Chlamydia trachomatis in the conjunctiva of children living in three rural areas in Mexico

Pablo Goldschmidt,[1] Virginia Vanzzini Zago,[2] Lidia Diaz Vargas,[3] Laura Espinoza Garcia,[3] Carlos Morales Montoya,[3] Beatríz Peralta,[4] and Mario Mercado[4]

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ABSTRACT

Objectives. Chlamydia trachomatis *infections, in the context of extreme poverty, may trigger trachoma. Because the levels of* C. trachomatis *eye infections in Mexico are unknown, this study sought to determine if* C. trachomatis *was present in the conjunctiva of children living in three poor, rural areas of the country.*
Methods. *Clinical diagnosis of conjunctival follicles in children was conducted during the 2004 visual acuity assessment campaigns in rural areas of the states of Chiapas, Oaxaca, and Zacatecas.* C. trachomatis *detection was carried out by sampling the children with follicles and examining the specimens after Giemsa or microimmunofluorescence (MIF) staining.*
Results. *A total of 941 children from 6 to 12 years of age were examined in 2004. Of the 484 in Chiapas, 30% were found to have follicles; of the 181 in Zacatecas, 22%; and of the 276 in Oaxaca, 42%.* C. trachomatis *was detected at levels ranging between 2% and 5%; positive by Giemsa in 4.5% of the children with follicles, and by MIF in 15.5%.*
Conclusions. *Considering that the chlamydiae sampling procedures and detection methods used in this study were not the most sensitive, the results underestimate the chlamydial eye infections and represent a conservative assessment of a potential risk for preventable visual impairment. Because* C. trachomatis *was detected here at levels similar to those reported for low-endemic trachoma areas, health authorities should be prepared to implement appropriate measures should it be confirmed that the visual health of Mexico's children is at risk.*

Key words *Chlamydia trachomatis*, trachoma, eye infections, conjunctivitis, Mexico.

Chlamydiae are obligate intracellular eubacteria that bind to squamocolumnar epithelial cells, where they replicate and persist (1). In the context of extreme poverty, the immune reactions induced by repeated or chronic infections trigger inflammatory responses that can lead to trachoma and blindness (2, 3). Trachoma is a major cause of visual impairment. It is still detected in areas without basic sanitation services—parts of Africa, the Middle East, Southwest Asia, the Indian subcontinent, and aboriginal communities in Australia. In addition, small focuses of blinding trachoma have been found in Central and South America (4–6).

Before the arrival of Europeans in the Americas, the Mayas described the existence of conjunctival granulations. However, at the 1986 Pan American Congress of Ophthalmology, it was reported that trachoma had been found among the European immigrants to Mexico, but not among the indigenous population (6–8). In 1906, however, trachoma had been found among the indigenous populations, in villages situated in the high valleys of Toluca and Texcoco (8). A high prevalence of tra-

1 Laboratoire du Centre National d'Ophtalmologie des Quinze Vingts, Paris, France. Send correspondence to: Pablo Goldschmidt, 28 rue de Charenton, Paris 75012, France; telephone: 331 40 02 17 95; fax: 331 40 02 16 99; e-mail: pablogol@aol.com.
2 Asociación para Evitar la Ceguera, Hospital Sanchez Bulnes, Coyoacán, Mexico.
3 Centro Médico Nacional La Raza, Mexico City, Mexico.
4 Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, Mexico City, Mexico.

choma (>70%) was also reported among Asian immigrants arriving through the port of Salina Cruz, Mexico, to work on the San Benito to Tapachula railway in the state of Chiapas (8, 9). By 1923, trachoma was reported in almost all of Mexico's states, as well as in several cities located on Guatemala's coasts (10).

Later in the century, severe forms of blinding trachoma were reported. By 1962, 60 000 cases were diagnosed in the Tzeltal ethnic group in the Altos de Chiapas area, state of Chiapas (7, 9). In 1966, the Dr. Torroella Center for Trachoma and Onchocercosis opened in the city of San Cristobal de las Casas, state of Chiapas. In 1983, it conducted a survey in the rural populations showing that, among those 0–14 years of age, the prevalence rate of active disease ran as high as 20%. Scars related to trachoma were found in more than half of the population over 50 years of age (11, 12).

