

Opinion

Toxoplasma gondii: An Underestimated Threat?Gregory Milne ^{1,2,*}, Joanne P. Webster,^{1,2} and Martin Walker^{1,2}

Traditionally, the protozoan parasite *Toxoplasma gondii* has been thought of as relevant to public health primarily within the context of congenital toxoplasmosis or postnatally acquired disease in immunocompromised patients. However, latent *T. gondii* infection has been increasingly associated with a wide variety of neuropsychiatric disorders and, more recently, causal frameworks for these epidemiological associations have been proposed. We present assimilated evidence on the associations between *T. gondii* and various human neuropsychiatric disorders and outline how these may be explained within a unifying causal framework. We argue that the occult effects of latent *T. gondii* infection likely outweigh the recognised overt morbidity caused by toxoplasmosis, substantially raising the public health importance of this parasite.

Toxoplasmosis: Malaria's Neglected Cousin

Evidence of exposure to the apicomplexan protozoan parasite *Toxoplasma gondii*, which is capable of infecting all warm-blooded animals, is found in approximately 30% of the world's human population [1]. However, seroprevalence shows marked global variability: for example, in highly endemic regions, such as parts of Africa, seroprevalence can reach almost 90% in certain demographic groups, whereas in some European populations it can reach 60% [2]. Felines are the only known **definitive host** (see [Glossary](#)) for the parasite, shedding in their faeces up to millions of **oocysts** per day, which sporulate and become infective in the environment [3]. While in domestic cats, oocyst shedding occurs for only 1–3 weeks after initial infection, in wild feline species shedding may potentially continue intermittently for life [3]. Ingestion of these sporulated oocysts, which contaminate crops, soil, and water sources [4,5], or consumption of **bradyzoites** from raw or undercooked meat comprise the two major horizontal routes of transmission. Indeed, these parasite stages are responsible for a substantial burden of postnatally acquired infections, causing both sporadic outbreaks of acute, symptomatic disease in immunocompetent adults [6] and severe toxoplasmosis in immunocompromised individuals including HIV/AIDS patients (following reactivation of bradyzoites into disseminating **tachyzoites**) [7].

In humans, congenital toxoplasmosis is acknowledged as a significant public health problem [8,9]. Vertical transmission of *T. gondii* from mother to foetus occurs most frequently following a primary maternal infection during pregnancy (although other means of vertical transmission are possible, including infection with an atypical genotype overriding acquired immunity from a prior nonatypical exposure [10]). While the likelihood of maternofetal transmission is highest in the third trimester, the severity of congenital disease has the inverse relationship with gestational age, such that first-trimester infections generally result in the most severe clinical symptoms in neonates, including spontaneous abortion or stillbirth [8]. While overall approximately 75% of congenital cases are subclinical, congenital infection, amongst those surviving infants, can nonetheless result in various craniocerebral, ocular, and/or cognitive abnormalities in early or later life (e.g., **chorioretinitis**, **intracranial calcifications**, and learning difficulties [8]).

Latent *T. gondii* infection, following postnatally acquired (acute) infection, has historically been considered benign or even asymptomatic in immunocompetent individuals [1,11,12]. Yet a

Highlights

Accumulating evidence suggests that latent infection with *Toxoplasma gondii* is associated with a variety of neuropsychiatric and behavioural conditions.

Many of the conditions associated with *T. gondii* can be explained by emerging understanding of the parasite's neurotropic activity.

The public health burden of latent infection may far outweigh that caused by acute and congenital toxoplasmosis.

More powerful epidemiological studies, in conjunction with further mechanistic investigations, are needed to strengthen the evidence base for *T. gondii* as a causal agent of a range of neuropsychiatric and behavioural disorders.

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burgeoning number of epidemiological studies suggest that the parasite can be associated with a number of long-term behavioural effects in hosts, including humans. In rodent **intermediate hosts**, *T. gondii* can cause a range of behavioural alterations, including altered activity levels, decreased neophobic behaviour and a 'fatal feline attraction' to cat urine, thereby increasing the efficiency of transmission to the definitive host [13]. Similar by-product (nonadaptive, or residual manipulative [14]) behavioural effects, from the subtle (e.g., changes in personality traits) to the severe (e.g., increased risk of schizophrenia), have also been identified in *T. gondii*-infected humans [15,16]. Here, we review the increasing number of human disorders that have been linked to *T. gondii* infection and argue that these occult effects raise the public health burden of this chronic parasitic infection far beyond that recognised by the overt burden of acute disease.

How Does *Toxoplasma* Cause Neuropsychiatric Disease?

The first studies describing potential associations between latent *T. gondii* infection and human neuropsychiatric disorders, in this case schizophrenia, were published in the 1950s [16] (even before the parasite's life cycle was completely understood [17]). After a lull in interest, the 21st century has seen a proliferation of studies – followed by a flurry of meta-analyses – on associations between *T. gondii* infection and a wide variety of cognitive and neuropsychiatric disorders, including Alzheimer's disease [18], bipolar disorder [19,20], epilepsy [21], and obsessive-compulsive disorder (OCD) [19] (Figure 1). Whilst these studies and meta-analyses have demonstrated consistent support for the link with schizophrenia [19,22,23], the strength of evidence for other disorders is variable (Figure 2).

