

Coxiella burnetii in Humans and Ticks in Rural Senegal

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Abstract

Background: Q fever is a worldwide zoonotic disease caused by *Coxiella burnetii*. Epidemiologically, animals are considered reservoirs and humans incidental hosts.

Methodology/Principal Findings: We investigated Q fever in rural Senegal. Human samples (e.g., sera, saliva, breast milk, feces) were screened in the generally healthy population of two villages of the Sine-Saloum region. Ticks were collected in four regions. Seroprevalence was studied by immunofluorescence, and all other samples were tested by two qPCR systems for detection of *C. burnetii*. Positive samples were genotyped (multispacer typing) by amplification and sequencing of three spacers. Strains were isolated by cell culture. We found that the seroprevalence may be as high as 24.5% (59 of 238 studied) in Dielmo village. We identified spontaneous excretion of *C. burnetii* by humans through faeces and milk. Hard and soft ticks (8 species) were infected in 0–37.6%. We identified three genotypes of *C. burnetii*. The previously identified genotype 6 was the most common in ticks in all studied regions and the only one found in human samples. Three strains of genotype 6 of *C. burnetii* were also recovered from soft tick *Ornithodoros sonrai*. Two other genotypes found in ticks, 35 and 36, were identified for the first time.

Conclusions/Significance: Q fever should be considered a significant public health threat in Senegal. Humans, similar to other mammals, may continuously excrete *C. burnetii*.

Citation: Mediannikov O, Fenollar F, Socolovschi C, Diatta G, Bassene H, et al. (2010) *Coxiella burnetii* in Humans and Ticks in Rural Senegal. PLoS Negl Trop Dis 4(4): e654. doi:10.1371/journal.pntd.0000654

Editor: Pamela L. Small, University of Tennessee, United States of America

Received: November 11, 2009; **Accepted:** March 2, 2010; **Published:** April 6, 2010

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Funding: No additional funding except from authors' affiliations (IRD, CNRS). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Q fever is a worldwide zoonotic disease caused by *Coxiella burnetii* [1]. The disease can be acute or chronic and exhibits a wide spectrum of clinical manifestations. The reported prevalence of Q fever is continuously increasing due to both true prevalence and improved quality of diagnostic tools together with the growing interest of physicians and epidemiologists focusing on this disease [2]. The natural cycle of this bacterium is not reported to include humans, who are considered incidental hosts. The true reservoir is wide and includes mammals, birds and arthropods, mainly ticks. Cattle, sheep and goats are most commonly identified as sources of human infection and the disease is prevalent in mostly rural areas worldwide. Other animals, however, including common pets such as cats, rabbits, pigeons and dogs [1] may also serve as sources. Q fever is usually transmitted by inhalation of aerosol [3]. Hard and soft ticks may be infected during feeding, may transmit *C. burnetii* transovarially and transstadially and excrete it *via* feces, saliva and coxal fluid [4–7]. Being reservoirs, ticks, however, are not considered as a vector for transmission of this disease to humans, although crushing an infected tick between the fingers has resulted in Q fever [8]. Although no human cases of Q fever developing after a tick bite have yet been reported, the role of ticks as vectors and reservoirs has been discussed since 1937 [9]. The reference strain

Nine Mile was isolated from a *Dermacentor andersoni* tick and was initially named *Rickettsia diaporica* [10].

The Q fever agent was subsequently identified either serologically or by strain isolation in many species of ticks. In the former USSR alone, 32 species of Ixodid ticks, 6 species of Argasid ticks (*Ornithodoros tartakovskyi*, *O. papillipes*, *O. alactagalis*, *Argas persicus*, *A. reflexus*, and *A. vespertilionis*) and 5 species of mites were found to harbor *C. burnetii* [7]. *Coxiella* infection in *O. moubata* was reported once [11]. Several strains from wild bed bugs (*Cimex lenticularis*) were isolated and experimental infection was shown to be stable [12].

Q fever has been reported all over the African continent with generally higher serological indices in the countries of West Africa [13–15]. A case of Q fever imported from Senegal was reported [16], and a strain was isolated from *Hyalomma truncatum* [17]. In the neighboring country, Guinea-Bissau (ancient Portuguese Guinea) several strains of *C. burnetii* were isolated in fifties from hard ticks, including *Amblyomma variegatum* and *Rhipicephalus senegalensis*, goat and cow milk [18].

