Am. J. Trop. Med. Hyg., 112(4), 2025, pp. 828–831
doi:10.4269/ajtmh.23-0565
Copyright © 2025 The author(s)

Testing for Antibodies to Four Parasites in Residual Blood Specimens from Trachoma Surveys in Kiribati, 2015–2019

AdeSubomi O. Adeyemo,[1,2]* Raebwebwe Taoaba,[3] Diana Martin,[1] E. Brook Goodhew,[1] Robert Butcher,[4] Caleb Mpyet,[5] Emma Harding-Esch,[4] Anasaini Cama,[6] Sarah Anne J. Guagliardo,[1] Sarah Gwyn,[1] Anthony W. Solomon,[7] Kabiri Tuneti Itaaka,[3] Ana Bakhtiari,[8] Cristina Jimenez,[9] and Rabebe Tekeraoi[3]

[1]*Centers for Disease Control and Prevention, Atlanta, Georgia;* [2]*Epidemic Intelligence Service, Centers for Disease Control and Prevention, Atlanta, Georgia;* [3]*Ministry of Health and Medical Services, South Tarawa, Kiribati;* [4]*London School of Hygiene & Tropical Medicine, London, United Kingdom;* [5]*Sightsavers, Nigeria Country Office, Kaduna, Nigeria;* [6]*The Fred Hollows Foundation, Melbourne, Australia;* [7]*Global Neglected Tropical Diseases Programme, World Health Organization, Geneva, Switzerland;* [8]*Task Force for Global Health, Decatur, Georgia;* [9]*Sightsavers, Haywards Heath, United Kingdom*

Abstract. To assess the prevalence of several parasitic infections in Kiribati, dried blood spots collected during trachoma prevalence surveys in the two major population centers in 2015, 2016, and 2019 were tested using multiplex bead-based serologic assays to detect IgG antibodies against four pathogens of public health interest: *Toxoplasma gondii* (*T. gondii*), *Taenia solium* (*T. solium*), *Strongyloides stercoralis* (*S. stercoralis*), and *Toxocara canis* (*T. canis*). In Kiritimati Island, the seroprevalences of *T. solium* recombinant antigen for detection of cysticercosis antibodies (T24H) and recombinant antigen for detection of taeniasis antibodies (ES33) were ≤4% in both surveys, whereas in Tarawa, the T24H seroprevalence was 2% (2016) and 7% (2019) and the ES33 seroprevalence was ≤3% in both surveys. At both sites, the seropositivity of *S. stercoralis* recombinant antigen for detection of *Strongyloides* was 0–4%, and for *T. canis*, the C-type lectin-1 antigen was 0–1% in all surveys. For *T. gondii*, the surface antigen glycoprotein 2A antigen seroprevalences on Kiritimati Island were 41% (2015) and 36% (2019), and in Tarawa, they were 36% (2016) and 22% (2019), suggesting that *T. gondii* infections are common in Kiribati, whereas the other pathogens are not.

Multiplex bead-based serologic assays (MBAs) can assess for antibodies to multiple pathogens in a single specimen. Testing specimens collected during single-disease surveys using MBAs can cost-effectively generate seroprevalence estimates for neglected diseases that might otherwise not be assessed, for example, because of a lack of data on the burden of infection or a lack of resources. In this study, we took advantage of dried blood spot (DBS) specimens collected during routine trachoma prevalence surveys in the remote island nation of Kiribati to assess for antibodies against antigens derived from *Strongyloides stercoralis* (*S. stercoralis*), *Toxocara canis* (*T. canis*), *Taenia solium* (*T. solium*), and *Toxoplasma gondii* (*T. gondii*) using MBA. Few data on the presence of these pathogens in Kiribati existed before our evaluation; therefore, public health officials were interested in obtaining prevalence estimates.