The association between *T. gondii* and neuropsychiatric conditions could partly be explained by the influence of the parasite on the expression of several neurotransmitters. Dopamine dysregulation, which has received the most research attention, results partly from the parasite's ability to synthesise tyrosine hydrolase (an enzyme involved in dopamine biosynthesis) [24]. *T. gondii* also alters the expression of a range of other neurotransmitters, including γ -aminobutyric acid (GABA), glutamate, serotonin, and norepinephrine [25]. These effects might be mediated by the encystment of bradyzoites in neural – and most often microglial or neuronal – cells, thereby causing considerable alterations, both in host neurobiochemistry and in the expression of specific receptors/transporters [24,25]. For example, it has been shown, in chronically infected mice, that *T. gondii* decreases the expression of the glutamate transporter GLT-1, resulting in a twofold increase in extracellular glutamate concentrations [26].

In humans, dysregulation of neurotransmitter expression is critically involved in many behavioural and neuropsychiatric conditions, including bipolar disorder, depression, drug addiction, OCD, schizophrenia, and suicide [25]. As examples, changes in dopamine-mediated neurotransmission have been proposed to be involved in the pathophysiology of both addictive and obsessive behaviours [27,28], and serotonin is thought to play a central role in the aetiology of mood disorders [29]. *T. gondii* is auxotrophic for tryptophan – the precursor to serotonin – and low plasma tryptophan concentrations have been associated with cases of severe depression [30,31].

A differing hypothesis is that changes in the endocrine system, particularly testosterone expression, may drive behavioural changes observed in *T. gondii*-infected hosts. The evidence to support this comes from *in vivo* studies showing that infected male rodents have higher concentrations of testosterone and reduced innate aversion to the odour of cat urine compared with uninfected controls (with castration prior to infection rescuing the aberrant behavioural phenotype) [32,33]. Furthermore, castrated male mice given exogenous testosterone display a reduced aversion to cat odour, which corresponds to changes in regulatory gene methylation patterns in the extended medial amygdala [33]. Hence, it is plausible that both neurotransmitter

Glossary

Bradyzoite: the slowly dividing, sessile parasite stage that is found within tissue cysts in various body regions, including skeletal and cardiac muscles, the eyes, and central nervous system (with a preference for neuronal and glial cells). Bradyzoites are associated with latent infection and cannot be cleared by any current antiparasitic drugs.

Chorioretinitis: inflammation of the choroid layer of the eye, superior to the retina and inferior to the sclera. Scarring, particularly in the macular region of the eye, and necrotic lesions are consistent features.

Definitive host: the organism(s) which supports the sexually reproducing stage of the parasite.

Effect size: the magnitude of an association between an exposure (e.g., infection) and an outcome (e.g., neuropsychiatric disease). Effect sizes are frequently expressed as RRs (or relative risks) or ORs, depending on the study design.

Intermediate host: the organism(s) which harbour the asexual parasite stage and which allow for transmission to the definitive host. In the case of *T. gondii*, examples include rodents and birds (domestic cat prey) and potentially wildebeest and zebra (large wild cat prey).

Intracranial calcifications: deposits of calcium within the brain, often associated with infection with TORCH agents (toxoplasmosis, *Cytomegalovirus*, and *Herpes simplex virus*). Infants with these lesions are suspected to be at increased risk of severe neuropsychiatric sequelae.

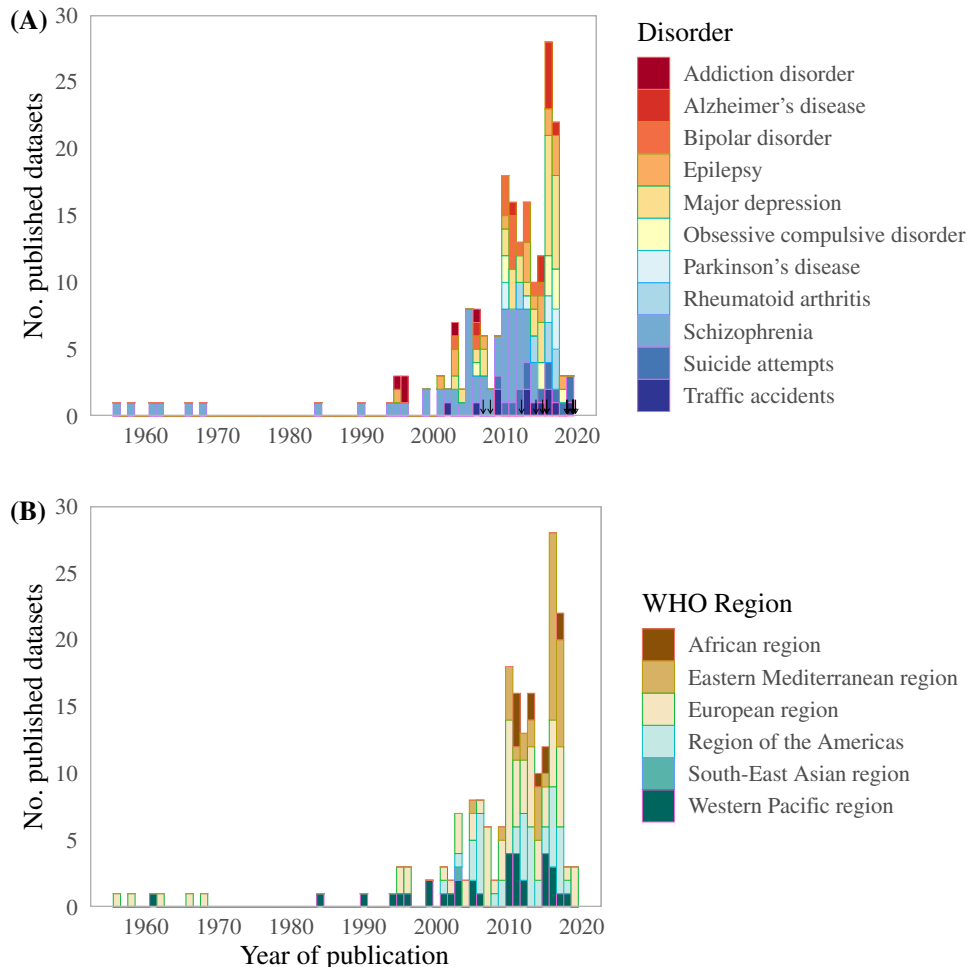
Latent *T. gondii* infection: the phase of *T. gondii* infection in which the parasite remains in tissue cysts as bradyzoites.

