Molecular detection of *Rickettsia felis* and *Candidatus Rickettsia Asemboensis* in Fleas from Human Habitats, Asembo, Kenya

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Abstract

The flea-borne rickettsioses murine typhus (*Rickettsia typhi*) and flea-borne spotted fever (FBSF) (*Rickettsia felis*) are febrile diseases distributed among humans worldwide. Murine typhus has been known to be endemic to Kenya since the 1950s, but FBSF was only recently documented in northeastern (2010) and western (2012) Kenya. To characterize the potential exposure of humans in Kenya to flea-borne rickettsioses, a total of 330 fleas (134 pools) including 5 species (*Xenopsylla cheopis*, *Ctenocephalides felis*, *Ctenocephalides canis*, *Pulex irritans*, and *Echidnophaga gallinacea*) were collected from domestic and peridomestic animals and from human dwellings within Asembo, western Kenya. DNA was extracted from the 134 pooled flea samples and 89 (66.4%) pools tested positively for rickettsial DNA by 2 genus-specific quantitative real-time PCR (qPCR) assays based upon the citrate synthase (*gltA*) and 17-kD antigen genes and the *Rfelis* qPCR assay. Sequences from the 17-kD antigen gene, the outer membrane protein (*ompB*), and 2 *R. felis* plasmid genes (pRF and pRFd) of 12 selected rickettsia-positive samples revealed a unique *Rickettsia* sp. (*n* = 11) and *R. felis* (*n* = 1). Depiction of the new rickettsia by multilocus sequence typing (MLST) targeting the 16S rRNA (*rrs*), 17-kD antigen gene, *gltA*, *ompA*, *ompB*, and surface cell antigen 4 (*sca4*), shows that it is most closely related to *R. felis* but genetically dissimilar enough to be considered a separate species provisionally named *Candidatus Rickettsia asemboensis*. Subsequently, 81 of the 134 (60.4%) flea pools tested positively for *Candidatus Rickettsia asemboensis* by a newly developed agent-specific qPCR assay, Rasemb. *R. felis* was identified in 9 of the 134 (6.7%) flea pools, and *R. typhi* the causative agent of murine typhus was not detected in any of 78 rickettsia-positive pools assessed using a species-specific qPCR assay, Rtyph. Two pools were found to contain both *R. felis* and *Candidatus Rickettsia asemboensis* DNA and 1 pool contained an agent, which is potentially new.

Key Words: *Rickettsia*—Fleas—PCR—Multilocus sequence typing—Surveillance.

Introduction

*Rickettsiae* are obligate intracellular gram-negative bacteria that are associated with various arthropod vectors, including ticks, mites, lice, and fleas, and can cause mild to life-threatening human disease (Kelly et al. 2002). The first rickettsia associated with fleas was *Rickettsia typhi*, the causative agent of murine typhus. *R. typhi*, a typhus group (TG) rickettsia, has been most commonly associated with *Xenopsylla cheopis* (oriental rat flea), although at least 10 other
species of fleas have been found to be naturally or experimentally infected with \textit{R. typhi}. In addition, other arthropods (lice and mites) have been found to be infected with \textit{R. typhi} (Azad 1990). Murine typhus is endemic to Kenya, but at a low level, especially in rural areas (Heisch and Harvey 1959, Maina et al. 2012). During the early 1960s it was reported that of batches of the fleas \textit{Ctenophthalmus cabirius}, \textit{Dinopsyllus lyapis}, and \textit{X. cheopis} collected from wild rodents only \textit{X. cheopis} were infected with \textit{R. typhi} (Heisch et al. 1962), although in experimental infections both \textit{X. cheopis} and \textit{Xenopsylla brianziensis} could be infected equally well with \textit{R. typhi} in the laboratory (Heisch 1969). More recently a serosurvey conducted in western Kenya showed that of a randomly selected subset of stored sera (\(n=357\)) collected from January, 2007, through October, 2008, from patients who presented to a rural health clinic with respiratory illness, jaundice, and acute febrile illness with or without a clinically apparent cause, 5 (1.4\%) of the serum specimens contained immunoglobulin G (IgG) antibodies that reacted against \textit{R. typhi}, as determined by an indirect fluorescence antibody assay (IFA) (Maina et al. 2012). This low prevalence of antibodies reactive to typhus group rickettsiae corroborates the earlier assertion by Heisch and Harvey (1959) that murine typhus is rare in Kenya, especially in rural areas.

