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Original research

Integrated serological surveillance of communicable diseases in the Paraguayan Chaco, 2019

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ABSTRACT

Objective. To establish baseline seroprevalence of soil-borne, waterborne, and foodborne diseases and to monitor diseases that are eliminated or on the path to elimination in the Paraguayan Chaco.

Methods. A total of 1 100 school-age children (6–15 years) were tested in urban and rural schools selected for a cross-cutting population-based survey using a two-stage probabilistic sample design in the three departments of the Paraguayan Chaco. Blood samples were taken on filter paper to measure IgG antibodies using a multiplex bead assay. Data collection was carried out through interviews with parents and caregivers. Access to basic sanitation and improved water was assessed. Differences in pathogen seropositivity and seroprotection were estimated by urban and rural areas.

Results. Seroprotection against measles was 62.9% and against rubella was 78.2%. Minimal diphtheria and tetanus seroprotection (≥0.01 IU/ml) was 92.9% and 98.3%, respectively. Seroprotective levels against these four vaccine-preventable diseases significantly decreased with increasing age ($p < 0.05$). The following pathogens and respective antigens showed significantly higher seroprevalence ($p < 0.05$) in rural areas compared with urban areas: *Cryptosporidium parvum* Cp17: 80.4% vs 64.6%, and Cp23: 60.6% vs 44.8%; *Giardia lamblia* VSP3: 26.9% vs 16.6%; *Strongyloides stercoralis* NIE: 11.5% vs 4.1%; and *Taenia solium* T24H: 7.1% vs 1.6%. Seroprevalence for these pathogens was also higher in Indigenous population when compared to non-Indigenous. Basic sanitation conditions showed significant differences ($p < 0.05$) between rural and urban areas: adobe and soil dwelling floor (65.3% vs 30.2%), use of pit latrine (90.3% vs 44.2%), availability of drainage or septic tank (8.7% vs 55.2%), access to safe water (19.7% vs 44.9%), and water treatment (6.8% vs 32.3%).

Conclusions. We identified high exposure to soil-borne, waterborne, and foodborne diseases in rural areas and Indigenous population in the Paraguayan Chaco. Low seroprotection against measles and rubella alerts about the risk of immunity gaps to maintain elimination targets.

Keywords Communicable diseases; neglected diseases; vector borne diseases; vaccine-preventable diseases; serology; public health surveillance; epidemiological monitoring; Paraguay.

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Serosurveillance monitors pathogen transmission patterns and characterizes immunity profiles that can be useful to prioritize at-risk populations and assess the impact of public health interventions (1, 2). Antibodies can be used as immunological markers to measure exposure to pathogens causing diseases of public health importance. Additionally, antibodies indicate the levels and duration of seroprotection following immunization for vaccine-preventable diseases (VPDs) in order to identify immunity gaps.

Integrated serosurveillance assesses the prevalence of antibodies for multiple pathogens in populations living in geographic areas where diseases overlap (3). Integrated serosurveillance can transcend the monitoring of a single disease, facilitates interprogrammatic work, and optimizes the use of resources to address multiple pathogens and interventions in populations living in areas facing common problems. This is especially critical in populations with limited access to health services, surveillance systems, safe water, housing, and sanitation (4).

Since 2016, the Pan American Health Organization (PAHO) in partnership with the United States Centers for Disease Control and Prevention (CDC) has been implementing a regional initiative to use integrated serosurveillance as a complementary tool for surveillance by using the multiplex bead assay (MBA) in different epidemiological scenarios to produce information to support public health decision-making (5). Multiplexed assays are more cost- efficient than serological methods such as enzyme-linked immunosorbent assay (ELISA), as it is possible to measure antibodies against multiple antigens (up to 500 in some instruments) simultaneously from less than 1 µl of serum; for example, a single drop of dried blood (~10 µl) eluted from filter paper (dried blood spot – DBS) is enough to run 40 assays (6, 7).

This article presents the results of an integrated serosurvey conducted in the Paraguayan Chaco in 2019. The Chaco region covers a large area of the Paraguayan territory, with dispersed populations and the largest proportion of Indigenous population in the country. The Chaco faces geographic access and communication barriers, which affects the quality of epidemiological surveillance and hinders the population's access to health services. The objectives of the survey were: (a) to establish baseline seroprevalence for five pathogens (*Strongyloides stercoralis*, *Taenia solium*, *Giardia lamblia*, *Cryptosporidium parvum*, and *Toxoplasma gondii*); and (b) to monitor population immunological status for diseases that are eliminated or on the path to elimination, such as measles, rubella, diphtheria, maternal and neonatal tetanus, and trachoma.

MATERIALS AND METHODS

Study population

The research questions, study population, geographical area, and antigens to analyze in the serosurvey were defined by a multidisciplinary and interprogrammatic team led by the General Directorate of Health Surveillance (DGVS) and the Central Laboratory of Public Health (LCSP) of Paraguay, and involved representatives of the Directorate of Communicable Disease Surveillance (DIVET), National Immunization Program (PAI), National Directorate of Child and Adolescent Health (DIRSINA), National Directorate of Health of Indigenous Peoples (DINASAPI), General Directorate of Planning and Evaluation, Health Regions of the Paraguayan Chaco, as well as PAHO and CDC.

The studied geographical area included the three departments of the Paraguayan Chaco: Presidente Hayes, Boquerón, and Alto Paraguay (Figure 1). The Paraguayan Chaco encompasses an area of 246 925 km^2 (60% of the national territory). It is located in the west of the country, separated from the rest of the country by the Paraguay River. In 2022, the total estimated population in the Paraguayan Chaco was 213 083 inhabitants (8), of which and 64 054 (30%) were classified as Indigenous population (9). This survey assessed children aged 6–15 years, enrolled in First through Ninth Grade in public, private-subsidized, and private schools in the Paraguayan Chaco.

