Research Square

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Molecular detection and characterization of emerging pathogens of Rickettsia- felis and felis-like organisms from peri-domestic fleas in Uganda

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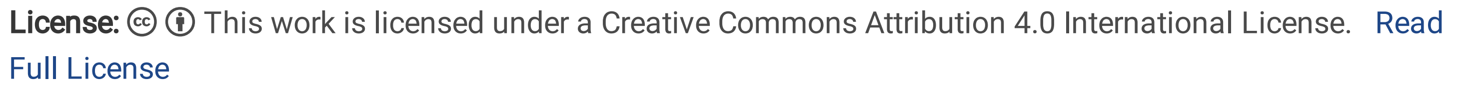

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Abstract

Background: Flea-borne spotted fever is an emerging zoonosis caused by *Rikecttsia felis*, a Gram-negative obligate intracellular bacterium. The agent is believed to be cosmopolitan, following the distribution pattern of its host and reservoir, *Ctenocephalides felis*. However, the epidemiology and public health risk it poses remains poorly understood in sub-Saharan Africa, including Uganda. *Yersinia pestis*, is primarily transmitted by rodent fleas, *Xenopsylla cheopis*, but other fleas, particularly *C. felis*, have vectoral capacity. They are neglected in Ugandan entomological surveillance and public health practices, particularly outside endemic foci of bubonic plague.

Methods: We collected 14,641 fleas from domestic animals, rodents and homestead environment; compared their diversity and abundance. Pooled into 714 flea pools by species, collection time, host, and host species, 172 pools were selected based on seasons and analyzed for *Yersinia pestis Pla* genes, while 62 pools were tested for Rickettsia species *gltA*, *ompA*, and *17kDA* genes by qPCR and Sanger sequencing.

Results: Five flea species were identified from the collections: *Ctenocephalides canis, C. felis, Echidnophaga gallinacea, Pulex irritans,* and *Xenopsylla cheopis*. *Ctenocephalides* was the predominant genus, accounting for 84.8% of fleas collected, mostly found on dogs and goats. Except for *P. irritans* (which was found in Gulu district) the other four flea species were found across all districts, year-round, with higher numbers collected in dry seasons compared to rainy seasons (c^2=47.64, *df*=20, *p*<0.001). *Rattus rattus* constituted 74% of rodents captured from human dwellings and was the only rodent species with fleas, where *X. cheopis* was the predominant species and *E. gallinacea* found on only three rodents. All 172 pools of fleas tested negative for *Yersinia pestis*. Of the 62 pools tested for *Rickettsia* spp., 29 (46.8%) were positive. Twenty-five PCR amplicons were successfully sequenced for *17kDa* and two for *ompA* genes. Based on *17kDa*, two were identified as *R. felis* from *C. canis* and 23 were *R. asembonensis* from multiple flea species, including *C. canis* collected goats and *C. felis* from cats.

Conclusion: Our survey identified a high pooled detection rate (~50%) of *Rickettsia* spp. in fleas tested, suggesting a potential risk of human exposure and infection. *Rickettsia felis* and *R. asembonensis* were the predominant flea-borne *Rickettsia* spp. identified, with this study also representing the first report of *Rickettsia* spp. in *E. gallinacea* in Uganda.

Introduction

Rickettsia felis is an obligate intracellular Gram-negative bacterium in the class alpha-proteobacteria. The Rickettsia genus is divided into four groups based on genotyping: the ancestral group, transitional group, typhus group, and the spotted fever group to which R. felis belongs [1]. Rickettsia felis is an emerging pathogen and is the causative agent of flea-borne spotted fever (FBSF). Since its first detection in humans in the US, it has since been detected in febrile patients worldwide, emerging as a common cause of acute febrile illness in sub-Saharan Africa [2-4]. Rickettsia felis-specific circulating DNA has been detected in up to 15% of febrile patients from Mali, Senegal, Gabon, and Kenya [5-7]. Through molecular genetics and surveillance of flea-borne rickettsial agents in fleas, R. felis-like organisms, such as Candidatus R. asemboensis, have also been detected in various flea species, although their pathogenicity remains unknown [8-10]. Diagnosis of flea-

borne rickettsioses is difficult as they cannot be cultured using conventional bacteriological methods due to their intracellular nature. As a result, they often go undiagnosed due to lack of access to advanced diagnostic techniques, which are often unavailable in resource-limited settings [11].

