African tick-bite fever is an emerging infectious disease caused by the spotted fever group Rickettsia africae and risk factors associated with infection, we conducted a cross-sectional study of persons in seven rural villages in distinct ecological habitats of Cameroon. We examined 903 plasma samples by using an indirect immunofluorescence assay for antibodies to R. africae and analyzed demographic and occupational data collected from questionnaires. Of the 903 persons tested, 243 (26.9%) had IgG/IgM/IgA reactive with R. africae. Persons from four of the seven village sites were significantly more likely to be seropositive (P < 0.05), and lowland forest sites tended to have higher seroprevalences. These results suggest that African tick-bite fever is common in adults in rural areas of Cameroon and that ecological factors may play a role in the acquisition of R. africae infection.
included in the large epidemiologic survey. Sites were selected
to represent different habitats, including one lowland coastal
forest site, one mangrove site, one lowland forest site, two
gallery highland forest sites, one lowland equatorial forest site,
and one forest-savanna mosaic site. The seven sites included
one in each of the Southwest, Northwest, West, Littoral,
Central, South, and East Provinces. The study protocol was
reviewed and approved by the Johns Hopkins Bloomberg
School of Public Health Committee for Human Research, the
Cameroon National Ethical Review Board, and the HIV Tri-
Services Secondary Review Board.

Serologic testing. Rickettsia africae (ESF strain) antigen
slides were prepared in the Ehrlichial and Rickettsial Diseases
Research Laboratory, Department of Pathology, University
of Texas Medical Branch (Galveston, TX). Rickettsiae were
cultured in Vero cells, and after an infection rate ≥ 70% was
achieved, cells were harvested and antigen slides prepared
according to standard procedures. Indirect fluorescent anti-
body assays were performed on the samples to detect anti-
bodies reactive with R. africae as described.17,21 Serial two-fold
dilutions (1:32–1:64) of human serum were prepared in
phosphate-buffered saline (PBS) containing 1% bovine serum
albumin (BSA) and 0.1% Tween 20. Antigen slides were
blocked in PBS containing 1% BSA and 0.01% sodium azide.
Ten microliters of each serum dilution were added to each
well of the antigen slide and incubated in a humid chamber
for 30 minutes at 37°C. Slides were subsequently washed
with PBS containing 0.1% Tween 20 for 10 minutes and then
washed twice in the same solution for 10 minutes. Fluorescein
isothiocyanate–conjugated goat anti-human IgA, IgG, and
IgM immune serum (Kirkegaard and Perry Laboratories,
Gaithersburg, MD) diluted 1:100 in PBS containing 1% BSA
and 0.01% Tween 20 was added to each well and incubated in
a humid chamber for 30 minutes at 37°C. Slides were washed
once with PBS containing 0.1% Tween 20 for 10 minutes and
once with PBS containing 0.1% Tween 20 and 0.01% Evans blue
for 10 minutes. The slides were blot-dried, mounted with gel
mount (Biomedica Corp, Foster City, CA), and observed under a
fluorescence microscope at 400× magnification. Serum samples
yielding distinctly fluorescent rickettsiae at a 1:64 dilution were
considered positive. Positive samples were further two-fold
serially diluted 1:128–1:4,096 to determine endpoint titers.

Statistical analysis. Descriptive statistics were calculated for
demographic, occupational, and behavioral variables. House
roofing material and ceiling condition (unfinished or finished)
were used to assess socioeconomic status. Occupations were
categorized by their risk of contact with ticks: agricultural
occupations, occupations requiring direct contact with animals,
agricultural and direct contact occupations, or neither.

The seroprevalence of antibodies to R. africæ was calcu-
lated overall and by village site, and a chi-square test was used
to evaluate heterogeneity of rates among the different villages.
To examine the associations between questionnaire variables
and R. africæ seropositivity, single covariate (unadjusted)
logistic regression models were generated to calculate odds
ratios (ORs) and 95% confidence intervals (CIs). All variables
significant at P < 0.10 were considered for inclusion in multi-
variable logistic regression models. The variables were added
sequentially to a model containing only R. africæ seropositi-
ity. Models were then evaluated using likelihood ratio tests in
a forward stepwise manner to identify a parsimonious mul-
tivariable model. To adjust for potential confounding, covari-
ates whose addition to the model changed the ORs by ≥ 10%
were included in the final model. An interaction term between
hunting wild game and age group was also assessed. All analy-
ses were conducted by using Stata/SE version 10.1 software
(StataCorp, College Station, TX).