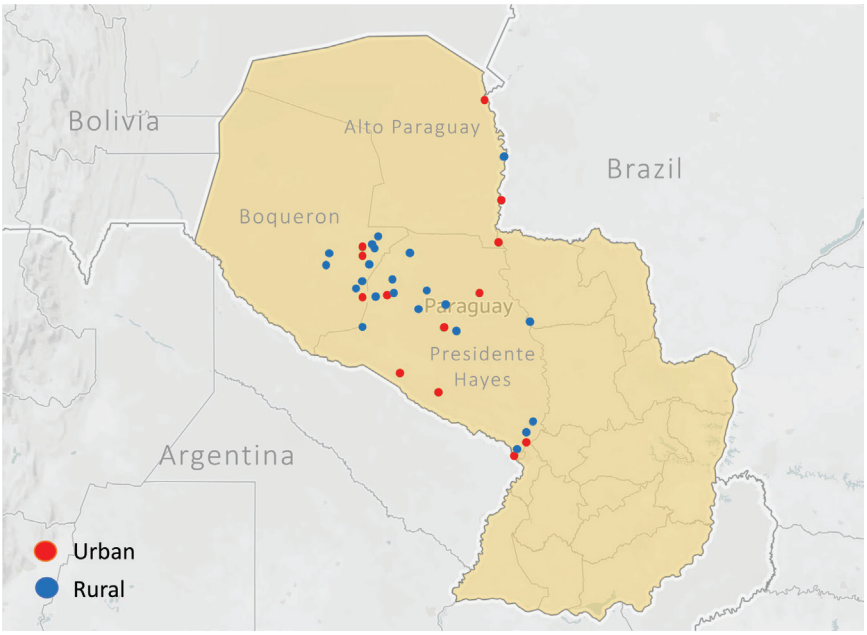
Sample design

A two-stage probabilistic cluster cross-cutting survey was designed. In the first stage, public and private schools were selected using the list of schools registered by the Ministry of Education and Sciences of Paraguay. Schools located in the Paraguayan Chaco region were identified and classified by department and stratified based on their location in urban or rural areas. Of the total number of schools selected, 4 were located in Alto Paraguay, 11 in Boquerón, and 20 in Presidente Hayes. In a second stage, a simple random selection of 36 children aged 6–15 years enrolled in the selected schools was carried out using an updated list of enrolled students from each selected school. The sample size (n = 1 234 children) was calculated considering seroprotection for VPD = 70% and an expected seroprevalence of 50% for the rest of the communicable diseases, standard error (SE) = 5%, design effect = 1.5, alpha error = 0.05, adding 10% for "non-response."

Data collection and ethical approvals

Prior to visiting the schools, the national coordinating team communicated with the school staff and informed parents and/or caregivers about the study. Field teams and supervisors were trained on the survey procedures, including interview, data, and sample collection. To interview the parents and caregivers of the children, a questionnaire was developed that included sociodemographic variables (gender, age, caregiver occupation and ethnicity, student scholar level), housing and basic sanitation characteristics (such as types of latrine and availability of improved water sources), history of deworming, and vaccination. Data were recorded in electronic forms using a tablet and sent daily to a server for review and cleaning, maintaining the confidentiality of the data. Before proceeding with the interview and blood sample collection, signed informed consent was obtained from each parent or caregiver of the children and an assent was obtained from each child aged 9–15 years. The study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the National Health Research Ethics Committee of Paraguay (Protocol No. 104/2019) and the PAHO Ethics Review Committee (PAHO-2019-01-0007) and reviewed by CDC. The study was conducted consistent with applicable federal laws and CDC policy.

FIGURE 1. Location of surveyed schools in urban and rural areas in the Paraguayan Chaco, 2019



Source: Prepared by the authors.

Laboratory

Blood samples were collected on TropBio filter paper (Cellabs, Sydney, Australia) by fingertip puncture and analyzed for detection of IgG antibodies using MBA. Segments of 6 mm diameter completely covered by blood were eluted overnight at 4 °C with Buffer B (1X PBS pH 7.2–7.4, 0.5% casein, 0.5% PVA, 0.8% PVP, 0.3% Tween 20, 0.02% NaN_3, 3 µg/ml of *Escherichia coli* extract) to a final estimated serum concentration of 1:400. For cases where there was not enough sample, 3 mm segments were punched from a region of the filter paper that was completely covered with blood.

Fifty µL of the eluted samples was incubated for 1.5 hours with the beads coated by the antigens NIE (*S. stercoralis*), rES33 and T24H (taeniasis and cysticercosis due to *T. solium*), Pgp3 and CT694 (*C. trachomatis),* Cp17 and Cp23 (*C. parvum),* VSP3 and VSP5 *(G. lamblia),* SAG2A (*T. gondii*), rubella whole virus, measles whole virus, diphtheria toxoid (*Corynebacterium diphtheriae*), and tetanus toxoid (*Clostridium tetani*) (additional information on the antigens included is available through the corresponding author). The concentration of beads used for each antigen was 1 250 beads/well, diluted in assay buffer (1X PBS, 0.5% BSA, 0.05% Tween 20, 0.02% NaN_3).

For detection, samples were incubated for 45 minutes with a mixture of biotinylated mouse anti-human IgG (50 ng/well) and IgG4 (40 ng/well) (Southern Biotech, Birmingham, AL, USA), and then 30 minutes with R-phycoerythrin conjugated to streptavidin (250 ng/well) (Invitrogen, Waltham, MA, USA) as a fluorescent marker. Incubations were carried out at room temperature in agitation, with triple washes with PBST buffer (PBS with 0.05% Tween) between each of the incubations, using a vacuum filtration system. The beads were resuspended in 100 µL PBS and the plates were stored at 4 °C until read the next day.

