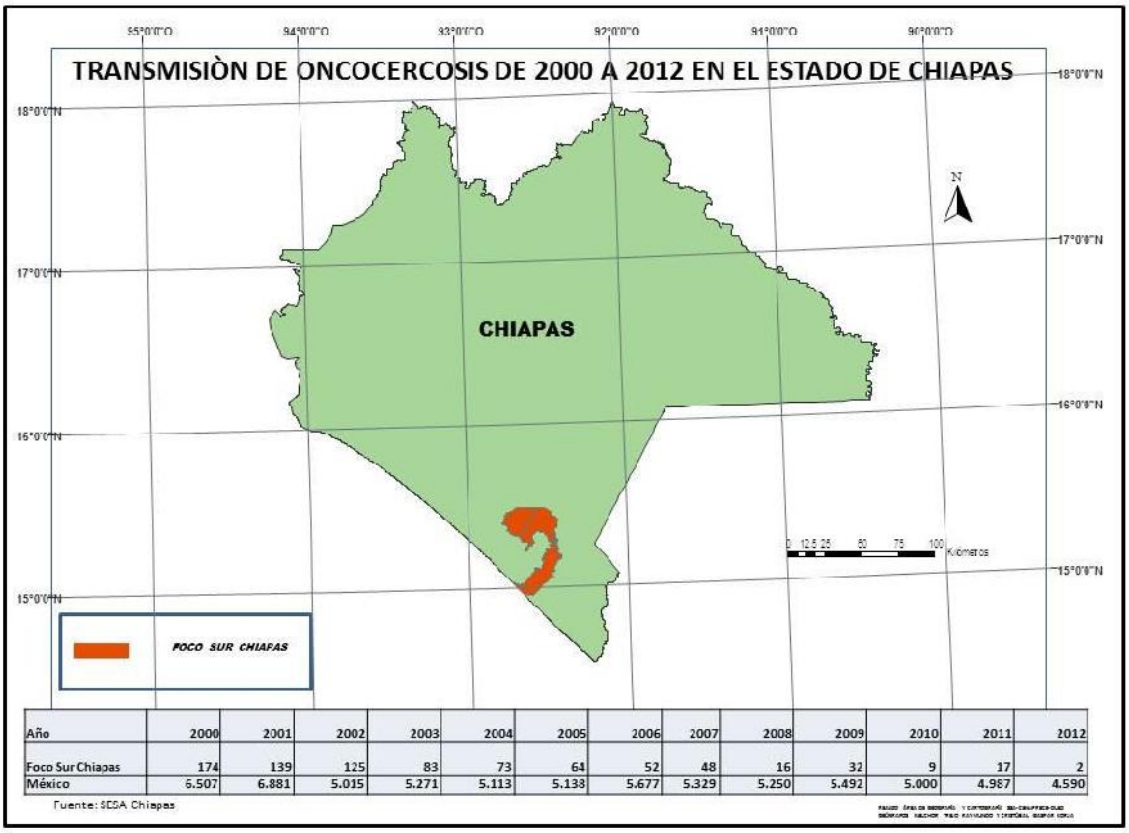


Año	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Foco Sur Chiapas	174	139	125	83	73	64	52	48	16	32	9	17	2
México	6.507	6.881	5.015	5.271	5.113	5.138	5.677	5.329	5.250	5.492	5.000	4.987	4.590

Fuente: SSA Chiapas

Anexo 8. Modelos de simulación matemática en México. (Fuente: OEPA)

Report on EUSIMON simulation

Date of report: June 7th 2011

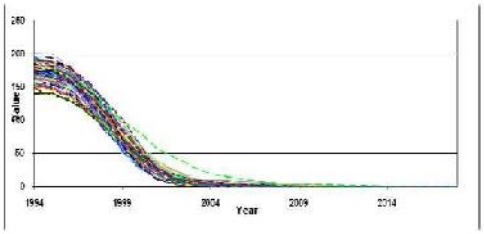
Community:	**Colonia Estrella Roja**
Focus:	**South of Chiapas**
Vector:	***S. ochraceum***
Year Treatments began:	**1995**
Name of ESOut.csv File:	**ESout-EstrellaRoja7Jun2011.esd**
Name of Demogr.csv File:	**DemogrEstrellaRoja.cvs**
Name of Params.csv File:	**ParamsEstrellaRoja.cvs**

Scenario: Real coverages were considered for this run until second round of 2009. Then default 85% was used for 2010. After 2010 no coverages were considered.

RESULTS

Disease:

Fig1. Number Infected



Prob of Zero in year	Lower 95% CL (exact)	Upper 95% CL (exact)	Year
88%	0.80	0.94	2009
94%	0.87	0.98	2010
99%	0.95	1.00	2011
99%	0.95	1.00	2012
99%	0.95	1.00	2013
100%	0.97	1.00	2014

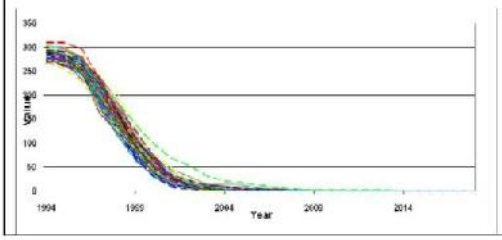
In 2011, 1 runs out of 100 (1 : 0 - 3) indicated the presence of infected persons in the population.

By 2014 no runs (0%-3%) indicated any infected persons in the population.

Look at the Maximum number infected as recorded by any of the 100 repeated runs, we get:

Year	2011	2012	2013	2014
Max No. Infected People	2	1	1	0

Fig 2. Worms



Prob of Zero in year	Lower 95% CL (exact)	Upper 95% CL (exact)	Year
94%	0.87	0.98	2010
99%	0.95	1.00	2011
99%	0.95	1.00	2012
99%	0.95	1.00	2013
100%	0.97	1.00	2014

In 2011, 1 runs out of 100 (1: 0 – 5%) indicated the presence of living worms in the population. By 2014 no runs (0%-3%) indicated any living worms in the population.

Maximum number of living worms in the human population recorded by any of the 100 repeated runs:

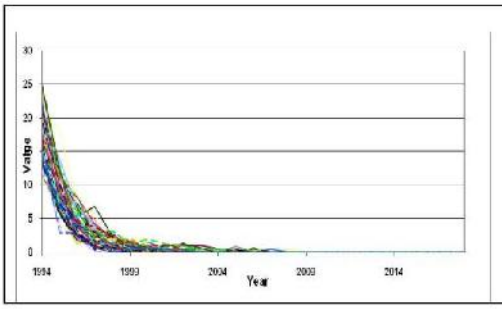
Year	2011	2012	2013	2014
Max # Worms	2	1	1	0

If we look at maximum numbers of fertile worms, there are even fewer:

Year	2011	2012	2013	2014
Max No. Fertile Worms	0	0	0	0

These low levels of infections are reflected in the Biopsy prevalence:

Fig. 3 Biopsy prevalence:

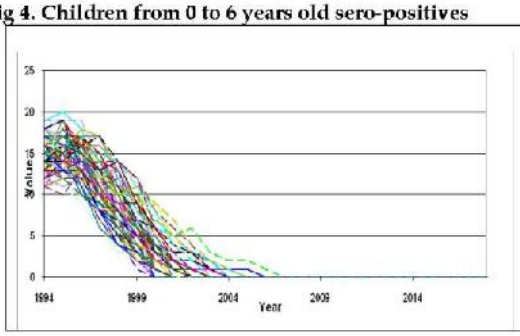


Prob of Zero in year	Lower 95% CL (exact)	Upper 95% CL (exact)	Year
90%	0.82	0.95	2005
95%	0.89	0.98	2006
98%	0.93	1.00	2007
99%	0.95	1.00	2008
100%	0.97	1.00	2009

By 2009, no runs out of 100 (0%: 0% -3%) indicated biopsy prevalence in the population.

Serology

Fig 4. Children from 0 to 6 years old sero-positives

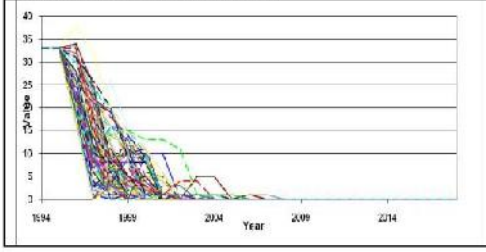


Prob of Zero in year	Lower 95% CL (exact)	Upper 95% CL (exact)	Year
94%	0.87	0.98	2004
96%	0.93	1.00	2005
99%	0.95	1.00	2006
100%	0.97	1.00	2007

By 2007 no runs (0%-3%) indicated any children from 0 to 6 years old sero-positives in the population.

Transmission

Fig. 5 Number of new worms



Prob of Zero in year	Lower 95% CL (exact)	Upper 95% CL (exact)	Year
92%	0.85	0.96	2005
97%	0.91	0.99	2006
98%	0.93	1.00	2007
100%	0.97	1.00	2008

In 2008, 0 runs out of 100 (0: 0 – 3) indicated the presence of new worms in the population.

Summary

1) **Disease**:

   a) **Living worms**: In 2011, 1 run out of 100 (1: 0 -5%) indicated the presence of living worms in the population. However **if we look at the maximum number of living worms it decreases from 2 at 2011 to 0 at 2014, and zero of them are female worms.** By 2014 no runs (0%-3%) indicated any living worms in the population.

   b) **Infected people:** By 2011, 1 run out of 100 (1: 0 -5%) indicated the presence of infected persons in the population. However if we look at **the maximum number predicted of infected persons it decreases from 2 at 2011 to 0 at 2014. By 2014 no runs (0%-3%) indicated any infected persons in the population.**

   c) **By 2009, no runs out of 100 (0%: 0% -3%) indicated biopsy prevalence in the population.**

2) **Serology**

   a) By 2007 no runs (0%-3%) indicated any children from 0 to 6 years old sero-positives in the population.

3) **Transmission**:
   a) In 2008, 0 runs out of 100 (0: 0 – 3) indicated the presence of new worms in the population.
   b) Transmission should have stopped at 2008.

4) **Recrudescence**:
   a) The tables following Fig. 2 show that at maximum of living worms in 2011 are 2. By 2014, the living worms have dropped to 0.
   b) Therefore the chance of recrudescence after 2011 is highly unlikely.

Report on EUSIMON simulation

Date of report: June 2011

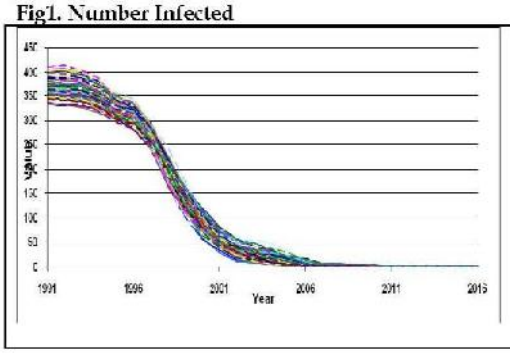
Community:	**Las Golondrinas**
Focus:	**South of Chiapas**
Vector:	***S. ochraceum***
Year Treatments began:	**1992**
Name of ESOut.csv File:	**ESoutSero-Golondrinas6Jun2011.esd**
Name of Demogr.csv File:	**DemogrLasGolondrinas.cvs**
Name of Params.csv File:	**ParamsLasGolondrinas.cvs**

Scenario: The coverages considered for this run were until first round 2011, the others rounds of 2011 were considered as 85% default.

RESULTS

Disease:

Fig1. Number Infected



Prob of zero	Lower 95%CL	Upper 95%CL	Year
80%	0.71	0.87	2011
85%	0.76	0.91	2012
89%	0.81	0.94	2013
91%	0.84	0.96	2014
95%	0.90	0.99	2015
97%	0.91	0.99	2016
100%	0.97	1.00	2017

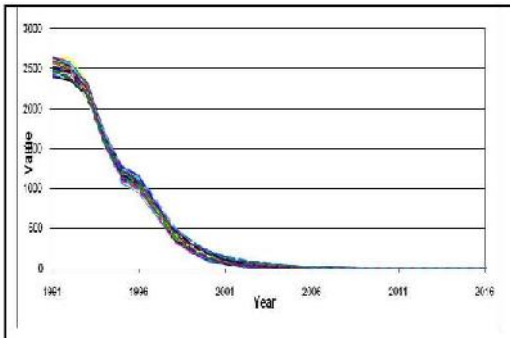
In 2011, 20 runs out of 100 (20 : 13 - 29) indicated the presence of infected persons in the population.

By 2017 no runs (0%-3%) indicated any infected persons in the population.

Look at the Maximum number infected as recorded by any of the 100 repeated runs, we get:

Year	2010	2011	2012	2013	2014	2015	2016	2017
Max No. Infected People	3	2	2	2	1	1	1	0

Fig 2. Worms



Prob of zero	Lower 95%CL	Upper 95%CL	Year
80%	0.71	0.87	2011
85%	0.76	0.91	2012
89%	0.81	0.94	2013
91%	0.84	0.96	2014
96%	0.90	0.99	2015
97%	0.91	0.99	2016
100%	0.97	1.00	2017

In 2011, 20 runs out of 100 (20: 13 - 31) indicated the presence of living worms in the population. By 2017 no runs (0%-3%) indicated any living worms in the population.

Maximum number of living worms in the human population recorded by any of the 100 repeated runs:

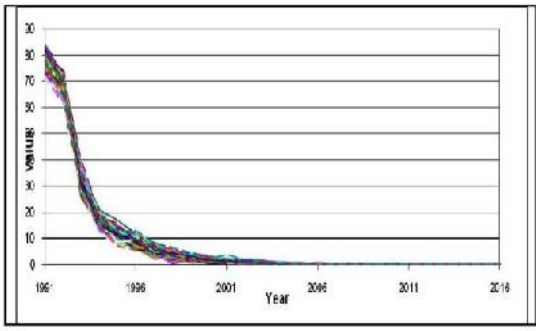
Year	2011	2012	2013	2014	2015	2016	2017
Max # Worms	2	2	2	1	1	1	0

If we look at maximum numbers of fertile worms, there are even fewer:

Year	2011	2012	2013	2014
Max No. Fertile Worms	2	1	1	0

Fig. 3 Biopsy prevalence:

3

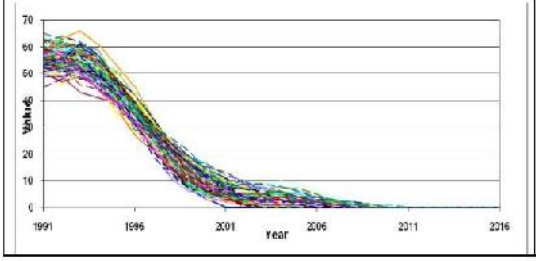


Prob of zero	Lower 95%CL	Upper 95%CL	Year
89%	0.81	0.94	2006
98%	0.90	0.99	2007
96%	0.90	0.99	2008
98%	0.93	1.00	2009
99%	0.95	1.00	2010
100%	0.97	1.00	2011

By 2011, no runs out of 100 (0%: 0% -3%) indicated biopsy prevalence in the population.

Serology

Fig 4. Children from 0 to 6 years old sero-positives



Prob of zero	Lower 95%CL	Upper 95%CL	Year
81%	0.72	0.88	2008
95%	0.89	0.98	2009
99%	0.95	1.00	2010
100%	0.97	1.00	2011

In 2010, 1 run out of 100 (3 : 1 - 5) indicated the presence of children from 0 to 6 years old sero-positives in the population.

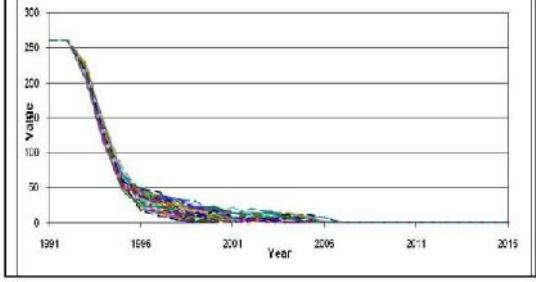
By 2011 no runs (0%-3%) indicated any children from 0 to 6 years old sero-positives in the population.

Look at the Maximum number children 0 to 6 years old sero-positives by any of the 100 repeated runs, we get:

Year	2008	2009	2010	2011
Max. # children sero-positives	2	1	1	0

Transmission

Fig. 5 Number of new worms



Prob of zero	Lower 95%CL	Upper 95%CL	Year
94%	0.87	0.98	2007
98%	0.93	1.00	2008
99%	0.95	1.00	2009
99%	0.95	1.00	2010
100%	0.97	1.00	2011

By 2011, no runs out of 100 indicated successful transmission.

95%CL indicate no significant difference between 2009 and 2010.

Summary

Community:	**Las Golondrinas**
Focus:	**South of Chiapas**
Vector:	***S. ochraceum***
Year Treatments began:	**1992**

1) **Disease**:
   a) In 2011, 20 runs out of 100 (20 : 13 - 29) indicated the presence of infected persons in the population. **However if we look at the maximum number of infected people it decreases from 3 at 2010 to 0 at 2017 and living worms decreases from 2 in 2010 to 0 in 2017. By 2017 no runs (0%-3%) indicated any infected people and living worms in the population.**

   b) By 2011, no runs out of 100 (0%: 0% -3%) indicated biopsy prevalence in the population.

2) **Serology**
   a) In 2010, 1 run out of 100 (3 : 1 - 5) indicated the presence of children from 0 to 6 years old sero-positives in the population. The maximum numbers of children under 6 years old ser-positives decreases from 2 at 2008 to 0 at 2011.

   b) By 2011 no runs (0%-3%) indicated any children from 0 to 6 years old sero-positives in the population.

3) **Transmission**:
   a) By 2011, no runs out of 100 indicated successful transmission.
   b) Transmission should have stopped at 2011.

4) **Recrudescence**:
   a) The tables following Fig. 2 show that at maximum of living worms in 2011 are 2. By 2017, the living worms have dropped to 0, but by 2014 there will be not female worms.
   b) Therefore the chance of recrudescence after 2011 is highly unlikely.

Report on EUSIMON simulation

Date of report: June 6th 2011

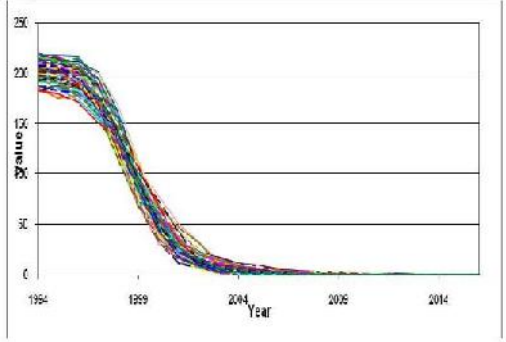
Community:	**Morelos**
Focus:	**South of Chiapas**
Vector:	***S. ochraceum***
Year Treatments began:	**1995**
Name of ESOut.csv File:	**ESout-Morelos6Jun2011.esd**
Name of Demogr.csv File:	**DemogrMorelos.cvs**
Name of Params.csv File:	**ParamsMorelos.cvs**

Scenario: The coverages considered for this run were until first round of 2011. Then default 85% was used for 2011. Since 2012 no coverages were considered.

