ORIGINAL ARTICLE

Molecular evidence of *Rickettsia slovaca* in spleen of wild boars in northeastern Algeria

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Abstract

Using molecular assays, *Rickettsia slovaca,* the agent of a spotted fever group rickettsiosis resulting in scalp eschar and neck lymphadenopathy after tick bite, was assessed in 92 spleens recovered from 117 wild boars hunted in the far northeast of Algeria. *Rickettsia slovaca* was detected in 5.4% of tested wild boar spleens. The presence of *R. slovaca* DNA in boar spleens questions the relationship that may exist between this bacterium and *Sus scrofa algira,* and its role in human infections.

Keywords: Algeria, *Haematopinus suis*, *Rickettsia slovaca*, Spleen, Wild boar
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Introduction

Wild animals play an important role in the epidemiology of infectious diseases as reservoirs of several zoonotic and non-zoonotic diseases [1]. It has been clearly shown that wild boars can act as reservoirs for a long list of zoonotic bacterial agents [2]. The wild boar, *Sus scrofa,* is one of the most widely distributed mammalian species, present in Europe, North Africa and many Asian countries as far as south as Indonesia [3]. Wild boars are omnivores that eat everything, including vegetables, mushrooms, seeds, larvae, reptiles, mammals, birds and their eggs, and even carrion. In addition, they are considered to be pests in agricultural fields, as they can cause significant damage to plants [4].

This ubiquitous omnivorous mammal is constantly expanding its territory in Algeria. In addition, the behaviour of wild boars is changing. Previously they were strictly nocturnal animals, taking their food in forests and mountains while hidden from the eyes of humans; today, they are able to live close to humans, and they even feed near cities [5]. Wild boars are a preferred host for several ectoparasite species, including lice (*Haematopinus suis*) and ticks (*Dermacentor marginatus*) [6,7]. In Algeria, adult wild boars (*Sus scrofa algira*) are parasitized by seven species of ticks (*Rhipicephalus turanicus, Dermacentor marginatus, Hyalomma marginatum, Ixodes ricinus, Rhipicephalus sanguineus, Rhipicephalus bursa* and *Haemaphysalis punctata*); indeed, they are considered to be among the most tick-parasitized animals [8,9].

Rickettsia slovaca, a spotted fever group rickettsia, was first isolated in 1968 from *D. marginatus* ticks in the former Czechoslovakia. To date, the *D. marginatus* tick is recognized as the main vector and reservoir for *R. slovaca* in Mediterranean areas, including southern Europe and North Africa [10]. Recently *R. slovaca* has been detected in other ectoparasites such as flies (*Melophagus ovinus*) [11] and lice (*H. suis*) [12].

Recreational hunting of wild boars and consumption of wild boar meat in some regions of the world has further provided ample opportunities for direct human contact with wild boars and has thus created an ideal environment for the transmission of pathogens between wild boars and domestic swine, and

