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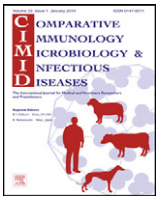

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Rickettsiae in arthropods collected from the North African Hedgehog (*Atelerix algirus*) and the desert hedgehog (*Paraechinus aethiopicus)* in Algeria☆

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ABSTRACT

Hedgehogs have become a popular pet despite their potential role in zoonotic disease transmission. We conducted an entomological study in a mountainous region of northeast Algeria in which we collected 387 fleas (*Archeopsylla erinacei*) and 342 ticks (*Rhipicephalus sanguineus* and *Haemaphysalis erinacei*) from *Paraechinus aethiopicus* and *Atelerix algirus* hedgehogs. Of the hedgehogs sampled, 77.7% and 91% were infested with fleas and ticks, respectively. Significantly more ticks and fleas were collected from *A. algirus* than from *P. aethiopicus*. *Rickettsia felis* was detected in 95.5% of fleas and *R. massiliae* was detected in 6.25% of *Rh. sanguineus* ticks by molecular tools. A new *Rickettsia* species of the spotted fever group was detected in 11.25% of *Rh. sanguineus* and in 77% of *H. erinacei* ticks. Overall, we show that hedgehogs can act as hosts for ectoparasites infected with several rickettsial agents. These data justify a more detailed investigation of animal reservoirs for *Rickettsiae*.
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Rickettsiae are small obligate intracellular bacteria of the family Rickettsiaceae and the order Rickettsiales and cause emergent or re-emergent diseases on all continents. They are frequently detected in arthropods, such as ticks and mites, and in other insects, including lice, fleas, beetles and homopterans; as well as in amoebae and leeches [1]. Hematophagous arthropods are considered to be the main vectors and reservoirs for *Rickettsiae*, although vertebrates may be secondary reservoirs in certain ecosystems that favor the persistence of the bacteria [2]. Humans may become accidentally infected [1].

Hedgehogs are one of the spiny mammals of the subfamily Erinaceinae and the order Erinaceomorpha, with seventeen species in five genera found throughout parts of Europe, Asia, Africa, and New Zealand (by importation). Hedgehogs can carry several tick and flea species, and the load of these ectoparasites can vary among individuals. Parasitization rates of hedgehogs in urban environments can be affected by heterogeneous landscape matrices effects [3], and tick infestation rates have been linked to odors related to the host's health status [4]. Recently, in Germany, *Borrelia burgdorferi* sensu lato and *Anaplasma*

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phagocytophilum were detected in the European hedgehog (*Erinaceus europaeus*) and its tick, *Ixodes hexagonus* [5–7]. Little is known about *Rickettsiae* in hedgehogs and their ectoparasites [8,9]. The aim of our work was to test hedgehog ectoparasites for rickettsial agents in Algeria, which is a region endemic for rickettsioses. Mediterranean spotted fever (MSF) is the most common and is transmitted by the brown dog tick *Rhipicephalus sanguineus* (2). However, one case of *R. sibirica mongolotimonae* and two cases of *R. aeschlimannii* infection have been reported [10,11].

1. Materials and methods

1.1. Study area

Field work was carried out in two areas: Hodna (M'sila) (35°42′07.00″N, 4°32′50.00″E) with twenty sampling sites and Bordj-Bou-Arreridj (36°04′16.29″N, 4°45′31.69″E) with two sampling sites (Belimor, Bordj El Ghidir). Hodna is a steppe area (semi-arid and continental climate) that covers 1,200,000 ha (63% of the total surface area) of the region receives an average annual rainfall of 200–250 mm. M'sila, an open area surrounded by mountains, has a low temperatures (18 °C mean annual), due to its high altitude. In Bordj-Bou-Arreridj, the altitude varies between 700 and 1741 m with annual precipitation ranging between 300 and 700 mm. The hedgehogs have been captured at 470 and 590 m altitude in Hodna (M'sila) area and at 927 m (Belimor) and 1200 m (Bords Laghdir) in Bordj-Bou-Arreridj area.

