Onchocerciasis remains an important public health problem throughout much of sub-Saharan Africa. Nigeria is the country whose population is most afflicted by onchocerciasis; however, little is known concerning the epidemiology of onchocerciasis in this country. Previous studies demonstrated that onchocerciasis in West Africa exists in two forms, which differ in their clinical and epidemiologic characteristics. This is believed to be due to the existence of 2 strains of *Onchocerca volvulus*, the causative agent of onchocerciasis. The O-150 polymerase chain reaction has been developed to differentiate these 2 strains, and this method has been used to map the distribution of the blinding and nonblinding strains of *O. volvulus* in Nigeria. The strain distribution is consistent with what is known concerning the ecology and epidemiology of onchocerciasis in this country. The results also suggest that migration may be affecting the historic distribution of the 2 strains of the parasite in Nigeria.

Onchocerciasis is endemic throughout most of sub-Saharan Africa and still represents a major public health problem in many African countries [1]. The causative agent for onchocerciasis, *Onchocerca volvulus*, is endemic throughout a wide belt of Africa, extending from Senegal in the east through Uganda in the west [1]. As a result of at least three factors, Nigeria is the country whose population is most afflicted with onchocerciasis. The first reason is that Nigeria is situated at the center of the area of the world where *O. volvulus* is most common. Second, Nigeria is the most populous country in West Africa. Third, the Onchocerciasis Control Programme (OCP) has succeeded in controlling onchocerciasis as a public health problem throughout much of West Africa [2]; however, for historical reasons, Nigeria was not included in the area covered by the OCP.

For most of this century, it has been recognized that onchocerciasis in West Africa exhibits two distinct epidemiologic and clinical patterns. In the savanna bioclime of West Africa, a blinding form of the disease predominates [3]. Here, blindness resulting from *O. volvulus* infection is common, and the prevalence of onchocercal ocular disease is linearly related to the intensity of infection in the community [3]. In areas where the population is afflicted with blinding onchocerciasis, ocular lesions affecting the anterior chamber of the eye, such as scle-rosering keratitis, are the most common clinical manifestation of ocular onchocerciasis. In contrast, in the rain forest areas of West Africa, a nonblinding form of the disease is found. Here onchocercal blindness is rare, and the prevalence of ocular disease is not related to the intensity of infection in the community [4]. Furthermore, the ocular lesions seen in the rain forest usually involve changes in the posterior chamber of the eye [5].

A number of studies have suggested that the differences seen in blinding and nonblinding onchocerciasis may be due to differences in the endemic parasite populations. Strain-specific DNA sequences have been identified [6, 7], and a polymerase chain reaction (PCR)–based assay has been developed that can differentiate *O. volvulus* collected from the forest and savanna areas [8]. This assay is based upon the amplification of a repeated sequence family specific to the genome of parasites of the genus *Onchocerca* (designated the O-150 family); amplification is followed by hybridization of the PCR products with strain-specific DNA probes [8]. In studies done using parasites collected in the OCP area, this assay predicted the pathogenic potential of a given parasite population with a high degree of accuracy [9].

Little is known concerning the distribution of *O. volvulus* strains in Nigeria despite the fact onchocerciasis is more endemic there than anywhere else in the world. Herein, we report on the use of the O-150 PCR assay to classify parasite isolates collected from several sites throughout Nigeria.

**Materials and Methods**

Skin-snip samples were collected, following standard procedures, from persons residing in 12 onchocerciasis-endemic villages throughout Nigeria [10]. As described above, the 2 parasite strains differ in their ability to induce the pathologic manifestations of onchocerciasis. To ensure that no biases were introduced into the
sampling process, we collected samples from individuals without regard to the presence or absence of clinical indications of onchocerciasis. The collection data are summarized in table 1.

The skin snip samples were placed in isopropanol and stored at ambient temperature. DNA was prepared from the skin snips as previously described [8]. O-150 PCR was done as previously described [11] to amplify the parasite DNA in the samples. In brief, the purified DNA was used as a template to amplify the O-150 repeat sequence, using the primers 5'-GATTYTTCCGRGAA-3' and 5'-GCNRTTRATCTATATC-3', where N = A, G, C, or T; R = A or G; and Y = C or T. Reactions consisted of 25 1-min cycles at 95°C, 2 min at 37°C, and 30 s at 72°C followed by a 7-min extension at 72°C in a solution containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 6 mM MgCl2 0.01% gelatin, 200 μM each dNTP, 0.5 μM each primer, and 1.25 U of Taq1 DNA polymerase (Applied Biosystems, Norwalk, CT). After PCR, we separated the products on a 2% agarose gel and transferred them to a nylon membrane. The bound products were classified on the basis of hybridization to the *O. volvulus*-strain specific DNA probes pFS-1 and pSS-1BT as previously described [9].

Results

Of the 261 samples included in this study, 126 (48%) produced positive results in the O-150 PCR. The proportion of blinding versus nonblinding parasite isolates found in each village is summarized in figure 1. The proportion of nonblinding isolates was greatest in southern Nigeria. The proportion of nonblinding parasites decreased markedly north of the Niger Delta area, and in central Nigeria (e.g., Kambre [site 7] in the Akwange local government area [LGA] and Arusu [site 9] in the Kaduna LGA), essentially all of the parasite isolates belonged to the blinding strain.

