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Original article

Clinical and Microbiological Assessment of Trachoma in the Kolofata Health District, Far North Region, Cameroon

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Abstract:
Background and aims: Trachoma is a sight-threatening process triggered by the infection of the conjunctiva with *Chlamydiae*. Blindness associated with trachoma was reported in Sahelian areas of Cameroon. However, data on the prevalence of this neglected infection in the Far North Region are not available. The aim of this study was a) to assess clinical trachoma and b) to detect *Chlamydia* in the conjunctiva of trachomatous populations living in the Far North Regions of Cameroon.
Methods: A total of 2,423 randomly selected children (1–10 years) and 1,590 women over 14 from randomly selected villages from the Kolofata Health District (115,000 inhabitants) were included in a cross-sectional study in February 2009. Trained staff examined and obtained conjunctival swabs from trachomatous subjects. DNA was extracted and amplified to detect *Chlamydia* DNA by real-time PCR. The quality of sampling was assessed by quantifying the number of epithelial cells.
Results: Children (2,397 or 98.9% of the predicted number) and women (1,543; 97.0%) were examined. The prevalence of follicular trachoma (TF) in children was 21% (95% CI 17.8–24.5) and of intense inflammatory trachoma (TI) 5.2% (95% CI 3.6–7.3). Among the women, trichiasis (TT) was observed in 3.4% (95% CI 2.4–4.7), corneal opacities (CO) in 1.4% (95% CI 0.8–2.3) and trachoma-related blindness in 0.9% (95% CI 0.4–1.8). Conditions related to income, illiteracy, latrines, water supply and animals wandering close to dwellings were similar in all the villages. PCR was positive in 35% of children with active trachoma and in 6% of adult females presenting TT and/or related corneal opacities.
Conclusion: The prevalence of trachoma and the severe trachoma *sequelae* found during this survey underline the urgent need to implement efficient blindness prevention interventions to improve the visual future of the people in the Sahelian region.
Key words: trachoma, survey, trichiasis, blindness, Cameroon, Sahel, Kolofata, *Chlamydia*

INTRODUCTION

Trachoma is a major public health issue that causes visual disability and dependency and impedes development [1–2]. The extreme north savannah of Cameroon is part of the west Africa Sahelian area where a high prevalence of trachoma was reported. In this area, over 90% of the population earns less than 1 US dollar a day, and the health infrastructure is poor, with widespread illiteracy, poor sanitation and lack of potable water [2–6]. Cases of trachomatous blindness were reported in 4.63% of the 3,326 cases of blindness, with a 1:2 male-female ratio in a limited area of northern Cameroon in the early 90s [7].

The World Health Organization (WHO) defines a public health problem as a condition where the prevalence of TF (trachomatous follicles) and TI (intense trachomatous

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inflammation) among children aged 1–9 years exceeds 10% and where entropion trichiasis (TT) and corneal opacities (CO) of at least 1% are observed in those aged 15 years or more [8–10]. Given the absence of reliable data from epidemiological trachoma surveys at the district level [1, 2, 11–12] in the Far North Region, we conducted this study with the support of the non-governmental organization Ophtalmo Sans Frontières, the Centre Hospitalier National d'Ophtalmologie des Quinze-Vingts in Paris, and local health authorities. The goal was to determine the extent of active trachoma (TF and TI) in randomized children from 1 to 10 years of age and in women over 14 years (TT and CO) living in rural villages of Kolofata district. The subjects diagnosed with trachoma were sampled to detect *Chlamydia* DNA by real-time PCR.

MATERIALS AND METHODS

Study population

This cross-sectional descriptive study was conducted during the dry season (February 2009) in compliance with the Declaration of Helsinki and the European Guidelines for Good Clinical and Laboratory Procedures in the Kolofata Health District, a rural zone of 108,625 inhabitants in the Sahelian area of the Far North Region of Cameroon (baseline population statistics were culled during the 2008 census) [13]. Local teachers and religious authorities actively sensitized the populations to participate in the survey. After explanation of the goals of the study, informed consent was obtained from each participant and confirmed by individual fingerprints. For children the parent/caretaker provided consent on their behalf. The consent of each participant was validated by the signature or fingerprint of the village chief and by the local health worker.

