Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Modelling anti-Ov16 Seroprevalence for the Control and Elimination of Onchocerciasis

Maria-Gloria Basáñez

m.basanez@imperial.ac.uk

Imperial College London https://orcid.org/0000-0001-5031-3361

Aditya Ramani

https://orcid.org/0009-0006-2120-4629

Jacob Stapley

Imperial College London https://orcid.org/0000-0003-0732-1600

Matthew Dixon

Imperial College London https://orcid.org/0000-0002-1710-6237

Jonathan Hamley

University of Bern

Luis Amaral

Imperial College London

Martin Walker

Royal Veterinary College, University of London https://orcid.org/0000-0001-8714-5365

Article

Keywords: onchocerciasis, Ov16 antigen, seroprevalence, microfilariae, ivermectin, surveillance, modelling

Posted Date: August 1st, 2025

DOI: https://doi.org/10.21203/rs.3.rs-7140160/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: There is **NO** Competing Interest.

Version of Record: A version of this preprint was published at Nature Communications on August 19th, 2026. See the published version at https://doi.org/10.1038/s41467-026-76562-9.

Abstract

The World Health Organization aims to verify the elimination of onchocerciasis transmission in one-third of endemic countries by 2030, principally through mass drug administration (MDA) of ivermectin. Serological assays that detect IgG4 antibodies against the *Onchocerca volvulus* Ov16 antigen are central to decision-making regarding start- and stop-MDA, post-treatment surveillance and post-elimination surveillance. Transmission models have a crucial role to play in understanding anti-Ov16 IgG4 seroprevalence dynamics, but uncertainty associated with the parasite stage(s) that stimulate(s) seroconversion and the duration of antibody persistence have hitherto hindered progress. Using the individual-based stochastic transmission model, EPIONCHO-IBM, we tested several hypotheses on the timing of anti-Ov16 IgG4 seroconversion and seroreversion. We fitted the model to data from treatment-naïve communities in Gabon and validated its predictions using data collected in Togo after 39 years of onchocerciasis interventions. The model best reproduced observed patterns when anti-Ov16 IgG4 seroconversion was elicited by near-patent/patent infection, marked by the production of *O. volvulus* microfilariae by a mating worm pair. The duration of antibody persistence remains uncertain. These findings support the use of mechanistic transmission models to refine serological thresholds for initiating and safely stopping MDA, and to aid interpretation of serological signals in post-treatment and post-elimination surveillance.

Introduction

Human onchocerciasis, caused by the filarial nematode *Onchocerca volvulus*, and transmitted among humans by the bites of female *Simulium* blackflies, is targeted by the World Health Organization (WHO) for elimination of transmission in 12 endemic countries by 2030[1]. Currently, 27 countries in SSA remain endemic[2]. Mass drug administration (MDA) of ivermectin is the principal intervention strategy employed to achieve this goal[1]. MDA has been used since the late 1980s in the Onchocerciasis Control Programme in West Africa (OCP, 1974–2002), and since the late 1990s in the African Programme for Onchocerciasis Control (APOC, 1995–2015)[3]. After the closure of APOC, ivermectin MDA has continued to be implemented by national programmes with technical support by the WHO's Expanded Special Project for Elimination of Neglected Tropical Diseases (ESPEN), established in 2016.[3]

Serological assays that detect IgG4 antibodies against the (recombinant) *O. volvulus* Ov16 antigen (anti-Ov16 IgG4) are central to programmatic decision making at both poles of the elimination pathway. On the elimination end, anti-Ov16 seroprevalence in children is used (together with infectivity prevalence in blackflies) to inform stop-MDA decisions, post-treatment surveillance (PTS) and confirming elimination in post-elimination surveillance (PES)[4]. To start-MDA in previously unidentified low-transmission foci, anti-Ov16 seroprevalence in adults is used following the procedures for onchocerciasis elimination mapping (OEM)[5]. Several serological diagnostics are recommended for these purposes, including enzyme-linked immunosorbent assays (Ov16 ELISAs) and rapid diagnostic tests (Ov16 RDTs)[4,5]. These

tests detect both current and past infection[6] and are less invasive than traditional skin-snip microscopy for detecting *O. volvulus* microfilariae[7,8].

The WHO recommends that MDA be initiated in communities where anti-Ov16 seroprevalence is ≥ 2% in adults aged ≥ 20 years, providing cut-off values for Ov16 RDT using dry blood spot (DBS) eluates, the Onchocerciasis Elimination Program for the Americas (OEPA) ELISA, or the alkaline phosphatase (AP) ELISA, according to their diagnostic performance[5]. It is considered that MDA can be safely stopped when seroprevalence—measured by Ov16 ELISA—is < 0.1% (at the upper 95% confidence limit) in children aged < 10 years (in addition to an infectivity prevalence < 0.05% in blackflies)[4]. While the former recommendation may lead to initiating MDA in populations previously considered non-endemic, the latter is statistically and operationally difficult to achieve without near-perfect serological specificity[9–11]. These challenges have motivated epidemiological[12] and transmission modelling studies[13–15] to scrutinize the robustness and appropriateness of these thresholds, with the goal of informing programmatic decision-making with the best available evidence.

Modelling work on serological thresholds is highly sensitive to underlying assumptions on age- and sex-dependent exposure patterns and population processes regulating *O. volvulus* abundance[15] and—critically—the parasite stage that stimulates the production of anti-Ov16 IgG4 antibodies[13] as well as the duration of antibody persistence after infection clearance (seroreversion)[16]. For example, a study using the ONCHOSIM onchocerciasis transmission model, indicated that an anti-Ov16 IgG4 seroprevalence < 2% in children aged 5–14 years may reflect unstable or interrupted transmission[14]. However, more recent modelling using EPIONCHO-IBM has shown that unstable low prevalence may persist for decades, challenging the validity of fixed serological thresholds for stopping MDA[17]. Hence, there is a critical need to better understand the immuno-dynamics of seroconversion and seroreversion to interpret reliably serological data for programmatic decision-making.

Early work in humans and chimpanzees found that total anti-Ov16 IgG seroconversion occurred during pre-patent infection, before detection of microfilariae in the skin[18]. A subsequent study in non-human primates found that detection of anti-Ov16 IgG4 corresponded with the detection of microfilariae, indicative of patent infection, with some evidence of seroreversion following parasite clearance[19]. Immuno-epidemiological studies have yielded similarly inconclusive results, with evidence showing persistent IgG4 responses in post-patent individuals and variable antigen recognition patterns across infection states[20]. Hence, the *O. volvulus* parasite stage(s) predominantly involved in IgG4 antibody seroconversion remain(s) incompletely understood. This has hindered the role that modelling of anti-Ov16 IgG4 serological dynamics can play to support decision-making on serological thresholds for onchocerciasis control and elimination.

Here, we test different hypotheses on the *O. volvulus* parasite stage(s) that result(s) in anti-Ov16 IgG4 antibody seroconversion—with and without seroreversion (i.e., loss of detectable anti-Ov16 IgG)—by fitting the individual-based EPIONCHO-IBM stochastic transmission model[21] to seroprevalence data

from a treatment-naïve setting in Gabon[22]. We then validate the parameterised model by comparing predictions (following simulated interventions) with empirical seroprevalence data collected after 39 years of onchocerciasis control in Togo[23]. Finally, we discuss how our results will strengthen the use of modelling to inform serological thresholds regarding start- and stop-MDA decisions, as well as PTS and PES for elimination of onchocerciasis transmission.

Results

Model calibration using microfilarial prevalence data

We calibrated EPONCHO-IBM[21] using pre-intervention microfilarial age-prevalence data collected from 4,257 participants living in 58 hypoendemic, treatment-naïve communities in Gabon[22] by estimating the annual biting rate (ABR, no. bites/person/year) of *S. damnosum sensu lato* (s.l.)[21,24] and the degree of inter-individual exposure heterogeneity, defined by parameter k_E[21]. The prior ranges and best-fit ABRs for k_E=0.2 and k_E=0.3 are shown in Supplementary Table S1 and Supplementary Fig. S1; the observed and fitted microfilarial age-prevalence profile are shown in Fig. 1. Using the best-fit ABR of 76 bites/person/year and k_E=0.2, the projected microfilarial prevalence in individuals aged ≥ 5 years was 8.0% (95% uncertainty interval, UI = 6.3%-9.5%), closely matching the observed prevalence of 7.9% (95% Wilson score confidence interval, CI = 7.1%-8.7%).

We also calibrated EPIONCHO-IBM to pre-intervention microfilarial prevalence estimates from four prefectures (administrative level 2) in the Savanes and Kara regions of Togo, where vector control had been implemented (during the OCP and subsequent Special Intervention Zone (SIZ) anti-vectorial operations) for 16 and 30 years, respectively, and ivermectin MDA since 1991[23,25]. The range of best-fit ABRs for each prefecture, assuming k_E=0.3[25] is shown in Supplementary Table S2.

Identifying the immunological stimulus for seroconversion

Using the calibrated model, we evaluated the goodness-of-fit of model-predicted versus observed age-seroprevalence profiles assuming various hypotheses on the *O. volvulus* parasite stage(s) that elicit(s) anti-Ov16 IgG4 seroconversion (Fig. 2, Table 1) and different diagnostic performance characteristics (sensitivity and specificity) of the SD BIOLINE Ov16 RDT using whole blood[22] (Supplementary Table S3).

The hypothesis that seroconversion is elicited by near-patent/patent infection (H4, Table 1) provided a better quantitative fit to the data (lower mean squared error, MSE), irrespective of the assumed sensitivity and specificity (Fig. 3). We could not distinguish between the no seroreversion hypothesis (i.e., lifelong immunological memory, H4i) and the finite seroreversion hypothesis (i.e., finite immunological memory, H4iii in Table 1) under assumptions of either a 40% sensitivity and 99% specificity or a 50% sensitivity and 99% specificity, respectively (Fig. 3; unnormalised MSEs and associated UIs are shown in Supplementary Table S4).

The observed and EPIONCHO-IBM-predicted age-seroprevalence profiles for these indistinguishable hypotheses (H4i and H4iii) are shown in Fig. 4. Both hypotheses were advanced for validation using the Togo seroprevalence data. Supplementary Figs. S2, S3 and S4 show the observed and predicted age-seroprevalence profiles for all seroconversion-seroreversion hypotheses (H1i-H4iii, Table 1) and diagnostic sensitivity/specificity combinations.

