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Presence of Chlamydiales DNA in ticks and fleas suggests that ticks are carriers of Chlamydiae

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ABSTRACT

The Chlamydiales order includes the Chlamydiaceae, Parachlamydiaceae, Waddliaceae, Simkaniaceae, Criblamydiaceae, Rhabdochlamydiaceae, Clavichlamydiaceae, and Piscichlamydiaceae families. Members of the Chlamydiales order are obligate intracellular bacteria that replicate within eukaryotic cells of different origins including humans, animals, and amoebae. Many of these bacteria are pathogens or emerging pathogens of both humans and animals, but their true diversity is largely underestimated, and their ecology remains to be investigated. Considering their potential threat on human health, it is important to expand our knowledge on the diversity of Chlamydiae, but also to define the host range colonized by these bacteria. Thus, using a new pan-Chlamydiales PCR, we analyzed the prevalence of Chlamydiales DNA in ticks and fleas, which are important vectors of several viral and bacterial infectious diseases. To conduct this study, 1340 *Ixodes ricinus* ticks prepared in 192 pools were collected in Switzerland and 55 other ticks belonging to different tick species and 97 fleas belonging to different flea species were harvested in Algeria. In Switzerland, the prevalence of Chlamydiales DNA in the 192 pools was equal to 28.1% (54/192) which represents an estimated prevalence in the 1340 individual ticks of between 4.0% and 28.4%. The pan-Chlamydiales qPCR was positive for 45% (25/55) of tick samples collected in Algeria. The sequencing of the positive qPCR amplicons revealed a high diversity of Chlamydiales species. Most of them belonged to the Rhabdochlamydiaceae and Parachlamydiaceae families. Thus, ticks may carry Chlamydiales and should thus be considered as possible vectors for Chlamydiales propagation to both humans and animals.

Introduction

The Chlamydiales order (Everett et al., 1999) currently includes the Chlamydiaceae, Parachlamydiaceae, Waddliaceae, Simkaniaceae, Criblamydiaceae, Rhabdochlamydiaceae, Clavichlamydiaceae, and Piscichlamydiaceae families (Corsaro and Greub, 2006; Greub, 2013). Members of the Chlamydiales order are strict intracellular bacteria that replicate within eukaryotic cells of different origins including humans, animals, and amoebae (Corsaro and Greub, 2006; Horn, 2008). Chlamydiales are characterized by a biphasic development cycle comprising infectious metabolically inactive elementary bodies and non-infectious metabolically active and replicating reticulate bodies (Moulder, 1991).

Chlamydiales bacteria have been identified in hosts covering the whole animal kingdom. Several Chlamydiaceae such as *Chlamydia trachomatis* and *C. pneumoniae* colonize humans. *Waddlia chondrophila* was isolated from an aborted bovine fetus (Rurangirwa et al., 1999), whereas *Waddlia malaysiensis* was isolated from fruit bats (Chua et al., 2005). Bacteria belonging to the Piscichlamydiaceae and the Clavichlamydiaceae families were detected in gills from fish exhibiting signs of epitheliocystis (Draghi et al., 2004; Karlsen et al., 2008). The 2 members of the Rhabdochlamydiaceae family, *Candidatus* Rhabdochlamydia porcellionis and *Candidatus* Rhabdochlamydia crassificans, were identified in arthropods by 16 sRNA gene sequence analysis and electron microscopy (Corsaro et al., 2007; Kostanjsek et al., 2004), but were never recovered and isolated. Similarly, 2 candidatus Chlamydiales species belonging to the Simkaniaceae family were detected in insects by DNA

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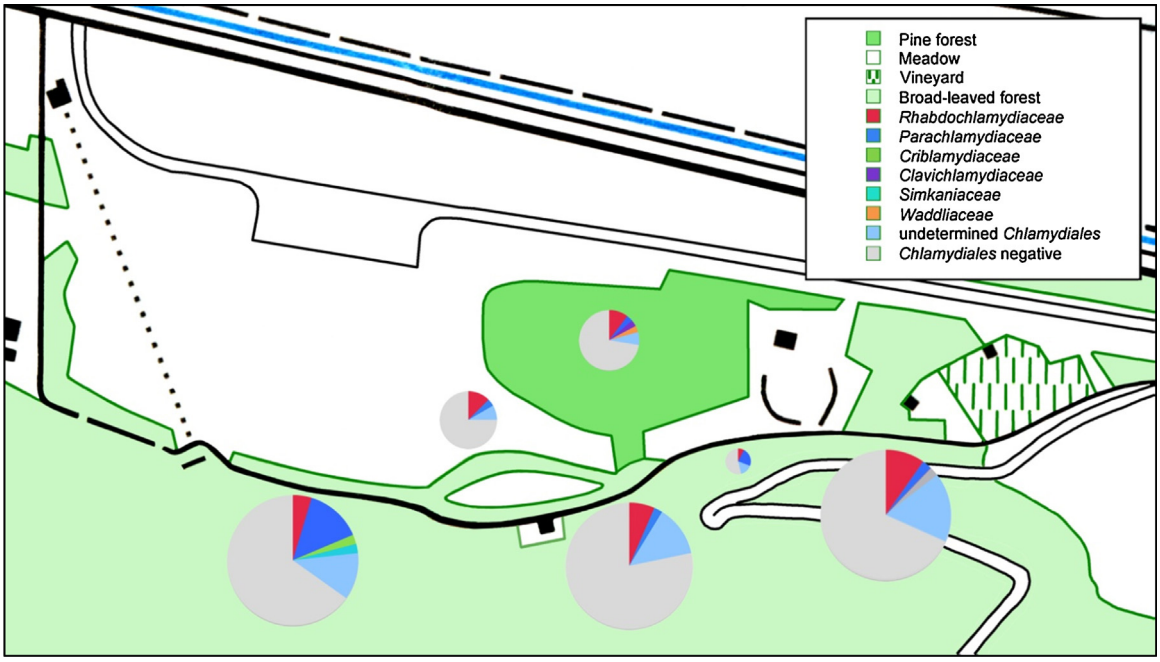

