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February 2016
Master's Degree Thesis

# First Detection and Identification of *Anaplasma phagocytophilum* from Mites and *Candidatus* Neoehrlichia from Striped Field Mouse in Korea

**Graduate School of Chosun University**

**Department of Medicine**

**Piyush Jha**

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# 한국의 등줄쥐와 털진드기에서 *Candidatus* Neoehrlichia 와 *Anaplasma phagocytophilum* 의 최초 검출과 동정

**February, 2016**
**Graduate School of Chosun University**
**Department of Internal Medicine**

**Piyush Jha**

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# First Detection and Identification of *Anaplasma phagocytophilum* from Mites and *Candidatus* Neoehrlichia from Striped Field Mouse in Korea

**Advisor: Prof. Dong-Min Kim, MD, PhD**

THESIS SUBMITTED TO THE DEPARTMENT OF MEDICINE, SCHOOL OF MEDICINE, CHOSUN UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF MEDICINE

October, 2015

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# ABBREVIATIONS AND SYMBOLS

| | |
|---|---|
| A | adenine |
| *ankA* | ankyrin-repeat protein |
| *16S rRNA* | 16S ribosomal RNA gene |
| BLAST | basic local alignment search tool |
| bp | base pairs |
| C.I. | confidence interval |
| dNTPs | deoxyribonucleic triphosphates |
| e.g. | example given |
| ELISA | enzyme linked immunosorbent assay |
| G | guanine |
| HGE | human granulocytic ehrlichiosis |
| *groEL* | heat-shock protein operon gene |
| Kb | kilobase (one thousand bases) |
| kDa | kilodalton |
| $km^2$ | kilometre squared |
| L(K) | likelihood distribution; estimates number of clusters in STRUCTURE |
| Mb | megabase pairs |



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| | |
|---|---|
| MLG (s) | multi-locus genotype (s) |
| MSPs | *A. marginale* major surface proteins o |
| OR | odds ratio |
| p p value: | type I error (probability of wrongly rejecting null hypothesis) |
| PBS | phosphate buffered saline |
| PCA | principal component analysis |
| PCR | polymerase chain reaction |
| PCR-RFLP PCR – | restriction fragment length polymorphism |
| PCV | packed cell volume |
| PRA | polymerase chain reaction - restriction analysis |
| qPCR | quantitative polymerase chain reaction |
| RAPD | random amplification of polymorphic DNA |
| ROK | Republic of Korea |
| rRNA | ribosomal ribonucleic acid |
| spp | species |
| SPSS | statistical package for social sciences |
| T | thymine |



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| | |
|---|---|
| Taq | Thermus aquaticus polymerase |
| TBDs | tick-borne diseases |
| T-cells | T-lymphocytes |
| Tm | Melting temperature |
| *msp2* | major surface protein 2 gene |
| VD | observed variance of mis-match values (linkage analysis |



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# ABSTRACT

## First Detection and Identification of *Anaplasma phagocytophilum* from Mites and *Candidatus* Neoehrlichia from Striped Field Mouse in Korea

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*Anaplasma phagocytophilum* and *Candidatus* Neoehrlichia are mainly considered to be tick-borne bacterial infections implicated in human health. Only a few studies have reported mites as a possible vector for *A. phagocytophilum*. Rodents are discussed to serve as main reservoir hosts for *C.* Neoehrlichia but not for *A. phagocytophilum* especially in Republic of Korea. Analyzing the prevalence of both pathogens in small mammals and their ectoparasite mites in endemic regions may help to elucidate possible transmission paths in small mammal populations and between small mammals and mites.

In 2014, small mammals were trapped at three different sites in suburban part of Gwangju City which is located in the south-west of Republic of Korea. A total of 810 Trombiculidae mites and 14 *Apodemas agrarius* mice collected were analyzed. DNAs were extracted and used as templates for PCRs targeting fragment genes of *groEL, ankA, msp2* and *16S rRNA* for *Anaplasma/Ehrlichia* detection. The amplified products were sequenced and analyzed.



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In this study, 0.5 % of Trombiculidae mites and 21.4% of the *Apodemus agrarius* mouse samples were positive for *A. phagocytophilum*. Also, 28.57% of the 14 mouse samples were positive for *C.* Neoehrlichia mikurensis. The *groEL* gene fragments amplified in 50% and *ankA* gene fragments amplified in 78.57% of the 14 mouse samples for *A. phagocytophilum* respectively.

The total of 810 mites shows that *Leptotrombidium scutellare* (71.23%) is the most common mite species in this local area followed by *Eushoengastia koreaensis* (10.88%) and *Leptotrombidium orientale* (9.13%) respectively. An interesting co-incidence is that two mite pools which were collected from two different mice shows the presence of *A. phagocytophilum*. This fact suggests that there might be a possibility of mites playing a role to transmit these agents in nature.

The detection of *C.* Neoehrlichia in striped field mouse and presence of *A. phagocytophilum* in Trombiculidae mites as well as in striped field mouse indicates its circulation and the potential risk of contracting a human infection in this country. This is the first detection and identification of *A. phagocytophilum* from mites and *C.* N mikurensis from striped field mouse in Republic of Korea.

**Keywords:** *Anaplasma phagocytophilum*, PCR, *Candidatus* Neoehrlichia, mites, stripped field mouse



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# 한 글 요 약

## 한국의 등줄쥐와 털진드기에서 *Candidatus* Neoehrlichia 와 *Anaplasma phagocytophilum* 의 최초 검출과 동정

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**Background**

*Anaplasma phagocytophilum* 와 *Candidatus* Neoehrlichia 는 인간에서 진드기를 매개로 하는 세균성 (리케챠)질환을 일으키는것으로 알려져 있다. *A. phagocytophilum* 의 매개체는 진드기이며, 털진드기가 매개체인 경우는 거의 보고되지 않았다. 특히 한국에서 *C.* Neoehrlichia 의 경우 주로 설치류가 보균동물이라고 논의되었지만, *A. phagocytophilum* 의 경우는 그렇지 않는다. 발병 지역에서의 작은 포유 동물과 거기에 기생하는 털진드기로 인한 병원균의 유병률을 분석하는 것은, 작은 포유동물과 털진드기 사이의 가능한 감염 전파 경로를 규명하는 데 도움이 될 수 있다.

**Methods**

2014 년, 한국의 남서쪽에 있는 광주광역시 근교의 세 개의 다른 장소에서 설치류를 포획하였다. 총 14 마리의 등줄쥐를 포획하였으며 이들 등줄쥐로부터 810 마리의 털진드기를 얻어 분석하였다. Genomic DNA 를 추출하여, *groEL, ank, msp2* 그리고



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16S rRNA 유전자를 대상으로 하여 *Anaplasma/Ehrlichia* 를 검출하는 conventional PCR 및 nested PCR 을 수행하였다. PCR 후 얻은 증폭산물은 염기서열분석을 수행하였다.

**Results**

본 연구 결과, 털진드기의 0.5 %, 등줄쥐의 21.4%가 *A. phagocytophilum* 에 대하여 양성을 보였다. 또한, 등줄쥐에서 28.57%가 *C.* Neoehrlichia mikurensis 양성으로 확인되었다

등줄쥐 검체에서, *groEL, ank* 유전자를 대상으로 각각 50%, 78.57%에서 *A. phagocytophilum* 양성으로 확인되었다. 수집된 810 마리의 털진드기(162 모음)는 *Leptotrombidium scutellare* (72.13%)가 가장 많았으며, *Eushoengastia koreaensis* (10.88%) 와 *Leptotrombidium orientale* (9.13%)가 그 다음으로 많이 수집되었다.

흥미로운 결과는 두 마리의 서로 다른 등줄쥐에서 수집한 각각의 털진드기모음이 모두 *A. phagocytophilum* 으로 동일하게 확인되었다는 것이다. 이 사실은 털진드기가 Anaplasmosis 전파에 역할을 할 수 있음을 암시한다.

**Conclusions**

한국의 등줄쥐에서의 *C.* Neoehrlichia 검출과 털진드기에서의 *A. phagocytophilum* 의 검출은 환자에서의 잠재적 감염 위험성의 가능성을 시사하는 소견으로 사료된다. 본 연구를 통해 한국의 등줄쥐에서 *C.* N mikurensis 가 확인되고, 털진드기에서 *A. phagocytophilu* 이 최초로 검출 및 동정되었다.



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# 1 Introduction

## 1.1 Basis of this study

This study shows the occurrence of two intracellular bacterial pathogens, *Anaplasma phagocytophilum* and *Candidatus* Neoehrlichia mikurensis in mites and stripped field mice in the Republic of Korea. Recently, we encountered two cases of Anaplasmosis in our University Hospital; both these patients had eschar which is a characteristic of mite bite. This triggered an interest to investigate whether mites play a role as a vector for Anaplasmosis or not. We collected trombiculide mites and striped field-mouse from the sub-urban areas Gwangju city which is located in south-western part of Republic of Korea.

This contributes to the understanding of the natural cycle of these bacteria. So far only a limited data has been available in the Republic of Korea, especially concerning animal hosts and its vector. We also describe the molecular techniques for detection of these pathogens. *A. phagocytophilum* is considered as a tick-borne disease and only on rare occasions it has been detected in mites (Skoracki *et al*. 2005). This is the first instant to detect *A. phagocytophilum* in mites and *Candidatus.* Neoehrlichia. mikurensis from small mammal hosts from Korea.

## 1.2 Aim of this study

In Republic of Korea *A. phagocytophilum* has been detected from ticks, striped field mice (Chae *et al*., 2003) and Korean water deer (Kang *et al*., 2011). The first case of Human Granulocytic Anaplasmosis (HGA) was been reported in 2013 from ROK (Kim *et al*., 2013). *C.* Neoehrlichia mikurensis has not been detected or reported either in vectors, rodents or humans from ROK untill date. The quill mites as a vector for *A. phagocytophilum* have been reported from Poland (Skoracki *et al*., 2005). Therefore, we conducted this study to detect the prevalence of *Anaplasma* and *C.* Neoehrlichia in mite vectors and rodent hosts from south-western part of ROK.

The aim of this study was to investigate the natural cycle of the new human pathogenic bacteria *A. phagocytophilum* and *Candidatus* Neoehrlichia mikurensis and in Gwangju city which is located in south-



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western part of Republic of Korea. Our study provides new data on the occurrence of these pathogens in rodent tissue samples, questing mites and engorged mites in suburban area of Gwangju city which is located in the south-western part of ROK.

## 1.3 Anaplasmosis

Anaplasmosis is a disease caused by a rickettsial parasite of ruminants, such as sheep, cattle and horses. *Anaplasma* spp. The microorganism is gram-negative obligate bacterium and occurs in the neutrophils. It is transmitted by natural means through a number of haematophagous species of ticks. Anaplasmosis can also be transmitted iatrogenically by the use of surgical, dehorning, castration, and tattoo instruments and hypodermic needles that are not disinfected between uses. Although the term is often associated with veterinary infection, it is also used to describe infection in humans (Bakken *et al*. 1996).

It was previously known as human granulocytic ehrlichiosis (HGE) and has more recently been called human granulocytic anaplasmosis (HGA). Anaplasmosis is known to be transmitted to humans by tick bites primarily from the black-legged tick (*Ixodes scapularis*) and the western black-legged tick (*Ixodes pacificus*). Of the four distinct phases in the tick life-cycle (egg, larvae, nymph, adult), nymphal and adult ticks are most frequently associated with transmission of anaplasmosis to humans. In humans, clinical manifestations range from a mild self-limited flulike illness to a life-threatening infection. Most human infection probably results in minimal or no clinical manifestation (Dumler *et al*. 2005).

Typical symptoms include fever, headache, chills, and muscle aches. Usually, these symptoms occur within 1-2 weeks of a tick bite. Anaplasmosis is initially diagnosed based on symptoms and clinical presentation, and later confirmed by the use of specialized laboratory tests. The first line treatment for adults and children of all ages is doxycycline.

### 1.3.1 Bacteria

*A. phagocytophilum* is an obligate Gram-negative intracellular bacterium. It has been a well-known pathogen among the domestic ruminants causing “tick-borne fever” but it is a generalist pathogen and can infect several other land-living vertebrate species (including humans) on the Northern hemisphere where



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ticks of the *I. ricinus* complex are endemic. Fatal infection cases were reported in sheep, horse, roe deer, dogs and humans. This bacterium infects and colonizes the neutrophils thus the pathogen decreases the number of the useful immune cells often leading to immunodeficiency (Stuen *et al*., 2013).

### 1.3.2 Direct Identification

Light microscopy of blood smears taken in the initial fever period is normally sufficient to state the diagnosis. Stained with MayGrünwald Giemsa, the organisms appear as blue cytoplasmic inclusions in monocytes and granular leucocytes, especially neutrophils (Foggie, 1951). Electron microscopy may also confirm the diagnosis of acute *Anaplasma* infection in blood or organs. Single or multiple organisms are then identified in clearly defined cytoplasmic vacuoles (Tuomi and von Bonsdorff, 1966; Rikihisa, 1991). Immuno-histochemistry on tissue samples could also be performed to confirm the diagnosis (Lepidi *et al*., 2000).

Morulae may be seen within neutrophils during cytologic examination of Romanowsky-stained peripheral blood smears. Although the finding of morulae within neutrophils in peripheral blood from a dog in an endemic area is highly suggestive of infection with *A. phagocytophilum*, the morulae cannot be distinguished from those of *Ehrlichia* spp. such as *E. ewingii*, and serology or PCR are needed to confirm that the organism is *A. phagocytophilum*. Morulae were detected within neutrophils and 100% of dogs in each of the 4 larger case series reported. The percentage of neutrophils containing morulae during acute infection has ranged from 7 to 32%. In experimentally infected dogs, morulae appear as early as 4 days after inoculation, and persist for 4–8 days (Carrade *et al*, 2009; Greig *et al*, 1996; Egenvall *et al*, 1998).

### 1.3.3 Poylmerase chain reaction (PCR) and Cultivation

Several PCR techniques (conventional, nested, and real-time) for the identification of *A. phagocytophilum* infection in blood and tissue samples have been established primarily on the basis of the *16S rRNA*, *groEL*, and *p44* genes (Chen *et al*., 1994; Courtney *et al*., 2004; Alberti *et al*., 2005). Multiple variants of *A. phagocytophilum* have been genetically characterized. Identification and stratification into phylogenetic subfamilies have been based on cell culturing, experimental infections, PCR and sequencing



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techniques (Dumler *et al*., 2007). Cultivation of *A. phagocytophilum* in cell cultures has been described for variants isolated from human, dog, horse, roe deer, and sheep (Goodman *et al*., 1996; Munderloh *et al*., 1999; Bjöersdorff *et al*., 2002; Woldehiwet *et al*., 2002; Silaghi *et al*., 2011c).

### 1.3.4 Serology

The presence of specific antibodies may support the diagnosis. A complement fixation test, counter-current immunoelectrophoresis test and an indirect immunofluorescent antibody (IFA) test can be used (Webster and Mitchell, 1988; Paxton and Scott, 1989). Several ELISA tests have also been developed (Ravyn *et al*., 1998; Magnarelli *et al*., 2001; Alleman *et al*., 2006; Woldehiwet and Yavari, 2012). A SNAP® 4Dx® ELISA test is commercially available for rapid in-house identification of *A. phagocytophilum* antibodies in dog serum, but the kit has also been used successfully on horse and sheep sera (Granquist *et al*., 2010a; Hansen *et al*., 2010).

### 1.3.5 Pathology

An enlarged spleen, up to 4–5 times the normal size with subcapsular bleedings, has been regarded as indicative of TBF in sheep for decades (Gordon *et al*., 1932; Øverås *et al*., 1993). No other typical pathological changes have been described (Munro *et al*., 1982; Campbell *et al*., 1994; Lepidi *et al*., 2000). An enlarged spleen with subcapsular bleedings has also been observed in roe deer and reindeer (Stuen, 2003). Relative sensitivity of the diagnostic tests used for laboratory diagnostic confirmation of *A. phagocytophilum* infection in humans is shown in Table 9.

### 1.3.6 *Anaplasma phagocytophilum* prevalence in ROK

The first case of HGA patient in ROK was been reported in 2013 (Kim *et al*. 2013). However, *A. phagocytophilum* has been detected in *Haemaphysalis longicornis*, *Ixodes nipponensis*, and *I. persulcatus* ticks (Kim *et al*. 2003, Chae *et al*. 2008) in ROK. Molecular epidemiologic studies detected *A. phagocytophilum* in 2.6% (5/196) of striped field mice (Chae *et al*. 2008 and Chae *et al*. 2008) and in 63.6% (42/66) of Korean water deer (Kang *et al*. 2011). Seroprevalence studies showed that 1.8% of serum



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samples from patients with acute fever were positive for *A. phagocytophilum* by an immunofluorescence antibody test (Heo *et al*. 2002).

## 1.4 Neoehrlichiosis

*C.* Neoehrlichia mikurensis was first reported to be a human pathogen in 2010 from Sweden (Welinder-Olsson *et al*. 2010). Until now, a total of fifteen cases have been published from Sweden, Germany, Switzerland, the Czech Republic, and China. A little more than half of the published cases concerned apparently healthy persons, while the remainders were immunocompromised patients.

### 1.4.1 Bacteria

*C.* Neoehrlichia mikurensis bacteria are small, gram negative pleomorphic cocci 0.5 to 1.2 m in diameter that are obligate intracellular organisms (Kawahara *et al*. 2004). They belong to the family Anaplasmataceae and lack serological cross-reactivity with other genera of the family, such as *Anaplasma* and *Ehrlichia* species (Kawahara *et al*. 2004). It was first detected in the late 1990's in *I. ricinus* in the Netherlands and Italy and later on it was also found China in a wild Norway rat of China (*Rattus norvegicus*). It was initially called Ehrlichia-like due to a diverging *16S rRNA* gene sequence (Schouls *et al.*, 1999). Further findings of the microorganism in rats and *Ixodes ovatus* ticks in Japan and the passaging of the agent in laboratory rats led to its description as the new species *C.* N. mikurensis in 2004 (Kawahara *et al*., 2004).