RESULTS

Study participants. Demographic, occupational, and
behavioral information for the 903 persons included in this
study is shown in Table 1. In the aggregate results, sexes were
represented approximately equally. Within the seven sites, sex
was similar with the exception of three sites: Mundemba,
Nyabisan, and Sobia, which had 81.3%, 10.0% and 36.3%
female participants, respectively. Study participants’ ages
ranged from 16 to 88, years and younger age groups (16–25
and 26–35 years) were more frequently represented. Most
participants had ≤ 9 years of formal education; lived in houses
with corrugated tin, tile, or tar roofs with unfinished ceilings;
and worked in subsistence or market agriculture. Although
few participants worked in occupations involving direct
contact with animals, a substantial percentage reported having
hunted wild game at some time. Among the participants who
reported ever having owned a wild pet, most owned a rodent
or a medium-to-large herbivore.

Seroprevalence of antibodies to R. africæ. Of the 903
persons, 243 (26.9%) had antibodies reactive with R. africæ
(Table 2). High seroprevalences were seen in Njikwa (51.8%),
Lomie (38%), Sobia (37%), and Nyabisan (28.7%) and
represented the gallery highland (Njikwa) and most of the
lowland village sites (Lomie, Sobia, and Nyabisan). The
lowest rate was seen in Mouanko (11.9%), a mangrove site.
Seropositivity rates for each of the seven villages by village
site and ecological characteristics are shown in Table 1. Seropositivity rates were significantly different among the
different villages (χ² = 93.3, P < 0.001). Most of the lowland
forest sites had high seropositivity rates. However, the highest
and lowest rates were found in highland gallery-forest sites.
The distribution of endpoint IgG/IgM/IgA titers for R. africæ
was 64 (16.8%), 128 (27.1%), 256 (22.1%), 512 (18.1%), 1,024
(9.8%), and 2,048 (6.2%).

Unadjusted analyses of demographic, occupational, and
behavioral characteristics associated with R. africæ sero-
positivity showed no significant differences in seropositivity
between females and males or across different age strata, with
the exception of persons 36–45 and > 55 years of age, who had
significantly higher odds of seropositivity (Table 3). In contrast,
increasing years of education (7–9 and > 9 years) were asso-
ciated with significantly lower odds of seropositivity. Lower
socioeconomic status as indicated by grass or thatched house
roofing material was associated with significantly higher odds
of seropositivity, as was missing house roofing material.

Four of the village sites, Lomie, Nyabisan, Sobia and Njikwa,
were associated with significantly higher odds of seropositivity.
Participants in high-risk occupations (occupations involving
only agricultural work or agricultural work and direct contact
with animals) and those who reported ever having hunted wild
game had significantly higher odds of seropositivity. Although
owning a wild pet had no significant effect on seropositivity,
those who reported having ever owned a medium-to-large
herbivore, primate, or rodent had significantly increased odds
of seropositivity.
had significantly higher odds of seropositivity; no other age
females and males. However, participants 36–45 years of age
There was no significant difference in seropositivity between
and ever hunted wild game were retained in the final model.
primate category includes monkey, chimpanzee and gorilla; rodent category includes porcu-
pine, hedgehog and rat; reptile category includes crocodile and tortoise.

In the multivariable analyses, only sex, age group, village site
and ever hunted wild game were retained in the final model.
There was no significant difference in seropositivity between
females and males. However, participants 36–45 years of age
had significantly higher odds of seropositivity; no other age
group showed this effect. Participants from the villages of
Lomie, Njikwa, Nyabisan, and Sobia also had significantly
higher odds of seropositivity. Those persons who reported
ever having hunted game had non-significantly elevated odds
of seropositivity. The addition of an interaction term between
age group and ever hunted wild game to the final model was
not significant ($P = 0.6; OR = 1.1, 95\% CI = 0.8–1.4$).

**DISCUSSION**

The results of this study suggest that ATBF is common in
adults in rural areas in Cameroon, and ecological factors associated
with the tick vector may increase the risk of *R. africae*
infection. The detection of IgG/IgM/IgA and titers as high as
1:2,048 in some participants most likely indicate recent infec-
tions with *R. africae* and therefore provide support for the
ongoing transmission of infection.

Although the four village sites associated with high odds
of *R. africae* seropositivity were widely distributed across
Cameroon, three of the four sites were located in lowland
rainforest habitats. Lowland rainforest habitats are ideal for
*A. variegatum* ticks because of their moderate canopy cover,
which provide microclimates favoring tick survival, and high
biological diversity, which increase the number of habitats
available to the ticks. In addition, lowland rainforest habitats
tend to have suitable soils for agriculture and consequently
may offer the ticks access to domestic hosts. In contrast, mangrove forest habitats are not preferred by the ticks because they have a low broken canopy cover, relatively lower biological diversity, and brackish or salt water, which provide fewer habitats for the ticks and fewer opportunities for encountering hosts. This study found the expected findings associated with these habitats with most of the lowland forest sites showing high seroprevalences and the mangrove site showing the lowest seroprevalence. However, it is unclear whether these findings are a function of canopy development, biological diversity, presence of domestic hosts, or a combination of these factors.