The median fluorescence intensity (MFI-bg) for each of the bead couplings was measured using a BioPlex200 instrument. Each run included wells with Buffer B without any sample as a blank, and a negative control and two positive controls of different concentrations, as quality control of the results. The final values were obtained by subtracting the Buffer B blank from the MFI values of each antigen (MFI-bg).

To estimate seroprotection against tetanus and diphtheria, MFI values were converted to international units per ml (IU/ml) (10). Cutoff points for each antigen were established using dilution curves from available international reference standards for VPDs, presumed non-exposed samples, or receiver operating characteristic curves with positive and negative samples (additional information on the cutoffs is available through the corresponding author).

Statistical analysis

STATA 17.0 ™ software was used for statistical analysis of the survey data. Data were weighted to estimate seroprevalence based on MFI-bg for the pathogens analyzed or IU/ml for measles, rubella, diphtheria, and tetanus. Percentages and intervals were calculated with 95% confidence for demographic variables and housing conditions, sanitation, and risk factors related to soil-borne, waterborne, and foodborne diseases. Given that trachoma is targeted for elimination as a public health problem, with transmission monitored in 1–9-year-olds, we therefore limited our analysis to children aged 6–9 years to best approximate this age range. For all serological results, binary seroprevalence variables were defined according to the cutoff values established for each antigen. To classify seroprotection against tetanus and diphtheria, two levels were applied: minimal seroprotection (≥0.01 IU/ml) and full seroprotection

(≥0.1 IU/ml) (11, 12). Differences in pathogen seropositivity and VPDs seroprotection (95% CI) were estimated by urban and rural areas, ethnicity, and age according to Pearson Chi-Square. A p value < 0.05 was considered statistically significant.

RESULTS

A total of 1 100 children were surveyed and had samples collected as part of this study. Adverse climatic conditions, such as floods and fires, as well as the migration of selected children to other districts, hindered reaching the target population of 1 234 students, resulting in a loss of 10.9%. Population living in rural areas differ significantly ($p < 0.05$) from the population living in urban areas in their household characteristics, basic sanitation situation, and ethnicity. In rural areas, 76.5% of the population was Indigenous vs 17.2% in urban areas. We found statistically significant differences in the household conditions: type of floors (65.3% in the rural areas vs 30.2% in urban have adobe and soil floors); type of sanitation facility (90.3% vs 44.2% use pit latrines and 8.6% vs 54.7% use flush toilets); and the availability of drainage or septic tanks (8.7% vs 55.2%). No statistically significant differences were found between rural and urban areas in children who were dewormed during the previous year (84.4% vs 91.8%), or risk factors such as the percentage of pig rearing (10.2% vs 12.0%), as shown in Table 1.

Serological data for all antigens are presented in Table 2. The highest seroprevalence for any of the pathogens studied was against *C. parvum*, with over 50% of children being seropositive. Approximately one-quarter of children were seropositive against *G. lamblia* or *T. gondii* antigens, while the lowest levels of seropositivity were against *S. stercoralis* (9.3%) and *T. solium* antigens (5.4% for T24H and 0.7% for rES33). When comparing seropositivity among school-age children according to areas, significantly higher percentages ($p < 0.05$) were found in rural vs urban populations for *C. parvum* (80.4% vs 64.6% for Cp17, and 60.6% vs 44.8% for Cp23), *G. lamblia* (VSP3: 26.9% vs 16.6%), *S. stercoralis* (NIE: 11.5% vs 4.1%), and *T. solium* (T24H: 7.1% vs 1.6%). Seropositivity in children aged 6–9 years against antigens for *C. trachomatis* was 5.5% for Pgp3 (95%) and 8.2% for Ct694 (95%), without statistically significant differences by area.

When analyzing VPDs, the overall seroprotection against measles was 62.9% (95% CI [58.2, 67.4]) and 78.2% (95% CI [73.6, 82.2]) against rubella. The minimal seroprotection (≥0.01 IU/ml) against diphtheria was 92.9% (95% CI [89.8, 95.2]) and 98.3% (95% CI [96.5, 99.0]) against tetanus, but when analyzing the level of full seroprotection (≥0.1 IU/ml) a lower value was observed for diphtheria (50%; 95% CI [44.0, 56.0]) compared to tetanus (91.2%; 95% CI [87.7, 93.8]). No statistically significant differences were found in the levels of seroprotection against VPDs when comparing urban and rural areas, with the exception of the proportion of seroprotection against rubella, which was significantly higher ($p < 0.05$) in urban areas than in rural areas.

Seroprevalences were significantly higher in the Indigenous population when compared to the non-Indigenous for the following pathogens: *C. parvum* Cp17: 82.8% vs 65.5%, and Cp23: 65.2% vs 42.7%; *G. lamblia* VSP3: 28.4% vs 17.3% and VSP5: 33.9% vs 23.2%; *S. stercoralis* NIE: 14.0% vs 2.7%; and *T. solium* T24H: 8.3% vs 1.4% (Figure 2).

Significantly lower levels of seroprotection ($p < 0.05$) were found against measles and rubella in the Indigenous population (58.3% and 71.7%, respectively) compared to non-Indigenous population (69.7% and 87.7%, respectively). No differences were found between minimal or full seroprotection against diphtheria and tetanus when comparing the population of urban and rural areas, and Indigenous and non-Indigenous ethnicity.

