## Use of Ov16-Based Serology for Post-Elimination Surveillance of Onchocerciasis in Ecuador

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*Abstract.* Onchocerciasis is a blinding disease caused by the filarial parasite *Onchocerca volvulus*, with a worldwide distribution. Onchocerciasis has been targeted for regional elimination based on annual and semiannual mass drug administration (MDA) with ivermectin in endemic communities over several years. This strategy in Ecuador led to the interruption of transmission and suspension of ivermectin MDA in 2009 with certification of elimination in 2014. In the present study, we analyzed sera collected in 2018 from 123 children aged 5–9 years from formerly hyperendemic communities in the Esmeraldas focus, Ecuador, for the presence of antibodies to 0v16 antigen. All samples were negative, indicating no evidence of transmission since MDA was stopped. Ov16-based serology offers an economic and practical alternative for measuring vector infectivity for post-certification surveillance in formerly endemic countries where expertise and capacity to reliably measure fly infectivity rates are costly to maintain.

Onchocerciasis, caused by the filarial parasite Onchocerca volvulus, infects an estimated 17 million people in endemic areas of Africa, the Americas, and the Eastern Mediterranean region, and 205 million people are considered to be at risk of infection.<sup>1</sup> Onchocerciasis is a neglected tropical disease targeted for regional elimination and eventual global elimination.<sup>1</sup> In Latin America, a strategy of community-based periodic mass drug administrations (MDAs) with ivermectin has led to certification of elimination in endemic foci in Colombia, Ecuador, Mexico, and Guatemala, although transmission is still active in an isolated rain forest focus on the Brazil-Venezuela border.<sup>2</sup> In Ecuador, annual or semiannual ivermectin was administered to endemic communities in the endemic foci in Esmeraldas Province from 1991 to 2009 when treatments were stopped following interruption of transmission.<sup>3,4</sup> Posttreatment surveillance (PTS) of black flies by PCR to detect O. volvulus DNA (O-150 PCR) in 2012 showed no evidence of active transmission,<sup>4</sup> and elimination of onchocerciasis in Ecuador was certified in 2014. Measurement of infectivity rates in vectors by O-150 PCR is considered the key parameter for the detection of interruption of and resurgence in transmission, but useful also is the evaluation of exposure to O. volvulus by measurement of antibodies to an O. volvulusspecific antigen, Ov16.5

In the present study carried out in June 2018, almost 4 years after certification of elimination, we collected 123 blood samples from children aged 5–9 years living in four formerly hyperendemic communities for onchocerciasis in Esmeraldas Province in Ecuador (Figure 1): Zapallo Grande (n = 34), San Miguel (n = 24), El Tigre (n = 30), and Corriente Grande (n = 35). The mean age of the 123 children sampled was 7.5 years (range 5–9 years), and 53.7% were male.<sup>6</sup> Sera were tested for the presence of IgG4 antibodies to Ov16 antigen using an ELISA test as described.<sup>7,8</sup> In brief, ELISA plates were coated with 100 µL of 2.0 µg/mL Ov16 antigen. Between each step,

the plates were washed with phosphate-buffered saline containing 0.1% Tween 20 (PBS-T), used also as diluent. Plate wells were incubated sequentially with 50  $\mu$ L of sera (diluted 1/50 in PBS-T containing 5% [w/v] bovine serum albumin) or negative or positive controls and incubated at room temperature (RT) for 2 hours. Then, the plates were incubated with antihuman IgG<sub>4</sub>-biotin conjugate (1:1,000) for 1 hour at RT and streptavidin-alkaline phosphatase (1:2,000) for 1 hour at RT. The plates were developed with p-nitrophenyl phosphate until the positive control had reached an optical density (OD) of 1.1. Reactions were stopped with 25 µL of 3 M NaOH and read after 5 minutes at 405 nm. All samples were tested in duplicate and considered putatively positive if the OD in duplicate wells differed by less than 10% and the mean OD of the wells from the sample was equal to or greater than the signal obtained from a 1/1,280 dilution of a pool of known positive sera. Putatively positive samples were retested using the same procedure. A sample was classified as a confirmed positive if it gave a positive result in the confirmatory assay and in the initial assay. None of the 123 samples were positive for Ov16specific IgG4 antibodies (95% CI: 0-3).