Plague, caused by Yersinia pestis, is a flea-borne bacterial zoonosis that is often fatal if appropriate antibiotic treatment is inadequate or delayed [12]. The majority of human plague cases in recent decades have occurred in Africa [13]. In Uganda, the West Nile region represents the primary epidemiologic foci where 255 human plague cases were reported between 2008—2016 [14]. Due to the epidemic nature of the disease, many studies about Y. pestis and its epidemiology have concentrated in this region, neglecting other areas in Uganda.

Fleas are obligatory hematophagous wingless ectoparasites of higher vertebrates, particularly mammals and birds [15]. Due to their blood feeding habits across a wide host range, fleas are important vectors for pathogens of both medical and veterinary importance [15-16]. The evolutionary success of fleas has enabled them to survive globally in a variety of landscapes and environmental conditions. The most famous cosmopolitan fleas from medical and veterinary perspectives are several pulicids (Pulex irritans, Xenopsylla cheopis, Ctenocephalides canis, C. felis), ceratophyllids (Nosopsyllus consimilis, N. fasciatus), and one leptopsyllid (Leptopsylla segnis) [15]. The ubiquitous distribution of these species is a result of their close interaction between humans, livestock, pets, and commensals (mice and rats). Despite their cosmopolitanism, the distribution of these fleas is not uniform [15]. Instead, they are distributed in patches that are characterized by the host and environmental conditions that are favorable for each given species. This may explain the focal occurrences of the diseases they transmit.

Ctenocephalides felis (cat fleas) are hosts and vectors of R. felis and R. asembonensis [3]. However, the detection of R. felis in other arthropods such as other flea species, ticks, and Anopheles mosquitoes have been reported [17]. Rodent fleas, particularly X. cheopis and X. brasiliensis, are the main vectors of Y. pestis [18-19]. However, C. felis has also been demonstrated to transmit plague in its early stages of feeding and is a potential vector in endemic areas or hotspots of re-emergence [20]. Ctenocephalides felis are indiscriminate feeders that parasitize domestic cats, dogs and other livestock including ruminants. A bird flea, E. gallinacea, is characterized as a stationary feeder, and like other fleas it also parasitizes mammals [15].

In Uganda, the epidemiology of R. felis and its role as potential cause of febrile illness has largely been neglected. Furthermore, the flea populations, their preferred hosts and the pathogens they carry in domestic and peri-domestic areas have been understudied outside plague-endemic foci of the West Nile region of Uganda. In this study, we sought to describe flea–host associations on animals in four regions of Uganda outside the West Nile region, determine the flea index on animals from homesteads, and screen fleas for flea-borne pathogens, specifically Rickettsia spp. and Y. pestis. This study adds critical epidemiological information on circulating flea-borne pathogens and can be used to improve prevention and control efforts.

Materials And Methods

Study sites

We collected fleas in five districts of Uganda, namely Jinja (Eastern Uganda), Kampala (Capital of Uganda), Kasese (Western Uganda), Gulu (Northern Uganda), and Luwero (Central Uganda) (Figure 1) between April

2017 and September 2018. The selected districts are considered major economic hubs in their respective regions and are both geographically and culturally diverse, with high levels of economic heterogeneity. Fleas were collected from livestock (cattle, goats, sheep, pigs, rabbits), companion animals (dogs and cats), rodents, and directly from the environment. In each of the study districts, 18 villages were selected, and from each village four animal owning households were selected for sample collection. In total, 360 households were visited over 18 months. Informed consent was obtained from the head of household for inclusion in the study.

Flea collection and Pooling

Most mammalian fleas do not attach to hosts during feeding and can easily escape when disturbed, so attempts to restrain and immobilize the fleas were made [15]. Pets with phlegmatic temperament and small ruminants were hand restrained. Aggressive pets were sedated with Xylazine 1mg/kg (Xyla, Interchemie, Holland) and Ketamine 6mg/kg (Rotex Medica, Tritau, Germany). Animals were placed on white plastic sheets, and the surface of their fur was sprayed with 70% ethanol to immobilize fleas. After one to four minutes, fleas under the fur emerged to the surface and were brushed onto the white sheet, picked with forceps, and transferred into 70% ethanol for preservation. Rodents were trapped using Sherman traps in houses occupied or frequently visited by people in the selected homesteads. Traps were baited and set in the evening with a mixture of ground peanuts and smoked fish, with captured rodents recovered and processed in the morning as previously reported [18]. Traps were transported in white aerated gunny bags to prevent fleas from escaping. The rodent species were identified by morphological characteristics including the length of their body, tail, ear, hind foot, weight [21]. Fleas were collected by combing the fur with a brush on to white gunny bags and placed in 70% ethanol for preservation. The flea index (# of fleas/host examined) for the animals was determined using established methods [22]. Fleas were also collected from earthen floor houses inhabited or frequented by people in the homesteads. We used trays containing water with grease or Vaseline smeared on the sides to prevent fleas from crawling as previously described [8]. Fleas were identified to the species level using published morphologic taxonomic keys under a stereomicroscope [16, 23-24]. A total of 14,641 individual fleas were collected and then pooled into 714 sample pools according to flea species, host, collection area and date of collection. Pool sizes ranged from 1-281 fleas.