Here we present our data from an investigation of the seroprevalence and roles of ticks and humans as reservoirs for Q fever. We based our study of human samples in two villages, Dielmo and Ndiop, of the Sine-Saloum region of Senegal. A longitudinal study [19,20] of malaria and tick-borne relapsing fever has been ongoing in these villages since 1990. It consists of

Author Summary

Q fever is a zoonotic disease known since 1937. The disease may be severe, causing pneumonia, hepatitis and endocarditis. Q fever agent has been described as a possible biological weapon. Animals—especially domestic cows, goats and sheep—are considered reservoirs for this infection. They are capable of sustaining the infection for long periods and excreting viable bacteria, infecting other animals and, occasionally, humans. Here we studied the distribution of Q fever in a poorly studied region, Senegal. We studied the agent of Q fever both in ticks parasitizing domestic animals and in humans (antibodies in serum, bacteria in feces, saliva and milk). We found from the studied regions the bacterium is highly prevalent in rural Senegal. Up to 37.6% of five different and most prevalent tick species may carry the bacterium. Humans living in such areas, as other mammals, may occasionally excrete Q fever agent through feces and milk.

long-term investigations of host-parasite relationships in the entire village population, which was enrolled in a longitudinal prospective study.

Materials and Methods

Tick collection and treatment

From October to December 2008, Ixodid ticks were collected manually from domestic animals (e.g., cattle, goats, sheep, horses, and donkeys) in three different regions of Senegal (Table S1, Figure 1). The collected ticks were preserved in 90% ethanol. Specimens of soft ticks were collected in May 2009 from two villages in the Kaffrine and Tambacounda regions (Table S1, Figure 1). Sampling was conducted in human dwellings in small mammal burrows. Soft ticks were collected by introducing a flexible tube inside burrows and aspirating the contents using a portable petrol-powered aspirator. The contents were exposed to sunlight on sorting trays, and selected soft ticks were stored in closed tubes until determination, isolation and DNA extraction. The species and sex of hard and soft ticks were identified according standard taxonomic keys for adult ticks [21]. DNA from adult ticks was extracted using a DNeasy Tissue Kit (Qiagen, Courtaboeuf, France) following the manufacturer's instructions and stored at 4°C until use in PCR amplifications.

Ten adult *O. sonrai* ticks were selected for bacterial culture. 40 days has passed between collection and isolation. Each tick was washed in a 10% water solution of commercial disinfectant-detergent (Amphomousse, Hydenet S.A., Sainghin-en-Melantois,