This randomized cluster study was carried out taking into account the fact that the population—of each village relative to the total district population—required the random selection of 30 villages (clusters) according to the principle of proportional probability [12, 14–16]. The calculation of sample size of each stratum was based on an expected prevalence of trichiasis-entropion of 2.5%, a precision of 1%, an alpha risk of 5% and a cluster effect of 1.5. Considering the demographic structure of Cameroon, in which women account for 29% of the population, a total of 4,810 subjects were required for each stratum. The sample population includes 2,076 children over 1 year and under 10 years of age or 69 children per cluster. For a presumed prevalence of 20%, the size of this subpopulation of children is consistent with a precision of 3.5%, an alpha risk of 5% and a cluster effect of 4 [12, 14–16].

Training

Training sessions (4 days) organized by WHO staff on site assured the standardization of procedures. Examiners who achieved at least 80% agreement proceeded to the field evaluation of 50 volunteers of various ages and sexes from villages not included in this survey. All signs associated with trachoma were represented in the training set of pictures for evaluation (trachomatous follicles, TF; intense trachomatous inflammation, TI; entropion trichiasis, TT, trachomatous scarring, TS, and corneal opacities, CO) [1, 16, 17].

Clinical examination and sampling for detection of genomes of *Chlamydia*

The personnel in charge of the clinical examination and collecting of samples first washed their hands with an appropriate disinfectant, put on gloves, and changed these upon the arrival of each subject. Clinical examinations of the conjunctiva for signs of trachoma in children (WHO trained staff) were performed using a flashlight (held by an independent operator) as a source of light and with the aid of binocular magnifying glasses (2.5 X). Each eye was examined and sampled separately by reverting the upper eyelid. To avoid cross contamination with chlamydial DNA, the person in charge of the clinical examination (wearing glasses, gloves, surgical mask, shoes and hair protection) was not allowed to touch flashlights, pens, tubes or files. The operator used a new pair of sterile gloves for each subject. The clinical diagnosis and comments were recorded by an independent member of the team. Trachoma was graded according to the grading card supplied by the Prevention of Blindness and Deafness Department of the World Health Organization. Women over 14 years were examined to assess the rates of TT (at least one eyelash rubbing against the cornea), or CO that covered most or the pupil. When such signs were found, visual acuity was measured according to the Snellen chart. Blindness and visual impairment were defined according to the 10th International Classification of Diseases [17].

Sampling

Samples were obtained from 628 children with active trachoma (TF or TF+TI, 1 to 10 years) and from 94 women with TT by vigorous scraping across the tarsal conjunctiva with Dacron swabs (at least 4 + 4 return movements rubbing the targeted epithelium), following procedures to avoid cross contamination. The swabs were introduced into a dry sterile tube, placed in a container, kept dry at 4–8°C for less than 8 h after sampling and stored at –20°C for DNA extraction. In each village, air controls were collected and labeled to ensure further masked testing. Frozen samples were

shipped for masked analysis to the laboratory of the Centre Hospitalier National d'Ophtalmologie des Quinze-Vingts, in Paris, France.

Assessment of environmental risk factors for trachoma

The present trial was conducted during the first 3 weeks of February 2009 during the dry season, when the outside temperature oscillated between 24 +/– 2°C in the early morning and 34 +/– 4°C at noon (the external temperature and/or the humidity levels may interfere with the clinical assessment of the trachomatous inflammatory signs: vasoconstriction due to cold +/– high humidity may reduce the number and/or size of the follicles) [18].

Availability of latrines and water and their use and maintenance

In each village, the head of the village or religious leader was questioned by local health workers about latrines and water wells/pumps or taps in the village. If latrines or pumps were reported to be available, they were visited by the team to verify that they were functioning. In villages where latrines were available, each parent or caretaker was asked whether or not the tested child used the latrine. In villages where there were no working latrines, the response was completed as "No" for all subjects.

Animals wandering close to the dwellings

The local health workers visited the dwellings and their surroundings to determine whether cows, sheep or goats were wandering free. The response was recorded as "Yes" where animals wandered within 5 m of the sleeping areas.

Availability of tarmac-covered roads

Availability was assessed as "Yes" for villages located 500 m or less from tarmac-covered roads. The presence of one or more motorcycles, one or more cell phones and electric wires was registered by the health personnel as "Yes".

