LEPTOSPIROSIS AND EBOLA VIRUS INFECTION IN FIVE GOLD-PANNING VILLAGES IN NORTHEASTERN GABON

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Abstract. An exhaustive epidemiologic and serologic survey was carried out in five gold-panning villages situated in northeastern Gabon to estimate the degree of exposure to leptospirosis and Ebola virus. The seroprevalence was 15.7% for leptospirosis and 10.2% for Ebola virus. Sixty years after the last seroepidemiologic survey of leptospirosis in Gabon, this study demonstrates the persistence of this infection among the endemic population and the need to consider it as a potential cause of hemorrhagic fever in Gabon. There was no significant statistical correlation between the serologic status of populations exposed to both infectious agents, indicating the lack of common risk factors for these diseases.

The humid and tropical climate in Gabon provides an ideal environment for the propagation of leptospirosis and Ebola viruses, both of which can cause lethal hemorrhagic fevers. Leptospirosis is an anthropozoonosis caused by a spirochete of the Leptospira type, a bacterium that has a variety of mammalian hosts, particularly rodents. It is transmitted to humans by direct or indirect contact with infected animals and their urine. It is a frequent cause of pathology in tropical zones, but has only rarely been described in Africa.1,2 Leptospirosis was well established in Gabon in the mid 1930s, as shown by the results of an epidemiologic survey,3 but appears thereafter to have become dormant, until recognized in several Europeans returning from Gabon in 1994 and 1995.4,5 This raised questions as to its endemic status at the present time.

The Ebola virus is endemic to central Africa,6–11 occurring in isolated epidemics throughout the region,12–14 specifically on three occasions between 1994 and 1996 in Gabon.15,16 Several strains of the virus have been characterized, some of which may cause a clinically atypical disease in an as yet unknown proportion of cases.17–19 The primary mode of infection of Ebola virus has yet to be elucidated, as has also the natural reservoir of the virus, although bats, insects, and primates have been postulated. Once the disease is established in a population, its mode of secondary transmission has been well documented. Rodents are known to transmit leptospirosis. Their role as a potential reservoir or vector for Ebola has also been suggested.

While carrying out a retrospective epidemiologic survey at the site of a recent epidemic of Ebola virus fever in Gabon, we expanded the range of the study to include the possible modes of transmission of both diseases, in view of the hypothesis that there is no link between the two diseases for any given population.

MATERIALS AND METHODS

The survey site included five gold-panning villages along the Nouna River in a remote forest area of the province of Ogooué-Ivindo in northeastern Gabon (Figure 1). At the beginning of 1995, these villages were at the center of a hemorrhagic fever epidemic caused by Ebola virus; nine deaths occurred in 19 registered cases in a population of 350 inhabitants.15 The study was reviewed and approved by the Ethical Committee of the Centre International de Recherches Médicales de Franceville (CIRMF), which was composed of scientists and representatives of the Gabonese Ministry of Health. All subjects four years of age and older who gave informed consent (that of the parents for the children) were included in the study. Between January 24 and February 4, 1996, two doctors and one nurse collected data on age, sex, nationality, ethnic group, occupation, history of clinical disease during the last 12 months, and a history of contacts they had with victims of the last Ebola virus fever epidemic. An individual in contact was defined as a subject having nursed or transported a victim. Venous blood samples were taken from the antecubital vein, and serum was separated and stored at −60°C before being transported to the laboratories of the CIRMF where all analyses were carried out.

Volunteers for this survey were also vaccinated against yellow fever.

Enzyme immunoassays were used to detect anti-Ebola virus antibodies. For IgG detection, antigens were obtained from Vero cells infected with the virus strain Gabon 95-39/3 (CIRMF). We used serum of the first victim of the previous Ebola virus fever outbreak as a positive control. A serum was considered positive for anti-Ebola virus IgG when the optical density (OD) was greater than the mean ± 2 standard deviations of three known negative control IgG samples. Anti-Ebola virus IgM was detected by immunocapture assay.20

Anti-Leptospira antibodies were detected by macroagglutination on slides (Leptospira antigen TR®; Sanofi-Pasteur, Marne-la-Coquette, France). This assay detects a thermostable antigen expressed by Leptospira biflexa patoc. It is highly sensitive for the Leptospira group but not specific for a given serotype.21

Data were collected and analyzed with the Epi-Info 6 program (Centers for Disease Control and Prevention, Atlanta, GA). Any differences were considered statistically significant for a 5% alpha risk and the relative risks were calculated with a confidence interval of 95%.