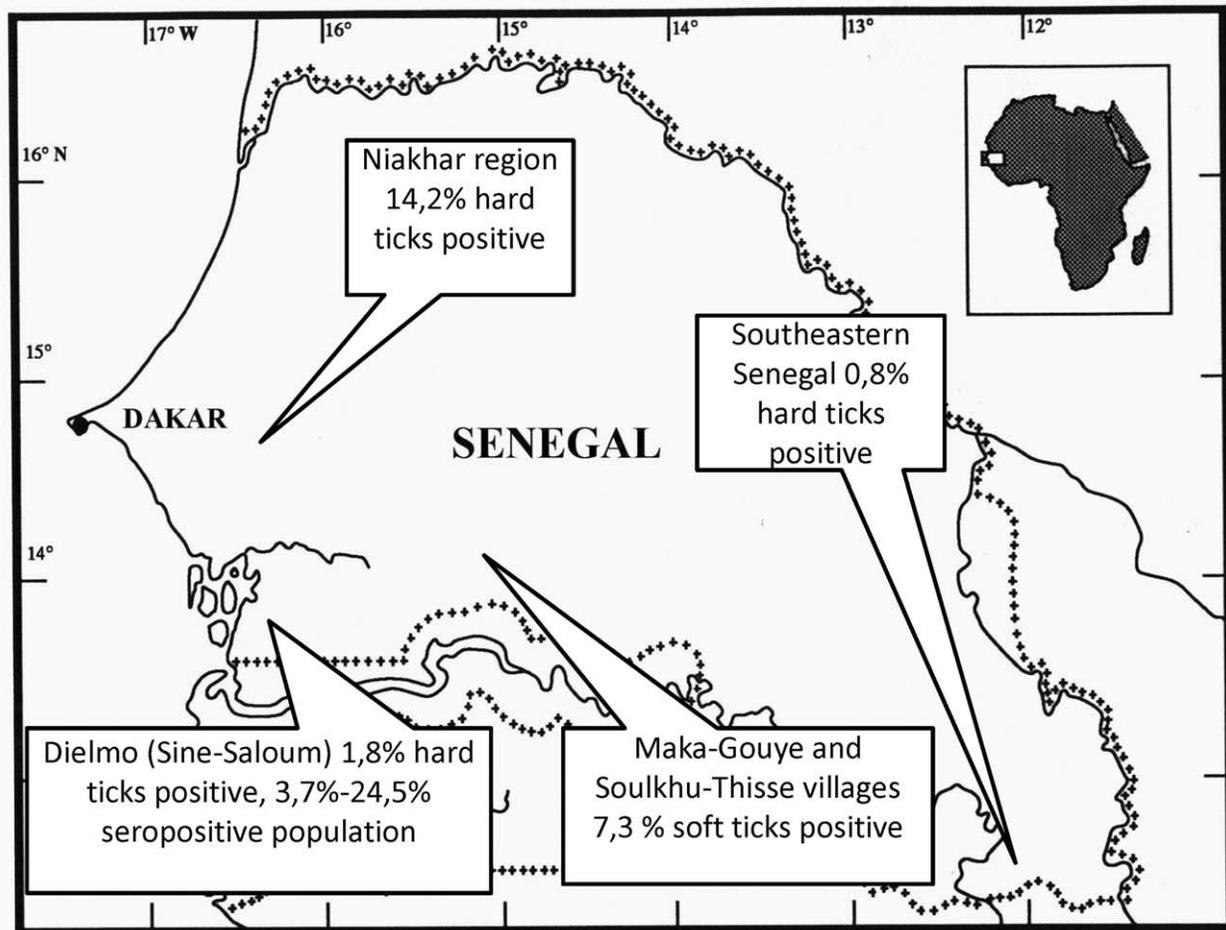


Figure 1. Map of Senegal with the locations of sample collection.

doi:10.1371/journal.pntd.0000654.g001

France), then rinsed in sterile water and placed in a 1% solution of sodium hypochlorite for 10 minutes. After rinsing with distilled water, a 15-minute incubation in 70% ethanol was performed. A final rinse in sterile phosphate-buffered saline preceded inoculation. Ticks were placed in a sterile 1.5 plastic tube, where they were triturated with a sterile micropestle in 600 μ l of Rinaldini solution. Isolation was carried out according to a well-known modified shell-vial technique [22]. We used 300 μ l of whole tick suspension for inoculation of each of two vials with monolayer of ISE6 (*Ixodes scapularis* hard tick) and DH82 (dog's macrophage) cells. After centrifugation, the supernatant was removed and conserved for future molecular identification. ISE6 cells were cultivated in special L15B medium [23], and DH82 cells in MEM supplemented with 5% of FBS. We did not use antibiotics in the medium.

Human sample collection and treatment

The project was approved by the National Ethics Committee of Senegal [19], and Local Ethics Committee (Marseille, France). Written individual informed consent was obtained from each participant, including the parents or legal guardians of all children at the beginning of the current study. All participants were questioned and examined before taking samples. Those who were unwell were not included in the present study. Dielmo and Ndiop villagers are settled agricultural workers; millet and peanut crops are cultivated during the rainy season, and market gardening is the main agricultural activity during the dry season.

For serological studies we used the samples collected in 2008 from the serological bank created for the above mentioned longitudinal study. In total, 238 serum samples collected in 2008 in Dielmo (mean age 26 ± 18 , range from 3 to 78, 117 men and 121 women) and 241 samples from Ndiop (mean age 25 ± 17 , range from 5 to 82, 112 men and 129 women) were tested.

The samples (1 ml) of human breast milk were collected in April 2009 in both villages (Dielmo: 26 samples, mean age 30 ± 5.5 , from 21 to 48; and Ndiop: 18 samples, mean age 29.5 ± 9 , from 20 to 49). Samples of human saliva from Dielmo (167 samples; 66 from men and 101 from women; mean age 26 ± 20 , range from 10 months to 87 years) and from Ndiop (200 samples; 93 from men and 107 from women; mean age 21 ± 18 , range from 2 to 83 years) and stool samples from Dielmo (221 samples, 103 from men and 118 from women; mean age 19.5 ± 19 , range from 6 months to 87 years) and from Ndiop (229 samples; 103 from men and 126 from women; mean age 19 ± 18 , range from 1 month to 83 years) were collected in sterile tubes. All samples were covered with absolute ethanol for conservation before DNA extraction. DNA was extracted from all samples using a DNeasy Tissue Kit (Qiagen) according manufacturer's instructions.

Molecular studies

Bacterial DNA was initially detected by *C. burnetii* specific semi-quantitative PCR with primers and probes designed for the amplification of IS1111 [24,25] and IS30A spacers. The quality of

DNA handling and extraction of samples of human saliva and milk was verified by real-time PCR for a housekeeping gene encoding beta-actin (Table 1). The specificity of *C. burnetii* detection systems was tested on DNA samples from 347 different bacterial species, including a number of phylogenetically closely related bacteria (Table S2). Sensitivity was quantified by comparison with a proposed earlier system based on a plasmid containing the *sodB* gene of *C. burnetii* [26] (Table S3). The reactions were performed using a LightCycler 2.0 and software (Roche Diagnostics GmbH, Mannheim, Germany). Master mixes were prepared by following the instructions of the manufacturer. The results were considered positive when confirmed by both spacers.