A second rickettsia associated with fleas, \textit{Rickettsia felis}, was discovered in \textit{Ctenocephalides felis} during the 1990s (Adams et al. 1990, Azad et al. 1992). \textit{R. felis}, initially characterized as a TG rickettsia because of its reactivity to guinea pig antiserum against \textit{R. typhi}, was subsequently placed in the spotted fever group (SFG) of rickettsiae due to presence of \textit{ompA} and phylogenetic studies. More recently, some have further separated \textit{R. felis} from both the TG and SFG rickettsiae into a transition group of rickettsiae (Gillespie et al. 2007). It was successfully cultivated in the XTC-2 cell line derived from \textit{Xenopus laevis} and fully described in 2001 (Raoult et al. 2001). Early on, \textit{R. felis} was determined to be a human pathogen (Shriefer et al. 1994) causing flea-borne spotted fever (FBSF) that now is frequently diagnosed around the world, especially in sub-Saharan Africa (Parola 2011). Two recent reports from our group have shown FBSF due to infection with \textit{R. felis} to be endemic to both the northeastern and western regions of Kenya (Richards et al. 2010, Maina et al. 2012). Risk factors of flea-borne rickettsioses in humans have not been fully characterized in those areas.

To identify the potential causative agent(s) of human flea-borne rickettsioses, we surveyed fleas collected from domestic and peridomestic animals and village homes for rickettsial agents. \textit{R. felis} and a new rickettsia, \textit{Candidatus Rickettsia asemboensis}, were identified among the fleas collected in Asembo, western Kenya.

Materials & Methods

Study site

The study was conducted in the Asembo division of Kakula District, Nyanza Province in western Kenya. This rural site on the eastern shore of Lake Victoria falls within a health and demographic surveillance system (HDSS) run by the Kenya Medical Research Institute (KEMRI) and the US Centers for Disease Control and Prevention (CDC) (Adazu et al. 2005). The Kenyan International Emerging Infections Program of KEMRI/CDC has conducted population-based infectious disease surveillance (PBIDS) of the human population in Asembo since late 2005 (Feikin et al. 2011). The PBIDS population is approximately 25,000 people living in 33 villages. The Asembo area is approximately 225 km² and is culturally homogeneous, with 95\% of people being ethnically Luo. It has a bimodal rainfall pattern, with rain seasons occurring from March to May and from September to November. The community lives in dispersed settlements, and houses are made of mud, cement, or brick, with roofs of iron sheet or thatch (Bi-gogo et al. 2010). The primary economic livelihood is subsistence farming and fishing. According to the demographic surveillance system (DSS) 2007/2008 census, the mean number of animals per compound was 2.6 cattle, 3.3 small ruminants, and 11 poultry (KEMRI/CDC, unpublished data).

Collection of fleas from domestic and peridomestic animals and human households

Three hundred livestock-owning compounds (LOC) were randomly selected from all known LOCs (\(n=4528\)) among the 33 PBIDS villages from January through May, 2009. The sampling frame of LOCs was compiled from livestock census data collected by the HDSS. A LOC was defined by ownership of 1 or more of the following animals—cattle, sheep, goats, and chickens. Where present, a maximum of 3 dogs and cats in each compound were surveyed for fleas. Fleas were also collected from 16 randomly selected houses from the 300 LOCs using light traps. The light traps were homemade and consisted of a hurricane lamp that was suspended a few inches above a tray containing water with dish soap and Vaseline smeared on the sides to prevent fleas from crawling out.

Additionally, peridomestic small mammal sampling was conducted in collaboration with the National Museums of Kenya using baited Sherman live traps over the period July 30 to August 7, 2009. Fifty compounds were randomly selected from the 300 LOCs. Informed consent for participation was obtained from household representatives prior to collection of animals. Five traps were placed within dwellings, in outbuildings, and outdoors in each compound for 4 consecutive nights, and the GPS locations of key points were recorded. Captured animals were euthanized using an overdose of halothane inhalant anesthesia (Rhodia Limited, Avonmouth, Bristol, BS119YF, UK). Fleas were collected from the animals by combing. Animal species identification was made on the basis of morphometric data at the National Museums of Kenya, where all specimens were submitted for archiving.