RESULTS

Disease:

Fig1. Number Infected



Prob of Zero in year	Lower 95% CL (exact)	Upper 95% CL (exact)	Year
86%	0.78	0.92	2009
92%	0.85	0.96	2010
95%	0.89	0.98	2011
98%	0.93	1.00	2012
100%	0.97	1.00	2013

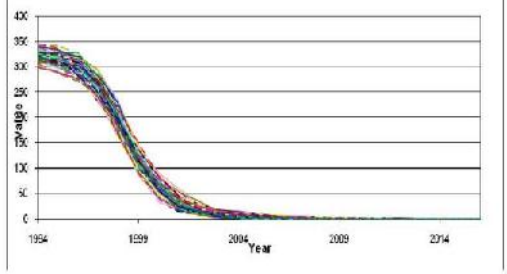
In 2012, 2 runs out of 100 (2 : 0%–7%) indicated the presence of infected persons in the population.

By 2013 no runs (0%-3%) indicated any infected persons in the population.

Look at the Maximum **number infected people** as recorded by any of the 100 repeated runs, we get:

Year	2008	2009	2010	2011	2012	2013
Max No. Infected People	3	3	1	1	1	0

Fig 2. Worms



Prob of Zero in year	Lower 95% CL (exact)	Upper 95% CL (exact)	Year
86%	0.78	0.92	2009
92%	0.85	0.96	2010
95%	0.89	0.98	2011
98%	0.93	1.00	2012
100%	0.97	1.00	2013

In 2011, 5 runs out of 100 (5: 2% - 11%) indicated the presence of living worms in the population.
By 2013 no runs (0%-3%) indicated any living worms in the population.

Maximum number of living worms in the human population recorded by any of the 100 repeated runs:

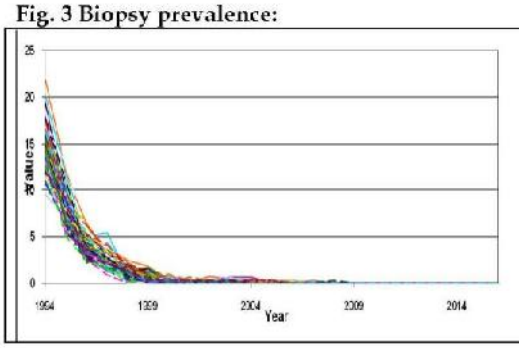
Year	2009	2010	2011	2012	2013
Max # Worms	3	1	1	1	0

If we look at maximum numbers of fertile worms, there are even fewer:

Year	2009	2010	2011	2012
Max No. Fertile Worms	2	1	1	0

These low levels of infections are reflected in the Biopsy prevalence:

Fig. 3 Biopsy prevalence:



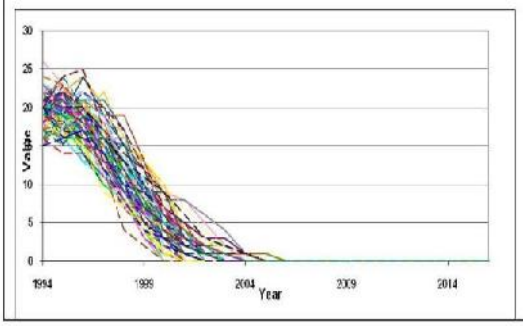
Accumulated frequency of attaining zero	Prob of zero	Lower 95%CL	Upper 95%CL	Year
97	0.97	0.91	0.99	2006
98	0.98	0.93	1.00	2007
98	0.98	0.93	1.00	2008
100	1.00	0.97	1.00	2009

In 2006, 3 runs out of 100 (3: 1 - 9) indicated biopsy prevalence in the population.
By 2009, no runs out of 100 (0%: 0% -3%) indicated biopsy prevalence in the population.

95%CL indicate no significant difference between 2007and 2009

Serology

Fig 4. Children from 0 to 6 years old sero-positives



Prob of Zero in year	Lower 95% CL (exact)	Upper 95% CL (exact)	Year
67%	0.57	0.76	2003
90%	0.82	0.95	2004
98%	0.93	1.00	2005
100%	0.97	1.00	2006

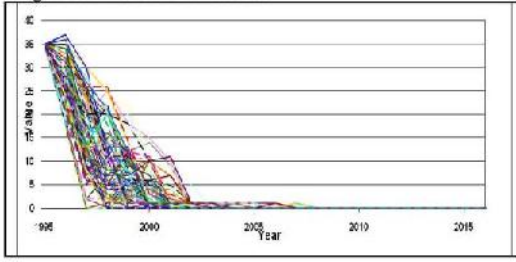
By 2006 no runs (0%-3%) indicated any children from 0 to 6 years old sero-positives in the population.

Look at the Maximum number children 0 to 6 years old sero-positives by any of the 100 repeated runs, we get:

Year	2003	2004	2005	2006
Max. # children sero-positives	4	1	1	0

Transmission

Fig. 5 Number of new worms



Prob of Zero in year	Lower 95% CL (exact)	Upper 95% CL (exact)	Year
88%	0.80	0.94	2004
90%	0.82	0.95	2005
91%	0.84	0.96	2006
96%	0.90	0.99	2007
100%	0.97	1.00	2008

By 2008, no runs out of 100 indicated successful transmission.

Summary

1) **Disease**:
   a) **Living worms**: In 2011, 5 runs out of 100 (5: 2% - 11%) indicated the presence of living worms in the population. However **if we look at the maximum number of living worms it decreases from 3 at 2009 to 0 at 2013.** By 2013 no runs (0%-3%) indicated any living worms in the population.

   b) **Infected people:** By 2012, 2 runs out of 100 (2: 0% - 7%) indicated the presence of infected persons in the population. However if we look at **the maximum number predicted of infected persons it decreases from 3 at 2008 to 0 at 2013**.

   c) **Biopsy prevalence:** By 2009, no runs out of 100 (0%: 0% -3%) indicated biopsy prevalence in the population.

2) **Serology**
   a) By 2006 no runs (0%-3%) indicated any children from 0 to 6 years old sero-positives in the population. If we take a look at the maximum number of children, it decreases from 4 at 2003 to 0 at 2006.

3) **Transmission**:
   a) By 2008, no runs out of 100 indicated successful transmission.
   b) Transmission should have stopped at 2007.

4) **Recrudescence**:
   a) The tables following Fig. 2 show that at maximum of living worms in 2010 is 1. By 2013, the living worms have dropped to 0.
   b) Therefore the chance of recrudescence after 2010 is highly unlikely.

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PLOS | NEGLECTED TROPICAL DISEASES

Time Series Analysis of Onchocerciasis Data from Mexico: A Trend towards Elimination

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Abstract

Background: In Latin America, there are 13 geographically isolated endemic foci distributed among Mexico, Guatemala, Colombia, Venezuela, Brazil and Ecuador. The communities of the three endemic foci found within Mexico have been receiving ivermectin treatment since 1989. In this study, we predicted the trend of occurrence of cases in Mexico by applying time series analysis to monthly onchocerciasis data reported by the Mexican Secretariat of Health between 1988 and 2011 using the software R.

Results: A total of 15,584 cases were reported in Mexico from 1988 to 2011. The data of onchocerciasis cases are mainly from the main endemic foci of Chiapas and Oaxaca. The last case in Oaxaca was reported in 1998, but new cases were reported in the Chiapas foci up to 2011. Time series analysis performed for the foci in Mexico showed a decreasing trend of the disease over time. The best-fitted models with the smallest Akaike Information Criterion (AIC) were Auto-Regressive Integrated Moving Average (ARIMA) models, which were used to predict the tendency of onchocerciasis cases for two years ahead. According to the ARIMA models predictions, the cases in very low number (below 1) are expected for the disease between 2012 and 2013 in Chiapas, the last endemic region in Mexico.

Conclusion: The endemic regions of Mexico evolved from high onchocerciasis-endemic states to the interruption of transmission due to the strategies followed by the MSH, based on treatment with ivermectin. The extremely low level of expected cases as predicted by ARIMA models for the next two years suggest that the onchocerciasis is being eliminated in Mexico. To our knowledge, it is the first study utilizing time series for predicting case dynamics of onchocerciasis, which could be used as a benchmark during monitoring and post-treatment surveillance.

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Competing Interests: The authors have declared that no competing interests exist.

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☯ These authors contributed equally to this work.

Introduction

Human onchocerciasis is caused by the filarial worm *Onchocerca volvulus*, which is transmitted by the bites of blackflies of *Simulium* species [1]. The symptomatology of onchocerciasis disease is characterized by clinical manifestations such as onchocerca skin diseases, onchocercomata, lymphadenopathy, and ocular lesions, including the irremediable terminal effect of blindness [2]. Onchocerciasis is the major cause of blindness and dermatitis in endemic areas, and it remains as an important public health problem in Africa. In Latin America, there are six countries (Brazil, Colombia, Ecuador, Venezuela, Guatemala, and Mexico) with scattered and small endemic onchocerciasis foci, where a population of 470, 222 individuals is currently estimated to be at risk [3].

The discovery of onchocerciasis in America was in 1915 by Rodolfo Robles in Guatemala; hence it was first named as Robles's disease. In Mexico, the first cases of onchocerciasis were documented in 1923 in Chiapas, originated as a consequence of active seasonal migration of coffee workers from the endemic areas between Guatemala and Mexico. The regions in Chiapas and Oaxaca of Mexico are associated with the presence of abundant vector populations [4]. The focus of Oaxaca and Northern Chiapas represented the expansion of onchocerciasis from Southern Mexico or Guatemala [5].

The onchocerciasis control program in Mexico was first established in 1930 and has worked continuously up to date. During 1930–1946, a sporadic larval vector control campaign using Creolin was carried out to eliminate vector populations from breeding sites together with nodulectomy (removal of nodules) campaigns [6]. The administration of diethylcarbamazine (DEC) began in 1947, followed by a sporadic application of DDT to eliminate the vector populations in 1952. In 1990, DEC was supplanted by ivermectin (Mectizan; Merck & Co., Inc., Whitehouse Station, NJ) [4]. In 1992, the Onchocerciasis Elimination Program for the Americas (OEPA) was launched [7], and has

Author Summary

Mexico is one of the countries where human onchocerciasis (river blindness) can be found in Latin America. In 1989, the onchocerciasis program in Mexico started the treatment with ivermectin only for symptomatic individuals and then mass distribution of ivermectin was initiated for all eligible residents from 1994, either annually, twice or four times a year in endemic foci, coordinated by Mexican Secretariat of Health (MSH). In our study, we used a statistical method to analyse the cases of the disease reported by MSH from 1988 to 2011. The analysis showed that the cases of the disease have marginally decreased since 1999. The results also predicted an extremely low number (absence) of cases between 2012 and 2013 in the Chiapas region, the last endemic area, suggesting that disease is on a trend towards elimination in Mexico. Meanwhile, it could provide a benchmark for surveillance after mass treatment has been halted in 2012.

successfully coordinated the efforts of the affected countries in Latin America. In 1989, the onchocerciasis program in Mexico started the treatment with ivermectin only for symptomatic individuals [4]. Later in 1997, ivermectin distribution was implemented twice a year for most eligible residents from all at-risk communities, followed by the distribution four times a year in the Chiapas foci as from 2003 upward, which was a successful strategy to accelerate the interruption of the parasite transmission [8]. As OEPA is preparing to wind up activites as from 2012, it is important to predict the possibility of future occurrence of new cases of the disease because there still exists the possibility of recrudesce due to the existence of potential infected vector population or multiple vectors [9].

The time series analysis has been applied in the field of epidemiological research on infectious diseases for the prediction of epidemiological spread tendency, which provided valuable information for making decisions in the control of such diseases [10–13]. For instance, the ARIMA models [14] as well as Seasonal Auto-Regressive Integrated Moving Average (SARIMA) [15] models were used to analyze time series data containing ordinary or seasonal trends of dengue cases to develop a forecasting model in endemic areas of Rio de Janeiro, Brazil and French West Indies, Guadeloupe respectively.

The univariate Auto-Regressive Integrated Moving Average (ARIMA) models are a kind of time series analysis for forecasting a time series data [16]. ARIMA models need a stationary series, that is, the mean and variances of the series are independent of time. Stationarity can be accomplished by data transformations and differencing. Once the series is stationary, it is fine-tuned through adding auto-regressive (AR) orders (lags of the differenced series) and/or moving average (MA) orders (lags of the forecast errors) as needed to remove any last hints of autocorrelation from the forecast errors. The usefulness of ARIMA models resides mostly in providing an estimate of the variability to be expected among future observations and depends on past values and random errors [16].

Herein, the cases of onchocerciasis reported by the Mexican Secretariat of Health (MSH) during the past two decades were analyzed using time series analysis. We adopted the ARIMA approach for describing the case dynamics of onchocerciasis in the endemic foci and predicted the tendency of occurrence of onchocerciasis cases in the immediate future.

Methods

Dataset

The official norm NOM-032-SSA2-2002 of MSH has defined that a case of onchocerciasis should comply with at least one of with the following requirements: demonstration of microfilariae through microscopic examination of superficial skin snips, identification of adult worms by removing nodules, observation of microfilariae in the cornea and anterior chamber of the eye, positive PCR and hybridization from skin snips or nodules. The individual should also present typical clinical manifestations of the disease, and inhabit or have resided in areas of active transmission. Monthly data of onchocerciasis cases between 1988 and 2010 were obtained from the MSH web site (http: www.dgepi.salud.gob.mx/anuario). Preliminary information on cases in 2011 was obtained from the weekly bulletin web site of MSH (http://www.dgepi.salud.gob.mx/boletin/).

Time Series Analysis

Time series analysis for identifying significant predictors as well as for forecasting monthly onchocerciasis cases were carried out using the statistical analysis ARIMA model. The data in 1990 for Oaxaca and in 2001 for Chiapas was not available. The cases of onchocerciasis in Chiapas and Oaxaca from 1988 to 1993 were recorded every two months, which could result in data bias (one month with 0 cases after one month with data). Considering that the month without data does not indicate no case occurrence but the cases not reported, and then the cases of that month accumulated in the data of next month, we thus decided to adjust the data by assigning the half part of cases of a month to the previous zero-case month. Because of disease control activities (ivermectin distribution), cases of infection have been greatly reduced, giving rise to an abundance of zeros in the monthly case data. It needs to stabilize the variance of the series before seeking the best model that fits each dataset. The square root (sqrt) transformation was applied to stationarize our datasets. After stabilizing the variance, the descriptive method procedure was performed for plotting the onchocerciasis data through the autocorrelation function (ACF) and partial autocorrelation function (PACF) to identify the order of differentiation as well seasonal and non-seasonal effects. The residuals of the models fitted were inspected with the ACF and PACF plots and further verified with the Ljung-Box test. The best ARIMA model was selected for analysis according to the lowest Akaike Information Criterion (AIC). The ARIMA models were represented by the form as (p, d, q) (P, D, Q)S, where p is the order of auto-regression, d is the order of differencing (or integration), and q is the order of moving-average for non-seasonal series. P, D, Q are their seasonal counterparts, and S is the seasonal period. If the parameters p and q or P and Q are together present in the non-seasonal or seasonal series, the model was termed as mixed ARIMA model. We estimated the parameters of ARIMA models with the "arima" function implemented in the software R [17] that compute the exact likelihood via a state-space representation of the ARIMA process by using the Kalman filter [18,19], "skipping" the missing observations in the computations, obtaining the maximum likelihood estimators of the model parameters. The model's fitted values were also graphically compared with the observed data. The fitted model was adopted to out-of-sample predict onchocerciasis cases for the next two years in the foci using the one-step ahead approach, that is, a forecast generated for the next observation only. For example, as the observed value for January 1998 was obtained in Oaxaca region, the data were updated to January 1998, re-estimated the parameters of the ARIMA model,

and computed the next 1-step ahead predicted value, February 1998. This process was continued until the end of the year 1999. The software R (version 2.11.1) was used for all statistical analyses and graphic displays [17]. The automatic algorithms implemented in software R were also used to aid in the selection of the ARIMA models [20].

Results

Onchocerciasis Disease Patterns in Mexico

The cases of onchocerciasis in Mexico from 1988 to 2011 were summarized in Table S1. The total number onchocerciasis cases in Chiapas, Oaxaca and other regions of Mexico during 1988 to 2011 were 15,584 cases. The highest number of cases was in 1988 with 3,197 cases and afterwards the number decreased gradually, with the lowest number in 2010 with just 15 cases. The recorded cases were predominantly from two regions, Oaxaca and Chiapas (Figure 1), and some sporadic cases from other regions. In the Oaxaca focus, the total reported cases were 1,628; the number of cases was highest in 1991 and later decreased marginally. The last case in Oaxaca was recorded in 1998. Therefore, this disease had been successfully eliminated from the Oaxaca region. The Chiapas foci had a total of 13,849 cases reported. The case number remained high before 1990 and maintained a little lower level from 1991 to 1997, with the second peak in 1994. Then the recorded cases stably reduced, until 12 cases in 2011. There were 107 cases reported in other states (mainly Northern Mexico) during 1988–1993, 2005–2007, and 2009–2011, which were imported cases of onchocerciasis according to case definition of MSH.

Time Series Analysis

In Oaxaca, there were no reported cases since 1999. This observation gave a good example for us to test if the Time Series Analysis describes well the dynamics of infection cases and predicted the approximate time of disease elimination in Oaxaca. The plot of sqrt-transformed onchocerciasis cases for Oaxaca showed a decreased trend since 1990 (Figure 2 A). The plot of ACF has positive autocorrelations out to a high number of lags, suggesting a nonstationary time series and a need for differencing (Figure 2 B). After stabilizing the series with the first order difference, we inspected the ACF and PACF plots, which suggests that non-seasonal and seasonal parameters are needed in the model (Figure S1 A, B). The negative ACF cutoff at lag 2, associated with the slow decay of PACF at lags 2–3 suggests that MA orders are needed in the model but the positive correlation at lag 12 associated with the PACF cutoff at lag 12 suggests that non-seasonal AR orders could be also added. In addition, the negative ACF cutoff at lag 32 associated with the slow decay of PACF at lags 28–31 suggested seasonal MA orders. Consequently, we fitted several ARIMA models with different Auto-Regressive orders, AR (p,P) and Moving Average orders, MA(q,Q), and excluded any models in which the residuals were not significant and had high AIC values. Thus, the best-fit model for Oaxaca was a mixed ARIMA $(0,1,2)\times(0,0,1)_{12}$ (AIC = 388.28) seasonal non-stationary model. All coefficients of ARIMA models for Oaxaca were significant (Table 1). The plots ACF and PACF of the residuals were almost located within the confidence limits (Figure 3 A, B). The Ljung-Box statistic test did not reject the null hypothesis of independence in the residuals time series (P value = 0.93). The model plot that fitted actual plot of dynamics of case data was shown in Figure 4 A. This model was then adopted for two-years-ahead prediction using the 1-step ahead approach. The forecast values for Oaxaca, showed a markedly decreasing trend and zero cases would occur from January 1998 to December 1999 (Figure 4 A), corresponding to the fact that the last case was reported from Oaxaca in 1998.