Ov16-based serological assays in young children can be used to monitor resurgence of transmission of O. volvulus: young children born following suppression and interruption of transmission in black fly vectors would be expected to be negative for Ov16 antibodies. This study, carried out in formerly hyperendemic communities for onchocerciasis in Ecuador, which were the last to receive ivermectin MDA and which would be the first in which resumption of transmission of onchocerciasis would be detected, confirms the absence of O. volvulus transmission in these communities for at least 9 vears (i.e., since the oldest child was born in 2009 around the time that ivermectin MDA was stopped in these communities). These findings are consistent with previous serological studies using the same assay in the Esmeraldas focus, where large samples of children aged up to 15 years were shown to be negative up to 2008.3

Current guidelines for the use of the Ov16 assay are to confirm the interruption of transmission as part of PTS when the results of fly infectivity rates show levels close to the

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## LOCATION OF THE FORMER ONCHOCERCOSIS HYPERDEMIC COMMUNITIES PROVINCE OF ESMERALDAS, ECUADOR

FIGURE 1. Location of the former onchocerciasis hyperdemic communities, province of Esmeraldas, Ecuador. This figure appears in color at www.ajtmh.org.

threshold for the interruption of transmission. Recommendations for post-elimination surveillance include periodic testing of fly infectivity by O-150 PCR until elimination is verified in all endemic countries in the same region.<sup>5</sup> However, conducting black fly collections and O-150 PCR following certification of elimination is a challenge for most formerly endemic countries including Ecuador with multiple competing demands for scarce health resources: maintaining such capacity requires continued investment in infrastructure and personnel. In the case of the latter, effective vector surveillance requires experience and expertise as well as historical knowledge of where and when to sample vectors to detect transmission. In the specific case of Ecuador, following certification of elimination, the program's entomological expertise was lost because of reassignment or loss of experienced staff and lack of an assigned budget for post-certification activities. The measurement of Ov16 antibodies offers potential advantages over studies of fly infectivity. First, it is much less costly and time-consuming to collect, transport, and test sera from schoolchildren than to conduct black fly collections that reguire maintaining specialized teams in isolated settings for prolonged periods. Furthermore, Ov16-based surveillance is not dependent on maintaining highly specialized personnel and infrastructure. Finally, measures of immunologic exposure by detection of specific IgG4 antibodies may be a more sensitive measure to detect indirect transmission occurring

over extended periods (particularly when transmission rates are low) than point estimates of black fly infectivity that may be greatly affected by vector-parasite variability in terms of time and site of sampling. The only previous report of postcertification surveillance was from a former focus in southern Chiapas in Mexico in which  $\geq$  60% of inhabitants of three communities were examined annually for onchocercomas (with surgical removal of suspicious masses) for 4 years after certification,<sup>9</sup> another setting where pool screening of black flies was presumably not viable: skin snips were taken from contacts of the only person found to have a viable female adult and from young children in the three communities who had never received ivermectin-the snips were examined by microscopy and O-150 PCR, and were found to be negative. The detection and removal of onchocercomas is time-consuming and expensive and carries the risk of complications relating to a surgical procedure. Ov16-based surveillance is, thus, likely to be preferable as well as being a more sensitive tool for monitoring recrudescence of transmission, albeit indirect, because many adult females are not found in palpable nodules and because of the delay between infection and nodule formation.

In conclusion, we have used Ov16-based serology to monitor potential resurgence of transmission of *O. volvulus* in formerly hyperendemic communities following the certification of elimination of onchocerciasis in Ecuador. Our data show no evidence of *O. volvulus* transmission since 2009 when MDA was stopped. We believe that Ov16-based surveillance offers practical advantages over measurements of vector infectivity for post-certification surveillance in resource-limited settings.

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