Detection of *Onchocerca volvulus* Infection by O-150 Polymerase Chain Reaction Analysis of Skin Scratches

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The standard assay for onchocerciasis diagnosis is microscopic detection of parasites in skin snips. Skin snipping is painful and may potentially transmit bloodborne infections. Thus, an alternative method for the diagnosis of onchocerciasis that does not require skin snipping is needed. A polymerase chain reaction (PCR)–based assay was shown to detect the presence of parasite DNA in superficial skin scrapings. Detection of parasite DNA in both skin snips and skin scrapings was found to be more sensitive for detecting low-density infections than was microscopic examination of skin snips. The skin scratch PCR assay is minimally invasive and painless and does not present the risk of transmitting bloodborne infections. These properties make the skin scratch an attractive alternative to the skin snip for detecting *O. volvulus* infection.

Onchocerciasis, or river blindness, is caused by infection with the filarial parasite *Onchocerca volvulus*. When measured in terms of the socioeconomic effects on afflicted communities, onchocerciasis is one of the most important infectious diseases worldwide [1]. As a result of the large socioeconomic impact of the disease, several international programs underway in Africa and the Americas have the goal to eliminate onchocerciasis as a socioeconomic and public health problem [1, 2].

In any disease control program, it is important to have safe and effective diagnostic methods available. The classical method of detecting *O. volvulus* infection consists of microscopically examining small skin biopsies (skin snips) for the presence of *O. volvulus* microfilariae [3]. However, this method is invasive, painful, and relatively insensitive.

Recent studies have shown that the detection of parasite DNA in extracts prepared from skin snips is more sensitive than microscopy [4]. This method is based on polymerase chain reaction (PCR) amplification of an *O. volvulus*–specific repeated sequence, designated O-150 [5]. However, this procedure still requires the collection of a skin snip. It is known that *O. volvulus* microfilariae reside in the uppermost layers of the dermis [6] and may be detected by skin scarification [7]. We therefore hypothesized that it might be possible to detect *O. volvulus* DNA in the skin of infected persons, without resorting to skin biopsy, by using the O-150 PCR.

**Materials and Methods**

The initial study was conducted in the village of Gnankoradji, Côte d’Ivoire. Gnankoradji is located in the southwestern corner of Côte d’Ivoire in the rain forest zone, and at the time of the study was outside of the Onchocerciasis Control Programme (OCP) control area. In total, 99 children and young adults were enrolled in the study. Children and young adults were chosen because they form the most suitable population for measuring incidence of *O. volvulus* infection in areas subject to control, due to the extremely long life span of the adult parasite [8]. The study population consisted of 55 male and 44 female subjects with a mean age of 8.5 years (range, 2–15). The geometric mean of microfilarial counts in the study population was 0.18 microfilariae per skin snip.

After the Gnankoradji study, similar studies were carried out in four villages in southern Burkina Faso (Habré, Diourao, Zoulo, and Founou). These villages are located in the savanna bioclimate of West Africa and are included within the OCP control zone. The study populations in these villages were similar in age and sex to the population in Gnankoradji. The geometric means of microfilariae counts per snip in these villages were as follows: Founou = 0.0, Zoulo = 0.06, Habré = 0.01, and Diourao = 0.05.

Four samples were obtained from each individual enrolled in the Gnankoradji study. Two of these were skin scratch samples, consisting of bilateral skin scrapings that were collected by scraping the hip area with the blunt edge of a disposable blood lancet. Care was taken to ensure that the skin was not broken during the scraping procedure. Only the superficial layer of the epidermis was removed, so as not to cause erythema at the sample site. The mean weight of each skin scratch sample was 120 μg. Lancets containing the scraped material were placed into a 1.5-
mL microcentrifuge tube and stored dry at ambient temperature. At the beginning of the sample collection procedure, and following the collection of samples from every 10 individuals enrolled in the study, skin scrapings were taken in an identical manner from two US nationals who had never been exposed to *O. volvulus*. Bilateral skin snips were also taken from each individual following standard techniques [3]. The samples were microscopically examined for the presence of microfilariae following a 30-min incubation in distilled water. The skin snip and associated liquid were then placed in a solution consisting of 50 mM EDTA (pH 8.0) and stored at ambient temperature.

DNA was prepared from the skin snips as previously described [9]. To prepare DNA from the skin scratch samples, 50 μL of 10 mM Tris-HCl (pH 8.0) plus 1 mM EDTA was added to the microcentrifuge tube containing the blood lancet, and the tube was mixed to elute the material adhering to the lancet. The liquid in the tube was collected by brief centrifugation, and the DNA was purified from the samples following the protocol developed for skin snips [9].