The highest seroprevalence (51.8\%) was recorded in
Njikwa, a village in the highland gallery-forest surrounded by

TABLE 1
Baseline characteristics of the study population (n = 903), Cameroon

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No (% )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>462 (51.2)</td>
</tr>
<tr>
<td>M</td>
<td>441 (48.8)</td>
</tr>
<tr>
<td>Age group (y)</td>
<td></td>
</tr>
<tr>
<td>16–25</td>
<td>236 (26.1)</td>
</tr>
<tr>
<td>26–35</td>
<td>223 (24.7)</td>
</tr>
<tr>
<td>36–45</td>
<td>177 (19.6)</td>
</tr>
<tr>
<td>46–55</td>
<td>118 (13.1)</td>
</tr>
<tr>
<td>&gt; 55</td>
<td>149 (16.5)</td>
</tr>
<tr>
<td>Formal education (y)</td>
<td></td>
</tr>
<tr>
<td>0–4</td>
<td>234 (25.9)</td>
</tr>
<tr>
<td>4–6</td>
<td>262 (29.0)</td>
</tr>
<tr>
<td>7–9</td>
<td>222 (24.6)</td>
</tr>
<tr>
<td>&gt; 9</td>
<td>185 (20.5)</td>
</tr>
<tr>
<td>House roofing material</td>
<td></td>
</tr>
<tr>
<td>Corrugated tin, tile, or tar with unfinished ceiling</td>
<td>423 (46.8)</td>
</tr>
<tr>
<td>Corrugated tin, tile, or tar with finished ceiling</td>
<td>199 (22.0)</td>
</tr>
<tr>
<td>Grass or thatched</td>
<td>92 (10.2)</td>
</tr>
<tr>
<td>Missing*</td>
<td>189 (20.9)</td>
</tr>
<tr>
<td>Village site</td>
<td></td>
</tr>
<tr>
<td>Mundemba</td>
<td>176 (19.5)</td>
</tr>
<tr>
<td>Mouanko</td>
<td>151 (16.7)</td>
</tr>
<tr>
<td>Lomie</td>
<td>142 (15.7)</td>
</tr>
<tr>
<td>Massangan</td>
<td>109 (12.1)</td>
</tr>
<tr>
<td>Njikwa</td>
<td>110 (12.2)</td>
</tr>
<tr>
<td>Nyabisan</td>
<td>80 (8.9)</td>
</tr>
<tr>
<td>Sobia</td>
<td>135 (14.9)</td>
</tr>
<tr>
<td>High-risk occupation†</td>
<td></td>
</tr>
<tr>
<td>Neither agriculture nor direct contact</td>
<td>154 (17.0)</td>
</tr>
<tr>
<td>Only agriculture</td>
<td>635 (70.3)</td>
</tr>
<tr>
<td>Only direct contact</td>
<td>6 (0.7)</td>
</tr>
<tr>
<td>Agriculture and direct contact</td>
<td>108 (12.0)</td>
</tr>
<tr>
<td>Ever hunted wild game</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>666 (73.7)</td>
</tr>
<tr>
<td>Yes</td>
<td>237 (26.3)</td>
</tr>
<tr>
<td>Ever owned a wild pet‡</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>823 (91.2)</td>
</tr>
<tr>
<td>Yes</td>
<td>79 (8.8)</td>
</tr>
<tr>
<td>Ever owned as a pet§</td>
<td></td>
</tr>
<tr>
<td>Wild bird</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>818 (90.6)</td>
</tr>
<tr>
<td>Yes</td>
<td>85 (9.4)</td>
</tr>
<tr>
<td>Medium-to-large herbivore</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>728 (80.6)</td>
</tr>
<tr>
<td>Yes</td>
<td>175 (19.4)</td>
</tr>
<tr>
<td>Pangolin</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>775 (85.8)</td>
</tr>
<tr>
<td>Yes</td>
<td>128 (14.2)</td>
</tr>
<tr>
<td>Primate</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>793 (87.8)</td>
</tr>
<tr>
<td>Yes</td>
<td>110 (12.2)</td>
</tr>
<tr>
<td>Rodent</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>695 (77.0)</td>
</tr>
<tr>
<td>Yes</td>
<td>208 (23.0)</td>
</tr>
<tr>
<td>Reptile</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>890 (98.6)</td>
</tr>
<tr>
<td>Yes</td>
<td>13 (1.4)</td>
</tr>
</tbody>
</table>

