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Suburban Transmission of Q Fever in French Guiana: Evidence of a Wild Reservoir

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The annual incidence of Q fever in French Guiana was found to have increased in 1996 and was 37/100,000 population over the last 4 years. Subsequent investigations in Cayenne and its suburbs indicated that a wild reservoir of the bacteria was responsible for the epidemiologic pattern. A case-control study showed that residence near a forest and occupations and activities that result in exposure to aerosols of dusts from the soil are risk factors for Q fever. By means of time-series analysis, a strong positive correlation between rainfall and the incidence of Q fever with a time lag of 1–3 months was found. The spatial distribution of the cases showed that transmission occurs widely throughout greater Cayenne, which is incompatible with a pinpoint source of contamination. Transmission from livestock and dissemination of the bacteria by the wind appeared to be unlikely, which strengthens the hypothesis that a wild reservoir is responsible for transmission.

Q fever, a zoonosis caused by *Coxiella burnetii*, is endemic worldwide in a variety of birds, wild and domestic mammals, and arthropods [1–3]. The bacterium is transmitted to mammals mainly during parturition [4], and *C. burnetii* is found at high concentrations in the placenta, amniotic fluid, and other parturition products of sheep, cattle, and goats. In most countries, humans are infected with *C. burnetii* by direct contact with aerosols generated during parturition of domestic ungulates. Nevertheless, contamination may occur some time after parturition, because *C. burnetii* is strongly resistant to desiccation and environmental degradation. Although domestic ungulates seem to constitute the main reservoir for the bacteria, other animals, such as dogs [5, 6], cats [7, 8], and pigeons [9], have been implicated in rare cases. Oral transmission during the consumption of raw milk has been suggested in some cases but is uncertain [10, 11].

The incubation period before the appearance of disease is ~20 days. Although many infections are asymptomatic, most acute cases constitute an influenza-like illness, hepatitis, and/or pneumonia, occasionally leading to a lethal respiratory distress syndrome [11]. Usually, patients develop protective immunity and have uneventful recoveries; in rare cases, chronic complications, such as endocarditis, may occur [12]. Tetracyclines are the usual treatment for Q fever; they must be administered for 15–21 days for acute cases and for much longer for chronic disease. Because of the large number of possible reservoirs, the strong resistance to desiccation in the environment, and the aerosol mode of infection, the epidemiology of Q fever is complex. Cases are usually sporadic, but epidemics have been described in farms around periods of parturition.

French Guiana, an overseas French administrative unit in the Amazonian forest complex, is located on the northeastern coast of the South American continent between Brazil and Surinam. Ninety percent of its surface of 90,000 km² is tropical rain forest; the remaining 10%, the northern part of the country, is a coastal plain where 90% of the 157,000 inhabitants live. The county town Cayenne and 2 adjacent towns, Remire-Montjoly and Matoury, constitute the main urban centers, with 80,000 inhabitants, representing ~50% of the population. People live mainly in individual houses and small buildings. Except in the center of Cayenne, many houses are near forests. Thus, the outskirts of Remire and Matoury are surrounded by secondary rain forest, and those of the Cayenne area by wooded hills, where wild mammals, such as small monkeys, sloths, agouti, paca, and other rodents, can still be seen. The climate consists of 2 rainy seasons and 2 dry seasons. The first dry season (in March) is short, and the second lasts from July to

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November. The annual rainfall in the littoral plain is 2500–3500 mm. The humidity remains high throughout the year, and the average monthly temperature in the littoral zone is 26°C, with little variation.

A serologic study conducted in 1954 (H. Floch and P. Giroud, unpublished data) among persons working in the slaughterhouse in Cayenne showed that *C. burnetii* has been present in French Guiana for a long time. Only sporadic cases were reported until 1996, when 3 patients were admitted to the intensive care unit of the Cayenne hospital for acute respiratory distress syndrome, and 1 died. At the same time, several cases of Q fever were reported in the general population among patients who did not have the usual risk factors. A retrospective seroepidemiologic study showed a significant increase in the incidence of Q fever in 1996 [13]. Interestingly, the higher rates were found among people living in Cayenne and its suburbs, which indicates that the epidemiology of Q fever was unusual in our region. We therefore undertook the following study, to determine the risk factors associated with infection by *C. burnetii* in French Guiana and to identify the reservoirs of the bacteria.