Odds ratio (OR): an effect size metric which is most frequently calculated from case-control studies to quantify the magnitude of association between an exposure and a disease.

Oocyst: an environmentally resistant parasite stage which contains the sexual stage sporozoites that are produced in intestinal cells of the felid definite hosts. Depending on environmental conditions (e.g., temperature and humidity), oocysts can survive for many years.

Population attributable fraction

(PAF): the burden of a particular outcome (e.g., a neuropsychiatric disorder) attributable to an exposure (e.g., infection with *T. gondii*).



Risk ratio (RR): an effect size metric typically calculated from cohort studies to quantify the risk of developing a disease associated with a particular exposure.

Secondary host: the organism(s) which harbour the asexual parasite stage. Unlike intermediate hosts, secondary hosts do not necessarily support transmission to the definitive host. In the case of *T. gondii*, examples include non-prey animal associates of the cat, for example, cow, sheep, and human.

Tachyzoite: the rapidly dividing parasite stage associated with acute infection. Tachyzoites disseminate infection throughout the body prior to the establishment of a latent infection and may reactivate from bradyzoite cysts in immunocompromised individuals.

Toxoplasmic encephalitis: inflammation of the brain which results most often from immunocompromise and subsequent reactivation of *T. gondii* bradyzoites in tissue cysts into tachyzoites, which invade and replicate in new cells.

Figure 1. Publication History of Datasets from Meta-Analyses Associating *Toxoplasma gondii* with Neuropsychiatric and Behavioural Disorders. Published datasets ($N = 202$) collected across 39 countries and cited in 15 systematic reviews, assessing the relationship between *T. gondii* infection and neuropsychiatric disorders, grouped by: (A) specific neuropsychiatric and behavioural disorders (arrows indicate years in which systematic reviews were published); (B) World Health Organization (WHO) global region in which the study was conducted (for all studied disorders combined).

and endocrine dysregulation have roles to play in the behavioural changes associated with *T. gondii* infection.

A notable exception to the neuromodulation-based (or endocrine-based) mechanistic explanation is the association of *T. gondii* infection with epilepsy. Development of epilepsy is likely primarily determined by the pattern and extent of cyst presence and/or rupture in the brain and the subsequent formation of scar tissue [34]. In agreement with a nonspecific infectious aetiology of some cases of epilepsy, a case-control study conducted in sub-Saharan Africa found the prevalence of active convulsive epilepsy to be higher among individuals seropositive for *T. gondii*, although also among those seropositive for a range of other parasitic infections, including *Onchocerca volvulus* and *Toxocara canis* (with numerous other studies reporting associations between epilepsy and the aetiological agent of neurocysticercosis, *Taenia solium* [35]) [36]. Interestingly, the