Skin snip samples were PCR-analyzed as described in detail elsewhere [10]. In brief, DNA purified from the skin snips served as templates to amplify the O-150 repeat sequence, using primers 5'-GATTYTTCCGRCGAAANARC-3' and 5'-GCRNRTRAAT-ATNTGNAATT-3'. Reactions consisted of 25 cycles of 1 min 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, each dNTP at 200 μM, each primer at 0.5 μM, and 1.25 U of Taq 1 DNA polymerase (Applied Biosystems, Norwalk, CT). Skin scratch samples were subjected to the same amplification conditions as the skin snips, with the exception that the number of cycles was increased from 25 to 40.

After PCR, the amplification products were detected by Southern blotting, followed by hybridization with the *O. volvulus*-specific oligonucleotide OVS-2, as previously described [10]. In the Gnankoradji study, 1 set of paired samples from each individual consisting of 1 skin snip and 1 skin scratch was analyzed in the OCP laboratory in Bouake, Côte d’Ivoire, while the second pair was analyzed at University of Alabama at Birmingham to confirm that the technique produced reproducible results in two independent laboratory settings. The subsequent samples collected in the four villages in Burkina Faso were analyzed solely by the OCP laboratory.

Results

The initial study to determine if it was possible to detect parasite DNA in the epidermis of infected persons was carried out in Gnankoradji, Côte d’Ivoire. We discovered that it was possible to amplify the O-150 repeat family from DNA extracted from the majority of the skin scratch samples collected (table 1, bottom). In contrast, all samples collected simultaneously from unexposed persons were negative on PCR amplification (data not shown). Results from the skin scratch PCR assay were compared with those from microscopic and O-150 PCR assays of the bilateral skin snip samples collected from all enrollees. As expected, 100% (12/12) of the skin snips found to contain microfilariae by microscopic examination were positive in the skin snip O-150 PCR assay (table 1, top), giving a calculated sensitivity of 100% relative to the skin snip. However, 85% of persons found to be negative by microscopic examination of the skin snip were positive in the O-150 skin snip PCR assay (table 1, top). Overall, microscopic examination of the skin snip produced an estimated prevalence of infection of 12% in the study population, while the O-150 PCR assay, when performed on DNA isolated from the skin snips, produced a prevalence of 87%.

The results produced from the PCR assay on the DNA prepared from the skin scratch samples were quite similar to those obtained from the PCR assay performed on the skin snip samples. In this case, 11 of the 12 individuals with microscopically detectable microfilariae were positive in the skin scratch PCR assay (table 1, bottom), giving a calculated sensitivity of 92% relative to the skin snip. Furthermore, 77% of those with no microscopically detectable microfilariae were positive in the skin scratch PCR (table 1, bottom). The PCR scratch assay predicted an overall prevalence of infection of 78% in the study population.

Because only a small number of individuals in the study population were negative by the skin scratch PCR, the assay was further evaluated on samples collected from a population residing in Birmingham, where *O. volvulus* is not endemic. None of the 40 skin scratch samples collected from this population produced a positive PCR result. Thus, the calculated specificity of the scratch assay, when tested on a known negative population, was 100%. This is consistent with previous studies, which have also demonstrated a high degree of specificity of the skin snip PCR assay for the detection of *O. volvulus* infection in humans [4].

Similar studies were then conducted on a total of 553 persons from four villages in Burkina Faso. These villages exhibited various prevalences of *O. volvulus* infection as measured by microscopic examination of skin snips and by skin scratch PCR assay (table 2). A linear relationship was found to exist between

| Table 1. Comparison of microscopic examination and the O-150 PCR assays. |
|-----------------|-----------------|-----------------|
| Microscopy result | Snip PCR positive | Snip PCR negative |
| Positive | 12 | 0 |
| Negative | 74 | 13 |

Scratch PCR positive | Scratch PCR negative
<table>
<thead>
<tr>
<th></th>
<th></th>
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<tbody>
<tr>
<td>Positive</td>
<td>11</td>
</tr>
<tr>
<td>Negative</td>
<td>66</td>
</tr>
</tbody>
</table>

Data are no. of samples.
Table 2. *O. volvulus* infection prevalences measured by microscopic examination of skin snips and skin scratch PCR.

<table>
<thead>
<tr>
<th>Country, village</th>
<th>Microfilariae prevalence*</th>
<th>Scratch PCR prevalence¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burkina Faso</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foungou</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Habré</td>
<td>1.2</td>
<td>15.4</td>
</tr>
<tr>
<td>Diourao</td>
<td>2.8</td>
<td>30.8</td>
</tr>
<tr>
<td>Zoulo</td>
<td>8.2</td>
<td>54</td>
</tr>
<tr>
<td>Côte d’Ivoire</td>
<td>13</td>
<td>79</td>
</tr>
<tr>
<td>Gnankoradji</td>
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* By microscopic examination of skin snips. ¹ By PCR analysis of skin scratch samples.