Table 1
Seroconversion and Seroreversion Hypotheses Tested

Seroconversion Hypothesis	**No Seroreversion[a] (Lifelong Immunological Memory)** **(i)**	**Immediate Seroreversion[b] (no Immunological Memory)** **(ii)**	**Finite Seroreversion[c] (Finite Immunological Memory)** **(iii)**
Exposure to L3 larvae (H1)	H1i	H1ii	H1iii
During the L4-L5 moult (H2)	H2i	H2ii	H2iii
Presence of any adult worm (of either sex) (H3)	H3i	H3ii	H3iii
Presence of microfilariae (from a mating worm pair), not necessarily detectable by skin-snip microscopy (H4)	H4i	H4ii	H4iii

[a] No seroreversion assumes that anti-Ov16 IgG4 antibodies persist for life (i.e., lifelong immunological memory).

[b] Immediate seroreversion assumes that anti-Ov16 IgG4 antibodies persist only while the parasite stage stimulating seroconversion is present (i.e., no immunological memory).

[c] Finite seroreversion assumes that anti-Ov16 IgG4 antibodies persist until all parasite stages (L3-L5, and mature worms) are cleared, irrespective of the seroconversion stimulus (i.e., finite immunological memory). Note that this assumes that microfilariae are never present in the absence of mature (reproductively active) worms.

Validating seroprevalence predictions under intervention

We used EPIONCHO-IBM, calibrated to pre-intervention microfilarial prevalence estimates from Togo[25], to predict anti-Ov16 IgG4 seroprevalence profiles using the best-fitting seroconversion hypothesis (H4:

near-patent/patent infection) and the two indistinguishable seroreversion hypotheses (H4i: no seroreversion; H4iii: finite seroreversion) identified from the Gabon data.

Model simulations covered 39 years of intervention in four prefectures (administrative level 2; Ôti/Kpendjal, Kéran and Bassar), including vector control from 1976–2007 (ending in 1993 in Ôti/Kpendjal)[25] and MDA from 1991 to 2015[23,25]. Intervention effectiveness was modelled in a prefecture-specific manner, as defined by Amaral et al.[25] based on vector control efficacy (proportional reduction in ABR for the duration of implementation), MDA coverage and systematic non-adherence (the proportion of individuals never participating in MDA). Supplementary Table S5 provides parameter values and categorizations of intervention effectiveness. A comparison of the model-predicted versus observed microfilarial prevalence estimates in 2015 is given in Supplementary Table S6.

The predictive performance of the prefecture-specific EPIONCHO-IBM seroprevalence projections, under the two seroreversion hypotheses and a range of assumed sensitivity and specificity values for the horseradish peroxidase (HRP) Ov16 ELISA used in Togo[23] (Supplementary Table S7), is shown qualitatively in Fig. 5 and quantitatively (using MSEs and UIs) in Supplementary Table S8. Model performance varied by prefecture, with no single seroreversion hypothesis consistently providing the best fit. In Kéran, the best predictive performance was obtained under the no seroreversion hypothesis (H4i) with 70% sensitivity and 85% specificity. In contrast, in Bassar, the finite seroreversion hypothesis (H4iii) yielded the best fit under the same diagnostic assumptions. Predictive performance in Ôti/Kpendjal was generally poorer across all diagnostic and seroreversion scenarios, with a tendency for the model to overpredict seroprevalence and produce larger absolute MSEs (Supplementary Table S8).

Aggregated predictive performance across all prefectures—summarised using normalized MSEs—is shown in Fig. 6, with corresponding unnormalised values and UIs in Supplementary Table S9. Overall, the finite seroreversion hypothesis (H4iii) provided the lowest MSE. However, this result was not robust to variation in diagnostic assumptions on the HRP Ov16 ELISA, as indistinguishable results were obtained assuming either 95% specificity and any sensitivity, or 85% specificity and 70% sensitivity (Supplementary Table S9). It is also important to note that this result was driven largely by the comparatively poor fit in Ôti/Kpendjal, and similar aggregate MSEs were obtained under other combinations (e.g., H4i with 95% specificity and 70% sensitivity, or H4iii with 85% specificity and 70% sensitivity). Hence, although H4iii yielded the best overall performance, it cannot be considered definitively superior across all settings.

The predicted versus observed age-seroprevalence profiles aggregated across all prefectures (prefecture-specific age-seroprevalence estimates were not available[23]) for H4i and H4iii, assuming 70% sensitivity and either 85% or 95% specificity, are shown in Fig. 7. Model predictions tended to overestimate seroprevalence in younger age groups (≤ 20 years), although the observed seroprevalence estimates fall within the UIs of the model predictions. In older age groups (> 20 years), predictions under H4i and H4iii tended to straddle the observed data. For visualization purposes, the midpoint of the two hypotheses is also shown in Fig. 7.

Discussion

Understanding the dynamics of anti-Ov16 IgG4 antibody responses is essential to support onchocerciasis elimination efforts, particularly as programmes have moved from parasitological to serological surveillance for decision-making. Since 2016, the WHO has recommended the use of anti-Ov16 IgG4 serology (together with blackfly vector xenomonitoring) to support decisions on stopping ivermectin MDA and proceeding with PTS and PES[4]. However, until now, modelling of seroprevalence dynamics has lagged behind modelling of microfilarial prevalence[25–27], owing to major uncertainties in the immunological stimulus for seroconversion and the persistence of IgG4 antibody responses against the Ov16 antigen[13–15].

Our findings indicate that the production of anti-Ov16 IgG4 antibodies is likely elicited by parasite lifecycle stages associated with near-patent/patent infection, around the time of microfilarial production by mating worm pairs. Using this parameterisation, EPIONCHO-IBM accurately reproduced age-seroprevalence profiles in two contrasting epidemiological and programmatic settings: a hypoendemic untreated setting in Gabon[22], and a highly endemic setting with nearly four decades of interventions in Togo[23]. This represents a remarkable advance in the use of transmission models to inform serological thresholds for key public health decisions such as initiating or stopping MDA, and informing the elimination of onchocerciasis transmission during the PTS and PES periods.

Experimental[18,19] and clinico-immunological[20,28] evidence on the stimulus that leads to the production of anti-Ov16 IgG4 antibodies has remained inconclusive since the first development of anti-Ov16 serology in the late 1980s (initially using total IgG)[18] and the precursors of the current Ov16 RDTs[29]. Our finding that IgG4 seroconversion is most likely stimulated by parasite stages surrounding the timing of patent infection aligns with the findings of Cama et al.[19] and is consistent with IgG4 antibodies being produced later than, for instance, IgE[28]. Moreover, microfilariae (and female worms) have been associated with transforming growth factor beta (TGF-β) expression (among other cytokines produced by regulatory T cells)[30,31], which in turn is implicated in the induction of IgG4 antibodies[30]. Previous modelling studies have assumed that seroconversion is initiated by pre-patent parasite stages[13–15], which we demonstrate yields substantially different and less accurate age-seroprevalence profiles.

Importantly, our modelled seroconversion mechanism does not require microfilariae to be detectable by skin-snip microscopy, recognising the limitations of this diagnostic in low-intensity infections[5,10,32]. This helps explain observations of seropositive but skin-snip negative children[18,28]. Moreover, our finding that near-patent/patent infection is the predominant stimulus for IgG4 seroconversion is consistent with other studies suggesting that anti-Ov16 IgG4 seropositivity is a good marker of active infection in children under 11 years of age[33]. Notably, this conclusion held across a range of assumed sensitivities and specificities of the Ov16 RDT protocol used in Gabon[22], which we varied to reflect the range of published estimates[22,34–36].

Limitations

Despite these advances, an important remaining source of uncertainty is the duration of persistence of anti-Ov16 IgG4 antibodies (i.e., the duration of immunological memory). In our analysis of the Gabon data, we could not distinguish between lifelong persistence (no seroreversion) and finite seroreversion occurring after clearance of all parasite stages. This is not unexpected as seroreversion and diagnostic sensitivity are confounded; higher sensitivity can compensate, to some extent, for declining antibody responses. This is clearly illustrated by the two best-fitting hypotheses from our fitting to the Gabon data, namely, no seroreversion with 40% sensitivity and finite seroreversion with 50% sensitivity (both with 99% specificity).

The seroprevalence data from Togo are potentially more informative on seroreversion dynamics since—after decades of intervention—transmission may be close to interruption in some of the surveyed villages[25]. A study in Guatemala estimated a mean IgG4 antibody decay rate of 0.20/year and a half-life of 3.3 years, with 73% of previously seropositive individuals seroreverting 2–3 years after transmission was interrupted and MDA halted[16]. When aggregated across the four prefectures in Togo, our findings point towards finite seroreversion providing a better prediction of the data. However, this conclusion is driven largely by results from Ôti/Kpendjal, where EPIONCHO-IBM consistently overpredicted seroprevalence under all assumptions. Thus, while we are confident that near-patent/patent infection is the predominant seroconversion stimulus, our findings remain inconclusive regarding the dynamics of seroreversion.

Several factors underlie this residual uncertainty. We implemented seroreversion as a binary (on/off) process rather than as a decay in antibody titres. This simplification may lack sufficient nuance to capture accurately observed age-seroprevalence patterns. Indeed, the no-seroreversion and finite seroreversion hypotheses qualitatively straddled the observed data, suggesting that a more refined approach to modelling seroreversion may be necessary. Furthermore, it is likely that villages from the different prefectures in Togo were (in 2015) differentially close to interruption of transmission[25], meaning that signals of seroreversion in some may have been masked by ongoing transmission in others. Notwithstanding, even with a more nuanced approach to modelling seroreversion and a village-level analysis, definitive inference may remain elusive given the substantial uncertainty around the performance of the HRP Ov16 ELISA used in Togo, for which variation in sensitivity and specificity can mimic effects of seroreversion.

The sensitivity and specificity of the HRP Ov16-ELISA used in Togo were previously estimated by comparing test results to skin-snip microscopy[23]. Given the low microfilarial prevalence across villages (0%-13.6% in 2015) and the reduced sensitivity of skin-snip microscopy in low-intensity infections[5,10,32], the reported specificity of the ELISA was most likely underestimated[23]. This explains why model predictions systematically overestimated seroprevalence when using a specificity of 70%—resulting in an excess of false positives—and improved with increasing specificity, up to 95%, consistent with other published estimates[6,33,34]. Conversely, the estimated 70% sensitivity of the HRP Ov16-ELISA—derived

from using the near-100% specificity of microfilarial detection—is probably unbiased, explaining why no improvement in predictive performance was observed when increasing the sensitivity. This broadly aligns with other published estimates of sensitivity, which are in the range of 50–95%[6,7,23,33,34,37,38]. (Details of the age ranges and comparators used for previous sensitivity and specificity estimates of the HRP Ov16 ELISA are given in Supplementary Table S7.)