DNA extracted from uninfected ticks in laboratory colonies at the Unite des Rickettsies, Marseille, France was used as a negative control and DNA extracted from L929 cell culture supernatant of *C. burnetii* served as a positive control. All positive samples with a Ct (the cycle number at the threshold level of log-based fluorescence) lower than 35 (≈ 10 –20 copies of spacer) from hard ticks of southeastern Senegal and the Sine-Saloum region were subjected to a simple PCR using three pairs of primers: Cox2F/Cox2R, Cox5F/Cox5R and Cox18F/Cox18R, manufactured by Eurogentec (Seraing, Belgium). These amplified three intergenic spacers that were later used for multispacer typing of *C. burnetii* strains [27]. Because of the large number of positive results, five samples from each species of hard tick from Ndiop were genotyped. All positive samples from humans were genotyped.

Polymerase chain reactions were performed in automated DNA thermal cyclers (GeneAmp 2400 and 9700; Applied Biosystems, Foster City, CA, USA). PCR products were visualized by electrophoresis on a 1.5% agarose gel, stained with ethidium bromide and examined using an ultraviolet transilluminator. The PCR products were purified using a QIAquick Spin PCR Purification Kit (Qiagen) according to the manufacturer's instructions. Sequencing of amplicons was performed using the BigDye Terminator Cycle Sequencing Kit (Perkin Elmer Applied Biosystems) with an ABI automated sequencer (Applied Biosystems). The obtained sequences were assembled (ChromasPro 1.49, Technelysium Pty Ltd, Tewantin, Australia) and edited [Genetyx-Win 5.1 (Software Development Co., Ltd., Japan)]. All three spacers from each positive sample were used for multispacer typing of *C. burnetii* using the freely available MST database (http://ifr48.timone.univ-mrs.fr/MST_Coxiella/mst). Sequences of three spacers from all available genotypes were concatenated and aligned with CLUSTAL W program, and a neighbor-joining phylogenetic tree was constructed with Geneious 4.7.6 software (Biomatters Ltd, Australia).

Serological studies

Titers of IgG, IgM, and IgA antibodies in serum samples obtained from each patient were determined using an indirect immunofluorescence assay with antigens generated in-house [28]. All serum samples were diluted at ratios of 1:25, 1:50 and 1:100, and were screened for total immunoglobulin. If the serological

Table 1. Target sequences, primers and probes used in a study.

Target sequence	Forward primer	Reverse primer	Probe
<i>C. burnetii</i> spacers IS1111	CAAGAAACGTATCGCTGTGGC	CACAGAGCCACCGTATGAATC	6' FAM-CCGAGTTCGAAACAATGAGGGCTG-TAMRA
IS30A	CGCTGACCTACAGAAATATGTCC	GGGGTAAAGTAAATAATACCTCTCGG	6' FAM-CATGAAGCGATTATCAATACGTGTATGC-TAMRA
Human beta actin	CATGCCATCTGCGTCTGGA	CCGTGCCATCTCTGTCTCG	6' FAM-CGGGAAATCGTGCCTGACATTAAG-TAMRA

doi:10.1371/journal.pntd.0000654.t001

screening for Q fever was positive, final titers for IgG, IgA, and IgM were determined for both anti-phase I and anti-phase II antibodies. The criteria for serological diagnosis of acute Q fever were considered to be a titer of anti-phase II IgG antibodies of 1:200 and a titer of anti-phase II IgM antibodies of 1:50 [29].

Statistical analysis

Data were analyzed using Epi Info software, version 3.4.1 (Centers for Disease Control and Prevention, Atlanta, GA, USA). Non-parametric values were compared using a χ^2 test. Statistical significance was defined as $p < 0.05$.

Results

Tick studies

In total, we collected 492 hard ticks of 6 species from the Sine-Saloum region, 2,494 ticks of 6 species from the Niakhar region and 376 ticks of 1 species from southeastern Senegal from domestic animals (Table 2). The majority of ticks in Sine-Saloum were *Rhipicephalus evertsi evertsi* (54.5%); *Amblyomma variegatum* constituted 17.5%; and two species of *Hyalomma* (*H. truncatum* and *H. marginatum rufipes*) constituted 15% and 11.8%, respectively. We found 5 (1%) *Rhipicephalus* (*Boophilus*) *annulatus* ticks and 1 (0.2%) *Rhipicephalus guilhoni* tick (Figure 2). In the region of Niakhar, *Rh. e. evertsi* were even more abundant (83.8%), followed by *A. variegatum* (5.3%), *H. m. rufipes* (4.6%), *H. truncatum* (2.7%), *Rh. guilhoni* (2%) and *Rhipicephalus* (*Boophilus*) *decoloratus* (1.6%). *A. variegatum* was the only species collected in the southeastern Senegal because of the small sampling focused only on bovines.

