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Decline in Total Serum IgE and Soluble CD30 in the Context of Soil-Transmitted Helminth Decline in Bolivia

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Abstract. In the Bolivian Chaco, recent surveys documented a dramatic decrease in the prevalence of soil-transmitted helminth (STH) infections as compared with the 1980s after thirty years of preventive chemotherapy (PC). Concomitant immunological rearrangements are expected. Because nematode infections are associated with increased levels of circulating IgE and glycoprotein CD30 soluble form (sCD30), this study aims to evaluate changes in serological markers of T helper (Th)2-cells activity between 1987 (high STH prevalence) and 2013 (low STH prevalence) in rural communities in the Bolivian Chaco area. We collected 151 sera during two different surveys in 1987 (n = 65) and 2013 (n = 86) and measured the concentration of total IgE and sCD30 by immunoassays. We found a statistically significant age-independent decrease in the total IgE ($P < 0.0001$) and sCD30 ($P < 0.0001$) from 1987 to 2013. The significant decrease in serological Th2 markers (IgE and sCD30) between 1987 and 2013 is consistent with the drop in STH prevalence in this geographical area during the same period of time. Further studies might elucidate the clinical and epidemiological impact of these serological rearrangements

INTRODUCTION

It is estimated that soil-transmitted helminthes (STHs), namely, the roundworm *Ascaris lumbricoides*, the whipworm *Trichuris trichiura*, and the hookworms *Necator americanus* and *Ancylostoma duodenale*, infect approximately one-fourth to one-fifth of the world's population. Children are particularly exposed: approximately 270 million preschool-age children (pre-SAC) and more than 550 million school-age children (SAC) are affected, mostly living in resource-deprived areas, where these parasites are extensively transmitted because of the low socio-sanitary conditions.[1] Moreover, 370 million persons are now estimated to be infected worldwide with another intestinal nematode, *Strongyloides stercoralis*, but the global prevalence of this parasite has long been underestimated, as it has been based on studies using poorly sensitive diagnostics.[2]

In 2001, the WHO endorsed a resolution to control STH infections through improved sanitation, health education, and preventive chemotherapy (PC), whereby anthelmintic drugs such as albendazole or mebendazole should be periodically administered to high-risk groups, such as preSAC, SAC, and women of reproductive age.[3] The global target is to eliminate morbidity due to soil-transmitted helminthiases in children by 2020, by regularly treating at least 75% of the children in endemic areas[1]; in 2017, the coverage has reached more than 69% of the SAC and preSAC groups.[4]

In Bolivia, PC based on 6-monthly single-dose mebendazole delivery to SAC has been implemented since 1985 and achieved impressive results, at least in the Chaco region, in the southeastern part of the country. Parasitological surveys conducted 26 years apart in rural areas of the Bolivian Chaco documented a dramatic decrease in STH prevalence between 1987 and 2013: the prevalence of hookworm dropped from up to 50% to 0.4–1.3%, *A. lumbricoides* from 19% to 0.9–1.5%, and *T. trichiura* from 19% to 0%.[5–7] Based on these findings, and according to WHO recommendations, in September 2016, local authorities interrupted PC delivery in this area and started a program of STH surveillance through annual cross-sectional parasitological surveys at sentinel sites. Two years later, surveillance confirmed the sustained STH reduction (< 1%) in the entire ecological zone.[8]

Concomitantly, a serosurvey evidenced a drop of anti–*S. stercoralis* antibody prevalence from 1987 to 2013 (from 16% to 6%), although PC is not effective against this parasite.[9] Conversely, protozoan parasitic infections did not show any substantial decreasing.[7]

