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International Journal of Infectious Diseases 28 (2014) 107–110

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

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

Coxiella burnetii-positive PCR in febrile patients in rural and urban Africa

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ARTICLE INFO

Article history:
Received 14 April 2014
Received in revised form 27 May 2014
Accepted 30 May 2014

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Q fever
Africa
Coxiella burnetii
Genotype
Multi-spacer sequence typing

SUMMARY

Objectives: Q fever has been reported throughout the African continent. The objective of this study was to detect the presence of *Coxiella burnetii* in febrile patients from Africa.
Methods: Blood samples from febrile and non-febrile patients from six African countries and from France were investigated retrospectively for Q fever infection by molecular assays targeting the *IS*1111 and *IS*30A spacers.
Results: We tested 1888 febrile patients from Senegal, Mali, Tunisia, Algeria, Gabon, and Morocco and found one male adult patient (0.3%) infected with *C. burnetii* in Algeria and six positive patients (0.5%) in Senegal. For one patient from Senegal we determined that the infection was caused by *C. burnetii* genotype 35. In Senegal, more patients were infected with *C. burnetii* in Keur Momar Sarr ($p = 0.002$) than in the other locations. Blood samples taken from 500 (51% males) non-febrile people from Senegal and France were all negative.
Conclusions: The installation of point-of-care laboratories in rural Africa can be a very effective tool for studying the epidemiology of many infectious diseases.
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1. Introduction

Fever is a common problem for which patients seek medical advice. Investigations of the spectrum of etiology of unknown fever in Africa have progressed rapidly during recent years, resulting in an improvement in knowledge about bacterial infection.[1] Most uninvestigated causes of death in Sub-Saharan Africa are the result of infectious diseases.[1] Moreover, travelers in Africa are exposed to various health risks in unfamiliar environments, and fever is a common problem in travelers.[2–4] Most cases are acquired in Sub-Saharan Africa, and spotted fever group (SFG) rickettsioses are second only to malaria as the most commonly diagnosed diseases in returnees with a systemic febrile illness.[2,3] In 2008, we started to investigate the causes of fever of unknown origin (FUO) in Africa, and particularly in Senegal, to identify appropriate strategies for case management in rural communities.[5–8]

Q fever is a worldwide zoonosis with many acute and chronic manifestations that is caused by the pathogen *Coxiella burnetii*.[9] Clinical findings in Q fever infection are often confusing, and primary infection is asymptomatic in approximately 60% of cases.[9] Infections with *C. burnetii* occur throughout Africa.[10] In some cases of Q fever, bacteria may persist for years despite antibiotic therapy, and it has been proposed that in endemic regions, humans may excrete *C. burnetii*, even without evident clinical signs, for a long time.[6] A high prevalence of Q fever was identified recently in several villages in Senegal, indicating that Q fever should be

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http://dx.doi.org/10.1016/j.ijid.2014.05.029
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considered a significant public health threat.[6] Moreover, several strains of *C. burnetii* have been isolated from soft ticks in Senegal.[6] Although more than 40 tick species have been found infected with *C. burnetii*, ticks are not considered essential in the natural cycle of *C. burnetii* in livestock.[9]

To better guide diagnostic approaches and empirical therapies in Africa, more studies are needed to assess the role of fastidious bacterial infections in systemic febrile illness. The improvement in molecular biology tools, especially the availability of full genome sequences and real-time (RT)-PCR assays, has made it possible to design specific and sensitive assays in a systematic strategic approach to the diagnosis of Q fever. The objective of this study was to detect the presence of *C. burnetii* infection in febrile populations in Africa, an area in which epidemiological and clinical studies of zoonoses are scarce, using molecular methods. We collected blood samples from febrile and non-febrile patients from France and six countries in North and Sub-Saharan Africa to investigate for *C. burnetii* infection using molecular assays.

2. Materials and methods

2.1. Patients

Patients from six African countries with fever were investigated retrospectively for Q fever infection (Figure 1). Health centers distributed throughout six rural villages in Senegal were included from June 2010 to March 2012. Patients were sampled in the Kenitra region (dispensaries) of Morocco in 2008, and in Mali (Diankabou, Kole) and Franceville, Gabon in 2011. Patients were sampled in Sfax (infectious diseases and pediatric departments), Tunisia, and in Oran (department of infectious diseases), Algeria in 2012. All patients from Senegal, Morocco, and Gabon originated from rural areas, whereas the patients from Tunisia, Algeria, and Mali originated from urban areas (Table 1). The study sites in Senegal covered various ecosystems, from the dry area in the north to the humid area in the south, with a rainy season from June through October. As controls, blood samples from non-febrile villagers in Senegal and from Marseille, France were tested. Interviews, sampling, and a medical examination by physicians were performed on each individual with fever (an axillary temperature >37.5 °C).