No further studies have contributed to the overall picture of trachoma prevalence in rural Mexico and the presence of *C. trachomatis* was never confirmed. Likewise, no data regarding levels of C. *trachomatis* eye infection in Mexico have been reported. The only relevant data available are from genital samples obtained from a female, rural population that had a positive rate of 8% (13, 14).

Persistent chlamydial infection in children with signs of active conjunctival inflammation is considered to be the community pool of active trachoma, (15–17). The aim of this trial was to break new ground by determining if *C. trachomatis* could be detected, with the tools locally available, in the conjunctiva of children presenting follicles in rural areas of three different states of Mexico: Chiapas, Oaxaca, and Zacatecas.

MATERIALS AND METHODS

Subjects

All the investigations were performed in accordance with World Medical Association's *Declaration of Helsinki on Ethical Principles for Medical Research involving Human Subjects* (18) and the *Ley General de Salud de Mexico* (19). The protocol for this study was approved by the Ethics Committee of the Instituto Mexicano del Seguro Social (IMSS), Mexico City.

The Proyecto Solidaridad (Solidarity Health Project), funded by the IMSS, offers primary health care services to poor populations living in remote, rural areas beyond the reach of more traditional health care. In 2004, the Children's Visual Health Project, part of the Solidarity Health Project, carried out ophthalmologic testing of school-age children in hospitals that were reachable by paved roadways. Corrective eyeglasses were delivered free of charge following visual acuity testing.

Dwellings and settlements outside the reach of conventional transportation were visited by local health promoters one week prior to testing. Inhabitants were informed of the study and its purpose. Each parent or caretaker had to give formal consent for the child's participation before the child was clinically examined and sampled.

Prior to commencing our research, the local health authorities had indicated that trachoma was absent from the indigenous population. The local health officials and the IMSS representatives authorized only visual acuity testing, followed by a supplementary, rapid clinical examination of the children's inferior conjunctivas for the assessment of follicles. Therefore, the present study should not be likened to a trachoma survey. These exams were performed by trained, volunteer physicians in their third year of residency in ophthalmology. Positive follicles were defined by detection of at least five round swellings of at least 0.5 mm in diameter that were lighter in color—white, gray, or yellow—than the surrounding conjunctiva.

To avoid cross-contamination, physicians changed gloves between subjects, and were not allowed to handle pens, flashlights, or clinical files, nor register any data. An independent assistant managed the identification of the children and other logistics. Clinical data were registered by an independent ophthalmologist.

Sampling procedure

Specimens were collected from children 6 to 12 years of age presenting follicles. After administration of 1% tetracaine eye drops, the inferior eye lid was reverted with sterile gauze, and the follicles in the inferior conjunctiva were scraped with sterile, disposable, apyrogenic, stainless-steel, Kimura-like spatulas. After sampling, the children received a local application of chloramphenicol ointment.

C. trachomatis detection

Although DNA extraction and amplification (NAATs) are known to be more sensitive than Giemsa staining and microimmunofluorescence (MIF) for the detection of *Chlamydia* in human conjunctiva (20–23), this study's funding would not cover the cost of the most sensitive options. Therefore, two samples were obtained from each child by scraping the conjunctiva. The samples were deposed on coded, multialveolar, black-Teflon slides; fixed with ice-cold acetone/ethanol (1:1) at –20°C for 30 minutes, and dried and conditioned separately in individual, paper envelopes. The fixed slides were kept at –20°C until staining for microscopic examination.

C. trachomatis detection was carried out on masked samples in two laboratories. In the first lab, staff experienced in the identification of typical, intracytoplasmic, epithelial cell basophilic inclusions examined smears stained with Giemsa (pH 7.4) and determined whether each was positive or negative. In the second lab, MIF was conducted by incubating the fixed specimens with a FITC-labeled monoclonal antibody diluted in a Blue Evans solution (counterstaining) according to manufacturer's instructions (Biomérieux, Lyon, France).

Two microbiologists with confirmed experience in direct diagnosis of chlamydial infections by MIF examined each sample and considered the result positive when 25 or more epithelial cells per smear were identified (counter stain) and associated with three or more green, fluorescent images (as appearing in the positive control slides). Yellowish images were considered negative; smears with fewer than 25 cells, noninterpretable.

Statistical analysis

Intergroup and intragroup differences were analyzed by analysis of variance (ANOVA). Non-parametric variables were studied by the Kruskal-Wallis test and correlation by Spearman's test.