Modelling 39 years of intervention in Togo necessarily made use of several assumptions. Due to the lack of village-level treatment and vector control history[23], we applied prefecture-level generalisations for MDA coverage and vector control efficacy, assuming that all villages within a prefecture shared the same history of intervention[25]. Additionally, due to limited geo-located, age-stratified data, we aggregated model predictions across prefectures. These assumptions may have contributed to our overprediction of seroprevalence in Ôti/Kpenjal, where vector control efficacy in some villages may have approached 100%[25,39], higher than the maximum of 90% considered here, albeit our microfilarial prevalence predictions were consistent with the observed data in all prefectures (Supplementary Table 6). Given the extended timelines of onchocerciasis programmes, such data limitations are not uncommon[25,26,40]. For example, in the absence of detailed entomological time series, it is not possible to capture potential secular changes owing to climate and/or environmental change and, consequently, only vector control was assumed to effect reductions in blackfly ABRs[25].

It is also important to acknowledge other key assumptions embedded within EPIONCHO-IBM. These include density-dependent parasite establishment in humans, whereby the efficiency of transmission from vectors to humans *increases* with *decreasing* transmission intensity[41], and age- and sex-specific exposure profiles[15,42]. Compared to the ONCHOSIM transmission model[40,43,44], EPIONCHO-IBM is parameterised to reflect higher exposure in children younger than 10 years[42], which could potentially overestimate seroprevalence in this age group (although it has successfully captured microfilarial loads in 5-10-year-old children in high-transmission settings[45]). Lont et al.[13] (using ONCHOSIM) showed that higher exposure can be offset by lower sensitivity to yield equivalent seroprevalence in children. A key advantage of our analysis is the use of all-age seroprevalence data from Gabon[22], allowing us to show that, irrespective of the assumed sensitivity and specificity, the near-patent/patent serovonversion hypothesis consistently outperforms those invoking pre-patent seroconversion.

Conclusions

As onchocerciasis elimination programmes continue transitioning away from using skin-snip microscopy to less invasive serological surveillance, it is essential that transmission models advance in parallel to remain useful for public health decision-making. Here, we have incorporated an immunologically motivated anti-Ov16 IgG4 seroconversion-seroreversion sub-model into EPIONCHO-IBM, indicating that seroconversion seems most likely elicited by near-patent/patent *O. volvulus* infection. The model captures observed age-seroprevalence profiles both in treatment-naïve and intervention settings. This is a major step forward for onchocerciasis transmission dynamics modelling,

providing a critical tool to support programmatic decisions on initiating MDA, stopping treatment safely, determining elimination, and responding to serological signals during PTS and PES. We hope that our work will pave the way towards characterising onchocerciasis elimination using serology as done for other neglected tropical diseases such as trachoma[46].

Methods

EPIONCHO-IBM

EPIONCHO-IBM is a stochastic individual-based onchocerciasis transmission model[21,47], based on its deterministic precursors[26,41,42]. The model tracks *O. volvulus* life stages within individual human hosts—pre-patent (L3, L4, L5), adult worms and microfilariae (per milligram of skin or per skin snip)—and the mean number of L3 per blackfly vector, with the dynamics of L1, L2 and L3 larvae within the vector modelled deterministically[26]. The dynamics of microfilariae within humans are also modelled deterministically[21]. Adult female worms transition between non-fertile and fertile states to capture the 3–4 reproductively active cycles that female worms undergo per year, for which they need to be re-inseminated[48,49]. Microfilarial production takes place when at least one adult male and a fertile female worm are present, i.e., assuming a completely polygamous mating system and a balanced sex ratio[21,48]. The mortality rate of adult worms and microfilarie increases and the fecundity of female worms decreases with parasite age[21]. Human age- and sex-dependent exposure patterns are parameterised using data from African savannah-*S. damnosum* s.l. epidemiological settings[42]. The model incorporates density-dependent establishment/development of ingested microfilariae to L3 larvae within blackfly vectors, parasite-induced vector mortality, and transmission intensity-dependent establishment/development of inoculated L3 larvae to adult worms within humans[41]. Individual exposure to vector bites varies according to a gamma distribution with shape = rate parameter = k_E., such that the mean exposure in the population is unity. This ensures that blackfly bites are distributed among hosts with an average exposure given by the annual biting rate (ABR, the number of bites per person per year)[21]. Mass drug administration is modelled assuming that a proportion of eligible individuals (aged ≥5 years) receive treatment with ivermectin, which exerts a microfilaricidal effect and a temporary embryostatic effect[49]. A cumulative, irreversible sterilizing effect per treatment dose[50] is assumed from the second round of treatment onwards[26,51]. EPIONCHO-IBM is fully described in Hamley et al.[21] and the code is available at: https://github.com/mrc-ide/EPIONCHO.IBM. All model simulations were run using R[52] and the Imperial College Research Computing Service[53].

Modelling anti-Ov16 IgG4 seroprevalence

We defined four hypotheses on the immunological (parasite stage) stimulus for production of detectable anti-Ov16 IgG4 antibodies (seroconversion): H1) following exposure to L3 larvae; H2) following the L4 to L5 moult (which occurs approximately 5 months following infection with L3)[54]; H3) following establishment of a single adult worm (of either sex); H4) following production of microfilariae (not

necessarily detectable by skin-snip microscopy) corresponding to the presence of a mating worm pair (i.e., a fertile female and at least one male worm, assuming completely polygamous mating). Hypotheses H1-H3 reflect seroconversion during pre-patent infection; hypothesis H4 reflects seroconversion elicited by near-patent/patent infection (Fig. 2)[19]. Although patency in onchocerciasis is defined by the presence of skin microfilariae, hypothesis H4 accounts for any microfilariae in the host, whether detectable by skin-snip microscopy or not, allowing for an individual to be anti-Ov16 seropositive but skin-snip negative. We also considered three seroreversion hypotheses: i) no seroreversion (i.e., lifelong persistence of IgG4 antibodies); ii) seroreversion immediately following absence of the relevant seroconversion stimulus (i.e., immediate seroreversion with no immunological memory), and iii) seroreversion immediately following absence of all (larval and adult) parasite stages within a human host (i.e., finite seroreversion, following the absence of all parasite stages). The latter accounts phenomenologically for a finite (not lifelong) immunological memory, characterised by a cessation of IgG4 antibody production following complete clearance of infection. Thus, in total, we considered 12 hypotheses: four seroconversion hypotheses and for each of these, three seroreversion hypotheses (Table 1).

Seroprevalence data

We used anti-Ov16 IgG4 seroprevalence data from Gabon[22] to identify the best-fitting seroconversion-seroreversion hypotheses, and data from Togo[23] for validation. From the treatment-naïve endemic setting in Gabon (i.e., in the absence of control intervention), 4,350 individuals (aged 5–85 years, from 67 communities) consented to undergo skin-snip microscopy, of which 4,257 (from 58 communities) also donated blood samples. The blood samples were tested in the field using an SD BIOLINE Ov16 RDT prototype. In the 4,257 individuals, mean microfilarial prevalence and seroprevalence (both measured in those aged ≥ 5 years) were 7.9% (95% CI: 7.1%-8.7%) and 9.0% (95% CI: 8.2%-9.9%), respectively. The reported sensitivity and specificity of the Ov16-RDT with whole blood, compared to skin-snip microscopy performed in the same individuals, were 52% and 95%, respectively[22]. Microfilarial prevalence and anti-Ov16 IgG4 seroprevalence (estimated using an HRP Ov16 ELISA) from Togo were extracted from a published study conducted in 2015[23], covering 11 villages (4 prefectures) located in the SIZ that followed the closure of the OCP[25]. The villages were: Pancerys (Kpendjal prefecture), Boutchakou and Koukoumbou (Ôti prefecture), Goulbi, Tchitchira, Koutougou Solla and Kpantiiyagou (Kéran prefecture), and Bawlesi, Mô-village, Katcha-Konkomba and Saboundi (Bassar prefecture)[23]. Kpendjal and Ôti prefectures are in the Savanes region; Kéran and Bassar prefectures are in the Kara region[25]. A total of 1,455 people (aged 5–80 years) were examined by skin-snip microscopy, of whom 644 were tested using an HRP Ov16 ELISA. The intervention history included vector control (1976–1993 in Savanes; 1976–2007 in Kara), and MDA from 1991[25]. The observed all-age microfilarial prevalence in 2015 was 5.7% (95% Wilson score CI: 4.5%-6.9%) and the anti-Ov16 IgG4 seroprevalence was 34.0% (95% CI: 30.3%-37.7%)[23]. Using skin-snip microscopy as the reference, the reported sensitivity and specificity of the HRP Ov16 ELISA used in Togo was 71.4% and 71.1%, respectively[23].

Model calibration

We calibrated EPIONCHO-IBM using the microfilarial data from Gabon (treatment-naïve, no prior MDA) and Togo (following 39 years of intervention). For Gabon, we ran 500 simulations for 100 years for each of a previously determined range of ABRs (which yielded endemic microfilarial prevalence between 0.05% and 21%) using two values of the inter-individual exposure heterogeneity parameter, k_E = 0.2 or k_E = 0.3 and their associated density dependence parameters[21,45]. We identified the ABR-k_E combinations that minimised the weighted MSE of the model predictions compared to age-stratified (sixteen 5-year age groups) microfilarial prevalence estimates (weighting squared errors by the proportion of the data within each age group; see Supplementary Methods S1 for further details).

For Togo, the model was calibrated by grouping villages into prefectures (three villages in Ôti/Kpendjal, four villages in Kéran, and three villages in Bassar) due to a lack of village-specific intervention histories. Pancerys (in the Kpendjal prefecture) was grouped with the two other villages in the Ôti prefecture due to its location on the same river basin (Ôti River Basin), its proximity to the Ôti prefecture, and a shared history of vector control efforts, which ended in 1993 in both prefectures[25]. The baseline (pre-intervention) microfilarial prevalence had not been recorded for most villages (excepting Tchitchira in Kéran, which was holoendemic at baseline with an 89.4% microfilarial prevalence, and Mô-village in Bassar, which was hyperendemic, with a 71.4% microfilarial prevalence), so model-derived estimates from Amaral et al.[25] were used instead (Supplementary Methods S1).

We ran 100 simulations for 100 years to reach endemic equilibrium for ABRs ranging from 100 to 60,000 bites/person/year using k_E=0.3. For each prefecture, we constructed a distribution of ABRs that matched (± 1%) the aggregated prefecture-specific pre-intervention microfilarial prevalence estimates that were either observed or model-derived for that prefecture[25]. The latter were derived using EPIONCHO-IBM to simulate the history of intervention—under various assumptions of effectiveness defined by vector control efficacy, total population treatment coverage, and systematic non-adherence with MDA—in each prefecture (Supplementary Methods S2) and inferring the pre-intervention microfilarial prevalence that was compatible with modelled and observed microfilarial prevalence trends from repeated cross sectional studies undertaken between 1975 and 2015[25]. Supplementary Methods S1 and Amaral et al.[25] provide further details.