2.2. Molecular diagnosis and multi-spacer sequence typing

A 200-μl sample of whole blood was collected in ethylenediaminetetraacetic acid (EDTA; Becton, Dickinson and Company, USA). The samples were handled under sterile conditions to avoid cross-contamination. DNA was extracted from the blood samples from France, Senegal, Algeria, Tunisia, and Morocco using a QIAamp kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. For Gabon, DNA was extracted from blood samples by DNA Blood Omega Bio-Tek E.Z.N.A method (Omega Bio-Tek, USA) as per the manufacturer's protocols. The genomic DNA of each sample was stored at −20 °C under sterile conditions. The genomic DNA of each sample was then sent to the World Health Organization (WHO) Collaborative Center for Rickettsial Diseases (Marseille) under sterile conditions at −20 °C. DNA was initially detected by *C. burnetii*-specific RT-PCR with primers and probes designed for the amplification of the *IS*1111 and *IS*30A spacers.[11] The quality of DNA handling and the extraction of samples was verified by RT-PCR for a housekeeping gene encoding the human beta-actin gene.[6,12] A sample was considered positive when PCR was positive for both *IS*1111 and *IS*30A spacers. Results were considered negative when PCR for *C. burnetii* was negative for both *IS*1111 and *IS*30A spacers and the cycle threshold (Ct) value of the beta-actin control gene was strongly positive ≤30 indicating an appropriate DNA load. DNA extracts were also tested by RT-PCR for the presence of *Tropheryma*

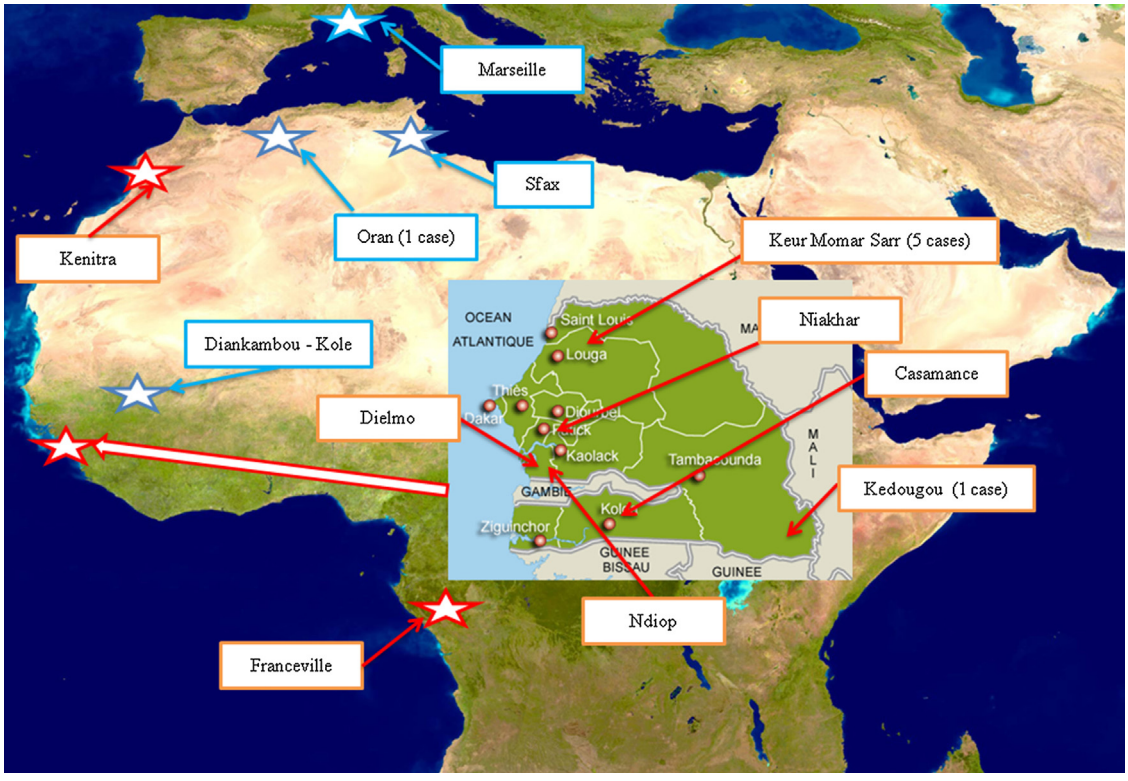

Figure 1. *Coxiella burnetii*-positive cases at the different health centers included in the current study (red color, rural areas; blue color, urban areas).

