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

Mario A. Rodríguez-Pérez,* Thomas R. Unnasch, Alfredo Domínguez-Vázquez, Alba L. Morales-Castro, Frank Richards Jr., Graciela P. Peña-Flores, María Eugenia Orozco-Algarra, and Gibert Prado-Velasco

Centro de Biotecnología Genómica, Instituto Politécnico Nacional, Reynosa, Tamaulipas, México; Global Health Infectious Disease Research Program, Department of Global Health, College of Public Health, University of South Florida, Tampa, Florida; Onchocerciasis Elimination Program for the Americas, Guatemala City, Guatemala; Onchocerciasis Elimination Program for the Americas, River Blindness Program of The Carter Center, Atlanta, Georgia; Dirección de Enfermedades Transmitidas por Vector, Centro Nacional de Vigilancia Epidemiológica y Control de Enfermedades, Secretaría de Salud, México Distrito Federal Instituto de Salud del Estado de Chiapas, Tuxtla Gutiérrez, Chiapas, México

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 Naicóna, Colombia.¹ 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.

*Address correspondence to Mario A. Rodríguez-Pérez, Centro de Biotecnología Genómica, Instituto Politécnico Nacional, Boulevard del Maestro esquina Elías Piña, Col. Narciso Mendoza, 88710, Reynosa, Tamaulipas, Mexico. E-mail: drmarrodriguez@hotmail.com

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 OEPA¹⁰ 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%).⁹ 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.¹⁰ 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}

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 Altigracia (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 (Altigracia 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

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.

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 described⁶ 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.¹³ 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.^{10,18} 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 corneosceral 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.¹⁹ 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 <i>S. ochraceum</i> 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.^{9,10} 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.⁸ 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 modeling²⁴ and from 7.6 to 18 L3/person/year using field observations.^{9,25} 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. examined/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 Altigracia. 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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Authors' addresses: Mario A. Rodríguez-Pérez, Centro de Biotecnología Genómica, Instituto Politécnico Nacional, Boulevard del Maestro esquina Elías Piña, Col. Narciso Mendoza, Reynosa, Tamaulipas, México, E-mail: drmarrodriguez@hotmail.com. Thomas R. Unnasch, Global Health Infectious Disease Research Program, Department of Global Health, College of Public Health, University of South Florida, Tampa, FL. Alfredo Domínguez-Vázquez and Alba L. Morales-Castro, Onchocerciasis Elimination Program for the Americas, Murano Center, Guatemala City, Guatemala. Frank Richards Jr., River Blindness Program, Carter Center, Atlanta, GA. Graciela P. Peña-Flores and María Eugenia Orozco-Algarra, Dirección de Enfermedades Transmitidas por Vector, Centro Nacional de Vigilancia Epidemiológica y Control de Enfermedades, Secretaría de Salud, Colonia Escandón, Delegación Miguel Hidalgo, México D.F., México. Gibert Prado-Velasco, Instituto de Salud de Chiapas, Edificio "C" Unidad Administrativa, Calzada a la Ciudad Deportiva, Col. Centro, Tuxtla Gutiérrez, Chiapas, México.

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