Baseline trachoma surveys were conducted in the two main population centers in Kiribati—Kiritimati Island (2015) and Tarawa (2016)— during which DBS specimens from children aged 1–9 years were collected.[1,2] These surveys were powered to detect a follicular prevalence of trachomatous inflammation of 10% in 1- to 9-year-olds with a precision of 3%.[3] Based on these survey results, interventions were implemented in 2017 and 2018 that included the mass drug administration of azithromycin (or tetracycline eye ointment for those in whom azithromycin was contraindicated), as well as efforts to enhance facial cleanliness and encourage environmental improvement. Follow-up trachoma impact surveys were conducted at each site in 2019 using a two-stage cluster-sampled population-based design adjusted for finite population size that was powered to detect a follicular prevalence of trachomatous inflammation of 4% in 1- to 9-year-olds with a precision of ±2%.[4] The islands are >3,000 km from each other; therefore, data from each evaluation unit (EU) were assessed separately. In the clean dataset, 382 and 658 Kiritimati children contributed data for 2015 and 2019, respectively, and 863 and 867 Tarawa children contributed data for 2016 and 2019, respectively.

Finger-prick blood samples were collected onto filter paper extensions calibrated to hold 10 μL of blood (TropBio Pty Ltd., Queensland, Australia). The papers were air-dried overnight, placed in sealed plastic bags containing desiccant, and stored at −20°C until they were shipped at ambient temperature. Samples from DBS extensions were tested with MBA using previously described methods.[5] The assay tested for total IgG antibodies to recombinant antigen for detection of *Strongyloides* (NIE) from *S. stercoralis*, recombinant antigen for detection of *Toxocara* (CTL-1) from *T. canis*, recombinant antigen for detection of cysticercosis (T24H) and recombinant antigen for detection of taeniasis (ES33) from *T. solium* (associated with the presence of tissue cysts and adult stage tapeworm, respectively),[6] and recombinant antigen for detection of acute *Toxoplasma* (SAG2A) from *T. gondii*. A Bio-Plex 200 instrument (Bio-Rad, Hercules, CA) was used to read plates using the Bio-Plex manager 6.0 software (Bio-Rad). The antibody levels of each antigen were reported as median fluorescence intensity, with background fluorescence subtracted. The median fluorescence intensity with background fluorescence subtracted data were converted to seropositive/seronegative outcomes using the following cutoffs: 1,515 (NIE), 348 (CTL-1), 61 (ES33), 261 (T24H), and 163 (SAG2A). For CTL-1, the cutoff was calculated as three standard deviations greater than the mean response of a panel of 86 sera from US nontravelers. For all other antigens, cutoffs were calculated with a receiver operator characteristic (ROC) analysis using panels of defined positive sera from confirmed cases for each pathogen (or the presence of cysts for T24H) and negative sera from US

* Address correspondence to AdeSubomi O. Adeyemo, Centers for Disease Control and Prevention, 1600 Clifton Rd. NE, Atlanta, GA 30333. E-mail: lqp4@cdc.gov

TABLE 1
Sensitivity and specificity for NIE, ES33, T24H, and SAG2 antigens

Antigen	Sensitivity (%)	95% CI	Specificity (%)	95% CI
NIE	83.9	72.8–91.0	100	95.7–100.0
ES33	90.6	75.8–96.8	98.9	93.8–99.9
T24H	92.6	84.8–96.6	96.5	90.2–99.1
SAG2	100	67.6–100.0	100	67.6–100.0

ES33 = recombinant antigen for detection of taeniasis antibodies; NIE = recombinant antigen for detection of *Strongyloides* antibodies; SAG2 = recombinant antigen for detection of acute *Toxoplasma* antibodies; T24H = recombinant antigen for detection of cysticercosis antibodies.

nontravelers. Based on these ROC panels, the sensitivity and specificity are provided in Table 1. Sensitivity and specificity values were not available for CTL-1 because of the lack of defined panels; however, based on a previously reported study using these antigens, the sensitivity to detect visceral larva migrans was 90% (95% CI 85–94%), the sensitivity to detect ocular larva migrans was 54% (95% CI 39–68%), and the specificity was 99% (95% CI 97–100%).[7] We used R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria) for the statistical analysis. The seroprevalences at the two time points (baseline and impact surveys) were assessed for the two EUs, Kiritimati Island and Tarawa. The seroprevalence among 1- to 9-year-olds was further assessed in 3-year age bands. A χ^2 test was used to compare the seroprevalences over time.