The predicted result that matches the observations in Oaxaca focus allows us to apply the same methodology to the Chiapas foci. Figure 2C showed the time series profile from 1988–2011 of onchocerciasis cases with the sqrt-transformation. As in Oaxaca, the plot of ACF showed a need for differencing because of a slow decay of positive autocorrelations out to a high number of lags (Figure 2 D). The ACF and PACF plots produced with the first order difference also suggest that non-seasonal and seasonal parameters are needed in the model (Figure S1 C, D). The negative ACF cutoff at lag 1, associated with the slow decay of PACF at lags 1–5, suggests non-seasonal MA orders. The positive ACF at lags 12 and 15 associated with the PACF sharp cutoffs at

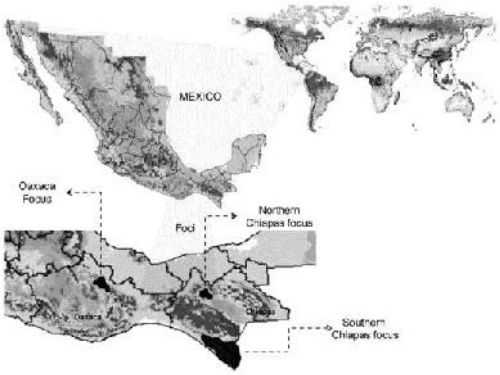

Figure 1. Map of the three endemic regions in the two southern Mexico states. The dark grey areas indicate the Oaxaca focus and the Northern and Southern Chiapas foci.
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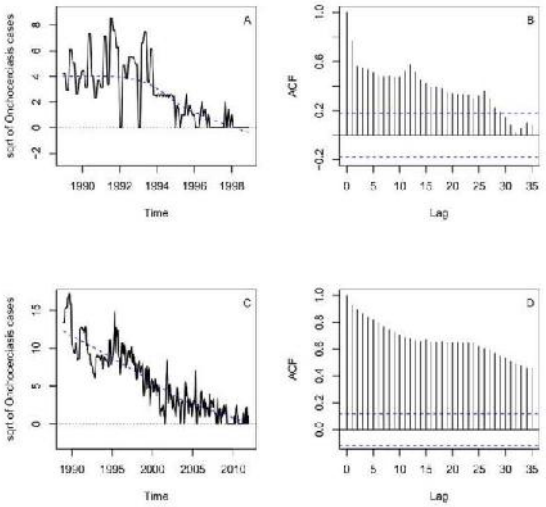

Figure 2. Time series profile and ACF plot for the data from Oaxaca and Chiapas. A and C) Time series profile of the square root values of onchocerciasis cases in Oaxaca and Chiapas. Dashed blue line indicates the trend of onchocerciasis series. B and D) Autocorrelation function (ACF) of onchocerciasis cases from Oaxaca and Chiapas. The x-axis represents the number of lags. Dashed blue line indicates 95% confidence interval.
doi:10.1371/journal.pntd.0002033.g002

lags 12 and 15 also suggests that non-seasonal and seasonal AR orders could be added. Then, several ARIMA models with different AR and MA orders were fitted, excluding any models in which the residuals exhibited higher autocorrelation, non-significant coefficients and high AIC values. The best-fitted model obtained for Chiapas was a mixed ARIMA $(1,1,1)\times(1,0,1)_{12}$ (AIC = 960.77). All the coefficients of the ARIMA model for Chiapas were significant (Table 1). The plots ACF and PACF of the residuals show no remaining temporal correlation (Figure 3 C, D). The Ljung Box statistic test did not reject the null hypothesis of independence in the residuals time series (P value = 0.34). Graphically the best-fitted model followed closely the decreasing trend of the observed series in Chiapas (Figure 4 B). The model was then used for two-years-ahead prediction using the 1-step ahead approach. It showed that the cases would continuously and markedly decrease in the recent years and the annual zero case could occur at the period from January of 2012 to December of 2013 in the Chiapas foci (Figure 4 B).

Discussion

The key aspect in the control of onchocerciasis disease in Latin America is the treatment with the drug ivermectin available to all the people at risk [4,8,21–24]. The onchocerciasis program in Mexico began treatment with ivermectin in 1989, initially treating only symptomatic individuals in hyperendemic communities [4]. In 1994, annual mass ivermectin treatment to eligible residents (i.e., those who were 5 years older and who had resided in the endemic community) in the at-risk communities was initiated. From 1997, the strategy was modified to provide mass treatments twice a year to every eligible resident in the at-risk communities [8]. In Oaxaca, the new cases had been controlled effectively and the last case occurred in 1998. The situation in Chiapas is more

Table 1. Estimate parameters of the ARIMA models.

Region	ARIMA Model	Parameter	Coefficient	Stnd. Error	t statistic	P-value
Oaxaca	$(0,1,2)\times(0,0,1)_{12}$	MA(1)	−0.2235	0.0867	−2.57783467	2.48e-03
		MA(2)	−0.3917	0.0855	−6.92046784	1.11e-17
		SMA(1)	0.286	0.1015	2.81773399	1.21e-03
Chiapas	$(1,1,1)\times(1,0,1)_{12}$	AR(1)	0.5862	0.078	7.51538461	1.37e-14
		MA(1)	−0.8974	0.0432	−20.7731481	0.00e+00
		SAR(1)	0.8675	0.0841	10.31510107	0.00e+00
		SMA(1)	−0.746	0.1149	−6.49260226	2.15e-11

ARIMA. Auto-Regressive Integrated Moving Average. **AR.** Auto-Regressive. **MA.** Moving Average. **SMA.** Seasonal Moving average. **SAR.** Seasonal Auto-Regressive.
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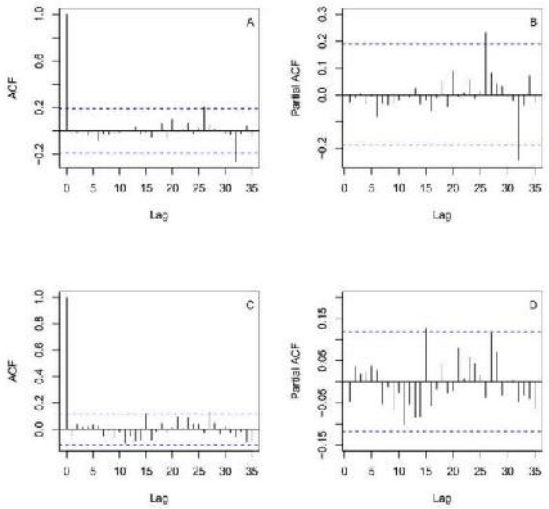

Figure 3. ACF and PACF plots of the residuals for the fitted models. A and B) Autocorrelation function (ACF) and Partial ACF (PACF) plot of the residuals of the ARIMA $(1,1,1)x(0,0,1)_{12}$ model fitted for Oaxaca. C and D) Autocorrelation function (ACF) and Partial ACF (PACF) plot of the residuals of the ARIMA $(1,1,1)x(1,0,0)_{12}$ model fitted for Chiapas. The x-axis represents the number of lags. Dashed blue lines indicate 95% confidence interval.
doi:10.1371/journal.pntd.0002033.g003

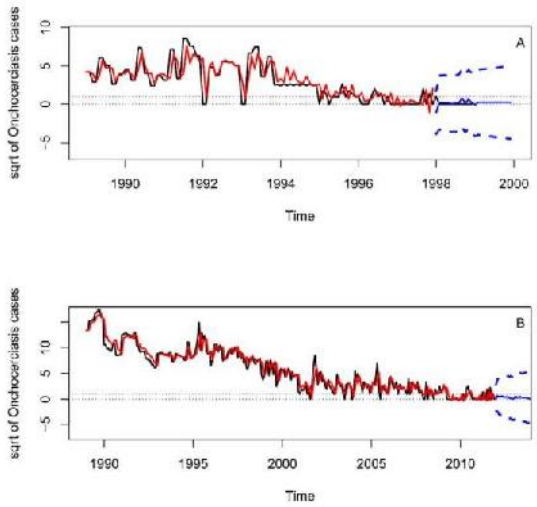

Figure 4. Time series profile for the observed data and for the fitted model. A). Black line: The square root curve of observed onchocerciasis cases in Oaxaca for the period 1988–1998. Solid red line: ARIMA $(0,1,2)x(0,0,1)_{12}$ model's fitted values (1988–1997) and 1-step ahead predicted values (year 1998–1999) with their 95% prediction intervals (dashed blue line). B) Black line: The square root curve of observed onchocerciasis cases in Chiapas for the period 1988–2011. Solid red line: ARIMA $(1,1,1)x(1,0,1)_{12}$ model's fitted values (1988–2011) and 1-step ahead predicted values (year 2012–2013) with their 95% prediction intervals (dashed blue lines).
doi:10.1371/journal.pntd.0002033.g004

complicated. It needs to note that the infection cases in Chiapas maintained a high platform before 1997 and there was a marked reduction from 953 cases in 1996 to 573 cases in 1997 and remained on a decreasing trend up to date.

In 2003, the biannual treatment strategy was modified in the majority of the formerly hyperendemic communities of Southern Chiapas focus by increasing treatment frequency to four times a year in order to accelerate the interruption of parasite transmission [25]. After this modification, the incidence was maintained at a low level (<100 cases) and reduced stably until the 12 cases in 2011. Thus, this observation indicates treatment of endemic communities with regimental distribution of Mectizan was a key to the elimination of onchocerciasis in Mexico, which is in agreement with a recent study [6]. At present, the transmission of onchocerciasis in Oaxaca and the Northern Chiapas has been eliminated, but the transmission was only interrupted in the Southern Chiapas focus as recently declared by OEPA [26]. Ivermectin treatment has been halted in the Southern Chiapas focus in 2012. Thus, all foci in Mexico are under epidemiological surveillance post-treatment which is within the fourth phase for certification of the elimination of onchocerciasis.

The above description shows that the elimination of onchocerciasis in humans is an arduous task. The evaluation of the current state and the prediction of future situations are germane for evaluating epidemiological patterns. Several mathematical models have been developed to simulate the onchocerciasis future in specific endemic zones [27–29]. These methods take into account mainly: the life cycle of the parasite, the type of treatment, the phenotypic characteristics of the vector, the microfilarial load in the skin, and the biting rate of the vectors. Two onchocerciasis transmission models were predominant in use that incorporated some of the above-mentioned variables [28,30–32]. These mathematical models were applied to explore the epidemiological consequences and the effects of control interventions on the parasite population dynamics. One of the statistical analyses useful to make predictions is the time series analysis that has been used to study vector-borne diseases [15,33,34]. In the present study, the ARIMA models based on statistical concepts were fitted to onchocerciasis data collected from the endemic regions to predict the cases for the coming years. The Oaxaca focus has recorded the systematic data and there is no case since 1999, which provided a good example for practicing the method on describing the variation of cases in the form and predicting the annual absence of infection case. A mixed ARIMA model was fitted to imitate the case variation data and predicted the absence of cases between 1998–1999, which coincides with the observed data, suggesting that the method is reliable. We then employed the similar ARIMA method for data from Chiapas, in which the mixed ARIMA model fitted well the observed data. Thus, this methodology could be considered to apply in other regions as the surveillance system for onchocerciasis. On the other hand, one goal of the onchocerciasis program in Mexico was to interrupt transmission of the parasite by the year 2012 [4]. Our model predicted that the values less than 1 case annually were located in the years 2012–2013. According to the World Health Organization (WHO) [35,36] and OEPA [37] criteria to declare a place free of onchocerciasis, a reduction of new infections to an incidence rate of less than one new case per 1,000 individuals (<0.1%) and an absence, or near absence, of infective-stage larvae of *O. volvulus* in the vector population (i.e., a rate of less than one infective fly per 1,000 parous flies) must be documented in such area. If the current trend of onchocerciasis cases is sustained, the declaration of onchocerciasis elimination in Mexico would be in the not far future.

In the present study, sqrt transformation was chosen for stationarizing the series. Actually, Anscombe transform [38] (Figure S2) and the natural *log* (*ln*) (Figure S3) have been also tried. As approaching to elimination, cases of infection have been greatly reduced, giving rise to an abundance of zeros in the monthly case data. With the application of *ln*-transformation (the most common use to stabilize the variance), we cannot use the number "1" to replace the zero in the dataset, as done in the previous report [38], because the model will consider it as one real infection case and the cases could never be less than 1 in the prediction. Thus, log-transformations of the data were performed by adding a constant (<1) to the entire dataset. The results, based on the datasets transformed respectively by each of the three methods, were evaluated by the root-mean-square of the errors (RMSE). The RMSE values with the application of sqrt or Anscombe transform were similar and much smaller than with *ln*-transformation (Table S2), indicating that both sqrt and Anscombe transform are better than *ln*- transformation, which corresponds to the comment that logarithmic transformation is generally not recommended to apply to count dataset [39], particularly to small count dataset. Although there is almost no difference between sqrt and Anscombe transform in present study, sqrt could be more practical way to stationarize such datasets because sqrt is much easier to operate.

One phenomenon needs to be mentioned. The predicted plot in both Oaxaca and Chiapas showed that all the data in the coming years are less than 1 case but with 95% prediction interval beyond 1 case. A prediction interval is always wider than a confidence interval because it is not only related to the value of the population mean, but also the data scatter. When it approaches the elimination of disease, the number of cases show as 0, 1, or >1, not as continuous data less than 1 but approaching to 0. It means that as much closer to elimination, the distribution data become much more discretized. In this situation, it could be difficult to expect the prediction of annual cases less than 1 with the 95% prediction intervals within one case, even though the zero cases were treated as 0.1. On the other hand, our test for the focus in Oaxaca demonstrated our prediction is acceptable despite of the 95% prediction intervals beyond 1 case. This phenomenon mentioned here could be common for times series analysis as approaching to elimination of diseases. Our analysis thus provided a good reference for such prediction of similar diseases.

We realize that the present model is adopted for predicting the cases under the same situation in the recent future. Since 2012, the mass treatments with ivermectin have been halted. Does it mean our prediction does not work? If ivermectin is the principal reason leading to reduction of cases and the transmission has been really interrupted, it is very possible that the tendency of case occurrence could keep reduction until to zero case. In other words, the mass treatments with ivermectin still keep the influence (the consequence of the treatment) and our prediction model has the condition to work. Otherwise, the mass treatments with ivermectin could need to be re-continued. To this sense, the application of this prediction model could be used as a benchmark during monitoring and surveillance after mass treatment has been withdrawn.

We are aware of the possible limitations of the present study. The data used in the current study rely on total clinical cases of onchocerciasis reported by the surveillance system of MSH, which may underestimate the true number of cases as earlier posited by various researchers [38,40,41]. Another limitation is the heterogeneity of the data used that could affect the time series analysis. However, the application of this method in Oaxaca focus indicated that our data analysis was adequate. Therefore, the time series analysis applied herein is acceptable.

In conclusion, onchocerciasis in Mexico was a serious public health problem in the past. ARIMA models predicted an extremely low (zero) expected cases of onchocerciasis for the next two years, implying that onchocerciasis is being eliminated. These results showed that time series analysis could be a practical method for predicting onchocerciasis case tendencies and could be used as a benchmark for monitoring and surveillance on the post ivermectin-mass-treatment duration. To our knowledge, it is the first study utilizing time series analysis for predicting the case dynamics of onchocerciasis.

Supporting Information

Checklist S1 STROBE checklist.
(DOC)

Figure S1 ACF and PACF plots produced with the first order difference. A and B) Autocorrelation function (ACF) and Partial ACF (PACF) plot for Oaxaca. C and D) Autocorrelation function (ACF) and Partial ACF (PACF) plot for Chiapas. The x-axis represents the number of lags. Dashed blue lines indicate 95% confidence interval.
(TIF)

Figure S2 Time series profile for the observed data and for the fitted model. A). Black line: The Anscombe transform curve of observed onchocerciasis cases in Oaxaca for the period 1988–1998. Solid red line: ARIMA $(0,1,2)x(0,0,1)_{12}$ model's fitted values (1988–1997) and 1-step ahead predicted values (year 1998–1999) with their 95% prediction intervals (dashed blue line). B) Black line: The Anscombe transform curve of observed onchocerciasis cases in Chiapas for the period 1988–2011. Solid red line: ARIMA $(1,1,1)x(1,0,1)_{12}$ model's fitted values (1988–2011) and 1-step ahead predicted values (year 2012–2013) with their 95% prediction intervals (dashed blue lines).
(TIF)

Figure S3 Time series profile for the observed data and for the fitted model. A). Black line: The natural *ln* curve of observed onchocerciasis cases in Oaxaca for the period 1988–1998. Solid red line: ARIMA $(2,1,1)x(0,0,1)_{12}$ model's fitted values (1988–1997) and 1-step ahead predicted values (year 1998–1999) with their 95% prediction intervals (dashed blue line). B) Black line: The natural *ln* transform curve of observed onchocerciasis cases in Chiapas for the period 1988–2011. Solid red line: ARIMA $(1,1,1)x(1,0,0)_{12}$ model's fitted values (1988–2011) and 1-step ahead predicted values (year 2012–2013) with their 95% prediction intervals (dashed blue lines).
(TIF)

Table S1 Annual onchocerciasis cases in Mexico from 1988–2011.
(DOC)

Table S2 The values of RMSE applied for evaluating the different data transformation methods.
(DOC)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: EELR MARP XG. Performed the experiments: EELR. Analyzed the data: EELR XG. Contributed reagents/materials/analysis tools: MEOA JIAJ. Wrote the paper: EELR MARP MAPR MAA XG.

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Anexo 9. Artículos científicos arbitrados de circulación internacional sobre la interrupción de la transmisión de *O. volvulus* en México. (Fuente: Dr. Mario A. Rodríguez Pérez-IPN)

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PLOS | NEGLECTED TROPICAL DISEASES

Interruption of Transmission of *Onchocerca volvulus* in the Southern Chiapas Focus, México

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Abstract

Background: The Southern Chiapas focus of onchocerciasis in Southern Mexico represents one of the major onchocerciasis foci in Latin America. All 559 endemic communities of this focus have undergone semi-annual mass treatment with ivermectin since 1998. In 50 communities of this focus, ivermectin frequency shifted from twice to four times a year in 2003; an additional 113 communities were added to the quarterly treatment regimen in 2009 to achieve a rapid suppression of transmission.

Methodology/Principal findings: In-depth epidemiologic and entomologic assessments were performed in six sentinel communities (which had undergone 2 rounds of ivermectin treatment per year) and three extra-sentinel communities (which had undergone 4 rounds of ivermectin treatment per year). None of the 67,924 *Simulium ochraceum* s.l. collected from this focus during the dry season of 2011 were found to contain parasite DNA when tested by polymerase chain reaction-enzyme-linked immunosorbent assay (PCR-ELISA), resulting in an upper bound of the 95% confidence interval (95%-UICI) of the infective rate in the vectors of 0.06/2,000 flies examined. Serological assays testing for *Onchocerca volvulus* exposure conducted on 4,230 children 5 years of age and under (of a total population of 10,280 in this age group) revealed that 2/4,230 individuals were exposed to *O. volvulus* (0.05%; one sided 95% confidence interval = 0.08%).

Conclusions/Significance: The in-depth epidemiological and entomological findings from the Southern Chiapas focus meet the criteria for interruption of transmission developed by the international community.