RESULTS

Visual acuity was assessed in a total of 941 children 6 to 12 years of age in three rural areas: Chiapas, 484; Zacatecas, 181; and Oaxaca, 276. Clinical assessment of inflammatory signs in the inferior conjunctiva showed follicles in 30%, 22%, and 42% of the children, respectively.

Tables 1, 2, and 3 show that although the number of children tested varied from area to area, age- and sex-distribution were the same. No significant differences were found for the overall results when comparing the age or the sex of the children ($P = 0.96$ and 0.81, respectively). Interaction between age/sex, age/state, and sex/state were statistically insignificant ($P = 0.99$, 0.23, and 0.28, respectively).

For the subgroup of children with follicles, the differences for age and sex were not statistically significant ($P = 0.68$ and 0.94, respectively) for the three groups (intra- and intersubgroup). No significant interactions were detected while comparing age/sex or sex/location ($P = 0.99$ and 0.37, respectively) for the whole subgroup of children with follicles. Nevertheless, in Oaxaca (where follicles were detected in 42%), a statistically significant difference in the number of affected children was found when comparing it with the other two areas (Chiapas, 21%; Zacatecas, 30%) ($P = 0.001$).

In all three states, the rate of children with follicles to the total number of children examined was similar in each of the seven age groups (one group for each year from 6 to 12 years) (Tables 1–3). In Zacatecas, all but one age group had a rate under 30%; in Oaxaca, all age groups had more than 28% with follicles; and in Chiapas, the rate was always greater than 25% in the children less than 10 years of age, and always less than 25% in those 10 or older. The interaction of age/state had a follicle rate that was statistically significant ($P = 0.002$).

The overall analysis shows that of the 245 children with follicles, *C. trachomatis* was detected by MIF in 15.5% of the samples and by Giemsa staining in only 4.5%. *C. trachomatis* detection rates by MIF were significantly higher ($P = 0.0001$) and showed a better correlation with the proportion of children with follicles (Spearman's rho: 0.522; $P = 0.001$) than the levels obtained with Giemsa staining (Spearman's rho: 0.273; $P = 0.107$). The difference in the number of positive samples was not statistically significant ($P = 0.650$) for the two techniques regarding sex ($P = 0.483$) or age ($P = 0.090$).

DISCUSSION AND CONCLUSIONS

Trachoma is thought to be endemic in rural Mexico; however, its extent, severity, and infection rate are unknown. No surveys were conducted during the late 1980s or 1990s, and the question of the real impact of ophthalmic chlamydial infections has remained unanswered. In this context, the Visual Health Program team was only allowed to conduct a rapid, clinical observation of the children's conjunctivas, far short of the World Health Organization (WHO) guidelines for trachoma assessment.

In addition to the visual acuity testing, the ophthalmologists examined the inferior conjunctival fornix to assess follicles and to sample when positive. Because bacteria other than *Chlamydia* such as, fungi, viruses, aller-

TABLE 1. Distribution by age, sex, presence of follicles, and detection of *Chlamydia trachomatis* by microimmunofluorescence (MIF) or Giemsa staining, of children 6 to 12 years of age in rural areas of the state of Chiapas, Mexico, 2004

Age (years)/Sex	Total	Follicles in inferior tarsus	MIF-positive	Giemsa-positive
6				
Female	24	11	2	0
Male	40	17	3	0
7				
Female	40	21	3	2
Male	25	9	0	0
8				
Female	39	16	3	0
Male	31	9	4	0
9				
Female	46	20	3	2
Male	29	16	2	0
10				
Female	31	5	1	0
Male	30	5	2	1
11				
Female	38	4	0	0
Male	32	7	1	0
12				
Female	40	4	0	0
Male	39	2	1	0
Total	484	146	25	5

TABLE 2. Distribution by age, sex, presence of follicles, and detection of *Chlamydia trachomatis* by microimmunofluorescence (MIF) or Giemsa staining, of children 6 to 12 years of age in rural areas of the state of Zacatecas, Mexico, 2004

