KALA-AZAR IN A HIGH TRANSMISSION FOCUS: AN ETHNIC AND GEOGRAPHIC DIMENSION


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Abstract. In 1994–1996, we studied a group of 58 game wardens stationed in an area known to be highly endemic for visceral leishmaniasis (kala-azar) for evidence of infection with Leishmania donovani. Leishmania DNA was detected by the polymerase chain reaction in the peripheral blood of cases of active kala-azar, former patients with visceral leishmaniasis, patients, and asymptomatic subjects. Using the cloned antigen rk39, antibodies were detected in 44.2% of the game wardens while leishmanin skin test result was positive in 77% of our sample. It was shown that certain tribes from northern Sudan were more likely to develop subclinical infections, while those of the Baria tribe from southern Sudan and those of the Nuba tribe from western Sudan were more likely to develop visceral leishmaniasis. Whether this is due to genetic factors or previous exposure to Leishmania parasites remains to be elucidated.

Dinder National Park is a game reserve and one of the last remaining havens of wild life in northern Sudan. In the past few years, increasing attention has been directed towards Dinder National Park following reports of visceral leishmaniasis (VL) (kala-azar) infection, even after short visits to the park. Kadaru reported 4 veterinary students who visited the park for a week and developed VL 1 week after returning to Khartoum, where there is no transmission of VL.

The disease has been particularly associated with game wardens recruited from southern Sudan who became infected with VL in 2 outbreaks (1988–1989 and 1994–1995) following their transfer from southern Sudan to Dinder National park. There is no leishmaniasis in western Equatoria Province in southern Sudan where the last group of wardens came from and were therefore presumably non-immune. The area directly north of the park along the Rahad River contain several human settlements and is endemic for VL. The disease is well documented in that area where we have been conducting a longitudinal study since 1990,2,3 In 1994–1995, we studied a group of game wardens who stayed in the park for various periods of time for prevalence of VL using several diagnostic methods. Our findings indicate that there is intense transmission in the park, and that certain tribal groups tend to develop frank VL more than others.

MATERIALS AND METHODS

Study area. Dinder National Park has been a protected game reserve since the middle of this century. It occupies the area between the Dinder and Rahad rivers, two small seasonal tributaries of the Blue Nile (Figure 1).

Subjects. The total number of game wardens working in the area is approximately 300, including 50 wardens recently transferred from southern Sudan, mainly from Equatoria province. Fifty-eight game wardens who spent most of the previous year in the park were studied after obtaining their informed consent in a cross-sectional survey. A prospective study was later carried out for those with leishmanin-negative results. This study was approved by the Ethical Committee of the Institute of Endemic Diseases of the University of Khartoum and the National Ethics Committee of Sudan.

Clinical history and examination. A detailed clinical history was obtained. Particular emphasis was made regarding previous or any form of leishmaniasis. Subjects were questioned about their ethnic and geographic origin and were examined for clinical manifestations of VL. In those suspected of having VL, a lymph node and/or bone marrow aspiration was performed and slides were stained with Giemsa for demonstration of Leishmania parasites. A peripheral blood film was examined for malaria parasites.

Leishmanin skin test. A leishmanin skin test was done by the intradermal injection of 0.1 ml of Leishmania infantum antigen (Dr. M. Gramiccia, Instituto Superiore di Sanita, Rome Italy) on the volar aspect of the right forearm. Controls consisted of 0.1 ml of diluent injected at least 10 cm away from the test antigen. The injection site was examined after 48–72 hr and induration was assessed using the ball-point pen method. A reaction with an induration > 5 mm in the absence of induration in the control site was considered positive.

Enzyme-linked immunosorbent assay. Detection of antibodies to Leishmania was carried out using a standard ELISA with k39, a cloned L. chagasi antigen (prepared in the laboratory of Dr. Steve Reed, Corixa Corp., Seattle, WA) on eluates of blood collected on Whatman (Maidstone, United Kingdom) no. 3 filter paper. An optical density > 0.13 was regarded as positive.

Polymerase chain reaction (PCR) and hybridization with an L. donovani-specific probe. Extraction of DNA from filter paper was performed on peripheral blood, lymph node, or bone marrow aspirates as described by Meredith and others. The PCR was carried out using genus-specific diagnostic primers AS3 and DB8: AS3, 5’-GGGGTTGGT-GTAGGGC-3’, DB8 5’-CCAGTTCCCGCCCGG-3’. Forty microliter reaction volumes consisting of 4 μl of PCR buffer (Promega, Madison, WI), 2 μl of 10 mM MgCl₂, 0.1 mM of each Dntp, 100 pmol of each primer and 1 unit of Taq DNA polymerase were subjected to 35 rounds of amplification (annealing at 60°C for 1 min, extension at 72°C for 2 min, and denaturation at 94°C for 30 sec) in a Perkin-Elmer (Norwalk, CT) thermal cycler and subsequently subjected to
electrophoresis on a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV illumination. Ten of the positive PCRs were confirmed by Southern blotting and subsequent hybridization with *L. donovani*-specific probe B4Rs.