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Introduction

Human onchocerciasis is caused by the filarial parasitic nematode *Onchocerca volvulus*, which in Latin America is transmitted by new world black flies (*Simulium* spp.) in six countries (Brazil, Colombia, Ecuador, Venezuela, Guatemala, and Mexico), where 525,548 individuals are at risk [1]. In Mexico, onchocerciasis was endemic in three distinct foci (Southern Chiapas, Northern Chiapas, and Oaxaca). The Southern Chiapas focus was the major focus in Mexico given its large size (12,579.70 km^2) and well-documented history of intense transmission. The Southern Chiapas focus contains 559 affected communities, 39 of which were hyperendemic for onchocerciasis before control efforts began, while 209 of the communities were mesoendemic, and 311 were hypoendemic. The population at risk in the Southern Chiapas focus (114,024 individuals) comprised about 21% of the total at-risk population in the Americas.

Since the 1990s, onchocerciasis control in Mexico has relied on the mass distribution of Mectizan (ivermectin) to the at-risk communities. Annual mass ivermectin distribution treating to all eligible residents from the at-risk communities began in 1994. In 1998 the strategy was modified to provide mass treatments every 6 months. In 50 communities from the Southern Chiapas focus, the ivermectin distribution frequency again shifted in 2003, from twice to four times a year. An additional 113 communities were added to the quarterly treatment regimen in 2009.

The goal of the Onchocerciasis Elimination Program for the Americas [1] is to eliminate new ocular morbidity caused by infection with *O. volvulus* and eventually eliminate transmission of the parasite in all 13 foci in Latin America. The World Health

Author Summary

The absence of infective larvae of *Onchocerca volvulus* in the black fly vector of this parasite and reduction of exposure to and new infections with *O. volvulus* are the criteria currently used to certify focal interruption of parasite transmission. In the present study, we report entomological and epidemiological assessments in the Southern Chiapas focus of Mexico that together indicates that transmission of *O. volvulus* has been interrupted in this focus. None of the *Simulium ochraceum* s.l. vector black flies collected from sentinel and extra-sentinel communities in this focus during the 2011 transmission season was found to contain parasite DNA when tested by PCR-ELISA, suggesting vector parasite contact was nearly nonexistent. In addition, there was a minimal exposure to the parasite in children 5 years of age and under, as measured by circulating antibody to a parasite-specific antigen. The Southern Chiapas focus was the major focus in Mexico and one of the largest in Latin America, with well-documented history of active transmission prior to the commencement of Mectizan mass distribution. This study demonstrates the interruption of transmission in geographically large focus in Latin America with a historically high intensity of transmission.

Organization (WHO) [2] and OEPA [3,4] have established a series of epidemiologic and entomologic criteria to be achieved to declare onchocerciasis eliminated. These include a reduction of new infections to an incidence rate of less than one new case per 1,000 individuals (<0.1%) [2,3] and an absence, or near absence, of infective-stage larvae of *O. volvulus* in the vector population (i.e., a rate of less than one infective fly per 1,000 parous flies). Practically, because polymerase chain reaction (PCR) using *O. volvulus*-specific DNA probes are generally applied to examine pools of flies, parity cannot be easily determined, so the threshold used is less than one infective fly per 2,000 flies tested (assuming a 50% parity rate) [2,3,4]. The data presented here report results of in-depth epidemiological and entomological studies conducted in 2007 through 2011 which, when taken together suggest that, based upon these criteria, that *O. volvulus* transmission has been interrupted in the Southern Chiapas focus.

Materials and Methods

Selection of communities and study area

In 1995, local health authorities selected six sentinel communities of the Southern Chiapas focus (Figure 1). These included Ampliación Las Malvinas (Escuintla municipality; 92°28′24″W, 15°20′36″N, elevation 1,000 masl), Estrella Roja and José María Morelos (Huixtla municipality; 92°28′48″W, 15°16′1.″N and 92°27′35″W, 15°13′48″N, elevation 660 and 1400 masl, respectively), Nueva Costa Rica (Mapastepec municipality; 92°48′46″W, 15°28′01.″N, elevation 600 masl), Nueva Reforma Agraria (Acacoyagua municipality; 92°45′04″W, 15°26′04″N, elevation 500 masl), and Primero de Mayo (Motozintla municipality; 15°14′31.″W, 92°18′04″N, elevation 1,300 masl). Following the addition of 50 communities to the quarterly treatment in 2003, two additional extra-sentinel communities were selected. These included Las Golondrinas (Acacoyagua municipality; 92°39′17″W, 15°26′06″N, elevation 920 masl) and Las Nubes II (Escuintla municipality; 92°31′49″W, 15°23′09″N, elevation 1,200 masl). In 2008, an additional extra-sentinel community was added; Nueva América (Huixtla municipality; 92°26′40″W, 15°.7′00″N, elevation 880 masl) (Figure 1).

These communities were the ones evaluated in this study. These communities have to varying extents been included in previous in-depth epidemiological evaluations (EEP) which have been carried out in the Southern Chiapas focus (Table 1). The EEPs in these communities have permitted the evaluation of the impact of mass treatment with ivermectin on transmission and have provided the baseline data employed to demonstrate that transmission has been interrupted.

Apart from the sentinel and extra-sentinel communities, a large-scale serological study was performed during 2010 on 4,230 children resident in 110 extra-sentinel communities with high historical endemicity. This sampled group represented 41% of all children five and under who were residents in these historically highly endemic communities.

In the Southern Chiapas focus, ivermectin distribution was commenced in 1990, but was initially only offered to registered onchocerciasis "clinical cases". In 1994, every eligible resident in the hyperendemic and mesoendemic communities was treated, but only 25% of the eligible residents from hypo-endemic communities were treated. From 1995 to 1997, the 40% of the eligible population in hypo-endemic communities was treated. Beginning in 1998, the entire eligible populations in all communities, regardless of their level of endemicity, were provided with semi-annual ivermectin treatments. In 50 communities of this focus, treatments were increased from twice to four times per year in 2003; the quarterly treatment regimen was extended to 113 communities in 2009. A total of 22 consecutive treatment rounds reaching >85% of the eligible population have been provided in the Southern Chiapas focus over the past 11 years. Ivermectin coverage of the eligible population has remained at a level greater than 85% every year from 1998 through 2011 (Figure 2).

Entomological survey

Black flies (*S. ochraceum* s.l) were collected in the sentinel and extra-sentinel communities using standardized procedures [5,6,7,8,9] during the peak *O. volvulus* transmission season, lasting from December to March. Collections were carried out during the first 50 minutes of each hour, beginning at 11:00 AM and ending at 4:50 PM [10].

Black flies were collected before they began feeding. The landing rate measured from the collections was taken as an estimate of the biting rate, although this probably overestimated the biting rate, because a proportion of the landing flies in a natural setting do not successfully obtain a blood meal. Thus, the transmission potential calculations provided below are likely to be overestimated by a factor proportional to the number of flies that land but do not bite.

Flies were combined into pools containing a maximum of 50 individuals per pool and the heads and bodies separated as previously described [5]. The separated head and body pools were tested for *O. volvulus* parasites by using a PCR assay specific for *O. volvulus*. Details of protocols for genomic DNA purification, primer sequences, PCR conditions, and detection of PCR products by enzyme-linked immunosorbent assay (ELISA) have been published elsewhere [5,11]. In brief, DNA extractions were carried out in sets of 20 samples each, with each set containing 18 fly pools and two sham extractions which served as contamination controls for the DNA extraction process. All PCRs were carried out in sets of 84 samples, in rows B–H of a PCR microtiter plate. Row A was reserved for 10 PCR-negative controls and two positive controls. One positive control contained the minimal amount of positive control DNA consistently detected by the PCR amplification conditions, as determined by an initial titration study. This control was carried out to ensure that all of the reactions were operating at

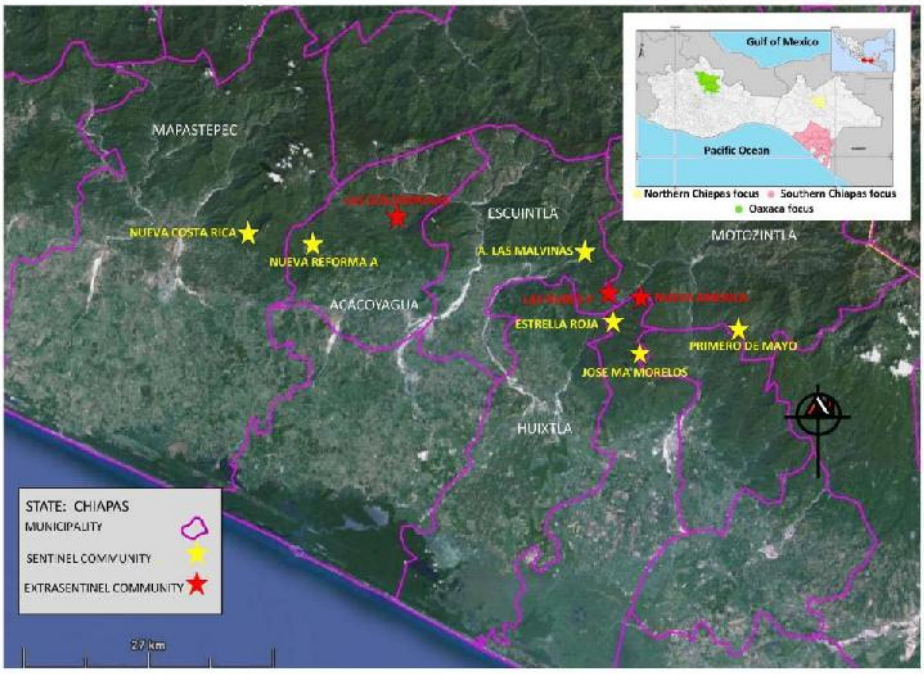

Figure 1. Map of the Southern Chiapas focus, México. The map of the Southern Chiapas focus shows the six sentinel (marked in yellow) and three extra-sentinel communities (marked in red) and the main rivers and tributaries that might serve as a source of black fly breeding. Map (right) of the Southern Mexico states showing the three onchocerciasis endemic foci.
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peak efficiency. The second positive control contained the same minimal amount of positive control DNA mixed with 2.5 μL of a DNA preparation from a fly pool that tested negative in a prior set of reactions. This control ensured that no inhibitors were present in the fly DNA preparations. Initial screenings focused on pools of bodies, as previous studies have shown that infection rates in bodies provide a more sensitive indicator of parasite-vector contact than testing heads [5,11]. All head pools were screened from any community found to have evidence of any vector-parasite contact based upon the body pool screens, providing an estimate of the prevalence of flies carrying infective larvae. PoolScreen v2.0 was used to estimate the prevalence of infected flies in the community and the associated 95% confidence intervals (CIs) [12].

Seasonal transmission potentials [STP] for each sentinel and extra-sentinel village were calculated as the product of the seasonal biting rate, the proportion of flies carrying L3 larvae in the transmission season (from December through March), and the average number of L3 larvae in each infective fly. As previously discussed, after multiple rounds of Mectizan treatment, the number of infective larvae present in each infective fly was assumed to be one [8,9,11].

The seasonal biting rate was calculated as the product of the geometric mean [13] of the number of flies collected per person per day and the total number of days in the transmission season, which included the months of December through March. The daily biting rate and the seasonal biting rate were estimated as

Table 1. In-depth epidemiologic assessments in sentinel and extra-sentinel communities of the Southern Chiapas focus.

Surveys	1995	1998	2000	2001	2004	2006	2008	2010	2011
Ophthalmological	x		x		x	x	x		
Parasitological	x	x	x		x	x	x		
Entomological				x	x	x	x	x	x
Serological					x	x	x	x	

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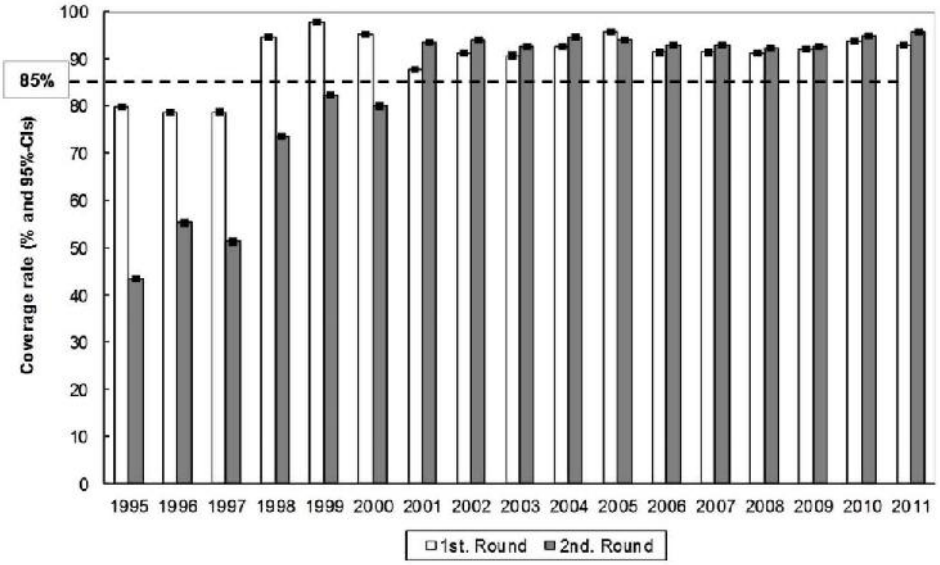

Figure 2. Coverage rate with ivermectin of the eligible population of the Southern Chiapas focus. The coverage rate, expressed in percent and the 95%-confidence intervals, CIs, surrounding point estimate, with ivermectin of the eligible population of the Southern Chiapas focus, 1995–2011. The line at 85% indicates the coverage needed in a sustained fashion to interrupt transmission.
doi:10.1371/journal.pntd.0002133.g002

previously described [8,9,14]. Because *S. ochraceum* s.l. females were not collected throughout the year, it was not possible to precisely calculate the annual transmission potential (ATP). However, given the paucity of vector black flies present outside the normal transmission season, the transmission potential outside of the peak transmission period is probably zero or near zero. The STP (transmission occurring during the peak transmission season of December through March) thus likely represented a fairly accurate estimate of ATP.

Serological survey

The prevalence of IgG4 antibodies to Ov16 [15,15], a recombinant antigen of *O. volvulus*, was determined from two populations of children in the Southern Chiapas focus: residents in the sentinel and extra-sentinel villages, and school children selected from the overall population sample in the focus. In 110 communities (including all previously mesoendemic and hypoendemic communities in the focus), blood spots were collected from 4,313 children 5 years of age and under. Sera from 4,230 children were screened for Ov16 antibodies.

Blood spots were collected by finger prick from each individual enrolled in the study, dried in the field, transported to the laboratory at 4°C, and kept refrigerated in sealed bags containing silica gel at −20°C until use, which occurred within a month of collection. Two 6-mm punches of blood saturated filter paper were placed in a phosphate-buffered saline-Tween (PBS-T) 0.05% and bovine serum albumin (BSA) 5% buffer and eluted overnight at 4°C. The elution was then run in duplicate in a standard ELISA [5], to detect IgG4 antibodies against the OV-16 recombinant antigen. A standard curve was used on each plate to identify positive samples and permit comparisons between plates and over days. The cut-off value was determined after analyzing OV-16 negative and OV-16 positive samples (from 10 parasitologically confirmed *O. volvulus* positive individuals). The cutoff was chosen as 40 arbitrary units by identifying the value that optimized both sensitivity and specificity. Any positive results were repeated before being reported as positive.

Ophthalmological survey

Ocular examinations were carried out by an ophthalmologist experienced in onchocerciasis ocular evaluations for OEPA. The examinations were done using a Topcon Optical SL-3D slit lamp (Kogaku Kikai KK, Tokyo, Japan). Exams focused on finding *O. volvulus* microfilariae in the cornea (MFC) and/or the anterior chamber of the eye (MFAC). Before the exam the patients kept a "head down position" (forehead in the lap) for 5 minutes to allow MFC and/or MFAC to settle in a visible position. In 2007 and 2008, a population of 1,418 and 326 residents, representing about 72% and 74% of the total population in the six sentinel and the three extra-sentinel communities were examined.

Parasitological survey

Nodulectomy campaigns have been undertaken in Mexico since 1932. Individuals that undergo nodulectomy received beneficial effects as their mf loads and skin pathology were reduced. Hence, nodulectomy was considered a routine treatment provided by the Mexican elimination program. The number of individuals with nodules of the Southern Chiapas focus was registered from 1995 through 2010. In parallel, the

number of new clinical cases (i.e., individuals diagnosed positive for nodules or skin microfilariae for the first time) were also registered from 1989 through 2010. The skin biopsies were taken from each patient using a 1.5- to 2.0-mm corneoscleral biopsy punch. Skin biopsies were incubated overnight in buffered saline, and emerging microfilariae were visualized and counted using an inverted microscope.

Ethics statement

The procedures were reviewed and approved by the Presidents of the Ethical and Bio-security Committees of the National Institute of Public Health (Cuernavaca, México) and the Health Secretariat of México (The city of México, D.F.). These are the equivalents of the Institutional Review Boards for Human Subject Research in the USA. Community meetings were held in all selected villages within the focus to explain the research procedures, and the right of each individual to decide whether or not to participate was explained. The individuals were also informed that they would be provided with the results of the tests upon request. Before each examination, each adult who had voluntarily come to the examination point and agreed to participate were provided with a capsule summary of the project and process and oral consent was obtained. Parents or guardians provided oral consent on behalf of all child participants. The Ethical Committee of the Health Secretariat of México approved the use of oral consent, given that the studies were conducted as part of the national onchocerciasis surveillance program and were therefore part of a routine public health monitoring program conducted by the Mexican government.

Community leaders were also consulted and approved the use of the selected locations in the village and river banks as vector catching points.

Statistical analysis

PoolScreen v2.0 was used to calculate a prevalence of infection and associated 95% CIs in the vector populations. The prevalence of infective flies was then combined with estimates of the biting rate (calculated from the fly collection data as described previously) to calculate an estimated STP. The proportion of individuals with mf in the cornea and/or anterior chamber of the eye was

Table 2. Entomological parameters in the focus of Southern Chiapas, México.