Parasitic infections are associated with type 2 immune responses, characterized by T helper (Th)-2 cells activity, eosinophilia, and elevated IgE levels.[10] The release by Th-2 cells of high amounts of soluble CD30 (sCD30), belonging to the tumor necrosis factor/nerve growth factor receptor superfamily, is also associated with type 2 response.[11] Type 2 immune response is also involved in allergic diseases, which can lead to debilitating or even fatal disorders. Studies in animals provide evidence that helminth infections downregulate parasitic-specific immune responses and protect against allergic airway inflammation.[12] According to the so-called hygiene hypothesis, the increase in allergic disorders observed in western countries in recent times would be related to the low exposure in childhood to microbes and parasites, which would modulate the human immune system growth.[13]

Taking advantage of the preferential expression of IgE and sCD30 as Th2 activation markers,[14] we investigated whether a

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change in serum concentration of IgE and sCD30, as a proxy for Th2 activation markers, had occurred over a 26-year period (from 1987 to 2013), among people living in two rural communities of the Bolivian Chaco area at the same time of the progressive disappearance of STH infections.

MATERIALS AND METHODS

Study area and population. The Bolivian Chaco is a semiarid and sparsely populated region, located in the southeast of Bolivia (longitude 64°300 and 58°500 west of the Greenwich meridian; latitude 17°580 and 22°200 south). Serum samples were collected within research activities conducted in 1987 in Javillo, a very small community (of about 110 inhabitants), and in 2013, in the rural community of Ivamirapinta (about 1,200 inhabitants). Both communities lie in the municipality of Gutierrez (Cordillera Province, Department of Santa Cruz).

Determination of total IgE and sCD30 in human sera. Sera were obtained from venous blood samples, stored at −20°C in Bolivia, shipped within 2–4 weeks to Florence, Italy, and stored here at −70°C until tested. Sera were anonymized and labeled with a unique code, allowing to retrieve demographic information.

The total IgE level in the human serum samples was determined via ImmunoCAP total IgE kit automatically processed into the Phadia 250 instrument (Thermo Scientific, Massachusetts). Inside the instrument, the anti-IgE antibody, covalently linked to the solid phase, reacts with the IgE in the serum samples. After incubation and a washing step, enzyme-labeled anti-IgE antibody is added to form a complex. A further washing step, necessary for the removal of unbound substances, is carried out before a second incubation period with the developing agent. Finally, the reaction is stopped and the eluate's fluorescence measured. The total IgE amount in each tested sample is then available in kU/L.

Sera-soluble CD30 concentrations were determined using Invitrogen ZyQuick sCD30 ELISA kit (Invitrogen Corporation, Camarillo, California), according to the manufacturer's instructions. Briefly, soluble CD30 in the samples and standards binds to the anti-sCD30 coated on the plate. A biotin-conjugated antibody is added to bind the sCD30 captured by the first antibody. After adding Streptavidin-HRP and the substrate solution, the colorimetric reaction is terminated by the addition of acid. Absorbance was measured at 450 nm with Multiskan GO spectrophotometer and analyzed with its SkanIT 3.2 software (Thermo Fisher Scientific, Massachusetts).

Statistical analysis. Statistical analysis was performed with STATA 11.0 (StataCorp, College Statio, TX). Two age categories were created: < 26 and ≥ 26 years of age (the time difference between the two surveys, so as to have a group of 2013 subjects who were not yet born when the first survey was conducted). Descriptive statistics was used for the calculation of means, SD, medians, and interquartile range of quantitative data. The normal distributions of test results for the two groups of sera were compared using the Student's *t*-test. Differences in age distribution were estimated by the Mann–Whitney test. Linear regression models were performed to establish the association between serum concentration of Th2 markers and year of collection, allowing for age (continuous). $P < 0.05$ was considered statistically significant.

Ethics statement. The studies were programmed and conducted in agreement with the Ministry of Health of the Plurinational State of Bolivia (within the Convenio Ministerio de Salud y Deportes, Estado Plurinacional de Bolivia/Cátedra de Enfermedades Infecciosas, Universidad de Florencia, Italia) and with the support of the Guaraní political organization (Asamblea del Pueblo Guaraní). The study was approved by a local Ethics Committee (Colegio Médico de Santa Cruz, TDEM CITE No. 028/2017).