Nucleic acid extraction

Dacron dry swabs were thawed, placed in 0.5 ml of phosphate buffer solution (PBS) and vigorously vortexed. The treatment of samples was carried out under a vertical laminar flow and the extraction of DNA was performed in a separated area. Aliquots of 200 µl of PBS suspensions were extracted using MagnaPure® (LC Robot, Roche Molecular System, France) with the LC Magnapure Nucleic Acid Isolation kit I® (Roche Molecular System, France) and eluted in 50 µl of buffer [19–20]. To monitor the extraction and amplification processes, 10 µl of a whole virus preparation of seal herpes virus used as internal control (IC) (gift from G. J. van Doornum, Dept. of Virology Erasmus MC, Rotterdam, The Netherlands) was added before the extraction.

Detection of *Chlamydia* DNA

Primers and probe

The primers were selected in order to bracket a highly conserved multicopy gene coding for the ribosomal RNA in the vast majority of sequences of *C. trachomatis*, *C. psittaci*, *C. pneumoniae*, *C. felis*, *C. pecorum*, *C. caviae*, *C. suis* and *C. muridarum* [17]. No cross-reactivity with genes of other microorganisms or with any other mammal gene was detected for the primers or for the probe. The sequences of forward and reverse primers are: (5'TCGAGAATCTTTC GCAATGGAC); (5'CGCCCTTTACGCCCAATAAA). The reporter (5'fluor, 6-FAM) and the quencher (TAMRA) dyes were attached to the 5' end and the 3' end of the probe respectively (FAM-AAGTCTGACGAAGCGACGCCGC) [17]. The sequences of the primers and probe used for the detection of the Internal Control (IC, PhHV) are: (5'GGGC GAATCACAGATTGAATC); (5'GCGGTTCCAAA CGTA CCAA) and (5'TET-TTTTTATGTGTCCGCCACCATCTG GATC) [19, 20].

Real-time TaqMan broad-spectrum PCR assay

PCR reactions were carried out in a final volume of 24 µl containing 2X TaqMan FAST Universal PCR Mastermix® (MNL 4352042 AB), forward primer (0.5 uM), reverse primer (0.5 uM), FAM-TAMRA probe (0.4 uM) of both chlamydial and phHSV and 12 µl of the isolated DNA. After incubation for 10 min at 95°C, the PCR cycling program consisted of 50 two-step cycles of 10 s at 95°C and 65 s at 60°C. The amplification and detection were carried with the ABI Prism® 7500 sequence detector system (AB). Each run contained negative controls with no template. The extraction performance of the device and equipment as well as the potential presence of PCR inhibitors in each sample was systematically assessed [21].

Quantification of human cells in the samples

The Quantifier Human DNA Kit (Applied Biosystems, France) was used according to the manufacturer's instructions to assess the quality of sampling. This test allows quantification by real-time PCR of the human telomerase reverse transcriptase gene (hTERT) present as a single copy in each human cell. Calibration curves for cell load of the samples were plotted using commercial standards and human fibroblast preparations from the laboratory [19, 21].

Statistical analysis

Statistical analysis was performed with the EPIINFO

version 6 software. Multivariate analysis was performed to estimate the potential association of trachoma with housing conditions, illiteracy, accessibility and income level. Descriptive statistics were generated with Stata™ 8.0. Confidence intervals for the point prevalence estimates were generated using the Huber/White sandwich estimator of variance to adjust for the clustering effects of trachoma at the household level. Age- and sex-adjusted prevalence was generated and applied to the population estimates for 2008 to assess the burden of TT and number of persons with active trachoma. The 95% confidence intervals of the adjusted prevalence estimates were multiplied by the population estimates to derive the lower and upper bounds [14–15].

RESULTS

This is the first large-scale survey of trachoma in children born and living in rural areas of the District of Kolofata in the Far North Region of Cameroon. Clinical examination was carried out in 2,397 children and 1,543 females > 14 years. Table 1 shows the participation and distribution of children by age and sex and the global figures of active trachoma in children, and trachoma-related entropion-trichiasis, corneal opacities and blindness among women over 14 years old. The participation was 98.9% (2,423) and 97% (1,590) of the forecasted figures for children and adults, respectively. A statistically significant under-representation of children 5–9 years old ($p < 0.001$) and of males in all groups ($p = 0.01$) was observed with respect to the general population of Cameroon (2008 national census). The statistically significant under-representation of boys is probably due to the reluctance of some families to report absent children working in the fields or otherwise occupied outside the home during this survey. The age distribution of women was similar to that reported for the general population. Nevertheless, we found an under-representation of the 14–24 group and a statistically significant over-representation of the 25–34 group with respect to the general population of Cameroon ($p < 0.001$), probably due to the subjective estimation of ages by the participants. Differences were not statistically significant for the other age groups.