Community (year of entomologic study)	Seasonal biting rate	Prevalence of infective flies/2,000*	Seasonal transmission potential*
Sentinel communities (2008)			
Primero de Mayo	86,009	**0** (0.3)	0 (12.9)
Ampliación Malvinas	14,111	**0** (0.5)	0 (3.7)
Estrella Roja	14,628	**0** (0.4)	0 (2.9)
José María Morelos	102,020	**0** (0.4)	0 (18.4)
Nueva Costa Rica	38,733	**0** (0.4)	0 (7.8)
Nueva Reforma Agraria	35,690	**0** (0.4)	0 (7.9)
Extra-sentinel communities (2008)			
Las Golondrinas	81,701	**0** (0.3)	0 (12.3)
Las Nubes II	83,237	**0** (0.3)	0 (12.5)
Nueva América	37,246	**0** (0.3)	0 (9.7)
Other communities (2009–2010)			
Brasil	60,183	**0.3** (0–0.7)	**7.5** (0–21.1)
Mezquito	83,038	**0.4** (0.2–1.0)	**16.6** (8.3–42.3)
Coronado Santa Rita	51,720	**0** (0.4)	0 (10.3)
Loma Bonita	14,278	**0** (0.9)	0 (6.4)
Montaña	9,390	**0** (1.3)	0 (6.1)
La Granja	3,142	**0** (4.1)	0 (6.4)
La Soledad	10,311	**0** (1.1)	0 (5.7)
Sentinel communities (2010–2011)			
Estrella Roja	7,530	**0** (0.5)	0 (2.3)
José María Morelos	85,637	**0** (0.3)	0 (12.8)
Other communities and Coffee fincas (2010–2011)			
Brasil	58,732	**0** (0.3)	0 (8.8)
Mezquito	81,338	**0** (0.3)	0 (12.2)
Finca La Victoria	50,282	**0** (0.3)	0 (7.5)
Finca Santa Amalia	36,250	**0** (0.4)	0 (7.3)

The seasonal *S. ochraceum* s.l. biting rate is the number of bites per person per season. Prevalence of infective flies is expressed as rate per 2,000 flies examined and seasonal transmission potential is the third stage larvae per person per season. These entomological parameters were estimated during 2008 through 2011 in the sentinel, extra-sentinel and other communities in the focus of Southern Chiapas.
*Value represents point estimate and value in parentheses represents 95%-confidence intervals. CIs (95% upper limit CI when zero) surrounding point estimate.
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Table 3. Prevalence of IgG4 antibodies to Ov16 in the focus of Southern Chiapas, México.

Sentinel communities (2008)						
Primero de Mayo	Ampliación Malvinas	Estrella Roja	José María Morelos	Nueva Costa Rica	Nueva Reforma Agraria	**Total**
0/37 (0.0%)	1/71 (1.4%)	2/119 (1.7%)	1/87 (1.1%)	0/227 (0.0%)	0/135 (0.0%)	**4/676 (0.6%)**
Extra-sentinel communities (2008)						
Las Golondrinas	Las Nubes II	Nueva América				
0/100 (0.0%)	0/38 (0.0%)	3/133 (2.3%)				**3/271 (1.1%)**
Other communities (2009)						
Ranchería Las Marías	Barrio El Retiro	La Paz de Sabinos	Col Libertad el Pajal	Montecristo de Guerrero	S. Antonio Miramar	
0/50 (0.0%)	0/48 (0.0%)	0/106 (0.0%)	0/128 (0.0%)	0/452 (0.0%)	0/20 (0.0%)	
El Vergel	Plan de Ayala					
1*/122 (0.8%)	0/120 (0.0%)					**1*/1,046 (0.09%)**
Coffee fincas (2009)						
Finca Santa Amalia	Finca Victoria	Finca Santa Fé				
2/224 (0.9%)	1/456 (0.2%)	0/143 (0.0%)				**3/823 (0.4%)**
110 communities within 4 districts (2010)						
Comitán	Villaflores	Tapachula	Tonalá			
0/918 (0.0%)	0/1,338 (0.0%)	2/1,869 (0.1%)	0/105 (0.0%)			**2/4,230 (0.05%)**

Prevalence of IgG4 antibodies to Ov16 in children <10 years of age and under from sentinel, extra-sentinel, and eight other communities, in migrant workers >15 years of age from three coffee fincas and in children 5 years of age and under from 4 districts in the focus of Southern Chiapas, México.
*Skin biopsies were taken and tested by PCR; they contained no parasite DNA (unpublished data).
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calculated as the number of positive individuals divided by the total number examined, and expressed as a percentage. The associated 95% exact CIs of the proportion of individuals harboring Ov16 antibodies from the large-scale serology study were determined using the method of Thompson [17]. The method of Miettinen (1970), as described in Armitage and Berry [18] was used to estimate the 95% exact CIs surrounding the point prevalence of Ov16 antibodies, MFC and MFAC of the sentinel and extra-sentinel communities.

Results

Entomological survey

In 2008, a total of 103,610 *S. ochraceum* s.l. (68,616 in the sentinel and 34,994 in the extra-sentinel communities) were subjected to analysis by PCR. The number of flies tested in each community was sufficient to comply with the WHO guideline of having at least 10,000 flies tested from each community. The results are summarized in Table 2. The point estimate of the prevalence of

Table 4. Total population and individuals examined by parasitology and ophthalmology in Southern Chiapas focus.

Community	Total population/Examined by parasitology/Examined by ophthalmology					
	1995	1998	2000	2004	2006	2008
Sentinel communities						
Primero de Mayo	213/175/80	226/118/ND	211/108/67	178/150/104	153/ND/103	137/126/ND
Ampliación Malvinas	184/120/85	204/139/ND	231/135/28	214/160/108	206/ND/96	227/199/ND
Estrella Roja	248/179/100	306/40/ND	351/146/ND	330/263/170	304/ND/234	305/264/ND
José María Morelos	358/138/75	358/181/ND	406/95/163	360/228/181	329/ND/221	322/305/ND
Nueva Costa Rica	542/520/276	722/305/ND	759/409/396	667/635/433	663/ND/500	729/653/ND
Nueva Reforma Agraria	305/233/135	372/254/ND	378/297/188	343/322/203	318/ND/234	385/340/ND
Extra-sentinel communities						
Las Golondrinas				303/285/186	323/317	333/325/273
Las Nubes II				137/111/73	118/105	121/113/95
Nueva América						439/386/326

The number of communities, number of people in total, and number of people examined by parasitology and ophthalmology in the sentinel and extra-sentinel communities of the Southern Chiapas focus, México.
ND = No data.
doi:10.1371/journal.pntd.0002133.t004

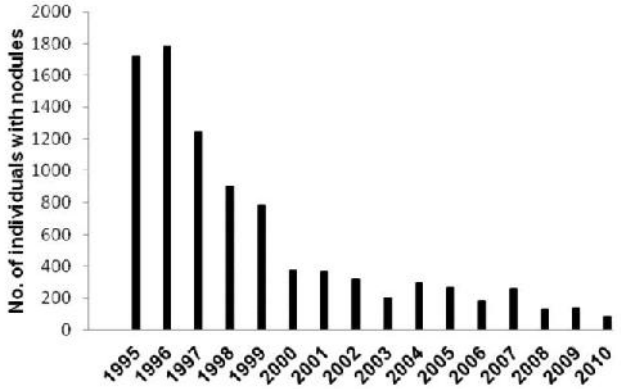

Figure 3. Number of individuals with nodules in the Southern Chiapas focus, Mexico, 1995–2010.
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infective flies and the associated 95%-upper limit confidence interval (ULCI) in all communities were both well below the threshold of 1/2,000 (maximum 0.07/2,000 and 0.1/2,000 flies examined, respectively).

In 2011, follow-up entomologic studies were carried out (Table 2). Two sentinel communities (José María Morelos and Estrella Roja) were included in the follow up study. A total of 67,924 flies were collected and tested from these two communities, and all body pools were negative for *O. volvulus* DNA. Similarly, flies were collected in two communities (Brasil and Mexiquito) where evidence of *O. volvulus* DNA in the vector was seen in entomological studies carried out in 2009–2010. None of the pools collected in 2011 were positive (Table 2). Finally, flies were collected and tested from two coffee fincas (La Victoria and Santa Amalia); these were also all found to be negative for parasite DNA (Table 2). Taken together, these data suggested no parasite-vector contact was occurring throughout the entire focus. The 95%-ULCI surrounding point prevalence of infective flies in all areas was well below the threshold of 1/2,000 (maximum 0.06/2,000 flies examined). The upper bound of the 95% CI for the STPs ranged from 0.0 to 1.0 L3 per person per season.

Serological survey

The results of the serological surveys are shown in Table 3. In 2010, only two children 5 and under from 110 communities within the focus were positive for Ov16 IgG4 antibodies out of a total of 4,230 children examined (point prevalence = 0.05%). The upper bound of the one sided 95% confidence interval (calculated taking into account that a large proportion of a finite population had been sampled) was 0.08%.

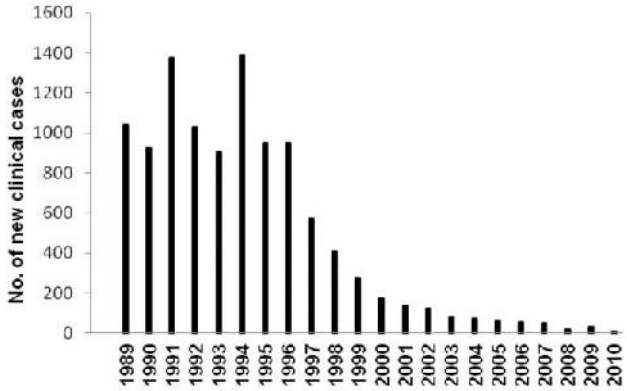

Figure 4. Number of new clinical cases of onchocerciasis in the Southern Chiapas focus, México. The number of new clinical cases of onchocerciasis (individuals diagnosed positive to Mazzotti reaction, nodules, or skin mf for the first time) in the Southern Chiapas focus, from 1989 to 2010.
doi:10.1371/journal.pntd.0002133.g004

Ophthalmological survey

No microfilariae in the cornea were observed; two individuals harboring microfilariae in the anterior chamber of the eye were identified in 1,744 residents examined during 2007 and 2008 in the sentinel and extra-sentinel communities, (point prevalence 0.07%; 95% CI = 0.001–0.4%; Table 4).

Parasitological survey

A steady decrease of the number of individuals harboring nodules in the Southern Chiapas focus was recorded in the 1995–2010 period (Figure 3). There was also a dramatic variation in the number of new clinical cases (i.e., individuals diagnosed positive for nodules or skin microfilariae for the first time) from 1,041 individuals identified with nodules or skin microfilariae in 1989 to just 9 such individuals in 2010 (Figure 4).

Discussion

The epidemiological and entomological parameters presented in this study strongly suggest that transmission of *O. volvulus* has been interrupted in the Southern Chiapas focus. Of the communities examined in this paper, no entomological evidence for ongoing transmission was detectable when the studies reported were completed in 2011. In Las Golondrinas and José María Morelos, where pre-control entomological data were available, Mectizan treatment has reduced transmission by greater than 99% when compared with the seasonal transmission potential of about 20 L3s per person per year that existed prior to initiating Mectizan treatment [5,7,19]. This meets the criterion proposed by WHO for a "near absence" of transmission for areas where pre-treatment data on transmission exist.

Given that pre-treatment data for the level of transmission do not exist for most other communities within this focus, it is not possible to quantify the effect of Mectizan on transmission in most foci as is possible for Las Golondrinas. However, as mentioned in the introduction, OEPA has set a threshold of less than one infective fly per 2,000 flies tested as the current criterion for interruption of transmission [2,3,4]. In 2008, the upper limit of the 95% confidence interval of the infective rate varied from 0.3 to 0.5 infective flies/2,000 examined in the sentinel and extra-sentinel communities, which is well below OEPA's criterion. This situation remained unchanged in the four communities (two of which are sentinels) that were re-tested in 2011 (ou average = 0.06 infective flies/2,000 tested), indicating that transmission remained interrupted in this focus.

In addition to the 1/2,000 infective fly threshold, OEPA recommends the use of Annual transmission potential (which in the present situation is equivalent to STP) to assess the status of onchocerciasis transmission, because these metrics take into account the biting rate and the prevalence of infective flies. Estimates of the ATP necessary to maintain the parasite population (the transmission breakpoint) range from 5 to 54 L3/person/year using mathematical modeling [20] and from 7.6 to 18 L3/person/year using field observations [2,21]. All point estimates of STP were zero, and the 95%-ULCI of potential STPs in all of the communities examined were at or below the estimated transmission breakpoint (2.3–18.4, Table 2). This suggests that if conditions remain unchanged, the parasite population is likely to be on the path to local extinction.

In 2010, a detailed assessment of the prevalence of *O. volvulus* antibodies in children in the Southern Chiapas focus was conducted, assessing over 40% of the population of children of age 5 and under who were residents of the afflicted communities. Only two children harbored Ov16 antibodies from 4,230 blood spot samples examined leading to a point estimate of exposure of 0.05% and a one-sided 95% confidence interval on the estimate of the prevalence of exposure of 0.08%, which was below the cutoff established by OEPA of 0.1%.

Additional data suggest that that clinical onchocerciasis has also been eliminated in the Southern Chiapas focus. A total of 1,719 individuals were identified with nodules in this focus in 1995; this declined to 82 individuals in 2010 (Figure 3). This decline in nodule rates was mirrored in the near absence of new clinically defined cases of onchocerciasis from reports of the local health officials in the Southern Chiapas focus during the last three years. Only nine new clinical cases (i.e., individuals diagnosed positive for nodules or skin microfilariae for the first time) were reported in 2010 (Figure 4). This finding suggests that endemic onchocerciasis no longer represents a serious health risk to the endemic community in Southern Chiapas focus.

Supporting Information

Checklist S1 STROBE Checklist.
(DOCX)

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Author Contributions

Conceived and designed the experiments: MARP ADV TRU. Performed the experiments: HKH KBRM ICRL FGPV. Analyzed the data: MARP ADV. Contributed reagents/materials/analysis tools: JTAJ MFOA. Wrote the paper: MARP TRU.

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Interruption of Transmission of *Onchocerca volvulus* in the Oaxaca Focus, Mexico

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Abstract. All endemic communities of the Oaxaca focus of onchocerciasis in southern Mexico have been treated annually or semi-annually with ivermectin since 1994. In-depth epidemiologic assessments were performed in communities during 2007 and 2008. None of the 52,632 *Simulium ochraceum* s.l. collected in four sentinel communities was found to contain parasite DNA when tested by polymerase chain reaction-enzyme-linked immunosorbent assay (PCR-ELISA), resulting in an upper bound of the infection rate in the vectors of 0.07/2,000. The prevalence of microfilariae (mf) in the cornea and/or anterior chamber of the eye was also zero (0 of 1,039 residents examined; 95%-UL = 0.35%). Similarly, all 1,164 individuals examined by skin biopsy were mf negative (95%-UL = 0.31%), and sera collected from 3,569 children from 25 communities did not harbor Ov16 IgG4 antibodies (95%-UL = 0.09%). These meet the criteria for absence of morbidity and parasite transmission in the Oaxaca focus. As a result mass treatments with ivermectin were halted in 2009.

INTRODUCTION

Human onchocerciasis is caused by the filarial parasitic nematode *Onchocerca volvulus*, which in Latin America is transmitted by new world black flies (*Simulium* spp.) in six countries (Brazil, Colombia, Ecuador, Venezuela, Guatemala, and Mexico), where 525,543 individuals are at risk.[1] In Mexico, onchocercerciasis occurs in three distinct foci (Southern Chiapas, Northern Chiapas, and Oaxaca) where *S. ochraceum* s.l. is the main vector. The Oaxaca focus contains 98 affected communities, none of which are hyperendemic (11 of the communities are mesoendemic, and 87 hypoendemic). The population at risk in Oaxaca (44,919 individuals) comprises about 10% of the total at risk population in the Americas (525,543 individuals). The predominant inhabitants of the focus are indigenous people of the Zapoteco, Chinanteco, and Cuicateco ethnic groups. The most important economic activity in the communities of the Oaxaca focus is coffee cultivation.

Introduction of the parasite into the Oaxaca focus probably resulted from human movements from Oaxaca to and from the endemic areas of Chiapas or Guatemala during religious pilgrimages to Esquipulas.[2] Historically, the first cases of onchocerciasis were discovered in 1924 in the community San Miguel Tiltepec in the Oaxaca focus.[3] Since then, there have been continuous efforts by residents of communities, operational workers, health authorities, and researchers to control the disease. In 1927, the first parasitological studies based on the analysis of nodules were performed in Oaxaca. In 1931, an onchocerciasis control program was launched based on mass identification and removal of onchocercomas, which are subcutaneous masses containing the adult worms. In 1947, Dr. Luis Mazzotti discovered the utility of diethylcarbamazine (DEC) for the diagnosis and treatment of onchocerciasis, and DEC treatments of patients was added to the nodulectomy program from 1948 through to the 1980s. Since the 1990s, onchocerciasis control in Mexico has relied on the mass distribution of Mectizan (ivermectin) to the at-risk communities. Annual mass ivermectin distribution treating to all eligible residents from the at-risk communities began in 1994, and in 1997 the strategy was modified to provide mass treatments every 6 months.

The goal of the Onchocerciasis Elimination Program for the Americas (OEPA) is to eliminate new ocular morbidity caused by infection with *O. volvulus* and interrupt transmission of the parasite by the year 2012. Ultimately, the goal of the program is to eliminate the parasite transmission in all affected countries of the region, which requires a 3-year period of surveillance after treatment interventions have stopped to verify no recrudescence of transmission occurs. The World Health Organization (WHO)[4] and OEPA[5] have established a series of epidemiologic and entomologic criteria to be achieved to declare onchocerciasis eliminated. World Health Organization/OEPA criteria include: 1) the elimination of new ocular morbidity (defined as a prevalence of < 1% of *O. volvulus* microfilariae [mf] in the cornea and/or anterior chamber of the eye), and 2) transmission criteria related to human epidemiological and vector entomological indices. A reduction of new infections to an incidence rate of less than one new case per 1,000 individuals (< 0.1%)[4] has been practically defined as lack of specific antibodies to *O. volvulus* in children.[6] The sample size required to calculate a one-sided 95% confidence interval (CI) for a point prevalence that excludes 0.1% is 3,000 children. WHO/OEPA entomological criterion for interruption of transmission is to show the absence, or near absence, of infective-stage larvae of *O. volvulus* in the vector population (i.e., a rate of less than one infective fly per 1,000 parous flies). Practically, because polymerase chain reaction (PCR) using *O. volvulus*-specific DNA probes are generally applied to examine pools of flies, parity cannot be determined, so the threshold used is less than one infective fly per 2,000 flies tested (assuming 50% of these are parous flies).[7,8] A minimum sample size of 10,000 flies is required to be examined by PCR collected per each monitored community to reach this standard.

The data presented here report the in-depth epidemiological follow-up study conducted throughout 2008 necessary to declare onchocerciasis ocular morbidity eliminated and transmission interrupted in the Oaxaca focus, following the criteria for elimination established by WHO/OEPA. As a result of these data, health authorities decided to halt treatments

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with ivermectin in 2009, and subsequently initiate the post-treatment surveillance activities in this focus.

MATERIALS AND METHODS

Study area. In 1995, local health authorities selected four sentinel communities of the Oaxaca focus (Figure 1). They were La Esperanza (17°37′40″N, 96°22′10″W, elevation 1,600 m above sea level), Santiago Lalopa (17°25′47″N, 96°14′54″W, elevation 1,200 m), Santiago Teotlaxco (17°26′45″N, 96°19′14″W, elevation 1,225 m), and Santa María La Chichina (17°26′23″N, 96°17′80″W, elevation 1,360 m). In addition to the study of the sentinel communities, a large-scale serological study was performed in children resident in 21 extra-sentinel communities of highest historical endemicity.