TABLE 1. Characteristics of the surveyed children by urban and rural areas,[a] Paraguayan Chaco, 2019

Characteristics	Total (N = 1 100)			Urban (n = 490)			Rural (n = 610)			p value
	n	%	95% CI	n	%	95% CI	n	%	95% CI	
Sex										
Male	553	49.4	46.5, 52.2	245	49.4	44.5, 54.4	308	49.4	45.9, 52.8	0.995
Female	547	50.6	47.8, 53.5	245	50.6	44.7, 55.5	302	50.6	47.2, 54.1	
Age (years)										
6–8	375	32.9	28.3, 37.9	167	32.9	26.4, 40.1	208	33.0	27.1, 39.4	0.845
9–11	395	35.2	32.0, 38.6	181	36.4	31.7, 41.3	214	34.7	30.6, 39.0	
12–15	330	31.9	25.9, 38.5	142	30.8	21.6, 41.9	188	32.4	25.0, 40.7	
Department										
President Hayes	606	52.4	33.8, 70.4	246	51.7	24.9, 77.6	360	52.7	29.5, 74.8	**0.001[b]**
Boquerón	357	36.6	20.2, 56.8	107	11.7	3.5, 33.1	250	47.3	25.2, 70.5	
Alto Paraguay	137	11.0	4.2, 25.8	137	36.6	14.0, 67.1	0	0.0	-	
Ethnicity										
Non-Indigenous	587	41.4	37.2, 45.6	414	82.6	76.2, 87.9	173	23.5	19.4, 28.0	**<0.001[b]**
Indigenous	513	58.6	42.2, 73.3	76	17.2	4.0, 50.9	610	76.5	53.2, 90.3	
Type of floor in the household										
Adobe/soil	511	54.7	45.7, 63.5	129	30.2	16.0, 49.6	382	65.3	54.4, 74.8	**0.0005[b]**
Cement	314	25.0	20.0, 30.9	173	33.1	22.2, 46.2	141	21.5	16.2, 27.9	
Tile/ceramic	245	17.5	11.7, 25.3	181	35.0	21.9, 51.0	64	9.9	4.9, 19.0	
Other	30	2.8	1.2, 6.4	7	1.7	0.4, 6.2	23	3.3	1.2, 8.6	

(*Continued*)

TABLE 1. (Cont.)

Characteristics	Total (*N* = 1 100)			Urban (*n* = 490)			Rural (*n* = 610)			*p* value
	n	%	95% CI	*n*	%	95% CI	*n*	%	95% CI	
Type of sanitation facility										
Pit latrine	740	76.4	67.6, 83.4	203	44.2	28.3, 61.5	537	90.3	82.6, 94.8	**<0.001**[b]
Flush toilet	347	22.5	15.4, 31.6	281	54.7	37.3, 71.0	66	8.6	4.1, 17.0	
None	11	1.1	0.5, 2.4	4	1.1	0.4, 3.0	7	1.1	0.4, 3.0	
Presence of drainage or septic tank										
No	741	77.3	68.2, 84.4	204	44.8	28.6, 62.3	537	91.3	82.9, 95.8	**<0.001**[b]
Yes	347	22.7	15.6, 31.8	281	55.2	37.7, 71.4	66	8.7	4.2, 17.2	
Type of drinking water source										
Public or private water network/ bottled	369	27.3	16.8, 41.1	226	44.9	23.1, 68.9	143	19.7	9.4, 36.8	0.06
Rain/cistern/well	641	64.6	50.4, 76.6	221	42.4	20.5, 67.7	420	74.2	57.3, 86.0	
River/cutwater	90	8.1	3.8, 16.4	43	12.7	3.2, 39.0	47	6.1	3.1, 11.9	
Water treatment										
No	903	85.5	76.8, 91.3	342	67.7	50.6, 81.1	561	93.2	82.9, 93.2	**0.002**[b]
Yes	190	14.5	8.7, 23.2	144	32.3	18.9, 49.4	46	6.8	2.5, 17.1	
Pig rearing										
No	980	89.2	81.2, 94.1	443	88.0	82.1, 92.2	537	89.8	77.3, 95.8	0.728
Yes	120	10.7	5.9, 18.8	47	12.0	7.8, 17.9	73	10.2	4.2, 22.7	
Dewormed during the last year										
Yes	954	86.6	76.6, 92.8	523	91.8	85.5, 95.5	518	84.4	69.8, 92.6	0.165
No	135	13.4	7.2, 23.5	81	8.2	4.5, 14.5	88	15.6	7.4, 23.4	
Brought vaccination card										
No	939	84.8	76.4, 90.5	429	88.9	75.7, 95.4	510	82.9	71.8, 90.3	0.378
Yes	150	15.2	9.5, 23.6	56	11.1	4.6, 24.3	94	17.1	9.7, 28.2	

Notes:
[a] Weighted data.
[b] Statistically significant ($p < 0.05$) differences between urban and rural areas according to Pearson Chi-Square.
Source: Prepared by the authors based on the study data.

TABLE 2. Seropositivity of soil-borne, waterborne, and foodborne pathogens and seroprotection (%, 95% CI) against vaccine-preventable diseases in children aged 6–15 years by urban and rural areas,[a] Paraguayan Chaco, 2019