Homogenization of the flea pools

Flea pools were placed in Eppendorf tubes containing RNA later (Sigma Life Science, Darmstadt, Germany) and disrupted using sterile disposable pestles attached to a motorized grinder (HLD-12, Ryobi, China). The fleas were then homogenized by passing them through 20-gauge needles. The homogenates were stored at -80°C until DNA extraction. A subset of 172 pools (2,457 fleas) consisting of 68 rodent flea pools (three pools of *E. gallinacea*, 65 *X. cheopis*) and 104 pools (pool size 1-63 fleas) from pets, livestock, and the environment were tested for *Y. pestis*. The flea pools selected for *Y. pestis* testing comprised the five flea species collected in the five districts and two seasons (dry and rainy seasons). Of the 172 pools, a subset of 62 pools (353 individual fleas) with pool sizes of 1-28 fleas selected based on seasons (dry and wet) and subsequently tested for *Rickettsia* spp.

DNA Extraction and PCR

Total DNA was extracted from all the flea homogenates using the Qiagen DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. Duplicate positive and negative controls were included during every batch of DNA extraction. The 62 flea pool DNA samples were screened for flea-borne *Rickettsia* spp. with primers amplifying the 74-bp citrate synthase (*gltA*) gene as previously described [25-26]. The primers were CS-F (5-TCGCAAATGTTCACGGTACTTT-3) and CS-R (5-TCGTGCATTTCTTTCCATTGTG-3). A second *Rickettsia* genus-specific qPCR amplified a 115-bp segment of the *17kDa* and *ompA* genes to confirm the initial PCR results as described previously [27]. *R. felis* DNA (provided by Walter Reed Army Institute of Research, Silver Spring, MD) was used as a positive control and ultrapure water as a negative control. To detect *Y. pestis* from flea pools, qPCR targeting the plasminogen activator gene (*Pla*) was performed using primers Yper_PLA_F (59- ATG-GAG-CTT-ATA-CCG-GAA-AC-39) and Yper_PLA_R (59-GCG-ATA-CTG-GCC-TGC-AAG-39) and probe Yper_PLA _P (6- FAM-TCC-CGA–AAG-GAG-TGC-GGG-TAA-TAGG- TAMRA) [28]. *Y. pestis* CSUR P100 strain DNA was used as positive control.

Sequencing and Phylogenetic Analysis

A 539 base pair amplicon for *17kDa* and a 650 base pair amplicon for *ompA* gene was amplified as previously described [29] using Platinum Taq (Thermo Fisher Scientific). PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's instructions. Agarose gel purified amplicons of the *17kDa* and *ompA* genes were sequenced on the SeqStudio (Thermo Fisher Scientific) using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) according to the manufacturer's recommendations. Forward and reverse reads were aligned using CLC Genomics Workbench (Qiagen), and a consensus sequence for each gene was generated for BLAST analysis. Sequences of *17kDa* and *ompA* genes and references from GenBank were imported and aligned in Geneious Prime 2022.11.0.14.1. The sequences were MAFFT aligned and exported to MEGA 10.2.6 [30]. Maximum likelihood trees were created using the Tamura-Nei model with bootstrap iterations set at 1,000.

Mapping

Descriptive maps with the collection sites, tick species, and pathogen location were created in QGIS 3.28 [31]. The Uganda district shapefile was accessed at https://data.unhcr.org/en/documents/details/83043.