Patients and Methods

Follow-up of new cases. All cases of Q fever that occurred in Cayenne and its suburbs between July 1996 and October 2000 were registered. We made all possible efforts to be exhaustive, including cases diagnosed in public hospitals, in private laboratories, and in the Institut Pasteur de la Guyane. The residences of all patients were localized through a geographic information system, to analyze the spatial distribution of the cases. The cases considered were those of patients who presented with symptoms compatible with a diagnosis of Q fever and who showed seroconversion from negative to positive or a 4-fold increase in IgG titer, with the presence of IgM in the second sample. Total antibodies to *C. burnetii* were detected by an immunofluorescence assay (Biomerieux) in serum samples diluted 1:80. All positive samples then were tested for IgG and IgM by the same method; the IgG and IgM titers were determined in a serial 2-fold dilution.

Because the epidemiologic curve showed seasonal variation, we hypothesized that infection is related to meteorologic factors. The curve for incidence was compared with those of various meteorologic parameters, especially temperature, precipitation, and wind. Correlations were explored by Almon distribution lag analysis by means of STATISTICA software [14]. The best coefficient of correlation and the best fit were sought while varying the length of the time lag.

Case-control study. Case patients were defined as patients living in Cayenne and its suburbs, divided into different areas, who presented with Q fever between 1 January 1998 and 1 June 2000. Q fever was diagnosed serologically, as described above. The control subjects comprised a random sample of patients who lived in Cayenne and its suburbs and who had been referred to the Arbovirus Laboratory at the Institut Pasteur de la Guyane for diagnosis of dengue fever and from whom a serum sample had been taken >14 days after the onset of fever. Serum taken during convalescence

was tested for antibodies to *C. burnetii*. Only patients with a titer of total antibodies <80 were included as control subjects. A priori, we considered that Q fever and dengue do not share any risk factors; bias introduced by the choice of control subjects who presented with a febrile illness is discussed below. Case patients and control subjects were given a questionnaire eliciting information on occupation, housing, contacts with animals, food, and leisure activities. At least 1 and, when possible, 2 control subjects, frequency-matched by age, sex, date of disease, and area of residence, were included for each case patient.

Statistical analysis was done in 2 steps. We first looked for factors associated with Q fever, by means of the χ^2 or Fisher's exact test for frequency comparisons and analysis of variance or the Kruskal-Wallis test, depending on the distribution of variables, for comparisons of averages. The potential explanatory variables identified were included in a second step in a multivariate analysis with the logistic regression model, with a backwards stepwise strategy.

Determination of reservoirs of *C. burnetii*. Serum samples were taken from domestic cattle, sheep, pigs, and goats during routine monitoring for brucellosis. Samples of serum from dogs and cats belonging to the case patients and control subjects also were taken. Wild rodents, marsupials, and bats were captured in various areas near the houses of case patients. Two species of swallows (*Hirundinidae*) were captured on the roof of the jail at Remire, where many cases of Q fever had been described among guards and prisoners. Amphibians (frogs and toads) were captured on the edge of the town.

The presence of anti-*C. burnetii* antibodies was determined by complement fixation in cattle (using a kit developed jointly by Rhone Mérieux and Sanofi Pasteur) and dogs and cats (using a kit from Institut Viron & Serion) [15]. Because complement fixation could not be used on most wild animal species, because of inadequate quantities of serum or lack of anticomplementary serum samples, antibodies to *C. burnetii* were tested by an immunofluorescence test. For *Proechimys* species, the fluorescence test was done as for human routine diagnosis, with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-rat IgG (Sigma Laboratories). For *Mus musculus*, FITC-conjugated rabbit anti-mouse IgG (Sanofi Diagnostic Pasteur) was used.