Pathogen	Antigen	Cutoff value[b]	Total (*n* = 1 100)		Area				*p* value
					Urban (*n* = 490)		Rural (*n* = 610)		
			n	%	*n*	%	*n*	%	
Strongyloides stercoralis	NIE	606	76	9.3% (5.2, 16.1)	17	4.1% (2.6, 6.8)	59	11.5% (5.9, 21.4)	**0.011**[f]
Cryptosporidium parvum	Cp17	24	787	75.6% (69.8, 80.6)	309	64.6% (53.4, 74.3)	478	80.4% (73.9, 85.5)	**0.007**[f]
	Cp23	816	568	55.8% (47.7, 63.7)	214	44.8% (37.3, 52.6)	354	60.6% (49.8, 70.4)	**0.020**[f]
Giardia lamblia	VSP3	114	243	23.8% (19.6, 28.5)	86	16.6% (11.2, 23.9)	157	26.9% (22.0, 32.5)	**0.023**[f]
	VSP5	190	304	29.5% (25.6, 33.9)	116	23.7% (16.0, 33.4)	188	32.1% (28.0, 36.5)	0.108
Taenia solium	rES33	67	6	0.7% (0.3, 1.5)	1	0.2% (0.0, 1.7)	5	0.9% (0.4, 1.5)	0.186
	T24H	117	44	5.4% (0.3, 7.6)	6	1.6% (0.3, 4.9)	38	7.1% (0.3, 10.7)	**0.009**[f]
Toxoplasma gondii	Sag2A	273	298	25.4% (20.1, 31.6)	153	31.2% (25.2, 37.8)	145	23.0% (16.3, 31.3)	0.109

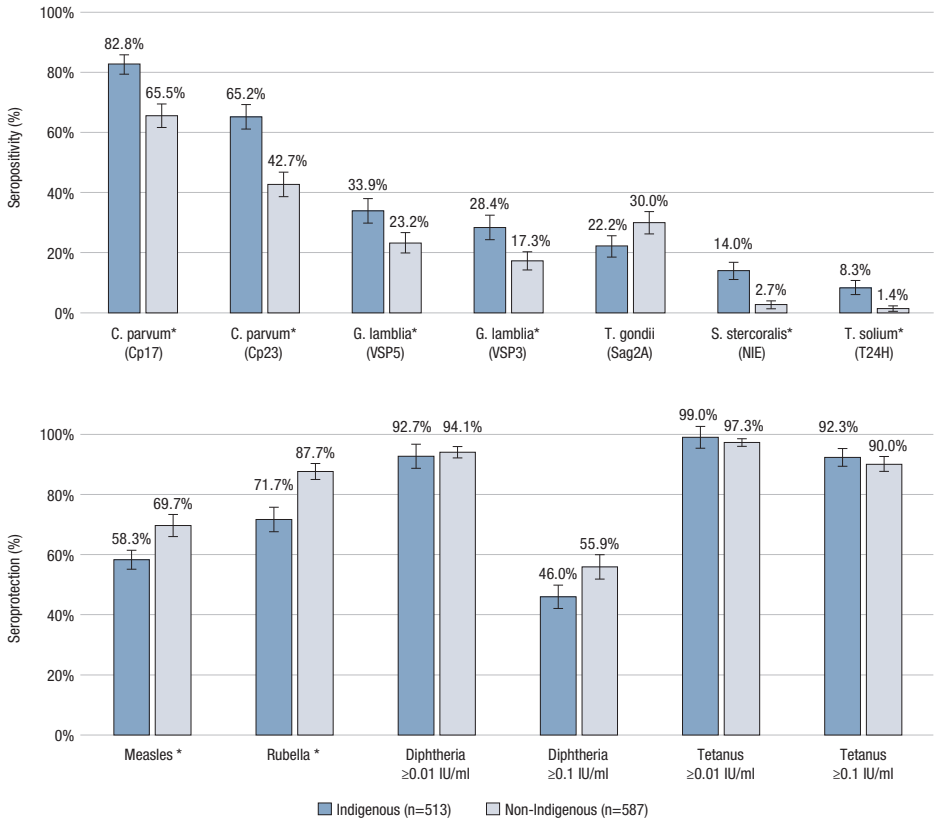
(*Continued*)

TABLE 2. (Cont.)

Pathogen	Antigen	Cutoff value[b]	Total (n = 1 100)		Area: Urban (n = 490)		Area: Rural (n = 610)		p value
			n	%	n	%	n	%	
Chlamydia trachomatis[c]	Pgp3	783	25	5.5% (3.3, 7.1)	14	5.5% (3.5, 9.6)	11	3.2% (2.2, 6.9)	0.715
	Ct694	102	43	8.2% (6.3, 11.2)	25	11.0% (7.2, 15.1)	18	6.9% (4.1, 10.1)	1.086
Measles	Whole virus	153 IU/ml	681	62.9% (58.2, 67.4)	314	68.3% (62.9, 73.2)	367	60.7% (54.4, 66.6)	0.061
Rubella	Whole virus	9.36 IU/ml	856	78.2% (73.6, 82.2)	395	85.3% (78.9, 90.1)	461	75.2% (69.2, 80.4)	**0.017**[f]
Diphtheria	Diphtheria toxoid	≥0.01[d] IU/ml	1 021	92.9% (89.8, 95.2)	460	95.0% (91.7, 98.0)	561	92.1% (89.8, 95.2)	0.182
		≥0.1[e] IU/ml	560	50.0% (44.0, 56.0)	264	55.2% (48.6, 61.6)	296	47.7% (39.9, 55.6)	0.149
Tetanus	Tetanus toxoid	≥0.01[d] IU/ml	1 078	98.3% (96.5, 99.0)	480	98.1% (96.5, 99.0)	598	98.4% (94.3, 99.6)	0.851
		≥0.1[e] IU/ml	1 002	91.2% (87.7, 93.8)	448	91.5% (86.2, 94.8)	554	91.2% (86.4, 94.4)	0.913

Notes:
[a] Weighted data.
[b] Values are reported in median fluorescence intensity (MFI-bg) for communicable disease pathogens and international units (IU)/ml for vaccine-preventable diseases.
[c] Seropositivity (%, 95% CI) to *C. trachomatis* was calculated in children aged 6–9 years.
[d] Minimal diphtheria or tetanus seroprotection (≥0.01 IU/ml).
[e] Full diphtheria or tetanus seroprotection (≥0.1 IU/ml).
[f] Statistically significant differences ($p < 0.05$) between urban and rural areas according to Pearson Chi-Square.
Source: Prepared by the authors based on the study data.