Annual mass ivermectin distribution, offered to every eligible resident in the 30 communities in the Oaxaca focus, was initiated in 1994; from 1997 to 2008 mass treatments were provided twice a year. A total of 26 treatment rounds have been provided in Oaxaca over the last 13 years. As shown in Figure 2, ivermectin coverage of the eligible population has remained at a level of greater than 85% every year from 2001 throughout 2008.

The consistently high coverage on the eligible population in the Oaxaca focus is the result of several favorable factors. First, the onchocerciasis program is well established, having been initiated in the 1930s, and throughout the intervening period has consistently maintained a staff of workers exclusively dedicated to onchocerciasis control. Staff members are organized into brigades, and they visit their assigned communities every 3 months. Thus, the brigades become very familiar with their assigned communities, maintaining a detailed census of the residents. Second, ivermectin distribution is regularly performed at a central location in the community. If someone in the community does not attend the distribution event, a home visit is then conducted by the brigades to attempt to convince the person to undergo treatment. Finally, ivermectin distribution has always been accompanied with health education activities. These are directed toward preserving the interest and participation in the community by emphasizing the benefits associated with ivermectin treatment to the individual, for the individual, and to the community.

Entomologic study. Black flies (52,632 *S. ochraceum*) were collected by using standardized procedures[6–8] during the peak *O. volvulus* transmission season lasting from December 2007 to

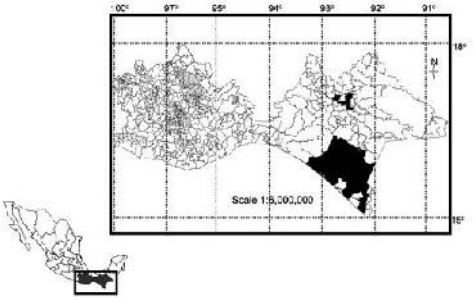

FIGURE 1. Map of the two southern Mexico states (marked in dark grey) showing the three endemic foci for onchocerciasis: The gray area indicates the Oaxaca focus, and the black areas indicate the Northern and Southern Chiapas foci.

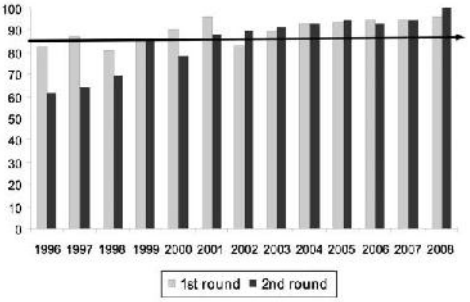

FIGURE 2. Coverage rate (percent) with ivermectin of the eligible population of the Oaxaca focus, Mexico, 1996–2008. The line at 85% indicates the coverage needed in a sustained fashion to interrupt transmission.

March 2008. In 2007, mass ivermectin distribution was conducted just before the peak transmission began. The collections were carried out during the first 50 minutes of each hour, beginning at 11:00 AM and ending at 4:50 PM.[9] Collectors received ivermectin 1 week before beginning the collection process. This procedure was reviewed and approved by the Ethics and Biosecurity Committee of the National Institute of Public Health of the Health Secretariat of Mexico (Cuernavaca, Mexico).

Black flies were collected before they began feeding. The landing rate measured from the collections was taken as an estimate of the biting rate, although this probably overestimated the biting rate, because a proportion of the landing flies in a natural setting do not successfully obtain a blood meal. Thus, the transmission potential calculations provided below are likely to be overestimated by a factor proportional to the number of flies that land but do not bite.

Flies were combined into pools containing a maximum of 50 individuals per pool and the heads and bodies separated as previously decribed.[6] The separated head and body pools were tested for *O. volvulus* parasites by using a PCR assay specific for *O. volvulus*. Details of protocols for genomic DNA purification, primer sequences, PCR conditions, and detection of PCR products by enzyme-linked immunosorbent assay (ELISA) have been published elsewhere.[9,10] The DNA extractions were carried out in sets of 20 samples each, with each set containing 18 fly pools and two sham extractions that served as contamination controls for the DNA extraction process. All PCRs were carried out in sets of 84 samples, in rows B–H of a PCR microtiter plate. Row A was reserved for 10 PCR-negative controls and two positive controls. One positive control contained the minimal amount of positive control DNA consistently detected by the PCR amplification conditions, as determined by an initial titration study. This control was carried out to ensure that all of the reactions were operating at peak efficiency. The second positive control contained the same minimal amount of positive control DNA mixed with 2.5 μL of a DNA preparation from a fly pool that tested negative in a prior set of reactions. This control ensured that no inhibitors were present in the fly DNA preparations.

The infected proportion in the vector population was calculated from the proportion of body (thorax plus abdomen) pools positive in the PCR assay and this proportion expressed as the number of positive flies per 2,000 flies examined. Head pools were not analyzed from the four sentinel villages in

which no evidence for infection in the vector bodies was found, as infection in bodies has previously been shown to be a more sensitive indicator of parasite vector contact than infection in heads.[9,10] Thus, if no bodies were found to be positive, it was assumed that no parasite vector contact was detected, and that the prevalence of infectious flies (i.e., flies with infective stages in the head capsule) would be zero. All of the body pools collected from that community was screened, and PoolScreen version 2.0 was used to estimate the prevalence of infected flies in the community and the associated 95% CIs.[11]

Seasonal transmission potentials (STP) for each sentinel village were calculated as the product of the seasonal biting rate, the proportion of flies carrying L3 larvae in the study season (from December 2007 through March 2008), and the average number of L3 larvae in each infective fly. As previously discussed, we assumed that after multiple rounds of Mectizan treatment, the number of infective larvae present in each infective fly would be close to one.[10]

The seasonal biting rate was calculated as the product of the geometric mean[12] of the number of flies collected per person per day and the total number of days in the transmission season, which included the months of December through March. The daily biting rate and the seasonal biting rate were estimated as previously described.[13] Because *S. ochraceum* s.l. females were not collected throughout the year, it was not possible to precisely calculate the annual transmission potential (ATP). However, in the Oaxaca focus, the level of transmission estimated during the peak of greatest transmission in 2008 was very low (because of the effect of 13 years of treatment with Mectizan). Therefore, the value of transmission potential outside of the peak transmission period is probably zero or near zero. The STP (transmission occurring during the peak transmission season of December through March) likely represents a fairly accurate estimate of ATP.

Serologic study. The prevalence of IgG4 antibodies to Ov16,[14,15] a recombinant antigen of *O. volvulus*, was determined from two populations of children in the Oaxaca focus: those residents in the four sentinel villages, and school children selected from the overall population sample in the focus. All 242 children 10 years of age and under living in the four sentinel communities were tested. In 21 "extra-sentinel" communities (including all previously mesoendemic communities in the focus) 3,327 school children were also screened. In our study, we included 36 schools that were randomly selected out of 91 schools present in the focus. On average, there were 105 children per school. To recruit participants, explanatory meetings were held with all the members of the school community—parents, teachers, and administrators who provided the lists of all the students enrolled. Considering that not all of the school children participated, the final number of children included was 3,327.

Blood was collected by finger prick from each individual enrolled in the study and dried in the field, transported to the laboratory at 4°C, and kept refrigerated in sealed bags containing silica gel at −20°C until use, within a month of collection. Two 6-mm punches of blood saturated filter paper were placed in a phosphate-buffered saline-Tween (PBS-T) 0.05% and bovine serum albumin (BSA) 5% buffer and eluted overnight at 4°C. The elution was then run in duplicate in a standard ELISA,[3] to detect IgG4 antibodies against the OV-16 recombinant antigen. A standard curve was used on each plate to identify positive samples and permit comparisons between plates and over days. The cut-off value was determined after analyzing OV-16 negative and OV-16 positive samples (from 10 parasitologically confirmed *O. volvulus* positive individuals). The cutoff was chosen as 40 arbitrary units by identifying the value that optimized both sensitivity and specificity. Any positive results were repeated before being reported as positive.

Ophthalmologic study. Ocular examinations were carried out by an ophthalmologist experienced in onchocerciasis ocular evaluations for OEPA. The examinations were done using a Topcon Optical SL-3D slit lamp (Kogaku Kikai KK, Tokyo, Japan). Exams focused on finding *O. volvulus* microfilariae in the cornea (MFC) and/or the anterior chamber of the eye (MFAC). Before the exam the patients kept a "head down position" (forehead in the lap) for 5 minutes to allow MFC and/or MFAC to settle in a visible position. A population of 1,039 residents, representing about 80% of the total population in the four sentinel communities, was examined.

Parasitologic study. A total of 1,164 individuals, representing 89% of the total population in the sentinel communities, participated in the survey. Two simultaneous skin biopsies were taken from each patient using a 1.5–2.0-mm corneoscleral biopsy punch, one from the left supra-scapular region and the right supra-iliac region. Skin biopsies were incubated overnight in buffered saline, and emerging mf was counted using an inverted microscope.

Statistical analysis. PoolScreen version 2.0 was used to calculate a prevalence of infection in the fly vector populations, together with the associated 95% CIs. The prevalence of infective flies was then combined with estimates of the biting rate (calculated from the fly collection data as described previously) to calculate an estimated STP. *Simulium ochraceum* s.l. were collected during the peak transmission period of December 2007 through March 2008 from four sentinel communities in the Oaxaca focus endemic for *O. volvulus*. The proportion of individuals positive to infection with mf in skin snips, and in the cornea and/or anterior chamber of the eye, was calculated as the number of positive individuals divided by the total number examined and expressed as a percentage. The associated 95% exact CIs of the proportion of individuals harboring Ov16 antibodies were determined using the method of Miettinen (1970), as described in Armitage and Berry.[16] The same method was used to estimate the 95% exact CIs surrounding the point prevalence of MFC, MFAC, and skin mf.

RESULTS

Entomologic study. A total of 52,632 flies were examined by PCR in 1,412 pools (La Esperanza: $N = 343$, Santa Maria La Chichina: $N = 356$, Santiago Teotlaxco: $N = 367$, and Santiago Lalopa: $N = 346$). The number of vectors collected was sufficient to comply with the WHO/OEPA guideline

TABLE 1

Prevalence of infective flies (expressed as rate per 2,000 flies examined) and seasonal transmission potential (third-stage larvae per person per season) estimated during 2008 in four sentinel communities in the focus of Oaxaca, Mexico*

Community	Seasonal biting rate	Prevalence of infective flies/2,000	Seasonal transmission potential
La Esperanza	48,427	0 (0.27)	0 (6.5)
Santa Maria la Chichina	31,836	0 (0.29)	0 (4.6)
Santiago Teotlaxco	32,686	0 (0.31)	0 (5.1)
Santiago Lalopa	72,794	0 (0.27)	0 (9.8)
Total	120,772	0 (0.07)	0 (4.2)

*Value represents point estimate and value in parentheses represents 95% upper limit confidence interval surrounding point estimate.

TABLE 2

Prevalence of IgG4 antibodies to Ov16 in four sentinel communities and 21* extra-sentinel communities in the focus of Oaxaca, Mexico

Santiago Lalopa	Santiago Teotlaxco	La Esperanza	Santa María La Chichina	Extra-sentinel communities*	Total
0/92 (3.92%)	0/86 (4.1%)	0/20 (15.8%)	0/44 (8.0%)	0/3,327 (1.5%)	0/3,569 (0.09)

*San Juan Yaee, San Bartolomé Yatoni, Teloxtlaco de Castro, San Juan Teavelo, San Pedro Teutila, San Juan Zapotitlán, San Pedro Stgo. papa, San Juan Zapotitlán, San Felipe de León, Villa Alta, Arroyo de Barco, Santiago Progreso, Talaeste de Zaragoza, San Isidro Reforma, Santa María Zoogochi, Santo Domingo Roayaga, Lachiroag, Ayxchiapeo, San Juan Yatzona, San Juan Yaluhui, and San Marcos Yelta.

Value represents point prevalence and value in parentheses represents 95% upper limit confidence interval surrounding point estimate.

of having at least 10,000 flies tested from each community. The results are summarized in Table 1. All body pools were negative for *O. volvulus* DNA, which suggested no parasite-vector contact was occurring. For this reason, head pools from these communities were not examined. Upper limit confidence limits of prevalence of infective flies in all areas were well below the threshold of 1/2,000 (maximum 0.31/2,000). Upper confidence interval limits of the STPs ranged from 4.6 to 9.8 L3 per person per season (overall, the maximum value of the STP in Oaxaca was 4.2 L3 per person per season).

Serologic study. The results of the serological studies are shown in Table 2. No child among a total of 3,569 examined in the four sentinel communities and the 21 extra sentinel communities was positive for Ov16 IgG4 antibodies (95%-UL = 0.09%).

Ophthalmologic study. No MFC or MFAC were found among the 1,039 residents (95%-UL = 0.35%) in the four sentinel communities.

Parasitologic study. None of the 1,164 residents examined by skin biopsy were found to have mf (95%-UL = 0.31%) in the four sentinel communities (Table 3).

DISCUSSION

The epidemiologic parameters of transmission and morbidity presented in this study strongly suggest that transmission of *O. volvulus* is permanently interrupted and that neither ocular nor skin disease is now attributable to the infection in the Oaxaca focus. Regular semi-annual treatment rounds with high coverage were very important to reach the OEPA's goal of interruption of transmission and the likely elimination of onchocerciasis in this area. Likewise, the regional program based on ivermectin has already made significant progress for elimination in other nearby foci including the focus of Northern Chiapas in Mexico, the foci of Santa Rosa, Huehuetenango, and Escuintla-Guatemala in Guatemala, and that of López de Micay in Colombia, where transmission has also been judged to have been interrupted.[5,17–21]

The entomological criteria for asserting interruption of transmission includes absence or near absence of infective-stage larvae of *O. volvulus* in the vector population, which has been operationally defined as < 1 infective fly/2,000. Martin-Tellaeche and others[22] showed that consecutive treatment with ivermectin resulted in a dramatic decrease of the prevalence of skin mf and nodules in Oaxaca. Despite this promising finding in the human population, during the first large-scale entomologic study of transmission in this area conducted in 2001 after 7 years of mass administration of ivermectin, three of four sentinel communities still had evidence for ongoing transmission.[10] During the second large-scale entomologic study carried out in this area, conducted in 2004 and after 10 years of the ivermectin program, parasite DNA was detected in one single pool of 50 vector heads out of 170 such pools (8,500 flies) examined in one of the sentinel communities. No evidence for transmission was found in the other communities in a total of 13,650 flies' examined.[23]

In addition to the 1/2,000 infective fly threshold, OEPA recommends the use of the ATP, (which in the present situation is equivalent to seasonal transmission potential) to assess the status of onchocerciasis transmission, because both of these measurements take into account the biting rate and the prevalence of infective flies. Annual transmission potential encompasses not only the vector competence and vector capacity, but also frequency of feeding on humans, longevity, biting density, how often it feeds, the size of the mf reservoir (prevalence, density, etc.). Unfortunately, the threshold ATP under which transmission does not occur is controversial. The estimated threshold ATP has ranged from 5 to 54 L3/person/year using mathematical modeling[24] and from 7.6 to 18 L3/person/year using field observations.[4,25] All 95%-UL of potential STPs in the four sentinel villages were within this range (4.6–9.8, Table 1) during 2008, which suggests that if conditions remain unchanged, the parasite population is likely to be on the path to elimination. It should be noted that these are overestimated STPs because our calculations used landing rate as a proxy for biting rate, whereas only a proportion of the flies that land would actually be successful in obtaining a blood meal.

During the first serologic studies in the sentinel communities, carried out in 2001, IgG4 antibodies to Ov16 were detected by using an unmarketed immunochromatographic card test (ICT, AMRAD, Sydney, Australia[26]) in a population of 210 participating persons. All 192 children (16 years of age and under) were negative, but 7/17 adults (41%) (17 years of age and over) harbored antibodies (Onchocerciasis Program in Oaxaca,

TABLE 3

Population (no. examined), prevalence of microfilaria in the cornea (MFC), and/or microfilariae (mf) in the anterior chamber of the eye (MFAC), and skin mf (by skin biopsy) in four sentinel communities in the focus of Oaxaca, Mexico*

Community	Population (no. examined)	Prevalence (%) of MFC and/or MFAC	Population (no. examined)	Prevalence of skin mf
La Esperanza	157 (112)	0 (3.23)	157 (121)	0 (2.9)
Santa María la Chichina	323 (225)	0 (1.6)	323 (238)	0 (1.5)
Santiago Teotlaxco	378 (307)	0 (1.19)	378 (359)	0 (0.97)
Santiago Lalopa	454 (395)	0 (0.92)	454 (446)	0 (0.8)
Total	1312 (1039)	0 (0.35)	1312 (1164)	0 (0.31)

Value represents point prevalence and value in parentheses represents 95% upper limit confidence interval surrounding point estimate. MFC = microfilariae in cornea; MFAC = microfilaria in anterior chamber.

unpublished data). In addition to ICT testing, 1,133 individuals of both sexes and ages participated in a seroconversion study using a tricocktail of recombinant antigens (OvMBP16, OvMBP11, and OvMBP10).[23] A cohort of 117 children 10 years of age and under, which were negative to antibodies in 2001, were re-tested in 2004 and none had seroconverted, which resulted in an estimated past exposure incidence of 0%.[23]

Subsequent serologic studies carried out in 2004 and 2008 also showed that 267 children 10 years of age or under did not harbor Ov16 antibodies (Onchocerciasis Program in Oaxaca, unpublished data and this work). This testing represented a serosurvey that encompassed all children from the sentinel communities. In 2008, we conducted a larger-scale serologic study to comply with the WHO requirements and reach the required statistical power to exclude 0.1% prevalence. We excluded children of preschool age (5 years of age and less) who are more difficult to access and unlikely to be infected or exposed in low transmission settings, the current situation in the Oaxaca focus. Because the durability of the Ov16 antibody is unknown, at the other end of the age spectrum adults greater than 20 years of age are likely to have antibody from previous exposure or infection. School-aged children seemed best to test, because the rate of new and detectable seroconversions is likely to rise most rapidly from 5 to 20 years of age.[3]

It is useful to consider previous impact study results from the Oaxaca focus. The number of individuals with nodules in the entire Oaxaca focus was *circa* 400 individuals in 1981, however, the last three individuals with nodules were seen in 1999 (Figure 3). The prevalence of MFAC and/or MFC in the four sentinel communities of the Oaxaca focus dropped from 1.7% to 4.1% in 1995–2000 to 0% in 2004–2008 (Figure 4). The Mectizan program also had a significant impact on the prevalence of skin mf (Figure 5). These results are similar to those observed in other endemic areas,[5,8,28] showing the strong effect of Mectizan on skin and eye mf, thus preventing later severe ocular and dermic pathologies.

Although migration of infected flies from neighboring communities, such as San José Yaee, Santa Ma. Yaviche, Santiago Yagayo, Santa Cruz Yagavila, San Juan Yagila, and Santa Ma. Zoogochi, could have occurred, this mechanism of re-introduction of infection is unlikely. The only communities located within the flight range of the vector (around 3.5 km)[29] have also been receiving ivermectin treatment at the same coverage levels as those of the sentinel communities, and consequently, the level of infection in migrating flies from these communities is likely to be similar to that in the local population. Similarly, the threat of infection from the focus of Southern and Northern Chiapas, the most proximal focus to Oaxaca is extremely small, given that these foci are separated by 540 km and 380 km (Figure 1), a distance that is over 100 times that of the flight range of the vector black fly.