RESULTS

Overall, 151 sera were collected from people living in two rural communities (age 4–70 years): Javillo (65 serum samples, collected in 1987) and Ivamirapinta (86 serum samples, collected in 2013). Table 1 summarizes the characteristics of the participants in the surveys. No significant difference in sex distribution was observed between 1987 and 2013 population; participants were significantly older in 2013 ($P < 0.0001$).

The mean serum total IgE concentrations were 205.4 kU/L (SD 55.8 kU/L, median 186.0 kU/L) and 77.9 kU/L (SD 21.3 kU/L, median 81.5 kU/L) in 2013 and 1987, respectively. The mean serum total sCD30 concentrations were 46.0 U/mL (SD 19.2 U/mL, median 41.0 U/mL) and 18.4 U/mL (SD 10.6 U/mL, median 15.0 U/mL) in 1987 and 2013, respectively. The difference was statistically significant for both total IgE and sCD30 ($P < 0.0001$) (Figure 1), both overall and in each of the age categories (SAC, and subjects aged < 26 and ≥ 26 years) (Table 2). By linear models, both IgE and sCD30 confirmed a significant decline from 1987 to 2013 ($P < 0.0001$), independent of age (Supplemental Table). No significant differences were found in serum concentration of either markers between SAC and between subjects aged < 26 and ≥ 26 years, both in 1987 and in 2013 (Table 2).

DISCUSSION

Our study shows a significant decrease in IgE and sCD30 concentrations ($P < 0.0001$) between sera collected 26 years apart in two rural communities of the Bolivian Chaco. These

TABLE 1
Demographic data of the 151 subjects surveyed in 1987 and 2013 in the Bolivian Chaco

		Javillo (1987)	Ivamirapinta (2013)
Gender	Total	*N* = 65	*N* = 86
	Male	48% (<I>N</I> = 31)	48% (<I>N</I> = 41)
	Female	52% (<I>N</I> = 34)	52% (<I>N</I> = 45)
Age (years)	Median (IQR) range	15.5 (10–30) 4–70	32 (13–48) 6–70
	< 26	71% (<I>N</I> = 46)	43% (<I>N</I> = 37)
	≥ 26	29% (<I>N</I> = 19)	57% (<I>N</I> = 49)
SAC		51% (<I>N</I> = 33)	29% (<I>N</I> = 25)

IQR = interquartile range; SAC = school-age children (4–15 years old).

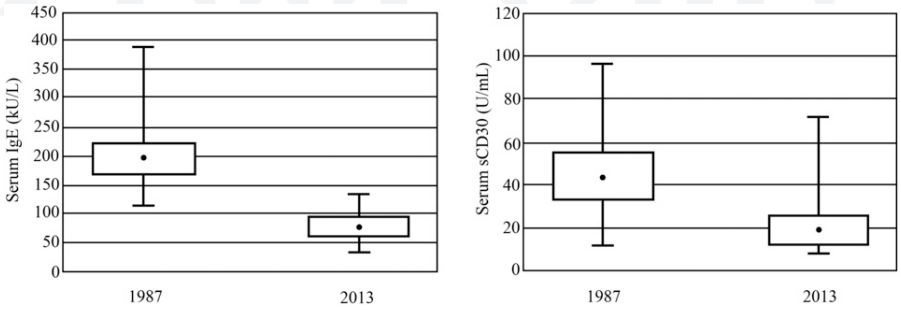

FIGURE 1. Box-plot of serum median IgE (left panel) and sCD30 (right panel) concentration distributions in patients from Javillo (1987) and Ivamirapinta (2013) communities.