Statistical analysis

The pooled prevalence rates, maximum likelihood estimation (MLE), and minimum infection rates (MIR) were calculated by collection district and flea species to assess the probability of *Rickettsia* spp. detection from the flea pools as described before [32]. MIR was calculated by dividing the number of infected pools by the total number of fleas in all of the pools and expressed as the number of infected pools per 100 fleas tested assuming that each positive pool only had one positive flea. The maximum-likelihood estimate (MLE) is the infection rate most likely observed given the test results and an assumed probabilistic model; which is a binomial distribution of infected individuals in a positive pool. The CDC's Mosquito Surveillance Software (https://www.cdc.gov/westnile/resourcepages/mosqSurvSoft.html) was used in Excel to obtain MLE and MIR estimates with their corresponding 95% confidence intervals accounting for individual pool sample size. A Pearson chi-squared test was used to detect any differences between the distributions of outcomes in

different groups, with a p-value of <0.05 considered significant. Data were analyzed using STATA software, version 16.1 (StataCorp, College Station, TX).

Results

Flea infestation of the hosts

A total of 14,641 individual fleas, comprising five species were collected from livestock, companion animals, rodents, and the environment during the sampling period (Table 1). Rodents and shrews (n=159) belonging to three species were collected from human dwellings in the five districts. Among rodents, fleas were only recovered from the *R. rattus* and none from shrews (*Crocidura* spp.) and mice (*Mus musculus*) (Additional File 1, Table 1). From *R. rattus,* 256 fleas belonging to two species, *X. cheopis* and *E. gallinacea* were recovered. A total of 119 fleas belonging to three species (*C. canis, E. gallinacea* and *P. irritans*) were collected from 13 domestic rabbits. *Echidnophaga gallinacea* was recovered from rodents, cats, dogs, goats, pigs, rabbits, and the environment, and *X. cheopis* was found on cats, dogs, and goats. The predominant fleas, *C. canis* (84.8% of all fleas collected), were collected on all animals except chickens and rodents. There were significant differences observed in flea diversity between districts (c^2=42.51, *df*=16, *p*<0.001) and seasons (quarters) (c^2=47.64, *df*=20, *p*<0.001) of collection (Additional File 2, Tables 1—4).

Infection rates of *Rickettsia* spp. and *Y. pestis* in the flea pools from the districts

Pool positivity rates for *Rickettsia* spp. from the 62 flea pools consisting of 363 fleas tested with qPCR for *gltA*, *17kDa,* and *ompA* gene primers is presented in Table 2. *Rickettsia* spp. was detected in all districts (Figure 2) with an overall pooled prevalence of 48.8% (29/62).

Overall, MLE for *Rickettsia* spp. for the districts was 11.5%, with the corresponding MIR of 8.2%. Gulu district had the highest MLE of 29.8% (95% CI: 16.4–50.5) with a corresponding MIR of 17.2% (95% CI: 7.9–26.4) while Luwero had the lowest MLE of 4.6% (95% CI: 1.7–10.4) with a MIR of 4.3% (95% CI: 0.2–9.0). In general, higher MLE values were obtained in the northern and eastern districts. *Rickettsia*-positive flea pools were from three genera, *Ctenocephalides* (82.8%), *Xenopsella* (10.3%) and *Echidnophaga* (6.9%). *Ctenocephalides canis* had the highest MLE and MIR across flea species, while other flea species had variable MLEs based on districts of collection (Table 2). The 172 flea pools consisting of 2,457 fleas from all five species collected tested negative for *Y. pestis Pla* genes.

Rickettsia spp. identified by nucleotide sequences and phylogenetic analysis

Of the 29 flea pools positive for *Rickettsia* spp., 25 pools of purified PCR amplicons were successfully sequenced using the *17kDa* and *ompA* genes. The nucleotide sequences obtained from *17kDa* and *ompA* were compared to those available on NCBI GenBank database by BLASTn analyses. The summary of the *Rickettsia* spp. identified by sequences and phylogenetic trees are presented in Table 3 and Figure 3. The predominant *Rickettsia* spp. identified was *R. asembonensis* (23/25). *R. asembonensis* was only detected in *C. canis* from multiple animal species (goat, dog, cat, and a rabbit). The comparison of the *17kDa* sequence from this study to GenBank sequences revealed that flea pools from Gulu, Jinja, Luwero and Kampala had identical homology with a sequence isolated from cat fleas in Peru. *Rickettsia felis* was also identified in two *C. canis* pools from a

goat and a dog respectively. A sequence of *R. felis* detected from a flea pool in Luwero district matched to fleas collected from wild animals in Brazil. Using the *ompA* gene, *R. felis* was detected in two flea pools, one of which had consensus with *17kDa* sequence. The *ompA* sequence comparison revealed *R. felis* from this study were identical to a sequence from a Brazilian flea.