### 1.4.2 Diagnosis

This novel bacterial species has been identified in blood, liver, and spleen samples from both laboratory and wild rats. The pathogen appears to reside within the cytoplasm of endothelial cells lining the venous sinuses, at least in rats (Kawahara *et al*. 2004). In humans, the pathogen's close relatives *Ehrlichia chaffeensis* and *A. phagocytophilum* selectively infect the professional phagocytes monocytes/macrophages and neutrophilic granulocytes, respectively (Dumler *et al*. 2007), which suggests that *C*. N. mikurensis may display tropism for leukocytes also. Until now, no one has been able to grow *C.* N. mikurensis (Kawahara *et al*. 2004), which explains why all the patient's found positive for this



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pathogen by other diagnostic methods their blood cultures remained negative. *E. chaffeensis* and *A. phagocytophilum* can be cultivated only in cells such as those of human leukemia cell lines (Dumler *et al*. 2007). In the acute phase of human ehrlichial infections, PCR analysis of EDTA- or citrate-anticoagulated whole blood is the preferred diagnostic method, followed by examination of blood films for cytoplasmic bacterial inclusions (called ehrlichial morulae) and IgM serology (Dumler *et al*. 2007).

#### 1.4.3 *Candidatus* Neoehrlichia mikurensis prevalence in ROK

*C.* N. mikurensis has not been so far detected in any vector, hosts or humans in South Korea. To the best of our knowledge this is the first instance of *C.* N. mikurensis reported from field stripped mice from ROK.

# 2 Materials and Methods

## 2.1 Study sites and collection of mice and mites

The wild rodents and mites were captured in a sylvatic habitat located within the west of the farmlands area in the south-west city of Gwangju of ROK (34°10′N; 126°55′E), in autumn from October to November 2014 using live-traps (Figure 1). We collected rodents with live-traps and after euthanizing the mice the bodies of the rodents were hung individually over a 1,000 ml beaker filled to a depth of 1 cm with tap water for harvesting the larval mites. The mites which fell into the water were removed with a fine brush and placed in 75% ethanol until mounted on slides with polyvinyl alcohol media. The larval mites were identified under a light microscope using morphological keys prepared by Ree et al.

in 2014. Small mammals were euthanized, tissue samples were collected and all the ectoparasites were removed and stored in 70% alcohol. We found relatively low overall prevalence of tick infestation (8%). Samples were analyzed for *A. phagocytophilum* and *Candidatus* N. mikurensis with Nested and conventional PCR targeting a part of heat shock protein (*groEL*) genes, major surface protein 2 (*msp2*), 16S rRNA and ankyrin-repeat protein (*ankA*) gene, respectively. The sampling area consisted of a mixed stand with a well-developed leaf litter layers. The live-traps were placed along five transects (each 45 cm



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long) spaced 150-200 m apart, depending on location and were monitored for the captured mouse next morning. The mites were removed from the mice by using a head-mounted magnifying glass and finally hanging the euthanized mice in a water pool for 4-6 hrs. The organ samples of the euthanized mice were stored at –20 ℃ until further experiment.

## 2.2 Identification of collected mice and mites

The larval mites were identified under a light microscope using morphological keys prepared by Ree *et al*. A total of 14 wild rodents captured were stripped field mice; all of them were identified as *Apodemas agrarius* (Figure 2). Of the 14 captured mice, 12 were captured in the month of October and 2 in the month of November. The entire mouse was numbered for convenience of the research (Table 1).

A total of 810 Trombiculid mites were collected from October to November, 2014. The collected mites were identified to the species level. The most predominant species of mite collected for this study was *Leptotrombidium scutellare* (N= 577, 72.13 %) followed by *Eushoengastia koreaensis* (N= 87, 10.88 %), *Leptotrombidium orientale* (N= 73, 9.13 %), *Leptotrombidium pallidum* (N= 29, 3.63 %), *Neotrombicula gardellai* (N= 28, 3.5 %), *Leptotrombidium palpale* (N= 15, 1.88 %) and *Leptotrombidium zetum* (N= 1, 0.13 %) (Table 2).

## 2.3 DNA extraction from mites and pooling of the sample

The individual mites were first placed in a 1.5 mL Eppendorf tube and mechanically homogenized with a sterile micropestle using the GenoGrinder 2000 homonizer (OPS Diagnostics, USA) and stored at –20 ℃. The Genomic DNA was extracted from 810 (162 pools) mite samples by using QIAamp DNA Blood & Tissue Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. One mite pool is made up of 5 individual mites which resulted in 162 pools. For the experimental convenience we further made pools of pool by mixing 5 pools into one which resulted in total of 33 pools of pool (Table 3).



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## 2.4 DNA extraction from mice spleen and kidney samples

The mice organ samples stored at –20 ℃ were homogenized by grinding with sterile Cell Strainer 70 µm Nylon and incubated overnight in water bath. The next morning the Genomic DNA was extracted from 14 spleen and 14 kidney samples by using QIAamp DNA Blood & Tissue Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. For DNA extraction, 10µg of spleen and kidney samples were used.

## 2.5 PCR amplifications

Conventional PCR and nested PCR were performed using specific primers for *A. phagocytophilum* and *Candidatus* Neoehrlichia (Table 4). The *Ehrlichia chaffeensis* genomic DNA was used as a positive control for detecting mite and mice pathogenic for *Anaplasma* and *Candidatus* Neoehrlichia species. The first and nested PCRs were performed in a total volume of 20 mL. Each PCR mixture consisted of 10 pmol/µL of primers, 10 µL of Master mix 2 µL of GC enhancer, 4 µL of distilled water, 1 µL of primers and 2 µL template of genomic DNA of samples for the first PCR and 2 mL of the first PCR product for the second PCR. The amplification was carried out in a AB thermal cycler (Applied Biosystem, Inc.). Table 4 shows the conditions of the PCRs. Amplified products were separated by electrophoresis on 1.5% agarose gel and visualized by ethidium bromide.

## 2.6 Nucleotide sequencing

The nucleotide sequence of the amplified region of the *groEL*, *ankA* and 16S rRNA gene was determined by sequencing the DNA of positive isolates of Trombiculidae mites and spleen and kidney samples of *Apodemas agrarius* (striped field mouse) and using the same primers. The PCR products were purified with QIAquick PCR purification kits (Qiagen). Sequencing was performed at the Medical Institute Research (Daejong, Republic of Korea). The obtained sequences were evaluated with Chromas software (Ver 2.33; www.technelysium.com .au/chromas.html) and were aligned using Clustal X (Ver 2.0; www.clustal.org/). The aligned sequences were examined with a similarity matrix. Relationships between individuals were assessed by the neighbor-joining method with nucleotide distance (p distance) for 1000



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replications with a bootstrap test. The phylogenetic tree was based on the sequences and determined using PAUP 4.0b software (Swofford 2002).

The 16S ribosomal RNA, *groEL*, *msp2*, and *ankA* gene sequences and specific genospecies sequences related to Anaplasmataceae pathogens for sequence comparisons are listed in Table 6- 10.

## 2.7 Phylogenetic analysis

Nucleotide sequences of *groEL*, *ankA*, and 16S rRNA loci were downloaded from GenBank, and analyzed using the program MEGA6 (Molecular Evolutionary Genetics Analysis Version 6.0) (Tamura *et al*. 2013). Sequences of each gene were aligned by ClustalW applying the IUB matrix. Alignments have been deposited in TreeBase (http://treebase.org/treebaseweb/home.html). Tree construction was achieved using the Neighbor-Joining method (Saitou and Nei, 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Zharkikh and Li, 1995).

# 3 Results

In total, 810 trombiculid mites representing 7 different species and 14 *Apodemas agrarius* (stripped field mice) were captured and studied during our survey. Trombiculid mites (generally 0.2–0.5mm in width and 0.7–1.4 mm in length) were obtained from 14 stripped field mouse representing seven species (Table 1).

*Leptotrombidium scutellare* (N=577, 72.13 %) was the most predominant species in our study site followed by *Eushoengastia koreaensis* (N=87, 10.88 %), *Leptotrombidium orientale* (N=73, 9.13 %), *Leptotrombidium pallidum* (N=29, 3.63 %), *Neotrombicula gardellai* (N=28, 3.5 %), *Leptotrombidium palpale* (N=15, 1.86 %) and *Leptotrombidium zetum* (N=1, 0.13 %) (Table 2).



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## 3.1 Nested PCR for *groEL* of mites sample

The nested PCR targeting the *groEL* gene of pools of pool the mite detected three positive pools of pool samples out of total 32 positive. The pools of pool no. 9, 10 and 23 were positive (Fig. 4 a, b). Primers and conditions used is mentioned in Table 4. Further, nested PCR of the individual pool targeting the *groEL* gene revealed that the 5 individual pools were positive for *Anaplasma*/*Ehrlichia*; these were individual pools 46, 47, 111, 113 and 114 (Fig. 5). These positive individual pools were sent for sequencing to SolGent Co., Ltd, ROK and all the 5 individual pools were identified as *A. phagocytophilum* (Table 6).

## 3.2 Nested PCR for *groEL* of mice organs

The nested PCR of mice spleen and kidney samples were done targeting the *groEL* genes, in which 7 spleen samples and 3 kidney samples out of the total 14 samples were detected positive for *Anaplasma*/*Ehrlichia* (Table 4). All the positive samples were sequenced in which 3 of the 7 spleen samples were identified as *A. phagocytophilum* (mouse-10-3, 11-12, 11-13) and 4 of them (mouse-10-4, 10-5, 10-6, 10-11) were identified as *C.* Neoehrlichia mikurensis. Similarly, one of the positive kidney sample was identified as *A. phagocytophilum* (mouse 10-3) and 2 of them were *C.* N mikurensis (mouse 10-5, 10-11) (Table 6).

## 3.3 Nested PCR for *ankA* of mice organ

AnkA (also known as Ank) is a unique protein of *A. phagocytophilum* that is high molecular weight (160 kDa), not associated with the bacterial membrane, but is found localized within nuclei of infected granulocyte hosts, presumably after secretion and transport through at least three distinct membranes (Caturegli *et al*., 2000). Although its close ultrastructural association with condensed chromatin and the presence of multiple ankyrin repeat domains tempts speculation about a role in affecting gene transcription or transcription factors, the actual binding targets in the nucleus are not known.

The ankyrin-repeat protein (*ankA*) gene targeting nested PCR was done and it resulted in detection of 11 positive spleen samples. The primers used for primary amplification were ANK-F1 and ANK-R1 (705 bp



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length) and for nested amplification ANK-F2 and ANK-R2 (664 bp length) were used. On sequencing it was identified that 10 out of the 11 positive samples were *A. phagocytophilum*. These were mouse 10-1, 10-2, 10-3, 10-4, 10-8, 10-10, 10-11, 10-13, 11-12, 11-13 (Figure 6).

### 3.4 Nested PCR for16S rRNA with mice organs

The 16S rRNA gene targeting nested PCR was performed using the primers AE1-F and AE1-R for primary amplification and for the nested amplification of *A. phagocytophilum* primers EE-3 and EE-4 (926 bp length) was used. Similarly, for the *E. chaffeensis* primers HE1 and HE3 (390 bp length) was used. The result of this amplification yielded 7 positive samples for *A. phagocytophilum* (mouse 10-3, 10-4, 10-5, 10-6, 10-11, 11-12, 11-13) and 3 positive samples *E. chaffeensis* (mouse 10-3, 11-12, 11-13). However, on sequencing the PCR product it identified only 3 of the samples for *A. phagocytophilum* and rest of the samples were unable to be detected.

### 3.5 Nested PCR for 16S rRNA with mice organ (Primers specific for *C.* Neoehrlichia)

The 16S rRNA gene targeting nested PCR was performed using the primers 16S-EC9-F and 16S-EC12A-R for primary amplification and for the nested amplification of *C.* Neoehrlichia, primers 16S-IS58-62f and 16S-IS58-594r (488 bp length) was used. The result of this amplification yielded 4 positive samples for Ca. Neoehrlichia (mouse 10-4, 10-5, 10-6, 10-11). On sequencing the PCR product it identified all the 4 samples as *Candidatus* Neoehrlichia mikurensis (Table 9).

### 3.6 Phylogenetic tree

Phylogenetic trees inferred from comparison of the *groEL* (330 bp) were constructed (Figure 3). Three of the spleen and 1 kidney isolates clustered around the *A. phagocytophilum* (NO007797) which is a clinical isolate. The *C.* N mikurensis from other spleen and kidney samples were closer to the *C.* N mikurensis from rodents of China and Russia as being in the same clade. The sequences provided similar topology and indicated a close relationship with '*Candidatus* N mikurensis'. Similar to previous studies (Kawahara *et al*., 2004; Brouqui *et al*., 2003), '*C.* N mikurensis' was distinct from other genera in the family



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*Anaplasmataceae* and formed a well-supported sister clade to the genus *Ehrlichia.* All of these current sequences from this group have been lumped together in a single candidate species ('*C.* N mikurensis').

## 4 Discussion

HGA was first identified in 1990 in USA, in patient who died with a severe febrile illness two weeks after a tick bite (Chen *et al*. 1994). In Europe, antibodies against *A. phagocytophilum* were described in 1995 in the Swiss population (Brouqui *et al*. 1995). First documented HGA case in Europe was reported in Slovenia (Petrovec *et al*. 1997). Anaplasmosis in domestic ruminants is also called tick-borne fever and has been mentioned in 1932 (Gordon *et al*. 1932). HGA agent is maintained in nature in a tick-ruminant-rodent cycle. Humans are involved only as accidental "dead-end" hosts (Blanco and Oteo 2002). The agent *A. phagocytophilum*, may cause infection in several animal species including humans. Anaplasmosis may cause high fever, loss of appetite, malaise, cytoplasmatic inclusions in granulocytic neutrophils, neutropenia and thrombocytopenia (Rikihisa 1991, Greig *et al*. 1996, Egenvall *et al*. 1997, Engvall and Egenvall 2002). Anaplasmosis is seldom fatal unless there are complications by other infection. In humans, clinical manifestations range from a mild self-limited flulike illness to a life-threatening infection. Most human infection probably results in minimal or no clinical manifestation (Dumler *et al*. 2005).

A. *phagocytophilum* was first described in 1994 in the USA (Bakken *et al*. 1994, Chen *et al*. 1994). The agent was recognized by molecular amplification and DNA sequencing and was initially named Human granulocytic ehrlichiosis (HGE) agent. Recently, the families Rickettsiaceae and Anaplasmataceae were complete revised and these bacteria: *Ehrlichia phagocytophilum*, *E. equi* and the HGE agent were reclassified as a single species, *A. phagocytophilum* (Dumler *et al*. 2001). *A. phagocytophilum* has been detected in ticks and mammals in many European countries (Strle 2004). Seroprevalence rates in European countries range from zero to up to 28% of the examined human population (Strle 2004). *A. phagocytophilum* is small (0.2 ~ 1.0 μm) obligate intracellular bacteria with a gram-negative cell wall



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(Walker and Dumler 1996, Parola and Raoult 2001). This bacterium resides in an early endosome in granulocytic neutrophils, where Anaplasma obtains nutrients for fission and grows into a cluster called morulae (Lin and Rikihisa 2003).

The main vector of *A. phagocytophilum* in Europe is *Ixodes ricinus* (Parola and Raoult 2001, Strle, 2004). The prevalence of *A. phagocytophilum* infection in *I. ricinus* in Europe varies from area to area and between development stages of the tick (Lillini *et al*. 2006). Occurrence in nymphs has been found to vary between 0.25~ 25% (Walker *et al*. 2001). Prevalence is usually higher in adult ticks than in nymphs and ranges from zero to 30% (Pusterla *et al*. 1999a, Liz *et al*. 2000, Strle 2004). Tick is infected after feeding on an infected host. The bacterium is passed transstadially but not transovarially (Dumler *et a*l. 2001). *A. phagocytophilum* has been associated with other ticks such as *Haemaphysalis punctata* (Barandika *et al*. 2008), *I. persulcatus* (Alekseev *et al*. 1998), *I. triangulicep*s (Ogden *et al*. 1998) and *Riphicephalus sanguineus* (Alberti *et al*. 2005). In the USA, *A. phagocytophilum* has been often associated with *I. scapularis* and with *I. pacificus*, and these may serve as the primary vectors (Barlough *et al*. 1997, Chang *et al*. 1998).

There is field evidence that sheep are natural hosts for *A. phagocytophilum* in the UK (Ogden *et al*. 1998, Ogden *et al*. 2002). In the USA rodents, particularly white-footed mice *P. leucopus* (Bunnell *et al.* 1998), and white-tailed deer *O. virginianus* (Belongia *et al*. 1997) are involved as natural reservoirs for *A. phagocytophilum*. Wild rodents in Europe have been suggested to be competent reservoirs for *A. phagocytophilum* (Ogden *et al*. 1998a, Liz *et al*. 2000). Long-tailed mice *A. sylvaticus*, yellow-necked mice *A. flavicollis*, common shrew *S. araneus*, and bank voles *C. glareolus* were found to be likely natural reservoir for *A. phagocytophilum* (Liz *et al*. 2000, Liz 2002).

Monitoring the environment for the incidence of *A. phagocytophilum* involves both classical and nested PCR, as these simple methods are very efficient. As markers, portions of the 16*S rRNA*, *ankA*, *groESL*, *msp2*, or *msp4* genes are used for analyses. The template comprises DNA collected from ticks (tick



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homogenates), or blood or pieces of other tissues collected from wild animals. Amplification is based on a one step PCR protocol for the genes *msp2, ankA*, or 16S rRNA, or a two-step PCR assay, which is recommended for the *groEL* or *msp4* genes. The genome of *A. phagocytophilum* is relatively small, consisting of a single circular chromosome (Dunning Hotopp *et al*., 2006). The complete genomic sequence of *A. phagocytophilum* estimated at 1 471 282 bp was submitted to GenBank in 2006 (NC007797). The established number of genesis 1 411, 1 264 of which encode proteins, 42 which encode structural RNAs and 27 of which are pseudo genes (www.ncbi.nlm.nih.gov). Sequencing the complete genome of *A. phagocytophilum* has greatly facilitated research into the diversity of these microorganisms.

The activity of the bacterial chaperonin g*roEL* and its cofactor *groES,* and the molecular machinery underlying this activity, has been investigated by several groups in recent years. The role of *groEL* is twofold. First, it binds partially folded or misfolded proteins in its central cavity, thus preventing their aggregation. Second, the central cavity works as an Anfinsen cage in which the isolated protein folding intermediate is actively folded, after being unfolded, if necessary, to states more committed toward correct folding (Corrales, F. J *et al*. 1996;  Clarke, A. R. 1996).