Hypothesis testing

We tested our 12 seroconversion-seroreversion hypotheses (Table 1) using EPIONCHO-IBM calibrated to the epidemiological data from Gabon by comparing the weighted MSEs of the predicted age-seroprevalence profiles against the observed age-stratified anti-Ov16 IgG4 seroprevalence estimates. We simulated the model 1,500 times for 100 years (using the best ABR-k_E combinations from the calibration) for each hypothesis and adjusted the predicted anti-Ov16 seroprevalence using different sensitivities (40%, 50%, 60%) and specificities (94%, 97%, 99%) of the Ov16 RDT. These values were informed by reported sensitivity and specificity of IgG4 Ov16 RDTs using whole blood in other studies

(Supplementary Methods S3, Supplementary Table S3). Adjustment of the model-predicted ('true') seroprevalence was undertaken in a stochastic manner: putative seropositive individuals were randomly assigned a number from 0 to 1 using a uniform probability distribution, and considered as testing positive if their number was below the sensitivity value (or negative if it was above, indicating a false negative result); putative seronegative individuals were considered positive if their number exceeded the specificity value (indicating a false positive result).

Model validation

We used the best fitting seroconversion-seroreversion hypotheses (smallest MSE) from hypothesis testing to compare the predicted age-seroprevalence profiles (under intervention) with the observed seroprevalence from Togo collected in 2015[23]. Using the distributions of ABRs from the model calibration, we simulated endemic equilibrium for each prefecture and projected forward to 2015, incorporating 39 years of interventions. These included the prefecture-specific history of vector control (from 1976–2007, except in Ôti/Kpendjal, where it ceased in 1993), MDA frequency between 1991 and 2015 (Supplementary Table S5), and intervention effectiveness—defined by vector control efficacy, MDA coverage, and the proportion of systematic non-adherence (i.e., individuals never treated)—as estimated by Amaral et al.[25] (Supplementary Table S6). A detailed description of the intervention histories and how they were modelled is given in Supplementary Methods S2.

We compared the prefecture-specific model predictions with the all-age anti-Ov16 IgG4 seroprevalence. Because the sensitivity of the HRP Ov16 ELISA used in Togo was estimated by comparing against presence of microfilariae—which has poor sensitivity in near-elimination settings[5,10,32]—we calculated the MSE of the model predictions using a range of sensitivities (70%, 80%, and 85%) and specificities (70%, 85%, and 95%) compiled from prior studies of HRP Ov16 ELISA protocols (Supplementary Methods S3). Supplementary Table S7 lists the studies used to inform these ranges of sensitivity and specificity. An age-stratified analysis at prefecture level was not possible as data disaggregated by age were not reported for each prefecture. Thus, we aggregated model outputs across prefectures, sampling individuals in proportion to the observed parasitological or serological data (324/1455 = 22% for microfilarial prevalence and 254/644 = 39% for anti-Ov16 IgG4 seroprevalence in Ôti/Kpendjal; 539/1455 = 37% and 355/644 = 55% in Kéran; 492/1455 = 41% and 35/644 = 5% in Bassar).

We adhered to the five principles of the Neglected Tropical Diseases (NTD) Modelling Consortium regarding Policy-Relevant Items for Reporting Models in Epidemiology of NTDs (PRIME-NTD), for good practice in NTD modelling[55] (Supplementary Table S10).

Declarations

Data availability

All information used for the analyses is contained in the figures, tables, and supplementary information. The data used to calibrate and validate the model are publicly available in the publications by Atsame et

al. 2024[22], Komlan et al. 2018[23] and Amaral et al.[25]

Code availability

The EPIONCHO-IBM model code and vignettes are available at: https://github.com/mrc-ide/EPIONCHO.IBM.

Acknowledgements

A.R. received a School of Public Health scholarship to pursue the MSc in Epidemiology at Imperial College London, UK. J.N.S. received funding from the UK Medical Research Council (MRC) MRes + PhD (1 + 3) Studentship in Infectious Disease Epidemiology (MR/S502388/1) at Imperial College London, UK. J.N.S., M.A.D. and M.-G.B. acknowledge funding from the MRC Centre for Global Infectious Disease Analysis (MR/X020258/1), funded by the UK Medical Research Council (MRC). This UK-funded award is carried out in the frame of the Global Health EDCTP3 Joint Undertaking. L.-J.A. was funded by La Caixa Foundation (B005782). A.R., J.N.S., M.A.D., M.-G.B. and M.W. also acknowledge funding by the Bill & Melinda Gates Foundation through the NTD Modelling Consortium (INV-030046). We thank Dr Katherine Gass at the Task Force for Global Health, USA and Dr Julienne Atsame at the Ministère de la Santé, Gabon for sharing the epidemiological data from Gabon with us. Dr Katherine Gass (TFGH), and Dr Paul T. Cantey at the Centers for Disease Control and Prevention, USA provided helpful discussions during the early stages of this work. Deepti Ramani helped to draw the blackfly silhouette in Fig. 2. This research was part of the MSc in Epidemiology final dissertation of A.R. at Imperial College London, UK. The funders did not have any influence in the study design, analysis, interpretation of results, the writing of the manuscript, or the decision to submit the paper for publication. The corresponding authors had final responsibility for the decision to submit for publication.

Author contributions

Conceptualisation: M.-G.B. and M.W. Formal analysis: A.R. Investigation and methodology: A.R., J.N.S., M.-G.B. and M.W. Software: A.R., M.A.D. and J.I.D.S. Data curation: A.R. and L.-J.A. Resources: J.N.S., M.A.D., L.-J.A. and M.-G.B. Visualisation: A.R., M.A.D., M.-G.B. and M.W. Validation: J.I.D.H., M.-G.B. and M.W. Supervision: M.-G.B. and M.W. Funding acquisition: A.R., M.-G.B. and M.W. Writing – Original draft: A.R., M.-G.B. and M.W. Writing – Review & Editing: A.R., J.N.S., M.A.D., J.I.D.H., L.-J.A., M.-G.B. and M.W.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information. The article contains supplementary material.

Correspondence and requests for materials should be addressed to Maria-Gloria Basáñez or Martin Walker or Aditya Ramani.

References

1. World Health Organization. Ending the neglect to attain the Sustainable Development Goals: a road map for neglected tropical diseases 2021–2030. Geneva: World Health Organization. https://www.who.int/publications/i/item/9789240010352 (2021).
2. World Health Organization. Status of endemicity of onchocerciasis. https://www.who.int/data/gho/data/indicators/indicator-details/GHO/status-of-endemicity-of-onchocerciasis (2024).
3. Lakwo, T., Ogutti, D., Ukety, T., Post, R. & Bakajika, D. Onchocerciasis elimination: progress and challenges. *Res. Rep. Trop. Med.* **11,** 81–95 (2020).
4. World Health Organization. Guidelines for stopping mass drug administration and verifying elimination of human onchocerciasis: criteria and procedures. Geneva: World Health Organization. https://www.who.int/publications/i/item/9789241510011 (2016).
5. World Health Organization. Onchocerciasis elimination mapping. A handbook for national elimination programmes. Geneva: World Health Organization. https://www.who.int/publications/i/item/9789240099227 (2024).
6. Golden, A. et al. Extended result reading window in lateral flow tests detecting exposure to *Onchocerca volvulus*: a new technology to improve epidemiological surveillance tools. *PLoS One.***8,** e69231 (2013).
7. Hotterbeekx, A. et al. Comparison of diagnostic tests for *Onchocerca volvulus* in the Democratic Republic of Congo. *Pathogens.***9,** 435 (2020).
8. Dieye, Y., et al. Feasibility of utilizing the SD BIOLINE Onchocerciasis IgG4 rapid test in onchocerciasis surveillance in Senegal. *PLoS Negl. Trop. Dis.* **11**, e0005884 (2017).
9. Gass, K. M. Rethinking the serological threshold for onchocerciasis elimination. *PLoS Negl. Trop. Dis.***12,** e0006249 (2018).
10. Unnasch, T. R., Golden, A., Cama, V. & Cantey, P. T. Diagnostics for onchocerciasis in the era of elimination. *Int. Health.***10,** i20–i26 (2018).
11. Richards, F. O. et al. Operational performance of the *Onchocerca volvulus* "OEPA" Ov16 ELISA serological assay in mapping, guiding decisions to stop mass drug administration, and posttreatment surveillance surveys. *Am. J. Trop. Med. Hyg.***99,** 749–752 (2018).
12. Dolo, H., et al. Progress towards elimination of onchocerciasis transmission in Mali: A "pre-stop MDA" survey in 18 transmission zones. *PLoS Negl. Trop. Dis.* **17,** e0011632 (2023).
13. Lont, Y. L et al. Modelling anti-Ov16 IgG4 antibody prevalence as an indicator for evaluation and decision making in onchocerciasis elimination programmes. *PLoS Negl. Trop. Dis.* **11,** e0005314 (2017).
14. Coffeng, L. E. et al. Predictive value of Ov16 antibody prevalence in different subpopulations for elimination of African onchocerciasis. *Am. J. Epidemiol.***188,** 1723–1732 (2019).