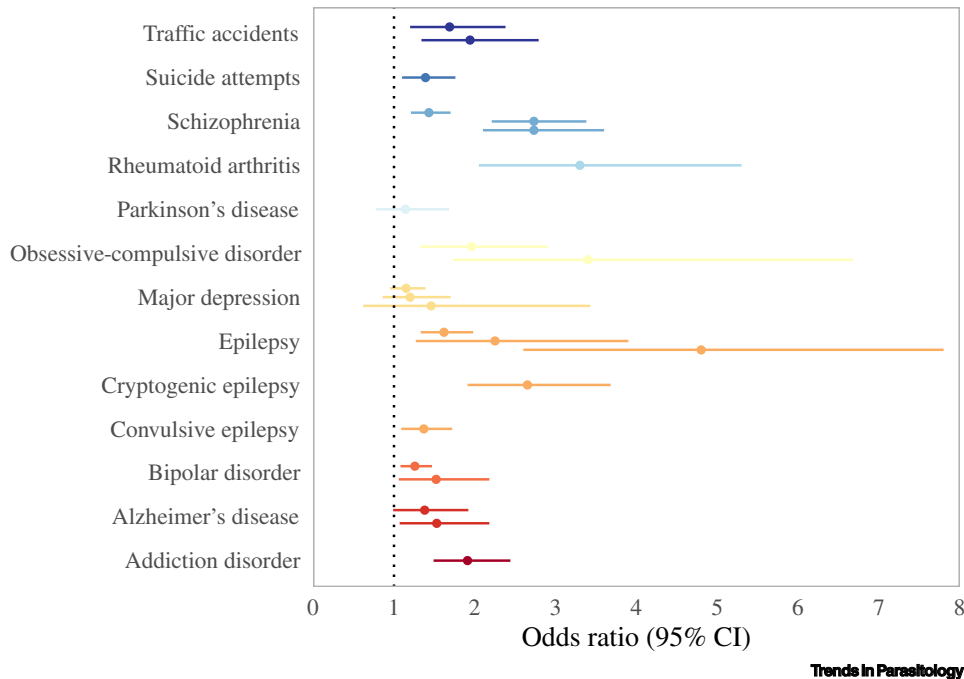


Figure 2. *Toxoplasma gondii* and Associated Human Behavioural and Neuropsychiatric Disorders. The association of *T. gondii* IgG seroprevalence in humans with various disorders compared with IgG seroprevalence in healthy control subjects. Each odds ratio (OR) shows the strength of association stated in a published systematic review and meta-analysis [18–21,23,46,47,71–77]. For some disorders more than one meta-analysis was published, and hence multiple ORs are presented in these cases. Note that the ORs for cryptogenic and convulsive epilepsy are calculated using subgroup analysis of one of the epilepsy meta-analyses [21]. Studies were found using PubMed and Google Scholar with terms “*Toxoplasma gondii* OR toxoplasmosis” AND [name of disorder]. Abbreviation: CI, confidence interval.

combined effects of certain coinfecting agents was more than additive [36], findings which are in agreement with the increasingly recognised notion that many human neuropsychiatric disorders may have a multi-infectious agent causation (Box 1) [37,38].

Another potential key and interrelated mechanistic factor in the propensity of *T. gondii* to cause behavioural alterations is the host's immune response. Following infection, immune cells in the small intestine, including innate lymphoid cells, are stimulated to produce a range of cytokines and transcription factors. Notable amongst these defences are cytokines, including interferon-gamma (IFN- γ), interleukin-12 (IL-12), and tumour necrosis factor-alpha (TNF- α), and chemokines, including CCL2 and CXCL2, which are produced by activated immune cells such as macrophages and dendritic cells, prompting further immune cell activation and anti-*T. gondii* gene expression (for a review see [39]). While this immune response mediates the effective control of acute infection, it also contributes to the maintenance of parasite latency and, potentially, to the development or aggravation of neurological sequelae. Studies using rodent models have observed excessive levels of proinflammatory cytokines, including TNF- α and IL-6, in the serum of mothers of offspring who later developed psychotic-like symptoms [40]. Interestingly, TNF- α and IL-6 have both been shown to promote bradyzoite formation in murine and cell culture models [41], and in humans, perinatal exposure to TNF- α and IL-8 has been linked to development of schizophrenia in offspring [42].

It is therefore likely that a number of mechanistic pathways, including endocrine and neurotransmitter dysregulation and the proinflammatory immune response to infection (including, potentially,

Box 1. A Multiagent Model of Neuropsychiatric Disease

Accumulating evidence suggests that the abnormal behaviour of *T. gondii*-infected intermediate/**secondary hosts** may result from coinfection with other neurotropic pathogen(s). For example, serological testing of schizophrenic patients and matched controls showed that schizophrenic patients more often had IgG antibodies to various coinfecting agents, including *T. gondii* but also *Chlamydia trachomatis*, human herpesvirus-6, and measles virus [37,38]. In agreement with these findings, our recent research has shown that UK foxes housed in sanctuaries with aberrant behaviours indicative of neuropsychiatric impairment have a higher prevalence of *T. gondii*/vulpine *Circovirus* (FoxCV) coinfections relative to wild foxes [64]. Thus, *T. gondii* infection and its interaction with other coinfecting neurotropic pathogens may be a factor contributing to the aetiology of human neuropsychiatric disorders like schizophrenia.

An extension to this model could include additional interactions with host and pathogen genetics [22]. In terms of host genetics, dysregulated expression or changes in the 'disrupted in schizophrenia' (DISC1) protein have been associated with a predisposition to schizophrenia [22]. Moreover, this protein has been implicated in the immune response against *T. gondii*, with individuals with altered DISC1 having higher titres of *T. gondii* antibodies [65]. Furthermore, the HLA-D alleles (e.g., HLA-DQ3, HLA-DQA1/B1) have been shown to play a key role in determining the likelihood and outcome of both *T. gondii* congenital disease and disease in immunocompromised patients [66]. These findings support a tripartite model of clinical disease outcome, which could be further extended given the addition of strain-specific differences in parasite pathogenicity.

Parasite genotype may partly determine patterns of neuropsychiatric disease [63]. For example, mothers infected with genotype I parasites have almost two times the odds of birthing a child who develops psychosis in later life, compared with seronegative mothers [67]. This pattern is not seen for other genotypes, perhaps suggesting differing parasite tropisms between lineages or differences in strain virulence factors, which could exacerbate the development of psychoses. Further, so-called atypical genotypes (those not captured by the traditional type I–III classification) circulating in the Americas have been associated with a greater burden and severity of ocular disease [63]. *T. gondii* epidemiology is therefore multifactorial, with different factors interacting and combining to alter the likelihood and outcome of clinical – and plausibly neuropsychiatric – disease. These factors likely include: (i) host genetics (and epigenetics); (ii) parasite genetics (types I–III, atypical); (iii) population disease endemicity (seroprevalence); and (iv) coinfecting neurotropic agents.

interactions between pathways [24]), act in parallel to explain the diversity of neuropsychiatric disorders associated with *T. gondii* infection (Figure 2). Indeed, emerging evidence from rodent models suggests that *T. gondii* may even transgenerationally modulate host behaviour, including anxious and depressive symptoms, via paternally inherited epigenetic changes [43].