For some species (bats, swallows, marsupials, and amphibians), no anti-IgG was available. In that case, total immunoglobulins were purified by precipitation with ammonium sulfate, followed by dialysis, as described elsewhere [16]. These immunoglobulins were used to immunize mice, to develop a mouse ascitic fluid specific for the immunoglobulins of each species. The specificity of the mouse ascitic fluids, cross-reactions in other species, and the dilutions were determined in preliminary ELISAs in which the immunoglobulins were coated onto Maxisorb plates. For the immunofluorescence test, serum samples diluted 1:50 in PBS were left for 30 min at 37°C; the slides were washed twice in PBS-Tween, were incubated for 30 min at 37°C with the specific mouse ascitic fluid diluted 1:100 in PBS, and were washed twice in PBS-Tween. The antibodies were revealed with FITC-conjugated rabbit anti-mouse IgG (Sanofi Diagnostic Pasteur).

The genome of *C. burnetii* was detected by polymerase chain reaction (PCR). DNA was extracted from the organs of the wild animals after preparation by a method described elsewhere [17], and PCR was done with Trans1 (5'-TATGTATCCACCGTAGCC-

AGTC-3'; forward) and Trans2 (5'-CCCAACAACACCTCCTTA-TTC-3'; reverse) derived from a transposon-like repetitive region of the *C. burnetii* genome [18], as described elsewhere [19]. A positive control provided by the National Reference Center for Rickettsiae (Marseille, France) was included in each assay. To test for the presence of inhibitors of Taq polymerase in the samples, positive DNA was mixed with some samples in each assay.

Results

Follow-up of new cases. During the 4 years of the study, 132 cases were identified, 93 in males and 39 in females. The mean age was 38.6 years (range, 6 months to 76 years). In comparison with census figures, the proportion of men (70.5%) was high ($P < .001$). Only 2 patients were <15 years old, and almost all patients had a pulmonary form of the disease. Among case patients, the median IgG titer was 1:640 (25th percentile, 1:320; 75th percentile, 1:1280), and the median IgM titer was 1:320 (25th percentile, 1:160; 75th percentile, 1:640).

Only 7 cases occurred in patients living outside the Cayenne area (figure 1). The cases in Cayenne and its suburbs were widely dispersed: many were geographically isolated, but we also observed clusters in space or in space and time. During the study period, the mean incidence of Q fever was 37/100,000 per year; however, as shown in figure 2, the incidence varied considerably, with peaks occurring once or twice a year at var-

ious periods and with an interruption in transmission between December and February. Graphically, a strong correlation was found between the incidence and the precipitation rate, showing that transmission was interrupted during the dry season, with a shift of roughly 2 months. The Almon distribution lag analysis showed that the correlation between incidence and monthly rainfall was statistically significant for a lag of 1–3 months, maximum at the second month ($r = .85$; $P < 10^{-3}$). No other correlation was found, for example, with wind, temperature, or sunshine.

Case-control study. Of the 132 cases diagnosed during the survey period, 60 were included in the study, and 105 people on the Arbovirus Laboratory registers were selected randomly as control subjects. Seven control subjects who had serum samples positive for Q fever were excluded. Because the control subjects were randomized by stratifying for age and sex, our populations of case patients and control subjects were similar with regard to these variables, with sex ratios (male:female) of 1.6 and 2.0 ($P = .55$) and mean ages of 41.0 years (range, 22–77 years) and 40.6 years (range, 22–69 years; $P = .86$) for case patients and control subjects, respectively.