1.2. Collection of ectoparasites

From March through October 2009, arthropods were collected from small wild hedgehogs that were captured with the aid of spotlights during nightly walks through parts of the study regions by one of us (MK). Each animal was weighed, sized, sexed and identified [12,13]. Six hedgehogs were anesthetized using ketamine and released into its natural habitat after full recovery. Two hedgehogs were found dead following road accidents. The others hedgehogs (No. 28) were maintained in the laboratory conditions in the context of a research project on hedgehog digestive parasites. This study on hedgehogs was authorized by local ethic committee and national legislation (le journal officiel n° 47 du 19 juillet 2006, http://www.iucnredlist.org/apps/redlist/details/40606/0; http://www.iucnredlist.org/apps/redlist/details/27926/0). All ectoparasites were collected with blunted clockmakers' forceps and immediately placed in 70% ethanol inside tubes labelled with the identification number of each hedgehog and the date of collection (two tubes by hedgehog, one for tick and one for fleas). We estimated the total number of ticks and fleas found on the ventral part of each animal, i.e., body parts not covered by spines, including the head. Initially, all samples were kept at room temperature in the *Laboratoire d'écologie,* M'sila University, Algeria. All samples were thereafter sent to the WHO Collaborative Center for Rickettsial Diseases and Other Arthropod-Borne Bacterial Diseases in Marseille, France, for morphological identification and molecular analyses. All ectoparasites were identified at the species level using morphological criteria within standard taxonomic keys by one of us (PP) [14,15]. The number of ticks and fleas collected in different hedgehog species were compared using χ^2 test (Epi Info software, version 3.4.1, CDC, Atlanta, USA). Statistical significance was defined as $p < 0.05$.

1.3. Rickettsial detection

Ticks and fleas were rinsed with distilled water for 10 min, dried on sterile filter paper in a laminar flow hood, and crushed individually in sterile Eppendorf tubes (Hamburg, Germany). DNA was extracted using the QIAamp Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. All DNA samples were screened by quantitative polymerase chain reaction (qPCR) targeting a fragment of *gltA* gene [16]. Positive samples from *Rhipicephalus* ticks were tested by *R. massiliae*-specific qPCR (new molecular system) with the following primers: R.massi_9666-F: 3′-CCA-ACC-TTT-TGT-TGT-TGC-AC-5′ and R.massi_9666-R: 3′-TTG-GAT-CAG-TGT-GAC-GGA-CT-5′ with probe R.massi_9666-s: 6FAM-CACGTGCTGCTTATACCAGCAAACA-TAMRA and *R. conorii*-specific qPCR [17]. For other tick-positive samples, *gltA* and *omp*A genes were amplified, sequenced, and analyzed as described [18]. Positive DNA samples of fleas for *Rickettsiae*-genus-specific qPCR were tested subsequently by *R. felis*-specific qPCR, targeting *bio*B gene [16].

Two negative controls were used for each test: sterile water and DNA extracted from non-infected ticks taken from a colony at the Unité des Rickettsies. For rickettsial screening, *R. montanensis* DNA served as a positive control. For species-specific qPCR detection, DNA from *R. conorii, R. massiliae,* and *R. felis* were used as positive controls.

2. Results

2.1. Ectoparasites collection

From the mountainous region of northeast Algeria, 36 hedgehogs in total were sampled, including 10 *Paraechinus aethiopicus*, the desert hedgehog (9 females and 1 male), and 26 *Atelerix algirus*, the North African Hedgehog (19 females and 7 males) (Table 1) [12,13]. All hedgehogs were found alive except for two found dead after road accidents (2 *P. aethiopicus* hedgehogs). Ticks and fleas were collected primarily around the neck and ears (Fig. 1). Fleas of the species *Archeopsylla erinacei* were found on 28 of the 36 hedgehogs (77.7%) with up to 67 fleas on an individual (means ± standard deviation (SD): 13.8 ± 16.3) [15]. Of the eight hedgehogs without fleas, four were *P. aethiopicus* and four were *A. algrirus*. In total, 36 *Archeopsylla erinacei* fleas were collected from *P. aethiopicus* hedgehogs and 351 were collected from *A. algrirus* hedgehogs (36/10 vs. 351/26, $p = 0.0006$).