The overall prevalence of trachoma during this survey conducted in the first 3 weeks of February (dry season with outside temperatures between 24°C and 34°C) was 32%. As shown in Table 1 the prevalence of active trachoma in children aged 10 or less was 26.2% and declined progressively after the age of 4 years. TF was diagnosed in 291 boys and in 327 girls (over representation; $p = 0.03$), and TI was diagnosed in 49 boys and 78 girls ($p = 0.015$). No significant differences were found among prevalence rates in the different villages between sex and age. TT without CO was detected in both eyes of 2 children aged 6 and 8 years. In women aged 14 and more the trachoma-related *sequelae* increase with age, peaking at 55–64 years. Corneal opacities were detected in the right eye of 20 women, of whom 17 were blind. Blindness associated with CO in both eyes was observed in 14 women (0.9%).

Table 1. Active trachoma (TF, TF/TI+) in children and trachoma-related entropion-trichiasis (TT), corneal opacities (CO) and blindness among women over 14 years old in Kolofata Health District

Children (participation: 98.9%)

Age	TF % from total TF (504 cases)	TI % from total TI (124 cases)
> 1	7.7	17.1
2	12.3	20.1
3	23.4	14.5
4	18.8	20.1
5	13.2	4.1
6	9.7	6.5
7	6	8.8
8	5.4	5.6
9	3.5	3.2
Prevalence	21% of examined children ($IC_{95\%}$: 17.8–24.5)	5.2% of examined children ($IC_{95\%}$: 3.6–7.3)

Adults (participation: 97%)

Age	TT		CO		Blindness	
	N	%	N	%	N	%
> 14–24	2	3.4	1	4.8	0	0
25–34	7	12	0	0	0	0
35–44	10	17.3	1	4.8	0	0
45–54	7	12	1	4.8	1	7.1
55–64	21	36.3	12	57	8	57
65–74	8	13.8	3	14.3	2	14.4
75 et +	3	5.2	3	14.3	3	21.5

	TT		CO	
	N	%	N	%
Right Eye	52	3.4% ($CI_{95\%}$ 2.5%–4.4%)	21	1.4% ($CI_{95\%}$ 0.8%–2.9%)
Left Eye	58	3.8% ($CI_{95\%}$ 2.9%–4.8%)	21	1.4% ($CI_{95\%}$ 0.8%–2.9%)
Both Eyes	53	3.4% ($CI_{95\%}$ 2.5%–4.4%)	21	1.4% ($CI_{95\%}$ 0.8%–2.9%)

Table 2 presents the results of *Chlamydia* genome detection by real-time broad range polymerase chain reaction (potential surrogate marker of active trachoma). The global positive *Chlamydia* PCR was 35%. Regarding the number

Table 2. Detection of *Chlamydiae* in children with clinical diagnosis of trachoma in randomized villages from the Far North Region of Cameroon

Village*	PCR+ in boys TF+/boys TF+	PCR+ in boys TI+/boys TI+	PCR+ in girls TF+/number of girls TF+	PCR+ in girls TI+/number of girls TI+
Double	4.23%	9.09%	14.08%	26.32%
Kodogo	6.90%	18.18%	13.79%	22.22%
Grea	7.14%	16.67%	17.86%	31.25%
Amchide	9.26%	20.00%	5.56%	10.34%
Talkomari	10%	18%	0%	0%
Ndjamena Karabia	10.94%	24.14%	25%	45.71%
Cheripouri	13.04%	42.86%	30.43%	43.75%
Garakawa	13.79%	30.77%	13.79%	25%
Katoua Houdiang	14.81%	57.14%	25.93%	35%
Mbanari	16.67%	35.71%	23.33%	43.75%
Blablin	16.67%	50.00%	33.33%	50%
Alagarno	19.05%	36.36%	11.90%	25%
Brouvari	19.30%	45.83%	29.82%	51.52%
Yegoua	21.74%	35.71%	17.39%	44.44%
Adanga Danga	22.22%	46.15%	40.74%	78.57%
Ndaba	22.73%	38.46%	9.09%	22.22%
Sanda Wajiri	27.27%	56.25%	27.27%	52.94%
Malika	39.13%	52.94%	17.39%	66.67%
Kerawa	44.44%	66.67%	22.22%	66.67%
X ± SD	18 ± 10	37 ± 16	20 ± 10	39 ± 20