RESULTS

Two hundred thirty-six subjects between four and 73 years of age participated in this survey (mean ± SD age = 33.5
 ± 12 years). Sample sizes for each age group were 14 for those 4–13 years old, 25 for those 14–23 years old, 89 for those 24–33 years old, 61 for those 34–43 years old, 32 for those 44–53 years old, 13 for those 54–63 years old, and two for those 64–73 years old. The sex ratio (M:F) was 2.03. Two hundred one (85.2%) were Gabonese, 17 (7.2%) were born elsewhere in central Africa, and 18 (7.6%) came from West Africa. One hundred sixteen (49.1%) belonged to the Bakouele ethnic group, 57 (24.2%) were Bakota, and the remaining 63 (26.7%) belonged to 23 different ethnic groups. One hundred thirty-three subjects (56.4%) were gold-panners and 36 (15.2%) were traders; the rest of the population included 33 housewives (14.0%), nine school-children (3.4%), eight fishermen (3.4%), eight farmers (3.4%), and nine subjects (3.8%) were unemployed. All fishermen and farmers were Bakouele. Eight individuals (6.9%) of this ethnic group were traders and 62 (53.4%) gold-panners. Among the Bakota, 41 (71.9%) were gold-panners and four (7.1%) were traders. Individuals from the other groups were gold-panners (30 subjects, 47.6%) or traders (23, 36.5%). All individuals reported fever and diarrhea at least once in a one-year period; none reported hemorrhagic symptoms.

Tests for detecting anti-<em>Leptospira</em> antibodies was carried out on 235 sera; 37 (15.7%) of which were positive. The average age of leptospirosis-seropositive subjects (31.9 years) was not different from that of seronegative subjects (33.9 years) (Kruskal Wallis test value = 0.477, <i>P</i> = 0.48). The youngest seropositive subject was seven years old and the oldest was 60 years old (Figure 2). Sixteen of the 78
women tested (20.5%) were seropositive compared with 21 of 157 men (13.1%), but this difference was not statistically significant ($\chi^2 = 2.00, P = 0.15$). There was a significant difference in rates of leptospirosis seroprevalence between the ethnic groups: 7.0% (4 of 57) among the Bakotas, 21.5% (25 of 116) among the Bakoueles, and 12.9% (8 of 62) among the other ethnic groups ($\chi^2 = 6.59, P < 0.05$). The seroprevalence rates were different with respect to the subject’s occupation: 19 of 132 gold-panners, three of 36 traders, five of 33 housewives, three of nine school children, two of eight farmers, and four of nine unemployed were leptospirosis seropositive. None of the eight fishermen were seropositive (Figure 3). The size of the sample was too small to determine if this observed difference arose by chance.

All the sera were tested for the presence of anti-Ebola virus antibodies. None of them contained anti-Ebola virus IgM, but 24 (10.2%) were positive for anti-Ebola virus IgG with an OD between 310 and 2,660 (Table 1). Only one victim of the epidemic was still on site; the OD of his serum was 1,110. Fifty-six subjects (23.7%) said that they had been in contact with the victims of the Ebola virus fever epidemic of 1995.

The average age of Ebola virus-seropositive subjects (34.6 years) was not significantly different from that of seronegative subjects (33.4 years) (Kruskal Wallis test value = 0.018, $P = 0.89$). The youngest Ebola virus-seropositive subject was 18 years old and the oldest was 61 years old (Figure 2). Six of the 78 women tested (7.7%) were seropositive for Ebola virus compared with 18 of 158 men (11.4%), but this difference was not statistically significant ($\chi^2 = 0.78, P = 0.37$). A total of 15.8% (9 of 57) among the Bakotas, 9.5% (11 of 116) among the Bakoueles, and 6.3% (4 of 63) among the other ethnic groups were Ebola virus seropositive; the difference observed between these percentages was not statistically significant ($\chi^2 = 3.04, P > 0.05$).

The risk of being seropositive for Ebola virus was three times greater for the 56 subjects (23.7%) who reported being in contact with victims of the 1995 Ebola virus fever epidemic (relative risk = 3.21, 95% confidence limit = 1.53–6.75).

The potential contamination sites and dates of presence on these sites were known for 217 subjects. At the time of the epidemic in 1995, 152 (70.0%) were on site. Among them, 19 (12.5%) were IgG positive compared with three (4.6%) of the 65 who were not (difference not significant; $\chi^2 = 3.11, P = 0.07$). For the part of the population that had not been in contact with victims of the epidemic, Ebola virus seroprevalence rates were lower: 8.2% (8 of 97) for those subjects on site and 3.7% (3 of 81) for those not present, but the difference was not significant ($\chi^2 = 1.57, P = 0.2$).