Candidate reservoirs migrating birds may be important in dispersal of *A. phagocytophilum* infected *I. ricinus* in Europe (Alekseev *et al*. 2001, Bjoersdorff *et al*. 2001, de la Fuente *et al*. 2005). A study from Slovenia revealed by PCR that red deer *Cervus elaphus* and roe deer *Capreolus capreolus* are infected with *A. phagocytophilum* in about 86% of cases, and the prevalence of IFA antibodies was found to be 35% and 94%, respectively (Petrovec *et al*. 2002). Red foxes *V. vulpes* and wild boar *Sus scrofa* were also found to be PCR positive (Petrovec *et al*. 2003). Infection by Anaplasma has been also identified in European bison (Grzeszcuk *et al*. 2003), donkey (de la Fuente *et al*. 2005b), and moose (Jenkins *et al.* 2001). Antibodies have been detected in hare (Groen et al. 2002) and Eurasian lynx (Ryser-Degiorgis *et al*. 2005). Domestic animals as hosts *A. phagocytophilum* is known to cause HGA in humans (Petrovec *et al*. 1997) and domestic animals such as horses (Bjoersdorff 1990, Engvall *et al*. 1996, Engvall and Egenvall 2002), dogs (Bellstrom 1989, Engvall *et al*. 1996, Engvall and Egenvall 2002, Skotarczak 2003,



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Lester *et al*. 2005, Poitout *et al*. 2005), cats (Bjoersdorff *et al*. 1999), cattle (Engvall *et al*. 1996), and llamas (Barlough *et al*. 1997b). *A. phagocytophilum* has been found to persist in sheep (Stuen *et al*. 1998).

Anaplasmosis signs in domestic animals include fever, fatigue, inappetence, lethargy, lameness, gastrointestinal and central nervous system signs (Rikihisa 1991, Greig *et al*. 1996, Egenvall *et al*. 1997, Engvall and Egenvall 2002). The related hematologic and biochemical abnormalities are anemia, thrombocytopenia, lymphocytopenia and elevated serum alkaline phosphatase activity (Greig *et al*. 1996, Goldman *et al*. 1998).

*A. phagocytophilum* often causes chronic disease in dogs with non-specific clinical findings. Clinical and haematological findings in dogs are fever, loss of appetite, joint swelling and pain, stiffness, neurologic inflammation, thrombocytopenia and neutropenia. Canine anaplasmosis was reported from many European countries (Sweden, Greece, Italy, Slovenia, Austria, Poland, Switzerland, and Czech Republic) (Lillini *et al*. 2006).

*A. phagocytophilum* in horses was reported in many European countries including Great Britain, Denmark, Sweden, Switzerland, France, Germany, Czech Republic and Italy. It was not only detected by serological and molecular methods, but also by positive findings in buffy coat smears. Hematological findings on Anaplasma-positive horses showed thrombocytopenia and leukocytosis (Bjoersdorff 1990, Bjoersdorff *et al.* 2002, Lillini *et al.* 2006, Zeman and Jahn 2009).

*A. phagocytophilum* causes a disease known as a tick-borne fever (TBF). *A. phagocytophilum* in sheep causes very high fever, reduced milk yield and abortion in pregnant animals. Laboratory findings are neutropenia and cytoplasmatic inclusion with more than 95% of neutrophils infected (Garcia-Perez *et al*. 2003).

The majority of Anaplasma infection is identified by indirect laboratory methods. The most frequent used method is the IFA test. While this test is often used, reactivity in samples with anticytoplasmic antibodies or with other autoimmune antibodies may confound interpretation, and the correlation of results from



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different laboratories and different commercial tests may be difficult (Blanco and Oteo 2002). *A. phagocytophilum* is visible as a cluster of small cocci in cytoplasm of neutrophils in the peripheral blood on a Wright- or Giemsa-stained smear. The characteristic cytoplasmatic inclusion in neutrophils can be detected in between 25% and 80% of patients during the active stage of HGA (Dumler and Brouqui 2004). This bacterium is an obligate intracellular parasite that can only be cultivated in the human HL-60 promyolocytic leukemia cell line derived from bone marrow myeloid progenitors, (Goodman *et al*. 1996). Cultivation may require several days to several weeks. Morphologic identification in culture still requires confirmation for example by PCR (Dumler and Brouqui 2004). Detection of *Anaplasma* genetic material by PCR demonstrates the presence of *A. phagocytophilum* nucleic acid in peripheral blood but not the presence of living agents. HGA is one of the rare infectious diseases that were first confirmed based upon molecular tests rather than culture or serology (Chen *et al*. 1994). The first tests used were based on an amplification of the highly conserved 16S rRNA gene, followed by a second stage that utilized primers suspected to anneal only to *A. phagocytophilum* rrs-specific sequence (Dumler and Brouqui 2004).

*C.* Neoehrlichia mikurensis was first reported to be a human pathogen in 2010 (Fehr *et al*. 2010, Welinder *et al*. 2010, von Loewenich *et al*. 2010). Until now, a total of 15 cases have been reported from Sweden (Welinder *et al*. 2010) Germany (von Loewenich *et al*. 2010) Switzerland (Fehr *et al*. 2010, Maurer *et al.* 2013) the Czech Republic (Pekova *et al*. 2011) and China (Li *et al*. 2012). A little more than half of the published cases concerned apparently healthy persons, (Fehr *et al*. 2010, von Loewenich *et al*. 2010, Li *et al*. 2012), whereas the remainders were immunocompromised patients (Welinder *et al*. 2010, von Loewenich *et al*. 2010, Maurer *et al*. 2013, Pekova *et al*. 2011).

*C.* N. mikurensis was named name in 2004, after it was discovered in ticks and rodents on the Japanese island of Mikura by means of PCR directed against conserved genes of the bacterial genome, for example, 16SrRNA and *groEL* sequences (Kawahara *et al*. 2004). Transmission electron microscopy of infected rats showed small cocci in the cytoplasm of endothelial cells (Kawahara *et al.* 2004). Phylogenetic analyses reveal it to be a new species belonging to the family Anaplasmataceae. Its closest relative is *C.*



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Neoehrlichia lotoris, which primarily infects raccoons (Yabsley *et al*. 2008). Other related species are *Ehrlichia ruminantium*, *Ehrlichia chaffeensis*, and *Anaplasma phagocytophilum* (Kawahara *et al*. 2004, Rar *et al*. 2011). All these bacterial species are strict intracellular pathogens that can only be cultivated in cell lines.

N. mikurensis is denominated "*Candidatus*" because no one to our knowledge has yet reported its cultivation. The target cells of Neoehrlichia infection in humans are yet to be defined although polymorphonuclear granulocytes (Pekova *et al*. 2011) and endothelial cells have been implicated (Rar *et al*. 2011). At present, the only diagnostic option is either pan-bacterial PCR (targeting the 16S rRNA gene) followed by sequence analysis, (Welinder *et al*. 2010) or a specific real-time polymerase chain reaction (RTPCR), performed on whole blood, plasma, or bone marrow (Maurer *et al*. 2013). No serological tests are available, and the lack of serological cross-reactivity with either *Anaplasma phagocytophilum* or *Ehrlichia chaffeensis* (von Loewenich *et al*. 2010) precludes the use of *Anaplasma*- or *Ehrlichia*-based indirect fluorescence antibody tests. In retrospect, it has become clear that others have reported on the same bacterial species prior to Kawahara's original report from 2004 but under other names (Alekseev *et al*. 2001, Brouqui *et al*. 2003, Jenkins *et al*. 2001, Pan *et al*. 2003, Schouls *et al*. 1999, Shpynov *et al*. 2006). *C*. N mikurensis is widely distributed among ticks including *Ixodes ricinus*, *I. persulcatus*, *I. ovatus*, *I. frontalis*, (Yabsley *et al*. 2008, Movila *et al*. 2013) rats, field mice, and voles in Northern (Jenkins *et al*. 2001, Andersson *et al*. 2011, Fertner *et al*. 2012), Central Europe (Capelli *et al*. 2012, van Overbeek *et al*. 2008, Vayssier *et al*. 2012, Lommano *et al*. 2012), and Eastern Europe (Movila *et al*. 2013, Movila *et al*. 2013, Spitalska *et al*. 2008), Asia (Kawahara *et al*. 2004, Li *et al*. 2013, Rar *et al*. 2011), and Africa (Kamani *et al* 2013). No reports exist from the Americas or Australia. Rodents appear to be healthy carriers of *C*. N mikurensis and may be viewed as the zoonotic reservoir (Andersson *et al*. 2011, Silaghi *et al*. 2012).

Besides humans the only other animal species that has been shown to become sick due to *C*. N mikurensis infection are dogs (Diniz *et al*. 2011). One peculiarity of Neoehrlichia infection in humans is



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the accumulation of cases among patients with B-cell malignancies or rheumatological diseases, many of whom have been splenectomized (Welinder-Olsson *et al*. 2010, Maurer *et al*. 2013, Pekova *et al*. 2011). Another distinguishing feature is the high prevalence of thromboembolic complications among these patients (Welinder-Olsson *et al*. 2010, von Loewenich *et al*. 2010, Maurer *et al*. 2013). Importantly, diagnosis of infection could be missed or severely delayed as the clinical picture of Neoehrlichia infection may be misinterpreted as noninfectious conditions, for example, arteriosclerotic thromboembolism with secondary fever, or systemic inflammation due to a new bout or recurrence of the underlying rheumatologic or hematologic disease.

## 5 Conclusion

All mite samples were identified and it was found that *Leptotrombidium scutellare* (71.23%) is the most dominant mite species in this local area followed by *Eushoengastia koreaensis* (10.88%) and *L. orientale* (9.01%) respectively. In this study, 0.5 % of mites and 21.4% of the mouse samples were positive for *A. phagocytophilum*. Also, 28.57% of the mouse samples were positive for *C.* N mikurensis. The *groEL* gene fragments amplified in 50% and *ankA* gene fragments amplified in 78.57% of the mouse samples for *A. phagocytophilum*. An interesting co-incidence is that mite for the pool 46 and 47 were collected from Mouse 10-3, and pool 111, 113, 114 were collected from Mouse 11-12 and both the mouse and mite set shows presence of *A. phagocytophilum*.

Our study revealed that granulocytic anaplasmosis and neoehrlichosis are important infectious agents found in wild rodents (stripped field mouse) and mites in ROK. This information on prevalence of intracellular bacterial pathogens such as *A. phagocytophilum* in mites and stripped field mouse and *C.* N mikurensis in the stripped field mouse suggests the possibilities of new hosts and vectors which were previously not considered.

To conclude, we hope to raise the awareness of these newly discovered microbes that may give rise to an infectious disease in local residents. Physicians should be aware of the prevalence of these new infectious



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agents in the new vectors previously not known and wild rodents; and draw their attention to the possibility of Anaplasmosis and Neoehrlichiosis in the ROK.

**Table 1 Mouse identification and entitled number**

| **Serial no.** | **Mouse identified name** | **Captured month** | **Number entitled** |
|---|---|---|---|
| 1. | Stripped field mouse (*Apodemas agrarius)* | October | 10-1 |
| 2. | Stripped field mouse (*Apodemas agrarius)* | October | 10-2 |
| 3. | Stripped field mouse (*Apodemas agrarius)* | October | 10-3 |
| 4. | Stripped field mouse (*Apodemas agrarius)* | October | 10-4 |
| 5. | Stripped field mouse (*Apodemas agrarius)* | October | 10-5 |
| 6. | Stripped field mouse (*Apodemas agrarius)* | October | 10-6 |
| 7. | Stripped field mouse (*Apodemas agrarius)* | October | 10-7 |
| 8. | Stripped field mouse (*Apodemas agrarius)* | October | 10-8 |
| 9. | Stripped field mouse (*Apodemas agrarius)* | October | 10-10 |
| 10. | Stripped field mouse (*Apodemas agrarius)* | October | 10-11 |
| 11. | Stripped field mouse (*Apodemas agrarius)* | October | 10-12 |
| 12. | Stripped field mouse (*Apodemas agrarius)* | October | 10-13 |
| 13. | Stripped field mouse (*Apodemas agrarius)* | November | 11-12 |
| 14. | Stripped field mouse (*Apodemas agrarius)* | November | 11-13 |



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**Table 2. Table showing the identified mites collected for this study**

| **Serial no.** | **Mite species name** | **Individual species in no.** | **Percentage (%)** |
|---|---|---|---|
| 1. | *Leptotrombidium scutellare* | 577 | 72.13 % |
| 2. | *Eushoengastia koreaensis* | 87 | 10.88 % |
| 3. | *Leptotrombidium orientale* | 73 | 9.13 % |
| 4. | *Leptotrombidium pallidum* | 29 | 3.63 % |
| 5. | *Neotrombicula gardellai* | 28 | 3.50 % |
| 6. | *Leptotrombidium palpale* | 15 | 1.88 % |
| 7. | *Leptotrombidium zetum* | 1 | 0.13 % |
| | Total | 810 | |



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**Table 3. Table showing the pooling of the mite sample**

| Number of rodents | | Number of mites | | Species |
|---|---|---|---|---|
| | | Number of Pool | Individual number | |
| 9 | 1 | p1 | 1 | *L. scutellare* |
| | | | 2 | *L. scutellare* |
| | | | 3 | ***N. gardellai*** |
| | | | 4 | *L. scutellare* |
| | | | 5 | *L. scutellare* |
| | | p2 | 6 | ***N. gardellai*** |
| | | | 7 | *L. scutellare* |
| | | | 8 | *L. scutellare* |
| | | | 9 | *L. scutellare* |
| | | | 10 | *L. scutellare* |
| | | p3 | 11 | *L. scutellare* |
| | | | 12 | *L. scutellare* |
| | | | 13 | *L. scutellare* |
| | | | 14 | *L. scutellare* |
| | | | 15 | *L. scutellare* |
| | | p4 | 16 | *L. scutellare* |
| | | | 17 | *L. scutellare* |
| | | | 18 | *L. scutellare* |
| | | | 19 | *L. scutellare* |
| | | | 20 | *L. scutellare* |
| | | p5 | 21 | *L. scutellare* |
| | | | 22 | *L. scutellare* |
| | | | 23 | *L. scutellare* |
| | | | 24 | *L. scutellare* |
| | | | 25 | *L. scutellare* |
| | | p6 | 26 | *L. scutellare* |
| 9 | 2 | | 1 | *L. scutellare* |
| | | | 2 | *L. scutellare* |
| | | | 3 | *L. scutellare* |
| | | | 4 | *L. scutellare* |
| | | p7 | 5 | *L. scutellare* |
| | | | 6 | *L. scutellare* |
| | | | 7 | *L. scutellare* |
| | | | 8 | *L. scutellare* |
| | | | 9 | *L. scutellare* |
| 9 | 3 | p8 | 1 | *N. gardellai* |
| | | | 2 | *E. koreaensis* |
| | | | 3 | *E. koreaensis* |
| | | | 4 | *N. gardellai* |
| | | | 5 | *E. koreaensis* |
| | | p9 | 6 | *E. koreaensis* |
| | | | 7 | *E. koreaensis* |
| | | | 8 | *E. koreaensis* |
| | | | 9 | *E. koreaensis* |
| | | | 10 | *E. koreaensis* |
| | | p10 | 11 | *N. gardellai* |
| | | | 12 | *N. gardellai* |
| | | | 13 | *L. scutellare* |
| | | | 14 | *E. koreaensis* |
| | | | 15 | *N. gardellai* |
| | | p11 | 16 | *E. koreaensis* |
| | | | 17 | *E. koreaensis* |
| | | | 18 | *L. scutellare* |
| | | | 19 | *N. gardellai* |
| | | p151 | 30 | *L. scutellare* |
| 11 | 22 | | 1 | *L. scutellare* |
| | | | 2 | *E. koreaensis* |
| | | p152 | 3 | *L. scutellare* |
| | | | 4 | *L. scutellare* |
| | | | 5 | *L. scutellare* |
| | | | 6 | *L. scutellare* |
| | | | 7 | *E. koreaensis* |
| | | p153 | 8 | *E. koreaensis* |
| | | | 9 | *L. scutellare* |
| | | | 10 | *E. koreaensis* |
| | | | 11 | *L. scutellare* |
| | | | 12 | *E. koreaensis* |
| | | p154 | 13 | *L. scutellare* |
| | | | 14 | *L. scutellare* |
| | | | 15 | *L. scutellare* |
| | | | 16 | *L. scutellare* |
| | | | 17 | *L. scutellare* |
| | | p155 | 18 | *L. pallidum* |
| | | | 19 | *L. scutellare* |
| | | | 20 | *L. pallidum* |
| | | | 21 | *L. scutellare* |
| | | | 22 | *L. scutellare* |
| | | p156 | 23 | *L. pallidum* |
| | | | 24 | *L. pallidum* |
| | | | 25 | *L. scutellare* |
| | | | 26 | *L. scutellare* |
| | | | 27 | *L. scutellare* |
| | | p157 | 28 | *L. scutellare* |
| | | | 29 | *L. scutellare* |
| | | | 30 | *L. scutellare* |
| 11 | 23 | | 1 | *L. scutellare* |
| | | | 2 | *L. pallidum* |
| | | p158 | 3 | *L. orientale* |
| | | | 4 | *L. orientale* |
| | | | 5 | *L. palpale* |
| | | | 6 | *L. pallidum* |
| | | | 7 | *L. orientale* |
| | | p159 | 8 | *L. orientale* |
| | | | 9 | *L. orientale* |
| | | | 10 | *L. scutellare* |
| | | | 11 | *L. scutellare* |
| | | | 12 | *L. palpale* |
| | | p160 | 13 | *L. orientale* |
| | | | 14 | *L. orientale* |
| | | | 15 | *E. koreaensis* |
| | | | 16 | *L. orientale* |
| | | | 17 | *L. pallidum* |
| | | p161 | 18 | *L. orientale* |
| | | | 19 | *L. scutellare* |
| | | | 20 | *L. scutellare* |
| | | | 21 | *L. pallidum* |
| | | | 22 | *L. orientale* |
| | | p162 | 23 | *L. scutellare* |
| | | | 24 | *L. orientale* |
| | | | 25 | *L. pallidum* |
| | | | 26 | *L. orientale* |
| | | | 27 | *L. scutellare* |