changes paralleled an impressive drop in STH prevalence in this area, from more than 50% to < 1%, likely the result of 30 years of PC.[8] Of note, no STH infections were identified in Ivamirapinta in 2013 during a parasitological survey, which involved 111 inhabitants (median age 30 years, range 1–73).[15] During the same period, a significant decrease in seroprevalence was documented also for *S. stercoralis*, although no large-scale control measures potentially affecting strongyloidiasis were implemented.[9] No significant change in intestinal protozoan parasite infections was observed during the same time interval, indicating socioeconomic conditions have not substantially improved during that period. In the two communities surveyed, the seropositivity for *S. stercoralis* observed in Javillo in 1987 was 12%, and 7% in Ivamirapinta in 2013 (unpublished data). No recent data are available on the prevalence of other helminth infections potentially affecting Th type 2 response, in the Bolivian Chaco. A high rate of *Mansonella ozzardi* microfilaremia (26%) was found in 1997, whereas in 1999, a 3% point prevalence for anti–*Trichinella spiralis* antibodies, and a high seroreactivity against *Taenia solium* and *Toxocara canis* antigens were detected in the population living in this area.[16,17]

Meanwhile, no specific interventions were implemented against these infections.

The capacity of helminth infection to modulate host immune response, and thus their impact on allergic and/or inflammatory diseases, has been a matter of a large volume of research since the 1970s.[18] The "hygiene hypothesis" links the reduction in endemic infections due to housing, sanitation, and health improvements in high-income countries with the concomitant rise in the prevalence of asthma and atopy in childhood.[19] In brief, the removal of the regulatory effect of microorganisms and other parasites, after millennia of coevolution and genetic adaptation with humans and other mammalian, might produce an imbalance in the host pro- and anti-inflammatory immune responses and an excess of immune-mediated diseases. However, little consensus exists among the studies that have explored the interaction between helminth infection and allergic diseases, including meta-analysis and systematic reviews.[20] For instance, as for the development of asthma, hookworm infection showed a strong protective role, but *T. trichiura*, *Enterobius vermicularis*, and *S. stercoralis* did not have an impact, whereas *A. lumbricoides* infection resulted a significant risk factor.[21] Most of the published studies agree that helminth infection protects against atopic sensitization and allergic skin reactivity, although these infections appear associated with increased allergen-specific IgE.[18,22] In recent years, deliberate infection of patients with worms has been proposed in non-endemic, high-income countries, as a possible treatment for inflammatory diseases, such as allergies, inflammatory bowel diseases, multiple sclerosis, rheumatoid arthritis, and psoriasis, with inconsistent results.[23]

TABLE 2
Serum concentrations of IgE (kU/L) and sCD30 (U/mL) of the human population surveyed in 1987 and 2013 in the Bolivian Chaco

		Javillo (1987)	Ivamirapinta (2013)	*P*-value
Serum IgE, mean (SD)	Total	205.4 (± 55.8)	77.9 (± 21.3)	$P < 0.001$
	SAC	206.3 (± 54.7)	79.9 (± 21.0)	$P < 0.001$
	< 26 years	199.1 (± 49.6)	80.0 (± 22.0)	$P < 0.001$
	≥ 26 years	220.7 (± 67.6)	76.3 (± 20.8)	$P < 0.001$
Serum IgE, median (IQR)	Total	186.0 (173.0–223.0)	81.5 (64.2–92.7)	$P < 0.001$
	SAC	188.0 (176.0–225.0)	84.0 (71.0–92.0)	$P < 0.001$
	< 26 years	186.0 (172.2–222.5)	84.0 (65.0–93.0)	$P < 0.001$
	≥ 26 years	186.0 (178.5–275.0)	76.0 (62.0–92.0)	$P < 0.001$
Serum sCD30, mean (SD)	Total	46.0 (± 19.2)	18.4 (± 10.6)	$P < 0.001$
	SAC	49.8 (± 19.7)	15.5 (± 7.2)	$P < 0.001$
	< 26 years	47.4 (± 18.4)	16.6 (± 7.6)	$P < 0.001$
	≥ 26 years	42.7 (±21.3)	19.7 (± 12.3)	$P < 0.001$
Serum sCD30, median (IQR)	Total	41.0 (33.0–57.0)	15.0 (11.0–25.0)	$P < 0.001$
	SAC	48.0 (34.0–61.0)	13.0 (11.0–17.0)	$P < 0.001$
	< 26 years	43.0 (36.0–57.7)	14.0 (11.0–24.0)	$P < 0.001$
	≥ 26 years	36.0 (27.5–52.5)	16.0 (11.0–27.0)	$P < 0.001$

IQR = interquartile range; SAC = school-age children (4–15 years old); sCD30 = soluble CD30.
Both overall and age-stratified comparison showed a significant decline from 1987 to 2013 ($P < 0.001$) (Student's *t*-test). No significant differences were found among the different age classes either in the 1987 or 2013 cohort.