We found a different percentage of ticks positive for *C. burnetii* among hard ticks in all three regions (Table 2). The prevalence in Sine-Saloum and southeastern Senegal varied from 0.7% to 6.8%

(except for *Rh. (B.) annulatus* from Dielmo, with one tick positive of the five studied), corresponding with the previously reported prevalence [30–32] of *C. burnetii* in ticks collected from domestic animals from other parts of the world. Despite the large number of ticks collected from small ruminants, donkeys and horses in Dielmo (data not shown), ticks positive for *C. burnetii* were only found on bovines.

The prevalence of infection in hard ticks from the Niakhar region at 14.2% was significantly higher than that in Sine Saloum (1.8%, $p < 10^{-3}$). The prevalence for each tick species in Niakhar varied from 10.2% for *Rh. guilhoni* up to 37.6% for *A. variegatum* (Table 2). In contrast to other regions, the ticks infected with *C. burnetii* were found on all kinds of studied domestic animals (sheep, goats, bovines, donkeys, horses), without a significant prevalence in any kind of animal (data not shown). Moreover, for *A. variegatum*, the only species collected in all three regions, we did not amplify *C. burnetii* DNA in the Sine-Saloum region, while 0.8% of ticks were infected in southeastern Senegal, and significantly more were infected in Niakhar (37.6%, $p < 10^{-3}$ when compared with the two other regions).

All soft ticks collected were *O. sonrai* (Figure 2). Among 109 studied samples, we amplified *C. burnetii* in 9 ticks (8.3%). We succeeded in isolating three strains of *C. burnetii* from *O. sonrai* soft ticks collected in rodent burrows inside human dwellings. These originated from the Soukhu-Thissé village in the Kaffrine region; one was isolated in ISE6 cell culture system, and the other two (from different ticks), in a DH82 cell line.

Human samples

Data on human sample tests are presented in Table 3.

Studies of serum samples of 241 Ndiop villagers showed that 9 (3.7%) individuals had serological evidence of infection with *C.*

Table 2. Results of PCR of hard ticks collected from domestic animals in Senegal.

Tick species	No	No positive for <i>C. burnetii</i> (%)	MST genotyping
Sine-Saloum region			
<i>Amblyomma variegatum</i>	86	0	
<i>Rhipicephalus</i> (<i>Boophilus</i>) <i>annulatus</i>	5	1 (20%)	Genotype 6
<i>Hyalomma marginatum rufipes</i>	58	1 (1.7%)	New genotype 36
<i>Hyalomma truncatum</i>	74	5 (6.8%)	New genotype 36
<i>Rhipicephalus evertsi evertsi</i>	268	2 (0.7%)	New genotype 36
<i>Rhipicephalus guilhoni</i>	1	0	
Total:	492	9 (1.8%)	
Niakhar region			
<i>Amblyomma variegatum</i>	133	50 (37.6%)	Genotype 6 (100%)
<i>Rhipicephalus</i> (<i>Boophilus</i>) <i>decoloratus</i>	40	12 (30.0%)	Genotype 6 (100%)
<i>Hyalomma marginatum rufipes</i>	115	24 (20.9%)	Genotype 6 (100%)
<i>Hyalomma truncatum</i>	67	21 (31.3%)	Genotype 6 (100%)
<i>Rhipicephalus evertsi evertsi</i>	2090	242 (11.6%)	Genotype 6 (100%)
<i>Rhipicephalus guilhoni</i>	49	5 (10.2%)	Genotype 6 (100%)
Total:	2,494	354 (14.2%)	
Southeastern Senegal			
<i>Amblyomma variegatum</i>	376	3 (0.8%)	New genotype 35 (67%) Genotype 6 (33%)
Kaffrine and Tambacounda regions			
<i>Ornithodoros sonrai</i>	109	8 (8.3%)	Genotype 6 (100%)

doi:10.1371/journal.pntd.0000654.t002



Figure 2. Ticks collected and studies. *Hyalomma marginatum rufipes*, male (A), female (B); *Rhipicephalus evertsi evertsi*, male (C), female (D); *Amblyomma variegatum*, male (E), female (F); *Rhipicephalus (Bophilus) decoloratus*, male (G), female (H); *Hyalomma truncatum*, male (I), female (J); *Rhipicephalus guilhoni*, male (K); *Ornithodoros sonrai* (L). doi:10.1371/journal.pntd.0000654.g002