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**Table 4. Oligonucleotide primers and probes used in this study**

| PCR assay | Primers and probe name (sequence) | Product size (bp) | Ref |
|---|---|---|---|
| *groEL* N-PCR (external primer) | GRO607F (5'- GAAGATGCWGTWGGWTGTACKGC-3')<br>GRO1294R (5'- AGMGCTTCWCCTTCWACRTCYTC-3') | 688 | Liz *et al*. (2000) |
| *groEL* N-PCR (internal primer) | GRO677F (5'- ATTACTCAGAGTGCTTCTCARTG -3')<br>GRO1121R (5'- TGCATACCRTCAGTYTTTTCAAC -3') | 445 | Liz *et al*. (2000) |
| *ankA* N-PCR (external primer) | ANK-F1 (5'- GAAGAAATTACAACTCCTGAAG -3')<br>ANK-R1 (5'- CAGCCAGATGCAGTAACGTG -3') | 705 | Massung *et al*. (2000) |
| *ankA* N-PCR (internal primer) | ANK-F2 (5'- TTGACCGCTGAAGCACTAAC -3')<br>ANK-R2 (5'- ACCATTTGCTTCTTGAGGAG -3') | 664 | Massung *et al*. (2000) |
| *msp2* C-PCR | MSP3F (5'- CCA GCG TTT AGC AAG ATA AGA G -3')<br>MSP3R (5'- GCC CAG TAA CAT CAT AAG C - 3') | 334 | Levin *et al*. (2002) |
| *16S* rRNA N-PCR (eternal primer) | AE1-F (5' -AAGCTTAACACATGCAAGTCGAA -3')<br>AE1-R (5'- AGTCACTGACCCAACCTTAAATG - 3') | 1406 | Oh *et al.* (2009) |
| *16S* rRNA N-PCR (internal primer) | EE-3 (5'- GTCGAACGGATTATTCTTTATAGCTTGC -3')<br>EE-4 (5'- CCCTTCCGTTAAGAAGGATCTAATCTCC - 3') | 926 | Barlough *et al.* (1996) |
| 16S rRNA N-PCR (internal primer) | HE1 (5'- CAATTGCTTATAACCTTTTGGTTATAAAT -3')<br>HE3 (5'- TATAGGTACCGTCATTATCTTCCCTAT- 3') | 390 | Barlough *et al*. (1996) |
| 16S rRNA N-PCR (external primer) | 16S-EC9-F (5'- TACCTTGTTACGACTT-3')<br>16-EC12-R (5'- TGATCCTGGCTCAGAACGAACG-3') | 1462 | Kawahara *et al.* (2006) |
| 16S rRNA N-PCR (internal primer) | 16S-IS58-62f (5'- GGA ATA GCT GTT AGA AAT GAC A-3')<br>16S-IS58-594r (5'- CTA TCC TCT CTC GAT CTC TAG TTT -3') | 488 | Kawahara *et al.* (2006) |



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**Table 5. PCR conditions used in this study**

| PCR assay | Name of primers | PCR conditions | | | | PCR product size (bp) |
|---|---|---|---|---|---|---|
| | | Denaturation (℃/min) | Annealing (℃/min) | Extension (℃/min) | Cycles | |
| groEL N-PCR (external primer) | GR0607F/ GR01294R | 95/0.5 | 54/0.5 | 72/1 | 30 | 688 |
| groEL N-PCR (internal primer) | GR0677F/ GR01121R | 95/0.5 | 57/0.5 | 72/1 | 30 | 445 |
| *ankA* N-PCR (external primer) | ANK-F1/ ANK-R1 | 95/0.5 | 53/0.5 | 72/1 | 35 | 705 |
| *ankA* N-PCR (internal primer) | ANK-F2/ ANK-R2 | 95/0.5<br>95/0.5 | 52/0.5<br>54/0.5 | 72/1<br>72/1 | 5<br>25 | 664 |
| *msp2* C-PCR | MSP3F/ MSP3R | 95/0.5<br>95/0.5 | 52/0.5<br>54/0.5 | 72/1<br>72/1 | 5<br>25 | 334 |
| *16S* rRNA N-PCR (eternal primer) | AE1-F/ AE1-R | 94/1 | 59/1 | 72/2 | 40 | 1406 |
| *16S* rRNA N-PCR (internal primer) | EE-3/ EE-4 | 94/0.5<br>94/0.5 | 54/0.5<br>56/0.5 | 72/1<br>72/1 | 5<br>30 | 926 |
| 16S rRNA N-PCR (internal primer) | HE1/ HE3 | 94/0.5<br>94/0.5 | 54/0.5<br>56/0.5 | 72/1<br>72/1 | 5<br>30 | 390 |
| 16S rRNA N-PCR (external primer) | 16S-EC9-F/ 16S-EC12-R | 94/0.5 | 41/1 | 72/0.5 | 40 | 1462 |
| *16S* rRNA N-PCR (internal primer) | 16S-IS58-62f/ 16S-IS58-594r | 94/0.5 | 54/1 | 72/0.5 | | 488 |



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**Table 6. Sequencing result and identification of A. phagocytophilum from mite pool**

**(by GROEL 1121R/GROEL667F; groEL N-PCR)**

| Sample Name | Length (bp) | Sequence (Gro1121R/GRO677F) | BLAST result |
|---|---|---|---|
| Mite pool 46 | 351 | CAGTTTTTTCAACTTCTAGATCCTTGAAGCCTTTGCTTTCTTCAACAGTTATAACACCGTCTTTACCGACTTCTTTAACACACTGTGCAATCTTACTTCCTATGTTCTTGTCTCCATTTGCAGACAATGTTGCAACTTGTGCAATTTCGTCTTCTTCTACTTCACGTCTCATAGACATCAGCGCTGTAAGAACCGCTTCCTTAGCCTTGAGAATACCATTCTTTATGCTAACAATATCAGATCCAGCAGCTTTCGCTTTTGAGACTTCTTCAATCACTTTTGCTGTCAGTATGGAGCATGTAGTAGTTCCATCTCCCACCTTATCATTACACTGAGAAGCATCATGAGTAA | gb\|JQ622144.1\| Uncultured Anaplasma sp. clone lpl1-2 heat shock ... 632 1e-177<br>gb\|HM752098.1\| Anaplasma phagocytophilum isolate KWDAPg GroEL ge... 632 1e-177<br>gb\|HM366578.1\| Anaplasma phagocytophilum isolate Kh-lp160 groESL... 632 1e-177<br>gb\|HM366577.1\| Anaplasma phagocytophilum isolate Kh-lp144 groESL... 632 1e-177<br>gb\|HM366575.1\| Anaplasma phagocytophilum isolate Kh-lp7 groESL o... 632 1e-177<br>gb\|HM366572.1\| Anaplasma phagocytophilum isolate Irk-lp662 groES... 632 1e-177<br>gb\|KJ832462.1\| Uncultured Anaplasma sp. clone MP-CH-61 heat shoc... 627 5e-176<br>gb\|KJ832461.1\| Uncultured Anaplasma sp. clone MP-CH-39 heat shoc... 627 5e-176<br>gb\|KJ832460.1\| Uncultured Anaplasma sp. clone MP-CH-16 heat shoc... 627 5e-176 |
| Mite pool 47 | 351 | CAGTTTTTTCAACTTCTAGATCCTTGAAGCCTTTGCTTTCTTCAACAGTTATAACACCGTCTTTACCGACTTCTTTAACACACTGTGCAATCTTACTTCCTATGTTCTTGTCTCCATTTGCAGACAATGTTGCAACTTGTGCAATTTCGTCTTCTTCTACTTCACGTCTCATAGACATCAGCGCTGTAAGAACCGCTTCCTTAGCCTTGAGAATACCATTCTTTATGCTAACAATATCAGATCCAGCAGCTTTCGCTTTTGAGACTTCTTCAATCACTTTTGCTGTCAGTATGGAGCATGTAGTAGTTCCATCTCCCACCTTATCATTACACTGAGAAGCATCATGAGTAA | gb\|JQ622144.1\| Uncultured Anaplasma sp. clone lpl1-2 heat shock ... 632 1e-177<br>gb\|HM752098.1\| Anaplasma phagocytophilum isolate KWDAPg GroEL ge... 632 1e-177<br>gb\|HM366578.1\| Anaplasma phagocytophilum isolate Kh-lp160 groESL... 632 1e-177<br>gb\|HM366577.1\| Anaplasma phagocytophilum isolate Kh-lp144 groESL... 632 1e-177<br>gb\|HM366575.1\| Anaplasma phagocytophilum isolate Kh-lp7 groESL o... 632 1e-177<br>gb\|HM366572.1\| Anaplasma phagocytophilum isolate Irk-lp662 groES... 632 1e-177<br>gb\|KJ832462.1\| Uncultured Anaplasma sp. clone MP-CH-61 heat shoc... 627 5e-176<br>gb\|KJ832461.1\| Uncultured Anaplasma sp. clone MP-CH-39 heat shoc... 627 5e-176<br>gb\|KJ832460.1\| Uncultured Anaplasma sp. clone MP-CH-16 heat shoc... 627 5e-176 |
| Mite pool 111 | 347 | CCGTCAGTTTTTTCAACTTCTAGATCCTTGAAGCCTTTGCTTTCTTCAACAGTTATAACACCGTCTTTACCGACTTCTTTAACACACTGTGCAATCTTACTTCCTATGTTCTTGTCTCCATTTGCAGACAATGTTGCAACTTGTGCAATTTCGTCTTCTTCTACTTCACGTCTCATAGACATCAGCGCTGTAAGAACCGCTTCCTTAGCCTTGAGAATACCATTCTTTATGCTAACAATATCAGATCCAGCAGCTTTCGCTTTTGAGACTTCTTCAATCACTTTTGCTGTCAGTATGGAGCATGTAGTAGTTCCATCTCCCACCTTATCATTACACTGAGAAGCATC | gb\|JQ622144.1\| Uncultured Anaplasma sp. clone lpl1-2 heat shock ... 627 5e-176<br>gb\|HM752098.1\| Anaplasma phagocytophilum isolate KWDAPg GroEL ge... 627 5e-176<br>gb\|HM366578.1\| Anaplasma phagocytophilum isolate Kh-lp160 groESL... 627 5e-176<br>gb\|HM366577.1\| Anaplasma phagocytophilum isolate Kh-lp144 groESL... 627 5e-176<br>gb\|HM366575.1\| Anaplasma phagocytophilum isolate Kh-lp7 groESL o... 627 5e-176<br>gb\|HM366572.1\| Anaplasma phagocytophilum isolate Irk-lp662 groES... 627 5e-176<br>gb\|KJ832462.1\| Uncultured Anaplasma sp. clone MP-CH-61 heat shoc... 621 2e-174<br>gb\|KJ832461.1\| Uncultured Anaplasma sp. clone MP-CH-39 heat shoc... 621 2e-174<br>gb\|KJ832460.1\| Uncultured Anaplasma sp. clone MP-CH-16 heat shoc... 621 2e-174 |
| Mite pool 113 | 340 | CAGTTTTTTCAACTTCTAGATCCTTGAAGCCTTTGCTTTCTTCAACAGTTATAACACCGTCTTTACCGACTTCTTTAACACACTGTGCAATCTTACTTCCTATGTTCTTGTCTCCATTTGCAGACAATGTTGCAACTTGTGCAATTTCGTCTTCTTCTACTTCACGTCTCATAGACATCAGCGCTGTAAGAACCGCTTCCTTAGCCTTGAGAATACCATTCTTTATGCTAACAATATCAGATCCAGCAGCTTTCGCTTTTGAGACTTCTTCAATCACTTTTGCTGTCAGTATGGAGCATGTAGTAGTTCCATCTCCCACCTTATCATTACACTGAGAAGC | gb\|JQ622144.1\| Uncultured Anaplasma sp. clone lpl1-2 heat shock ... 623 6e-175<br>gb\|HM752098.1\| Anaplasma phagocytophilum isolate KWDAPg GroEL ge... 623 6e-175<br>gb\|HM366578.1\| Anaplasma phagocytophilum isolate Kh-lp160 groESL... 623 6e-175<br>gb\|HM366577.1\| Anaplasma phagocytophilum isolate Kh-lp144 groESL... 623 6e-175<br>gb\|HM366575.1\| Anaplasma phagocytophilum isolate Kh-lp7 groESL o... 623 6e-175<br>gb\|HM366572.1\| Anaplasma phagocytophilum isolate Irk-lp662 groES... 623 6e-175<br>gb\|KJ832462.1\| Uncultured Anaplasma sp. clone MP-CH-61 heat shoc... 617 3e-173<br>gb\|KJ832461.1\| Uncultured Anaplasma sp. clone MP-CH-39 heat shoc... 617 3e-173<br>gb\|KJ832460.1\| Uncultured Anaplasma sp. clone MP-CH-16 heat shoc... 617 3e-173 |
| Mite pool 114 | 341 | CAGTTTTTTCAACTTCTAGATCCTTGAAGCCTTTGCTTTCTTCAACAGTTATAACACCGTCTTTACCGACTTCTTTAACACACTGTGCAATCTTACTTCCTATGTTCTTGTCTCCATTTGCAGACAATGTTGCAACTTGTGCAATTTCGTCTTCTTCTACTTCACGTCTCATAGACATCAGCGCTGTAAGAACCGCTTCCTTAGCCTTGAGAATACCATTCTTTATGCTAACAATATCAGATCCAGCAGCTTTCGCTTTTGAGACTTCTTCAATCACTTTTGCTGTCAGTATGGAGCATGTAGTAGTTCCATCTCCCACCTTATCATTACACTGAGAAGCA | gb\|JQ622144.1\| Uncultured Anaplasma sp. clone lpl1-2 heat shock ... 625 2e-175<br>gb\|HM752098.1\| Anaplasma phagocytophilum isolate KWDAPg GroEL ge... 625 2e-175<br>gb\|HM366578.1\| Anaplasma phagocytophilum isolate Kh-lp160 groESL... 625 2e-175<br>gb\|HM366577.1\| Anaplasma phagocytophilum isolate Kh-lp144 groESL... 625 2e-175<br>gb\|HM366575.1\| Anaplasma phagocytophilum isolate Kh-lp7 groESL o... 625 2e-175<br>gb\|HM366572.1\| Anaplasma phagocytophilum isolate Irk-lp662 groES... 625 2e-175<br>gb\|KJ832462.1\| Uncultured Anaplasma sp. clone MP-CH-61 heat shoc... 619 8e-174<br>gb\|KJ832461.1\| Uncultured Anaplasma sp. clone MP-CH-39 heat shoc... 619 8e-174<br>gb\|KJ832460.1\| Uncultured Anaplasma sp. clone MP-CH-16 heat shoc... 619 8e-174<br>gb\|KJ832458.1\| Uncultured Anaplasma sp. clone CRL3 heat shock pr... 619 8e-174 |



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**Table 7. Sequencing result and Identification of *A. phagocytophilum* and *C.* Neoehrlichia from 7 spleen samples (by GROEL 1121R/GROEL 667F; *groEL* N-PCR)**