It is possible that the high number of positives seen in our studies were a result of amplification of a sequence similar to the O-150 family present in an undefined organism found on the skin of rural African children. If this was the case, one would expect to find a poor relationship between the prevalence of infection measured by the skin scratch PCR and that measured by microscopy. However, there was a good relationship between the prevalence as measured by skin scratch PCR and that measured by microscopy.

Finally, it is possible that the PCR tests are a much more sensitive method of detecting *O. volvulus* infection than the skin snip. This has previously been documented to be the case for skin snip PCR [4], except in cases where the skin snip is carefully examined for the presence of microfilariae following collagenase digestion [11]. This finding has been corroborated by earlier studies, which show that the microscopic skin snip assay is relatively insensitive for the detection of low-density infections [12, 13].

The results presented above suggest that the skin scratch PCR is capable of detecting low-density infections of *O. volvulus*. However, it is clear that the pathologies associated with *O. volvulus* infection are strongly related to the density of microfilariae in the individual and the intensity of infection in the community as a whole [14]. The contribution that low-density infections make to the intensity of transmission is also unclear. Thus, more research will be needed to determine the potential contribution of low-density infections to transmission and the potential pathogenic burden in communities with endemic *O. volvulus*.

Discussion

The results we present demonstrate that the O-150 PCR assay may be used to detect *O. volvulus* DNA in skin scrapings collected from infected persons. This assay is not as sensitive as performing the O-150 assay directly on skin snips. However, the assay overcomes the disadvantages inherent in the collection of skin snips and is less invasive and painful than even the collection of the fingerprick blood samples needed to carry out serologic assays. Furthermore, since the assay relies on the use of disposable blood lancets, and the integrity of the skin of the individual tested is not breached, the assay carries no risk for the transmission of bloodborne infections. This is in contrast to the skin snip, which requires a relatively expensive sclerodermal punch that needs to be sterilized following sample collection from each individual. The advantages, when taken together, make the skin scratch assay a potentially valuable alternative to assays that rely on the collection of skin snips.

The prevalence of infection measured by either PCR assay was roughly 5-fold greater than that determined by microscopic examination of the skin snips. It is possible that the high number of positives seen in the PCR assay may have been due to cross contamination of the samples. This is unlikely, since negative control samples collected simultaneously showed no evidence of cross contamination. The results presented here, together with those of previous studies [4], further suggest that the O-150 PCR assay is highly specific when used on samples collected from populations in areas where *O. volvulus* is not endemic.

Acknowledgments

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References

Humoral Immune Responses of Africans to Cysteine Protease–Related Antigens of *Plasmodium falciparum*

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The *Plasmodium falciparum* serine repeat antigen (SERA) and serine repeat protein homologue (SERPH) contain highly conserved domains that appear to encode cysteine proteases or related proteins. Humoral immune responses against the protease domains of SERA and SERPH were evaluated. Malaria-immune Africans, but not nonimmune controls, demonstrated potent humoral responses against the protease domains. As the SERA and SERPH protease domains are likely accessible to circulating antibody, these results suggest that humoral responses to the domains may contribute to antimalarial immunity.

Despite extensive efforts, the development of a broadly effective malaria vaccine remains an elusive goal. Contributing to difficulties in vaccine development, malarial proteins that are exposed to the human immune response are often antigenically diverse, and immunologic pressure appears to elicit antigenic variation [1]. It seems appropriate, therefore, to consider as vaccine components malarial proteins that are targets of the host immune response but that are antigenically conserved. Among such proteins should be essential enzymes that are being studied as potential vaccine components [1]. SERA appears to be located in the parasitophorous vacuole surrounding the intraerythrocytic schizont [3] and on the surface of free merozoites [4], so it is likely accessible to circulating antibodies and other mediators of the host immune response. Supporting the consideration of SERA as a vaccine component, immunization with intact SERA homologues also induced protective immunity in monkeys. The role of the carboxy-terminal portion of SERA in immune responses has been relatively little studied, although a hybrid vaccine that included carboxy-terminal SERA fragments induced protective immunity in mice immunized by serial infection and drug cure demonstrated humoral and cellular immune responses against the fragments, and naïve mice immunized with one of the fragments were partially protected against lethal malaria [9].

Sequence comparisons of SERA [2] and a related schizont protein, the serine-rich protein homologue (SERPH) [10], show...