Discussion

The main objective of this study was *to detect emerging flea-borne pathogens and to describe flea-host associations in four regions of Uganda outside a plague-endemic area. Understanding flea-host associations and the pathogens they carry is critical in determining site specific outbreak potential of flea-borne febrile illnesses, which in turn can be used to guide prevention and control.* Although there are surveys on fleas of small mammals in plague endemic areas and Tunga fleas in Busoga and Karamoja regions of Uganda [18, 33-36] there are no comprehensive surveys of fleas parasitizing domestic animals in Uganda. We therefore report the on diversity of flea species on livestock, pets, rodents, and environment in human dwellings in Uganda.

Ctenocephalides canis was the most abundant flea species (84.8%), parasitizing dogs, goats, rabbits, pigs, cattle, sheep, and cats, as well as from the dry soil environment in homesteads. This flea occurs worldwide and is the predominant flea found on dogs in Greece [37], Ireland [38], Chile [39], Albania [40] and Hungary [41] among other countries. However, our findings differ from others that found *C. felis* as the dominant flea on both dogs and cats in parts of sub-Saharan Africa [36, 42]. Cat fleas, the second most abundant flea on domestic livestock and pets in our study are vectors of several zoonotic bacteria, including *Bartonella* spp., *Yersinia* spp., and *R. felis* [43-45]. The flea diversity on animals in close contact with humans is an important factor in predicting potential emergence of such zoonotic diseases. Animals that graze in the bush or hunt in the wild such as cats and dogs are likely to bridge the gap of circulation of certain diseases in the sylvatic cycle and domestic environments. In our study, we identified the rodent flea *X. cheopis* on domestic cats and dogs which could increase the potential risk of spillover to humans.

Ecological factors, human-livestock interactions, and commensals (rats and mice) affect the flea population and diversity in an area [15-16]. These dynamic changes in host type and flea diversity are associated with the shift in foci of zoonotic diseases. For example, the epidemiologic focus for plague in the 1930s was in the southern portion of Uganda but shifted in the early 2000s to the current west Nile region where there are routine outbreaks [46]. As a result of this change, small mammal populations have also adapted [18, 46-47]. During intensive sampling of small mammals and their fleas in plague foci of southern and West Nile regions in 1937-1938, only one *R. rattus* was caught in the West Nile region. *Mastomys natalensis* was the most abundant rodent in human dwellings while *X. cheopis* was the most abundant flea. But now, *R. rattus* is the most abundant rodent in human dwellings in the West Nile region with the same predominant flea, *X. cheopis*. This agrees with our study, where *R. rattus* accounted for 74% of all rodent collections in human dwellings in all the five districts mainly infested with *X. cheopis.*

Rickettsia-positive fleas were detected from every district, with varying MLEs. Higher MLEs and pool-positivity rates were in the Gulu district (northern Uganda) and Jinja district (eastern Uganda) suggesting potential hotspots for *Rickettsia* spp., warranting further investigation. Our study found a low pool prevalence of *R. felis* in *C. canis*, which is the causative agent of flea-borne spotted fever (FBSF), an important emerging but

neglected agent of febrile illness in sub-Saharan Africa [48]. Although *C. felis* is a known competent vector for *R. felis* [49], the presence of this bacterium in *C. canis* poses a risk to human health due to its relative abundance and poor host specificity. Moreover, *R. felis* transmission to humans has been associated with *Anopheles* mosquitos [6] and its DNA has been previously detected in other flea species [50]. Furthermore, *R. felis*, has been identified in febrile patients in East Africa [48] and should be considered in diagnostic testing of febrile illness in these regions of Uganda. *Rickettsia asembonensis* belongs to the *R. felis*-like genotype group and its pathogen potential remains unknown. First detected in Asembo (Siaya County, Western Kenya) and putatively named *Candidatus* R. asemboensis [8], it has since been recorded in other parts of Africa, including Uganda [10] and South Africa [51]. The organism has also been found in fleas infesting domestic and wild animals in central and South America [9, 52].

Yersina pestis was not detected in any flea pool, which still warrants reporting, given repeated plague outbreaks in neighboring West Nile region [14, 22]. Yersiniosis often causes epidemics with high case fatality rates in untreated patients drawing public health attention [14]. Even within plague endemic areas in Uganda, rodent fleas in quiescent plague periods had no *Y. pestis* detected [53]. Our finding agrees with the lack of human cases in other regions of Uganda despite a long history of cases in the West Nile region.