Analysis of data on travel within the administrative area did not show areas outside the Cayenne area of increased risk for contamination with *C. burnetii*. Furthermore, diet did not appear to be associated with infection (table 1). Greater differ-

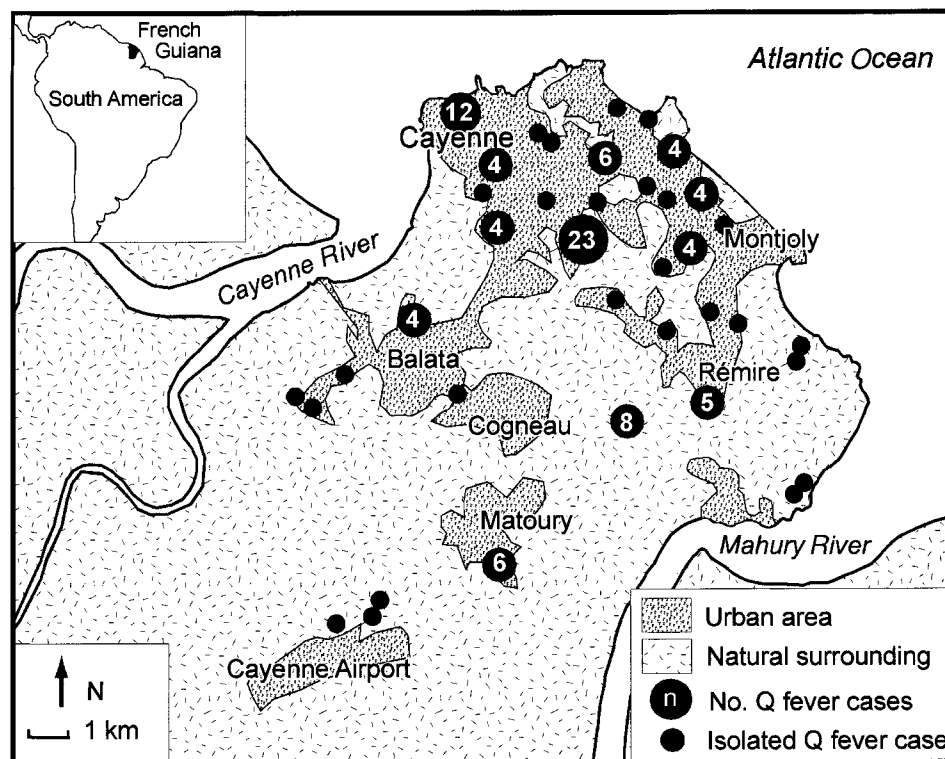


Figure 1. Locations of cases of Q fever in French Guiana, July 1996 through October 2000