Age (years)/Sex	Total	Follicles in inferior tarsus	MIF-positive	Giemsa-positive
6				
Female	9	1	0	0
Male	13	2	0	0
7				
Female	14	4	1	1
Male	16	4	1	1
8				
Female	12	1	0[a]	0
Male	12	2	0	0
9				
Female	14	2	0[a]	0
Male	16	3	0	0
10				
Female	12	3	0[a]	0
Male	12	3	1	1
11				
Female	13	2	1	0
Male	15	4	0	0
12				
Female	11	2	0	0
Male	12	6	0	0
Total	181	39	4	3

[a] Noninterpretable results (less than 50 cells/smear).

gens, and pollutants, may have induced the formation of follicles in the inferior fornix, the prevalence rates for children with more than five follicles cannot be considered in the present study as a predictor for trachoma nor can they be compared to results reported in trachoma surveys conducted according to WHO recommendations.

The present study is the first to show that *C. trachomatis* is present at levels ranging between 2% and 5% in the conjunctiva of children ages 6 to 12 years old living in three rural areas of Mexico, where low income and limited sanitation are widespread.

It should be recognized that the study results underestimate the actual rate of chlamydial eye infections for four reasons. First, a thorough evaluation of conjunctival inflammation could not be performed due to a lack of appropriate equipment (i.e., magnifying glass, flashlight). Second, trachoma sampling and testing are ideally carried out in the upper tarsal conjunctiva by reverting the upper-lid and assessing the number, morphology, and size of follicles with the help of a magnifying glass. Even so, in this study, where sampling and testing were carried out on the inferior conjunctival fornix and assessed without a magnifying device, the number of children with follicles and carrying *C. trachomatis* in the conjunctiva was substantial. Third, the rate of chlamydial eye infections may have been underestimated by the sampling procedure. The superficial epithelium of the inferior fornix—scraped during the sampling—may not have accurately represented the bacterial load of the conjunctiva, and therefore, would not have efficiently recovered *Chlamydia.* Finally, the most sensitive methods for *C. trachomatis* diagnosis are based on nucleic acid amplification technology (NAAT). Such tests can detect as few as one organism per assay, whereas the limit of detection for conventional nonculture methods is 10 or more organisms (20–26).

Performing NAAT-based testing requires at least one clean room for DNA extraction, and amplification equipment that is specifically dedicated to chlamydial DNA diagnosis. However, in the absence of appropriate conditions and equipment, *C. trachomatis* can be detected by direct, cytological examination of conjunctival swabs. Reading of Giemsa-stained specimens requires

TABLE 3. Distribution by age, sex, presence of follicles, and detection of *Chlamydia trachomatis* by microimmunofluorescence (MIF) or Giemsa staining, of children 6 to 12 years of age in rural areas of the state of Oaxaca, Mexico, 2004

Age (years)/Sex	Total	Follicles in inferior tarsus	MIF-positive	Giemsa-positive
6				
Female	22	9	0	0
Male	15	7	1	1
7				
Female	14	6	1	0
Male	14	7	1	0
8				
Female	15	8	1	0
Male	18	9	0[a]	0[a]
9				
Female	21	9	2	1
Male	19	7	0[a]	0
10				
Female	24	12	1	1
Male	32	14	0[a]	0
11				
Female	12	7	1	0
Male	14	4	1	0
12				
Female	28	9	0	0
Male	28	8	0	0
Total	276	116	9	3

[a] Noninterpretable results (less than 50 cells/smear).

only a light microscope, but has reduced sensitivity. Staining the smears with specific, fluorescent-tagged, monoclonal antibodies improves sensitivity, but requires a fluorescence microscope (23–25). In India, where slides were examined to diagnose clinically suspected cases of trachoma, MIF was found to be 2.52 times more sensitive than Giemsa staining for confirming *C. trachomatis* (22). In the United States of America, a prospective comparison of four currently available diagnostic tests for *C. trachomatis* infection in clinically suspected cases of chlamydial conjunctivitis showed that detecting inclusions by Giemsa staining had a sensitivity and specificity of 43% and 100%, respectively, while the monoclonal fluorescent antibody had 57% and 81%, respectively (cell culture on McCoy cells was the reference method) (23).