Conclusions

We confirm the presence of *R. felis* in Gulu district, which suggest a potential risk of human infection. We also demonstrate the wide distribution of *R. asembonensis* as the predominant *Rickettsia* spp. in multiple flea species in the four regions of Uganda. Given that these animals were collected near homesteads, there is a risk for human disease exposure, with future efforts expanding differential diagnosis to include *R. felis* and *R. asembonensis* in acute febrile patients with unknown etiology. As the first detection of *Rickettsia* spp. in *E. gallinacea,* expanded monitoring of homesteads in other areas of Uganda would contribute to the characterization of potential flea-borne disease threats and causes of febrile illness throughout the country. While we did not detect *Y. pestis,* the wide distribution of competent vectors across study sites is of concern, should *Y. pestis* be introduced to these districts.

Abbreviations

PCR: Polymerase Chain Reaction; qPCR: quantitative realtime PCR; *gltA*: Citrate synthase gene, *17kDA*: gene encoding for Outer Membrane protein with molecular mass of 17 kilo Daltons, OmpA: Outer membrane protein A gene, MIR: Minimum infection rate, MLE: Maximum likelihood estimate; FBSF: Flea-borne spotted fever.

Declarations

Supplementary Information

Additional File 1: Table S1. Number of rodents captured from homesteads different quarters (seasons).

Additional File 2: Table S1. Flea pools collected by quarters (seasons). **Table S2**. Flea pools collected by district. **Table S3:** Fleas (unpooled) collected by quarters (seasons). **Table S4:** Fleas (unpooled) collected by district.

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Data availability statement

The datasets used or analyzed in the current study are available with the corresponding author on reasonable request. The sequences from this study are available on GenBank under the accession numbers: OP974449-OP974471. The tables and figures are incorporated in the manuscript. Further supplementary files are attached as a separate file.

Author Contributions

DKB, JWK, HK, EM, RT and WE: Conceived and designed the study. WE, BE, AMB, GA, TT, QAU, NGC, performed the experiments. WE, NGC, MEvF, DKB, analyzed the data. All the authors participated in drafting and writing the manuscript.

Ethical approval and consent to participate

This study was performed under Protocol "Acute Febrile Illness in Uganda" approved by Makerere University School of Public Health Research and Ethics Committee (MakSPH-REC #369), Makerere University College of Veterinary Medicine Research Committee (SBLS/HDRC/19/004), Uganda National Council for Science and Technology (UNSCT # 2029) and Walter Reed Army Institute of Research (WRAIR #2327).

Consent for Publication

Not applicable

Competing Interests

The authors have declared that no competing interests exist.

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Tables

Table 1: Flea index and collection number on animals and the environment in five districts from April 2017-Sept 2018

	Total number of fleas collected (Number of fleas/host examined)					
Host (Numbers checked)	*C. canis*	*C. felis*	*E. gallinacea*	*P. irritans*	*X. cheopis*	Total
Livestock						
Cattle (3)	31 (Nd)	0 (Nd)	0 (Nd)	0 (Nd)	0 (Nd)	31 (Nd)
Goat (1196)	6286 (5.26)	0 (0.00)	13 (0.01)	0 (0.00)	8 (0.01)	6307 (5.27)
Sheep (12)	86 (Nd)	0 (Nd)	0 (Nd)	0 (Nd)	0 (Nd)	86 (Nd)
Pig (18)	115 (Nd)	0 (Nd)	8 (Nd)	0 (Nd)	0 (Nd)	123 (Nd)
Rabbit (13)	116 (8.92)	0 (0.00)	2 (0.15)	1 (0.08)	0 (0.00)	119 (9.15)
Chicken (8)	0 (0.00)	0 (0.00)	366 (45.75)	0 (0.00)	0 (0.00)	366 (45.75)
Domestic animal						
Dog (343)	5703 (16.66)	0 (0.00)	428 (1.25)	0 (0.00)	23 (0.07)	6154 (17.94)
Cat (122)	3 (0.02)	604 (5.00)	407 (3.34)	0 (0.00)	3 (0.02)	1017 (8.34)
Rodent						
Rattus rattus (118)	0 (0.00)	0 (0.00)	32 (0.27)	0 (0.00)	224 (1.90)	256 (2.17)
Crocidura spp. (9)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Mus musculus (32)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Environment						
Earthen floor house (124)	82 (Nd)	0 (Nd)	100 (Nd)	0 (Nd)	0 (Nd)	182 (Nd)
Total Fleas	12422	604	1356	1	258	14641

Nd: flea index not determined. For cattle, only calves were fully checked because mature cattle were not restrained for a complete examination due to the risk of bloat. Pigs were not entirely searched for fleas because they were not fully restrained by hand. Sheep were not considered in this estimate as it was not practical to comb for all fleas due to variable amounts of wool.