All fleas were preserved in 70\% ethanol until delivery to the laboratory and then stored at ~80°C. Fleas were identified using the entomological taxonomic keys of Segerman (1995), then pooled by individual host animal, method of collection, and flea species (49.3\% of pools contained a single flea, the remainder contained 2–14 fleas per pool).

DNA extraction and rickettsiae detection from fleas

Fleas were washed in molecular-grade water and mechanically disrupted using a bead mill (QIAGEN TissueLyser LT, Valencia, CA). Genomic DNA was extracted using QIAamp blood and tissue kits (QIAGEN) according to the manufacturer’s instructions, using a final elution volume of 50 μL.

A qPCR assay that amplifies and detects a 74-bp segment of the citrate synthase (\textit{gltA}) gene (Stenos et al. 2005) was used to
screen flea specimens for the presence of rickettsial DNA. A second Rickettsia genus-specific qPCR assay that amplifies and detects a 115-bp segment of the 17-kD antigen gene (Rick17b) (Jiang et al. 2012) was used to confirm the results.

**Identification of rickettsiae in fleas by species-specific qPCR assays**

To identify which rickettsiae were present in the rickettsia-positive flea pools, DNA samples were subsequently tested by 2 species-specific qPCR assays: (1) The Rfelis qPCR assay, in which a 129-bp fragment of the R. felis ompB was amplified (Henry et al. 2007), and (2) the Rtyph qPCR assay, in which a 122-bp fragment of the R. typhi ompB was amplified (Henry et al. 2007).

**Confirmation of rickettsiae species and identification of new rickettsiae by multilocus sequence typing**

To confirm the identity of the flea-borne rickettsiae, PCR and sequencing were performed on a subset of selected samples using the 17-kD antigen gene, ompB and R. felis plasmid genes pRF and pRF6. At least one of these positive samples was randomly selected from each of the different arthropod vectors, hosts, and method of collection.

To identify novel rickettsiae, we conducted multilocus sequence typing (MLST) using segments of 5 rickettsial genes, rrs, gltA, ompB, ompA, and sca4 (Fournier et al. 2003). Standard and nested PCR were carried out using primers and procedures previously described (Roux and Raoult 2000, Jiang et al. 2005) and new primers listed in Table 1. No positive controls were used in these PCR and nested PCR procedures to decrease chances of contamination; however, a negative control (molecular biology-grade water; Life Technologies) was run with the samples, and they were consistently negative in all runs.

PCR products were purified using a QIAquick PCR purification kit (QIAGEN). Sequencing reactions were performed for both DNA strands using a Big Dye Terminator v3.1 Ready Reaction Cycle Sequencing Kit (Life Technologies) according to the manufacturer’s instructions. After purification of sequencing products using Performa DTR Gel Filtration Cartridges (Edge BioSystems, Gaithersburg, MD), sequencing was run in an ABI Prism 3130xl Genetic Analyzer (Life Technologies). Sequences were assembled with Vector NTI Advance 11 software (Life Technologies), and BLAST searches were performed on the National Center for Biotechnology Information website (http://blast.ncbi.nlm.nih.gov/).

Phylogenetic analyses were performed using MEGA version 5 (Tamura et al. 2011) based on the multialignment of rrs, gltA, ompA, ompB, and sca4 sequences from the flea isolates and other rickettsial isolates from GenBank. The phylogenetic trees were constructed using the neighbor-joining method, and the bootstrap analyses were performed with 1000 replications.

**Development of the agent-specific qPCR assay (Rasemb) for the new flea-borne rickettsia, Candidatus R. asemboensis**

A 4311-bp fragment of ompB amplified from Candidatus R. asemboensis was aligned with the ompB from 28 different Rickettsia species obtained from GenBank using MEGA version 5; a unique 24-bp sequence fragment was identified to be the target of the probe. To insure the specificity of the assay, the probe Rasem2893BP (6-FAM-CCGCAGCTCCTCAATACCTTCCGCTAAAGCCATATGCGG-BHQ-1) was designed as a molecular beacon with the first and the last 6-bp sequences as the stem. The primers Rasem2828F (5′-CACACTTACCGGCCTTGATTC) and Rasem2939R (5′-AAGTGTATAGTCTGTTACTAAGACCG) amplified a 112-bp fragment of ompB.

