Rickettsia felis Infection in Febrile Children, Ghana

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Abstract. Rickettsial infections are an underrecognized cause of febrile illness in sub-Saharan Africa. To evaluate the epidemiology and clinical features of rickettsial disease in pediatric patients in Ghana, we screened blood samples from febrile children aged less than 15 years presenting to an outpatient department in Ghana's Ashanti Region for the presence of rickettsial DNA. We detected *Rickettsia felis* in 7/470 (1.5%) blood samples, using two independent real-time polymerase chain reactions. No other *Rickettsia* species were found. *R. felis* was detected repeatedly in one patient, and coinfection with *Plasmodium falciparum* was found in 3/7 samples. Symptoms apart from fever included cough (6/7) and vomiting (4/7). None of the *R. felis*-positive patients reported a rash. This study is the first report on *R. felis* in Ghana and adds to the growing evidence for its widespread occurrence with and without malaria coinfection in sub-Saharan Africa.

Systemic bacterial infections have been found to contribute substantially to the causes of febrile illnesses in sub-Saharan Africa (SSA). In blood cultures, *Salmonella enterica*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Escherichia coli* are the most common pathogens.¹ Bacteria requiring more elaborate diagnostic techniques such as *Rickettsia*, are still underdiagnosed despite their public health importance.² Besides typhus group and spotted fever group rickettsiae, *Rickettsia felis* has recently been found to be potentially widespread in SSA, where *R. felis*-specific circulating DNA has been detected in the blood of up to 15% of febrile patients from Mali, Senegal, Gabon, and Kenya.^{3–5} However, its role as a pathogen is still unclear and data on the geographical distribution, epidemiology, and clinical features of *R. felis* are limited. In West Africa, *R. felis* has only been reported in patients from the Sahel-zone countries of Mali and Senegal.^{4–6} Since appropriate diagnostic methods are largely unavailable and standard antibiotic regimens usually do not cover *Rickettsia* spp.,⁷ further data are critical to aid physicians in the empirical management of febrile illness.

We screened blood samples from febrile children to evaluate the epidemiology and clinical features of rickettsial disease in Ghana.

Recruitment took place at St. Michael's Hospital, Pramso, located in the suburban belt of Kumasi, the capital of Ghana's Ashanti Region, where malaria is endemic with high transmission intensity throughout the year.⁸ Venous blood was taken from all children below 15 years of age visiting the outpatient department with fever $\geq 38^{\circ}\text{C}$ (tympanic) between January and December 2012. Blood cultures and malaria microscopy (thick and thin films) were done at each presentation and a blood sample (ethylenediaminetetraacetic acid [EDTA]) was stored for further diagnostics. *Rickettsia* polymerase chain reaction (PCR) was

performed on 470 randomly selected blood samples, from patients with negative blood cultures and reporting regular contact with domestic animals.

For molecular diagnosis of *Rickettsia* spp., total DNA was extracted from EDTA-blood and tested by real-time PCR targeting the rickettsial *gltA* gene, using previously reported oligonucleotides.⁹ The amplicons were bidirectionally sequenced (SeqLab, Göttingen, Germany) and were 100% identical with *R. felis* upon BLAST analysis (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Species identification was confirmed using a second real-time PCR specific for the *ompB* sequence of *R. felis* (see Supplemental Material for details on molecular methods).¹⁰ No other *Rickettsia* species were found.

In total, 470 blood samples from 431 individuals were tested. Of the 431 individuals, 216 (50.1%) were females and median age at presentation was 38 months (interquartile range [IQR]: 12–71). 204 (43%) samples were malaria positive. *R. felis* was found in seven (1.5%) samples from six (1.4%) individuals (three males and three females) out of whom one tested positive twice during the study period. The median age of positive patients was 35 months (IQR: 12–59), with the youngest positive patient 1 month and the oldest 87 months of age (Table 1).

Apart from fever, *R. felis*-positive patients complained of cough (six out of seven visits) and vomiting (four out of seven visits); however, there were no reports of rash. Malaria coinfection was found in the three oldest positive patients and was absent in patients younger than 36 months. None of the *R. felis*-positive patients had started antibiotic or antimalarial treatment before presentation and one patient was admitted for treatment. No patient received empirical antimicrobial therapy covering *Rickettsia* spp. (Table 1).