**Follow-up of the leishmanin-negative subjects.** Nine of 13 leishmanin-negative individuals identified in the first survey were available for follow-up study, which consisted of a clinical examination and a repetition of the leishmanin test. The results showed that 3 developed VL during the follow-up period. Five leishmanin-negative individuals converted and became leishmanin-positive when examined in December 1996 without showing symptoms of kala-azar (Table 1).

**Visceral leishmaniasis in the various tribal groups.** A disproportionately high number of kala-azar cases were noted in 2 ethnic groups within the game wardens: the Nuba from western Sudan and the Nilotics from southern Sudan.

**Results of clinical examination, skin test, serology, and PCR.** At the time we conducted our surveys, the 58 subjects were clinically healthy showing no evidence of VL, except for 1 subject who was diagnosed parasitologically as having VL on subsequent examination at Soba Teaching Hospital. Eight subjects had a history of VL. One subject contracted the diseases 9 years ago in the park while the other 7 became ill from 1993 to 1994. They were recruits transferred to the park from Equatoria Province. Only 3 individuals from north Sudan reported a history of cutaneous leishmaniasis.

A positive leishmanin skin test result was obtained in 77% of all game wardens, irrespective of their time spent in the park. However, the percentage of leishmanin-positive individuals among those who stayed less than a year in the park (18 individuals) was 28.4% compared with 85.4% for the 40 individuals who stayed more than a year in the area. The difference in the rate of leishmanin positivity between those who stayed for a year or more and those who stayed for less than a year was highly significant (*P* = 0.00002).

Antibodies against the rk39 leishmanial antigen were detected by the ELISA in 44.2% of those tested (n = 52), again indicating intense transmission in the area. However, there was no significant difference between those who spent more than a year and those who spent less than a year in the park (*P* = 0.4149).

*Leishmania* DNA in the peripheral blood was detected by the PCR in 27.4% of the game wardens tested (n = 51). The identity of parasite was assigned to the *L. donovani* complex by the size of the PCR product (800 basepairs) and by subsequent hybridization of a subset of 10 of these products to the *L. donovani*-specific probe B4Rs.

**Follow-up (1 year).** Ten of the 13 leishmanin-negative individuals identified in the first survey were available for follow-up study, which consisted of a clinical examination and a repetition of the leishmanin test. The results showed that 3 developed VL during the follow-up period. Five leishmanin-negative individuals converted and became leishmanin-positive when examined in December 1996 without showing symptoms of kala-azar (Table 1).

**Table 1: Results of clinical examination, skin test, serology, and PCR**

<table>
<thead>
<tr>
<th>Ethnic group</th>
<th>VL†</th>
<th>K39†</th>
<th>Leishmanin†</th>
<th>PCR†</th>
<th>Follow-up (1 year)</th>
<th>Hospital records of VL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuba</td>
<td>2 (11)</td>
<td>6 (9)</td>
<td>7 (11)</td>
<td>1 (11)</td>
<td>2 (2)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Southerners</td>
<td>4 (14)</td>
<td>6 (12)</td>
<td>10 (14)</td>
<td>4 (9)</td>
<td>1 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Others</td>
<td>2 (33)</td>
<td>11 (31)</td>
<td>27 (32)</td>
<td>9 (31)</td>
<td>0 (4)</td>
<td>3 (4)</td>
</tr>
<tr>
<td><em>P</em> value</td>
<td>0.057</td>
<td>0.10</td>
<td>0.12</td>
<td>0.50</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Total</td>
<td>8 (58)</td>
<td>23 (52)</td>
<td>44 (57)</td>
<td>14 (51)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>%</td>
<td>13</td>
<td>44.2</td>
<td>77</td>
<td>27.4</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

† The cross-sectional survey includes the identification of cases of visceral leishmaniasis (VL) serology, leishmanin, and polymerase chain reaction (PCR). The *P* value (by Fisher’s exact test) for the association of the different parameters of infection with the main subdivisions of the game wardens into Southerners/Nuba and others is shown for the cross-sectional study. The follow-up data consist of leishmanin testing and identification of VL cases while the retrospective data consist only of hospital records of VL. The total positivity for each parameter and the percentage are shown in the bottom rows. Denominators are in parentheses. NA = not applicable.