Ebola virus seroprevalence rates also differed with respect to the subject’s occupation: 11.3% (15 of 33) for the gold-

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**Table 1**

Sociodemographic characteristics and history of previous contacts with victims of the Ebola virus fever epidemic of 1995 for 24 subjects seropositive for Ebola virus in Gabon in 1996*

<table>
<thead>
<tr>
<th>Serum No.</th>
<th>OD</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Ethnic group</th>
<th>Occupation</th>
<th>Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>52</td>
<td>310</td>
<td>M</td>
<td>31</td>
<td>Bakouele</td>
<td>Fisherman</td>
<td>Y</td>
</tr>
<tr>
<td>166</td>
<td>310</td>
<td>M</td>
<td>29</td>
<td>Bakota</td>
<td>Gold-panner</td>
<td>Y</td>
</tr>
<tr>
<td>180</td>
<td>320</td>
<td>M</td>
<td>23</td>
<td>Bakouele</td>
<td>Trader</td>
<td>N</td>
</tr>
<tr>
<td>50</td>
<td>340</td>
<td>F</td>
<td>46</td>
<td>Bakouele</td>
<td>Farmer</td>
<td>N</td>
</tr>
<tr>
<td>53</td>
<td>350</td>
<td>F</td>
<td>28</td>
<td>Bakota</td>
<td>Housewife</td>
<td>N</td>
</tr>
<tr>
<td>146</td>
<td>360</td>
<td>M</td>
<td>26</td>
<td>Bakouele</td>
<td>Trader</td>
<td>N</td>
</tr>
<tr>
<td>20</td>
<td>380</td>
<td>M</td>
<td>37</td>
<td>Sake</td>
<td>Gold-panner</td>
<td>N</td>
</tr>
<tr>
<td>68</td>
<td>450</td>
<td>M</td>
<td>40</td>
<td>Bakouele</td>
<td>Gold-panner</td>
<td>Y</td>
</tr>
<tr>
<td>98</td>
<td>490</td>
<td>F</td>
<td>31</td>
<td>Bakouele</td>
<td>Gold-panner</td>
<td>N</td>
</tr>
<tr>
<td>66</td>
<td>500</td>
<td>M</td>
<td>28</td>
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<td>Gold-panner</td>
<td>Y</td>
</tr>
<tr>
<td>118</td>
<td>520</td>
<td>M</td>
<td>54</td>
<td>Trader</td>
<td></td>
<td>N</td>
</tr>
<tr>
<td>138</td>
<td>610</td>
<td>M</td>
<td>28</td>
<td></td>
<td>Gold-panner</td>
<td>N</td>
</tr>
<tr>
<td>74</td>
<td>680</td>
<td>M</td>
<td>18</td>
<td>Bakouele</td>
<td>Gold-panner</td>
<td>N</td>
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<tr>
<td>54</td>
<td>700</td>
<td>M</td>
<td>61</td>
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<td>Fisherman</td>
<td>Y</td>
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<tr>
<td>86</td>
<td>730</td>
<td>F</td>
<td>26</td>
<td>Bakota</td>
<td>Housewife</td>
<td>Y</td>
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<tr>
<td>97</td>
<td>940</td>
<td>F</td>
<td>36</td>
<td>Bakouele</td>
<td>Gold-panner</td>
<td>N</td>
</tr>
<tr>
<td>90</td>
<td>1,110</td>
<td>M</td>
<td>30</td>
<td>Bakota</td>
<td>Gold-panner</td>
<td>Y</td>
</tr>
<tr>
<td>2</td>
<td>1,130</td>
<td>M</td>
<td>24</td>
<td>Sake</td>
<td>Gold-panner</td>
<td>N</td>
</tr>
<tr>
<td>85</td>
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<td>M</td>
<td>32</td>
<td>Bakota</td>
<td>Gold-panner</td>
<td>Y</td>
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<td>Bakouele</td>
<td>Gold-panner</td>
<td>Y</td>
</tr>
<tr>
<td>30</td>
<td>1,480</td>
<td>M</td>
<td>46</td>
<td>Bakota</td>
<td>Gold-panner</td>
<td>Y</td>
</tr>
<tr>
<td>102</td>
<td>2,630</td>
<td>F</td>
<td>36</td>
<td>Mahongue</td>
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<td>Y</td>
</tr>
<tr>
<td>78</td>
<td>2,650</td>
<td>M</td>
<td>48</td>
<td>Bakota</td>
<td>Gold-panner</td>
<td>N</td>
</tr>
<tr>
<td>31</td>
<td>2,660</td>
<td>M</td>
<td>39</td>
<td>Bakota</td>
<td>Gold-panner</td>
<td>N</td>
</tr>
</tbody>
</table>