Table 1
Coxiella burnetii cases in the health centers that participated in this study

	Health center	Population size	No. of patients (% males)	Climate/vegetation		Positive
Febrile patients						
Sub-Saharan Africa						
Senegal						
	Keur Momar Sarr S_3	70 743	223 (43%)	Sahelian/steppe-type	Rural	5 (2.2%)
	Niakhar S_4	69 446	316 (45%)	Sahelo-Sudanian/wooded steppe	Rural	0
	Casamance S_5	57 505	411 (51%)	Sub-Guinean/primary and secondary gallery forests	Rural	0
	Kedougou S_6	20 021	288 (49%)	Sudano-Guinean/woodland, wooded savannah	Rural	1 (0.3%)
Mali	Diankabou – Kole	14 833	100 (50%)	Sahelian/savannas, forest	Urban	0
Gabon	Franceville	56 000	50 (46%)	Equatorial/ savannah and tropical forest	Rural	0
North Africa						
Algeria	Oran	1 584 607	268 (63%)	Mediterranean climate	Urban	1 (0.3%)
Morocco	Kenitra	1 859 540	48 (27%)	Mild Mediterranean climate	Rural	0
Tunisia	Sfax	2 256 320	184 (59%)	Mediterranean climate	Urban	0
Total			1888 (53%)			7
Non-febrile patients						
Senegal						
	Dielmo S_1		200 (53%)	Sudanian/wooded savannah		0
	Ndiop S_2		200 (40%)	Sudanian/wooded savannah		0
France	Marseille		100 (71%)	Mediterranean climate		0
Total			500 (51%)			0

whipplei, *Bartonella spp*, *Rickettsia spp*, *Francisella tularensis*, *Staphylococcus aureus*, *Escherichia coli*, and *Plasmodium falciparum*.[13] Samples were also tested by 16S rRNA and 18S rRNA gene amplification and sequencing.[13,14]

We determined the multi-spacer sequence typing (MST) genotypes of *C. burnetii* in the positive *C. burnetii* samples from Senegal and Oran, as described previously.[15]

2.3. Statistical analysis

For data comparison, the Fisher's exact test was performed using EpiInfo version 6.0 software (Centers for Disease Control and Prevention, Atlanta, GA, USA). A *p*-value of 0.05 was considered to be significant.

2.4. Ethics statement

This study was done after ethical approval was obtained from the national ethics committees of Senegal, Gabon, and France (No. 0-00.87MSP/DS/CNERS and No. 001380MSP/DS/CNERS). Written individual informed consent was obtained from each participant, including the parents or legal guardians of all minors. For Morocco, Algeria, Tunisia, and Mali our study design conformed to directives concerning the conduct of clinical trials for these countries.

3. Results

Overall, we tested 1238 febrile patients from Senegal (50% males), 100 from Mali (50% males), 50 from Gabon (46% males), 184 from Tunisia (59% males), 268 from Algeria (63% males), and 48 from Morocco (27% males) (Table 1). All patients were negative for the infectious agents tested except for *C. burnetii*, which was found in six febrile patients (0.4%) in Senegal and in one (0.3%) in Algeria (Table 2). We did not find patients infected with *C. burnetii* in Mali, Morocco, Gabon, or Tunisia. Four of the *C. burnetii*-positive patients (66%) from Senegal were adolescents (<18 years old), one was an adult male, and surprisingly, one was a 1-year-old child. Fever and pulmonary manifestations were the most common clinical manifestations in these patients (Table 2). Contact was lost with these patients and none received doxycycline treatment. All non-febrile people from Marseille and from Dielmo and Ndiop were negative for *C. burnetii*.

In Keur Momar Sarr (Senegal), we tested 223 patients (43% males) and found five febrile patients (2.2%; four males and one female) infected with *C. burnetii*. Four cases occurred during the dry season, whereas one case occurred during the rainy season. In Kedougou, we tested 288 patients (49% males) and detected one female patient (0.3%) infected with *C. burnetii* during the dry season. Significantly more patients were infected with *C. burnetii* in Keur Momar Sarr than in the other locations in Senegal ($p < 0.001$). Based on our data, we estimate that the incidence of *C. burnetii*-positive cases was 480/100 000 febrile patients in Keur Momar Sarr. However, we could not estimate the incidence of *C. burnetii* for the other locations in Senegal.

