Acta Tropica 172 (2017) 255–262

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Are brucellosis, Q fever and melioidosis potential causes of febrile illness in Madagascar?

Ides Boone[a,*], Klaus Henning[b], Angela Hilbert[b,1], Heinrich Neubauer[b], Vera von Kalckreuth[c], Denise Myriam Dekker[d], Norbert Georg Schwarz[d], Gi Deok Pak[c], Andreas Krüger[e], Ralf Matthias Hagen[e], Hagen Frickmann[e,f], Jean Noël Heriniaina[g], Raphael Rakotozandrindrainy[g], Jean Philibert Rakotondrainiarivelo[g], Tsiry Razafindrabe[g], Benedikt Hogan[d], Jürgen May[d], Florian Marks[c], Sven Poppert[d,h], Sascha Al Dahouk[a,i]

[a] German Federal Institute for Risk Assessment, Department of Biological Safety, Diedersdorfer Weg 1, 12277 Berlin, Germany
[b] Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Bacterial Infections and Zoonoses, Naumburger Straße 96a, 07743 Jena, Germany
[c] International Vaccine Institute, SNU Research Park, 1-Gwanak-ro, Gwanak-gu, Seoul 08226, Republic of Korea
[d] Bernhard Nocht Institute for Tropical Medicine, Bernhard Nocht-Straße 74, 20359 Hamburg, Germany
[e] Department of Tropical Medicine at the Bernhard Nocht Institute, Bundeswehr Hospital of Hamburg, Bernhard Nocht-Straße 74, 20359 Hamburg, Germany
[f] University Medicine Rostock, Schillingallee 70, 18057 Rostock, Germany
[g] Department of Microbiology and Parasitology, University of Antananarivo, B.P.175, Antananarivo, Madagascar
[h] University Medical Center Hamburg-Eppendorf, Martinistraße 52, 20246 Hamburg, Germany
[i] RWTH Aachen University Hospital, Pauwelsstraße 30, 52074 Aachen, Germany

ARTICLE INFO

Keywords:
Zoonoses
Brucellosis
Q fever
Melioidosis
Febrile illness
Madagascar

ABSTRACT

Brucellosis, Q fever and melioidosis are zoonoses, which can lead to pyrexia. These diseases are often underascertained and underreported because of their unspecific clinical signs and symptoms, insufficient awareness by physicians and public health officers and limited diagnostic capabilities, especially in low-resource countries. Therefore, the presence of *Brucella* spp., *Coxiella burnetii* and *Burkholderia pseudomallei* was investigated in Malagasy patients exhibiting febrile illness. In addition, we analyzed zebu cattle and their ticks as potential reservoirs for *Brucella* and *C. burnetii*, respectively. Specific quantitative real-time PCR assays (qPCRs) were performed on 1020 blood samples drawn from febrile patients. In total, 15 samples (1.5%) were *Brucella*-positive, mainly originating from patients without travel history, while DNA from *C. burnetii* and *Bu. pseudomallei* was not detected.

Anti-*C. burnetii* antibodies were found in four out of 201 zebu serum samples (2%), whereas anti-*Brucella* antibodies could not be detected. *Brucella* DNA was detected in a single zebu sample. Three out of 330 ticks analyzed (1%) were positively tested for *C. burnetii* DNA but with high Ct values in the qPCR assay. Our data suggest that zebus as well as *Amblyomma* and *Boophilus* ticks have to be considered as a natural reservoir or vector for *C. burnetii*, but the risk of cattle-to-human transmission is low. Since bovine brucellosis does not seem to contribute to human infections in Madagascar, other transmission routes have to be assumed.

1. Introduction

Brucella spp., *Coxiella burnetii* and *Burkholderia pseudomallei* are Gram-negative pathogens, which cause brucellosis, Q fever, and melioidosis, respectively. They may considerably affect human and animal health, especially in low-resource areas. While Q fever and brucellosis are classical zoonotic diseases, melioidosis is a saprozoonosis, indicating that the source of infection is the abiotic environment (e.g. contaminated soil or water) (Hubalek, 2003). In areas endemic for malaria and typhoid fever, zoonotic infections, often characterised by