One patient tested positive for *Rickettsia* at two out of three visits: *Plasmodium falciparum* alone was detected on January 25, 2012; *R. felis* was detected on June 12, 2012 with no concurrent malaria infection identified; at the third presentation on August 17, 2012, a clinical diagnosis of severe malaria was made and tests were positive for both *P. falciparum* and *R. felis*. The patient was hospitalized, treated for severe malaria, and discharged 5 days later.

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TABLE 1
Clinical characteristics of *Rickettsia felis*-positive patients

Patient sex	Age at presentation (months)	Fever (°C)	<i>Plasmodium falciparum</i> (per µL)	Symptoms	Treatment
1 ♂	1	38.1	0	Cough, vomiting	ACT
2 ♂	12	39.9	0	Constipation, cough, vomiting	ACT ceftriaxone, cefuroxime
3 ♀	26	38.2	0	Cough, vomiting	ACT metronidazole
4 ♀	35	38.4	0	Cough	ACT amoxicillin, cefuroxime
	37	38.9	1,083,760	–	Quinine gentamycin, ceftriaxone, metronidazole, cefuroxime
5 ♂	59	40.2	54,046	Cough, stomach pain	ACT cefuroxime
6 ♀	87	39.6	173,630	Constipation, cough, vomiting	ACT cefuroxime

ACT = artemisinin-based combination therapy.

R. felis has been associated with febrile infections and symptoms similar to murine typhus, and was implicated as the causative agent of vesicular fever ("yaaf").^{5,6,11,12} It occurs worldwide, but data on its distribution in Africa are limited. Here, we report the first detection of *R. felis* in Ghana with 1.5% of febrile pediatric patients being infected. No other rickettsial pathogen was detected. Although infections with the primarily tick-borne spotted fever group rickettsiae appear to be common in West Africa, as suggested by high seroprevalences in Côte d'Ivoire (35%) and Burkina Faso (36%),⁹ *R. felis* as a member of the transitional group rickettsiae has only been detected in West Africans from the Sahel-zone countries of Senegal and Mali, where up to 24% of febrile patients were PCR positive.⁴

Malaria coinfection was present in three out of seven *R. felis*-positive patients, and all coinfecting patients were older than 36 months. Although these observations need to be interpreted with care, they are consistent with similar findings from Senegal, where coinfection was commonly observed and mostly occurred in older children.⁴ Similar age and seasonality patterns of infection, along with the recently reported potential of *Anopheles gambiae* mosquitoes to transmit *R. felis*, have given rise to a new hypothesis on possible common transmission routes of *R. felis* and malaria.^{4,5,13}

The clinical significance of *R. felis* infection and malaria-*R. felis* coinfection has been controversially discussed. In our study, all patients were febrile, but additional symptoms were nonspecific. No rash was found, which is consistent with other reports from Africa.⁵ Remarkably, some authors have detected *R. felis* DNA in blood samples of afebrile individuals, albeit at lower percentages than in febrile patients,^{3,4} thus challenging its role as an obligate pathogen. None of our *R. felis*-positive patients received empirical antimicrobial treatment covering *Rickettsia* spp. and the course of disease could not be monitored as patients were treated on an outpatient basis. Our study design did not allow the definitive determination of *R. felis* as the disease-causing agent. It also remains unclear whether a coinfection with malaria alleviated or aggravated the course of disease.

The significance of repeated detection of *R. felis*, as seen in one of our patients and also observed in other studies, is subject to ongoing discussion and may be explained either by persistence or reinfection.^{4,12,14,15} The detection of *R. felis* in afebrile individuals has furthermore led to the hypothesis that humans could be a natural reservoir of *R. felis*.^{3,4,12,16} However, as *R. felis* DNA has also been detected on the skin of healthy Senegalese villagers and in the feces of several arthropod vectors such as *Ctenocephalides felis* or *Liposcelis bostrychophila*, it is still unclear to which extent these findings may be influenced by skin contamination.^{5,12,17}

In conclusion, our study is the first report on the detection of *R. felis* in febrile children in Ghana, and thus adds to the growing evidence for a widespread occurrence of *R. felis* in SSA. To better understand the pathogenesis of *R. felis* infections, future studies should longitudinally monitor the presence of *R. felis* DNA and specific antibodies following infection, and use control groups to determine the clinical significance of *R. felis*, both in the absence and presence of malaria coinfection as well as in asymptomatic patients.

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REFERENCES

- Reddy EA, Shaw AV, Crump JA, 2010. Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis. *Lancet Infect Dis* 10: 417–432.