FIGURE 2. Seropositivity of soil-borne, waterborne, and foodborne pathogens and seroprotection (%, 95% CI) against vaccine-preventable diseases in school children aged 6–15 years by ethnicity, Paraguayan Chaco, 2019



Note: * Statistically significant differences ($p < 0.05$) between Indigenous and non-Indigenous populations according to Pearson Chi-Square.
Source: Prepared by the authors based on the study data.

TABLE 3. Seroprotection (%, 95% CI) against measles, rubella, tetanus, and diphtheria of children aged 6–15 years by age group, Paraguayan Chaco, 2019

Disease	Total (*N* = 1 100)		Age group (years) 6–8 (*n* = 375)		9–11 (*n* = 395)		12–15 (*n* = 330)		*p* value
	n	%	*n*	%	*n*	%	*n*	%	
Measles	681	62.9% (58.2, 67.4)	274	72.9% (67.5, 77.7)	248	65.1% (58.8, 71.0)	159	50.3% (41.7, 58.8)	<0.001[c]
Rubella	856	78.2% (73.6, 82.2)	331	89.1% (84.6, 92.4)	306	78.3% (71.1, 84.1)	219	67.0% (59.5, 73.8)	<0.001[c]
Diphtheria[a] ≥0.01 IU/ml	1 021	92.9% (89.8, 95.2)	364	97.0% (93.5, 98.7)	369	94.5% (90.1, 97.0)	288	87.0% (80.9, 91.4)	0.0012[c]
Diphtheria[b] ≥0.1 IU/ml	560	50.0% (44.0, 56.0)	223	60.8% (54.1, 67.0)	204	51.0% (43.5, 58.3)	133	37.8% (26.1, 51.2)	0.0041[c]
Tetanus[a] ≥0.01 IU/ml	1 078	98.3% (96.5, 99.0)	370	98.7% (95.8, 99.9)	392	99.5% (98.1, 99.9)	316	96.5% (92.4, 98.4)	0.0001[c]
Tetanus[b] ≥0.1 IU/ml	1 002	91.2% (87.7, 93.8)	354	95.2% (91.1, 97.4)	360	93.1% (89.2, 95.6)	281	85.2% (78.8, 89.9)	0.0002[c]

***Notes*:**
[a] Minimal diphtheria or tetanus seroprotection (≥0.01 IU/ml).
[b] Full diphtheria or tetanus seroprotection (≥0.1 IU/ml).
c Differences are statistically significant ($p < 0.05$) between age groups according to Pearson Chi-Square.
***Source*:** Prepared by the authors based on the study data.

Seroprotection against measles, rubella, diphtheria, and tetanus seems to decrease significantly with increasing age (Table 3). For measles, seroprotection showed a decrease from 72.9% (95% CI [67.5, 77.7]) in children aged 6–8 years to 50.3% (95% CI [41.7, 58.8]) in the 12–15 age group. Similarly, seroprotection against rubella was 89.1% (95% CI [84.6, 92.4]) in the younger group and seemed to drop to 67.0% (95% CI [59.5, 73.8]) in older children. This association was more pronounced for diphtheria compared to tetanus, especially when analyzing the level of full seroprotection (≥0.01 IU/ml), as it decreases from 60.8% (95% CI [54.1, 67.0]) to 37.8% (95% CI [26.1, 51.2]), compared to 95.2% (95% CI [91.1, 97.4]) to 85.2% (95% CI [78.8, 89.9]) for tetanus.

DISCUSSION

Integrated serosurveillance was initiated in Paraguay in 2016, and through this approach the country obtained seroprevalence data for pathogens for which there was no baseline information, either due to lack of available diagnostic tests, access to health services, or limitations of epidemiological surveillance systems. The piloting of integrated serosurveillance in Paraguay provided useful public health information about exposure to communicable diseases and access to vaccination in populations living in remote areas of the country. Notably, Indigenous populations showed higher exposure to soil-borne, waterborne, and foodborne diseases and lower levels of seroprotection against some VPDs.

The finding of low levels of seroprotection against measles (62.9%) and rubella (78.2%) in the studied population provides critical evidence on potential vaccination coverage issues that should be analyzed with other data from the immunization program in order to better understand the gaps and improve vaccination strategies. The seroprotection levels for VPDs are significantly lower in Indigenous population, emphasizing the need to develop strategies to reach these populations already living in vulnerable conditions. The low estimated seroprotection against VPDs could be related to programmatic factors such as low vaccination coverage, problems in data quality of the reported vaccination coverage, both numerator and denominator, or difficulties in maintaining the cold chain that affect the efficacy of vaccines in the Paraguayan Chaco (13). Routine measles and rubella (MR/MMR) coverage in these age cohorts fluctuated from 78% to 94% throughout the study period. In addition, these cohorts of children were vaccinated during national follow-up campaigns implemented in 2009 and 2014, but the reported coverages in the Chaco were not homogeneous by age (range: 82–93% in 2009 and 86–100% in 2014). It is also important to consider intrinsic host factors because it has been reported that malnourished children may have lower antibody responses to measles and tetanus vaccination (14). The higher levels of seroprotection against *C. diphtheriae* and *C. tetani* in younger children, with values that decrease with age, reflects the importance of applying tetanus and diphtheria toxoid boosters throughout the life course. This is crucial to achieve lifelong protection against these diseases and to maintain maternal and neonatal tetanus elimination (15–17).