* Corresponding author.
E-mail addresses: Idesbald.Boone@bfr.bund.de (I. Boone), Klaus.Henning@fli.de (K. Henning), angela.hilbert@havelland.de (A. Hilbert), Heinrich.Neubauer@fli.de (H. Neubauer), vera.vkalckreuth@daad-alumni.de (V. von Kalckreuth), dekker@bni-hamburg.de (D.M. Dekker), schwarznorbert@bni-hamburg.de (N.G. Schwarz), gdpak@ivi.int (G.D. Pak), krueger@bnitm.de (A. Krüger), hagen@bnitm.de (R.M. Hagen), frickmann@bnitm.de (H. Frickmann), herijean007@yahoo.fr (J.N. Heriniaina), rakrapha13@yahoo.fr (R. Rakotozandrindrainy), rakotophilibert@yahoo.fr (J.P. Rakotondrainiarivelo), rakouttsa@yahoo.fr (T. Razafindrabe), hogan@bnitm.de (B. Hogan), may@bnitm.de (J. May), fmarks@ivi.int (F. Marks), sven@poppert.eu (S. Poppert), Sascha.Al-Dahouk@gmx.de (S. Al Dahouk).
[1] Present address: Landkreis Havelland, Amt für Landwirtschaft, Veterinär- und Lebensmittelüberwachung, Goethestraße 59-60, 14641 Nauen, Germany.

http://dx.doi.org/10.1016/j.actatropica.2017.05.013
Received 22 December 2016; Received in revised form 9 May 2017; Accepted 10 May 2017
Available online 11 May 2017

non-specific clinical symptoms such as fever, headache, and fatigue, are underdiagnosed (Crump et al., 2013; Halliday et al., 2015). Hence, physicians should be aware of zoonotic diseases as a cause of fever of unknown origin (FUO) (Cleri et al., 2007).

Worldwide, more than 500,000 human brucellosis cases are reported annually, with high incidences in the Middle East and Central Asia (e.g. Syria, Iran, Iraq, Mongolia, Kirgizstan) and North Africa (e.g. Algeria) (Pappas et al., 2006). Human disease is mainly caused by *Br. melitensis*, *Br. abortus* and *Br. suis* which are transmitted to humans through direct contact with infected livestock (i.e. sheep, goat, cattle, pig), their excretions (faeces, urine, placenta and aborts) or by ingestion of contaminated food products (mainly unpasteurized dairy products and raw meat). In livestock farming, brucellosis leads to tremendous economic losses due to decreased milk production, abortions and limited fertility (McDermott and Arimi, 2002).

With the exception of New Zealand and French Polynesia, Q fever has a worldwide distribution (Million and Raoult, 2015). The largest Q fever outbreak so far has been notified in the Netherlands with more than 4000 human cases between 2007 and 2010 (Schneeberger et al., 2014). According to a recent review covering 51 studies, Q fever seroprevalence in the general African population is less than 8% with slightly higher rates in children (10–17%). However, it varies widely among geographic regions and a much higher seroprevalence was found in Egypt (32%) (Vanderburg et al., 2014). The main reservoirs of *C. burnetii* are mammals and arthropods, with ruminants (sheep, goat, cattle) as the most common source of human infections. The disease is usually transmitted through inhalation of aerosols from amniotic fluid or placenta material of infected animals, although ingestion, transfusion and sexual intercourse may sporadically occur (Million and Raoult, 2015). Acute, chronic (endocarditis) or subclinical courses have been described after infection with *C. burnetii*. In livestock, Q fever can lead to a loss of productivity mostly due to increased abortion rates (Vanderburg et al., 2014).

Melioidosis is a tropical disease, which is predominantly reported in Southeast Asia (i.e. Thailand, Singapore, Vietnam, Malaysia) and North Australia, although sporadic cases also occur elsewhere. In a recent study on the global burden of melioidosis, a total of 165,000 cases including 89,000 fatalities per year were estimated worldwide. These estimates were associated with substantial uncertainty (Limmathurotsakul et al., 2016). The reported number of cases in tropical low-resource countries are likely to be underestimated due to insufficient disease awareness and absence of diagnostic facilities (Hoffmaster et al., 2015). The host species spectrum of *Bu. pseudomallei* is broad including pigs, sheep, goats, horses, dogs and cats. A wide range of clinical symptoms have been described in humans, e.g. fever, headaches, muscle pain, pneumonia with chest pain and cough, abscesses. Humans are infected through contact with contaminated surface water and mud or indirectly through the inhalation of contaminated aerosols (Heymann, 2008).