*Question not included at the time of questionnaire administration.
†High-risk occupation includes agricultural occupations for subsistence or market, or occupations requiring direct contact with animals, such as butcher, animal breeder, hunter, or veterinary assistant.
‡Excludes one person with missing data.
§Medium-to-large herbivore category includes hare, antelope, deer, gazelle, and buffalo; primate category includes monkey, chimpanzee and gorilla; rodent category includes porcupine, hedgehog and rat; reptile category includes crocodile and tortoise.

TABLE 2
*Rickettsia africae* seroprevalence by village site and habitat type, Cameroon

<table>
<thead>
<tr>
<th>Village site</th>
<th>Habitat type</th>
<th>No. samples</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mundemba</td>
<td>Coastal lowland forest</td>
<td>176</td>
<td>26 (14.8)</td>
</tr>
<tr>
<td>Mouanko</td>
<td>Mangrove</td>
<td>151</td>
<td>18 (11.9)</td>
</tr>
<tr>
<td>Lomie</td>
<td>Congo Basin lowland forest</td>
<td>142</td>
<td>54 (38.0)</td>
</tr>
<tr>
<td>Massangan</td>
<td>Highland gallery-forest</td>
<td>109</td>
<td>14 (12.8)</td>
</tr>
<tr>
<td>Njikwa</td>
<td>Highland gallery-forest</td>
<td>110</td>
<td>57 (51.8)</td>
</tr>
<tr>
<td>Nyabisan</td>
<td>Atlantic equatorial lowland forest</td>
<td>80</td>
<td>23 (28.7)</td>
</tr>
<tr>
<td>Sobia</td>
<td>Lowland forest-savanna mosaic</td>
<td>135</td>
<td>51 (37.8)</td>
</tr>
</tbody>
</table>

In the southern part of the country, where cattle are
recorded in the southern part of the country, where cattle are
A. variegatum and therefore provide support for the
ongoing transmission of infection.

Although the four village sites associated with high odds
of *R. africae* seropositivity were widely distributed across
Cameroon, three of the four sites were located in lowland
rainforest habitats. Lowland rainforest habitats are ideal for
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biological diversity, which increase the number of habitats
available to the ticks. In addition, lowland rainforest habitats
tend to have suitable soils for agriculture and consequently
may offer the ticks access to domestic hosts. In contrast, mangrove forest habitats are not preferred by the ticks because they have a low broken canopy cover, relatively lower biological diversity, and brackish or salt water, which provide fewer habitats for the ticks and fewer opportunities for encountering hosts.

This study found the expected findings associated with these habitats with most of the lowland forest sites showing high seroprevalences and the mangrove site showing the lowest seroprevalence. However, it is unclear whether these findings are a function of canopy development, biological diversity, presence of domestic hosts, or a combination of these factors.

The highest seroprevalence (51.8%) was recorded in
Njikwa, a village in the highland gallery-forest surrounded by
cattle-rearing areas. Because Njikwa is not located in the ideal
lowland rainforest habitat, this high seroprevalence indicates
that the presence of cattle, the preferred host of *A. variegatum*,
may be more closely linked with *R. africae* seropositivity
than habitat type. In a serosurvey analyzing antibodies reactive
to *R. conorii* in Zimbabwe, the highest seroprevalence was
recorded in the southern part of the country, where cattle are
reared and *A. variegatum* ticks are commonly encountered.9

Thus, environmental factors may be less important for *R. africae* seropositivity in areas where domestic hosts are readily available.
It is also possible that an unrecognized tick vector may be transmitting *R. africae* in central Africa. In our previous study, we detected antibodies to spotted fever group rickettsiae in 35% of febrile patients from different localities, particularly urban and suburban communities, where cattle-rearing is rarely practiced and the prevalence of *A. variegatum* is low. Although the persons examined in that study were a biased group (febrile patients), the results suggest that a vector other...
than *A. variegatum* could also be transmitting infections in the urban and suburban areas where *A. variegatum* prevalence is low. Recently, *R. rickettsii*, the agent of Rocky Mountain spotted fever, and known to be transmitted by *Dermacentor* species was isolated from the dog tick *Rhipicephalus sanguineus* in Arizona, where febrile patients were diagnosed with Rocky Mountain spotted fever and *Dermacentor* ticks are uncommon. Therefore, it could be important to examine other tick species as probable vectors of *R. africae*, especially if the habitats are less hospitable for *A. variegatum*.