Table 2: Maximum Likelihood Estimates (MLE) and Minimum Infection Rate (MIR) with corresponding 95% confidence intervals for detection rates of *Rickettsia* spp. in all flea pools

District	Flea species	Positive pools (%)		Total fleas	MLE			MIR		
					Point	Low	High	Point	Low	High
Gulu	*C. canis*	7/9	(77.8%)	42	30.59	14.25	63.58	16.67	5.40	27.94
	E. gallinacean	2/4	(50.0%)	7	29.01	6.11	68.39	28.57	0.00	62.04
	P. irritans	0/1	(0%)	1	0.00	0.00	79.35	0.00	-	-
	X. cheopis	2/3	(66.7%)	14	16.10	4.25	66.80	14.29	0.00	32.62
	Total	11/17	(64.7%)	64	29.76	16.41	50.50	17.19	7.95	26.43
Kasese	*C. canis*	4/6	(66.7%)	55	11.85	3.99	34.53	7.27	0.41	14.14
	X. cheopis	0/3	(0%)	13	0.00	0.00	15.62	0.00	-	-
	Total	4/9	(44.4%)	68	8.33	2.74	21.76	5.88	0.29	11.48
Kampala	*C. canis*	2/4	(50.0%)	32	8.01	1.85	40.69	6.25	0.00	14.64
	C. felis	1/1	(100%)	7	-	-	-	14.29	0.00	40.21
	E. gallinacean	0/1	(0%)	1	0.00	0.00	79.35	0.00	-	-
	X. cheopis	0/5	(0%)	12	0.00	0.00	19.15	0.00	-	-
	Total	3/11	(27.3%)	52	7.98	2.15	23.69	5.77	0.00	12.11
Jinja	*C. canis*	6/7	(85.7%)	53	18.36	9.20	18.36	11.32	2.79	19.85
	X. cheopis	1/6	(16.7%)	24	3.85	0.25	3.85	4.17	0.00	12.16
	Total	7/13	(53.8%)	77	12.59	5.97	24.90	9.09	2.67	15.51
Luwero	*C. canis*	4/11	(36.4%)	90	4.65	1.74	10.57	4.44	0.19	8.70
	X. cheopis	0/1	(0%)	2	0.00	0.00	54.55	0.00	-	-
	Total	4/12	(33.3%)	92	4.56	1.69	10.36	4.35	0.18	8.52
	Overall total	29/62	(46.8%)	353	11.55	8.15	16.07	8.22	5.35	11.08

Table 3: Identity table comparing *Rickettsia* spp. sequences from this study to GenBank sequences by gene and district. The length of the *17kDa* sequences were 326-425bp and *ompA* sequences were 545-612bp.

Gene	*Rickettsia* spp.	District	GenBank[a]	Accession ID	Identity
17kDa	*R. asembonensis*	Luwero	OP974462	MK923739.1	100%
		Gulu	OP974452-OP974457, OP974463	KY445736.1	100%
		Kampala	OP974449-OP974451	KY445736.1	99.0%
		Kasese	OP974461, OP974467-OP974468, OP974470	MK923739.1	100%
		Jinja	OP974458-OP974460, OP974464-OP974466	KY445736.1	100%
	R. felis	Luwero	OP974469	MT012727.1[b]	100%
	Rickettsia spp.	Luwero	OP974471	MT012728.1	100%
ompA	*R. felis*	Luwero	OP985656, OP985657	KY172883.1[c]	100%

[a] Sequences from this study submitted to GenBank

[b] and [c] are *ompA* gene sequences recovered from fleas and the blood of animals from Brazil respectively.