*: Results are reported regarding positive cases of trachoma. “Villages” refers to the randomly selected central village and the surrounding dwellings with people sharing the wells in the village.

of TF and TI cases among boys and girls in each randomized village of the district, boys presenting TF positive PCR levels varied between 4.2% and 44% and girls between 9% and 67% ($p < 0.05$). Positive PCR levels ranged between 0% and 41% among boys presenting TI and between 0 and 67% among girls.

In Talkomari, 10% of the samples obtained from boys presenting TF and 18% presenting TI were PCR positive, but surprisingly none of the samples obtained from girls were positive. The levels of positivity for PCR from trachomatous children cannot be attributed to inhibitors of the chlamydial DNA amplification process, because controls for the inhibition of the polymerization were amplified and detected simultaneously with the target DNA.

PCR was positive in 6% of the samples obtained from women aged 14 or more with TT or CO (1 aged 60 presenting unilateral TT associated with unilateral CO; 1 aged 60 presenting bilateral TT and bilateral CO and 1 aged 68 presenting bilateral TI). One sample obtained from a female aged 43 who shared her dwelling and bed with 5 trachomatous children (with conjunctivitis but no signs of active trachoma) produced a weak and reproducible positive PCR signal (Ct: 38).

Table 3. Conjunctival epithelial cells and rates of positive *Chlamydia* PCR

Number of cells sample	% of positive PCR
0–100	8.51
100–500	23.02
500–1000	45.00
1000–1500	42.50
1500–2000	40.00
2000–5000	40.97
5000–10000	40.54
10000–20000	30.91
20000–60000	26.67

Real-time PCR for the internal controls was run simultaneously with that for *Chlamydia*. PCR inhibitors were present in 65 samples (9% of the total), and 28 (3.9% of the total) became positive for *Chlamydia* (true positives) when the inhibited DNA extracts were diluted (1/10) in distilled water. For the others (5.1%), the inhibition was eliminated by dilution of the extracts but the samples remained negative (true negatives). No significant correlation was found

Table 4. Trachoma risk factor univariate analysis (19 villages)

Risk factors	TF [p] and/or TF/TI	TT and/or CO
Accessibility / tarmac covered roads [a]	ns	ns
Latrines (used and maintained)	ns	ns
Cows and goats wandering in the village	ns	ns
Significantly low income*	ns	ns
Parental analphabetism [b]	ns	ns
Herd close to babies (< 10 meters from houses)	ns	ns
Water supply [c;d]	ns	ns
Wells in the village	ns	ns
> 1 cell phone in the village	ns	ns
Visible electric wires	ns	ns
> 1 motorcycle in the village	ns	ns

[a]: tarmac covered roads for the 19 villages at 30 or more km
*: according to local standards and compared with the income in big cities (≤ 1 US/day)
[b]: Parents unable to read French, English or any of the 6 indigenous languages
[c]: Free or paying wells at < 15 minutes walk from the dwellings
[d]: Free or paying wells at > 15 minutes walk from the dwellings

between age, sex, prevalence of TF or TI and positive PCR or load of *Chlamydia* (number of copies of chlamydial DNA). However, the comparison of PCR positive results with the number of epithelial cells shows that only 8.5% of PCR positive samples contained 100 cells/μl or less. Hence, specimens containing $10^{1.5}$ epithelial cells/sample or less should be considered as non-interpretable instead of negative (Table 3). The contamination controls were negative for all the samples from: a- the air (carried out in all the villages with swabs and swab containers kept open during clinical examination); b- from 25 adults with no clinical signs of trachoma and c- with the potentially exposed operators' fingers.

Income levels (1 US dollar or less/day) were similar throughout the villages and could not be analyzed individually as risk factors. The use and maintenance of latrines (< 33% of the villages), the presence of herds close to dwellings (> 75%), and the water supply (< 25%) were also not significantly different in the villages and had no direct impact on the prevalence of trachoma (Table 4).

