Portuguese hosts for *Ornithodoros erraticus* ticks.

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Abstract

The haematophagous soft tick *Ornithodoros erraticus* feeds nocturnally on multiple warm-blooded vertebrate hosts. This tick is often found living buried in soil of traditional pigpens. *O. erraticus* is an important infectious disease vector both for humans and animals. In the Iberian Peninsula, this tick serves as the vector of human tick-borne relapsing fever caused by the spirochaete *Borrelia hispanica*. The natural ecosystems maintaining this spirochaete remain defectively understood with details of competent vertebrate reservoirs and tick–host interactions poorly understood.

Investigation of arthropod blood meal composition provides evidence linking the vector to specific hosts, providing insights into possible disease reservoirs. Ticks collected from two pigpens located in Southern Portugal were subjected to blood meal analysis. PCR amplification of vertebrate cytochrome b was used to disclose the original host from which 349 ticks had derived their previous blood meal. Host origins for blood-meal analysis from 79 of 349 ticks revealed that 46.8% had previously fed from pigs, 35.4% human, 13.9% bovine, 5.1% sheep, 1.3% rodent and 1.3% from birds. Three samples revealed mixed blood-meals, namely, human-pig (1.3%), sheep-pig (1.3%) and bovine-pig (1.3%). The major role of pigs as hosts is consistent with fieldwork observation and underlines the importance of pigs for maintaining *O. erraticus* tick populations. Humans serve as accidental hosts, frequently confirmed by reports from both producers and veterinarians. Other livestock species and wildlife prevalent in the region appear only to have a minor role in maintaining this tick. The results demonstrate the importance of blood-meal analysis to determine tick hosts providing a tool for investigation of sylvatic cycle for *B. hispanica*.

Introduction

Ornithodoros erraticus (Lucas, 1849) is a soft tick (Argasidae) and haematophagous nocturnal feeder. *Ornithodoros erraticus* was first reported in the Iberian Peninsula during the 1940's (David de Morais et al., 2007) and is usually associated with swine pigpens, living buried in soil or within crevices (Encinas Grandes et al., 1999; Goddard, 2003). Recent studies have confirmed the persistence of this tick in some Portuguese regions (Boinas, 1994; Palma et al., 2011). *Ornithodoros,* though often residing in pigpens, can feed upon various warm-blooded vertebrates such as small rodents, pigs, porcupines, bats, birds (David de Morais et al., 2007; Assous and Wilamowski, 2009). Argasid ticks only attach to their host briefly, usually when hosts are resting (Boinas, 1994; Encinas Grandes et al., 1999). *O. erraticus* is an important infectious disease vector for men and animals through its transmission of tick-borne relapsing fever spirochaetes (TBRF) caused by *Borrelia hispanica* for humans, (Sarih et al., 2009; Toledo et al., 2010), and African swine fever virus among pigs (Sánches Botija, 1982).

Despite the absence of clinical case reports of TBRF in Portugal since 1961 (David de Morais et al., 2007), *B. hispanica* was recently identified in 2.2% of *O. erraticus* ticks from a pigpen (Palma et al., 2011). The transmission cycles and the natural reservoirs of *B. hispanica* and *B. crocidurae* are poorly understood, largely as a result of the diversity of potential hosts for these ticks (David de Morais et al., 2007; Assous and Wilamowski, 2009). Tick blood meal analyses can link the vector to specific hosts and thus providing an insight into possible vertebrate disease reservoirs. We used this approach to identify vertebrate species serving as hosts for *O. erraticus* that might serve as possible reservoirs for TBRF in this endemic region.

The sensitivity of this method of blood-meal analyses can be influenced by several factors such as DNA extraction methods, the time elapsed since the last meal, and the meal size (Kent, 2009). Several cytochrome b specific primers, with different host specificities, have been published (Molaei et al., 2006; Kent, 2009) and considered in this study.

Material and Methods

Ticks:

O. erraticus were collected from two pigpens located in Southern Portugal during 2009 and 2010, selected as earlier studies had established that these sites were infested with *O. erraticus* infected with *B. hispanica* (Palma et al., 2011). *O. erraticus* were washed (Palma et al., 2011) and DNA was extracted individually, from adults and large nymphal stages, using ammonia method as described (Schouls et al., 1999).