The concentrations of the primers, the probe, and MgCl2, were optimized, as well as the annealing temperature using Platinum Quantitative PCR SuperMix-UDG (Life Technologies), and run on a StepOnePlus Real-Time PCR platform (Life Technologies). Each 25-μL reaction contained 0.7 μM of each forward and reverse primer, 0.3 μM of probe, and 7 mM of MgCl2. Two microliters of template DNA was used in each reaction. The cycler parameters included incubation for 2 min at 50°C, initial denaturation for 2 min at 95°C and 45 cycles of denaturation for 5 s at 95°C, and annealing/elongation for 30 s at 59°C.

The specificity of Rasemb assay was confirmed by testing a panel of bacterial nucleic acid preparations (Jiang et al. 2012) representing genetically closely and distantly related bacteria, including R. felis URRWXCaI2 and 19 other Rickettsia species and 12 nonrickettsial bacteria.

**GenBank accession numbers**

The sequences of the new rickettsial molecular isolates (F30, obtained from Ctenocephalides canis; F82, obtained from C. felis) have been deposited in GenBank with accession numbers JN315967 to JN315977 for rrS, gltA, 17-kD antigen, sca4, ompA, and ompB, respectively.

**Ethical review**

The collection of arthropod specimens from human dwellings and animals was approved by the KEMRI and CDC Animal Care and Use Committees (protocols nos. 1191 and 1562BRETBDX, respectively). Written informed consent was obtained from all animal owners before specimen collection.

**Results**

**Vertebrate hosts and residences surveyed:**

Of the 300 LOCs identified, 149 and 26 compounds owned at least 1 dog or a cat, respectively. Fleas were searched for on

<table>
<thead>
<tr>
<th>Table 1. New Primers Used for PCR and Sequencing</th>
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<tbody>
<tr>
<td><strong>Gene</strong></td>
</tr>
<tr>
<td>rrs</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>17 kD</td>
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<td></td>
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<tr>
<td>ompA</td>
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<tr>
<td>ompB</td>
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</tbody>
</table>
299 domestic dogs and 26 cats from a total of 149 compounds. Thirty-nine (17%) dogs and 9 (34.6%) cats were found to be infested with fleas. Other domestic animals noted but not surveyed for fleas at the 300 LOC included cattle (n = 463), goats (n = 378), sheep (n = 159), and chickens (n = 760).

One or more peridomestic small mammals were trapped at 27 of the 50 compounds (54%). Fifty-five rodents and shrews were trapped from a total of 1142 trap placements (4.8%) and 18 (32.7%) were found to host fleas. The 55 peridomestic mammals were identified as Crocidura olivieri (n = 20), Rattus rattus (n = 17), Mastomys natalensis (n = 15), Lemniscomys striatus (n = 2), and Mus minutoides (n = 1).

All of the 16 randomly selected LOC dwellings were infested with fleas. A total of 88 fleas were trapped, with an average of 5.5 fleas/home (range 1–26 fleas) during a single night’s collection using a single light trap/household.

Invertebrate hosts and rickettsia detection

A total of 330 fleas were collected and identified as 5 flea species: X. cheopis (n = 77), C. canis (n = 7), C. felis (n = 193), Echidnophaga gallinacea (n = 45), and Pulex irritans (n = 8). DNA preparations were obtained from 134 flea pools, of which 66 (49.3%) consisted of a single individual flea. Overall 89 of 134 (66.4%) flea pools were positive for rickettsiae by using gltA, Rick17b, and Rfelis qPCR assays, which included all 4 (100%) C. canis pools, 75 (100%) C. felis pools, 8 (22.9%) X. cheopis pools, 1 (20%) P. irritans pool, and 1 (6.7%) E. gallinacea pool (Table 2). Seventy-eight of the 89 rickettsia-positive pools were tested by the R. typhi qPCR assay, and all of those pools were negative.