Ticks were collected from 33 of 36 hedgehogs (91.6%) with up to 98 ticks on an individual (means ± SD: 10.3 ± 17.7). Ticks were not found on one *A. algirus* and two *P. aethiopicus* hedgehogs. In total, 46 ticks were collected from *P. aethiopicus* hedgehogs and 296 ticks were collected from *A. algrirus* hedgehogs (46/10 vs. 296/26, $p = 0.02$). Ticks

Table 1
Collection of ectoparasites from *Atelerix algirus* and *Paraechinus aethiopicus* in Algeria.

Hedgehog				Fleas (*Archeopsylla erinacei*)			Ticks (*Haemaphysalis erinacei* and *Rhipicephalus sanguineus*)		
Species	Individuals sampled	Hodna (M'Sila)	Bordj-Bou-Arreridj	Infested hedgehogs (%)	Collected fleas	Mean ± SD on an individual	Infested hedgehogs (%)	Collected ticks	Mean ± SD on an individual
North African Hedgehog (*Atelerix algirus*)	26 (19F, 7M)	22 (15F, 7M)	4 (F)	22/26 (84.6%)	351	15.9 ± 17.7	25/26 (96%)	296	11.8 ± 20.2
Desert hedgehog (*Paraechinus aethiopicus*)	10 (9F, 1M) (including 2F dead)	10 (9F, 1M)	0	6/10 (60%)	36	6 ± 5.1	8/10 (80%)	46	5.75 ± 3.7
Total	36 (28F, 8M)	32 (24F, 8M)	4 (F)	28/36 (77.7%)	387	13.8 ± 16.3	33/36 (91.6%)	342	10.3 ± 17.7

Fig. 1. (A) *Rhipicephalus sanguineus* on the inside of the right ear of a female *Atelerix algirus* at the Ouled Mansour site (M'sila). (B) *Rhipicephalus sanguineus* on the outside of the left ear pavilion of a female *Atelerix algirus* at the Ouanougha site (M'sila). (C) Engorged ticks on the backside of *Atelerix algirus* hedgehog at the Hammam Dalaa (M'sila).

were identified as either *Haemaphysalis erinacei* adults or *Rhipicephalus sanguineus* adults [14].

2.2. Rickettsial detection

A total of 342 ticks and 387 fleas were collected on animal. A total of 212 ticks and 331 fleas were tested in the present study. Other specimens were kept for other studies in our arthropod collection. Fifty-two *H. erinacei* and 160 *Rh. sanguineus* ticks were individually screened by qPCR. Sixty-eight of 212 tick samples were positive for Rickettsial DNA, including 28 of 160 (17.5%) *Rh. sanguineus* ticks and 40 of 52 (77%) *H. erinacei* ticks (Table 2). The 28 *Rh. sanguineus* samples that were tested positive were further tested by *R. massiliae*- and *R. conorii*-specific qPCR. Ten of these 28 DNA samples tested positive for *R. massiliae* DNA (10/160, 6.25%), and no tick samples were positive for *R. conorii* DNA. Sequence analysis of the *ompA* gene of 18 DNA samples negative for *R. massiliae* showed 98.36% (542/551) similarity with *Rickettsia* sp. FUJ98 (GenBank

Table 2
Rickettsial detection in ectoparasites (fleas and ticks) collected from hedgehogs in Algeria.