15. Hamley, J. I. D. et al. Structural uncertainty in onchocerciasis transmission models influences the estimation of elimination thresholds and selection of age groups for seromonitoring. *J. Infect. Dis.***221(Suppl 5),** S510–S518 (2020).
16. Cama, V. A. et al. Changes in anti–Ov-16 IgG4 responses to onchocerciasis after elimination of transmission in the central endemic zone of Guatemala. *Am. J. Trop. Med. Hyg.***110,** 943–950 (2024).
17. Stapley, J. N., Hamley, J. I. D., Basáñez, M.-G. & Walker, M. Modelling transmission thresholds and hypoendemic stability for onchocerciasis elimination. *PLoS Comput. Biol.***21,** e1013026 (2025).
18. Lobos, E. et al. An immunogenic *Onchocerca volvulus* antigen: a specific and early marker of infection. *Science***251,** 1603–1605 (1991).
19. Cama, V. A. et al. Evaluation of an Ov-16 IgG4 enzyme-linked immunosorbent assay in humans and its application to determine the dynamics of antibody responses in a non-human primate model of *Onchocerca volvulus* infection. *Am. J. Trop. Med. Hyg.***99,** 1041–1048 (2018).
20. Soboslay, P. T. et al. The diverse expression of immunity in humans at distinct states of *Onchocerca volvulus* infection. *Immunology***90,** 592–599 (1997).
21. Hamley, J. I. D., Milton, P., Walker, M. & Basáñez, M.-G. Modelling exposure heterogeneity and density dependence in onchocerciasis using a novel individual-based transmission model, EPIONCHO-IBM: implications for elimination and data needs. *PLoS Negl. Trop. Dis.* **13,** e0007557 (2019).
22. Atsame, J., et al. Comparison of diagnostic tools to assess the feasibility of programmatic use of rapid diagnostic tests for onchocerciasis: a dataset from Gabon. *Data Brief.***57,** 110901 (2024).
23. Komlan, K., et al. *Onchocerca volvulus* infection and serological prevalence, ocular onchocerciasis and parasite transmission in northern and central Togo after decades of *Simulium damnosum* s.l. vector control and mass drug administration of ivermectin. *PLoS Negl. Trop. Dis.* **12,** e0006312 (2018).
24. Fobi, G. et al. L'onchocercose dans la région de Lastourville, Gabon. Aspects cliniques et entomologiques. *Bull. Soc. Pathol. Exot.***99,** 269–271 (2006).
25. Amaral, L.-J. et al. Reaching elimination of onchocerciasis transmission with long-term vector control and ivermectin treatment in West Africa: the example of Togo. *Nat. Commun.* (under review). Preprint at https://doi.org/10.21203/rs.3.rs-6284820/v1 (2025).
26. Walker, M. et al. Modelling the elimination of river blindness using long-term epidemiological and programmatic data from Mali and Senegal. *Epidemics*. **18,** 4–15 (2017).
27. Michael, E. et al. Data-driven modelling and spatial complexity supports heterogeneity-based integrative management for eliminating *Simulium neavei*-transmitted river blindness. *Sci. Rep.* **10,** 4235 (2020).
28. Gbakima, A. A., et al. Immunoglobulin G subclass responses of children during infection with *Onchocerca volvulus*. *Clin. Diagn. Lab. Immunol.***3,** 98–104 (1996).
29. Weil, G. J. et al. A rapid-format antibody card test for diagnosis of onchocerciasis. *J. Infect. Dis.* **182,** 1796–1799 (2000).

30. Adjobimey, T. & Hoerauf, A. Induction of immunoglobulin G4 in human filariasis: an indicator of immunoregulation. *Ann. Trop. Med. Parasitol.***104,** 455–464 (2010).
31. Korten, S., Kaifi, J. T., Büttner, D. W. & Hoerauf, A. Transforming growth factor-beta expression by host cells is elicited locally by the filarial nematode *Onchocerca volvulus* in hyporeactive patients independently from *Wolbachia*. *Microbes Infect.***12,** 555–564 (2010).
32. Bottomley, C. et al. Modelling Neglected Tropical Diseases diagnostics: the sensitivity of skin snips for *Onchocerca volvulus* in near elimination and surveillance settings. *Parasit. Vectors.***9,** 343 (2016).
33. Golden, A. et al. Analysis of age-dependent trends in Ov16 IgG4 seroprevalence to onchocerciasis. *Parasit. Vectors.***9,** 338 (2016).
34. Neglected Tropical Diseases Support Center, Taskforce for Global Health. Decatur, GA, USA, May 2–3, 2016. Ov-16 Meeting Notes. https://www.cor-ntd.org/resources/ov-16-meeting-notes (2016)
35. Nikièma A. S. et al. Field sensitivity and specificity of the SD BIOLINE onchocerciasis IgG4 Rapid Diagnostic Test in children <10 years old from endemic areas in Burkina Faso. *Parasite Epidemiol. Control***25,** e00352 (2024).
36. Ogawa, G. M. et al. Improved performance of a serology rapid diagnostic test for onchocerciasis by using dried blood spots. Abstract 530, 68th Annual Meeting of the American Society of Tropical Medicine and Hygiene, Maryland, USA, November 20–24, 2019. *Am. J. Trop. Med. Hyg*. **101 (5 Suppl),** 162 (2019).
37. Ogunrinade A. F., Chandrashekar R., Eberhard M. L. & Weil G. J. Preliminary evaluation of recombinant *Onchocerca volvulus* antigens for serodiagnosis of onchocerciasis. *J. Clin. Microbiol.***31,** 1741–1745 (1993).
38. Djune-Yemeli L., et al. Relationship between skin snip and Ov16 ELISA: two diagnostic tools for onchocerciasis in a focus in Cameroon after two decades of ivermectin-based preventive chemotherapy. *PLoS Negl. Trop. Dis.***16,** e0010380 (2022).
39. Boatin, B. et al. Patterns of epidemiology and control of onchocerciasis in west Africa. *J. Helminthol.***71,** 91–101 (1997).
40. Coffeng, L. E. et al. Elimination of African onchocerciasis: modeling the impact of increasing the frequency of ivermectin mass treatment. *PLoS One***9,** e115886 (2014).
41. Basáñez, M.-G. et al. River blindness: mathematical models for control and elimination. *Adv. Parasitol.* **94,** 247–341 (2016).
42. Filipe, J. A. N. et al. Human infection patterns and heterogeneous exposure in river blindness. *Proc. Natl. Acad. Sci. U. S. A*. **102,** 15265–15270 (2005).
43. Plaisier, A. P., van Oortmarssen, G. J., Habbema, J. D. F., Remme, J. & Alley, E. S. ONCHOSIM: a model and computer simulation program for the transmission and control of onchocerciasis. *Comput. Methods Programs Biomed.* **31,** 43-56 (1990).
44. Stolk, W. A., Walker, M., Coffeng, L. E., Basáñez, M.-G. & de Vlas, S. J. Required duration of mass ivermectin treatment for onchocerciasis elimination in Africa: a comparative modelling analysis. *Parasit. Vectors* **8,** 552 (2015).

45. Stapley, J.N. et al. Modelling onchocerciasis-associated epilepsy and the impact of ivermectin treatment on its prevalence and incidence. *Nat. Commun.***15,** 6275 (2024).
46. Kamau, E. et al. Characterizing trachoma elimination using serology. *Nat. Commun*. **16,** 5545 (2025).
47. Walker, M. et al. Designing antifilarial drug trials using clinical trial simulators. *Nat. Commun.***11,** 2685 (2020).
48. Schulz-Key, H. & Karam, M. Periodic reproduction of *Onchocerca volvulus*. *Parasitol. Today*. **2,** 284–286 (1986).
49. Basáñez, M.-G. et al. Effect of single-dose ivermectin on *Onchocerca volvulus*: a systematic review and meta-analysis. *Lancet Infect. Dis.***8,** 310–322 (2008).
50. Plaisier, A. P. et al. Irreversible effects of ivermectin on adult parasites in onchocerciasis patients in the Onchocerciasis Control Programme in West Africa. *J. Infect. Dis*. **172,** 204–210 (1995).
51. Turner, H. C., Walker, M., Churcher, T. S. & Basáñez, M. G. Modelling the impact of ivermectin on River Blindness and its burden of morbidity and mortality in African Savannah: EpiOncho projections. *Parasit. Vectors***7,** 241 (2014)
52. R Core Team. R: A lamguage and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www/R-project.org/ (2025).
53. Imperial College Research Computing Service. Imperial College London, UK. https://doi.org/10.14469/hpc/2232 (2025).
54. Eberhard, M. L. et al. Experimental *Onchocerca volvulus* infections in mangabey monkeys (*Cercocebus atys*) compared to infections in humans and chimpanzees (*Pan troglodytes*). *Am. J. Trop. Med. Hyg.***44,** 151–160 (1991).
55. Behrend, M. R. et al. NTD Modelling Consortium. Modelling for policy: the five principles of the Neglected Tropical Diseases Modelling Consortium. *PLoS Negl. Trop. Dis.***14,** e0008033 (2020).

Figures

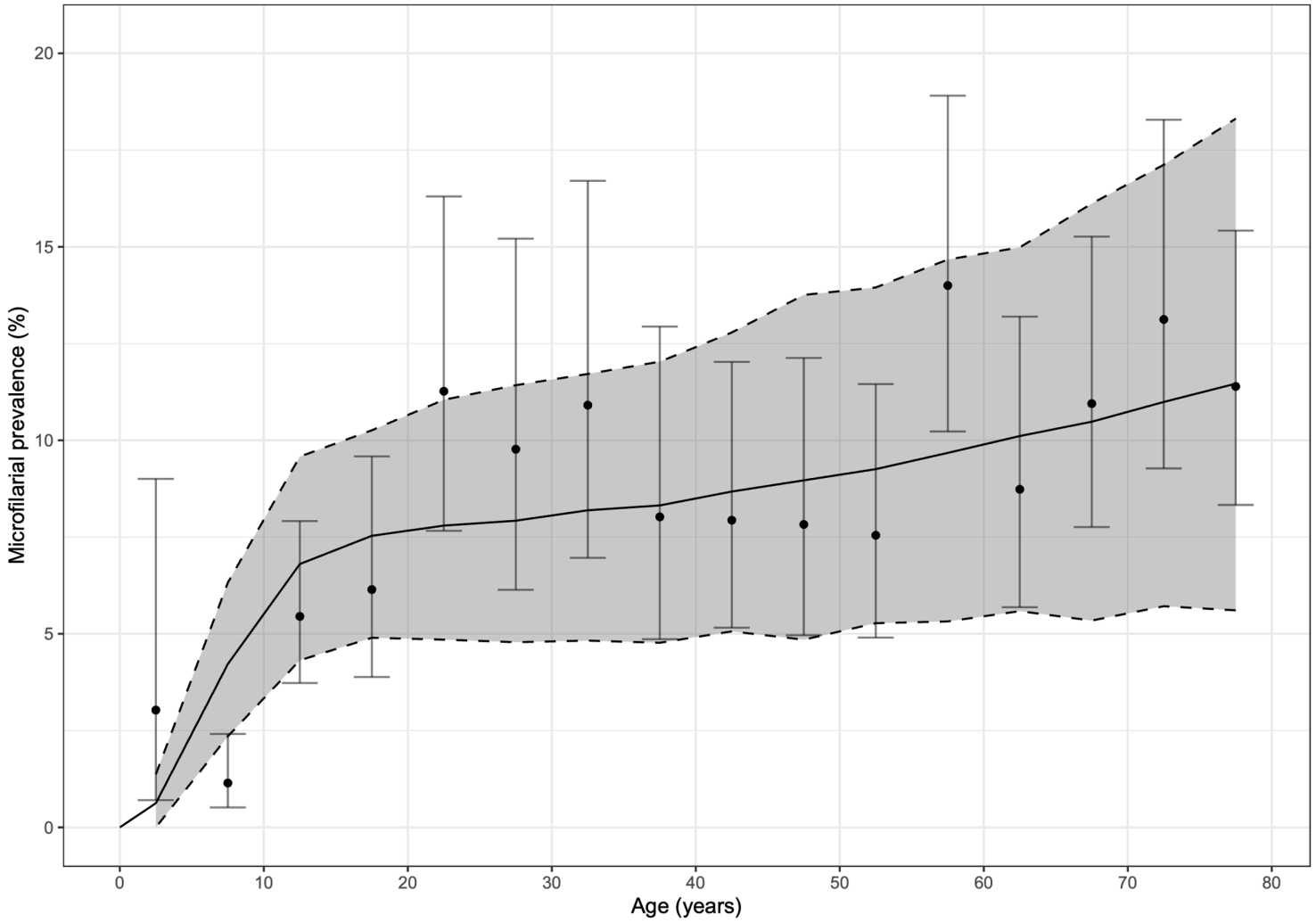

Figure 1

Observed and fitted microfilarial age-prevalence profiles for Gabon. The black solid line represents the modelled mean microfilarial prevalence using EPIONCHO-IBM calibrated with an annual biting rate (ABR) of 76 bites/person/year and an individual exposure heterogeneity parameter, k_E=0.2. The grey shaded area is the 95% uncertainty interval (2.5th to 97.5th quantile of the simulated mean) from 1,500 stochastic repeats. The black circles are the observed microfilarial prevalence estimates[22] for 4,257 individuals from 58 communities binned into 5-year age groups; the error bars are the associated 95% Wilson score confidence intervals.