burnetii (Table 3). The average age was 20.2±17.4 years with a range of 6.8 to 46.4 years. Eight had only a low titer (1/50) of total antibodies. One had serological evidence of chronic Q fever with titers of IgG of 1/1600 and 1/3200 with *C. burnetii* I and II phase respectively. We also tested 35 serum samples from the same patient (a girl born in 2001) collected regularly from July 2003 to June 2009. Seroconversion occurred between March 2007 and August 2007. In Dielmo villagers the seroprevalence was significantly higher ($p < 10^{-3}$), with 59 (24.8%) being positive: 22 (average age 21±16.2, from 5.6 to 67.4) had a titer of total Ig of 1/50 and 37 (15.9±10.9) were above this. The mean age of the seropositive population (17.8±13.2) was lower ($p < 0.003$) than the mean age of the studied population (26±18). Nine villagers had serological data suggesting recent infection with the presence of IgM (1/100 or higher), and their average age (14.9±7.5 years, 8 to

32.6) was younger ($p < 0.01$) than that of all those studied. All except one were younger than 20 years, which may indicate early contact with infection. Four (14.9±6 years old) had an IgG titer phase I $\geq 1/800$ without IgM, corresponding to the serological criteria for chronic Q fever [29].

The search for *C. burnetii* DNA in saliva was not successful with Ct values not lower than 35, despite all samples being highly positive for the beta actin gene, indicating that handling and DNA extraction of the samples was appropriate. None of 18 actin-positive human breast milk samples from the Ndiop village (Sine-Saloum region) was positive for *C. burnetii*. A 33-year-old woman from Dielmo, however, was positive with both real-time PCR systems. An attempt at coxiella genotyping was not successful. The serum taken at the same time with the milk was negative for both antigens.

Table 3. Identification of *C. burnetii* DNA in human samples.

	Dielmo	Ndiop
Total number of seropositives	59/238 (24.8%)	9/241 (3.7%)
Age	4.5 to 48.1 years (mean 17.8±13.2)	6.8 to 46.4 years (mean 20.2±17.4)
Titer of 1/50	22/238 (9.3%)	8/241 (3.3%)
Titer of 1/100 end higher	37/238 (15.5%)	1/241 (0.4%)
Positive saliva samples	0/167	0/200
Positive stool samples	4/221 (1.8%)	3/229 (1.3%)
Positive milk samples	1/26 (3.8%)	0/18

doi:10.1371/journal.pntd.0000654.t003

There were 4 of 221 (1.8%) stool samples from Dielmo and 3 of 229 (1.3%) from Ndiop positive by qPCR using both systems for the presence of *C. burnetii* DNA. In Dielmo, positive samples were obtained from three women and one man, with a mean age of 12.5 ± 15.1 years (range from 1 to 33 years). Interestingly, the same woman who gave a positive breast milk sample also showed the presence of *C. burnetii* in stool. In Ndiop, positive samples were obtained from one man and two women (23.3 ± 19.1 , from 2 to 39 years). Unfortunately, only one male villager from Dielmo whose stool samples were found to be positive for *C. burnetii* had his serum tested. The serum was positive when tested with *C. burnetii* antigens for the presence of total Ig, with a titer of 1/50.

Genotyping

We performed MST genotyping of *C. burnetii* strains and positive samples. Genotype 6 was identified in all hard ticks positive for *C. burnetii* from Niakhar, in one from Sine-Saloum and in one from southeastern Senegal, in addition to all three isolates of soft ticks from the Kaffrine region and 3 stool samples from Dielmo (2) and Ndiop (1). This genotype was previously detected in a patient with endocarditis in France without epidemiological data available. Genotype 36 (a new combination of already known alleles of intergenic spacers) was found in all except one tick from Dielmo. Another new genotype (genotype 35) was found in southeastern Senegal (Table 2).

A neighbor-joining phylogenetic tree based on concatenated sequences (Figure 3) shows that the newly found genotypes do not

group together. Genotype 36 clusters mostly with European strains (genotypes 1–4) and a strain from Namibia, while genotype 35 is situated inside a very heterogeneous group of genotypes from all over the world. Genotype 19 was previously isolated from a patient with endocarditis from Senegal and is quite remote from the new genotypes.

Discussion

In our study, we tried to identify the incidence of Q fever in tick reservoirs and in a generally healthy rural population in Senegal and to evaluate spontaneous bacterial excretion by humans in the endemic area. Serological studies performed in two villages situated in close proximity, Dielmo and Ndiop, from the same region of Sine-Saloum showed a high seroprevalence, with Dielmo villagers having a significantly higher seroprevalence (24.5% versus 3.7% in Ndiop; $p < 10^{-3}$).