	Ectoparasite species (No.)	SFG *Rickettsiae* qPCR (positive/tested)	*R. felis* qPCR (positive/tested)	*R. massiliae* qPCR (positive/tested)	*R. conorii* qPCR (positive/tested)	Amplification and sequencing of *ompA* gene	Amplification and sequencing of *gltA* gene
Fleas	*Archeopsylla erinacei* (331)	316/331 (95.5%)	316/316 (100%)	–	–	–	–
Ticks	*Haemaphysalis erinacei* (52)	40/52 (77%)	–	–	–	98% (617/629) *Rickettsia* sp. FUJ98 (AF169629) 95.57% (604/632) *R. japonica* Inha1 (DQ019319)	99.74% (770/772) *Rickettsia* sp. LON-13 (AB516964) 99.74% (770/772) *R. heilongjiangensis* (AF178034)
	Rhipicephalus sanguineus (160)	28/160 (17.5%)	–	10/28 (35.7%)	0/28	98.36% (542/551) *Rickettsia* sp. FUJ98 (AF169629) 97.74% (521/533) *Candidatus* R. davousti (DQ402517) 95.3% (528/554) *R. japonica* Inha1 (DQ019319)	99.74% (770/772) *Rickettsia* sp. LON-13 (AB516964) 99.74% (770/772) *R. heilongjiangensis* (AF178034)