| Sample Name | Length (bp) | Sequence (Gro1121R/GRO677F) | BLAST result |
|---|---|---|---|
| w/t mouse-10-3 spleen | 346 | ATGCTTCTCAGTGTAATGATAAGGTGGGAGATGGAACTACTA<br>CATGCTCCATACTGACAGCAAAAGTGATTGAAGAAGTCTCAA<br>AAGCGAAAGCTGCTGGATCTGATATTGTTAGCATAAAGAATG<br>GTATTCTCAAGGCTAAGGAAGCGGTTCTTACAGCGCTGATGT<br>CTATGAGACGTGAAGTAGAAGAAGACGAAATTGCACAAGTT<br>GCAACATTGTCTGCAAATGGAGACAAGAACATAGGAAGTAA<br>GATTGCACAGTGTGTTAAAGAAGTCGGTAAAGACGGTGTTAT<br>AACTGTTGAAGAAAGCAAAGGCTTCAAGGATCTAGAAGTTG<br>AAAAAACTGACGG | gb\|JQ622144.1\| Uncultured Anaplasma sp. clone lpl1-2 heat shock ... 627 5e-176<br>gb\|HM752098.1\| Anaplasma phagocytophilum isolate KWDAPg GroEL ge... 627 5e-176<br>gb\|HM366578.1\| Anaplasma phagocytophilum isolate Kh-lpl60 groESL... 627 5e-176<br>gb\|HM366577.1\| Anaplasma phagocytophilum isolate Kh-lpl44 groESL... 627 5e-176<br>gb\|HM366575.1\| Anaplasma phagocytophilum isolate Kh-lp7 groESL o... 627 5e-176<br>gb\|HM366572.1\| Anaplasma phagocytophilum isolate lrk-lp662 groES... 627 5e-176<br>gb\|KJ832462.1\| Uncultured Anaplasma sp. clone MP-CH-61 heat shoc... 621 2e-174<br>gb\|KJ832461.1\| Uncultured Anaplasma sp. clone MP-CH-39 heat shoc... 621 2e-174<br>gb\|KJ832460.1\| Uncultured Anaplasma sp. clone MP-CH-16 heat shoc... 621 2e-174<br>gb\|KJ832458.1\| Uncultured Anaplasma sp. clone CRL3 heat shock pr... 621 2e-174<br>gb\|KJ832457.1\| Uncultured Anaplasma sp. clone MP-CH-34 heat shoc... 621 2e-174<br>gb\|KJ832453.1\| Uncultured Anaplasma sp. clone MP-CH-15 heat shoc... 621 2e-174<br>gb\|KJ832451.1\| Uncultured Anaplasma sp. clone MP-CH-17 heat shoc... 621 2e-174<br>gb\|KJ832450.1\| Uncultured Anaplasma sp. clone MP-CH-33 heat shoc... 621 2e-174 |
| w/t mouse-10-4 spleen | 352 | CTTCTCAGTGTAATGACAAAGTTGGTGATGGAACTACAACAT<br>GTTCTATTTTAACAGCTAAAGTAATCGAGGAAGTATCTAAAG<br>CTAAAGCTGCTGGAGCAGATATTATTAGTATCAAAAATGGTA<br>TCTTAAAAGCAAAGGAATTAGTATTAGAATCTTTACTTTCTAT<br>GAAACGTGATGTTTCTTCAGAAGATGAAATAGCACAGGTTGC<br>AACAATTTCTGCAAATGGAGATAAAAACATAGGTAGCAAAA<br>TTGCACAATGCGTAAAGGAAGTTGGTAAAGATGGTGTTATTA<br>CAGTTGAAGAAAGTAAGGGATTTAAAGAATTAGAAGTTGAA<br>AAAACTGACGGGTATGC | gb\|JQ359062.2\| Candidatus Neoehrlichia mikurensis isolate 2010HL... 632 1e-177<br>gb\|JQ359072.1\| Candidatus Neoehrlichia mikurensis isolate 2005JN... 632 1e-177<br>gb\|FJ966359.1\| Candidatus Neoehrlichia mikurensis clone lrk-lp65... 632 1e-177<br>gb\|KJ663733.1\| Candidatus Neoehrlichia mikurensis strain 192/08 ... 621 2e-174<br>gb\|KJ561571.1\| Candidatus Neoehrlichia mikurensis clone HU808 he... 621 2e-174<br>gb\|KJ561570.1\| Candidatus Neoehrlichia mikurensis clone MgUR hea... 621 2e-174<br>gb\|KF803997.1\| Candidatus Neoehrlichia mikurensis clone HU L10 h... 621 2e-174<br>gb\|KF447527.1\| Candidatus Neoehrlichia mikurensis heat shock pro... 621 2e-174<br>gb\|KF312363.1\| Candidatus Neoehrlichia mikurensis isolate IRK2/W... 621 2e-174<br>gb\|JQ669946.1\| Candidatus Neoehrlichia mikurensis isolate 104/08... 621 2e-174<br>gb\|EU810406.1\| Candidatus Neoehrlichia mikurensis GroEL (groEL) ... 621 2e-174<br>gb\|EU432375.1\| Candidatus Neoehrlichia mikurensis strain Canis h... 621 2e-174<br>gb\|KF849343.1\| Candidatus Neoehrlichia mikurensis heat shock pro... 617 3e-173 |
| w/t mouse-10-5 spleen | 344 | GATGCTTCTCAGTGTAATGACAAAGTTGGTGATGGAACTACA<br>ACATGTTCTATTTTAACAGCTAAAGTAATCGAGGAAGTATCT<br>AAAGCTAAAGCTGCTGGAGCAGATATTATTAGTATCAAAAAT<br>GGTATCTTAAAAGCAAAGGAATTAGTATTAGAATCTTTACTT<br>TCTATGAAACGTGATGTTTCTTCAGAAGATGAAATAGCACAG<br>GTTGCAACAATTTCTGCAAATGGAGATAAAAACATAGGTAGC<br>AAAATTGCACAATGCGTAAAGGAAGTTGGTAAAGATGGTGTT<br>ATTACAGTTGAAGAAAGTAAGGGATTTAAAGAATTAGAAGTT<br>GAAAAAAC | gb\|JQ359062.2\| Candidatus Neoehrlichia mikurensis isolate 2010HL... 627 5e-176<br>gb\|JQ359072.1\| Candidatus Neoehrlichia mikurensis isolate 2005JN... 627 5e-176<br>gb\|FJ966359.1\| Candidatus Neoehrlichia mikurensis clone lrk-lp65... 627 5e-176<br>gb\|KJ663733.1\| Candidatus Neoehrlichia mikurensis strain 192/08 ... 616 1e-172<br>gb\|KJ561571.1\| Candidatus Neoehrlichia mikurensis clone HU808 he... 616 1e-172<br>gb\|KJ561570.1\| Candidatus Neoehrlichia mikurensis clone MgUR hea... 616 1e-172<br>gb\|KF803997.1\| Candidatus Neoehrlichia mikurensis clone HU L10 h... 616 1e-172<br>gb\|KF447527.1\| Candidatus Neoehrlichia mikurensis heat shock pro... 616 1e-172<br>gb\|KF312363.1\| Candidatus Neoehrlichia mikurensis isolate IRK2/W... 616 1e-172<br>gb\|JQ669946.1\| Candidatus Neoehrlichia mikurensis isolate 104/08... 616 1e-172<br>gb\|EU810406.1\| Candidatus Neoehrlichia mikurensis GroEL (groEL) ... 616 1e-172<br>gb\|EU432375.1\| Candidatus Neoehrlichia mikurensis strain Canis h... 616 1e-172 |
| w/t mouse-10-6 spleen | 348 | TGCTTCTCAGTGTAATGACAAAGTTGGTGATGGAACTACAAC<br>ATGTTCTATTTTAACAGCTAAAGTAATCGAGGAAGTATCTAA<br>AGCTAAAGCTGCTGGAGCAGATATTATTAGTATCAAAAATGG<br>TATCTTAAAAGCAAAGGAATTAGTATTAGAATCTTTACTTTCT<br>ATGAAACGTGATGTTTCTTCAGAAGATGAAATAGCACAGGTT<br>GCAACAATTTCTGCAAATGGAGATAAAAACATAGGTAGCAA<br>AATTGCACAATGCGTAAAGGAAGTTGGTAAAGATGGTGTTAT<br>TACAGTTGAAGAAAGTAAGGGATTTAAAGAATTAGAAGTTG<br>AAAAAACTGACGG | gb\|JQ359062.2\| Candidatus Neoehrlichia mikurensis isolate 2010HL... 632 1e-177<br>gb\|JQ359072.1\| Candidatus Neoehrlichia mikurensis isolate 2005JN... 632 1e-177<br>gb\|FJ966359.1\| Candidatus Neoehrlichia mikurensis clone lrk-lp65... 632 1e-177<br>gb\|KJ663733.1\| Candidatus Neoehrlichia mikurensis strain 192/08 ... 621 2e-174<br>gb\|KJ561571.1\| Candidatus Neoehrlichia mikurensis clone HU808 he... 621 2e-174<br>gb\|KJ561570.1\| Candidatus Neoehrlichia mikurensis clone MgUR hea... 621 2e-174<br>gb\|KF803997.1\| Candidatus Neoehrlichia mikurensis clone HU L10 h... 621 2e-174<br>gb\|KF447527.1\| Candidatus Neoehrlichia mikurensis heat shock pro... 621 2e-174<br>gb\|KF312363.1\| Candidatus Neoehrlichia mikurensis isolate IRK2/W... 621 2e-174<br>gb\|JQ669946.1\| Candidatus Neoehrlichia mikurensis isolate 104/08... 621 2e-174<br>gb\|EU810406.1\| Candidatus Neoehrlichia mikurensis GroEL (groEL) ... 621 2e-174<br>gb\|EU432375.1\| Candidatus Neoehrlichia mikurensis strain Canis h... 621 2e-174<br>gb\|KF849343.1\| Candidatus Neoehrlichia mikurensis heat shock pro... 617 3e-173 |
| w/t mouse-10-11 spleen | 357 | TACTCATGATGCTTCTCAGTGTAATGACAAAGTTGGTGATGG<br>AACTACAACATGTTCTATTTTAACAGCTAAAGTAATCGAGGA<br>AGTATCTAAAGCTAAAGCTGCTGGAGCAGATATTATTAGTAT<br>CAAAAATGGTATCTTAAAAGCAAAGGAATTAGTATTAGAATC<br>TTTACTTTCTATGAAACGTGATGTTTCTTCAGAAGATGAAATA<br>GCACAGGTTGCAACAATTTCTGCAAATGGAGATAAAAACATA<br>GGTAGCAAAATTGCACAATGCGTAAAGGAAGTTGGTAAAGA<br>TGGTGTTATTACAGTTGAAGAAAGTAAGGGATTTAAAGAATT<br>AGAAGTTGAAAAAACTGACGG | gb\|JQ359062.2\| Candidatus Neoehrlichia mikurensis isolate 2010HL... 632 1e-177<br>gb\|JQ359072.1\| Candidatus Neoehrlichia mikurensis isolate 2005JN... 632 1e-177<br>gb\|FJ966359.1\| Candidatus Neoehrlichia mikurensis clone lrk-lp65... 632 1e-177<br>gb\|KJ663733.1\| Candidatus Neoehrlichia mikurensis strain 192/08 ... 621 2e-174<br>gb\|KJ561571.1\| Candidatus Neoehrlichia mikurensis clone HU808 he... 621 2e-174<br>gb\|KJ561570.1\| Candidatus Neoehrlichia mikurensis clone MgUR hea... 621 2e-174<br>gb\|KF803997.1\| Candidatus Neoehrlichia mikurensis clone HU L10 h... 621 2e-174<br>gb\|KF447527.1\| Candidatus Neoehrlichia mikurensis heat shock pro... 621 2e-174<br>gb\|KF312363.1\| Candidatus Neoehrlichia mikurensis isolate IRK2/W... 621 2e-174<br>gb\|JQ669946.1\| Candidatus Neoehrlichia mikurensis isolate 104/08... 621 2e-174<br>gb\|EU810406.1\| Candidatus Neoehrlichia mikurensis GroEL (groEL) ... 621 2e-174<br>gb\|EU432375.1\| Candidatus Neoehrlichia mikurensis strain Canis h... 621 2e-174<br>gb\|KF849343.1\| Candidatus Neoehrlichia mikurensis heat shock pro... 617 3e-173<br>gb\|JX392803.1\| Candidatus Neoehrlichia mikurensis clone MYT-01 6... 616 1e-172<br>gb\|HM045824.1\| Uncultured Candidatus Neoehrlichia sp. clone 1 he... 610 5e-171<br>dbj\|AB204864.1\| Candidatus Neoehrlichia mikurensis GroEL gene fo... 582 1e-162<br>dbj\|AB074461.1\| Candidatus Neoehrlichia mikurensis GroEL gene fo... 566 1e-157<br>gb\|JQ359067.1\| Candidatus Neoehrlichia mikurensis isolate 2006VN... 560 5e-156 |
| w/t mouse-10-12 spleen | 353 | TGCTTCTCAGTGTAATGATAAGGTGGGAGATGGAACTACTAC<br>ATGCTCCATACTGACAGCAAAAGTGATTGAAGAAGTCTCAAA<br>AGCGAAAGCTGCTGGATCTGATATTGTTAGCATAAAGAATGG<br>TATTCTCAAGGCTAAGGAAGCGGTTCTTACAGCGCTGATGTC<br>TATGAGACGTGAAGTAGAAGAAGACGAAATTGCACAAGTTG<br>CAACATTGTCTGCAAATGGAGACAAGAACATAGGAAGTAAG<br>ATTGCACAGTGTGTTAAAGAAGTCGGTAAAGACGGTGTTATA<br>ACTGTTGAAGAAAGCAAAGGCTTCAAGGATCTAGAAGTTGA<br>AAAAACTGACGGGTATGCAA | gb\|JQ622144.1\| Uncultured Anaplasma sp. clone lpl1-2 heat shock ... 632 1e-177<br>gb\|HM752098.1\| Anaplasma phagocytophilum isolate KWDAPg GroEL ge... 632 1e-177<br>gb\|HM366578.1\| Anaplasma phagocytophilum isolate Kh-lpl60 groESL... 632 1e-177<br>gb\|HM366577.1\| Anaplasma phagocytophilum isolate Kh-lpl44 groESL... 632 1e-177<br>gb\|HM366575.1\| Anaplasma phagocytophilum isolate Kh-lp7 groESL o... 632 1e-177<br>gb\|HM366572.1\| Anaplasma phagocytophilum isolate lrk-lp662 groES... 632 1e-177<br>gb\|KJ832462.1\| Uncultured Anaplasma sp. clone MP-CH-61 heat shoc... 627 5e-176<br>gb\|KJ832461.1\| Uncultured Anaplasma sp. clone MP-CH-39 heat shoc... 627 5e-176<br>gb\|KJ832460.1\| Uncultured Anaplasma sp. clone MP-CH-16 heat shoc... 627 5e-176<br>gb\|KJ832458.1\| Uncultured Anaplasma sp. clone CRL3 heat shock pr... 627 5e-176<br>gb\|KJ832457.1\| Uncultured Anaplasma sp. clone MP-CH-34 heat shoc... 627 5e-176<br>gb\|KJ832453.1\| Uncultured Anaplasma sp. clone MP-CH-15 heat shoc... 627 5e-176 |
| w/t mouse-10-13 spleen | 345 | TGCTTCTCAGTGTAATGATAAGGTGGGAGATGGAACTACTAC<br>ATGCTCCATACTGACAGCAAAAGTGATTGAAGAAGTCTCAAA<br>AGCGAAAGCTGCTGGATCTGATATTGTTAGCATAAAGAATGG<br>TATTCTCAAGGCTAAGGAAGCGGTTCTTACAGCGCTGATGTC<br>TATGAGACGTGAAGTAGAAGAAGACGAAATTGCACAAGTTG<br>CAACATTGTCTGCAAATGGAGACAAGAACATAGGAAGTAAG<br>ATTGCACAGTGTGTTAAAGAAGTCGGTAAAGACGGTGTTATA<br>ACTGTTGAAGAAAGCAAAGGCTTCAAGGATCTAGAAGTTGA<br>AAAAACTGACGG | gb\|JQ622144.1\| Uncultured Anaplasma sp. clone lpl1-2 heat shock ... 627 5e-176<br>gb\|HM752098.1\| Anaplasma phagocytophilum isolate KWDAPg GroEL ge... 627 5e-176<br>gb\|HM366578.1\| Anaplasma phagocytophilum isolate Kh-lpl60 groESL... 627 5e-176<br>gb\|HM366577.1\| Anaplasma phagocytophilum isolate Kh-lpl44 groESL... 627 5e-176<br>gb\|HM366575.1\| Anaplasma phagocytophilum isolate Kh-lp7 groESL o... 627 5e-176<br>gb\|HM366572.1\| Anaplasma phagocytophilum isolate lrk-lp662 groES... 627 5e-176<br>gb\|KJ832462.1\| Uncultured Anaplasma sp. clone MP-CH-61 heat shoc... 621 2e-174<br>gb\|KJ832461.1\| Uncultured Anaplasma sp. clone MP-CH-39 heat shoc... 621 2e-174<br>gb\|KJ832460.1\| Uncultured Anaplasma sp. clone MP-CH-16 heat shoc... 621 2e-174<br>gb\|KJ832458.1\| Uncultured Anaplasma sp. clone CRL3 heat shock pr... 621 2e-174 |



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**Table 8. Sequencing result and Identification of *A. phagocytophilum* from mouse spleen sample (by EE-4/EE-3; 16S rRNA N-PCR)**