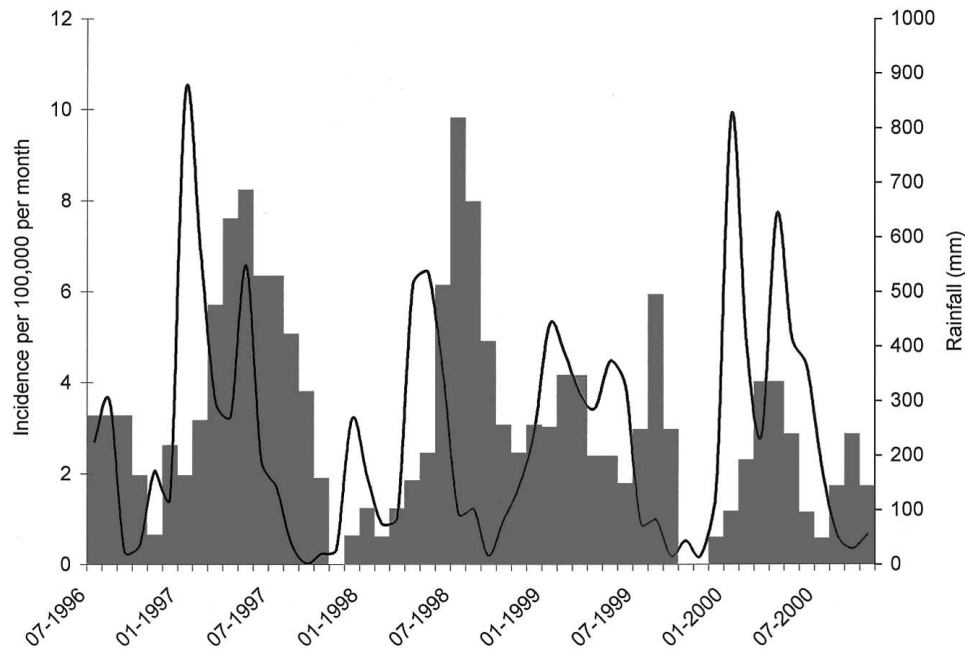


Figure 2. Plot of incidence of Q fever per 100,000 population per month (shaded area) against rainfall (line), French Guiana, July 1996 through October 2000.

ences were found between case patients and control subjects with regard to environmental features of their residences. Living near a forest was found to be a risk factor (odds ratio [OR], 3.8), as was frequent sighting of bats (OR, 3.0), marsupials (OR, 2.1), or other wild mammals (OR, 4.9) near the residence. Terracing work was more frequent near the houses of case patients at the time of illness (OR, 3.1); people who gardened were also found to be at risk for disease (OR, 2.5). Similarly, people working in the building trade or in public works appeared to be more heavily exposed (OR, 3.6). More case patients than control subjects owned air-conditioned vehicles (OR, 2.4). All these differences were statistically significant.

The same variables were included in a logistic regression model, except for 2 (gardening and terracing) for which there were too many missing values. The results (table 2) seem to confirm the greater risk of people working in the building trade or public works (OR, 3.5). Living close to the forest (OR, 2.7) and frequently seeing bats (OR, 2.6) and other wild mammals (OR, 3.1) remained risk factors after adjustment. The model confirmed the results of the univariate analysis and showed no interactions among variables.

Determination of the reservoir of *C. burnetii*. *C. burnetii* was not detected in the DNA of lung, liver, or intestine of any of the wild animals. The serologic test results are summarized in table 3.

Of the 355 cows, 50 sheep, 21 goats, and 25 pigs tested, only 6 cows (1.7%) had antibodies to *C. burnetii*. Of the 57 dogs and 6 cats belonging to 21 case patients and 24 control subjects, 25

dogs and the 6 cats belonged to case patients. Antibodies to *C. burnetii* were found in 7 dogs (12.3%), of which 2 belonged to case patients and 5 to control subjects. All the cats were serologically negative. The seroprevalence among pets was thus not statistically different among case patients and control subjects.

Of the 117 rodents, 42 marsupials, 86 bats, 69 birds, and 47 batrachians captured around Cayenne and tested, only 4 *Proechimys* species, 4 *Philander opossum*, 1 *Didelphis marsupialis*, and 1 *Progne chalybea* had antibodies to *C. burnetii* (table 3). High titers were found in *Proechimys* species, but the results for birds were doubtful.

Discussion

Although a study conducted in 1996 [13] indicated that the incidence of Q fever in French Guiana had increased in previous years and that the main areas of transmissions were located in Cayenne and its suburbs, in this preliminary study we found no link with the classical sources of contamination, that is, domestic ungulates (cattle, sheep, and goats): the serologic study conducted among ungulates showed little contamination, and the results of the case-control study do not indicate a role of livestock in transmission.

Although a role of pets has been suggested in other studies [5–7], the prevalence in dogs belonging to case patients was comparable with that of control subjects' dogs and was high, which suggests that dogs are in contact with the bacteria. We did not observe any epidemic in families with puppies' birth,

Table 1. Case-control study, results of univariate analysis: percentages and odds ratios (ORs) for risk factors for contracting Q fever in French Guiana, January 1998 through June 2000.