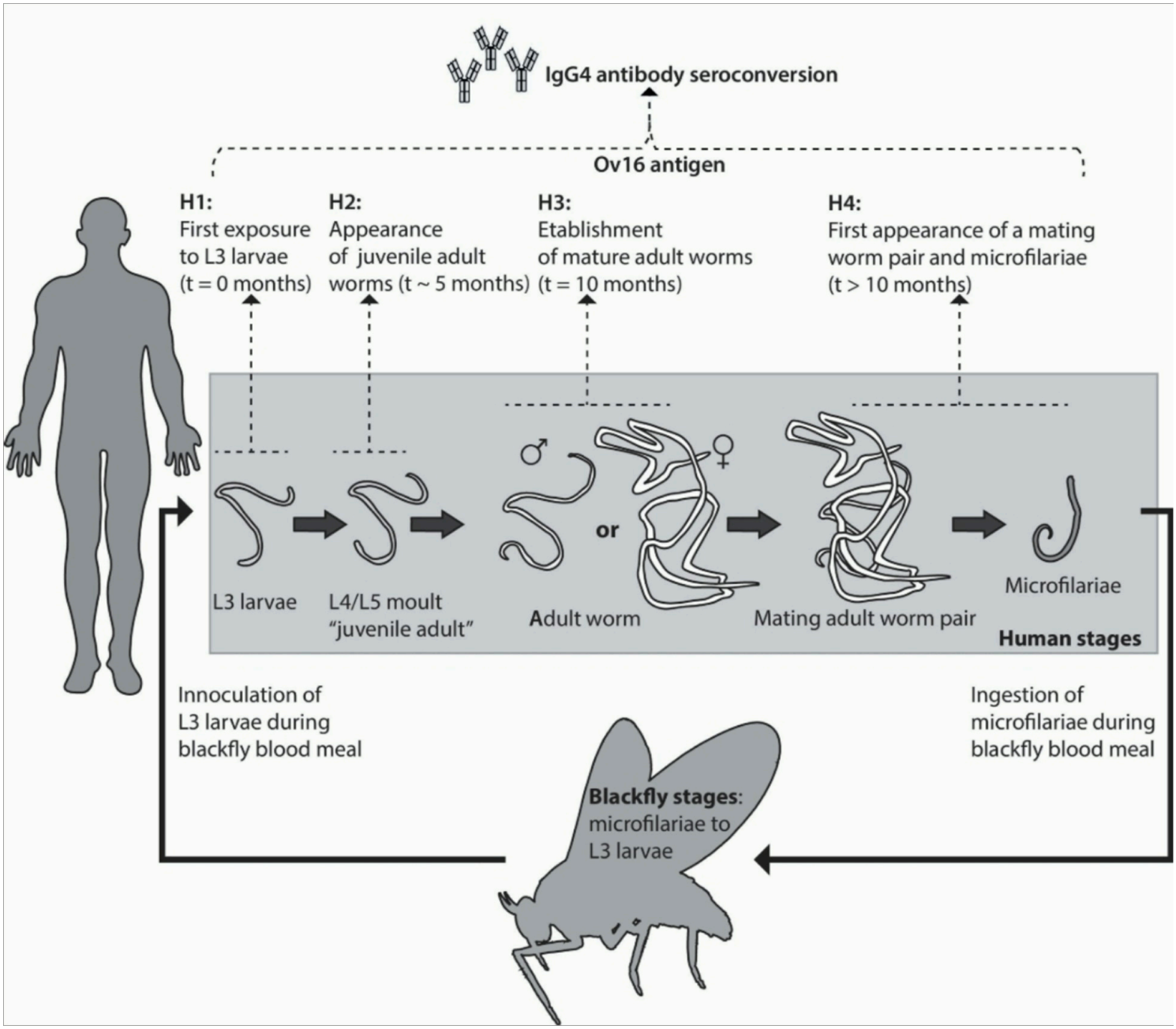

Figure 2

Lifecycle of *Onchocerca volvulus* showing seroconversion hypotheses incorporated into the onchocerciasis transmission model, EPIONCHO-IBM. The parasite stages resulting in the production of anti-Ov16 IgG4 antibodies are: L3 larvae (hypothesis H1); L5 (juvenile) worms following the L4-L5 moult (hypothesis H2); any mature adult *O. volvulus* (hypothesis H3), or the production of microfilariae by a mating worm pair (hypothesis H4).

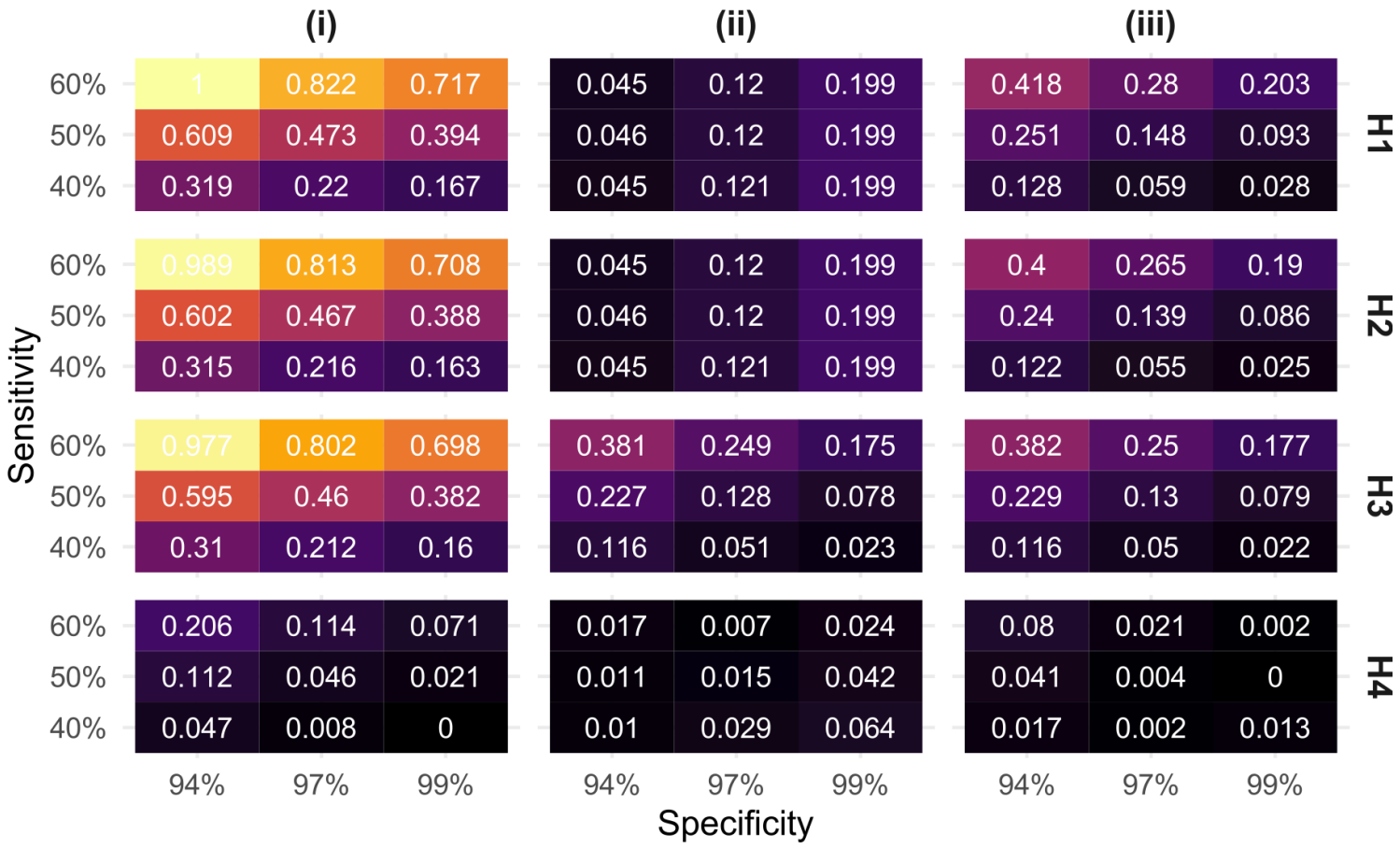

Figure 3

Quantitative assessment of goodness-of-fit of anti-Ov16 IgG4 seroconversion-seroreversion hypotheses under different assumptions on the sensitivity and specificity of the Ov16 rapid diagnostic test (Ov16-RDT). Each tile shows the normalized mean squared error (MSE), calculated by comparing EPIONCHO-IBM-predicted age-seroprevalence profiles with observed anti-Ov16 IgG4 seroprevalence estimates from 4,257 individuals living in 58 ivermectin-naïve, hypoendemic communities in Gabon[22]. The MSEs were calculated as the arithmetic mean of 1,500 stochastic repeats, with squared errors for each age group weighted by the proportion of observations within the given age group and then normalized to the interval [0,1] (unnormalised values and uncertainty intervals are shown in Supplementary Table S4). For each seroconversion (H1-H4) and seroreversion (i-iii) hypothesis (Table 1), MSEs were calculated under assumed diagnostic sensitivities of 40%, 50% and 60%, and specificities of 94%, 97% and 99%. Darker tiles correspond to lower (better) normalized MSEs. Hypotheses H4i (seroconversion initiated by near-patent/patent infection with no seroreversion, assuming 40% sensitivity and 99% specificity) and H4iii (seroconversion initiated by near-patent/patent infection with finite seroreversion, assuming 50% sensitivity and 99% specificity) provided the best quantitative fit to the data. These two hypotheses are statistically indistinguishable based on overlapping uncertainty intervals (Supplementary Table S4).