We identified spontaneous *C. burnetii* shedding in human breast milk and feces but not in saliva of generally healthy humans. Shedding of Q fever in milk has been proposed in one study as a possible mode of contamination in humans [33]. Our data show that randomly collected samples of feces may contain *C. burnetii* (1.8 and 1.2% for Dielmo and Ndiop, respectively) with the same being true for human breast milk (3.8%). Moreover, the same person without evident clinical signs may excrete bacteria in milk and feces. Thus, we have found that in endemic areas, humans similar to other mammals may become chronic excretors of *C. burnetii* via feces and milk. Found rates of shedding are, however,

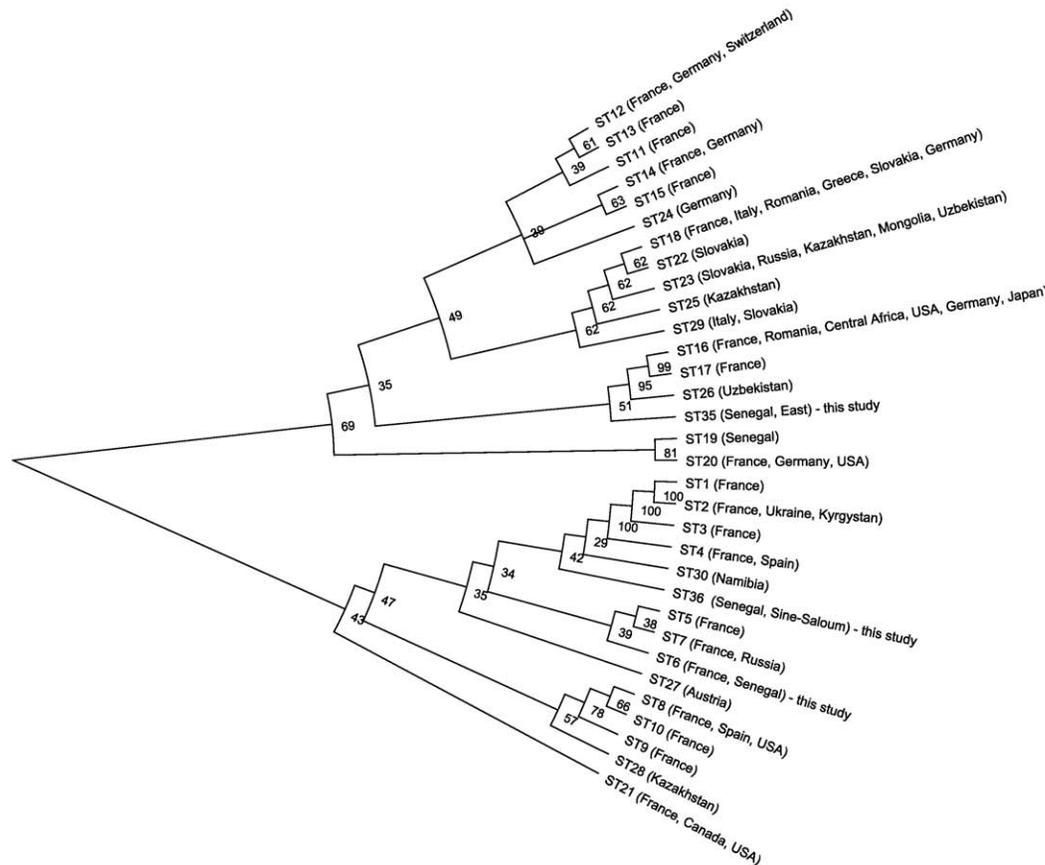


Figure 3. NJ semicircular unrooted tree with proportionally transformed branches showing the relationship of genotypes identified in this study with other *C. burnetii* genotypes.
doi:10.1371/journal.pntd.0000654.g003

not high and may not be compared with those identified in domestic animal. This may be partially explained by the human social habits, where continuous re-infection by Q fever agent is much less probable than that in domestic animals, so lesser infection rate were noted. Salivary glands and oral cavity is not, probably, the place of *C. burnetii* propagation in humans.

The role of this shedding in the epidemiology of Q fever in these villages remains unknown. The possibility of other modes of excretion, such as in urine and vaginal secretions in humans should be studied in the future, although studies of spontaneous bacterial shedding in vaginal samples may be difficult due to ethical issues.

At this stage it is not possible to differentiate whether humans excreting bacteria are subclinically infected individuals or real carrier/reservoir of Q fever agent. It is well-known that in some cases of chronic Q fever bacteria may persist during years, even despite of antibiotic therapy. We speculate that *C. burnetii* may be continuously excreted by humans even in the absence of clear clinical symptoms. This may suggest that humans may, as other mammals, be reservoirs and/or long-term excretors of these bacteria.