Toxoplasma gondii was the only pathogen for which a substantial seroprevalence was identified, with more than one-third of 1- to 9-year-olds testing seropositive for antibodies to SAG2A at baseline in both EUs (Table 2). In Tarawa, there was a significant decrease in antibody seroprevalence to *T. gondii* SAG2A, from 36% in 2016 to 22% in 2019 ($\chi^2 = 40.8$; *P*-value <0.001). A slight but not significant decrease in *T. gondii* SAG2A seroprevalence was observed on Kiritimati Island (Table 2). There was, however, a significant decrease in *T. gondii* SAG2A seroprevalence among the youngest children in both EUs (Table 3). Kiribati has a high feral cat population, which may lead to high rates of *T. gondii* infection through the ingestion of untreated water or raw or undercooked food.[8] Although previous information on acute toxoplasmosis in Kiribati is unavailable, among primary school children aged ≤9 years in the Pacific Island nation of the Marshall Islands, there was an acute/recurrent infection rate of 40.74% ($n = 81$).[9] The study in the Marshall Islands noted a positive but not statistically significant association of *T. gondii* infection among primary school children residing in homes with cats.[9] Children in the 1–3 years age range in Kiribati may have been the most likely to have lower exposure to *T. gondii*, and hence lower seroprevalence, because of efforts to control the feral cat population, improved practices to prevent the accidental ingestion of contaminated soil, and (possibly) interventions for trachoma because azithromycin has modest effects against *T. gondii*, and hygiene interventions may have limited transmission.[10]

The seroprevalences of *T. solium* T24H on Kiritimati Island increased modestly between 2015 and 2019 (2% versus 4%; $P = 0.09$) and more substantially in Tarawa between 2016 and 2019 (2% versus 7%; $\chi^2 = 26.1$; $P < 0.001$; Table 2). These prevalences fall at the lower end of those observed in sero-epidemiological studies using an ELISA or enzyme-linked immunoelectrotransfer blot.[11] However, no serological thresholds define *T. solium* as a public health concern. Because the prevalence of symptomatic neurocysticercosis increases with age in endemic areas,[12,13] this study may underestimate the overall seroprevalence in the population. We found low seroprevalences (1–3%) of *T. solium* ES33 for both EUs. Although both estimates fall within the specificity range of the assay, in 2019, Tarawa demonstrated a 3% seroprevalence of the infection marker ES33 and a 7% seroprevalence of the T24H antigen, which has been associated with the presence of cysts. Therefore, follow-up may be indicated in Tarawa, particularly among people with epilepsy. Seropositivity was low for antibodies against NIE from *S. stercoralis* (≤4%) and CTL-1 from *T. canis* (≤1%) at both sites in each survey (Table 2). We found little evidence suggesting the significant transmission of either infection in Kiribati. Again, noting the specificity range of the assays concerned, the antibody signals observed against *S. stercoralis* and *T. canis* antigens could be attributable to false positives.

There are some limitations to these analyses. The age range examined is limited because of the nature of trachoma surveys, and because these pathogens are also prevalent in adult populations, these data may underestimate the seroprevalence in the total population. Second, although most of these antigens are well-characterized, ES33 has limited validation data. The CTL-1 antigen has poor sensitivity for ocular larva migrans; thus, antibodies against this antigen may

TABLE 2
Seroprevalence for antibodies to parasitic diseases antigens in 1- to 9-year-olds at trachoma baseline (Kirimati 2015, Tarawa, 2016) and impact (2019) surveys