Confirmation of rickettsial identification by 17-kD antigen gene, ompB, pRF, and pRFα PCR sequencing

To confirm the identity of the rickettsia, 12 of the 89 rickettsia-positive samples were selected and assessed by PCR and sequencing of 17-kD antigen gene and/or ompB, as well as the plasmid genes pRF and pRFα. A 390-bp fragment of 17-kD antigen gene and/or a 599-bp fragment of ompB were amplified from all 12 samples. The sequences of 17kD and ompB from 1 of the 12 samples was 100% identical to R. felis URRWXCal2 (GenBank accession no. CP000053). The R. felis-positive sample was further tested for the presence of plasmids. The plasmid pRF, but not the pRFα, was detected by PCR in this sample. The 429-bp pRF amplicon was sequenced and was determined to be 100% identical to that of R. felis URRWXCal2. The plasmids from the other 11 samples were identical to each other for both genes. BLAST searches revealed a unique sequence of ompB that was different from all rickettsial ompB sequences in GenBank. However, the 371-bp fragment of the 17-kD gene sequence was identical (100%) to Rickettsia sp. SE313 isolated from E. gallinacea in Egypt. The plasmids pRF and pRFα were not detected in any of these 11 samples.

MLST results (rrs, gltA, ompB, ompA, sca4)

To determine the identification of and to more fully characterize the novel flea-borne rickettsial agent, sequences for rrs, gltA, ompA, ompB, and sca4, from 6 of the 11 novel rickettsia-positive samples were determined and compared to those available on GenBank. The sequences of the 1395-bp fragment of rrs and 1130-bp of gltA from the new isolate were closely related to R. felis URRWXCal2 (99.5% and 98.0%, respectively) and R. sp. RF2125 (99.6% for gltA only), indicating that this new isolate belongs within the genus Rickettsia. A 1517-bp fragment of ompA (3’ end) was amplified, providing evidence that the new molecular isolate is a member of the SFG of rickettsiae. The fragment of the 5’ end of ompA was not amplified by a SFG universal primer set, which suggests that the gene might be truncated or the sequence was not recognized by 1 or both of the primers. The sequence of the 1517-bp fragment of ompA was most similar to R. felis URRWXCal2 (92.3%). The 4311-bp fragment of ompB sequence from the new agent was most similar to R. felis URRWXCal2 (94.8%), although the sequence of a 790-bp fragment was most similar to an ompB sequence (P. Parola, unpubl. data; GenBank #JX183538) from Rickettsia RF2125 (99.7%), suggesting a very close relationship between these 2 new flea-borne rickettsiae. The sequence of a 1034-bp fragment of sca4 from the novel rickettsia was most similar to R. felis URRWXCal2 (95.5%). Because of the nucleotide dissimilarity between this new agent and the most homologous validated species R. felis, this novel agent should be considered for further studies to characterize it as a new Rickettsia species. Therefore, we propose this isolate be identified as Candidatus R. asemboensis until it is grown in culture and its biology is described more completely.

Phylogenetic relationships between Candidatus R. asemboensis and other validated rickettsial species were analyzed by constructing phylogenetic trees using rrs (1395 bp), gltA (1130 bp), ompA (1517 bp), ompB (1481 bp), and sca4 (1034 bp). Candidatus R. asemboensis was either clustered with or placed closely to R. felis URRWXCal2 for all 5 genes (Fig. 1). Candidatus R. asemboensis closely related to R. felis matched up with the results from the blast searches.

Development of the Rasemb qPCR assay

In determining the specificity of the Rasemb qPCR assay, it was found that only the 6 MLST-identified Candidatus

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Table 2. Rickettsia qPCR Results by Flea Species

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Number of pools</th>
<th>Number of positive qPCR</th>
<th>Percent positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dogs (n = 39 dogs)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ctenocephalides felis</td>
<td>58</td>
<td>58</td>
<td>100</td>
</tr>
<tr>
<td>Echidnophaga gallinacea</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cats (n = 9 cats)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ctenocephalides canis</td>
<td>1</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Ctenocephalides felis</td>
<td>5</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>Echidnophaga gallinacea</td>
<td>8</td>
<td>1</td>
<td>12.5</td>
</tr>
<tr>
<td>Rodents (n = 18 rodents)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Echidnophaga gallinacea</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Xenopsylla cheopis</td>
<td>26</td>
<td>7</td>
<td>26.9</td>
</tr>
<tr>
<td>Light trap (n = 16 households)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ctenocephalides canis</td>
<td>3</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>Ctenocephalides felis</td>
<td>12</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>Echidnophaga gallinacea</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pulex irritans</td>
<td>5</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Xenopsylla cheopis</td>
<td>9</td>
<td>1</td>
<td>11.1</td>
</tr>
<tr>
<td>Total</td>
<td>134</td>
<td>89</td>
<td>66.4</td>
</tr>
</tbody>
</table>

*For each species, n represents the number of animals or houses.
R. asemboensis DNA preparations were positive, whereas R. felis and 19 near-neighbor rickettsial DNA preparations (Jiang et al. 2012) were negative.