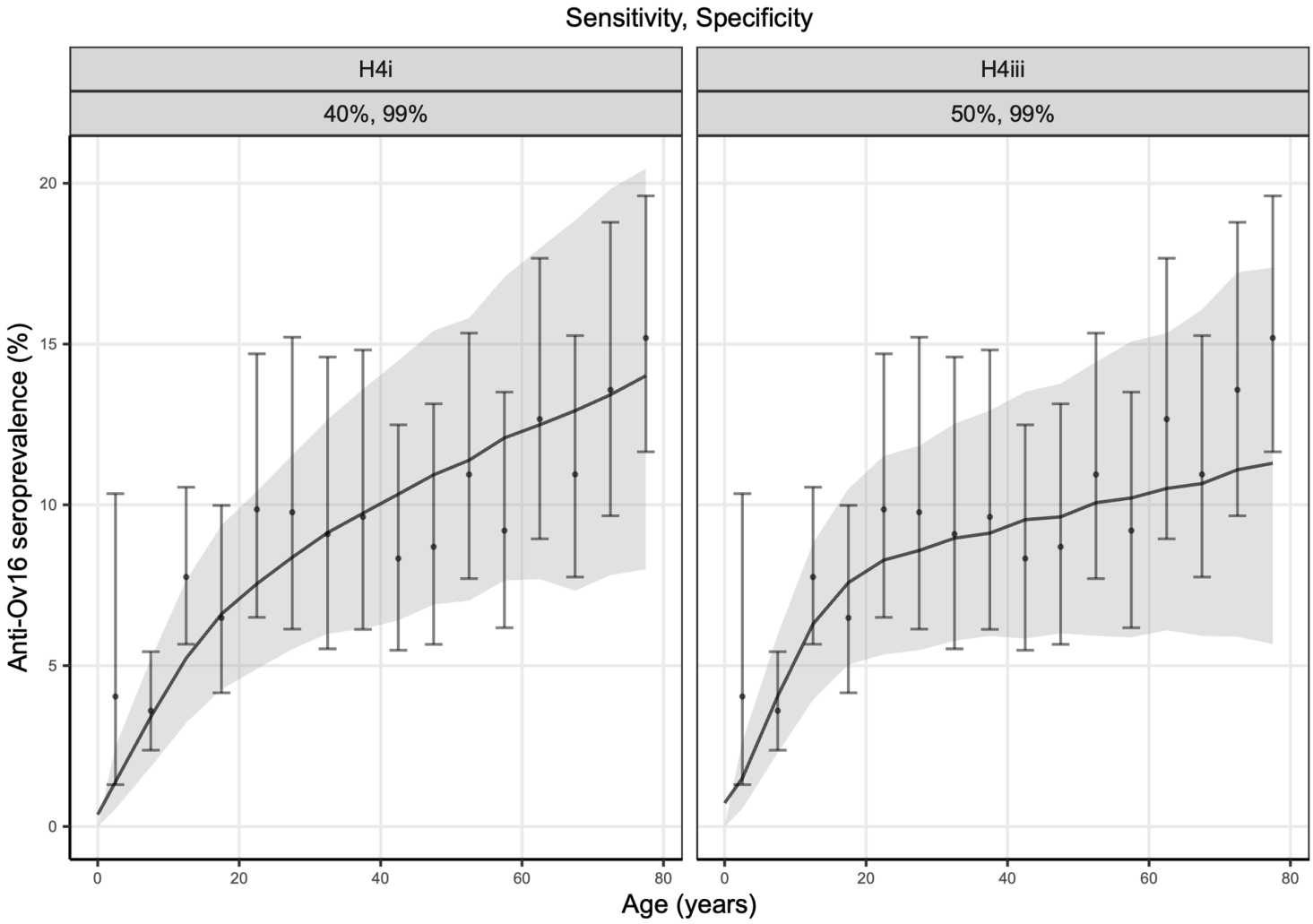

Figure 4

Observed and predicted anti-Ov16 IgG4 age-seroprevalence profiles for 4,257 individuals living in 58 ivermectin-naïve communities in Gabon. Solid lines represent the mean predicted age-seroprevalence profiles generated by EPIONCHO-IBM, calibrated to observed microfilarial prevalence estimates (Fig. 1)[22] using an annual biting rate of 76 bites/person/year and an inter-individual exposure heterogeneity parameter k_E = 0.2. Predictions assume that anti-Ov16 IgG4 seroconversion is elicited by near-patent/patent infection (hypothesis H4) with either no seroreversion (H4i, left panel) or finite seroreversion (H4iii, right panel), and are adjusted for the assumed diagnostic performance of the SD BIOLINE Ov16 rapid diagnostic test using whole blood. Assumed diagnostic parameters: 40% sensitivity and 99% specificity (left panel); 50% sensitivity and 99% specificity (right panel). Black circles show observed seroprevalence estimates binned into 5-year age groups; error bars represent 95% Wilson score confidence intervals. Shaded areas depict 95% uncertainty intervals (2.5th to 97.5th quantiles of the simulated mean), based on 1,500 stochastic repeats.

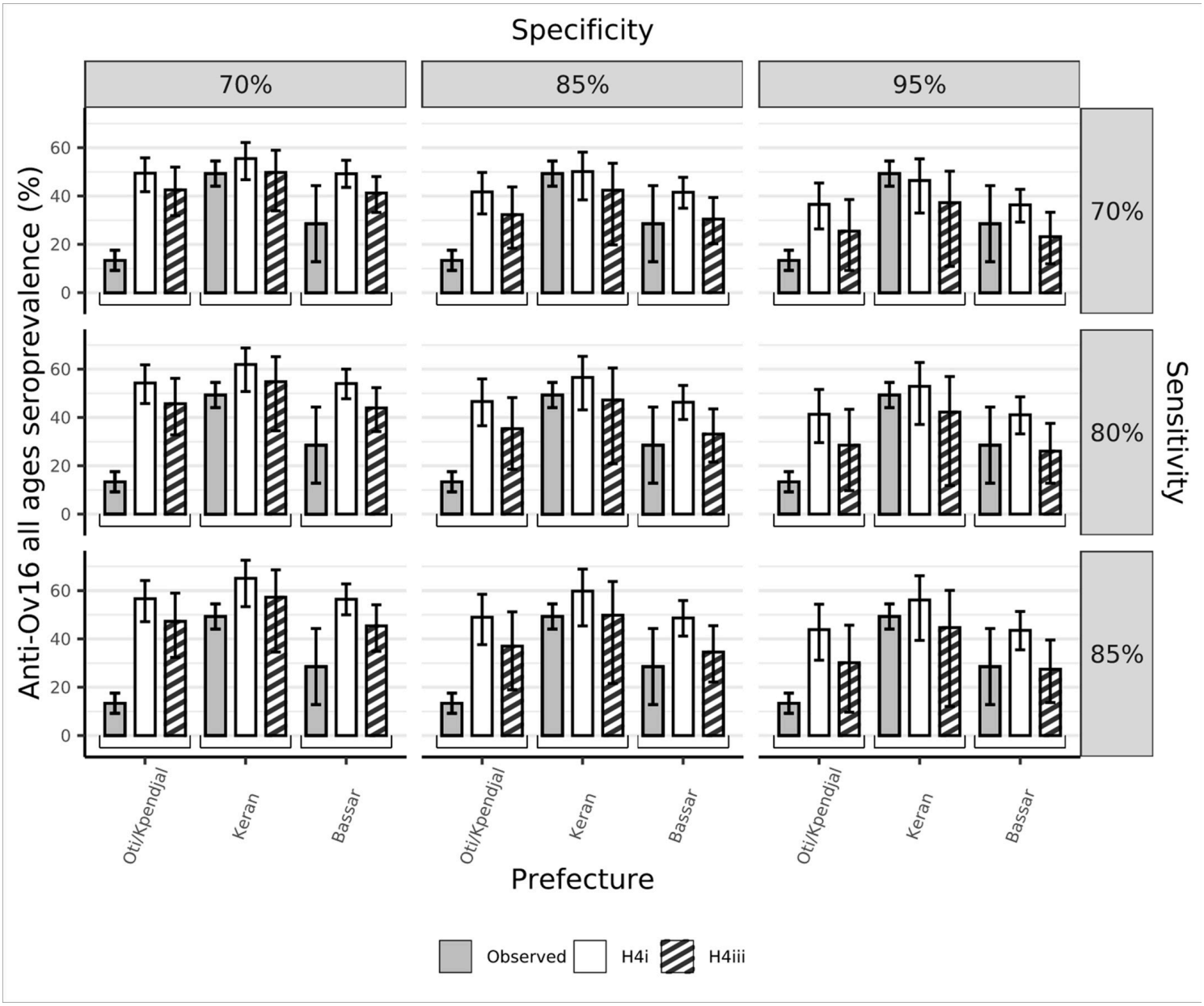

Figure 5

Observed and predicted anti-Ov16 IgG4 seroprevalence among 644 individuals (all ages) in four Togo prefectures (Ôti/Kpendjal, Kéran, and Bassar) after 39 years of anti-vectorial and anti-parasitic intervention, under assumptions of no seroreversion or finite seroreversion. Grey bars show observed seroprevalence estimates in 2015 and associated 95% Wilson score confidence intervals[23]. White and hashed bars represent mean predicted (all-age) seroprevalence generated by EPIONCHO-IBM, calibrated to model-derived pre-intervention microfilarial prevalence estimates (with inter-individual exposure heterogeneity parameter, $k_E = 0.3$)[25] using the best-fitting seroconversion hypothesis (anti-Ov16 IgG4 antibody production elicited by near-patent/patent infection) identified from the Gabon dataset[22]. Predictions are shown for different assumptions on the sensitivity (70%, 80% and 85%) and specificity (70%, 85% and 95%) of the horseradish peroxidase, HRP Ov16 ELISA used in Togo[23].White bars indicate model predictions assuming no seroreversion (hypothesis H4i); hatched bars indicate predictions

assuming finite seroreversion (hypothesis H4iii). Black error bars denote 95% uncertainty intervals (2.5th to 97.5th quantiles of simulated means) based on 1,500 stochastic repeats per prefecture (i.e., 4,500 simulations in total). Historical interventions included vector control (aerial larviciding of *Simulium damnosum* s.l. breeding sites: 1976–1993 in Ôti/Kpendjal; 1976–2007 in Kéran and Bassar) and ivermectin mass drug administration (1991–2015)[25]. Intervention effectiveness was modelled as 'minimal' in Bassar, 'enhanced' in Kéran, and a mix of 'minimal', 'reference', and 'enhanced' in Ôti/Kpendjal. See Supplementary Table S6 for parameters defining intervention effectiveness categories.