Fig. 1. Prevalence and distribution of Chlamydiales families DNA in *Ixodes ricinus* ticks collected in different areas of Rarogne, Switzerland. The 1340 ticks were collected in different geographical areas (pine forest, meadow, and broad-leaved forest) in the region of Rarogne. The prevalence and the distribution of sequences belonging to different Chlamydiales families differed between the different areas of sample collection.

and electron microscopy analysis, but were not isolated from their hosts (Everett et al., 2005). One member of the Simkaniaceae family, *Simkania negevensis* was isolated as a culture contaminant of human and simian cells (Kahane et al., 1999). Finally, several Chlamydiales bacteria belonging mainly to the Parachlamydiaceae and Criblamydiaceae families are considered symbionts of amoebae (Amann et al., 1997; Greub and Raoult, 2002; Horn et al., 2000) or associated with amoebae (Lienard et al., 2011b; Thomas et al., 2006), indicating the important role that these latter organisms play in the ecology of these obligate intracellular bacteria (Fritsche et al., 1993; Horn, 2008). However, the diversity of Chlamydiales bacteria is still likely underestimated, and their ecological distribution remains to be further investigated.

Such investigations are especially warranted since many Chlamydiales have been recognized as human and animal pathogens or are seriously considered pathogenic microorganisms (Corsaro and Venditti, 2004; Corsaro and Greub, 2006; Longbottom and Coulter, 2003; Senn et al., 2005). *Chlamydia trachomatis* is the causative agent of trachoma, the most frequent infectious cause of blindness (Burton, 2007) and is the most common cause of bacterial sexually transmitted diseases (Beagley and Timms, 2000). *Chlamydia pneumoniae* is a causative agent of pneumonia, and *Chlamydia psittaci* is the causative agent of the zoonotic infection called psittacosis which is often characterized by an interstitial pneumonia (Lamoth and Greub, 2010b). There is also clear evidence supporting the role of *Parachlamydia acanthamoebae* as a human respiratory pathogen (Greub, 2009). Thus, several serological and molecular studies have demonstrated a pathogenic role of *P. acanthamoebae* mainly in immunocompromised and intensive-care patients suffering from pneumonia (reviewed in Lamoth and Greub, 2010b). Finally, *Waddlia chondrophila* is an emerging pathogen which is considered a possible causative agent of abortion in both ruminants (Dilbeck-Robertson et al., 2003) and humans (Baud et al., 2007, 2011). Due to the intracellular lifestyle of chlamydiae, classic culture methods are ineffective to identify any members of the Chlamydiales order. Thus, the pathogenic potential of several of these bacteria still remains largely unexplored.

Chlamydiales bacteria belonging to the Rhabdochlamydiaceae family have been identified in arthropods including the cockroach *Blatta orientalis* and the terrestrial isopod *Porcellio scaber* (Corsaro et al., 2007; Kostanjsek et al., 2004). Arthropods represent thus a possible important reservoir for Chlamydiales bacteria that need to be investigated. Among arthropods, fleas and ticks are important vectors of both viral and bacterial infectious diseases. Lyme borreliosis caused by *Borrelia burgdorferi* sensu lato and tick-borne encephalitis (TBE) are the major tick-borne diseases affecting humans. In addition, several less frequent additional tick-borne infectious agents can cause severe diseases in humans including *Francisella tularensis* (tularemia), *Rickettsia* spp. (spotted fever), and *Anaplasma phagocytophilum* (anaplasmosis) (Brouqui et al., 2004). Similarly, fleas have been identified as vectors of transmission of numerous important human diseases including bubonic plague caused by *Yersinia pestis* (Wimsatt and Biggins, 2009). Finally, 2 studies suggested that ticks could play a role in the transmission of chlamydiae to cattle (Caldwell and Belden, 1973; McKercher et al., 1980). Thus, using a pan-Chlamydiales PCR, the prevalence and sequence diversity of Chlamydiales 16S rDNA were analyzed in *Ixodes ricinus* ticks collected in Switzerland and in several tick and flea species collected in Algeria.

Materials and methods

Tick collecting in Switzerland

The field work was conducted from May to July 2010. *Ixodes ricinus* ticks (adults and nymphs, $n = 1340$) were collected using a 1-m^2 white cotton towel which was dragged over the vegetation. Every 10 m, the operator stopped to count and put attached ticks into tubes, which were stored at −80 °C until further analysis. Ticks were collected on the site of Mutt-Rarogne (Fig. 1), which was chosen because of its small size and its clear demarcation due to the topology of the area.

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