In Oran, Algeria, we found one patient infected with *C. burnetii*. The patient was a 21-year-old male with asthenia who suffered from a persistent fever and myalgia for 6 days. The patient had respiratory symptoms including cough, with radiographic results compatible with atypical pneumonia at the time of sample collection. Laboratory values revealed a white cell count of 9.6×10^9/l (73% lymphocytes) and increased aspartate aminotransferase and alanine aminotransferase (66 and 104 U/l,

Table 2
Patients with *Coxiella burnetii* infection

Patient	Place	Sex	Age	Season	Area	Clinical manifestations	Doxycycline treatment
1	Keur Momar Sarr	Male	10	Dry	Rural	Fever	No
2	Keur Momar Sarr	Male	12	Dry	Rural	Fever	No
3	Keur Momar Sarr	Male	15	Dry	Rural	Persistent fever	No
4	Keur Momar Sarr	Male	56	Rainy	Rural	Persistent fever and vomiting	No
5	Keur Momar Sarr	Female	17	Dry	Rural	Fever and cough	No
6	Kedougou	Female	1	Dry	Rural	Pneumonia, vomiting	No
7	Oran	Male	21	Summer-dry	Urban	Persistent fever	No

respectively). The patient received treatment with azithromycin for 3 days, and the fever resolved. However, contact was then lost with the patient.

3.1. Multi-spacer sequence typing

Although seven MST genotyping studies were attempted, only one genotype was determined; *C. burnetii* genotype 35 was identified in one patient from Keur Momar Sarr. All attempts to genotype the other positive samples were unsuccessful, possibly because of low DNA load.

4. Discussion

In this study, we found that *C. burnetii* was the cause of fever in six patients from Senegal and one from Algeria. We believe our findings are reliable, because each positive result was confirmed by the amplification of a second specific gene of *C. burnetii* to avoid false-positive results.[11,12] Although in Gabon the local laboratory at the time of the study used a different DNA extraction assay than the other laboratories, this did not have an impact on our assays because the quality of DNA extraction was verified for all samples and the analysis of all controls yielded expected results. Our specific RT-PCR assay for the detection of *C. burnetii* was capable of detecting 10^2 bacteria/ml.[11] A limitation of our study was that the diagnosis was based only on molecular and not on serological data. Microimmunofluorescence (IFA) is the reference technique for diagnosing Q fever.[16,17] However this was not performed because IFA requires highly experienced technicians,[9] and such expertise was not available in the local laboratories that participated in this study. As a result, in the absence of serological data, we could not determine if our *C. burnetii*-positive patients were suffering from acute Q fever or Q fever endocarditis.

We found *C. burnetii* genotype 35 in a patient from Senegal. This genotype was recently found in ticks from Senegal,[6] but this is the first time that this genotype has been detected in a human. In a previous study, *C. burnetii* genotypes 6, 35, and 36 were identified in ticks from Senegal.[6] Moreover, *C. burnetii* genotype 19 was detected previously in a patient with endocarditis from Senegal.[6] In contrast, a larger biodiversity of strains has been observed in samples from patients in metropolitan France, where 21 genotypes circulate.[15,18] However, in the recent Q fever outbreak in the Netherlands, it appears that a single strain (genotype 33) was responsible for the epidemic.[19,20] Recently, a new *C. burnetii* genotype (genotype 51) was identified in an aortic valve sample from a patient with Q fever endocarditis in Saudi Arabia.[21]

In a previous study, a high seroprevalence of Q fever was found in West, Central, and southern Africa.[10] People in these rural areas are owners of a high number of domestic ruminants, and *C. burnetii* is also present in the household environment.[22] In Senegal, a high incidence rate was recently reported among people living in the villages of Dielmo (73/100 000 person-years) and Ndiop (223/100 000 person-years).[22] In the present study, the incidence of acute Q fever in Keur Momar Sarr was 480/100 000 febrile patients. If the incidence of fever is comparable to that in the villages of Dielmo and Ndiop, the current incidence may be 150/100 000 inhabitants. If our hypothesis is true, Senegal may be the country with the highest Q fever incidence among the tested countries. However, the fact that we tested fewer patients in Morocco, Mali, Gabon, Tunisia, and Algeria and that patients in Tunisia, Mali, and Algeria we primarily from urban areas could possibly explain why we found only one *C. burnetii*-positive patient in all these countries.

The installation of point-of-care (POC) laboratories in rural Senegal is a very effective tool for studying the epidemiology of many infectious diseases.[1,23] Molecular methods play a primary role in the diagnosis of many infectious diseases, and the widespread use of RT-PCR, which is less expensive than conventional PCR, can reduce the delay in diagnosis of infections. For this study, all blood samples were initially collected in the rural health dispensaries and were then sent to our reference center in Marseille for molecular analysis. As a result, a lot of time was lost in obtaining the diagnosis and contact was lost with all the *C. burnetii*-infected patients.

In conclusion, we showed that *C. burnetii* may be a cause of fever primarily in children and young adolescents living in Senegal. Patients living in rural areas of Africa or travelers returning from these areas with unexplained fever should be tested for *C. burnetii* infection. Molecular methods provide a convenient tool for the diagnosis of *C. burnetii*, and as a result, we believe that the presence of POC laboratories in rural areas of Africa is critical.

Funding: No funding sources declared.

Conflict of interest: None.

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