2. Prasad N, Murdoch DR, Reyburn H, Crump JA, 2015. Etiology of severe febrile illness in low- and middle-income countries: a systematic review. *PLoS One* 10(6): e0127962.
3. Maina AN, Knobel DL, Jiang J, Halliday J, Feikin DR, Cleaveland S, Ng'ang'a Z, Junghae M, Breiman RF, Richards AL, Njenga MK, 2012. *Rickettsia felis* infection in febrile patients, western Kenya, 2007–2010. *Emerg Infect Dis* 18: 328–331.
4. Mediannikov O, Socolovschi C, Edouard S, Fenollar F, Mouffok N, Bassene H, Diatta G, Tall A, Niangaly H, Doumbo O, Lekana-Douki JB, Znazen A, Sari M, Ratmanov P, Richet H, Ndiath MO, Sokhna C, Parola P, Raoult D, 2013. Common epidemiology of *Rickettsia felis* infection and malaria, Africa. *Emerg Infect Dis* 19: 1775–1783.
5. Angelakis E, Mediannikov O, Parola P, Raoult D, 2016. *Rickettsia felis*: the complex journey of an emergent human pathogen. *Trends Parasitol* 32: 554–564.
6. Mediannikov O, Fenollar F, Bassene H, Tall A, Sokhna C, Trape J-F, Raoult D, 2013. Description of “yaaf”, the vesicular fever caused by acute *Rickettsia felis* infection in Senegal. *J Infect* 66: 536–540.
7. Crump JA, Morrissey AB, Nicholson WL, Massung RF, Stoddard RA, Galloway RL, Ooi EE, Maro VP, Saganda W, Kinabo GD, Muiruri C, Bartlett JA, 2013. Etiology of severe non-malaria febrile illness in northern Tanzania: a prospective cohort study. *PLoS Negl Trop Dis* 7: e2324.
8. UNICEF, 2013. At a glance: Ghana—Statistics. Available at: http://www.unicef.org/infobycountry/ghana_statistics.html. Accessed February 8, 2015.
9. Mediannikov O, Diatta G, Fenollar F, Sokhna C, Trape JF, Raoult D, 2010. Tick-borne rickettsioses, neglected emerging diseases in rural Senegal. *PLoS Negl Trop Dis* 4: e821.
10. Henry KM, Jiang J, Rozmajzl PJ, Azad AF, Macaluso KR, Richards AL, 2007. Development of quantitative real-time PCR assays to detect *Rickettsia typhi* and *Rickettsia felis*, the causative agents of murine typhus and flea-borne spotted fever. *Mol Cell Probes* 21: 17–23.
11. Schriefer ME, Sacci JB, Dumler JS, Bullen MG, Azad AF, 1994. Identification of a novel rickettsial infection in a patient diagnosed with murine typhus. *J Clin Microbiol* 32: 949–954.
12. Brown LD, Macaluso KR, 2016. *Rickettsia felis*, an Emerging Flea-Borne Rickettsiosis. *Curr Trop Med Rep* 3: 27–39.
13. Dieme C, Bechah Y, Socolovschi C, Audoly G, Berenger J-M, Faye O, Raoult D, Parola P, 2015. Transmission potential of *Rickettsia felis* infection by *Anopheles gambiae* mosquitoes. *Proc Natl Acad Sci USA* 112: 8088–8093.
14. Labruna MB, Walker DH, 2014. *Rickettsia felis* and changing paradigms about pathogenic rickettsiae. *Emerg Infect Dis* 20: 1768–1769.
15. Socolovschi C, Mediannikov O, Sokhna C, Tall A, Diatta G, Bassene H, Trape JF, Raoult D, 2010. *Rickettsia felis*-associated unruptive fever, Senegal. *Emerg Infect Dis* 16: 1140–1142.
16. Mourembou G, Lekana-Douki JB, Mediannikov O, Nzondo SM, Kouna LC, Essone JCBB, Fenollar F, Raoult D, 2015. Possible role of *Rickettsia felis* in acute febrile illness among children in Gabon. *Emerg Infect Dis* 21: 1808–1815.
17. Mediannikov O, Socolovschi C, Million M, Sokhna C, Bassene H, Diatta G, Fenollar F, Raoult D, 2014. Molecular identification of pathogenic bacteria in eschars from acute febrile patients, Senegal. *Am J Trop Med Hyg* 91: 1015–1019.