| Sample Name | Length (bp) | Sequence (EE-4/EE-3) | BLAST result |
|---|---|---|---|
| w/t mouse-10-3 spleen | 897 | GTTTTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGC<br>GGGTCCCCGTCAATTCCTTTGAGTTTTAGTCTTGCGACCGTAGTCCC<br>CAGGCGGAGTGCTTAACGCGTTAGCTACAACACAGAGATAAAAAAT<br>CCCCACATTCAGCACTCATCGTTTACAGCGTGGACTACCAGGGTATC<br>TAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGCGTCAGTACCGG<br>ACCAGATAGCCGCCTTCGCCACTGGTGTTCCTCCTAATATCTACGAA<br>TTTCACCTCTACACTAGGAATTCCGCTATCCTCTCCCGGACTCTAGT<br>CTGGCAGTATTAAAAGCAGCTCCAGGGTTAAGCCCTGGCATTTCAC<br>CTTTAACTTACCGAACCGCCTACATGCCCTTTACGCCCAATAATTCC<br>GAACAACGCTTGCCCCCTCCGTATTACCGCGGCTGCTGGCACGGAG<br>TTTGCCGGGACTTCTTCTGTAGGTACCGTCATTATCTTCCCTACTGA<br>AAGAGTTTTACAACCCTAAGGCCTTCCTCACTCACGCGGCATAGCT<br>GGATCAGGCTTGCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCC<br>GTAGGAGTCTGGACCGTATCTCAGTTCCAGTGTGGCTGATCATCCTC<br>TCAGACCAGCTATAGATCATCGCCTTGGTAGGCCTTTACCCTACCAA<br>CTAGCTAATCTAACATAGGCTCATCTAATAGCGATAAATCTTTCCCC<br>CGCAGGGATTATACAGTATTACCCACCATTTCTAGTGGCTATCCCAT<br>ACTACTAGGTAGATTCCTATGCATTACTCACCCGTCTGCCACTAATT<br>ATCCTCCATAGCAAGCTATAAAGAATAATCCGTTCGACTTGCATGT<br>GTTAAGCTT | gb\|AF205140.1\|AF205140 Ehrlichia sp. 'HGE agent' 16S ribosomal R... 1657 0.0<br>emb\|AJ242784.1\| Ehrlichia sp. 16S rRNA gene, partial, Swedish Ti... 1646 0.0<br>gb\|KC800983.1\| Anaplasma phagocytophilum isolate S40 16S ribosom... 1640 0.0<br>gb\|AF241532.1\|AF241532 Ehrlichia sp. 'LGE agent' 16S ribosomal R... 1640 0.0<br>emb\|AJ242783.1\| Ehrlichia sp. 16S rRNA gene, partial, Swedish Ti... 1640 0.0<br>gb\|CP006618.1\| Anaplasma phagocytophilum str. Dog2 genome 1635 0.0<br>gb\|CP006617.1\| Anaplasma phagocytophilum str. JM, complete genome 1635 0.0<br>gb\|CP006616.1\| Anaplasma phagocytophilum str. HZ2, complete genome 1635 0.0<br>gb\|KC800985.1\| Anaplasma phagocytophilum isolate 9B13 16S riboso... 1635 0.0<br>gb\|KC470064.1\| Anaplasma phagocytophilum strain HN 16S ribosomal... 1635 0.0<br>gb\|AY055469.3\| Anaplasma phagocytophilum 16S ribosomal RNA gene... 1635 0.0<br>gb\|AY527213.1\| Anaplasma phagocytophilum strain Susy 16S ribosom... 1635 0.0<br>gb\|CP000235.1\| Anaplasma phagocytophilum HZ, complete genome 1635 0.0<br>emb\|AJ242785.1\| Ehrlichia sp. 16S rRNA gene, partial, Swedish Ti... 1635 0.0<br>ref\|NR_044762.1\| Anaplasma phagocytophilum strain Webster 16S ri... 1635 0.0 |
| w/t mouse-11-12 spleen | 895 | TAAGCTTAACACATGCAAGTCGAACGGATTATTCTTTATAGCTTGCT<br>ATGGAGGATAATTAGTGGCAGACGGGTGAGTAATGCATAGGAATCT<br>ACCTAGTAGTATGGGATAGCCACTAGAAATGGTGGGTAATACTGTA<br>TAATCCCTGCGGGGGAAAGATTTATCGCTATTAGATGAGCCTATGTT<br>AGATTAGCTAGTTGGTAGGGTAAAGGCCTACCAAGGCGATGATCTA<br>TAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGATACGG<br>TCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGG<br>CGCAAGCCTGATCCAGCTATGCCGCGTGAGTGAGGAAGGCCTTAGG<br>GTTGTAAAACTCTTTCAGTAGGGAAGATAATGACGGTACCTACAGA<br>AGAAGTCCCGGCAAACTCCGTGCCAGCAGCCGCGGTAATACGGAG<br>GGGGCAAGCGTTGTTCGGAATTATTGGGCGTAAAGGGCATGTAGGC<br>GGTTCGGTAAGTTAAAGGTGAAATGCCAGGGCTTAACCCTGGAGCT<br>GCTTTTAATACTGCCAGACTAGAGTCCGGGAGAGGATAGCGGAATT<br>CCTAGTGTAGAGGTGAAATTCGTAGATATTAGGAGGAACACCAGTG<br>GCGAAGGCGGCTATCTGGTCCGGTACTGACGCTGAGGTGCGAAAGC<br>GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAA<br>CGATGAGTGCTGAATGTGGGGATTTTTTATCTCTGTGTTGTAGCTAA<br>CGCGTTAAGCACTCCGCCTGGGGACTACGGTCGCAAGACTAAAACT<br>CAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTT<br>AATTCGATGCAACGCGAAA | gb\|AF205140.1\|AF205140 Ehrlichia sp. 'HGE agent' 16S ribosomal R... 1652 0.0<br>emb\|AJ242784.1\| Ehrlichia sp. 16S rRNA gene, partial, Swedish Ti... 1640 0.0<br>gb\|KC800983.1\| Anaplasma phagocytophilum isolate S40 16S ribosom... 1635 0.0<br>gb\|AF241532.1\|AF241532 Ehrlichia sp. 'LGE agent' 16S ribosomal R... 1635 0.0<br>emb\|AJ242783.1\| Ehrlichia sp. 16S rRNA gene, partial, Swedish Ti... 1635 0.0<br>gb\|CP006618.1\| Anaplasma phagocytophilum str. Dog2 genome 1629 0.0<br>gb\|CP006617.1\| Anaplasma phagocytophilum str. JM, complete genome 1629 0.0<br>gb\|CP006616.1\| Anaplasma phagocytophilum str. HZ2, complete genome 1629 0.0<br>gb\|KC800985.1\| Anaplasma phagocytophilum isolate 9B13 16S riboso... 1629 0.0<br>gb\|KC470064.1\| Anaplasma phagocytophilum strain HN 16S ribosomal... 1629 0.0<br>gb\|AY055469.3\| Anaplasma phagocytophilum 16S ribosomal RNA gene,... 1629 0.0<br>gb\|AY527213.1\| Anaplasma phagocytophilum strain Susy 16S ribosom... 1629 0.0<br>gb\|CP000235.1\| Anaplasma phagocytophilum HZ, complete genome 1629 0.0<br>gb\|AF189153.1\|AF189153 Ehrlichia sp. 'HGE agent' 16S ribosomal R... 1629 0.0<br>emb\|AJ242785.1\| Ehrlichia sp. 16S rRNA gene, partial, Swedish Ti... 1629 0.0 |
| w/t mouse-11-13 spleen | 896 | GTTTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCG<br>GGTCCCCGTCAATTCCTTTGAGTTTTAGTCTTGCGACCGTAGTCCCC<br>AGGCGGAGTGCTTAACGCGTTAGCTACAACACAGAGATAAAAAATC<br>CCCACATTCAGCACTCATCGTTTACAGCGTGGACTACCAGGGTATCT<br>AATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGCGTCAGTACCGGA<br>CCAGATAGCCGCCTTCGCCACTGGTGTTCCTCCTAATATCTACGAAT<br>TTCACCTCTACACTAGGAATTCCGCTATCCTCTCCCGGACTCTAGTC<br>TGGCAGTATTAAAAGCAGCTCCAGGGTTAAGCCCTGGCATTTCACC<br>TTTAACTTACCGAACCGCCTACATGCCCTTTACGCCCAATAATTCCG<br>AACAACGCTTGCCCCCTCCGTATTACCGCGGCTGCTGGCACGGAGT<br>TTGCCGGGACTTCTTCTGTAGGTACCGTCATTATCTTCCCTACTGAA<br>AGAGTTTTACAACCCTAAGGCCTTCCTCACTCACGCGGCATAGCTG<br>GATCAGGCTTGCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCG<br>TAGGAGTCTGGACCGTATCTCAGTTCCAGTGTGGCTGATCATCCTCT<br>CAGACCAGCTATAGATCATCGCCTTGGTAGGCCTTTACCCTACCAAC<br>TAGCTAATCTAACATAGGCTCATCTAATAGCGATAAATCTTTCCCCC<br>GCAGGGATTATACAGTATTACCCACCATTTCTAGTGGCTATCCCATA<br>CTACTAGGTAGATTCCTATGCATTACTCACCCGTCTGCCACTAATTA<br>TCCTCCATAGCAAGCTATAAAGAATAATCCGTTCGACTTGCATGTGT<br>TAAGCTT | gb\|AF205140.1\|AF205140 Ehrlichia sp. 'HGE agent' 16S ribosomal R... 1653 0.0<br>emb\|AJ242784.1\| Ehrlichia sp. 16S rRNA gene, partial, Swedish Ti... 1642 0.0<br>gb\|KC800983.1\| Anaplasma phagocytophilum isolate S40 16S ribosom... 1637 0.0<br>gb\|AF241532.1\|AF241532 Ehrlichia sp. 'LGE agent' 16S ribosomal R... 1637 0.0<br>emb\|AJ242783.1\| Ehrlichia sp. 16S rRNA gene, partial, Swedish Ti... 1637 0.0<br>gb\|CP006618.1\| Anaplasma phagocytophilum str. Dog2 genome 1631 0.0<br>gb\|CP006617.1\| Anaplasma phagocytophilum str. JM, complete genome 1631 0.0<br>gb\|CP006616.1\| Anaplasma phagocytophilum str. HZ2, complete genome 1631 0.0<br>gb\|KC800985.1\| Anaplasma phagocytophilum isolate 9B13 16S riboso... 1631 0.0<br>gb\|KC470064.1\| Anaplasma phagocytophilum strain HN 16S ribosomal... 1631 0.0<br>gb\|AY055469.3\| Anaplasma phagocytophilum 16S ribosomal RNA gene,... 1631 0.0<br>gb\|AY527213.1\| Anaplasma phagocytophilum strain Susy 16S ribosom... 1631 0.0<br>gb\|CP000235.1\| Anaplasma phagocytophilum HZ, complete genome 1631 0.0<br>gb\|AF189153.1\|AF189153 Ehrlichia sp. 'HGE agent' 16S ribosomal R... 1631 0.0 |



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**Table 9. Sequencing result and identification of A. phagocytophilum from mouse spleen sample (by ANK-R2/ANK-F2; ankA N-PCR)**

| Sample Name | Length (bp) | Sequence (ANK-R2/ANK-F2) | BLAST result |
|---|---|---|---|
| w/t mouse-10-1 spleen | 632 | ACTAACGAATGGTCAATATAATGTACTGAAGACGTTAA<br>TTAAAAACAGTGCTGATGTAAATGCGTCTCCAGAACCA<br>GCTATTTCTTTGGGTATACAAGGAGGGTGCTTTCAGGG<br>GGGTAAAGCTATAAAGCATTTAACGCGTGTTGTAGAAG<br>CTGGGGCATCTATAAATGCTCCTACCGGATCTATGAGCC<br>CTTTAGCTGCTGCAGTTCAAGCGGCAAATGAGACAAG<br>TAACCCTAAAGCGGCTAATAGGATTGTAAATTTCCTTTT<br>ACAGAGGGGTGCAGATCTTTCGTCTACGGATCACAGT<br>GGAACTCCAGCCTTACATTTAGCAACAGCCGCTGGCA<br>ACCATAAGACTGCTAGGTTGCTCTTGGATAAAGGGGCT<br>CCAGCAACGCAGAGAGATGCTAGTGGTAGGACGGCTT<br>TACATGTAGCAGCTGCCAATGGTGACGGTAAGCTATAT<br>AAGTTCATTGCGAAAAAATGCCCAGATAGCTGTCAACC<br>ACTACATTCTCATATGGGATATACAGCGTTACATGAGGC<br>TTTATATTCTGATAAGGTTACAGAAAAATGCTTTTTAAA<br>GATGCTTAAAGAGTCTCGAAAGCATTTGACAACATCAT<br>CCCTTAAAGACTTGCTTAATGCTC | gb\|AY502606.1\| Anaplasma phagocytophilum ankyrin-related protein... 1068 0.0<br>gb\|AY502605.1\| Anaplasma phagocytophilum ankyrin-related protein... 1068 0.0<br>gb\|KJ677106.1\| Anaplasma phagocytophilum isolate gw1 ankyrin-rel... 1042 0.0<br>gb\|GU236735.1\| Anaplasma phagocytophilum isolate bison_8 AnkA (a... 974 0.0<br>gb\|KC740482.1\| Anaplasma phagocytophilum isolate cow_A262 AnkA (... 968 0.0<br>gb\|GU236718.1\| Anaplasma phagocytophilum isolate red_deer_473 An... 968 0.0<br>gb\|KJ832286.1\| Uncultured Anaplasma sp. clone RA-DO-02 ankyrin-r... 963 0.0<br>gb\|KJ832276.1\| Uncultured Anaplasma sp. clone NO-BO-22 ankyrin-r... 963 0.0<br>gb\|KJ832245.1\| Uncultured Anaplasma sp. clone BR-BO-27 ankyrin-r... 963 0.0<br>gb\|KJ832243.1\| Uncultured Anaplasma sp. clone BR-BO-22 ankyrin-r... 963 0.0<br>gb\|HQ629926.1\| Anaplasma phagocytophilum strain Rus30-13 ankryin... 963 0.0<br>gb\|GU391597.1\| Anaplasma phagocytophilum strain Violetti ankyrin... 963 0.0<br>gb\|GU236863.1\| Anaplasma phagocytophilum isolate horse_S3041_06 ... 963 0.0<br>gb\|GU236857.1\| Anaplasma phagocytophilum isolate horse_4_FR AnkA... 963 0.0<br>gb\|GU236851.1\| Anaplasma phagocytophilum isolate dog_54 AnkA (an... 963 0.0<br>gb\|GU236837.1\| Anaplasma phagocytophilum isolate dog_29 AnkA (an... 963 0.0 |
| w/t mouse-10-2 spleen | 635 | AGCACTAACGAATGGTCAATATAATGTACTGAAGACG<br>TTAATTAAAAACAGTGCTGATGTAAATGCGTCTCCAG<br>AACCAGCTATTTCTTTGGGTATACAAGGAGGGTGCTT<br>TCAGGGGGGTAAAGCTATAAAGCATTTAACGCGTGTT<br>GTAGAAGCTGGGGCATCTATAAATGCTCCTACCGGAT<br>CTATGAGCCCTTTAGCTGCTGCAGTTCAAGCGGCAAA<br>TGAGACAAGTAACCCTAAAGCGGCTAATAGGATTGTA<br>AATTTCCTTTTACAGAGGGGTGCAGATCTTTCGTCTAC<br>GGATCACAGTGGAACTCCAGCCTTACATTTAGCAACA<br>GCCGCTGGCAACCATAAGACTGCTAGGTTGCTCTTGG<br>ATAAAGGGGCTCCAGCAACGCAGAGAGATGCTAGTG<br>GTAGGACGGCTTTACATGTAGCAGCTGCCAATGGTGA<br>CGGTAAGCTATATAAGTTCATTGCGAAAAAATGCCCA<br>GATAGCTGTCAACCACTACATTCTCATATGGGATATA<br>CAGCGTTACATGAGGCTTTATATTCTGATAAGGTTAC<br>AGAAAAATGCTTTTTAAAGATGCTTAAAGAGTCTCGA<br>AAGCATTTGACAACATCATCCCTTAAAGACTTGCTTA<br>ATGCTC | gb\|AY502606.1\| Anaplasma phagocytophilum ankyrin-related protein... 1074 0.0<br>gb\|AY502605.1\| Anaplasma phagocytophilum ankyrin-related protein... 1074 0.0<br>gb\|KJ677106.1\| Anaplasma phagocytophilum isolate gw1 ankyrin-rel... 1042 0.0<br>gb\|GU236735.1\| Anaplasma phagocytophilum isolate bison_8 AnkA (a... 979 0.0<br>gb\|KC740482.1\| Anaplasma phagocytophilum isolate cow_A262 AnkA (... 974 0.0<br>gb\|GU236718.1\| Anaplasma phagocytophilum isolate red_deer_473 An... 974 0.0<br>gb\|KJ832286.1\| Uncultured Anaplasma sp. clone RA-DO-02 ankyrin-r... 968 0.0<br>gb\|KJ832276.1\| Uncultured Anaplasma sp. clone NO-BO-22 ankyrin-r... 968 0.0<br>gb\|KJ832245.1\| Uncultured Anaplasma sp. clone BR-BO-27 ankyrin-r... 968 0.0<br>gb\|KJ832243.1\| Uncultured Anaplasma sp. clone BR-BO-22 ankyrin-r... 968 0.0<br>gb\|HQ629926.1\| Anaplasma phagocytophilum strain Rus30-13 ankryin... 968 0.0<br>gb\|GU391597.1\| Anaplasma phagocytophilum strain Violetti ankyrin... 968 0.0<br>gb\|GU236863.1\| Anaplasma phagocytophilum isolate horse_S3041_06 ... 968 0.0 |
| w/t mouse-10-3 spleen | 635 | AGCACTAACGAATGGTCAATATAATGTACTGAAGACG<br>TTAATTAAAAACAGTGCTGATGTAAATGCGTCTCCAG<br>AACCAGCTATTTCTTTGGGTATACAAGGAGGGTGCTT<br>TCAGGGGGGTAAAGCTATAAAGCATTTAACGCGTGTT<br>GTAGAAGCTGGGGCATCTATAAATGCTCCTACCGGAT<br>CTATGAGCCCTTTAGCTGCTGCAGTTCAAGCGGCAAA<br>TGAGACAAGTAACCCTAAAGCGGCTAATAGGATTGTA<br>AATTTCCTTTTACAGAGGGGTGCAGATCTTTCGTCTAC<br>GGATCACAGTGGAACTCCAGCCTTACATTTAGCAACA<br>GCCGCTGGCAACCATAAGACTGCTAGGTTGCTCTTGG<br>ATAAAGGGGCTCCAGCAACGCAGAGAGATGCTAGTG<br>GTAGGACGGCTTTACATGTAGCAGCTGCCAATGGTGA<br>CGGTAAGCTATATAAGTTCATTGCGAAAAAATGCCCA<br>GATAGCTGTCAACCACTACATTCTCATATGGGATATA<br>CAGCGTTACATGAGGCTTTATATTCTGATAAGGTTAC<br>AGAAAAATGCTTTTTAAAGATGCTTAAAGAGTCTCGA<br>AAGCATTTGACAACATCATCCCTTAAAGACTTGCTTA<br>ATGCTC | gb\|AY502606.1\| Anaplasma phagocytophilum ankyrin-related protein... 1074 0.0<br>gb\|AY502605.1\| Anaplasma phagocytophilum ankyrin-related protein... 1074 0.0<br>gb\|KJ677106.1\| Anaplasma phagocytophilum isolate gw1 ankyrin-rel... 1042 0.0<br>gb\|GU236735.1\| Anaplasma phagocytophilum isolate bison_8 AnkA (a... 979 0.0<br>gb\|KC740482.1\| Anaplasma phagocytophilum isolate cow_A262 AnkA (... 974 0.0<br>gb\|GU236718.1\| Anaplasma phagocytophilum isolate red_deer_473 An... 974 0.0<br>gb\|KJ832286.1\| Uncultured Anaplasma sp. clone RA-DO-02 ankyrin-r... 968 0.0<br>gb\|KJ832276.1\| Uncultured Anaplasma sp. clone NO-BO-22 ankyrin-r... 968 0.0<br>gb\|KJ832245.1\| Uncultured Anaplasma sp. clone BR-BO-27 ankyrin-r... 968 0.0<br>gb\|KJ832243.1\| Uncultured Anaplasma sp. clone BR-BO-22 ankyrin-r... 968 0.0<br>gb\|HQ629926.1\| Anaplasma phagocytophilum strain Rus30-13 ankryin... 968 0.0<br>gb\|GU391597.1\| Anaplasma phagocytophilum strain Violetti ankyrin... 968 0.0 |