Tip of the Iceberg: An Underestimated Burden of Disease?

Notwithstanding the general scarcity of data on the causal nature of *T. gondii* infection and human neuropsychiatric disorders (with the exception of some cases of schizophrenia [13,22]), it is possible to approximate the **population attributable fraction (PAF)** from the **effect sizes (odds ratios, ORs)** estimated from meta-analyses (Box 2). This approach has been used to estimate that 21.4% (13.7–30.6%) of schizophrenia cases are associated with *T. gondii* infection [44]. Assuming a global incidence of schizophrenia of 15.2 per 100 000 [45], this means that between 150 000 and 335 000 cases per year may be attributable to *T. gondii*. While this PAF is based on an OR of 2.71 from an older systematic review [46,47] (higher than a more recent review [19]; Figure 2), these numbers nonetheless highlight the potentially significant global burden of *T. gondii*-associated schizophrenia. Indeed, this is particularly the case considering that the psychopathology of *T. gondii*-related schizophrenia has been reported to be more severe and of longer duration than schizophrenia unrelated to *T. gondii* [48].

Another recent study estimated, assuming equivalence of ORs and risk ratios (RRs) (Box 2), that *T. gondii* may account for approximately 17% of traffic accidents (6–29%) and 10% of suicide attempts (3–19%) [23]. The World Health Organization (WHO) estimates that there are 20–50 million non-fatal traffic-related injuries per year [49]. Taking the central value of 35 million accidents, *T. gondii* may therefore be associated with between 2.1 and 10.2 million non-fatal traffic accidents per year. A similar illustrative calculation is possible for suicides and non-fatal suicide attempts (NFSAs). For every suicide fatality there are roughly 20 attempts [50] and

therefore, given average annual global suicide rates of 9.94 per 100 000 [50], between 0.46 million and 2.91 million NFSAs per year may be attributable to infection with *T. gondii*.

How do these numbers compare with what is known about the overt burden of disease? It is estimated that between 179 300 and 206 300 cases of congenital toxoplasmosis (CT) occur annually [51], which result in an estimated 5900 cases of foetal loss and neonatal death, 24 700 cases of chorioretinitis in the first year of life, and 9300 cases of hydrocephalus and other central nervous system (CNS) abnormalities [52]. Disease in immunocompromised individuals also poses a considerable burden. For example, a recent meta-analysis estimated that there are 13.1 million HIV patients coinfecting with *T. gondii* [7]. While this figure is substantial, current data nonetheless suggest that the now routine use of combination antiretroviral therapy (cART) in individuals with HIV diagnoses has significantly mitigated the risk of *T. gondii*-associated sequelae [including **toxoplasmic encephalitis (TE)**] [53]. Furthermore, acute outbreaks of acquired toxoplasmosis, the largest of which resulted in 1400 human infections, occur locally and sporadically [6]. The case numbers of the overt manifestations of *T. gondii* infection are therefore likely dwarfed by those of neuropsychiatric disorders, non-fatal traffic accidents, suicide, and NFSAs associated with latent *T. gondii* infection.

While a sizeable (yet inestimable) burden of sequelae may still be present amongst immunocompromised/HIV-positive subpopulations either not seeking healthcare nor complying

Box 2. How Well Can the Odds Ratio Approximate the Risk Ratio?

Epidemiological studies are designed to determine whether there exists an association between an exposure (e.g., infection with *T. gondii*) and an outcome (a particular neuropsychiatric disease). They can be broadly split into observational or experimental study designs. Observational designs include case-control studies and cohort studies.

Case-control studies are particularly useful when an outcome is rare in a population. Case participants (with the disease) and controls (without the disease) are included, and the exposure status of each participant is ascertained retrospectively. The proportion of cases with the exposure of interest is then compared with the corresponding proportion of controls, usually expressed as an OR. A large majority of studies that have identified associations between neuropsychiatric disorders and exposure to *T. gondii* have used case-control designs.

Cohort studies are an alternative to case-control studies, with the key advantage that they measure exposure before the outcome of interest, a more powerful indicator of causality. Cohort studies involve tracking groups of participants (cohorts), either retrospectively or prospectively, with and without the exposure of interest and measuring the frequency (incidence) of the disease in each group. The relative frequency of disease occurrence in exposed and nonexposed cohorts is typically expressed as a **risk ratio (RR)**.