Figures

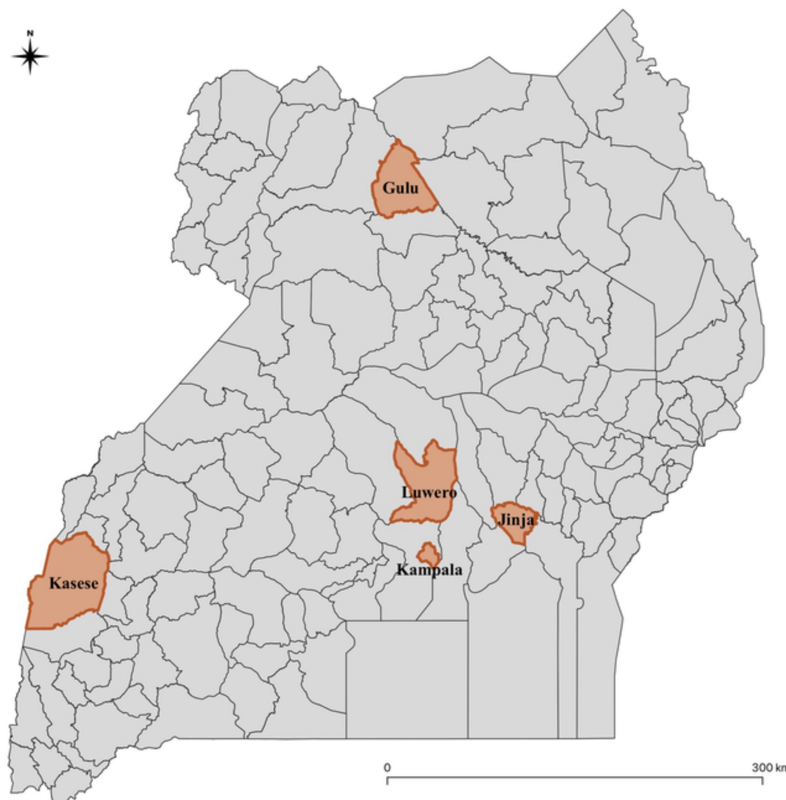

Figure 1

Study districts (shown in orange) for acute febrile illness surveillance and arthropod vectors in Uganda.

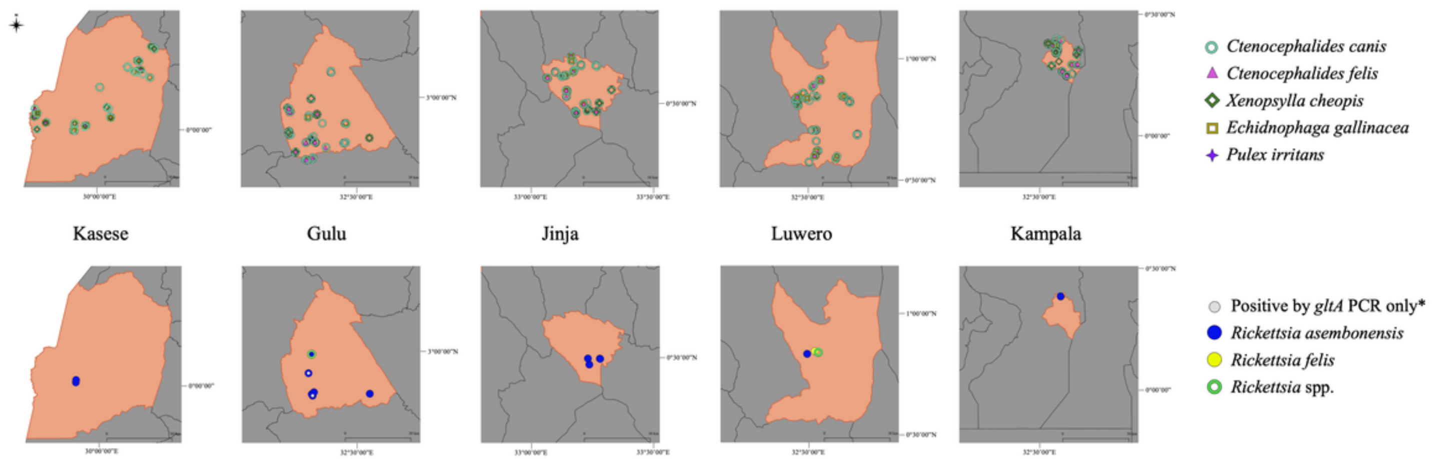

Figure 2

Flea species and positive Rickettsia spp. detections by districts. *Positive by initial *gltA* PCR but no sequence was obtained using *17kDa* and *ompA* PCR.

Figure 3

Maximum likelihood tree of the 17kDa Rickettsia spp. gene with sequences ranging from 362 to 425bp. Values less than 70% were excluded from the tree. GenBank numbers in bold were from this study.

Supplementary Files

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