		Kiritimati Island						Tarawa					
Parasite	Antigen	Year	*n*	%	95% CI	χ^2; df = 1	*P*-Value	Year	*n*	%	95% CI	χ^2; df = 1	*P*-Value
Toxoplama gondii	SAG2A	2015	382	41	37–46	3.2	0.07	2016	863	36	33–39	40.8	<0.001*
		2019	658	36	32–39			2019	867	22	19–25		
Taenia solium	ES33	2015	382	2	1–3	0	1	2016	863	1	1–2	2.39	0.12
		2019	658	1	1–3			2019	867	3	2–4		
	T24H	2015	382	2	1–4	2.9	0.09	2016	863	2	1–3	26.1	<0.001*
		2019	658	4	3–6			2019	867	7	6–9		
Strongyloides stercoralis	NIE	2015	382	0	0–1	0.1	0.73	2016	863	4	3–5	2.5	0.11
		2019	658	0	0–1			2019	867	2	1–3		
Toxocara canis	CTL	2015	382	0	0–1	N/A	N/A	2016	863	1	0–2	0.3	0.61
		2019	658	0	0–1			2019	867	1	1–2		

CTL = recombinant antigen for detection of *Toxocara* antibodies; df = degrees of freedom; ES33 = recombinant antigen for detection of taeniasis antibodies; NIE = recombinant antigen for detection of *Strongyloides* antibodies; SAG2 = recombinant antigen for detection of acute *Toxoplasma* antibodies; T24H = recombinant antigen for detection of cysticercosis antibodies.
* Significantly different seroprevalence for antibodies between baseline and impact surveys.

TABLE 3
Seroprevalence for antibodies to *Toxoplasma gondii* SAG2A antigen for baseline (Kirimati 2015, Tarawa, 2016) and impact (2019) surveys among children aged 1–9 years, by age group

	Kiritimati Island						Tarawa					
Age Group	Year	*n*	%	95% CI	χ^2; df = 1	*P*-Value	Year	*n*	%	95% CI	χ^2; df = 1	*P*-Value
1–3 years	2015	137	28	21–36	15.44	<0.001*	2016	234	17	12–22	11.82	<0.001*
	2019	233	11	8–16			2019	368	7	5–10		
4–6 years	2015	119	43	34–52	0.63	0.43	2016	381	37	34–42	7.72	0.005*
	2019	248	38	32–44			2019	316	27	22–32		
7–9 years	2015	126	55	46–63	2.47	0.12	2016	248	52	46–58	3.78	0.05
	2019	177	64	57–71			2019	183	42	35–49		

df = degrees of freedom; SAG2 = recombinant antigen for detection of acute *Toxoplasma* antibodies.
* Significantly different seroprevalence for antibodies between baseline and impact surveys.

underestimate transmission.[7] Additionally, the foci of transmission for these pathogens may have been missed, although the data are strengthened by two independent cross-sectional sampling frames, which increases the combined population coverage.

Despite these limitations, we were able to generate the first published estimates of seroprevalence of *T. gondii*, *T. solium*, *S. stercoralis*, and *T. canis* in Kiribati via secondary testing of specimens from what otherwise would have been single-disease surveys. We were unable to identify any previous studies that assessed the impact of trachoma control and elimination programs on *T. gondii* seroprevalence or disease. However, through this study, we demonstrated that almost one-third of children in Kiribati were exposed to *T. gondii* and that there were marginal but significant decreases in *T. gondii* seroprevalence between baseline and follow-up. These decreases are potentially due to the antibiotic and hygiene interventions implemented for trachoma elimination, although any attribution of causality could only be speculative. The potential application of MBA is broad; assays have been explored for the simultaneous surveillance of infectious diseases, including HIV, viral hepatitis, syphilis, and herpes.[14] Multiplex assays have also been explored to simultaneously screen for vaccine-preventable diseases, including measles and rubella, to guide immunization activities, including identifying lapses in coverage in a population.[15,16] Through this study, we were able to demonstrate the value of adding multiplex serological testing to routine programmatic activity in assessing diseases of public health interest. Future larger-scale studies involving children and adult populations in Kiribati could further support the effectiveness of utilizing MBA to detect multiple pathogens while providing a representative sample of parasitic infections in the country.

