通感染症である。ヒトに感染を引き起こすのは数種に限られているが、*Babesia microti* (ネズミ寄生種) によるものが最も多い。ヒトバベシア症例は長らくヨーロッパ、アメリカ合衆国一部地域に限局して発生していたが、1990 年後半に、台湾、日本で単発例が相次いで報告された。いずれも *B. microti* または *B. microti* 様原虫によるものと考えられた。先立って、台湾、日本では野ネズミに *B. microti* 様原虫の寄生が報告されていた。アジアの他地域からは、確実なヒトバベシア症例報告はない。しかし、ヒトバベシア症はマラリアと酷似しており、鑑別が困難である。従って、マラリア流行地では、ヒトバベシア症がマラリアと診断され看過されている可能性がある。ところが、これまでに、アジアの他地域から、ヒトバベシア症を最もよく引き起こす *B. microti* または *B. microti* 様原虫についての調査報告はほとんどなかった。

そこで、本研究では、タイでのヒトバベシア症の発生、潜在の可能性を検討するため、タイのマラリア流行地を調査地として、ネズミのバベシア感染状況について調査した。

方法

2000 年～2002 年に、タイ、チェンマイ/メホソンプロビンスの数地域で野ネズミを捕獲した。血液塗抹標本（ギムザ染色）の顕微鏡観察で、赤血球内原虫の有無を調べた。赤血球内原虫陽性ネズミについては、血液 DNA を抽出、真核生物の small subunit ribosomal RNA 遺伝子(SSUrDNA)に共通の塩基配列に基づく primers を用い、原虫のほぼ全長 SSUrDNA を PCR 増幅し、塩基配列を決定した。得られた塩基配列を基に、近隣結合法、最尤法で分子系統樹解析を行った。

結果と考察

*Bandicota indica* 30 匹、*Rattus exulans* 17 匹、計 47 匹の野ネズミが捕獲され、*Bandicota indica* 17 匹にバベシア様赤血球内原虫を認めた。陽性ネズミ 12 匹にのみマダニ

*Haemaphysalis doenitzi* が外寄生していた。

血液塗抹標本上、原虫は非常に多型的であり、典型的な“Maltese cross”は認められなかったが、形態学的に *B. microti* との鑑別は難しかった。

分子系統樹解析で、本原虫は *B. microti* (小型バベシアの代表) と全く異なるグループに属し、*B. canis* (イヌ寄生種; 代表的な大型バベシア) に最も近縁の寄生種であることが示された。

本調査で、タイの野ネズミにバベシア寄生が初めて示された。このバベシア原虫は形態学的には *B. microti* と区別できないが、分子系統樹解析で大型バベシアに属することが示され、新しいタイプのネズミバベシアと考えられた。

ヒトバベシア症発生国台湾で、同じ *Bandicota indica* にバベシア寄生が報告されている。今後、タイの *Bandicota indica* に寄生するバベシア原虫と、台湾の *Bandicota indica* に寄生するバベシア原虫との異同を明らかにし、ヒトへの感染性について検討する必要があるが、現時点では、タイにおいても、ヒトバベシア症の発生の可能性を念頭に、日常診療に臨む必要があると考える。

以上、本研究は、バベシア原虫とバベシア症について調べたものであるが、従来ほとんど解析されていなかった東南アジア地域のバベシア原虫を詳細に調べて、タイの野ネズミに新しいタイプのバベシア原虫が寄生していることを初めて示し、ヒトバベシア症の発生、潜在の可能性を示唆する新知見を得たものとして、価値ある集積であると認める。よって、本研究は、博士(医学)の学位を得る資格があると認める。