Subsequently the Rasemb qPCR assay was used to assess all 134 DNA preparations. All 45 of the rickettsia-negative preparations were also negative for the Rasemb qPCR assay. Of the 89 rickettsia-positive samples, 81 samples (which include the 6 MLST-confirmed Candidatus R. asemboensis DNA preparations and 2 pools that had both Candidatus R. asemboensis and R. felis) were positive (Table 3) and 8 were negative. The identities of the 8 negative samples were determined by PCR and sequencing a 599-bp fragement of *ompB*. Seven of the 8 were determined to be 100% identical to *R. felis*. One sample’s *ompB* sequence (from a DNA preparation of *F. cheopis*), was determined to be a unique rickettsial sequence that had only 98.3% similarity to *R. felis* and 92.0% similarity to Candidatus *R. asemboensis* (GenBank # JX183537). Thus, in addition to our near-neighbor panel of DNA preparations, the Rasemb qPCR assay was shown to be specific to Candidatus *R. asemboensis* using this study’s sample of flea DNA preparations.

**Discussion**

With the recently determined risk of FBSF to citizens of and travelers to western Kenya (Maina et al. 2012), we investigated the breadth, diversity, and prevalence of flea vectors containing *R. felis* in Asembo, because effective mitigation measures would certainly depend upon informed preventive medicine decisions. Of 300 LOCs selected, 149 had 1 or more dogs and cats, vertebrate hosts to *C. felis*, *C. canis*, and *P. irritans*, fleas known to be infected with *R. felis* (Venzal et al. 2006). Due to the ubiquitous nature of peridomestic rodents also known to harbor fleas infected with *R. felis* (Jiang et al. 2006), we included them in our investigation. Therefore, we surveyed from 300 randomly selected LOCs 50 compounds for fleas from domestic and peridomestic mammals that might harbor fleas infected with *R. felis*. From these locations 299 dogs, 26 cats, and 55 rodents were obtained and searched for fleas. In addition, 16 randomly selected residences were assessed for the presence of rickettsia-containing fleas by use of a light trap. Results showed that, 17% dogs (39/299), 34.6% cats (9/26), 32.7% rodents (18/55), and 100% homes (16/16)
FIG. 1. (Continued)
The prevalence of rickettsial infections within the vectors was determined by 2 genus-specific qPCR assays and 1 presumed species-specific qPCR assay, RfELs (Henry et al. 2007). Most notable was the high prevalence of rickettsiae found in dog (100%) and cat (97.3%) fleas (Ctenocephalides spp.). When combined with the frequent detection of these species on domestic dogs and cats, and within households, it is clear that the potential for human exposure to rickettsiae in these communities is very high.

When identities of the rickettsiae were confirmed by sequencing small fragments of the 17-kD antigen gene and ompB gene, it was determined that of the 12 samples assessed, only 1 was R. felis; the other 11 samples contained DNA of novel rickettsiae. Further characterization by MLST (Fournier et al. 2003) of this unique agent determined that it was most closely related to the validated species R. felis. However, it did not have enough nucleotide homology to R. felis to be considered to be that species. Therefore, by molecular techniques, this agent was found to represent a new rickettsial species that has some nucleotide similarity to members of a recently described, but poorly characterized, rickettsial genotype.