Received August 18, 2024. Accepted for publication September 1, 2024.

Published online February 11, 2025.

Acknowledgments: We thank the community members and field teams who respectively donated and collected the dried blood spots that made this work possible.

Financial support: We alone are responsible for the views expressed in this article; they do not necessarily represent the views, decisions, or policies of the institutions with which they are affiliated or of the United States Government. The fieldwork for the baseline surveys in Kiritimati was supported by the Global Trachoma Mapping Project grant from the United Kingdom's Department for International Development (ARIES: 203145) to Sightsavers, which led a consortium of nongovernmental organizations and academic institutions to support health ministries to complete baseline trachoma mapping worldwide; the Wellcome Trust (098521); and the Fred Hollows Foundation (1954-0). The GTMP was also funded by the United States Agency for International Development through the ENVISION project implemented by RTI International under cooperative agreement number AID-OAA-A-11-00048 and the END in Asia project implemented by FHI360 under cooperative agreement number OAA-A-10-00051. The fieldwork for the baseline surveys in Tarawa was funded by the Fred Hollows Foundation (1954-0).

Disclosures: A. Bakhtiari is employed by the International Trachoma Initiative, which receives an operating budget and research funding from Pfizer Inc., the manufacturer of Zithromax® (azithromycin). Pfizer had no role in the study design, conduct, analysis, or interpretation of results. E. Harding-Esch receives salary support from the International Trachoma Initiative. Impact surveys were funded by the Queen Elizabeth Diamond Jubilee Trust (QEDJT) to R. Tekeraoi. The salaries of A. Cama and R. Tekeraoi were shared by the QEDJT and the Commonwealth 2018–2020 Fund. R. Butcher's salary is funded by the Wellcome Trust (206275/Z/17/Z). A. W. Solomon is a staff member of the WHO. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The other authors of this manuscript declare no relevant conflicts of interest or financial relationships to disclose. Ethical approval for the surveys and all serological testing was given by the London School of Hygiene & Tropical Medicine and the Kiribati Ministry of Health and Medical Services, as outlined previously.[1,2] United States CDC researchers were determined to be nonengaged in human subject research.

Current contact information: AdeSubomi O. Adeyemo, Centers for Disease Control and Prevention, Atlanta, Georgia, E-mail: lqp4@cdc.gov. Raebwebwe Taoaba, Kabiri Tuneti Itaaka, and Rabebe Tekeraoi, Government of the Republic of Kiribati Ministry of Health and Medical Services, South Tarawa, Kiribati, E-mails: raebwebwetaoaba1971@gmail.com, dr_kabtun@yahoo.com.au, and rtekeraoi@gmail.com. Diana Martin, E. Brook Goodhew, Sarah Anne J. Guagliardo, and Sarah Gwyn, Centers for Disease Control and Prevention, Atlanta, GA, E-mails: hzx3@cdc.gov, isd6@cdc.gov, ywc2@cdc.gov, and yme8@cdc.gov. Robert Butcher and Emma Harding-Esch, London School of Hygiene & Tropical Medicine, London, United Kingdom, E-mails: robert.butcher@lshtm.ac.uk and emma.harding-esch@lshtm.ac.uk. Caleb Mpyet, Sightsavers, Nigeria Country Office, Kaduna, Nigeria, E-mail: cmpyet@sightsavers.org. Anasaini Cama, The Fred Hollows Foundation, Melbourne, Australia, E-mail: acama@hollows.org. Anthony W. Solomon, World Health Organization, Geneva, Switzerland, E-mail: solomona@who.int. Ana Bakhtiari, Task Force for Global Health, Atlanta, GA, E-mail: abakhtiari@taskforce.org. Cristina Jimenez, Sightsavers, Haywards Heath, United Kingdom, E-mail: cjimenez@sightsavers.org.