* OD = optical density. Italics indicate subjects absent during the epidemic.
† West Africans.
panners, 8.3% (3 of 36) for the traders, 9.1% (3 of 33) for the housewives, 25% (2 of 8) for the fishermen, and 12.5% (1 of 8) for the farmers. None of the nine school children and none of the nine unemployed individuals were Ebola virus seropositive (Figure 3).

Fishermen had the greatest seroprevalence for Ebola virus (25%), but this rate was not significantly different of that of the others ($\chi^2 = 0.41$, degrees of freedom = 1). Three of these eight fishermen had been in contact with sick persons as they evacuated them in their boats to the nearest dispensary, which was two days travel time from the site of the epidemic. Among the 180 subjects having no contact, farmers had the greatest seroprevalence (12.5%) but the difference was not significant compared with the remaining population ($P = 0.4$, by Fisher's exact test).

There was no statistical association between the serologic status for leptospirosis and that for the Ebola virus: among the 37 leptospirosis-seropositive individuals, five were also positive for Ebola virus compared with 19 among the 198 leptospirosis-seronegative individuals (Yates' corrected $\chi^2 = 0.18$, $P = 0.67$). When only the 180 subjects who had not been in contact with cases of Ebola virus fever and who were unlikely to be cases of secondary contamination were considered, no significant association between the two serologic status appeared: four Ebola virus seropositive among the 35 leptospirosis serositive compared with eight Ebola virus seropositive among the 145 leptospirosis seronegative ($P = 0.18$, by Fisher's exact test). Among people with direct exposure to Ebola virus, two (3.6%) of 55 were leptospirosis seropositive compared with 35 (19.4%) of 180 among those without contact ($\chi^2 = 7.93$, $P < 0.01$). This relationship disappeared after stratification by occupation.

**DISCUSSION**

The villages surveyed were remote, isolated communities, with the provincial capital being several days journey by boat, and their economy was entirely dependent on gold-panning. The villages resembled temporary encampments with almost no sanitary facilities. Unlike traditional Gabonese villages, there were few domestic animals, particularly dogs. Any food supplies from outside the encampments arrived only sporadically, and diet was supplemented by hunting and fishing. Gold-bearing concessions are exploited by craftsmen and their families. Because of a total lack of machinery, excavation work is carried out manually in stagnant muddy water. Thus, the study population is obviously not representative of the general population living elsewhere in the province of the Ogooué-Ivindo. Men make up the majority of the community, with children younger than 15 years and foreigners being over-represented (14.8% versus 4.4% for the province as a whole). Half of the foreigners come from West Africa and are traders. Gold-panning is the main occupation of more than half of the subjects, but is also a secondary activity for a large part of the rest of the population, especially for those families registered as gold-panners and unemployed people. The nine children did not usually live on the site because there was no school available.

Studies of Van Riel in the Belgian Congo in 1946 have already emphasized the high prevalence of leptospirosis among workers on gold-bearing sites.\(^2\) Thus, we expected to find similar results on the present site because of a similar tropical climate, repeated contacts of the population with stagnant water, the lack of hygiene, and the proximity to rodents. Leptospirosis has not been widely studied in Africa and there are few data on seroprevalence in the general population: 33% in humid forest areas in Ghana in 1986\(^2\) and 18% among volunteers in Nigeria in 1991.\(^2\) In central Africa, the last focus of leptospirosis appears to have been described in Cameroon in 1976 when an epidemic caused 95 cases, including 63 serologically confirmed cases, within two years.\(^2\) Sixty years after the last survey of leptospirosis seroprevalence in Gabon, our study emphasizes the persistence of this endemic disease.

The epidemiology of leptospirosis is well-known. Generally, poor living conditions and exposure to contaminated water and rodents may explain why there is no difference in the rates of prevalence according to sex and age. Infection can occur at an early age, at least from seven years onwards. The seroprevalence of leptospirosis is three times higher among the Bakoueules than the Bakotas. Both ethnic groups are closely related to each other culturally and geographically, but the Bakoueules are traditionally boatmen and fishermen while the Bakotas are traditionally hunters. However, there are insufficient data to determine statistically whether occupation has an influence on the seroprevalence of leptospirosis. Fishermen working from boats were less exposed than the others. The fact that more than 20% of the children less than 13 years of age and 30% of those of school age but who did not usually live on the survey sites were leptospirosis seropositive indicates that gold-panning is not the main risk factor for this disease.