† The denominator is the total number of game wardens in the area (approximately 300).
Between 1993 and 1995, 49 patients, most with parasitologically proven VL (27 from southern Sudan and 9 from the Nuba Mountains), were admitted to hospitals in Khartoum (Table 1). Eighteen of these patients died either before or during treatment. The other 31 patients were successfully treated and discharged in good health. Detailed information on the ethnicity of the individuals is not available because the ward administration does not keep such records. In the first year of the follow-up, of 5 leishmanin-negative individuals of the Nuba/Nilotic group, 3 developed VL. Excluding hospital records, these 2 groups (Nuba and Southern Nilotics) accounted for 36.3% and 45.4%, respectively, of the VL cases, although they represented only 19% and 24%, respectively, of the total sample of 58 individuals. In our cross-sectional survey, association of the disease with the 2 main ethnic groups was not significant at a 95% confidence limit (P = 0.057, by Fisher’s exact test). However, at the end of the study, a marked association between a higher rate of disease and the Nuba/Nilotic group was observed (relative risk = 5.9, P = 0.005, by Fisher’s exact test). There was no significant difference between the 2 groups for other infection parameters shown in Table 1.

**DISCUSSION**

Our data show that there is a high degree of transmission of *L. donovani* in the Dinder National Park in eastern Sudan. This conclusion is based on the prevalence of disease and the results of serology and the PCR among game wardens, as well as the results of the leishmanin skin test. Indirect evidence of ongoing transmission was deduced from the high percentage of the leishmanin-positive game wardens among those who stayed more than a year in the park compared with those who stayed for less than 1 year. Direct evidence for active and intensive transmission of *L. donovani* was seen in our follow-up data; 8 of 9 of the follow-up subjects showed conversion to leishmanin positivity and died. Using the PCR, we were also able to detect *Leishmania* DNA in the peripheral blood of game wardens who displayed a wide clinical spectrum. These included both leishmanin-negative and leishmanin-positive healthy individuals, active kala-azar patients, and previous kala-azar patients who had been successfully treated 8 months before. In 12 of 14 of the cases, the parasite involved was shown to belong to the *L. donovani* complex by the size of the amplified minicircle product following the PCR and subsequent Southern blotting using the B4Rsa *L. donovani*-specific probe.

Our data strongly suggest that humans can serve as reservoirs for *Leishmania* infection since significant numbers of asymptomatic carriers were observed. *Leishmania* parasites, particularly *L. donovani*, were shown by the PCR to circulate in the peripheral blood of healthy individuals as well as in previous kala-azar patients. This has also been shown by Schaefer and others’ using the PCR in a sample of kala-azar patients, individuals with subclinical infections, and healthy controls in the Baringo District in Kenya. Since the study area is a game reserve with rich Acacia habitat and few human settlements, and a strong association between kala-azar in Sudan with wild animals and sylvatic fauna has been reported by Hoogstraal and Heyneman, a possible role for wild animals acting as reservoirs deserves further study.

The most striking finding in this study is the marked vulnerability of 2 ethnic groups (Nilotic Baria and Nuba) to VL in the park. Mortality and morbidity due to VL in these tribal groups was comparable only with the high level of mortality among the Nilotic Nuer in the Upper Nile province in southern Sudan. An epidemic of VL in southern Sudan has moved northward into western Sudan with less grave consequences. This difference has been attributed to the difference in nutritional status between the nomadic Bagara of western Sudan and the famine-stricken Nuer tribe. However, this was unlikely in the game warden since they shared the same living conditions and diet.

With few exceptions, the question of genetic predisposition to VL in relation to the epidemiology of disease has not been adequately addressed. In trying to explain our results, two possibilities arise. The first is that the susceptibility of the 2 groups may reflect a lack of immunologic exposure to *Leishmania* antigens, especially among the Baria tribe who inhabit an area where leishmaniasis is not present. It has been shown that previous infection with *L. major* may confer protection against *L. donovani*. *Leishmania major* is known to be endemic in various parts of Sudan, but not in the southern Sudan. However, previous exposure to only *L. major* does not adequately explain the situation in the park, since there is no evidence that game wardens of other ethnic groups possess a higher rate of leishmanin positivity when they first arrived at the park, in addition to the fact that leishmaniasis is endemic to parts of the Nuba mountains. The second possibility is that differences in susceptibility are due to genetic variation(s) among the different groups. While we believe that the difference in susceptibility to VL among tribal groups is more likely explained by genetic differences in predisposition to disease, other factors cannot be completely ruled out.

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**REFERENCES**


observations on the epidemiology of kala-azar in the eastern and central states of the Sudan. Trop Geogr Med 47: 151–156.