The lack of access to clean water and adequate sanitation facilities is reflected in the high prevalence of antibodies to enteric pathogens such as *C. parvum* and *G. lamblia*. These enteric protozoa can cause diarrhea, abdominal pain, vomiting, malaise, and intestinal malabsorption (18). Initial exposure to enteric pathogens often occurs within the first two years of life, but these infections tend to become chronic if left untreated. After infection, many enteric pathogens elicit an elevated transient antibody response that decreases over time, as there is evidence that chronic giardia infection leads to immunotolerance and a decrease in detectable IgG levels (19). Baseline serological results of these pathogens can be useful for monitoring the effectiveness of public health measures such as chlorination or filtration of water sources, before and after their implementation (20). The results of this serosurvey reinforce the need for water, sanitation, and hygiene (WASH) interventions in the Paraguayan Chaco, in particular in rural areas.

There is limited information available about the prevalence of soil-borne, waterborne, and foodborne diseases in the Paraguayan Chaco. This survey found a high seroprevalence of antibodies to *T. gondii*, which can be acquired congenitally or from the ingestion of undercooked or poorly processed meat from infected animals, or from the ingestion of water or food contaminated by the environmentally resistant form that infects cats and that is shed into the environment. A systematic review and meta-analysis developed in Paraguay to estimate the dual impact of 20 zoonotic diseases on both the human health and animal health sector and the societal burden of such diseases, reported that *T. gondii* accounted for 1 139 (1 003–1 342) and cysticercosis accounted for 688 (369–1 259) disability-adjusted life-years (DALYs) in the country (21). Our data align with the results from this meta-analysis reporting human toxoplasmosis as one of the five pathogens causing the highest burden of zoonotic diseases in Paraguay.

Our study shows low seroprevalence to the antigens ES33, associated with taeniasis (infection), and T24H, associated with the presence of cysts. Low levels of taeniasis antigens can be explained by the fact that only 10.7% of the families in this study kept pigs, which are critical intermediate hosts for this infection (22, 23). Nevertheless, higher seropositivity of T24H (7.1%) was found in rural areas when compared to urban areas (1.6%). Considering the low sensitivity of taeniasis microscopy diagnosis and the high sensitivity and specificity reported for taeniasis antigens (24), serology results could be used to identify risk areas in which to implement rapid field assessments and confirmatory diagnosis (25). In Paraguay, the intensification of pig production contributed to the increase in consumption per capita, and the creole pig, locally called "kure saite," is found in free-range systems in some areas of Paraguarí, Misiones, and Ñeembucú, located in the eastern region of the country, not the western region of the Chaco (26).

There are no data available on prevalence of strongyloidiasis in Paraguay, for which serology is a good diagnostic method (27). It is estimated that between 30 million and 100 million people are infected with *S. stercoralis* worldwide, and like other soil-transmitted helminths, the risk of infection is associated with poor sanitation conditions and children are a vulnerable group for infection. Strongyloidiasis can be serious and even life-threatening in cases of immunodeficiency. Despite this, strongyloidiasis is often called the most neglected of the neglected tropical diseases and lacks a dedicated elimination program (28). One barrier to this is the lack of international parameters or recommendations to indicate at what level of seroprevalence strongyloidiasis could be considered a public health problem, which exists for many other communicable diseases. These data therefore may be useful to both understand the burden of strongyloidiasis in the Chaco and help inform the global burden of *S. stercoralis* to help determine the interventions to be implemented.

We assessed diseases targeted for elimination, such as trachoma. The seroprevalence of antibodies to *C. trachomatis* antigens was very low, coinciding with the negative results of rapid trachoma assessments carried out in 2018–2019 in Paraguay that reported lower seroprevalence in young children (6–9 years) (data provided by the Ministry of Health of Paraguay), which seems to indicate that ocular trachoma is not a public health problem in the population of the Paraguayan Chaco. A limitation of our study is the lack of inclusion of 1–5-year-olds, since trachoma surveillance is recommended to be conducted in children 1–9 years of age. However, because antibody seropositivity increases with age in trachoma-endemic settings, an antibody prevalence of <9% in older children is suggestive of low transmission and little increase in seroconversion with age, since in endemic settings, 6–9-year-olds typically exhibit >25% antibody seropositivity (29, 30).

In addition to serology information that can help inform a number of disease programs in the Paraguayan Chaco, this study was a successful experience about how to integrate serosurveillance using multiplexed assays to measure historical exposure to multiple pathogens and immunity to VPDs in hard-to-reach populations. Serosurveillance provides useful information to reduce access barriers to diagnosis and interventions, to carry out surveillance of diseases whose incidence is unknown in populations with weak surveillance systems, where clinical and laboratory diagnosis is limited, and to monitor diseases that are in the post-elimination surveillance phase (3). Nonetheless, this study has some limitations. Sample losses (10.9%) due to adverse climatic conditions and migration may have introduced bias. However, the 10% loss was accounted for in the sample size calculation, and the observed loss rate remained within the expected range.

Author contributions. PG, CH, GS, GRB, MPA, AM, MISD, GC, and DM conceived and designed the study. PG, CH, VO, AM, MISD, GC, and DM performed the study. PG, CH, VO, SA, VTP, VDE, RM, SGIO, AM, MISD, GRB, PB, DM, and GC organized and analyzed data. PG, CH, SA, VDE, MPA, GRB, PB, AL, RM, SGIO, AM, MISD, GC, and DM wrote the paper. All authors reviewed and edited the paper. All authors reviewed and approved the final version.

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Data availability statement. Data are unavailable due to privacy consideration, as datasets include global positioning system coordinates that might enable identification of location of study subjects. Only restricted individuals had access to the datasets for analysis purposes only.

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Vigilancia serológica integrada de enfermedades transmisibles en el Chaco paraguayo, 2019

RESUMEN

Objetivo. Determinar la seroprevalencia actual de referencia de las enfermedades transmitidas por el agua, los alimentos y el suelo, así como realizar un seguimiento de las enfermedades eliminadas o en proceso de eliminación en el Chaco paraguayo.