Variable	Case patients (n = 60)	Control subjects (n = 98)	OR	P
Profession				
Building trade or public works	21.7	7.1	3.60	.011
Administration	31.7	42.9	0.62	
Technical sector	16.7	20.4	0.78	
Military	10.0	13.3	0.73	
Other	6.7	6.1	1.28	
Diet				
Raw meat	24.0	34.7	0.59	
Milk	70.0	74.5	0.80	
Cheese	93.3	94.9	0.75	
Yogurt	80.0	88.8	0.51	
Smoked meat or fish	67.3	59.2	1.42	
Barbecue	57.1	70.1	0.57	
Environment around residence				
Proximity to forest	79.7	51.0	3.76	<.001
Animals in false ceiling	53.3	37.8	1.85	
Have a garden	80.0	79.6	1.03	
Practice gardening	40.5	21.2	2.53 ^a	.039
See marsupials near house	40.0	24.5	2.06	.041
See bats near house	75.0	50.0	3.00	.002
See other wild mammals near house	33.3	9.2	4.94	<.001
See birds near house	16.7	14.3	1.20	
See reptiles near house	33.3	33.7	0.98	
Earth-moving activity near house	58.5	30.8	3.18 ^a	.004
Pets				
Cat at home	26.7	24.5	1.12	
Dog at home	55.0	42.9	1.63	
Entered forest during preceding month				
Tick bite in past	21.7	25.5	0.81	
Tick bite during past month	30.8	38.0	0.72	
Tick bite during past month	5.7	5.4	1.06	
Air-conditioning				
At work	55.0	63.3	0.71	
In vehicle	39.0	21.1	2.40	.017
At home	28.3	25.5	1.15	

NOTE. Data are percentages, except where noted.

^a Data available for subgroup; not used for multivariate analysis because of missing values.

as described elsewhere [6], and all 4 veterinarians tested serologically were seronegative. Few cats were tested serologically, but none was seropositive, and the results of the case-control study show no indication of their involvement. We consider that house pets are probably not a source of contamination in French Guiana.

The large majority of cases occurred in Cayenne and its suburbs, where 50% of the population of the administrative unit lives. In the hyperendemic focus described in the south of France, the incidence was 35/100,000 [20], which is comparable to that observed in French Guiana. Physicians at the hospitals in Kourou and St Laurent du Maroni, situated on the coastline 50 and 250 km from Cayenne, respectively, have recorded no cases of Q fever, although they are aware of the situation in

Cayenne and carry out serologic testing for *C. burnetii* systematically in the presence of atypical pulmonary infection.

In 1997, we concluded that the patients were equally distributed throughout the Cayenne area. After 4 years of follow-up, however, it appears that the numbers of cases in the various districts are not proportional to the population density, but that cases are proportionally more numerous in the peripheral zones of the agglomeration. We also observed many cases isolated in space, whereas others were grouped in zones where cases are observed each year. This distribution is not in favor of dissemination of the bacteria by the wind from a point source (ranch or slaughterhouse), as described in other urban epidemics [21, 22]. The intensity and direction of the wind vary little during the year, the trade wind blowing mainly from the northeast to the southeast at 10–20 knots.

The case-control study indicates 2 types of risk factors. The first are associated with the environment around the residence, and the second with activities resulting in aerosols. Thus, people who live in the zones between the city and the forest are most heavily at risk for contamination, as indicated by the fact that sighting of wild mammals close to home was a risk factor in our survey. This result is consistent with observations on the spatial distribution of the cases. Furthermore, professions and activities that result in exposure to aerosols of dusts from the soil appear to be risk factors. Although gardening and exposure to earth moving could not be included in the multivariate analysis because of too many missing values, the results of the univariate analysis suggest that the bacterium is present on the soil and that contamination occurs aeri ally. Because the route of infection has been shown to determine the clinical manifestation (respiratory versus gastrointestinal) [23] of acute Q fever in animal models, this result is consistent with the fact that essentially the pulmonary form of the disease is observed in French Guiana.

The use of frequency matching on age and sex in the case-control study did not allow us to study these 2 variables in terms of ORs. However, by comparison with the structure of the population of French Guiana, we can conclude that Q fever affects mainly adult men, as observed in other studies [11, 24, 25]. Occupational or leisure exposure to aerosols could explain this observation.