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| | | | |
|---|---|---|---|
| w/t mouse-10-4 spleen | 596 | TTATTAAAACAGTGCTGATGTAAATGCGTCTCCAGAA<br>CCAGCTATTTCTTTGGGTATACAAGGAGGGTGCTTTCA<br>GGGGGGTAAAGCTATAAAGCATTTAACGCGTGTTGTA<br>GAAGCTGGGGCATCTATAAATGCTCCTACCGGATCTA<br>TGAGCCCTTTAGCTGCTGCAGTTCAAGCGGCAAATGA<br>GACAAGTAACCCTAAAGCGGCTAATAGGATTGTAAAT<br>TTCCTTTTACAGAGGGGTGCAGATCTTTCGTCTACGGA<br>TCACAGTGGAACTCCAGCCTTACATTTAGCAACAGCC<br>GCTGGCAACCATAAGACTGCTAGGTTGCTCTTGGATA<br>AAGGGGCTCCAGCAACGCAGAGAGATGCTAGTGGTA<br>GGACGGCTTTACATGTAGCAGCTGCCAATGGTGACGG<br>TAAGCTATATAAGTTCATTGCGAAAAAATGCCCAGAT<br>AGCTGTCAACCACTACATTCTCATATGGGATATACAG<br>CGTTACATGAGGCTTTATATTCTGATAAGGTTACAGA<br>AAAATGCTTTTTAAAGATGCTTAAAGAGTCTCGAAAG<br>CATTTGACAACATCATCCCTTAAAGACTTGCTTAATGC<br>TC | gb\|KJ677106.1\| Anaplasma phagocytophilum isolate gw1 ankyrin-rel... 1026 0.0<br>gb\|AY502606.1\| Anaplasma phagocytophilum ankyrin-related protein... 1009 0.0<br>gb\|AY502605.1\| Anaplasma phagocytophilum ankyrin-related protein... 1009 0.0<br>gb\|GU236735.1\| Anaplasma phagocytophilum isolate bison_8 AnkA (a... 920 0.0<br>gb\|AF153716.2\|AF153716 Ehrlichia phagocytophila ankyrin protein-... 920 0.0<br>gb\|AF100882.1\|AF100882 Ehrlichia equi ankyrin (ank) gene, comple... 920 0.0<br>gb\|CP006618.1\| Anaplasma phagocytophilum str. Dog2 genome 915 0.0<br>gb\|CP006617.1\| Anaplasma phagocytophilum str. JM, complete genome 915 0.0<br>gb\|JN005741.1\| Anaplasma phagocytophilum isolate PZA-2 AnkA (ank... 915 0.0<br>gb\|JN005740.1\| Anaplasma phagocytophilum isolate PZA-1 AnkA (ank... 915 0.0<br>gb\|GU236806.1\| Anaplasma phagocytophilum isolate human_03HE AnkA... 915 0.0<br>gb\|GU236718.1\| Anaplasma phagocytophilum isolate red_deer_473 An... 915 0.0 |
| w/t mouse-10-8 spleen | 643 | CACTAACGAATGGTCAATATAATGTACTGAAGACGTT<br>AATTAAAAACAGTGCTGATGTAAATGCGTCTCCAGAA<br>CCAGCTATTTCTTTGGGTATACAAGGAGGGTGCTTTCA<br>GGGGGGTAAAGCTATAAAGCATTTAACGCGTGTTGTA<br>GAAGCTGGGGCATCTATAAATGCTCCTACCGGATCTA<br>TGAGCCCTTTAGCTGCTGCAGTTCAAGCGGCAAATGA<br>GACAAGTAACCCTAAAGCGGCTAATAGGATTGTAAAT<br>TTCCTTTTACAGAGGGGTGCAGATCTTTCGTCTACGGA<br>TCACAGTGGAACTCCAGCCTTACATTTAGCAACAGCC<br>GCTGGCAACCATAAGACTGCTAGGTTGCTCTTGGATA<br>AAGGGGCTCCAGCAACGCAGAGAGATGCTAGTGGTA<br>GGACGGCTTTACATGTAGCAGCTGCCAATGGTGACGG<br>TAAGCTATATAAGTTCATTGCGAAAAAATGCCCAGAT<br>AGCTGTCAACCACTACATTCTCATATGGGATATACAG<br>CGTTACATGAGGCTTTATATTCTGATAAGGTTACAGA<br>AAAATGCTTTTTAAAGATGCTTAAAGAGTCTCGAAAG<br>CATTTGACAACATCATCCCTTAAAGACTTGCTTAATGC<br>TCCTCAAGAGCC | gb\|AY502606.1\| Anaplasma phagocytophilum ankyrin-related protein... 1077 0.0<br>gb\|AY502605.1\| Anaplasma phagocytophilum ankyrin-related protein... 1077 0.0<br>gb\|KJ677106.1\| Anaplasma phagocytophilum isolate gw1 ankyrin-rel... 1042 0.0<br>gb\|GU236735.1\| Anaplasma phagocytophilum isolate bison_8 AnkA (a... 989 0.0<br>gb\|KC740482.1\| Anaplasma phagocytophilum isolate cow_A262 AnkA (... 983 0.0<br>gb\|GU236718.1\| Anaplasma phagocytophilum isolate red_deer_473 An... 983 0.0<br>gb\|KJ832286.1\| Uncultured Anaplasma sp. clone RA-DO-02 ankyrin-r... 977 0.0<br>gb\|KJ832245.1\| Uncultured Anaplasma sp. clone BR-BO-27 ankyrin-r... 977 0.0<br>gb\|HQ629926.1\| Anaplasma phagocytophilum strain Rus30-13 ankryin... 977 0.0<br>gb\|GU391597.1\| Anaplasma phagocytophilum strain Violetti ankyrin... 977 0.0<br>gb\|GU236863.1\| Anaplasma phagocytophilum isolate horse_S3041_06 ... 977 0.0<br>gb\|GU236857.1\| Anaplasma phagocytophilum isolate horse_4_FR AnkA... 977 0.0<br>gb\|GU236851.1\| Anaplasma phagocytophilum isolate dog_54 AnkA (an... 977 0.0 |
| w/t mouse-10-10 spleen | 635 | AGCACTAACGAATGGTCAATATAATGTACTGAAGACG<br>TTAATTAAAAACAGTGCTGATGTAAATGCGTCTCCAG<br>AACCAGCTATTTCTTTGGGTATACAAGGAGGGTGCTT<br>TCAGGGGGGTAAAGCTATAAAGCATTTAACGCGTGTT<br>GTAGAAGCTGGGGCATCTATAAATGCTCCTACCGGAT<br>CTATGAGCCCTTTAGCTGCTGCAGTTCAAGCGGCAAA<br>TGAGACAAGTAACCCTAAAGCGGCTAATAGGATTGTA<br>AATTTCCTTTTACAGAGGGGTGCAGATCTTTCGTCTAC<br>GGATCACAGTGGAACTCCAGCCTTACATTTAGCAACA<br>GCCGCTGGCAACCATAAGACTGCTAGGTTGCTCTTGG<br>ATAAAGGGGCTCCAGCAACGCAGAGAGATGCTAGTG<br>GTAGGACGGCTTTACATGTAGCAGCTGCCAATGGTGA<br>CGGTAAGCTATATAAGTTCATTGCGAAAAAATGCCCA<br>GATAGCTGTCAACCACTACATTCTCATATGGGATATA<br>CAGCGTTACATGAGGCTTTATATTCTGATAAGGTTAC<br>AGAAAAATGCTTTTTAAAGATGCTTAAAGAGTCTCGA<br>AAGCATTTGACAACATCATCCCTTAAAGACTTGCTTA<br>ATGCTC | gb\|AY502606.1\| Anaplasma phagocytophilum ankyrin-related protein... 1074 0.0<br>gb\|AY502605.1\| Anaplasma phagocytophilum ankyrin-related protein... 1074 0.0<br>gb\|KJ677106.1\| Anaplasma phagocytophilum isolate gw1 ankyrin-rel... 1042 0.0<br>gb\|GU236735.1\| Anaplasma phagocytophilum isolate bison_8 AnkA (a... 979 0.0<br>gb\|KC740482.1\| Anaplasma phagocytophilum isolate cow_A262 AnkA (... 974 0.0<br>gb\|GU236718.1\| Anaplasma phagocytophilum isolate red_deer_473 An... 974 0.0<br>gb\|KJ832286.1\| Uncultured Anaplasma sp. clone RA-DO-02 ankyrin-r... 968 0.0<br>gb\|KJ832276.1\| Uncultured Anaplasma sp. clone NO-BO-22 ankyrin-r... 968 0.0<br>gb\|KJ832245.1\| Uncultured Anaplasma sp. clone BR-BO-27 ankyrin-r... 968 0.0<br>gb\|KJ832243.1\| Uncultured Anaplasma sp. clone BR-BO-22 ankyrin-r... 968 0.0<br>gb\|HQ629926.1\| Anaplasma phagocytophilum strain Rus30-13 ankryin... 968 0.0<br>gb\|GU391597.1\| Anaplasma phagocytophilum strain Violetti ankyrin... 968 0.0<br>gb\|GU236863.1\| Anaplasma phagocytophilum isolate horse_S3041_06 ... 968 0.0<br>gb\|GU236857.1\| Anaplasma phagocytophilum isolate horse_4_FR AnkA... 968 0.0 |
| w/t mouse-10-11 spleen | 610 | TTAAAACAGTGCTGATGTAAATGCGTCTCCAGAACCA<br>GCTATTTCTTTGGGTATACAAGGAGGGTGCTTTCAGG<br>GGGGTAAAGCTATAAAGCATTTAACGCGTGTTGTAGA<br>AGCTGGGGCATCTATAAATGCTCCTACCGGATCTATG<br>AGCCCTTTAGCTGCTGCAGTTCAAGCGGCAAATGAGA<br>CAAGTAACCCTAAAGCGGCTAATAGGATTGTAAATTT<br>CCTTTTACAGAGGGGTGCAGATCTTTCGTCTACGGATC<br>ACAGTGGAACTCCAGCCTTACATTTAGCAACAGCCGC<br>TGGCAACCATAAGACTGCTAGGTTGCTCTTGGATAAA<br>GGGGCTCCAGCAACGCAGAGAGATGCTAGTGGTAGG<br>ACGGCTTTACATGTAGCAGCTGCCAATGGTGACGGTA<br>AGCTATATAAGTTCATTGCGAAAAAATGCCCAGATAG<br>CTGTCAACCACTACATTCTCATATGGGATATACAGCG<br>TTACATGAGGCTTTATATTCTGATAAGGTTACAGAAA<br>AATGCTTTTTAAAGATGCTTAAAGAGTCTCGAAAGCA<br>TTTGACAACATCATCCCTTAAAGACTTGCTTAATGCTC<br>CTCAAGAAGCAAATGGT | gb\|AY502606.1\| Anaplasma phagocytophilum ankyrin-related protein... 1035 0.0<br>gb\|AY502605.1\| Anaplasma phagocytophilum ankyrin-related protein... 1035 0.0<br>gb\|KJ677106.1\| Anaplasma phagocytophilum isolate gw1 ankyrin-rel... 1026 0.0<br>gb\|GU236735.1\| Anaplasma phagocytophilum isolate bison_8 AnkA (a... 952 0.0<br>gb\|AF153716.2\|AF153716 Ehrlichia phagocytophila ankyrin protein-... 952 0.0<br>gb\|AF100882.1\|AF100882 Ehrlichia equi ankyrin (ank) gene, comple... 952 0.0<br>gb\|CP006618.1\| Anaplasma phagocytophilum str. Dog2 genome 946 0.0<br>gb\|CP006617.1\| Anaplasma phagocytophilum str. JM, complete genome 946 0.0<br>gb\|JN005741.1\| Anaplasma phagocytophilum isolate PZA-2 AnkA (ank... 946 0.0<br>gb\|JN005740.1\| Anaplasma phagocytophilum isolate PZA-1 AnkA (ank... 946 0.0<br>gb\|GU236806.1\| Anaplasma phagocytophilum isolate human_03HE AnkA... 946 0.0<br>gb\|GU236718.1\| Anaplasma phagocytophilum isolate red_deer_473 An... 946 0.0 |



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| | | | |
|---|---|---|---|
| w/t mouse-10-13 spleen | 632 | ACTAACGAATGGTCAATATAATGTACTGAAGACGTTA<br>ATTAAAAACAGTGCTGATGTAAATGCGTCTCCAGAAC<br>CAGCTATTTCTTTGGGTATACAAGGAGGGTGCTTTCA<br>GGGGGGTAAAGCTATAAAGCATTTAACGCGTGTTGTA<br>GAAGCTGGGGCATCTATAAATGCTCCTACCGGATCTA<br>TGAGCCCTTTAGCTGCTGCAGTTCAAGCGGCAAATGA<br>GACAAGTAACCCTAAAGCGGCTAATAGGATTGTAAAT<br>TTCCTTTTACAGAGGGGTGCAGATCTTTCGTCTACGGA<br>TCACAGTGGAACTCCAGCCTTACATTTAGCAACAGCC<br>GCTGGCAACCATAAGACTGCTAGGTTGCTCTTGGATA<br>AAGGGGCTCCAGCAACGCAGAGAGATGCTAGTGGTA<br>GGACGGCTTTACATGTAGCAGCTGCCAATGGTGACGG<br>TAAGCTATATAAGTTCATTGCGAAAAAGTGCCCAGAT<br>AGCTGTCAACCACTACATTCTCATATGGGATATACAG<br>CGTTACATGAGGCTTTATATTCTGATAAGGTTACAGA<br>AAAATGCTTTTTAAAGATGCTTAAAGAGTCTCGAAAG<br>CATTTGACAACATCATCCCTTAAAAACTTGCTTAATGC<br>TC | gb\|AY502606.1\| Anaplasma phagocytophilum ankyrin-related protein... 1057 0.0<br>gb\|AY502605.1\| Anaplasma phagocytophilum ankyrin-related protein... 1057 0.0<br>gb\|KJ677106.1\| Anaplasma phagocytophilum isolate gw1 ankyrin-rel... 1037 0.0<br>gb\|GU236735.1\| Anaplasma phagocytophilum isolate bison_8 AnkA (a... 963 0.0<br>gb\|KC740482.1\| Anaplasma phagocytophilum isolate cow_A262 AnkA (... 957 0.0<br>gb\|GU236718.1\| Anaplasma phagocytophilum isolate red_deer_473 An... 957 0.0<br>gb\|KJ832286.1\| Uncultured Anaplasma sp. clone RA-DO-02 ankyrin-r... 952 0.0<br>gb\|KJ832276.1\| Uncultured Anaplasma sp. clone NO-BO-22 ankyrin-r... 952 0.0<br>gb\|KJ832245.1\| Uncultured Anaplasma sp. clone BR-BO-27 ankyrin-r... 952 0.0<br>gb\|KJ832243.1\| Uncultured Anaplasma sp. clone BR-BO-22 ankyrin-r... 952 0.0<br>gb\|HQ629926.1\| Anaplasma phagocytophilum strain Rus30-13 ankryin... 952 0.0<br>gb\|GU391597.1\| Anaplasma phagocytophilum strain Violetti ankyrin... 952 0.0 |
| w/t mouse-11-12 spleen | 637 | GAAGCACTAACGAATGGTCAATATAATGTACTGAAGAC<br>GTTAATTAAAAACAGTGCTGATGTAAATGCGTCTCCAG<br>AACCAGCTATTTCTTTGGGTATACAAGGAGGGTGCTTT<br>CAGGGGGGTAAAGCTATAAAGCATTTAACGCGTGTTGT<br>AGAAGCTGGGGCATCTATAAATGCTCCTACCGGATCTA<br>TGAGCCCTTTAGCTGCTGCAGTTCAAGCGGCAAATGA<br>GACAAGTAACCCTAAAGCGGCTAATAGGATTGTAAATT<br>TCCTTTTACAGAGGGGTGCAGATCTTTCGTCTACGGAT<br>CACAGTGGAACTCCAGCCTTACATTTAGCAACAGCCG<br>CTGGCAACCATAAGACTGCTAGGTTGCTCTTGGATAAA<br>GGGGCTCCAGCAACGCAGAGAGATGCTAGTGGTAGGA<br>CGGCTTTACATGTAGCAGCTGCCAATGGTGACGGTAAG<br>CTATATAAGTTCATTGCGAAAAAATGCCCAGATAGCTG<br>TCAACCACTACATTCTCATATGGGATATACAGCGTTACA<br>TGAGGCTTTATATTCTGATAAGGTTACAGAAAAATGCTT<br>TTTAAAGATGCTTAAAGAGTCTCGAAAGCATTTGACAA<br>CATCATCCCTTAAAGACTTGCTTAATGCTC | gb\|AY502606.1\| Anaplasma phagocytophilum ankyrin-related protein... 1077 0.0<br>gb\|AY502605.1\| Anaplasma phagocytophilum ankyrin-related protein... 1077 0.0<br>gb\|KJ677106.1\| Anaplasma phagocytophilum isolate gw1 ankyrin-rel... 1042 0.0<br>gb\|GU236735.1\| Anaplasma phagocytophilum isolate bison_8 AnkA (a... 983 0.0<br>gb\|KC740482.1\| Anaplasma phagocytophilum isolate cow_A262 AnkA (... 977 0.0<br>gb\|GU236718.1\| Anaplasma phagocytophilum isolate red_deer_473 An... 977 0.0<br>gb\|KJ832286.1\| Uncultured Anaplasma sp. clone RA-DO-02 ankyrin-r... 972 0.0<br>gb\|KJ832276.1\| Uncultured Anaplasma sp. clone NO-BO-22 ankyrin-r... 972 0.0<br>gb\|KJ832245.1\| Uncultured Anaplasma sp. clone BR-BO-27 ankyrin-r... 972 0.0<br>gb\|KJ832243.1\| Uncultured Anaplasma sp. clone BR-BO-22 ankyrin-r... 972 0.0<br>gb\|HQ629926.1\| Anaplasma phagocytophilum strain Rus30-13 ankryin... 972 0.0<br>gb\|GU391597.1\| Anaplasma phagocytophilum strain Violetti ankyrin... 972 0.0 |
| w/t mouse-11-13 spleen | 635 | AGCACTAACGAATGGTCAATATAATGTACTGAAGACG<br>TTAATTAAAAACAGTGCTGATGTAAATGCGTCTCCAG<br>AACCAGCTATTTCTTTGGGTATACAAGGAGGGTGCTT<br>TCAGGGGGGTAAAGCTATAAAGCATTTAACGCGTGTT<br>GTAGAAGCTGGGGCATCTATAAATGCTCCTACCGGAT<br>CTATGAGCCCTTTAGCTGCTGCAGTTCAAGCGGCAAA<br>TGAGACAAGTAACCCTAAAGCGGCTAATAGGATTGTA<br>AATTTCCTTTTACAGAGGGGTGCAGATCTTTCGTCTAC<br>GGATCACAGTGGAACTCCAGCCTTACATTTAGCAACA<br>GCCGCTGGCAACCATAAGACTGCTAGGTTGCTCTTGG<br>ATAAAGGGGCTCCAGCAACGCAGAGAGATGCTAGTG<br>GTAGGACGGCTTTACATGTAGCAGCTGCCAATGGTGA<br>CGGTAAGCTATATAAGTTCATTGCGAAAAAATGCCCA<br>GATAGCTGTCAACCACTACATTCTCATATGGGATATA<br>CAGCGTTACATGAGGCTTTATATTCTGATAAGGTTAC<br>AGAAAAATGCTTTTTAAAGATGCTTAAAGAGTCTCGA<br>AAGCATTTGACAACATCATCCCTTAAAGACTTGCTTA<br>ATGCTC | gb\|AY502606.1\| Anaplasma phagocytophilum ankyrin-related protein... 1074 0.0<br>gb\|AY502605.1\| Anaplasma phagocytophilum ankyrin-related protein... 1074 0.0<br>gb\|KJ677106.1\| Anaplasma phagocytophilum isolate gw1 ankyrin-rel... 1042 0.0<br>gb\|GU236735.1\| Anaplasma phagocytophilum isolate bison_8 AnkA (a... 979 0.0<br>gb\|KC740482.1\| Anaplasma phagocytophilum isolate cow_A262 AnkA (... 974 0.0<br>gb\|GU236718.1\| Anaplasma phagocytophilum isolate red_deer_473 An... 974 0.0<br>gb\|KJ832286.1\| Uncultured Anaplasma sp. clone RA-DO-02 ankyrin-r... 968 0.0<br>gb\|KJ832276.1\| Uncultured Anaplasma sp. clone NO-BO-22 ankyrin-r... 968 0.0<br>gb\|KJ832245.1\| Uncultured Anaplasma sp. clone BR-BO-27 ankyrin-r... 968 0.0<br>gb\|KJ832243.1\| Uncultured Anaplasma sp. clone BR-BO-22 ankyrin-r... 968 0.0<br>gb\|HQ629926.1\| Anaplasma phagocytophilum strain Rus30-13 ankryin... 968 0.0<br>gb\|GU391597.1\| Anaplasma phagocytophilum strain Violetti ankyrin... 968 0.0<br>gb\|GU236863.1\| Anaplasma phagocytophilum isolate horse_S3041_06 ... 968 0.0 |