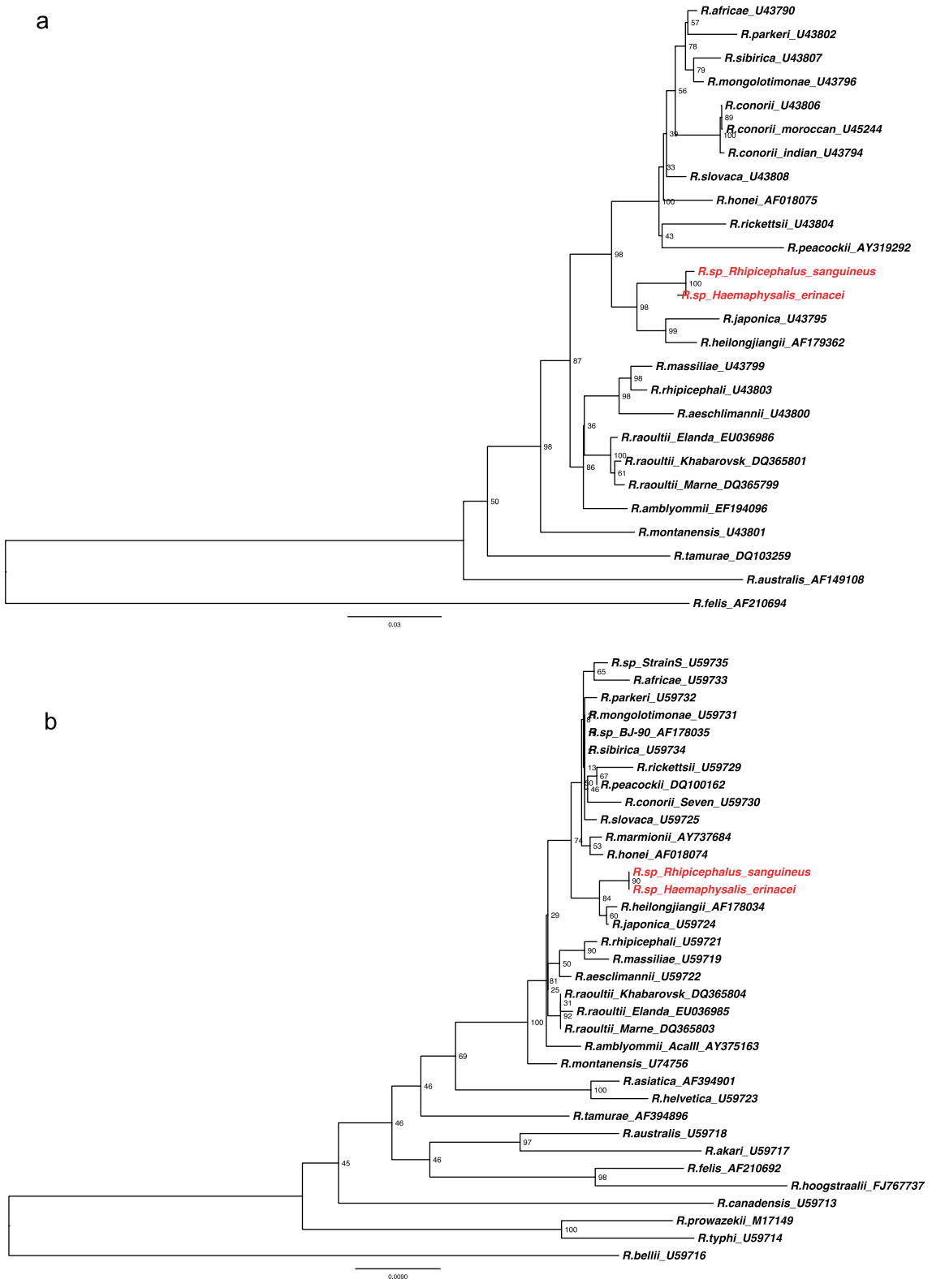

Fig. 2. Phylogenetic tree showing the relationships among the new rickettsial species detected in our study in *Rhipicephalus sanguineus* and *Haemaphysalis erinacei* ticks collected on Algerian hedgehogs and other validated rickettsial species, as inferred from sequence analysis of *omp*A (A) and *glt*A (B) genes by the maximum-parsimony method, as described [20]. Bootstrap values are indicated at the nodes.

accession no. AF169629), 97.74% (521/533) similarity with *Candidatus* Rickettsia davousti (DQ402517), and 95.3% (528/554) identity with *R. japonica* Inha1 (DQ019319). Sequence analysis of the *gltA* gene of three samples negative for *R. massiliae* showed 99.74% (770/772) similarity with *Rickettsia* sp. LON-13 (AB516964) and *R. heilongjiangensis* (AF178034) (Fig. 2). The nucleotide sequence of the *glt*A and *omp*A fragments of *Rickettsia sp.* was deposited in the GenBank database under accession numbers JN943293 and JN943295, respectively.

PCR and sequence analysis of the *ompA* gene for all *Rickettsia* DNA of *H. erinacei* samples revealed 98% (617/629) sequence identity with *Rickettsia* sp. FUJ98 (AF169629) and 95.57% (604/632) identity with *R. japonica* Inha1 (DQ019319). Sequence analysis of the *gltA* gene from the three samples showed 99.74% (770/772) identity with *Rickettsia* sp. LON-13 (AB516964) and *R. heilongjiangensis* (AF178034). The nucleotide sequence of the *glt*A and *omp*A fragments of *Rickettsia sp.* was deposited in the GenBank database under accession numbers JN943294 and JN943296, respectively. The ompA fragments of *Rickettsia* spp. detected in *H. erinacei* and in *Rh. sanguineus* ticks (negative for *R. massiliae*) have been aligned and analyzed with the CLUSTALX program. All sequence fragments were similar and come from the same *Rickettsia* sp. organism (Fig. 2).

Screening of individual *A. erinacei* fleas by qPCR revealed Rickettsia DNA in 316 of the 331 fleas tested (95.5%). The mean Ct value of *glt*A amplification by qPCR of positive *A. erinacei* flea samples was 22.66 ± 2.68 (means $\pm$ SD; min: 17.39 ct). All positive *A. erinacei* flea samples were also positive for a *R. felis*-specific qPCR with the mean Ct value of 27 ± 2.96 (means $\pm$ SD; min: 21.18 ct, max: 35.92 ct).