This is an open-access article distributed under the terms of the Creative Commons Attribution (CC-BY) License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

REFERENCES

1. Butcher R, et al., 2020. Ocular *Chlamydia trachomatis* infection, anti-Pgp3 antibodies and conjunctival scarring in Vanuatu and

Tarawa, Kiribati before antibiotic treatment for trachoma. *J Infect 80:* 454–461.

2. Cama A, et al.; Global Trachoma Mapping Project, 2017. Prevalence of signs of trachoma, ocular *Chlamydia trachomatis* infection and antibodies to Pgp3 in residents of Kiritimati Island, Kiribati. *PLoS Negl Trop Dis 11:* e0005863.
3. Solomon AW, et al., 2015. The Global Trachoma Mapping Project: Methodology of a 34-country population-based study. *Ophthalmic Epidemiol 22:* 214–225.
4. World Health Organization, 2018. *Design Parameters for Population-Based Trachoma Prevalence Surveys: Strategic and Technical Advisory Group for Neglected Tropical Diseases, Working Group on Monitoring and Evaluation*. Geneva, Switzerland: WHO.
5. Priest JW, Moss DM, 2020. Measuring cryptosporidium serologic responses by multiplex bead assay. *Methods Mol Biol 2052:* 61–85.
6. Salim L, Ang A, Handali S, Tsang VC, 2009. Seroepidemiologic survey of cysticercosis-taeniasis in four central highland districts of Papua, Indonesia. *Am J Trop Med Hyg 80:* 384–388.
7. Anderson JP, et al., 2015. Development of a Luminex bead-based assay for diagnosis of toxocariasis using recombinant antigens Tc-CTL-1 and Tc-TES-26. *PLoS Negl Trop Dis 9:* e0004168.
8. Pierce R, Brown D, VanderWerf E, Taabu K, 2018. *Guidelines for Monitoring Birds and Invasive Species at Kiritimati, Kiribati*. Available at: https://kiribati-data.sprep.org/system/files/2h._pierce_et_al_2012_cxi_guidelines_bird_monitoring__revised_2018_%20%281%29.pdf. Accessed September 23, 2022.
9. Fu CJ, Chuang TW, Lin HS, Wu CH, Liu YC, Langinlur MK, Lu MY, Hsiao WW, Fan CK, 2014. *Toxoplasma gondii* infection: Seroprevalence and associated risk factors among primary school children in the capital area of the Republic of the Marshall Islands. *Jpn J Infect Dis 67:* 405–410.
10. Wei HX, Wei SS, Lindsay DS, Peng HJ, 2015. A systematic review and meta-analysis of the efficacy of anti-*Toxoplasma gondii* medicines in humans. *PLoS One 10:* e0138204.
11. Coral-Almeida M, Gabriël S, Abatih EN, Praet N, Benitez W, Dorny P, 2015. *Taenia solium* human cysticercosis: A systematic review of sero-epidemiological data from endemic zones around the world. *PLoS Negl Trop Dis 9:* e0003919.
12. Singhi P, 2011. Neurocysticercosis. *Ther Adv Neurol Disord 4:* 67–81.
13. Fleury A, Dessein A, Preux PM, Dumas M, Tapia G, Larralde C, Sciutto E, 2004. Symptomatic human neurocysticercosis – Age, sex and exposure factors relating with disease heterogeneity. *J Neurol 251:* 830–837.
14. Yufenyuy EL, et al., 2022. Development of a bead-based multiplex assay for use in multianalyte screening and surveillance of HIV, viral hepatitis, syphilis, and herpes. *J Clin Microbiol 60:* e0234821.
15. Coughlin MM, Smits G, Matson Z, van Binnendijk R, Bankamp B, 2024. Multiplex bead assay for the serological surveillance of measles and rubella. *Methods Mol Biol 2808:* 225–246.
16. Coughlin MM, et al., 2021. Development of a measles and rubella multiplex bead serological assay for assessing population immunity. *J Clin Microbiol 59:* e02716-20.