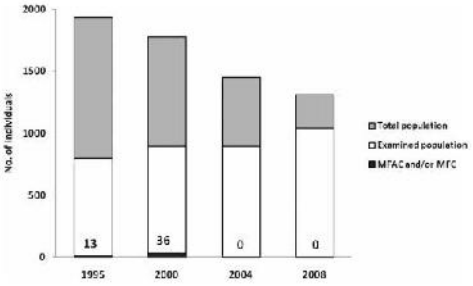

FIGURE 4. Overall and examined population for microfilariae (mf) in the cornea and/or anterior chamber of the eye in participants from the four sentinel communities of the Oaxaca focus, Mexico, 1995–2008.

It is extremely difficult to predict with certainty when treatments may be safely stopped, because transmission may rebound if the pressure on the parasite population is removed. As a result, the process for certification of onchocerciasis consists of four phases.[4,5] Phase I includes ivermectin treatment until transmission is suppressed. In phase II, suppression is maintained through treatment of the mean reproductive lifespan of the adult female. In phase III, which the data suggest represent the current situation in Oaxaca, the adult parasite population should have died by senescence and removal of invermectin treatment will not result in a resumption of transmission. To help guide the certification of elimination of the *O. volvulus* infection (phase IV), studies on parasite transmission in the post-treatment era in the Oaxaca focus will be needed. These are currently underway.

In conclusion, based on the entomologic and epidemiologic assessments presented in this study, the WHO/OEPA criteria indicating elimination of *O. volvulus* transmission have been met in the Oaxaca focus. In light of these results, local and federal Mexican health authorities agreed that ivermectin should be suspended in the Oaxaca focus in 2009, thus commencing an intensive epidemiological surveillance program to document that transmission will not re-develop.

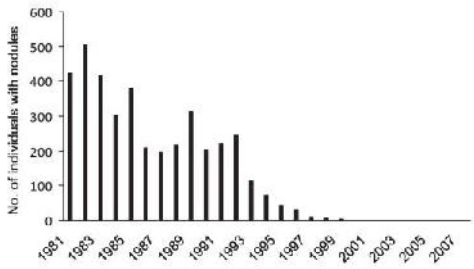

FIGURE 3. Number of individuals with nodules in the Oaxaca focus, Mexico, 1981–2007.

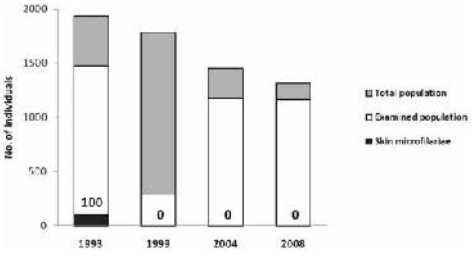

FIGURE 5. Overall and examined population for microfilariae (mf) in skin snips in participants from the four sentinel communities of the Oaxaca focus, Mexico, 1993–2008.

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Lack of Active *Onchocerca volvulus* Transmission in the Northern Chiapas Focus of Mexico

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Abstract. The northern Chiapas onchocerciasis focus has undergone 11 years of ivermectin mass treatment. No evidence of microfilariae in the cornea and/or anterior chamber of the eye or in skin snips was seen in residents examined in 2006 in two sentinel communities (upper limit of the 95% confidence interval [UL 95% CI] = 0.5% and 0.3%, respectively). In children 10 and under, 0 of 305 were found to harbor antibodies to Ov16, a marker of parasite exposure; 0 of 4,400 *Simulium ochraceum* s.l. collected in 2005 contained parasite DNA, giving an UL 95% CI for the infective rate of 0.9/2,000, and an UL 95% CI of the seasonal transmission potential of 1.2 L3/person. These data, assumed to be representative of the focus as a whole, suggest that there is no ongoing transmission of *Onchocerca volvulus* in the northern Chiapas focus. Community-wide treatments with ivermectin were halted in 2008, and a post-treatment surveillance phase was initiated.

INTRODUCTION

Human onchocerciasis is a debilitating disease caused by infection with the filarial parasite *Onchocerca volvulus*. It has historically been one of the leading causes of infectious blindness worldwide, and it remains an important public health problem. Human onchocerciasis is endemic in six countries in Latin America (Brazil, Colombia, Ecuador, Venezuela, Guatemala, and Mexico), where 510,947 individuals are currently estimated to be at risk.[1] In Mexico, three endemic foci have been defined: Oaxaca, northern Chiapas, and southern Chiapas. The onchocerciasis program in Mexico began treatment with ivermectin (Mectizan; Merck & Co., Inc., Whitehouse Station, NJ) in 1989, initially treating only symptomatic individuals in hyperendemic communities. From 1991 to 1997, biannual treatments were extended to symptomatic residents of mesoendemic and hypoendemic communities as well. In 1997, this strategy was modified to provide mass treatment to every eligible resident in all of the at-risk communities, regardless of endemicity.

There is no precise information about when the northern Chiapas endemic focus of onchocerciasis was discovered, but it was unearthed after the other two foci in Mexico were identified, most likely in 1951 when health personnel began providing systematic health care in this area.[2,3] The at-risk population in the northern Chiapas focus consists of indigenous people representing a mixture of diverse ethnic groups (mainly tzeltal and tzotzil). In 1979, de Castro[2,3] suggested that the onchocerciasis in the northern Chiapas focus resulted from the annual seasonal migration of the tzeltal and tzotzil coffee workers to and from coffee plantations in the southern Chiapas focus, an area where, historically, the transmission levels were high. The number of endemic communities in northern Chiapas as detected by clinically defined cases of onchocerciasis (i.e., individuals with nodules, positive skin biopsies, or a positive Mazzotti reaction) has been decreasing over time, probably because of the impact of control measures. For example, in 1989, there were 72 endemic communities with 351 clinical cases of onchocerciasis. In 1993, 42 endemic communities with 180 clinical cases were reported by the local programs. In 2000, this dropped to 13 endemic communities with 83 clinical cases, and in 2007, only 13 endemic communities were found, containing a total of 77 clinically defined cases. Currently, only 13 communities are considered to be at risk in the entire focus of northern Chiapas (Figure 1). In the last 20–30 years, the social and economic structure of the indigenous population of the northern Chiapas focus has been changing: young people have been abandoning the endemic area and migrating to industrialized urban cities, particularly in northern Mexico and the United States. In contrast, the seasonal migration of workers from northern Chiapas to the coffee plantation areas of southern Chiapas has declined. This latter decline of seasonal migration into the onchocerciasis endemic areas of southern Chiapas may have had an impact on the transmission of onchocerciasis, because no individuals harboring nodules have been observed in northern Chiapas since 1996. It has been hypothesized that onchocerciasis infections in the northern focus are solely a result of this seasonal migration and that no independent transmission of the parasite occurred in this focus. This hypothesis is supported by the fact that the indigenous populations in the northern Chiapas focus are generally free of the infection if they have not traveled outside the endemic area.[2–5] However, entomologic and serologic studies carried out in 2001 and 2005 indicated that autochthonous transmission of *O. volvulus* might be occurring in the northern Chiapas focus, albeit at a low level.[6–8]

The conclusion that transmission of *O. volvulus* is rare or non-existent in northern Chiapas is supported by the clinical incidence reports from the local health officials. The last five new clinical cases (i.e., individuals diagnosed positive for nodules or skin microfilariae [mf] for the first time) were reported in 1996 (Figure 2), when four individuals who had traveled outside of northern Chiapas were found to contain mf in skin snips.

Northern Chiapas is the second smallest of 13 foci of onchocerciasis in Latin America after Naicioná, Colombia.[9] It falls under the mandate of the Onchocerciasis Elimination Program for the Americas (OEPA). The ultimate goal of OEPA is to eliminate onchocerciasis from the Americas.

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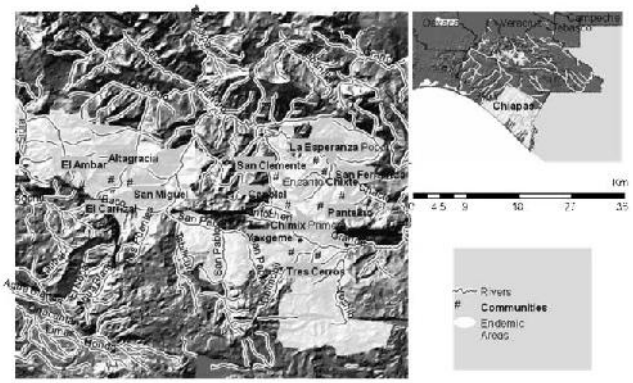

FIGURE 1. Map of the northern Chiapas focus showing the 13 endemic communities and the main rivers and tributaries that might serve as a source of black fly breeding. Map (right) of the southern Mexico states showing the three onchocerciasis endemic foci.

In efforts to reach this goal, OEPA has established interim goals to eliminate new cases of onchocerciasis-induced ocular morbidity caused by infection with *O. volvulus* and to interrupt transmission of the parasite by 2012. The World Health Organization (WHO)[9] and OEPA[10] have established a series of epidemiologic and entomologic criteria for onchocerciasis to be declared as eliminated. The WHO/OEPA criteria include three points. (1) The elimination of new ocular morbidity (defined as a prevalence of < 1% of *O. volvulus* microfilariae in the cornea and/or anterior chamber of the eye). (2) A reduction of new infections to an incidence rate of less than 1 new case per 1,000 individuals (< 0.1%).[3] This criterion has been operationally interpreted as lack of specific antibodies to *O. volvulus* in children under the age of 10. The sample size required to calculate a one-sided 95% confidence interval (CI) for a point prevalence that excludes 0.1% is 3,000 children.[11] In foci in which fewer than 3,000 children under the age of 10 reside, it is expected that all of the children of that age in the focus will be tested and that none will bear evidence of parasite exposure. (3) The absence, or near absence, of infective-stage larvae of *O. volvulus* in the vector population. This has been operationally interpreted as an upper bound to the prevalence of infective flies of less than 1/2,000.[9,10]

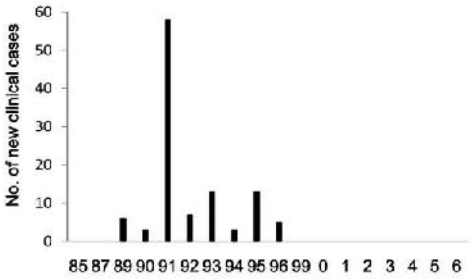

FIGURE 2. Number of new clinical cases of onchocerciasis (individuals diagnosed positive to Mazzotti reaction, nodules, or skin mf for the first time) in the northern Chiapas focus, Mexico, from 1985 to 2006.

In the first entomologic study of transmission in the northern Chiapas focus carried out in 2001, evidence for a low level of transmission was found.[8] In a follow-up study conducted in 2005, none of the bodies of the 4,400 flies collected carried parasite DNA, suggesting that vector–parasite contact had ceased.[8] It was assumed that if the bodies were negative, then the heads would be so also. However, that approach did not allow the researchers to definitively state that the flies did not contain infectious-stage parasites.

Here, we report data obtained from an in-depth epidemiological follow-up study of onchocerciasis in northern Chiapas conducted throughout 2006. We also report data showing an absence of infective larvae in the flies collected in the entomological study conducted in 2005.

MATERIALS AND METHODS

Study area. In 1995, local health authorities selected two sentinel communities of the northern Chiapas. Historically, these communities exhibited the highest prevalence of clinical onchocerciasis cases in the region. The sentinel communities included Altagracia (17°01′51″ N, 92°46′02″ W; elevation = 1,300 m above sea level) and El Ámbar (17°01′29″ N, 17°01′29″ W; elevation = 1,610 meters). The overall prevalence in the entire focus in 1995 was 0.8% (146 clinical cases of onchocerciasis in a total population of 18,891 individuals divided among 42 communities). These two sentinel communities were epidemiologically classified as hypoendemic for onchocerciasis, similar to the other communities of this focus. The present in-depth epidemiological assessment was conducted in 2005 and 2006 in these sentinel communities. The population of these two communities in 2006 totaled 1,391 individuals (Altagracia contained 578 individuals and El Ámbar contained 813 individuals).

Entomologic study. Black flies were collected by using standard procedures[6,11] from February to May 2005 coinciding with the peak *O. volvulus* transmission season.[12] The collections were carried out during the first 50 minutes of

each 1 hour, beginning at 7:00 AM and ending at 4:50 PM. Collectors received ivermectin 1 week before beginning the collection process. This procedure was reviewed and approved by the Ethics and Biosecurity Committee of the National Institute of Public Health of the Health Secretariat of Mexico (Cuernavaca, Mexico).

Black flies were collected before they began feeding. The landing rate measured from the collections was taken as an estimate of the biting rate; however, this probably overestimated the biting rate, because a proportion of the landing flies in a natural setting do not successfully obtain a blood meal. Thus, the transmission-potential calculations provided below are likely to be overestimated by a factor proportional to the number of flies that land but do not successfully obtain a blood meal.

The heads were separated from the bodies of the flies as previously described[6] and were combined into pools containing a maximum of 50 heads per pool. The separated head pools were tested for *O. volvulus* parasites by using a polymerase chain reaction (PCR) assay specific for *O. volvulus*. Details of protocols for genomic DNA purification, primer sequences, PCR conditions, and detection of PCR products by enzyme-linked immunosorbent assay (ELISA) have been published elsewhere.[6,7] PoolScreen version 2.0 was used to estimate the infective proportion in the vector population of the community and the associated 95% CIs.[12] This proportion was expressed as the number of positive flies per 2,000 flies examined.

Seasonal transmission potentials (STPs) for each sentinel village were calculated as the product of the seasonal biting rate, the proportion of flies carrying L3 larvae in the study season (from February to May 2005), and the average number of L3 larvae in each infective fly. Before Mectizan distribution, the average number of L3 larvae present in each infective *S. ochraceum* fly was estimated to be in the range of 1.5–2.0.[7] It has been shown that the number of L3s in each infected fly decreases as the skin microfilarial load is reduced by Mectizan treatment.[11] For these reasons, in calculating the upper bound of the STPs, we estimated that after 8 years of ivermectin mass treatment, the number of infective larvae present in each infective fly would be close to 1.[7,11]

The seasonal biting rate was calculated as the product of the geometric mean of the number of flies collected per person per day and the total number of days in the months of February through May.[14] The daily biting rate and the seasonal biting rate were estimated as previously described.[15] Because *S. ochraceum* s.l. females were not collected throughout the year, it was not possible to precisely calculate the annual transmission potential (ATP). However, in the northern Chiapas focus, the level of transmission estimated during the peak of greatest transmission in 2005 was very low because of the effect of 8 years or 16 rounds of mass semiannual treatment with Mectizan on the parasite population. Therefore, the value of transmission potential outside of the peak transmission period is probably 0 or near 0. Thus, the STP (transmission occurring during the peak transmission season of February through May) likely represents a fairly accurate estimate of the actual ATP.

Serologic study. The prevalence of IgG4 antibodies reacting with Ov16,[16,17] a recombinant antigen of *O. volvulus*, was determined from a population of 305 children 10 years of age and under in the two sentinel communities during 2006. This population represents the total number of children residing in the two sentinel communities. Although the number of individuals examined was less than the 3,000 necessary to ensure that the prevalence was less than 0.1%, the study did enroll all children in the sentinel communities, thus meeting the established criteria of the WHO and OEPA for estimating incidence. For this reason, additional communities were not included to increase the sample size as reported elsewhere.[7,10] Blood was collected by finger prick from each individual enrolled in the study, dried in the field, transported to the laboratory at 4°C, and kept refrigerated in sealed bags containing silica gel at −20°C until use. Blood collections and IgG 4-Ov16 ELISA assays to monitor exposure in the under 10 cohort were carried out as previously described.[10]

Ophthalmologic study. Ocular examinations were carried out by an ophthalmologist experienced in onchocerciasis ocular evaluations. The examinations were done using a Topcon Optical SL-3D slit lamp (Kogaku Kikai KK, Tokyo, Japan). Exams focused on finding *O. volvulus* mf in the cornea (MFC) and/or the anterior chamber of the eye (MFAC). Before the exam, the patients kept a head-down position (forehead in the lap) for 5 minutes to allow MFC and/or MFAC to settle in a visible position. A population of 682 individuals 10 years of age and over, representing about 50% of the total population in the two sentinel communities, were examined during 2006.

Parasitologic study. A total of 986 individuals representing 71% of the total population in the two sentinel communities participated in the survey. Two simultaneous skin biopsies were taken from each patient using a 1.5- to 2.0-mm corneoscleral biopsy punch: one biopsy from the left supra-scapular region and one biopsy from the right supra-iliac region. Skin biopsies were incubated overnight in buffered saline, and emerging microfilariae were counted using an inverted microscope.

Statistical analysis. Poolscreen v2.0 was used to calculate a prevalence of infectivity in the fly-vector populations together with the associated 95% CIs. The prevalence of infective flies was then combined with estimates of the biting rate (calculated from the fly-collection data as described above) to calculate an estimated STP. *S. ochraceum* s.l. were collected during the peak transmission period of February through May 2005 from two sentinel communities in the northern Chiapas focus endemic for *O. volvulus*. The proportion of individuals positive to infection with mf in skin snips and mf in the cornea and/or anterior chamber of the eye was calculated as the number of positive individuals divided by the total number examined, and expressed as a percentage. The associated exact 95% CIs of the proportion of individuals harboring Ov16 antibodies were determined using the Miettinen method described by Armitage and Berry.[13] The same method was used to estimate the 95% exact CIs surrounding the point prevalence of MFC, MFAC, and skin mf.

RESULTS

Entomological study. A total of 4,400 host-seeking *S. ochraceum* s.l. females were collected during the peak transmission period of February through May 2005 from two sentinel communities in the northern Chiapas focus endemic for *O. volvulus*. A total of 88 head pools, each containing 50 heads (Altagracia: $N = 71$; El Ambar: $N = 17$) were examined by PCR. The results of this analysis are summarized in Table 1. All head pools were negative for *O. volvulus* DNA. Thus, the point estimate of the prevalence of infective flies was zero and

TABLE 1

Transmission intensity of *O. volvulus* in two sentinel communities of northern Chiapas, Mexico

Community	Seasonal biting rate (no. of *S. ochraceum* bites per person per season)	Prevalence of infective flies*	Seasonal transmission potential*
Altagracia	2,227	0 (1.08)	0 (1.2)
El Ambar	394	0 (4.4)	0 (0.9)
Total	2,671	0 (0.9)	0 (1.2)

*The value represents the point estimate, and the value in parentheses represents the 95% upper limit CI surrounding the point estimate.

had an upper bound on the 95% CI of 0.9/2,000. Similarly, the upper bound of the 95% CI for the STP was 1.2 L3/season.

Serological study. Of the 305 children 10 years and under (representing all the children in that age group in the sentinel communities) that were tested for IgG4 antibodies, 0 were positive. Therefore, the estimated prevalence of *O. volvulus* exposure in the sentinel communities was 0% (Table 2).