Blood meal analyses:

A total 349 DNA samples were tested by PCR (Apperson et al., 2002; Molaei et al., 2006), screening vertebrate cytochrome b using generic Mammalian c (Molaei et al., 2006) and Avian a primers sets (Cícero and Johnson, 2001), with High Fidelity PCR Master Kit (Roche Applied Science, Mannheim, Germany), according to manufacturer's instructions. Negative and ambiguous samples with both above primer sets were then tested with Mammalian a (Ngo and Kramer, 2003), Mammalian b (Molaei et al., 2006) and Avian b (Sorenson et al., 1999) primers. All positive samples were sequenced in an ABI automated DNA capillary sequencer (Applied Biosystems, USA) by using ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems). Positive identification and host species assignment were attributed when exact or highly similar results (>95%) were obtained by BLAST within GenBank database (Altschul et al., 1997). A blood-meal was classified as mixed if two

different species were identified in separate PCR from the template and/or when chromatograms from each PCR demonstrated doublenucleotide peaks.

Results

The blood-meal host was identified for 79 (22.6%) of 349 samples tested (Table 1), in which, 37 (46.8%) were pig *(Sus scrofa*), 28 (35.4%) were human (*Homo sapiens*), 11 (13.9%) were bovine (*Bos taurus*), 4 (5.1%) were sheep (*Ovis aries*), 1 (1.3%) was rodent (Family Muridae), and 1 (1.3%) was warbler (*Hippolais* spp.). Three samples were mixed with two vertebrate hosts identified with one of each of human-pig, sheep-pig and bovine-pig (each representing 1.3% of total positive samples).

Table 1. Positive blood-meals identified from *Ornithodoros erraticus* collected in Portugal.

Species	No*	% from total (n = 79)
Pig *(Sus scrofa)*	37	46.8
Human *(Homo sapiens)*	28	35.4
Bovine *(Bos taurus)*	11	13.9
Sheep *(Ovis aries)*	4	5.1
Warbler *(Hippolais* spp)	1	1.3
Rodent (Fam. Muridae)	1	1.3
***Includes 3 specimens from which dual blood-meals were identified.**		

Discussion

Blood-meal analyses revealed six groups of vertebrate host for *O. erraticus* ticks. Most common hosts were pigs (46.8%) and humans (35.4%), followed by bovine and sheep. We accept that the collection site for ticks used in this study might have resulted in a bias towards both pigs and humans. Indeed pigs are the most readily available host in these premises, resting overnight in shelters where ticks cohabit. Man, through contact with swine and proximity with premises, frequently serves as an accidental host. Indeed during fieldwork, veterinarians and livestock producers frequently recall having been bitten by *O. erraticus* in infested facilities. Other animals like bovines and sheep are uncommon in pigpens and their surroundings, thus unsurprisingly the percentage of positive blood-meals was lower. Rodents and birds were less commonly represented. Others have frequently quoted role of rodents as preferential hosts for *O. erraticus* ticks and consequently potential reservoirs for *B. hispanica* (Rebaudet and Parola, 2006; David de Morais *et al*., 2007; Diatta et al.,2012), this role could not be substantiated by our findings. This could be due to rodent lifestyle whereby as a result of summer high temperatures, rodents tend to be more active at night (MacDonald and Barrett, 1993). Despite the ability of birds to serve as hosts, there are no records of infested nests in the vicinity of pig dwellings (Gooders and Harris, 1996). Nevertheless, during fieldwork some swallow nests were identified inside pig dwellings that were infested with *O. erraticus* (unpublished data). The method applied successfully disclosed information regarding the identity of previous tick hosts (and for some, multiple hosts). However, only 22.6% of ticks generated data through which the host identity could be determined. This poor sensitivity might have arisen from a combination of factors such as DNA extraction method used, the size of the meal and the time elapsed since the last meal (Kent, 2009). Future methodological refinements should be considered to optimize

this technique to further increase the percentage of successful host identification. Others have successfully utilized methods such as reverse line-blot hybridization against host species-specific probes (Kent, 2009).

Our results indicate that *O. erraticus* feeds from a variety of vertebrate hosts, but that *Sus scrofa* plays a prominent role as a preferred vertebrate host; consistent with observations from field studies and underscoring the major role of *Sus scrofa* in maintaining high population densities of *O. erraticus* and consequently *O. erraticus* associated zoonoses. The apparent contributions of other hosts to pathogen transmission show the need for a community approach to understand this vector-borne zoonosis. Moreover, the number of ticks that tested positive for human blood underscore their potential for zoonotic transmission. Recent changes in pig farm husbandry have contributed to decreased contact between ticks and humans with concomitant reduction of pathogen transmission. Despite this, pig farmers often report being bitten by *O. erraticus* and describe some symptoms of the disease, suggesting that infection remains active, albeit at a very low prevalence. These results demonstrate the value of blood-meal analysis to determine the host origin for blood meals in soft ticks and thus providing valuable information concerning other vertebrate hosts that could serve as reservoirs for propagation of *B. hispanica* as part of the natural ecology of this tick-borne infection in Portugal.

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Disclosure Statement

No competing financial interests exist.

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