In the present study, the MIF detection rates for *C. trachomatis* were also almost three times higher than the Giemsa staining rates. However, the *C. trachomatis* rate in rural Mexican children is not different from that found in the low trachoma prevalence areas of other countries (5, 6, 20, 26–28). Nevertheless, predictions of potential clinical degradation of visual function cannot be made just because *C. trachomatis* can be detected in the conjunctiva of asymptomatic children (3, 4, 15–17). The mere presence of *C. trachomatis* in conjunctival epithelial cells is not enough to provoke trachoma and blindness. Host factors associated with poverty and hygiene play a key role in the establishment and course of clinical disease. The prognosis for chlamydial infection for the area in which a child is born, can be different from that of the area in which he grows up.

In industrialized countries, chlamydial eye infections in children do not lead to blindness because hygiene, medical care, and appropriate antibiotic treatment can be administered on time, limiting the fibrotic effects of a prolonged infection (17, 29–30). For example, *C. trachomatis* was detected by MIF in 8% of the 162 newborns at 14 primary health centers from public hospitals in the city of Santiago de Chile, Chile (31) and in 41% of 180 babies with uni- or bilateral neonatal conjunctivitis in the south of Italy (32), but there has not been a single case of trachoma reported in either of these countries for over 30 years.

In the state of Chiapas, however, the detection of *C. trachomatis* in children's conjunctivas requires a different approach because in the early 1980s trachoma was reported in the state's Chaonil community in 24% of 475 children examined and in 8% of the trachomatous population with trichiasis (no data regarding chlamydial infections were produced) (11, 12, 33).

The children participating in the present study came from villages in the mountainous terrain of the state of Chiapas, at an elevation of at least 2 400 meters above sea level, with cool and damp weather year-round. Their homes were dispersed over a large area, isolated in the mountains. Out of reach by any kind of ground transportation, most children participating in the study arrived to the IMSS health centers by boat or by foot after walking for more than 60 minutes. None of the children examined in Chiapas, who belonged to the Chamula, Tzotzil, and Tzental ethnic groups spoke Mexico's official national language (Spanish) or had ever been seen by a physician.

A far different picture was found in Zacatecas and Oaxaca, where trachoma has not been reported recently. In these two study areas, almost all the children came from dwellings that had electricity and that were accessible by paved roads. In addition, either the children themselves or their parents had some level of formal education, had been seen by a physician or health promoter at least once, and reported having been vaccinated.

The results of this study represent a minimal assessment of the level of chlamydial infection in the conjunctivas of children living in rural areas of Mexico. Even with its limitations, this study should prompt health authorities to carry out comprehensive surveys, according to international standards, and engage in preventive and curative actions that will ensure the future ocular health of children in Mexico.

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RESUMEN

Chlamydia trachomatis en la conjuntiva de niños de tres zonas rurales de México

Objetivos. En un contexto de pobreza extrema, la infección por *Chlamydia trachomatis* puede desencadenar el tracoma. Debido a que se desconocen los niveles de infección ocular con *C. trachomatis* en México, el objetivo de este estudio fue determinar la presencia de *C. trachomatis* en la conjuntiva de niños de tres zonas rurales pobres de México.
Métodos. El diagnóstico clínico de folículos conjuntivales en los niños se llevó a cabo durante la campaña de evaluación de la agudeza visual en áreas rurales de los estados de Chiapas, Oaxaca y Zacatecas en 2004. Para la detección de *C. trachomatis* se tomaron muestras de los niños con folículos y se analizaron mediante la tinción de Giemsa o microinmunofluorescencia (MIF).
Resultados. En total se examinaron 941 niños de 6 a 12 años de edad en 2004. Se observaron folículos en 30% de los 484 niños de Chiapas, en 22% de los 181 de Zacatecas y en 42% de los 276 niños de Oaxaca. Se detectó *C. trachomatis* en niveles entre 2% y 5%; de los niños con folículos, 4,5% resultaron positivos por Giemsa y 15,5% por MIF.
Conclusiones. Estos resultados subestiman el nivel de infección ocular por clamidia, ya que los procedimientos de muestreo y los métodos de detección de clamidia empleados en este estudio no eran los más sensibles, por lo que representan una valoración conservadora del riesgo de trastornos visuales prevenibles. Como los niveles de *C. trachomatis* encontrados son similares a los informados para áreas de baja endemia de tracoma, las autoridades de salud deben estar listas para implementar medidas apropiadas si se confirmaran los riesgos para la salud visual de los niños mexicanos.

Palabras clave *Chlamydia trachomatis*, tracoma, infecciones del ojo, conjuntivitis, México.