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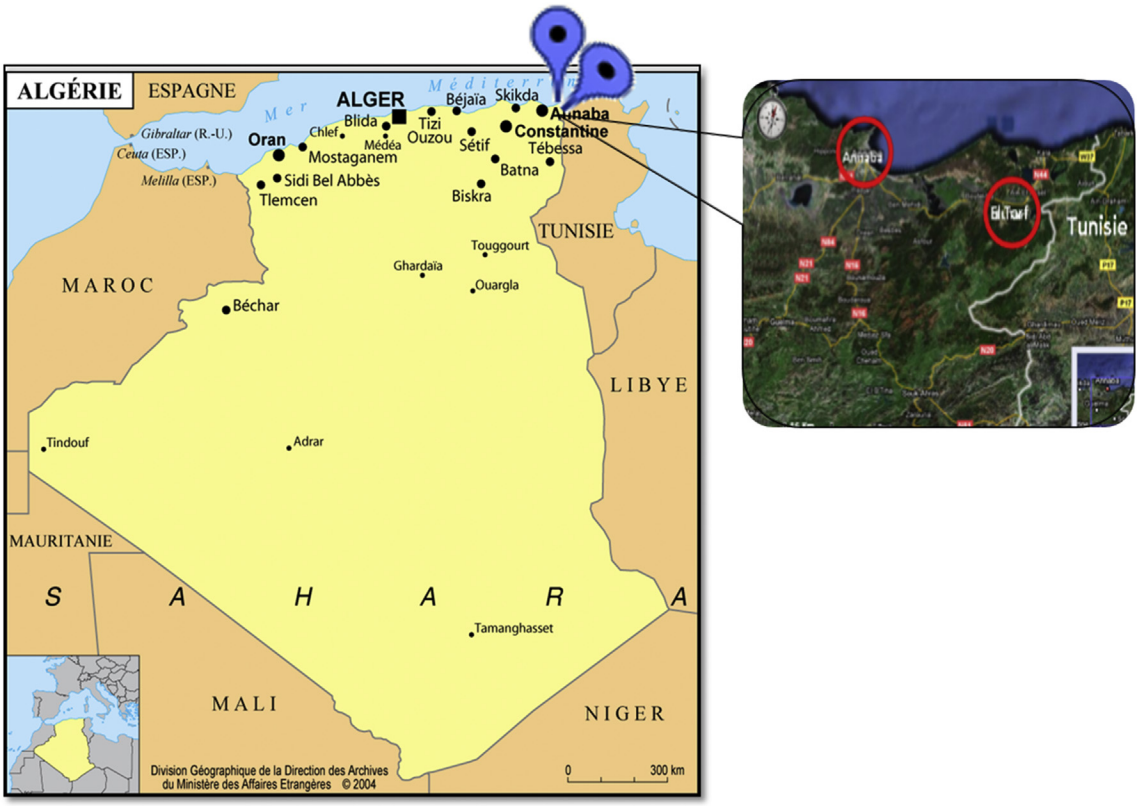

FIG. 1. Study sites.

between wild boars and humans [13]. It is therefore important to screen for the presence of infectious disease such as rickettsiae in wild boar (mainly *Sus scrofa algira*) tissue.

Materials and methods

Positive results were confirmed by using a standard PCR specific for the *ompA* gene of *Rickettsia* spp. Bacteria-free DNA of *Rhipicephalus sanguineus* ticks reared in our laboratory was used as a negative control, while DNA extracted from *Rickettsia montanensis* was used as a positive control.

Between April 2011 and April 2015, samples were removed by laparotomy from 117 wild boars killed by an approved hunting association in Annaba and El Tarf, Northeastern Algeria (Fig. 1). Once recovered from the boars, spleens were conserved in 70° alcohol and forwarded to the Vitrome Laboratory in Marseille. Total genomic DNA was isolated by the QIAamp Tissue Kit (Qiagen, Hilden, Germany) and the BioRobot EZ1 (Qiagen) as described by the manufacturer. DNA was used as template for quantitative real-time PCR. The RKND03 primer system, which is specific for the *gltA* gene of *Rickettsia* spp., was used to screen for the presence of *Rickettsia* spp. Real-time PCRs were performed using the CFX96 Real Time System C1000 Touch Thermal Cycler (Bio-Rad Laboratories, Singapore).

PCR amplicons were purified using a NucleoFast 96 PCR plate (Macherey-Nagel EURL, Hoerdt, France) as recommended by the manufacturer. Purified PCR products were sequenced using PCR primers, the BigDye version 1-1 Cycle Ready Reaction Sequencing Mixture (Applied Biosystems, Foster City, CA, USA) and an ABI 31000 automated sequencer (Applied Biosystems). Sequences were assembled and analysed by ChromasPro 1.34 software (Technelysium, Tewantin, Australia).