The most striking feature of our study is the strong correlation between rainfall and incidence, with a time lag of 1–3

Table 2. Case-control study, results of multivariate analysis: adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for risk factors for contracting Q fever in French Guiana, January 1998 through June 2000.

Variable	OR	CI	P
Work in building trade or public works	3.54	1.1–11.0	.03
See bats near house	2.58	1.2–5.6	.02
See other wild mammals near house	3.07	1.2–8.1	.02
Proximity to forest	2.71	1.2–6.3	.02

Table 3. Results of serologic testing for *Coxiella burnetii* in wild animals captured in French Guiana, September 1997 through September 2000.

Animal	No. tested	No. positive
Rodent		
<i>Mus musculus</i>	58	0
<i>Proechimys</i> species ^a	26	4
<i>Rattus rattus</i>	17	0
Other	16	0
Marsupial		
<i>Philander opossum</i>	36	4
<i>Didelphis marsupialis</i>	4	1
Other	2	0
Chiropters		
<i>Molossus molossus</i>	57	0
<i>Phyllostomus hastatus</i>	17	0
Other	12	0
Birds ^b	69	1
Batrachians		
<i>Buffo marinus</i>	21	0
<i>Leptodactylus pentadactylus</i>	20	0
Other	6	0

^a *P. cuvieri* and *P. cayennensis*.

^b *Progne chalybea* and *Progne tapera*.

months and a maximum at the second month. It is surprising that transmission is maximal during the rainy season, which is not favorable for aerosols; however, this observation is in favor of a wild reservoir whose activity is dependent on rain.

The 2 groups of risk factors identified in the case-control study, the location in space and the correlation with rainfall, lead logically to a search for a wild reservoir of *C. burnetii*. Antibodies to *C. burnetii* have been found in various species, but particularly in *Proechimys* species, small rodents that appear to be more frequently in contact with the bacteria than other species.

We looked for *C. burnetii* in batrachians and especially in frogs, because explosive breeding among some species is observed at the beginning of the rainy season and 2 or 3 times a year afterward. In the absence of any evidence of infection of batrachians with the bacteria, other explanations must be found. A correlation between disease and rain is frequently seen for diseases that are transmitted by arthropods. As the number of arthropods explodes during the rainy season, some could transmit the disease from animals to humans, even if the rate of transmission is low. Ticks have been proposed as a vector, but ticks bites are common in French Guiana, and we found no more bites among case patients than among control subjects.

The main potential bias in the study derives from the choice of control subjects who were patients admitted for a viral illness, including dengue, and viral syndromes related to influenza and arboviruses. They had therefore been exposed to factors conducive to development of a viral syndrome. In view of the ubiquity of such infections in French Guiana, we consider that these people were representative of the population of Cayenne and its suburbs. People who have been living in French Guiana

for a long time have a lower risk for developing viral syndromes, because they have usually developed protective antibodies against the viruses circulating in the country. There is therefore a lower probability that they would be included as control subjects. Nevertheless, the dengue 3 serotype was introduced recently into the country, and the entire population is therefore susceptible. In addition, antibodies to many viruses responsible for viral syndromes are only temporary. Moreover, in 70% of cases, the cause of the viral syndrome for which the patient was sent to the National Arbovirus Reference Center cannot be identified by serologic tests or viral culture. Furthermore, the fact that they developed a febrile illness permitted us to obtain the information elicited by our questionnaire. This choice also allowed us to test the control subjects for Q fever without taking other blood samples.

This survey confirms that the epidemiology of Q fever has many faces. Although we were unable to answer all the outstanding questions about Q fever in French Guiana, especially the reservoir of the bacteria, some risk factors appear to be clear. First, transmission of Q fever occurs mainly near the forest; second, the seasons of transmission can be predicted; and, third, some workers seem to be at greater risk for this disease, especially those involved in earth moving. The results of this study may therefore help the physicians of French Guiana to identify cases of Q fever and begin early, adapted treatment.

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