This study is not without limitations. One is that the two surveys took place in different communities—however, the Bolivian Chaco is a homogeneous ecological zone, where rural populations share the same hygienic and sanitary conditions, and no substantial differences in infestation rates are expected between two neighboring communities. Another limitation is that no parasitological data are available from Javillo—however, a parasitological survey conducted in 1987 in different rural communities of the Chaco region showed a homogenously high STH prevalence.[5] A third concern would be that the population of the second survey is older—however, different analyses concur to show the observed changes are age independent. Last and importantly, no information on allergic symptoms experienced by the participants and other epidemiological information on allergy-related clinical syndromes or immune-mediated diseases in the population are available. Further studies are warranted to address the clinical and epidemiological correlation of the serological rearrangements in populations where STH infestation has virtually disappeared. Longitudinal studies carried out in settings where mass deworming programs are ongoing may help elucidate whether the removal of helminth infections would lead to an increased prevalence of allergic and other immune-mediated diseases in the long term.

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Note: Supplemental table appears at www.ajtmh.org.

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S1 Table. Age-adjusted linear regressions of IgE (panel A) and sCD30 (panel B) levels, by year (1987 vs 2013)

A

IgE	Coef	Coef St. Err	[95%CI]	p-value
Age	-0.15	0.19	-0.51; – 0.22	0.433
Year	- 125.67	6.99	-139.49; -111.86	0.000

R-Sq = 71.8% R-Sq(adj) = 71.4%

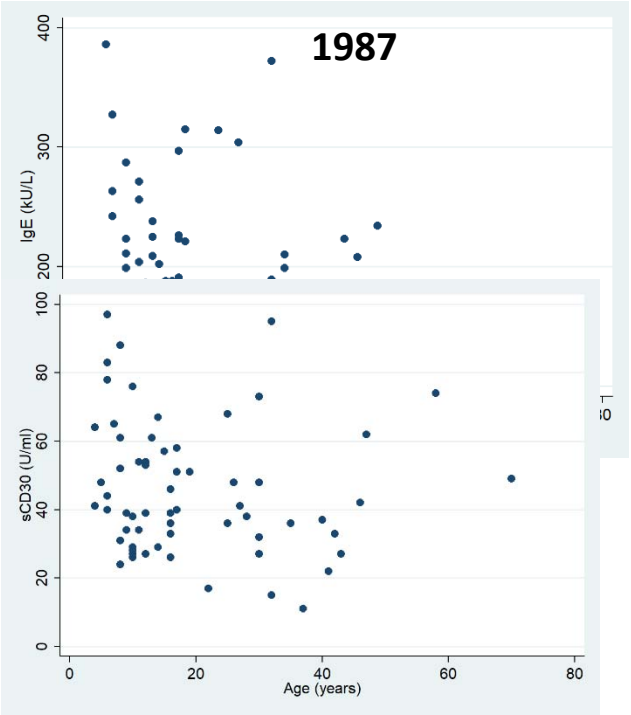
B

sCD30	Coef	Coef St. Err	[95%CI]	p-value
Age	-0.13	0.07	-0.15; 0.12	0.847
Year	-27.48	2.61	-32.64; -22.31	0.000

R-Sq = 46.1% R-Sq(adj) = 45.4%

S2 Figure. Scatter plot of the IgE (panel A) and sCD30 (panel B) levels distribution by age in 1987 and 2013.

A



B