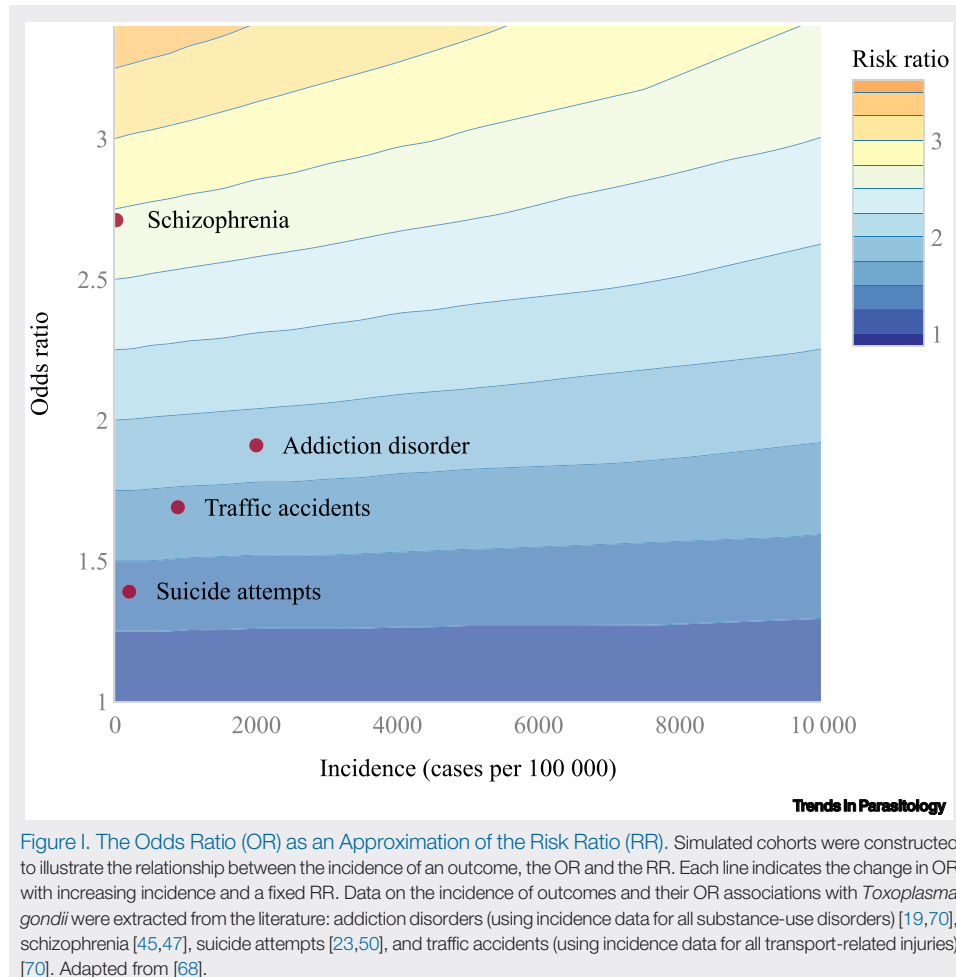
The PAF can be calculated to determine what proportion of cases in a population can be attributed to the exposure of interest and is given by:

$$PAF = \frac{P_E(RR-1)}{P_E(RR-1) + 1} \quad [I]$$

where P_E is the proportion of the population exposed (e.g., to *T. gondii*). To calculate the PAF using the results from a case-control study, one can either assume equivalence of the OR and RR, or adjust the OR using an estimate of the absolute risk of the outcome in the unexposed population, R_U [68],

$$RR = \frac{OR}{(1-R_U) + R_U \times OR} \quad [II]$$

If a disease is uncommon in the population, the denominator in Equation II tends to unity and gives equivalence of the RR and the OR (the so-called rare disease assumption [69]). For diseases like schizophrenia, with a worldwide incidence of 15.2 per 100 000 [45], the OR will provide a good approximation of the RR. For more common outcomes, like addiction disorder, with an incidence of approximately 2000 per 100 000 [19,70], an OR >1 will provide a biased overestimate of the RR and can be adjusted using Equation II [68] (Figure 1).



with cART or TE prophylaxis [54], it remains plausible that, without accounting for the burden of *T. gondii*-associated neuropsychiatric and behavioural conditions, we may be seeing only the tip of the iceberg of *T. gondii*'s effects on human populations.

Implications and Applications for Public Health

While accurate estimates of the burden of latent *T. gondii* infection are required, so too are parallel efforts towards improving public health interventions. Current interventions against *T. gondii* primarily focus on prevention of congenital transmission and treatment of acute disease. Prevention is largely limited to health advice on avoiding exposure during pregnancy and, in some countries, antenatal screening programmes that offer treatment with spiramycin to women who seroconvert during pregnancy. Treatment of acute disease is with pyrimethamine and sulfadiazine, although treatment failures are significant [55].

Whether current treatment and prevention practices have any tangible effect on neuropsychiatric sequelae will crucially depend on how the age of infection influences the likelihood of developing neuropsychiatric disease. For example, if congenital acquisition of infection was particularly likely

to cause neuropsychiatric sequelae later in life, then antenatal screening and treatment programs may be an effective intervention strategy.

By contrast, if the development of neuropsychiatric sequelae was mostly associated with (horizontally) acquired infections, treatment of acute cases may be ineffectual since the vast majority of such cases are unidentified because of mild and vague symptomology. While different strategies [11] could be employed to reduce the burden of horizontally acquired infections, a tailored response – one that efficiently distributes control resources – would require setting-specific information on the predominant route of infection (bradyzoite vs oocyst). Such information could be estimated using recent developments in sporozoite-specific serology [56].