We also tested 3,362 ticks from different areas of Senegal and identified a very high prevalence of *C. burnetii* infection of Ixodid ticks collected from domestic animals.

The soft tick *O. sonrai*, currently known mostly as a vector of human relapsing fever [34], harbored *C. burnetii* in 8.3% of ticks sampled. We have isolated three strains of typical *C. burnetii* from alive soft ticks that stayed without contact with potential reservoir for at least 40 days. It may indicate that at least viable bacteria may survive for a long time in a soft tick. It was repeatedly shown that *C. burnetii* may stay alive in soft ticks for several years [6], may transmit transovarially and transstadially and be excreted via feces, saliva and coxal fluid [4–6]. As *O. sonrai* feed predominantly on rodents and occasionally bite humans, the isolation may signify that rodents living inside human dwellings, soft ticks or both may harbor the Q fever agent. Although the possibility of transmission of Q fever by this tick was not studied, we identified *O. sonrai* as a new species of soft tick playing the role in epidemiology of Q fever.

We identified strikingly high (up to 37.6% for *A. variegatum* in the Niakhar region) level of infection of hard ticks with *C. burnetii*. It was higher than in Sine-Saloum or southeastern Senegal ($p < 10^{-3}$). The prevalence in other tick species was also higher in Niakhar compared to Sine-Saloum (Dielmo and Ndiop) and was statistically significant for *Rh. e. evertsi* ($p = 0.001$), *H. m. rufipes* ($p < 10^{-3}$), and *H. truncatum* ($p < 10^{-3}$). Taking these data into consideration, we believe that the prevalence of Q fever in Niakhar villagers should be investigated in future studies. The high prevalence of *C. burnetii* in ticks, especially in *A. variegatum* and *O. sonrai*, supports the idea that hard and soft ticks play a role in the circulation of Q fever agents.

We identified three genotypes of Q fever agents circulating in Senegal. Genotype 6 had already been found once in a patient diagnosed in France. In our study, it was identified in all four studied regions and was the only genotype found in patients. The existence of the same dominating genotype of *C. burnetii* in soft and

hard ticks and humans indicates low host-specificity at a subtype (genotype) level. Apparently, genotype 6 is epidemic and likely plays a major role in the origin of Q fever and in public health in Senegal. Two other genotypes, 35 and 36, were recovered here for the first time. They are probably less frequent since we found them in only solitary regions. Q fever is an important re-emerging disease caused by a strictly intracellular bacterium that can survive in the environment for at least several weeks [35]. The data presented from screening randomly collected samples show that serological and/or molecular evidence of contact/excretion of Q fever agents can be found quite often in a generally healthy rural population in Senegal.

In this work, we showed that in endemic region (with high seroprevalence in humans and high infection of ticks) humans may excrete the agent of Q fever even without evident clinical signs and during a long time. This gives us a possibility to speculate that humans, like other mammals, may be reservoirs of *C. burnetii*. Unexpectedly found high level of infection in hard and soft ticks together with high seroprevalence may indicate the active endemicity of studied regions, especially Niakhar. In summary, Q fever should be considered as an emerging disease in Senegal and local healthcare and veterinary workers must be aware of this infectious disease.

Supporting Information

Table S1 Geographic coordinates of tick collection sites.

Found at: doi:10.1371/journal.pntd.0000654.s001 (0.04 MB DOC)

Table S2 List of bacteria tested for specificity with two spacer-based semi-quantitative PCR systems designed for detection of *Coxiella burnetii*.

Found at: doi:10.1371/journal.pntd.0000654.s002 (0.28 MB DOC)

Table S3 Comparison of sensitivity of already quantified *C. burnetii* detection system based on amplification of sodB gene (24) with two semiquantitative PCR systems used in this study. Extrapolation allowed to calculate the sensitivity of IS 1111 system in revealing of 11–15 copies of spacer and IS 30A in revealing of 10–20 copies.

Found at: doi:10.1371/journal.pntd.0000654.s003 (0.04 MB DOC)

Acknowledgments

We thank M. Bedotto, S. Buffet, M. Sylla and D. Pyak for technical help; A. Tall and A.B. Ly from Pasteur Institute of Senegal for the help with the project realization and E. Botelho for the translation from Portuguese.

Author Contributions

Conceived and designed the experiments: OM FF CS JFT DR. Performed the experiments: OM FF HB. Analyzed the data: OM FF CS GD JFM CS JFT DR. Contributed reagents/materials/analysis tools: OM CS GD HB JFM CS JFT DR. Wrote the paper: OM FF CS GD JFT DR.

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