Método. Se realizaron pruebas a un total de 1100 menores de 6 a 15 años de escuelas urbanas y rurales seleccionadas para una encuesta transversal, de base poblacional, mediante un diseño de muestreo probabilístico en dos etapas en los tres departamentos del Chaco paraguayo. Se tomaron muestras de sangre en papel de filtro para determinar los anticuerpos IgG mediante un ensayo múltiple de microesferas. La recogida de datos se llevó a cabo mediante entrevistas con progenitores y tutores. Se evaluó el acceso al saneamiento básico y al agua de mejor calidad. Se calcularon las diferencias de seropositividad y seroprotección para diversos agentes patógenos, desglosadas por zonas urbanas y rurales.

Resultados. La seroprotección fue del 62,9% contra el sarampión y del 78,2% contra la rubéola. La seroprotección mínima contra la difteria y el tétanos (≥0,01 UI/ml) se observó en el 92,9% y el 98,3% de los casos, respectivamente. Los niveles de seroprotección contra estas cuatro enfermedades prevenibles mediante vacunación disminuyeron significativamente con la edad (p <0,05). Los siguientes agentes patógenos y sus respectivos antígenos mostraron una seroprevalencia significativamente mayor (p <0,05) en las zonas rurales en comparación con las urbanas: *Cryptosporidium parvum* Cp17: 80,4% frente a 64,6% y Cp23: 60,6% frente a 44,8%; *Giardia lamblia* VSP3: 26,9% frente a 16,6%; *Strongyloides stercoralis* NIE: 11,5% frente a 4,1%; y *Taenia solium* T24H: 7,1% frente a 1,6%. La seroprevalencia de estos agentes patógenos también fue mayor en la población indígena que en la no indígena. Las condiciones de saneamiento básico mostraron diferencias significativas (p <0,05) entre zonas rurales y urbanas: viviendas con suelo de adobe o de tierra (65,3% frente a 30,2%), uso de letrinas de pozo (90,3% frente a 44,2%), disponibilidad de desagües o fosas sépticas (8,7% frente a 55,2%), acceso a agua potable (19,7% frente a 44,9%) y tratamiento del agua (6,8% frente a 32,3%).

Conclusiones. Observamos una gran exposición a enfermedades transmitidas por el agua, los alimentos y el suelo en las zonas rurales y en la población indígena del Chaco paraguayo. La seroprotección baja contra el sarampión y la rubéola alerta sobre el riesgo de brechas en materia de inmunidad si se pretenden mantener los objetivos de eliminación.

Palabras clave Enfermedades transmisibles; enfermedades desatendidas; enfermedades transmitidas por vectores; enfermedades prevenibles por vacunación; serología; vigilancia en salud pública; monitoreo epidemiológico; Paraguay.

Vigilância sorológica integrada de doenças transmissíveis na região do Chaco paraguaio, 2019

RESUMO

Objetivo. Estabelecer a soroprevalência basal de doenças transmitidas pelo solo, pela água e por alimentos e monitorar as doenças já eliminadas ou em vias de eliminação na região do Chaco paraguaio.

Métodos. Um total de 1 100 crianças em idade escolar (6 a 15 anos) foram testadas em escolas urbanas e rurais selecionadas para um inquérito transversal de base populacional usando um método de amostragem probabilística em duas fases nos três departamentos do Chaco paraguaio. Foram coletadas amostras de sangue em papel-filtro para medir anticorpos IgG usando um ensaio multiplex com microesferas. A coleta de dados foi feita por meio de entrevistas com pais e cuidadores. Avaliou-se o acesso a saneamento básico e a fontes melhoradas de água. As diferenças na soropositividade para os patógenos e na soroproteção contra eles foram estimadas separadamente para áreas urbanas e rurais.

Resultados. A soroproteção contra o sarampo foi de 62,9% e contra a rubéola, de 78,2%. A soroproteção mínima contra a difteria e o tétano (≥0,01 UI/mL) foi de 92,9% e 98,3%, respectivamente. Os níveis de soroproteção contra essas quatro doenças imunopreveníveis diminuíram significativamente com a idade ($p < 0,05$). Os seguintes patógenos e respectivos antígenos apresentaram soroprevalência significativamente maior ($p < 0,05$) nas áreas rurais em comparação com as áreas urbanas: *Cryptosporidium parvum* Cp17: 80,4% vs. 64,6% e Cp23: 60,6% vs. 44,8%; *Giardia lamblia* VSP3: 26,9% vs. 16,6%; *Strongyloides stercoralis* NIE: 11,5% vs. 4,1%; e *Taenia solium* T24H: 7,1% vs. 1,6%. A soroprevalência desses patógenos também foi maior na população indígena em comparação com a não indígena. Houve diferenças significantes ($p < 0,05$) nas condições de saneamento básico entre as áreas rurais e urbanas: piso de barro e terra batida nas residências (65,3% vs. 30,2%), uso de latrina de fossa (90,3% vs. 44,2%), disponibilidade de drenagem ou fossa séptica (8,7% vs. 55,2%), acesso a água potável (19,7% vs. 44,9%) e tratamento da água (6,8% vs. 32,3%).

Conclusões. Identificamos uma alta exposição a doenças transmitidas pelo solo, pela água e por alimentos em áreas rurais e na população indígena do Chaco paraguaio. A baixa soroproteção contra o sarampo e a rubéola é um alerta para o risco das lacunas de imunidade para a manutenção das metas de eliminação.

Palavras-chave Doenças transmissíveis; doenças negligenciadas; doenças transmitidas por vetores; doenças preveníveis por vacina; sorologia; vigilância em saúde pública; monitoramento epidemiológico; Paraguai.