3. Discussion

Our results suggest that hedgehogs carry ectoparasites infected with several *Rickettsia* species, including *R. felis* in *A. erinacei* fleas, *R. massiliae* in *Rh. sanguineus* ticks, and a novel *Rickettsia* species in *H. erinacei* and *Rh. sanguineus* ticks. In the last few years, the purchase of domesticated hedgehogs has increased considerably, and this hedgehogs' potential for infestation with ticks and fleas may increase human exposure to zoonotic diseases. *Atelerix algirus*, the Algerian hedgehog, is surveyed and monitored by EU Habitats and Species Directive. It is endemic to the Mediterranean region, occurring in Spain, the Mediterranean islands, France (by importation), and across North Africa from Morocco to Libya. This species is sometimes taken from the wild to be kept as a pet and can be locally caught and eaten across the Mediterranean region [19]. In our work, significantly more ticks and fleas have been collected from the Algerian hedgehog than from the desert hedgehog *Paraechinus aethiopicus*, which is endemic to North Africa and the Arabian Peninsula.

The new OmpA and gltA sequences detected in our work correspond to a member of the genus *Rickettsia* and the spotted fever group. Indeed, they exhibit >92.7% homology with many of the 20 known *Rickettsia* species and possesses the *omp*A gene [20]. Also they exhibit less than 99.9% and 98.8% degrees of nucleotide similarity with the most homologous validated species for the *glt*A and *omp*A genes, respectively. As per the guidelines for classification of a new *Rickettsia* species, we need to isolate this bacterium and to characterize the five *Rickettsia*-specific genes (rrs, *glt*A, *omp*A, *omp*B, and geneD) [20]. Thus, further study is necessary to characterize this new *Rickettsia* species. Interestingly, this *Rickettsiae* has been detected in two tick species (*Rh. sanguineus* and *H. erinacei*). It is not yet understood if both ticks are associated with this *Rickettsiae*, or if they have been infected by feeding on bacteremic hedgehogs, or have been infected each other by cofeeding.

Our results confirm the presence of *R. felis,* the agent of flea borne spotted fever, in fleas with a high rate of infection (95.5%) and the presence of *R. massiliae*, the agent of spotted fever, in ticks collected in Algeria. Previous studies have shown that all *A. erinacei* fleas (four of four) collected from an *A. algirus* hedgehog in Algeria [9] and a single *A. erinacei* flea collected from an *Erinaceus europaeus* hedgehog in Portugal were tested positive for *R. felis* [21]. *R. massiliae* has been detected in one *Rh. turanicus* and four *Rh. sanguineus* ticks collected on a hedgehog from Algiers, Algeria [8]. Extensive epidemiological studies in Africa are lacking; we would predict that flea-borne spotted fever would be endemic in all countries. Misdiagnosis of *R. felis* infection for other rickettsiosis, such as murine typhus or spotted fever group rickettsiosis, without appropriate laboratory tests might be a factor contributing to the underestimation of human arthropod-borne spotted fever incidence in Africa [22,23].

Hedgehogs were suggested to be a potential reservoir of *Rickettsia conorii conorii*, which is the infectious agent of MSF [1] and of *R. conorii caspia*, which is the infectious agent of Astrakhan spotted fever [1]. *R. conorii conorii* was detected in one *Rh. sanguineus* collected from a hedgehog in Algeria [8]. To date, the reservoir for *R. massiliae*, *R. conorii conorii*, and *R. conorii caspia* has not been definitively described. In addition, *R. sibirica sibirica*, the infectious agent of Siberian tick typhus, was isolated from hedgehogs collected in a suburb of Beijing, China and was detected by molecular tools in its ticks [24]. The authors [23] suggested that horizontal transmission of the *Rickettsiae* between ticks and hedgehogs creates the potential for a hedgehog reservoir. The presence of these *Rickettsia* species in hedgehogs and in their ectoparasites suggest that this animal can act as a reservoir for these bacteria, but additional investigation is needed to confirm this hypothesis.

Competing interests

The authors declare that they have no competing interest.

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