The R. felis-like genotype group includes molecular isolates with partial sequences of a few conserved rickettsial genes (17-kD gene, rrs, and gltA) and have been found not only among various fleas species, but in mites, ladybird (Coccidula rufa), soft ticks (Ornithodoros moubata), and louse flies (Khalidi et al. 2012, Mediannikov et al. 2012, Roucher et al. 2012). The rickettsiae of this R. felis-like genotype group were initially identified in the 2000s and included Rickettsia RF2125 and Rickettsia RF31, which were detected in C. canis and C. felis (Parola et al. 2003) following a survey in Kanchanaburi Province, Thailand, near the Myanmar border. Rickettsia RF2125 and Rickettsia RF31 were distinct from other rickettsiae but most similar to R. felis (97.9 and 97.4%, respectively, for gltA). Subsequently, Reeves et al. (2005) identified, using molecular techniques, these 2 rickettsiae in C. felis collected from animals killed by automobiles in the Piedmont and foothills of South Carolina. A similar agent named Rickettsia sp. SE313, similar to RF2125, was also discovered in 12 of 12 Echidnophaga gallinacea (sticktight fleas) and in 7 of 120 (5.8%) pools of Ornithonyssus bacoti (tropical rat mites) obtained from rats live-trapped in Egypt (Loftis et al. 2006, Reeves et al. 2007). In a survey of ectoparasites and associated pathogens of free-roaming and captive animals in zoos of South Carolina, Rickettsia RF2125 was found in C. felis removed from a zoo-keeper and a grizzly bear (Nelder et al. 2009). In addition, Rickettsia RF2125 was identified in P. irritans (human flea) obtained from a dog in Hungary (Hornok et al. 2010). Most recently, an agent similar to RF2125 (99.7% identical sequence to a 728-bp portion of the conserved gltA gene) has been identified in Susternus pallidus fleas from Dielmo village, Senegal (Roucher et al. 2012). Collectively, these references indicate that other flea-borne rickettsiae exist throughout the world and that a much more thorough characterization of these agents by molecular and biological means is needed, especially in regard to pathogenicity.

The high prevalence of Candidatus R. asemboensis among fleas known to bite humans and to transmit agents that cause human disease raises the potential that this newly identified rickettsia could be an important cause of human illness. However, in the same area of Asembo where 50 of 699 (7.2%) fever patients were determined to have FB5F, 21 of 21 molecular isolates from the 50 positive blood samples had sequence-confirmed R. felis DNA (Maina et al. 2012). Similarly, in a study of non-malaria fever patients, 6 of 163 (3.7%) patients had evidence of FB5F, and all 6 samples were sequence verified to have R. felis DNA in the serum samples (Richards et al. 2010). Candidatus R. asemboensis was not detected in any patients’ samples from those areas. However, before concluding that Candidatus R. asemboensis is not pathogenic for humans, screening of specimens from additional febrile patients will be done while ensuring that illnesses from a distribution of age groups and during different months of the year and different years will be needed.

In contrast to the high prevalence of Candidatus R. asemboensis in arthropods obtained from animals and human dwellings was the low prevalence of R. felis (5.97%), a demonstrated human pathogen, among the same arthropods. This low prevalence of R. felis seems contrary to the 7.2% prevalence of FB5F among fever patients in Asembo (Maina et al. 2012) and the 4.4% and 3.7% prevalence among malaria-negative febrile patients in Senegal (Socolovschi et al. 2010) and northeastern Kenya (Richards et al. 2010), but more in line with the sporadic nature of this disease reported from non-sub-Saharan locations (only 68 cases from 20 countries on 6 continents; Parola 2011).

In conclusion, we report our findings that fleas collected from Asembo, Kenya, contain in addition to the rickettsial pathogen R. felis (the causative agent of flea-borne spotted fever), a new rickettsia, similar to the previously described Rickettsia RF2125 from Thailand, South Carolina, Egypt, Hungary, and Senegal. This new rickettsia, provisionally referred to as Candidatus R. asemboensis, is commonly found.

Table 3. Rickettsia Detected in 5 Flea Species from Asembo

| Female | Male |
|--------|------|--------|------|--------|------|------|------|------|------|
| 77     | 3     | 1     | 2     | 3     | 3     | 1     | 2     | 57     | 4     | 33    |
| Positive % | 22.9  | 6.7   | 66.4  | 100   | 100   | 100   | 100   | 89     | 100   | 100   |

No. species | 5 |
within fleas known to vector \textit{R. felis} in western Kenya where flea-borne spotted fever is endemic. Although we did not find evidence of human infection with this new rickettsia, more testing on human specimens will be needed to determine whether the organism is pathogenic for humans.

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Author Disclosure Statement

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