Future Directions

Ultimately, to achieve better control of *T. gondii* and reduce its public health burden, it will be important to further basic science research (including behavioural studies on non-human primates with more similar neurological structures to humans) to better understand the mechanisms driving neuropsychiatric associations, the role of host and parasite genetics on sequelae (Box 1), and to conduct more powerful epidemiological research to strengthen the evidence base for these associations (Box 2).

As the majority of epidemiological studies to date have been either cross-sectional or case-control, they cannot determine the temporality of an association (Box 2). Cohort studies, by contrast, can provide supportive evidence of causation by identifying the presence of exposure (infection) prior to the onset of the outcome (neuropsychiatric disease). Ideally, this is achieved by following individuals for many years measuring exposure and outcome through time. However, in the case of schizophrenia, as the average age of onset is 23 years [16], such a long time to disease onset realistically precludes the use of prospective cohorts. This limitation could be ameliorated through the improvement of assays which provide estimates of the timing of infection by measuring *T. gondii*-specific antibodies, for example, by improving the temporal resolution obtained from IgG avidity tests. Nevertheless, in the absence of such advances, retrospective cohorts, which are less resource-intensive and costly designs, could potentially be constructed to identify temporality of the exposure–outcome relationship.

Randomised controlled trials (RCTs) can test the efficacy of *T. gondii* treatment on the alleviation of neurological symptoms, an approach that has been taken before [57,58]. At least five RCTs have been performed in *T. gondii*-positive schizophrenic patients to assess the impact of adjunctive antiparasitic drugs on schizophrenic symptoms [57,58], although none have documented any impact on the severity of schizophrenic symptoms. This finding could be due to the choice of adjunctive treatment: azithromycin, trimethoprim, artemisinin, artemether, and valproate have no demonstrated *in vivo* efficacy against bradyzoites [57]. It is also possible that treatment did not affect existing symptoms because behavioural changes resulting from cyst formation are irreversible, even in instances when cysts degrade [59].

Recently, spiramycin combined with metronidazole (a blood–brain barrier efflux pump inhibitor) has been shown to significantly reduce bradyzoite cysts in latently infected mice [60] (several other candidates, including miltefosine and guanabenz, have also shown promise; for a review of antibradyzoite drug targets see [61]). This combination may therefore overcome drug brain penetration issues, allowing antiparasitics to reach therapeutic concentrations. Nonetheless, if behavioural abnormalities are indeed irreversible [59], future efforts should instead focus on preventative measures such as cat, human, and livestock vaccines. While an effective vaccine for preventing *T. gondii*-induced sheep and goat abortions exists, the development of cat vaccines

is in its infancy, and no human vaccine exists [though promising targets include, amongst others, hroptry proteins and surface antigen (SAG) proteins] [62].

Ensuring representation of different geographic regions in which epidemiological studies are conducted – particularly where high-quality data remain scarce, such as in South America and sub-Saharan Africa (Figure 1B) – will also be important for several reasons. Firstly, parasite genotype appears to be geographically heterogeneous and may possibly be predicted to drive differences in the likelihood and severity of various clinical disease outcomes (Box 1) [63]. Secondly, host genetics and gene–environment interactions (including epigenetics [43]) may also play a role in determining disease burden (Box 1). Finally, criteria for diagnosis of different neuropsychiatric disorders are likely highly geographically variable, making outcome ascertainment differ by global region. Therefore, to verify the robustness of these associations, it is imperative that new epidemiological studies be performed in currently under-represented populations.

Concluding Remarks

The burgeoning number of associations of *T. gondii* with various neuropsychiatric disorders – alongside compelling causal explanations supporting such links – suggest that the impact of this pervasive parasite on global populations has been greatly underestimated. More research is needed to strengthen the epidemiological evidence base for these associations, but crucially also to improve understanding of the causative mechanisms (see Outstanding Questions). Ultimately, better prevention, treatment, and transmission control will be required to reduce the public health burden of *T. gondii*. There currently exists no effective treatment for latent *T. gondii* infection; acute disease treatments, and prophylaxis to prevent vertical transmission, are imperfect and there are no human or cat vaccines. Together with its complex epidemiology, *T. gondii* is a substantial and incompletely understood global public health challenge.