In addition to ecological factors associated with seropositivity, this study found that persons 36–45 years of age were more likely to be seropositive than persons of all other age groups. This finding appears to signify the role of certain behavioral or occupational factors pertaining to this age group. Of the three vector-contact-associated activities investigated (wild game hunting, wild pet ownership, and occupations involving agricultural work or direct contact with animals), only game hunting was associated with a moderately increased odds of seropositivity in the multivariable analysis, although it did not reach the level of significance. The interaction between game hunting and age group did not show any significant differences in seropositivity. Thus, game hunting may only partially explain the increased odds of seropositivity in this group of middle-aged adults. The high odds of seropositivity caused by ecological factors as represented by village site may have obscured any smaller significant differences in odds of seropositivity associated with the other vector-contact-associated activities. Nonetheless, these findings suggest that risk factors for ATBF may be similar in indigenous populations in Africa compared with those for international travelers, for whom game hunting has been shown to lead to a 10-fold increased odds of ATBF.

This study had some limitations. Although we analyzed samples for reactivity with *R. africae* only, four rickettsial agents (*R. africae, R. conorii, R. aeschlimannii*, and *R. sibirica mongolotimoniae*) have been detected in Africa. *Rickettsia conorii* is associated with *Rhipicephalus sanguineus* ticks and is mainly encountered in urban areas, and *R. africae* is reported in semi-rural and rural areas. Although *R. africae* has not been isolated in Cameroon, we limited this serosurvey to *R. africae* because previous serologic and molecular surveys suggest that *R. africae* rather than *R. conorii* is the rickettsial agent infecting humans in Cameroon. In addition, there is evidence that the prevalence of *R. africae* may be as high as 75% in *A. variegatum* ticks in Cameroon.

The strengths of this study include the sample size, which is the largest seroepidemiologic study of *R. africae* infection in native populations in Africa to date, and the evaluation of risk factors for exposure in healthy adults. Previous studies have focused on international travelers and indigenous patients with acute febrile illnesses. Thus, this study extends the findings of these studies to the general population.

Although this serologic evidence further supports previous findings of *R. africae* in Cameroon, isolation of the agent in patients and the vector remains to be achieved. Few studies have analyzed antibodies to *R. africae* by age. Thus, further research is needed to clarify whether high antibody titers are associated with recent infection or the accumulation of antibodies through years of exposure. Active surveillance studies remain important for better determining the magnitude and distribution of African tick bite fever in indigenous populations in Africa.

Received March 31, 2010. Accepted for publication October 14, 2010.

Acknowledgments: We thank Dr. Donald Bouyer (Ehrlichial and Rickettsial Research Laboratory, Department of Pathology, University of Texas Medical Branch, Galveston, TX) for providing the antigen slides.

Financial support: This study was supported by awards from the U.S. Military HIV Research Program, the National Institutes of Health Director’s Pioneer Award (grant DP1-OD000370), the W.W. Smith Charitable Trust, and grants from the National Institutes of Health Fogarty International Center (International Research Scientist Development Award grant 3 K01 TW00003-05), AIDS International Training and Research Program (grant 2 D 43 TW00010-17-AITRP), Johns Hopkins Bloomberg School of Public Health, Center for AIDS Research (grant P30 AI42855), and the National Geographic Society Committee for Research and Exploration (grant #7762-04). The U.S. Embassy also provided support through the Humanitarian Assistance Program, as did google.org and the Skoll Foundation.

Authors’ addresses: Lucy M. Ndip, Landry E. Nfonsam, and Marie A. Bissong, Department of Biochemistry and Microbiology, University of Buea, Buea, South West Province, Cameroon. Hope H. Biswas, Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD. Matthew LeBreton, Emmanuel Mpoudi-Ngole, Cyrille Djoko, and Ubald Tamoufe, Johns Hopkins Cameroon Program/CRESAR, Yaoundé, Cameroon. Roland N. Ndip, Department of Biochemistry and Microbiology, Faculty of Science and Agriculture, University of Fort Hare, Alice, Republic of South Africa, A. Tassy Prosperi, Department of International Health, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD. Donald S. Burke, Department of Epidemiology, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA. Nathan D. Wolfe, Global Viral Forecasting Initiative, San Francisco, CA and Program in Human Biology, Stanford University, Stanford, CA, E-mail: nwolfe@gvfi.org.

Reprints requests: Nathan D. Wolfe, Global Viral Forecasting Initiative, San Francisco, CA and Program in Human Biology, Stanford University, Stanford, CA, 94305.

REFERENCES