Previous studies have noted that a relatively large proportion of parasite isolates classified as belonging to the blinding strain (i.e., those that did not hybridize to the nonblinding strain-specific probe pFS-1) were also negative when tested with pSS-1BT, a probe developed from a sequence thought to be specific for the blinding strain of the parasite [7]. To determine whether this observation extended to parasites collected in Nigeria, we tested parasites classified as belonging to the blinding strain (classified on the basis of their hybridization pattern to pFS-1) for their ability to hybridize to pSS-1BT. Parasites from only two sites hybridized to pSS-1BT. In Ezzen Sarki (site 8), 21% of the parasites hybridized to pSS1-BT, while in Arusu (site 9), 69% of the parasite isolates hybridized.

Discussion

The results of this study suggest that the nonblinding strain of *O. volvulus* is found mainly in southern Nigeria, while in central and northern Nigeria, the blinding strain predominates. This pattern of distribution follows the local ecology of the area. Southern Nigeria is a rain forest bioclime, which begins to change to a forest savanna mosaic just north of Enugu (site 1). This finding is consistent with previous studies from other countries in West Africa where nonblinding parasites are generally found in the rain forest regions and blinding parasites predominate in the savanna regions [12].

The strain distribution is also consistent with what is known concerning the epidemiology and clinical presentation of ocular onchocerciasis throughout Nigeria. For example, previous studies conducted in Jago (site 2) have suggested that while this area has a high prevalence of infection with *O. volvulus*, the prevalence of onchoercal blindness is quite low (Ogunrinade A, unpublished data). Furthermore, studies of ocular onchocerciasis in the Enugu LGA have demonstrated that the ma-

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**Table 1.** Collection sites and polymerase chain reaction (PCR) results for skin snip samples from onchocerciasis-endemic villages throughout Nigeria.

<table>
<thead>
<tr>
<th>Site no.</th>
<th>Village name</th>
<th>Local government area</th>
<th>Total no. of snips</th>
<th>Total no. positive (PCR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Enugu</td>
<td>Enugu</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>Jago</td>
<td>Ona-Ara</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>Nibango</td>
<td>Gashaka</td>
<td>29</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>Garbage</td>
<td>Gashaka</td>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>Rubochi</td>
<td>Toto</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>Kwanwai</td>
<td>Bokkos</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>Kambre</td>
<td>Akwange</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>Ezzen Sarki</td>
<td>Nassawara</td>
<td>24</td>
<td>19</td>
</tr>
<tr>
<td>9</td>
<td>Arusu</td>
<td>Kaduna</td>
<td>48</td>
<td>26</td>
</tr>
<tr>
<td>10</td>
<td>New Bussa</td>
<td>Koro</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>Kokona</td>
<td>Kokona</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>Kaduna</td>
<td>Kaduna</td>
<td>48</td>
<td>28</td>
</tr>
</tbody>
</table>
Majority of ocular lesions were localized to the posterior chamber of the eye [13]. This is consistent with the pattern noted in other areas endemic for the nonblinding strain of *O. volvulus* [1].

Previous epidemiologic surveys have suggested that the area surrounding Kaduna (site 12) is endemic for the blinding strain of *O. volvulus* [14]. In villages located just south of Kaduna (e.g., sites 8, 9, and 11), only 1 of 47 parasite isolates examined was classified as belonging to the nonblinding strain. However, 21% of the parasite isolates collected in Kaduna itself (site 12) were classified as belonging to the nonblinding strain. Kaduna is a large city located on one of the major north-south trading routes in Nigeria. It is likely that the high proportion of nonblinding strain parasites seen in Kaduna proper resulted from migration of individuals from southern Nigeria, where this strain is commonly found. This finding underscores the importance of migration in disturbing previously existing patterns of strain distribution for *O. volvulus* in West Africa, an issue of some concern to the OCP in West Africa [15].

Previous studies noted that only a proportion of the parasites classified as belonging to the blinding strain hybridized to the blinding strain probe, pSS-1BT. There were two possible explanations for this finding. The first was that the sequence present in pSS-1BT was specific to a subpopulation of the blinding strain parasites. Alternatively, it was possible that the DNA sequence detected by pSS-1BT was distributed throughout the blinding strain population but is not found in all individuals. In the current study, parasites from only two villages were found to hybridize to pSS-1BT. This suggests that the sequence recognized by pSS-1BT is not evenly distributed throughout the savanna strain population in Nigeria. Furthermore, even in areas where it is found, it is present only in a variable proportion of individuals.

In summary, the classification of parasites from Nigeria using the nonblinding strain-specific DNA probe sequence pFS-1 corresponded well with what is currently known concerning the ecology and epidemiology of onchocerciasis in Nigeria. These results suggest that, similar to what has been found in the countries of the OCP [12], the strain-specific PCR assay may be a useful tool in differentiating blinding and nonblinding *O. volvulus* in Nigeria. Such a tool may prove useful in assisting the various control projects in prioritizing their efforts in Nigeria. The O-150 PCR assay may also prove to be a useful tool for following the effect of migration on the distribution of the 2 *O. volvulus* strains, both in Nigeria and throughout West Africa.

References