Results

Ninety-two boar spleens (Fig. 2) were screened for the presence of *Rickettsia* spp. Overall, 5.4% (5/92) of spleens were positive for *Rickettsia* spp. Sequencing of PCR amplicons identified *R. slovaca* (100% similarity, 760/760 bp; GenBank accession no. HM161787.1).

Discussion and conclusion

Wild boars, *Sus scrofa,* are considered as potential reservoirs of several zoonotic diseases [4]. Our study shows that this well-known animal in Algeria carries *R. slovaca,* the agent of scalp eschar and neck lymphadenopathy after tick bite in humans.

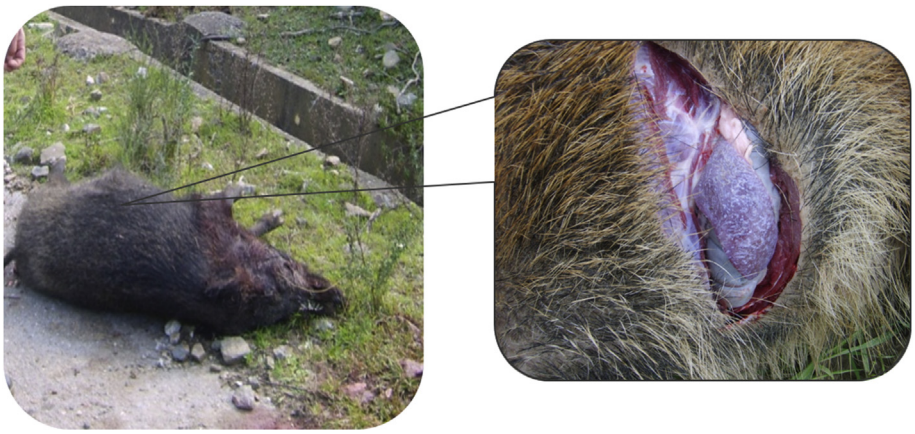

FIG. 2. Laparotomy and recovery of wild boar spleen.

Rickettsia slovaca was first isolated in 1968 from *D. marginatus* in the Czech Republic [14]. Between 1977 and 1982, antibodies to *R. slovaca* were detected in the blood of small terrestrial mammals, larger wild and domestic animals, as well as humans in the Sumava area of the same country [15]. The human disease caused by *R. slovaca* was later described as the association of an eschar to the scalp and cervical lymph nodes, and was initially termed tick-borne lymphadenopathy [16].

In Algeria, *R. slovaca* was detected for the first time in *D. marginatus* ticks collected from the vegetation of the Blida region [17]. It was found also detected in *D. marginatus* collected on *Sus scrofa algira* in Souk Ahras [9] and in *H. suis* lice from wild boars [12]. This pathogen was also detected in *D. marginatus* ticks in Morocco in 2008 [18]. In Europe, *R. slovaca* has also been found to be associated with *D. marginatus* ticks in a majority of countries [10,19]. *Rickettsia slovaca* has also been found in *D. marginatus* ticks in the Kurgan region (Ural) of Russia [20] and in Georgia [21]. In China, the bacterium has been detected in 6.5% of *Dermacentor silvarum* ticks [22]. In Spain, seropositivity in wild boars has suggested that these animals are exposed to *R. slovaca* infection [23]. In this country, *R. slovaca* has also been detected in sheep, goats and bullfighting cattle [24]. Finally, a high prevalence (12.63%) of *R. slovaca* has been demonstrated in *M. ovinus* flies from the Taklimakan Desert in China [11].

We report for the first time the presence of *R. slovaca* DNA in the spleens of wild boars. Our findings extend the range of spotted fever group rickettsiae detected in wild animals in Algeria. Our results suggest the circulation of *R. slovaca* in the wild life cycle. Given that humans have increasing contact with wild boars, further studies should be conducted to define their role in the maintenance of *R. slovaca* infection.

Conflict of interest

None declared.

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