The primary aim of the survey was to demonstrate the existence of the exposure to leptospirosis in the population. To achieve this, we used a macroagglutination test that was highly sensitive but not specific for a given species of the bacterium causing this disease. However, it will be necessary to carry out a second test to determine the precise nature of the infecting agent since clinical expression can differ according to the species concerned and some of them do not cause clinical symptoms, especially in Africa.\(^2\) Data concerning the subject's history of clinical disease were useless because in this particular context, pain, fever, and diarrhea are very common and even elementary diagnoses are not made. Minor hemorrhagic symptoms can go unnoticed. Nevertheless, it is worth noting that the first leptospirosis epidemic in Cameroon in 1975 was initially considered an epidemic of yellow fever.\(^2\) Patients presented with clinical syndromes associated with alga (in 84% of the cases), fever (79%), conjunctival hyperemia (53%), and hemorrhaging (hematuria bloody diarrhea, hematemesis subconjunctival hemorrhages) in 20% of the cases, with a mortality rate of 3.2%. One year later, the Ebola virus was isolated following an epidemic of hemorrhagic fever in Sudan, and the association of similar clinical symptoms in African subjects now appears evocative of such an etiology.\(^2\)

The seroprevalence of the Ebola virus in the gold-panning villages along the Nouna River is similar to previous findings reported elsewhere in tropical forest areas of Central Africa without periods of acute epidemics.\(^9,10,26\) However, under similar epidemiologic conditions, Gonzales and others in 1989 reported an Ebola virus fever seroprevalence two times
higher in the capital of the same province, 150 km from the Nouna area.7 In this last survey, an indirect immunofluorescent assay was used with reference strains from Zaire and Sudan, which may be less specific than the ELISA used in this study. The lack of any increase in seroprevalence just one year after an epidemic can be partially explained by modifications in population structure. The number of inhabitants decreased by one-third after the outbreak and has been altered by the recent arrival of 70 new inhabitants.

Among the Ebola virus-seropositive subjects, two had been present in the area and the OD of their serum was very high (> 2,000), and two others, natives of Ogoué-Ivindo, had not been present in the area at the time and had had no contact with sick persons. Thus, the presence or absence on site, with or without contact with sick persons, at the time of the epidemic does not influence the Ebola virus seroprevalence. These facts favor the continual presence of the Ebola virus among the population of the province.

The influence of gender on Ebola virus seroprevalence has not yet been defined. Some investigators have shown a relationship,9,12 whereas others have not.7,26 The role of age is as uncertain; some have demonstrated the role,9,26 whereas others have not.7 Previous findings have also shown a relationship between serologic status and ethnic group.9,26 In our survey, serologic status was independent of sex, age, and ethnic group. The epidemic context, with its high incidence of disease, has been able to even out differences observed elsewhere in the absence of any clinical symptoms. Moreover, it is known that the various strains of the virus possess different structures and degrees of pathogenicity;27 thus, we may infer that some of their epidemiologic characteristics could also be different. Fishermen seem to be more frequently infected but they may also have had more contact with the disease since they evacuated sick individuals in their boats. When we considered only those subjects who had no contact, farmers were the most frequently infected. It is possible that clearing the forest to create plantations puts them in more frequent contact with the virus; however, neither of these two categories of population was significantly more infected than the remaining groups. Thus, the mode of infection does not appear to be linked with occupation.

The recent, repeated epidemics of Ebola virus fever in central Africa have caused a problem in the implementation of specific epidemiologic control measures. It appears important, therefore, when considering the occurrence of clustered cases of hemorrhagic fevers, to include the possibility of leptospirosis, a bacterial infection that seemed to have disappeared from Gabon, but which in fact remains endemic. We observed a relationship between the serologic status for leptospirosis and a previous history of contact with victims of the Ebola virus fever outbreak; however, occupation is certainly a confounding factor. Some occupations that increased the risk of contact with infected individuals play a less important role in leptospirosis (fishermen). Moreover, this relationship disappeared after stratification by occupation. The serologic status of individuals for leptospirosis and for Ebola virus, particularly among subjects who are unlikely to have been secondarily infected by an patient infected with Ebola virus, do not appear to be linked. This emphasizes the lack of common risk factors for both infections since rodents are apparently not implicated in the epidemiology of the Ebola virus.

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