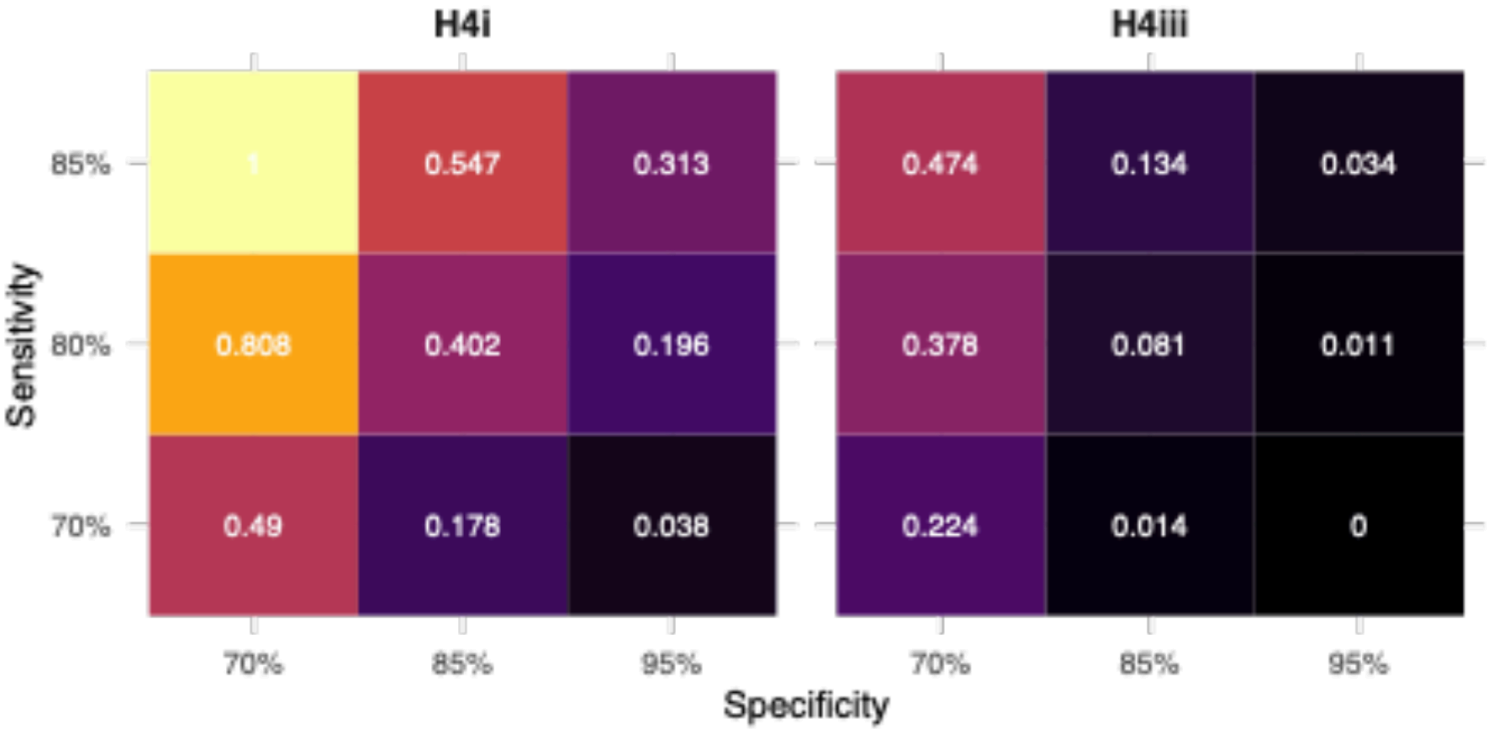

Figure 6

Quantitative assessment of EPIONCHO-IBM predictive performance under two seroreversion hypotheses and varying assumptions for the sensitivity and specificity of the horseradish peroxidase (HRP) Ov16 ELISA. Each tile shows the normalized mean squared error (MSE), calculated by comparing model-predicted anti-Ov16 IgG4 seroprevalence with observed seroprevalence data from 576 individuals living in four Togo prefectures after 39 years of anti-vectorial and anti-parasitic intervention[23]. The MSEs were calculated as the arithmetic mean of 1,500 stochastic repeats, with the squared errors for each prefecture weighted by the proportion of observations in that prefecture, and then normalized to the interval [0,1] (unnormalised values and uncertainty intervals are shown in Supplementary Table S9). For each seroreversion hypothesis—H4i (no seroreversion) and H4iii (finite seroreversion)—the MSE was calculated under assumed diagnostic sensitivity values of 70%, 80%, or 85%, and specificity of 70%, 85%, or 95%. Both hypotheses assume anti-Ov16 IgG4 seroconversion is initiated by near-patent/patent infection (Table 1). Darker tiles indicate lower (better) normalized MSEs. The best quantitative fit to the data was achieved under hypothesis H4iii (seroconversion elicited by near-patent/patent infection with finite seroreversion), assuming 70% sensitivity and 95% specificity.

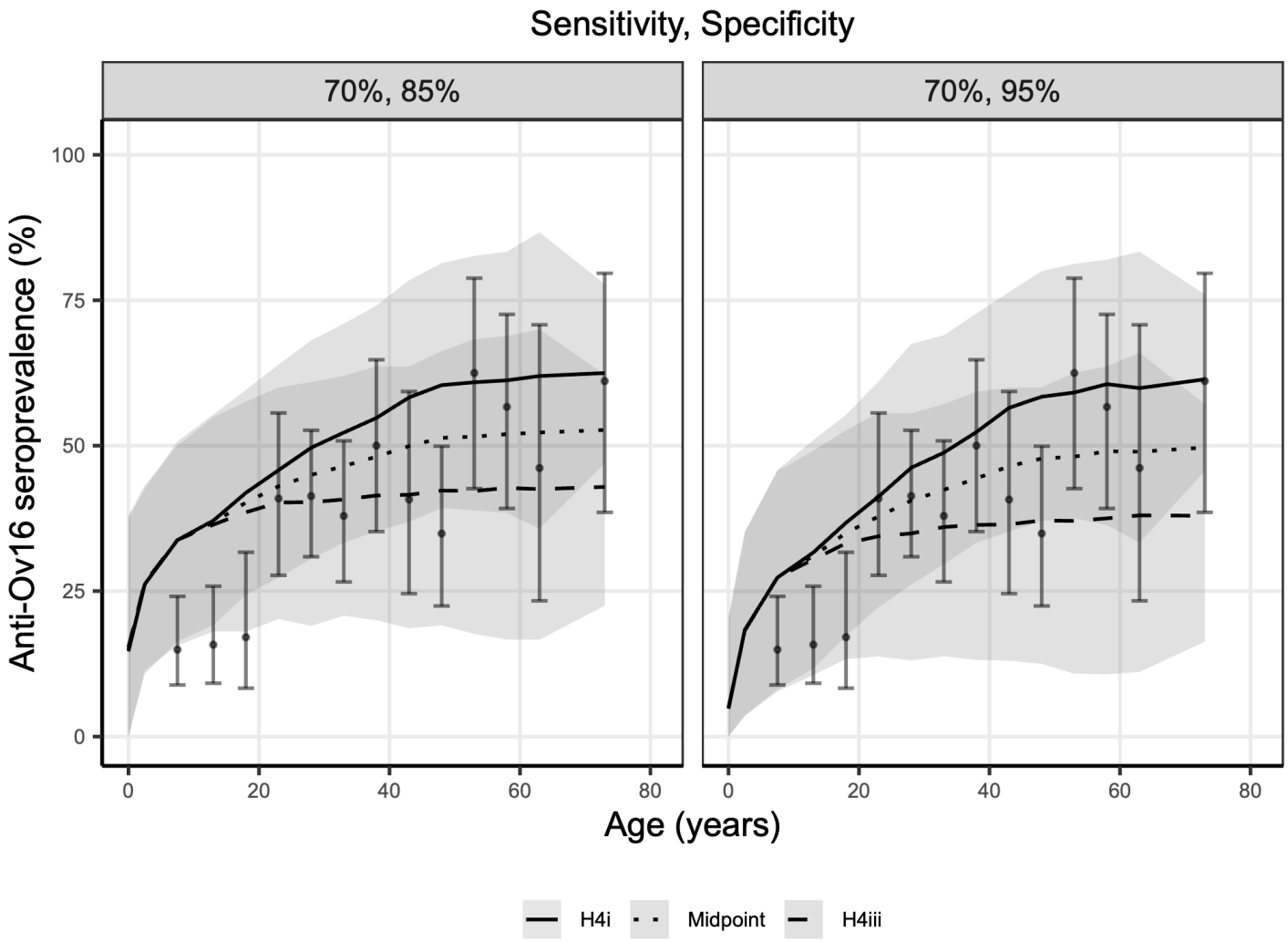

Figure 7

Observed and predicted anti-Ov16 IgG4 age-seroprevalence profiles for 576 individuals (aged 5-80 years) in four Togo prefectures (aggregated) after 39 years of anti-vectorial and anti-parasitic intervention. The solid, dashed and dotted lines represent mean predicted age-seroprevalence profiles generated by EPIONCHO-IBM, calibrated to inferred pre-intervention microfilarial prevalence estimates[25] (with inter-individual exposure heterogeneity parameter, k_E = 0.3) and using the best-fitting seroconversion hypothesis (anti-Ov16 IgG4 elicited by near-patent/patent infection) identified from the Gabon seroprevalence data[22]. Predictions are shown under two assumptions for the sensitivity and specificity of the horseradish peroxidase (HRP) Ov16 ELISA used in Togo: 70% sensitivity and 85% specificity (left panel); 70% sensitivity and 95% specificity (right panel). Solid lines indicate predictions assuming no seroreversion (hypothesis H4i), dashed lines correspond to predictions assuming finite seroreversion (hypothesis H4iii), and dotted lines show the mean of these two hypotheses. Shaded areas depict 95% uncertainty intervals (2.5th to 97.5th quantiles of simulated means) based on 1,500 stochastic repeats per prefecture (i.e., 4,500 simulations in total). Historical interventions included vector

control (aerial larviciding of *Simulium damnosum* s.l. breeding sites: 1976–1993 in Ôti/Kpendjal; 1976–2007 in Kéran and Bassar) and ivermectin mass drug administration (1991–2015)[25]. Intervention effectiveness was modelled as 'minimal' in Bassar, 'enhanced' in Kéran, and a mix of 'minimal', 'reference', and 'enhanced' in Ôti/Kpendjal. See Supplementary Table S6 for parameters defining intervention effectiveness categories.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Ramanietal.Ov16SerologySupplementaryFinal16.07.2025.pdf