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**Table 10. Sequencing result and identification of C. Neoehrlichia mikurensis from 4 mouse spleen sample (by 16S-IS58-62f and 16S-IS58-594r; 16S rRNA N-PCR)**

| Sample Name | Length (bp) | Sequence (16S-IS58-62f/ 16S-IS58-594r) | BLAST result |
|---|---|---|---|
| w/t mouse 10-4 | 462 | ATCTCTAGTTTAAATAGTATTAAAAGCAGYTCCAAGGTTA AGCCCTGGTATTTCACTTTTAACTTATTAAACCGCCTACAT GCCCTTTACGCCCAATAATTCCGAACAACGCTTGCCCCCT CCGTATTACCGCGGCTGCTGGCACGGAGTTTGCCGGGACT TCTTCTGCAGGTACCGTCATTATCTTCCCTGCTGAAAGAG TTTTACAACCCTAAGGCCTTCTTCACTCATGCGGCATAGC TGGATCAGGCTTGCGCCCATTGTCCAATATTCCCCACTGC TGCCTCCCGTAGGAGTCTGGACCGTATCTCAGTTCCAGTG TGGCTGATCGTCCTCTCAGACCAGCTATAGATCATAGCCT TGGTAAGCCATTACCTTACCAACTAGCTAATCTAACATAG GCTCATCTAATAGCGATAAATCTTTCCCCCGCAGGGATTA TACGGTATTACCTGTCATTTC | gb\|JQ359045.1\| Candidatus Neoehrlichia mikurensis isolate 2010HL... 850 0.0<br>gb\|JN378917.1\| Candidatus Neoehrlichia mikurensis isolate 4MBK2M... 850 0.0<br>gb\|GQ501090.2\| Uncultured Candidatus Neoehrlichia sp. clone 2 16... 850 0.0<br>gb\|FJ966360.1\| Candidatus Neoehrlichia mikurensis clone Irk-Ip65... 850 0.0<br>gb\|EU810404.1\| Candidatus Neoehrlichia mikurensis 16S ribosomal ... 850 0.0<br>dbj\|AB196305.1\| Candidatus Neoehrlichia mikurensis gene for 16S ... 850 0.0<br>dbj\|AB213021.1\| Candidatus Neoehrlichia mikurensis gene for 16S ... 850 0.0<br>gb\|AF104680.1\|AF104680 Ehrlichia-like sp. 'Schotti variant' 16S ... 850 0.0<br>gb\|AY098730.1\| Ehrlichia sp. Belluno 16S ribosomal RNA gene, par... 845 0.0 |
| w/t mouse 10-5 | 464 | CGATCTCTAGTTTAAATAGTATTAAAAGCAGCTCCAAGGT TAAGCCCTGGTATTTCACTTTTAACTTATTAAACCGCCTAC ATGCCCTTTACGCCCAATAATTCCGAACAACGCTTGCCCC CTCCGTATTACCGCGGCTGCTGGCACGGAGTTTGCCGGGA CTTCTTCTGCAGGTACCGTCATTATCTTCCCTGCTGAAAG AGTTTTACAACCCTAAGGCCTTCTTCACTCATGCGGCATA GCTGGATCAGGCTTGCGCCCATTGTCCAATATTCCCCACT GCTGCCTCCCGTAGGAGTCTGGACCGTATCTCAGTTCCAG TGTGGCTGATCGTCCTCTCAGACCAGCTATAGATCATAGC CTTGGTAAGCCATTACCTTACCAACTAGCTAATCTAACAT AGGCTCATCTAATAGCGATAAATCTTTCCCCCGCAGGGAT TATACGGTATTACCTGTCATTTC | gb\|JQ359045.1\| Candidatus Neoehrlichia mikurensis isolate 2010HL... 857 0.0<br>gb\|JN378917.1\| Candidatus Neoehrlichia mikurensis isolate 4MBK2M... 857 0.0<br>gb\|GQ501090.2\| Uncultured Candidatus Neoehrlichia sp. clone 2 16... 857 0.0<br>gb\|FJ966360.1\| Candidatus Neoehrlichia mikurensis clone Irk-Ip65... 857 0.0<br>gb\|EU810404.1\| Candidatus Neoehrlichia mikurensis 16S ribosomal ... 857 0.0<br>dbj\|AB196305.1\| Candidatus Neoehrlichia mikurensis gene for 16S ... 857 0.0<br>dbj\|AB213021.1\| Candidatus Neoehrlichia mikurensis gene for 16S ... 857 0.0<br>gb\|AF104680.1\|AF104680 Ehrlichia-like sp. 'Schotti variant' 16S ... 857 0.0<br>gb\|AY098730.1\| Ehrlichia sp. Belluno 16S ribosomal RNA gene, par... 852 0.0 |
| w/t mouse 10-6 | 469 | TTCGATCTCTAGTTTAAATAGTATTAAAAGCAGCTCCAAG GTTAAGCCCTGGTATTTCACTTTTAACTTATTAAACCGCCT ACATGCCCTTTACGCCCAATAATTCCGAACAACGCTTGCC CCCTCCGTATTACCGCGGCTGCTGGCACGGAGTTTGCCGG GACTTCTTCTGCAGGTACCGTCATTATCTTCCCTGCTGAA AGAGTTTTACAACCCTAAGGCCTTCTTCACTCATGCGGCA TAGCTGGATCAGGCTTGCGCCCATTGTCCAATATTCCCCA CTGCTGCCTCCCGTAGGAGTCTGGACCGTATCTCAGTTCC AGTGTGGCTGATCGTCCTCTCAGACCAGCTATAGATCATA GCCTTGGTAAGCCATTACCTTACCAACTAGCTAATCTAAC ATAGGCTCATCTAATAGCGATAAATCTTTCCCCCGCAGGG ATTATACGGTATTACCTGTCATTTCTAA | gb\|JQ359045.1\| Candidatus Neoehrlichia mikurensis isolate 2010HL... 865 0.0<br>gb\|JN378917.1\| Candidatus Neoehrlichia mikurensis isolate 4MBK2M... 865 0.0<br>gb\|GQ501090.2\| Uncultured Candidatus Neoehrlichia sp. clone 2 16... 865 0.0<br>gb\|FJ966360.1\| Candidatus Neoehrlichia mikurensis clone Irk-Ip65... 865 0.0<br>gb\|EU810404.1\| Candidatus Neoehrlichia mikurensis 16S ribosomal ... 865 0.0<br>dbj\|AB196305.1\| Candidatus Neoehrlichia mikurensis gene for 16S ... 865 0.0<br>dbj\|AB213021.1\| Candidatus Neoehrlichia mikurensis gene for 16S ... 865 0.0<br>gb\|AF104680.1\|AF104680 Ehrlichia-like sp. 'Schotti variant' 16S ... 865 0.0<br>gb\|AY098730.1\| Ehrlichia sp. Belluno 16S ribosomal RNA gene, par... 859 0.0 |
| w/t mouse 10-11 | 464 | GATCTCTAGTTTAAATAGTATTAAAAGCAGCTCCAAGGTT AAGCCCTGGTATTTCACTTTTAACTTATTAAACCGCCTAC ATGCCCTTTACGCCCAATAATTCCGAACAACGCTTGCCCC CTCCGTATTACCGCGGCTGCTGGCACGGAGTTTGCCGGGA CTTCTTCTGCAGGTACCGTCATTATCTTCCCTGCTGAAAG AGTTTTACAACCCTAAGGCCTTCTTCACTCATGCGGCATA GCTGGATCAGGCTTGCGCCCATTGTCCAATATTCCCCACT GCTGCCTCCCGTAGGAGTCTGGACCGTATCTCAGTTCCAG TGTGGCTGATCGTCCTCTCAGACCAGCTATAGATCATAGC CTTGGTAAGCCATTACCTTACCAACTAGCTAATCTAACAT AGGCTCATCTAATAGCGATAAATCTTTCCCCCGCAGGGAT TATACGGTATTACCTGTCATTTCT | gb\|JQ359045.1\| Candidatus Neoehrlichia mikurensis isolate 2010HL... 857 0.0<br>gb\|JN378917.1\| Candidatus Neoehrlichia mikurensis isolate 4MBK2M... 857 0.0<br>gb\|GQ501090.2\| Uncultured Candidatus Neoehrlichia sp. clone 2 16... 857 0.0<br>gb\|FJ966360.1\| Candidatus Neoehrlichia mikurensis clone Irk-Ip65... 857 0.0<br>gb\|EU810404.1\| Candidatus Neoehrlichia mikurensis 16S ribosomal ... 857 0.0<br>dbj\|AB196305.1\| Candidatus Neoehrlichia mikurensis gene for 16S ... 857 0.0<br>dbj\|AB213021.1\| Candidatus Neoehrlichia mikurensis gene for 16S ... 857 0.0<br>gb\|AF104680.1\|AF104680 Ehrlichia-like sp. 'Schotti variant' 16S ... 857 0.0<br>gb\|AY098730.1\| Ehrlichia sp. Belluno 16S ribosomal RNA gene, par... 852 0.0 |



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**Table 11. Relative sensitivity of diagnostic tests for *A. phagocytophilum* infection in humans (modified after Bakken and Dumler, 2006)**

| Duration of illness (days) | Blood smear microscopy | HL-60 cell culture | PCR | IFAT |
|---|---|---|---|---|
| 0-7 | Medium | Medium | High | Low |
| 8-14 | Low | Low | Low | Medium |
| 15-30 | | | Low | High |
| 31-60 | | | | High |
| >60 | | | | High |

PCR: Polymerase Chain Reaction; IFAT: Immunofluorescen Antibody Test; HL-60 cell: Human promyelocytic leukemia cells



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**Figure 1. Map showing the location of study site, Gwangju city which is located in south-west part of ROK**



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**Figure 2. The entire captured mice were stripped field mouse (*Apodemas agrarius*)**



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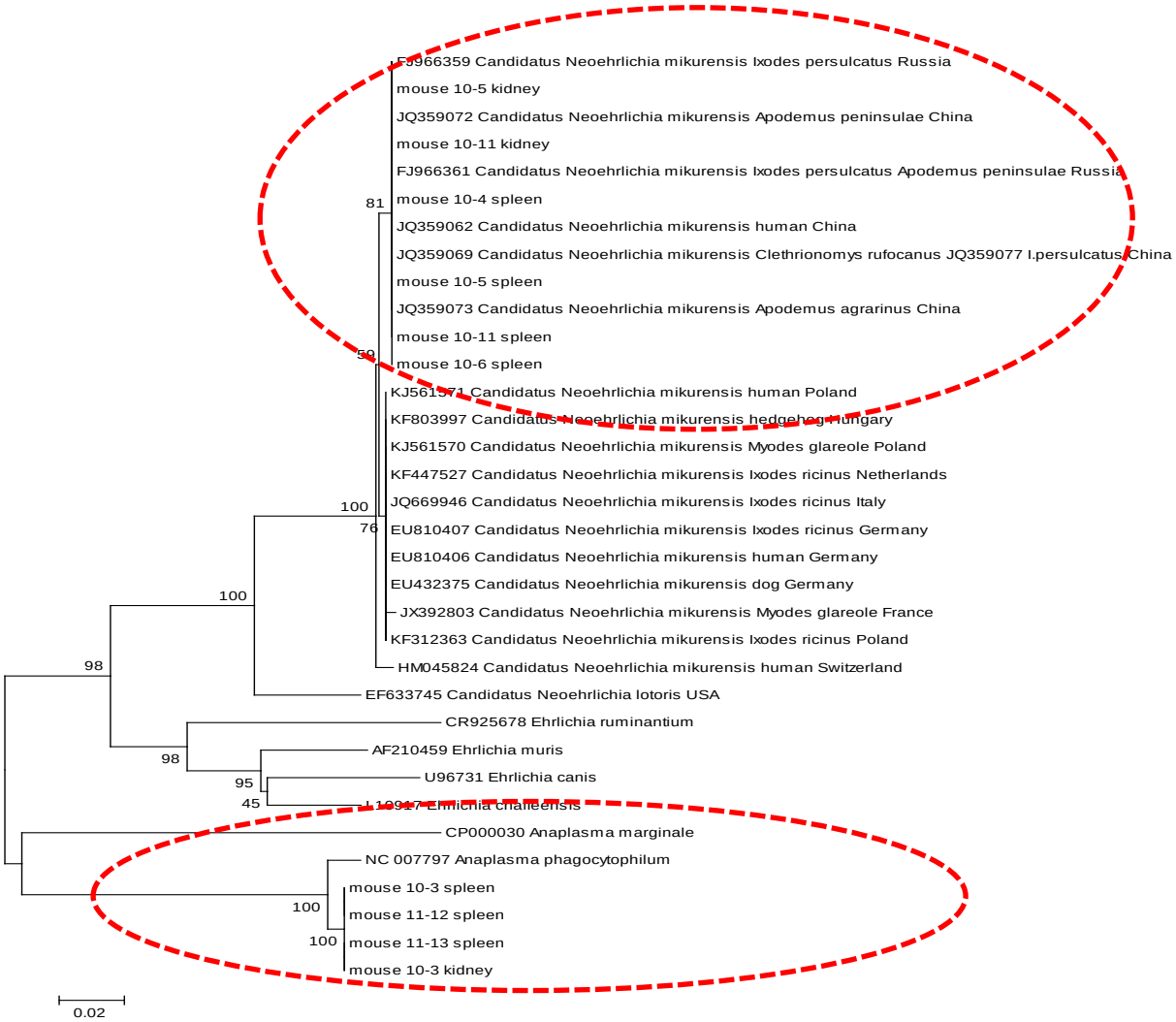


**Figure 3. Phylogenetic tree for *groEL* (330 bp) gene sequences of isolates obtained from mouse spleen and kidney samples**



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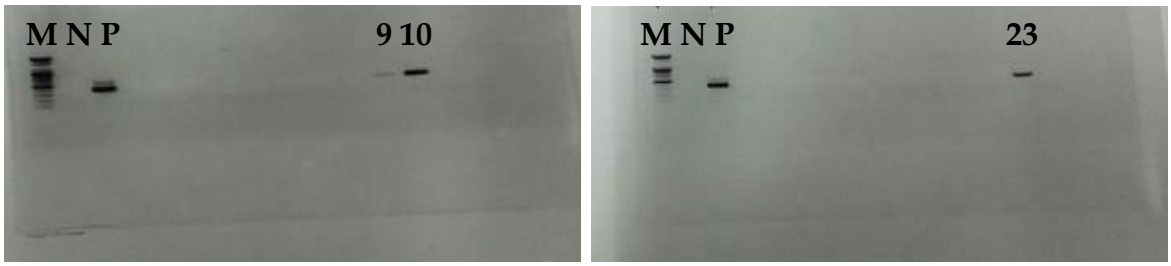


**Figure 4. A 445 bp product following PCR amplification of *A. phagocytophilum* DNA using GRO667F and GRO1121R primers; M= molecular weight marker, N= negative control (no DNA template added), P= positive control (*A. phagocytophilum* template added), 9,10 23 = analysed samples (mite gDNA), in which the presence of bacterial DNA has been detected (bands corresponds to the predicted product size)**

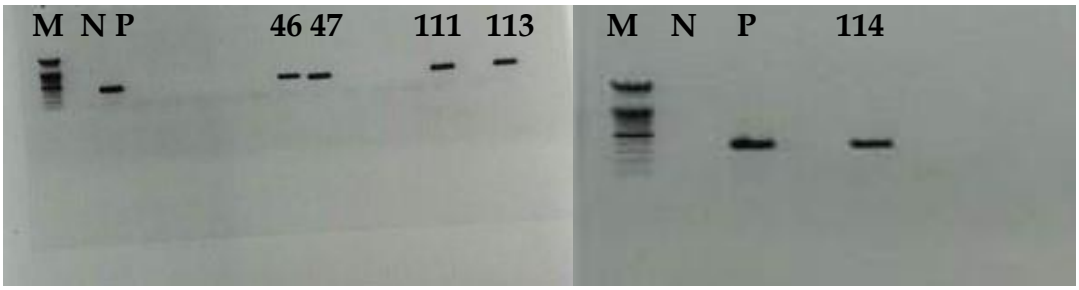


**Figure 5. A 445 bp product following PCR amplification of *A. phagocytophilum* DNA using GRO667F and GRO1121R primers; M= molecular weight marker, N= negative control (no DNA template added), P= positive control (*A. phagocytophilum* template added), 46, 47, 111, 113 and 114 = analysed samples (mite gDNA), in which the presence of bacterial DNA has been detected (bands corresponds to the predicted product size)**



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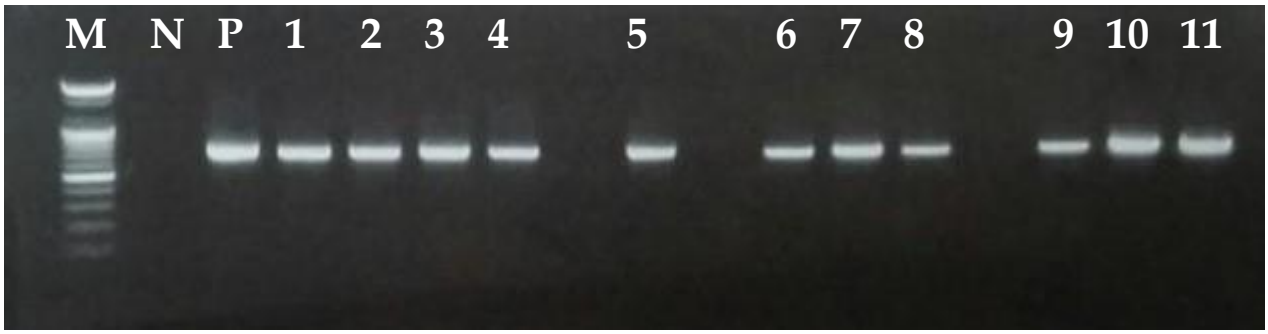


**Figure 6. A 664 bp product following PCR amplification of *A. phagocytophilum* DNA using ANK-F2 and ANK-R2 primers; M= molecular weight marker, N= negative control (no DNA template added), P= positive control (*A. phagocytophilum* template added), 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 = analysed samples (mouse spleen gDNA), in which the presence of bacterial DNA has been detected (bands corresponds to the predicted product size).**



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