Madagascar is a large island situated in the Indian Ocean, 500 km east of Mozambique, with an area of 587,295 km^2. The island is divided into 22 regions with a total population of 23,400,000 (INSTAT, 2016). Its climate is tropical along the coast, temperate in the Central Highlands and arid in the South. Livestock is mainly composed of cattle (ca. 10 million), goats (ca. 1,470,000), sheep (ca. 840,000) and pigs (ca. 1,500,000) (Data 2012: FAOSTAT (2015)). Recent epidemiological data on the presence of brucellosis (McDermott and Arimi, 2002) and Q fever (Vanderburg et al., 2014) in Madagascar do not exist and only sporadic cases of melioidosis have been reported (Garin et al., 2014).

Therefore, our objective was to investigate the presence of *C. burnetii*, *Brucella* and *Bu. pseudomallei* in Malagasy patients suffering from febrile illness as a potential sentinel symptom for zoonotic diseases. Furthermore, we aimed to identify potential sources of human infections, by screening zebus for *Brucella* and both zebus and ticks for *C. burnetii*.

2. Materials and methods

2.1. Human study population and survey area

The International Vaccine Institute (IVI) collected blood samples within the Typhoid Fever Surveillance in Africa Program (TSAP). This network aims to generate data on the burden of typhoid fever and other invasive *Salmonella* infections through standardized surveillance and disease burden studies in ten African countries (von Kalckreuth et al., 2016).

The TSAP survey sites in Madagascar consisted of public health care facilities in central Madagascar, both in a rural environment (Imerintsiatosika) and urban slums (Isotry, located in the capital of Madagascar Antananarivo) with a catchment population of 46,000 and 70,000 inhabitants, respectively. In the rural catchment area, cattle husbandry and rice farming are predominant and the people live in close contact with their animals. In total, 18% and 9% of the population in Imerintsiatosika and Isotry sought care for fever in the surveillance facilities (Panzner et al., 2016). A total of 4500 whole blood EDTA samples were collected in Madagascar between 2011 and 2013 within the framework of TSAP from patients with unknown diagnosis at presentation and a body temperature $\geq 37.5\,^{\circ}C$. From these, a subset of 1020 samples taken from patients with pyrexia $\geq 38.5\,^{\circ}C$ was screened for *C. burnetii*, *Brucella* spp. and *Bu. pseudomallei*. The selection of the subset was motivated in anticipation of a greater chance to detect bacteraemia in patients with a higher body temperature. However, we cannot exclude that in the samples not analyzed ($\geq 37.5\,^{\circ}C$ and $< 38.5\,^{\circ}C$) we might have missed subclinical or chronic infections. Samples were stored at $-20\,^{\circ}C$ prior to testing. Demographic data (age, gender), place of residence, travel history, clinical signs and symptoms as well as medication use were recorded.

Informed written consent was obtained from the patients, their parents or their legal guardians. The study was approved by the Malagasy Ethical Committee (no. 045 MSANP/CE) and the IVI Institutional Review Board (no. IVI IRB #2011-001).

2.2. Sampling of zebu and ticks

In October 2012, a total of 215 zebu cattle were sampled in three slaughterhouses in the municipality of Bemasoandro (district of Antananarivo-Atsimondrano). Origin of the cattle, age, sex, health and nutritional status were recorded.

Both whole blood and serum samples were taken, 214 samples could be used for bacteriology and qPCR, and 201 samples for serology.

In total, 1822 ticks (1090 *Amblyomma variegatum* and 732 *Rhipicephalus* (*Boophilus*) *microplus* were collected with a maximum of 10 ticks sampled from each cattle as described previously by Keller et al. (2016). A random sample of 330 ticks (including 199 *Amblyomma variegatum* and 131 *Rhipicephalus microplus* ticks) from 80 cattle was used for screening.

2.3. Molecular and serological tests

2.3.1. DNA preparation from blood samples and ticks

Genomic DNA was prepared from 1 ml of human EDTA blood samples using the FlexiGene DNA Kit (Qiagen, Hilden, Germany). For zebu blood samples either FlexiGene DNA Kits (Qiagen) or High Pure Template Preparation Kits (Roche Diagnostics GmbH, Mannheim, Germany) were used. After mechanical disruption DNA was extracted from ticks by the QIAamp DNA Mini Kit (Qiagen) as previously described (Keller et al., 2016).

2.3.2. Brucella spp. detection

Quantitative real-time PCR assays (qPCR) were performed to detect brucellae in human and zebu samples by targeting the genus specific marker sequences *bcsp31* and IS711 (Al Dahouk et al., 2007; Cloeckaert