Ophthalmologic study. No MFC and/or MFAC were found among the 682 residents tested of 1,391 individuals in the two sentinel communities (Table 2; 95% upper limit [UL] = 0.5%).

Parasitologic study. Of 986 residents examined by skin biopsy (total population = 1,391 individuals), 0 were found to have microfilariae in the two sentinel communities (Table 2; 95% UL = 0.3%).

DISCUSSION

The absence of new clinically defined cases of onchocerciasis in the entire northern Chiapas focus during the last decade (Figure 2) provides indirect support to the hypothesis that parasite transmission is no longer ongoing in this focus. This finding suggests that endemic onchocerciasis no longer represents a serious health risk to the endemic community in northern Chiapas. However, it is still possible that new cases might be introduced into this region from migration from other areas of Mexico where transmission is still ongoing, such as southern Chiapas. This risk is likely to be less than past levels, because the population in the northern Chiapas focus no longer conducts seasonal migration to southern Chiapas at the levels seen historically. However, the risk of reinfection from southern Chiapas, although decreased from historic levels, still exists.

No evidence for infective parasites in the vector, or for new parasite exposure in the human population, was found in the two sentinel communities examined in this study, supporting the conclusion that there is currently no ongoing transmission of *O. volvulus* in the northern Chiapas focus. As a result, community-wide treatments with ivermectin were halted in northern Chiapas in 2008, and post-treatment surveillance activities in this focus were initiated. Thus, northern Chiapas joins the five other foci in Latin America where transmission seems to have been interrupted: Oaxaca in Mexico, Santa Rosa, Huehuetenango, and Escuintla-Guatemala in Guatemala, and López de Micay in Colombia.[10,18,20–23]

The entomological criteria for asserting interruption of transmission includes the absence or near absence of infective-stage larvae of *O. volvulus* in the vector population, which has been operationally defined as < 1 infective fly per 2,000 examined.[5,10] During the first entomologic study carried out in Altagracia of the northern Chiapas focus, which was conducted in 2001 after 4 years of mass semiannual treatment with Mectizan, parasite DNA was detected in a single pool of 50 vector heads out of a total of 125 pools examined (6,250 flies total). The prevalence of infective flies was 0.32/2,000 (95% CI = 0.008–1.65/2,000), leading to an STP of 1.5 L3 (95% CI = 0.04–7.7) per person per season.[3] In the present study, the prevalence of infective flies collected in 2005 was 0/2,000 for both sentinel communities. The maximum value of the 95% CI surrounding the zero point estimate was 0.9/2,000, which is below the threshold of 1/2,000.

In addition to the 1/2,000 infective fly threshold, OEPA, during an OEPA-convened meeting of entomologists in September 2006, recommended the use of the ATP (which, in the present situation, is equivalent to STP) to assess the status of onchocerciasis transmission. This is because the true rate of exposure of an at-risk population is dependent not only on the prevalence of infection in the vector population but also on the degree of contact between the vector and at-risk populations. Furthermore, in some areas where the vector density is low, it may be difficult or impossible to collect sufficient vector insects to conclusively show that the prevalence of infection is below a set threshold value. Using the ATP or STP as a metric, therefore, more accurately represents the level of exposure of the at-risk community to the parasite, because it takes into account both the level of infection and the degree of host–vector contact. Unfortunately, the threshold level of the ATP below which the parasite population is unable to sustain itself has not been accurately defined. Estimates for this threshold value for the ATP have ranged from 5 to 54 L3/person/year using mathematical modeling[24] and from 7.6 to 18 L3/person/year using field observations.[5,25] In northern Chiapas, the estimated upper bound for the STP was below even the lowest of these estimates (1.2 L3/person/year). This suggests that if conditions remain unchanged, any parasite population remaining in this focus will be unable to recover; therefore, the population is on the path to elimination.

The epidemiological data presented above also suggest that transmission in northern Chiapas is no longer occurring. During the first large-scale serologic study carried out in this area in 2001, IgG4 antibodies to Ov16 were detected using a prototype immunochromatographic card test (ICT; AMRAD, Sydney, Australia)[26] in a population of 922 individuals aged 16 years and younger from the sentinel communities described above, as well as 10 additional endemic and non-endemic communities. None of these samples contained detectable IgG4

TABLE 2

O. volvulus infection and exposure in the human population in two sentinel communities of northern Chiapas, Mexico

Community	No. cases/total population	Prevalence of MFC and/or MFAC*	Population (no. examined)	Prevalence of skin mf*	Prevalence of IgG4 antibodies to Ov16 (no. examined)
Altagracia	225/578	0 (1.6%)	578 (317)	0 (1.1%)	0/225
El Ambar	457/813	0 (0.8%)	813 (669)	0 (0.5%)	0/80
Total	682/1,391	0 (0.5%)	1,391 (986)	0 (0.3%)	0/305

*The value represents the point estimate, and the value in parentheses represents the 95% upper limit CI surrounding the point estimate.

antibody to Ov16, leading to a post-exposure incidence of 0% (95% UL = 0.3%). The subsequent serologic studies reported above examined all children 10 years and younger in the sentinel communities. Again, none were positive. When these studies are combined, 1,241 individual serum samples were examined, and none were positive, leading to an upper bound of the 95% CI of a maximum prevalence of 0.2% in this area; this suggests that parasite contact among children is no longer occurring. Although the number of individuals examined was less than the 3,000 necessary to ensure that the prevalence was less than 0.1%, the study did enroll all children in the sentinel communities, thus meeting the WHO and OEPA criteria for incidence in this situation.

Although migration of infected flies from neighboring communities could occur after treatment is stopped, this mechanism of reintroduction of infection is unlikely. The only two formerly endemic communities located within the flight range of the vector (around 3.5 km)[27] are El Carrizal and San Miguel (Figure 1). Both of these communities have also been receiving ivermectin treatment at the same coverage levels as those of El Ambar and Altagracia. Consequently, the level of infection in migrating flies from these communities is likely to be similar to that in the sentinel communities. The other 11 endemic communities are outside the flight range of the vector, extending as far away as 30 km from the sentinel communities. Similarly, the threat of vector-borne infection from the other foci in Mexico, Oaxaca and southern Chiapas, is very slight; these foci are separated by 380 km and 120 km, respectively, from northern Chiapas (Figure 1), distances that are over 100 times that of the flight range of the vector black fly.

The process for certification of the elimination of onchocerciasis consists of four phases.[9,15] Phase I includes ivermectin treatment until transmission is suppressed. In Phase II, suppression is maintained through treatment of the mean reproductive lifespan of the adult female. In Phase III, the adult parasite population should have died by senescence, and removal of ivermectin treatment will not result in a resumption of transmission. The data suggest that this is the current situation in the northern Chiapas focus. To help guide the certification of elimination of the *O. volvulus* infection (Phase IV), studies on parasite transmission in the post-treatment era in the northern Chiapas focus will be needed for 3 years after treatment has ceased. These studies are currently underway.

Taken together, the entomologic and epidemiologic data presented in this study suggest that currently there is no ongoing transmission of *O. volvulus* in the northern Chiapas focus. Although some of the sample sizes collected were insufficient to satisfy the most stringent criteria promulgated by the OEPA and WHO because of the small population sizes of the sentinel communities and the low density of the vectors in the area, no evidence for ongoing transmission was detected in the samples. In light of these results, local and federal Mexican health authorities agreed with the OEPA steering committee's (the Program Coordinating Committee) recommendation that community-wide ivermectin distribution should be suspended in the northern Chiapas focus in 2008. This is to be followed by an intensive surveillance program to assure that transmission will not reemerge in this area of limited focus.

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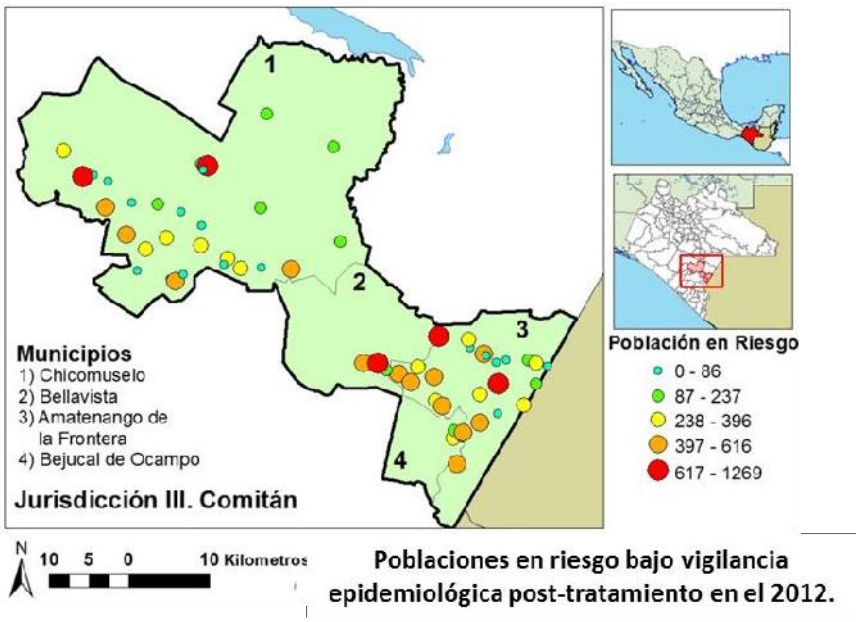
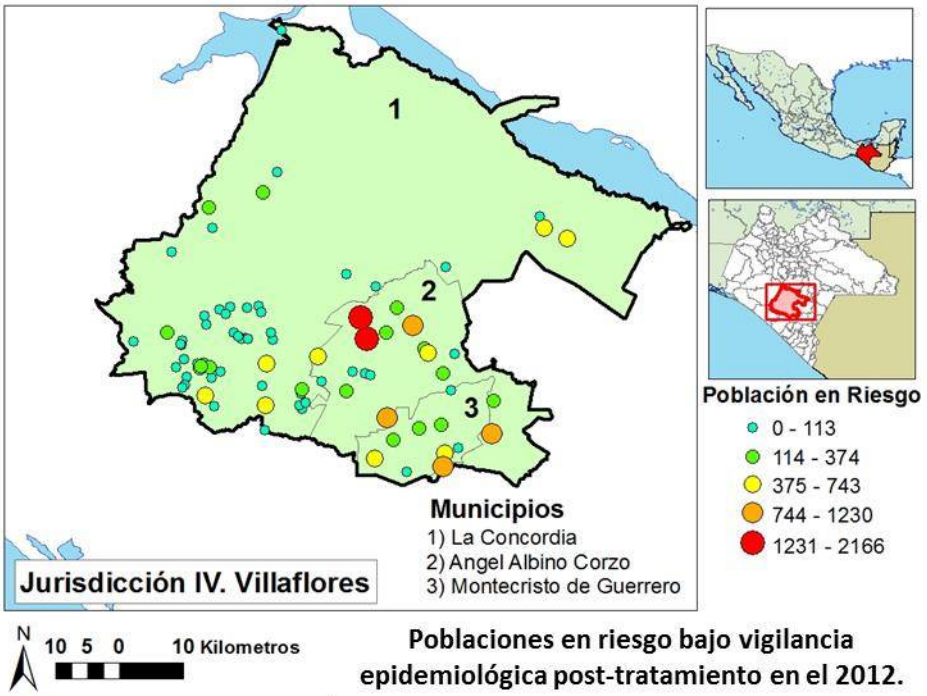
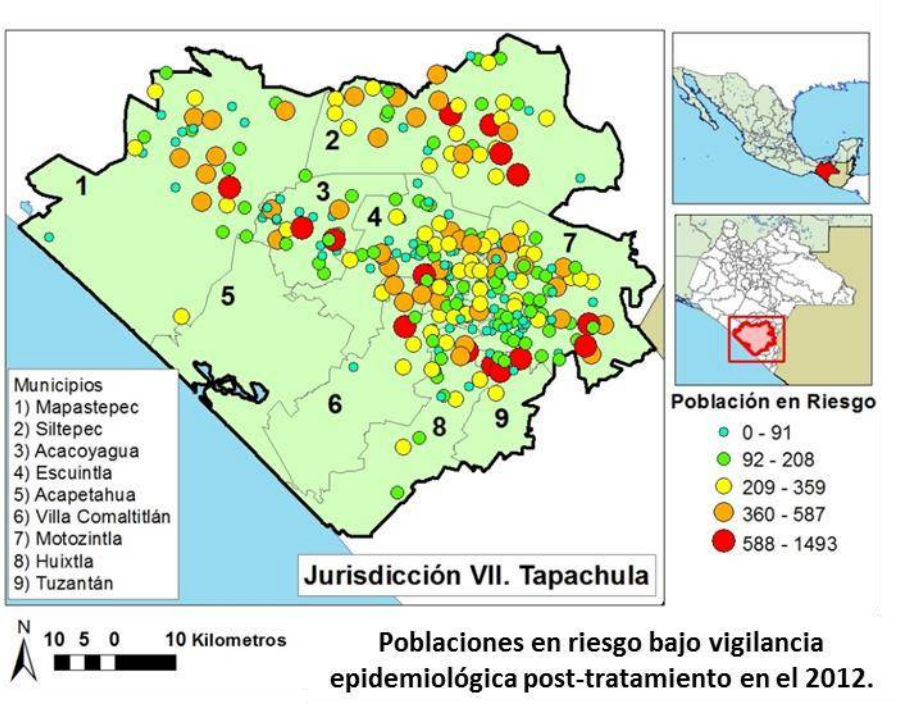
Anexo 10. Poblaciones en riesgo que estuvieron bajo vigilancia epidemiológica post-tratamiento en Chiapas. (**Fuente:** Servicios de Salud de Chiapas. Dirección de Prevención y Promoción de la Salud. Programa de Oncocercosis)



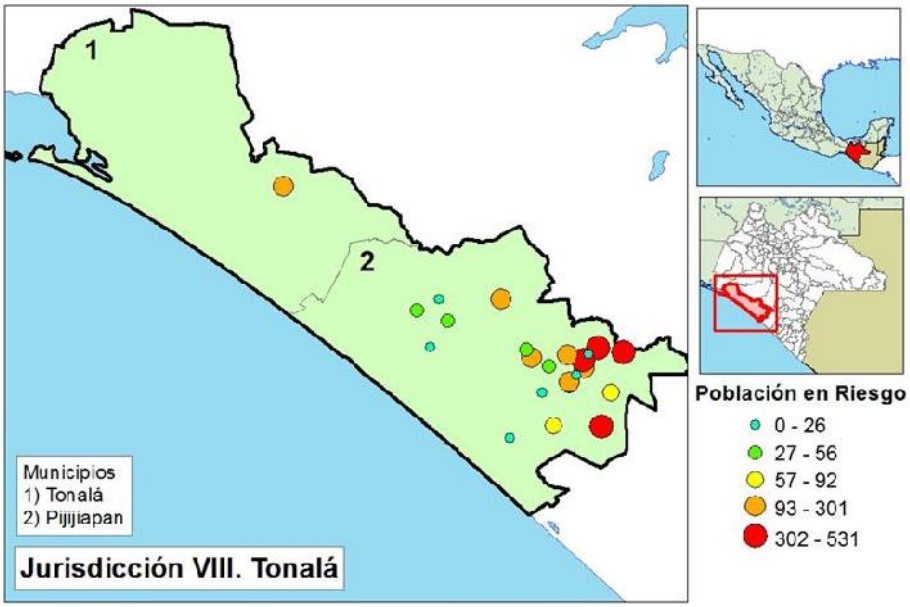
Poblaciones en riesgo bajo vigilancia epidemiológica post-tratamiento en el 2012.



Poblaciones en riesgo bajo vigilancia epidemiológica post-tratamiento en el 2012.



Poblaciones en riesgo bajo vigilancia epidemiológica post-tratamiento en el 2012.



Poblaciones en riesgo bajo vigilancia epidemiológica post-tratamiento en el 2012.

ADENDUM

(Información no disponible durante el desarrollo del Dossier)

Sistema de Apoyo Informático para la Eliminación en México de la Oncocercosis

(SAIEM - ONCO)

El Sistema de Información denominado SAIEM-Onco fue desarrollado en el año 2001 con el objetivo de fortalecer el PNEO sobre la automatización del procesamiento de información de los registros nominales recabados en campo. Además, con la finalidad de apoyar, de forma eficaz, el manejo de la información para su evaluación y control.

El desarrollo y consultoría de la creación del SAIEM-Onco requirió de diversas reuniones de trabajo con el deasrrollador del SAIEM-Onco, ISC. Roberto de Jesús Aguilar Dillmann, los representantes estatales, los jefes de jurisdicciones y las brigadas. La actividad fue recabar información que cimentara la lógica y funcionalidad del SAIEM-Onco hasta lograr su consolidación. Se formularon los formatos en campo que fueron la fuente principal para la alimentación del SAIEM-Onco.

El resultado obtenido fue la homogenización de los formatos de campo, la herramienta de trabajo primordial del personal de las brigadas, lo que permitió la recolección de información de la población afectada por oncocercosis. Igualmente, permitió la calendarización de las actividades y acciones orientadas hacia la educación para la salud. Los formatos de campo fueron denominados de la siguiente manera:

calendarización, prontuarios, carpetas familiares, inmigrantes, tratamiento a eventuales, nodulectomias, reacciones adversas y biopsias.

A partir de la implementación del SAIEM-Onco, el equipo multidiciplinario integrado por la ISC. Nadia Melissa Burgara Escobar, responsable del SAIEM-Onco en Chiapas y C. Josefa Guadalupe Rodríguez Jimenez e Ing. Miguel Enrique Farrera Nañez, capturistas, supervisados por el Ing. Roberto Dillmann, estaba a cargo de las evaluaciones, la operatividad, el manejo y el procesamiento de la información. Las actividades de trabajo que se realizaban con el SAIEM-Onco estaban en constante comunicación con el personal de las brigadas ya que la entrega e intercambio de información de campo hacia las oficinas centrales fue de manera periódica mediante una calendarización de actividades. El concentrado de información en SAIEM-Onco fue evaluado, en primer instancia, por las autoridades estatales, seguido por el CENAPRECE y, finalmente, por el OEPA. Se elaboraron reportes de coberturas de tratamientos trimestrales, desglosados por Jurisdicción, generándose asimismo un concentrado global por año de las actividades de tratamiento con Mectizan®. El concentrado anual se presentó en las diversas reuniones de la IACO, en lo correspondiente al estado de la situación de la oncocercosis en México.

Cuadro. Casos de ceguera por oncocercosis en Oaxaca y Chiapas durante el período 1981 - 2010. **Fuente**: CENAPRECE

Año	Chiapas	Oaxaca	Total
1981	65	59	124
1982	65	59	124
1983	65	53	118
1984	70	54	124
1985	47	49	96
1986	47	54	101
1987	60	56	116
1988	60	54	114
1989	60	52	112
1990	60	49	109
1991	58	49	107
1992	58	49	107
1993	58	48	106
1994	58	48	106
1995	58	43	101
1996	58	24	82
1997	42	23	65
1998	42	19	61
1999	32	18	50
2000	32	18	50
2001	32	17	49
2002	26	17	43
2003	25	15	40
2004	23	14	37
2005	23	13	36
2010			12