DISCUSSION

This is the first large-scale survey of trachoma in children living in rural areas of the district of Kolofata in the Far North Region of Cameroon carried out according to the WHO recommendations. The very high rates of participation (> 97%) in this survey are attributable to the vigorous information campaign conducted in all the villages. Among the randomized children over 1 year and less than 10 years in this district, 26.2% showed signs of active trachoma. Among women over 14 years the prevalence of TT was 3.4%, trachoma-associated corneal opacities 1.4%, and trachoma associated blindness (bilateral) 0.9%. The surveys carried out in areas of Nigeria, Niger and Chad (bordering Kolofata), which have similar ethnic, educational and socioeconomic indicators and the same topography and climatology, showed levels of active trachoma similar to those found in the present study [22–25].

The correlation between the clinical diagnosis and the detection of *Chlamydia* genomes by PCR was not statistically significant. The detection rates cannot be attributed to an unknown inhibitor of the *Chlamydia* DNA amplification agent present in the samples because the internal controls were extracted, amplified and have been detected simultaneously in the same tube as the target DNA. Strict sampling procedures were followed to avoid cross contamination, and the rates of positivity obtained in this study are in the range of what could have been expected from previous reports. *Chlamydiae* are intracellular bacteria and their detection requires cell-rich samples. The reduced number of positive PCR obtained here could be the result of not rubbing the swabs against the conjunctival epithelium vigorously enough to recover the infected epithelial cells. This was not the case because most of the samples contain 100 epithelial cells or more.

In addition, the samples from villages with high rates of positive PCR were retested in parallel with samples from villages with 0% PCR positivity, and no differences nor correlation between the number of cells in the samples and the positivity of PCR were found. Accordingly, if the conjunctival samples obtained with Dacron swabs from subjects

with active trachoma represent the sanctuary where *Chlamydia* replicate, the low rate of positive real-time PCR suggests that their detection using the most sensitive real-time PCR cannot be regarded as the gold standard for the diagnosis of trachoma. The association of clinical active trachoma with PCR results is under discussion especially because the lack of detection of chlamydial genomes (surrogate marker of infection) may not be related to the chronic inflammatory process leading to blindness [26, 27].

The detection of *Chlamydia* by Amplicor reported in most of the epidemiological studies targets a sequence of a *C. trachomatis* cryptic plasmid that is not required for growth *in-vitro* and that can be altered or absent in viable strains. Moreover, most of the commercial diagnosis tests for *Chlamydia* reported had monospecies spectrums and may produce negative results for other species of *Chlamydia* associated with conjunctival signs [21, 27–29]. To avoid these technical limitations we used the broad-spectrum real-time PCR that detects the entire genus *Chlamydia* [19].

Because proper conjunctival scraping and broad-spectrum testing for *Chlamydia* produces less than 50% PCR positivity in children presenting clinical active trachoma, the predictive value of the nucleic-acid amplification techniques (PCRs) used as surrogate markers for diagnosis and therapeutic follow up of trachoma require a comprehensive revision. Finally, the overall rate of positivity for PCR (35%) in the trachomatous children studied here suggests that extreme poverty (lack of water, lack of latrines, lack of physical separation between animals and people, low income and illiteracy) perpetuated the initial bacterial infection in a chronic inflammatory process.

CONCLUSION

The immune responses triggered by the intracellular chronic chlamydial infection and the lack of sanitation may lead to blindness. Trachoma preferentially affects neglected communities where the infection of children's conjunctival cells with *Chlamydia* evolves into an irreversible inflammatory process. The screening for trachoma in randomly selected villages in the District of Kolofata, Far North Region of Cameroon showed an overall prevalence of 30% with positive *Chlamydiae* PCR in 35% of the samples from trachomatous individuals. The income levels, quality of roads, access to latrines/potable water and illiteracy could not be analyzed as independent pejorative risk factors. The WHO, estimating that approximately 5.9 million persons have suffered from severe vision-loss as a result of trachoma while another 10 million are at high risk, proposed the SAFE (surgery for trichiasis/entropion, antibiotics to treat the active disease, facial cleanliness, and environmental changes to minimize transmission) strategy for trachoma control [1–4]. The results found during this survey should alert health authorities as to the urgent need to implement the SAFE strategy to improve the visual prognosis of these populations.

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