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References

- Montoya, J. and Liesenfeld, O. (2004) Toxoplasmosis. *Lancet* 2004, 1965–1976
- Molan, A. et al. (2019) Global status of *Toxoplasma gondii* infection: systematic review and prevalence snapshots. *Trop. Biomed.* 36, 898–925
- Kaushik, M. et al. (2014) What makes a feline fatal in *Toxoplasma gondii*'s fatal feline attraction? Infected rats choose wild cats. *Integr. Comp. Biol.* 54, 118–128
- Mangiacavchi, B.M. et al. (2016) Salivary IgA against sporozoite-specific embryogenesis-related protein (TgERP) in the study of horizontally transmitted toxoplasmosis via *T. gondii* oocysts in endemic settings. *Epidemiol. Infect.* 144, 2568–2577
- Boyer, K. et al. (2011) Unrecognized ingestion of *Toxoplasma gondii* oocysts leads to congenital toxoplasmosis and causes epidemics in North America. *Clin. Infect. Dis.* 53, 1081–1089
- Pinto-Ferreira, F. et al. (2019) Patterns of transmission and sources of infection in outbreaks of human toxoplasmosis. *Emerg. Infect. Dis.* 25, 2177–2182
- Wang, Z.D. et al. (2017) Prevalence and burden of *Toxoplasma gondii* infection in HIV-infected people: a systematic review and meta-analysis. *Lancet HIV* 4, e177–e188
- McAuley, J.B. (2014) Congenital toxoplasmosis. *J. Pediatr. Infect. Dis. Soc.* 3, S30–S35
- Saadatnia, G. and Golkar, M. (2012) A review on human toxoplasmosis. *Scand. J. Infect. Dis.* 44, 805–814
- Lindsay, D.S. and Dubey, J.P. (2011) *Toxoplasma gondii*: the changing paradigm of congenital toxoplasmosis. *Parasitology* 138, 1829–1831
- Opsteegh, M. et al. (2015) Intervention strategies to reduce human *Toxoplasma gondii* disease burden. *Clin. Infect. Dis.* 60, 101–107
- Villard, O. et al. (2016) Serological diagnosis of *Toxoplasma gondii* infection: Recommendations from the French National Reference Center for Toxoplasmosis. *Diagn. Microbiol. Infect. Dis.* 84, 22–33
- Webster, J.P. et al. (2013) *Toxoplasma gondii* infection, from predation to schizophrenia: can animal behaviour help us understand human behaviour? *J. Exp. Biol.* 216, 99–112
- Flegel, J. et al. (2011) Fatal attraction phenomenon in humans – cat odour attractiveness increased for *Toxoplasma*-infected men while decreased for infected women. *PLoS Negl. Trop. Dis.* 5, e1389
- Flegel, J. (2013) Influence of latent *Toxoplasma* infection on human personality, physiology and morphology: Pros and cons of the *Toxoplasma*–human model in studying the manipulation hypothesis. *J. Exp. Biol.* 216, 127–133
- Flegel, J. (2013) How and why toxoplasma makes us crazy. *Trends Parasitol.* 29, 156–163
- Innes, E. (2010) A brief history and overview of *Toxoplasma gondii*. *Zoonoses Public Health* 57, 1–7

Outstanding Questions

What are the mechanisms underlying epidemiological associations between latent *T. gondii* infection and specific neuropsychiatric disorders?

To what extent does parasite genotype influence the onset, maintenance, and type of neuropsychiatric disease?

How does age of infection influence the likelihood of developing neuropsychiatric disease?

Do interactions between *T. gondii* and other neurotropic agents affect the likelihood of developing neuropsychiatric disease?

How effective are antiparasitic drugs at reducing the symptoms of psychiatric illness?

18. Bayani, M. *et al.* (2019) *Toxoplasma gondii* infection and risk of Parkinson and Alzheimer diseases: A systematic review and meta-analysis on observational studies. *Acta Trop.* 196, 165–171
19. Sutherland, A.L. *et al.* (2015) Beyond the association. *Toxoplasma gondii* in schizophrenia, bipolar disorder, and addiction: Systematic review and meta-analysis. *Acta Psychiatr. Scand.* 132, 161–179
20. de Barros, J.L.V.M. *et al.* (2017) Is there any association between *Toxoplasma gondii* infection and bipolar disorder? A systematic review and meta-analysis. *J. Affect. Disord.* 209, 59–65
21. Sadeghi, M. *et al.* (2019) An updated meta-analysis of the association between *Toxoplasma gondii* infection and risk of epilepsy. *Trans. R. Soc. Trop. Med. Hyg.* 113, 453–462
22. Webster, J.P. *et al.* (2015) The *Toxoplasma gondii* model of schizophrenia. In *Modeling the Psychopathological Dimensions of Schizophrenia* (1st edn) (Pletnikov, M. and Waddington, J., eds), pp. 225–241, Academic Press
23. Sutherland, A.L. *et al.* (2019) Driving us mad: The association of *Toxoplasma gondii* with suicide attempts and traffic accidents - a systematic review and meta-analysis. *Psychol. Med.* 49, 1608–1623
24. McConkey, G.A. *et al.* (2013) *Toxoplasma gondii* infection and behaviour – location, location, location? *J. Exp. Biol.* 216, 113–119
25. Chaudhury, A. and Ramana, B.V. (2019) Schizophrenia and bipolar disorders: the *Toxoplasma* connection. *Trop. Parasitol.* 9, 71–76
26. David, C.N. *et al.* (2016) GLT-1-dependent disruption of CNS glutamate homeostasis and neuronal function by the protozoan parasite *Toxoplasma gondii*. *PLoS Pathog.* 12, e1005643
27. Kalivas, P.W. and Volkow, N.D. (2005) The neural basis of addiction: a pathology of motivation and choice. *Am. J. Psychiatry* 162, 1403–1413
28. Koo, M.S. *et al.* (2010) Role of dopamine in the pathophysiology and treatment of obsessive-compulsive disorder. *Expert. Rev. Neurother.* 10, 275–290
29. Kishi, T. *et al.* (2013) The serotonin 1A receptor gene confer susceptibility to mood disorders: Results from an extended meta-analysis of patients with major depression and bipolar disorder. *Eur. Arch. Psychiatry Clin. Neurosci.* 263, 105–118
30. Cowen, P. and Browning, M. (2015) What has serotonin to do with depression? *World Psychiatry* 14, 158
31. Marino, N.D. and Boothroyd, J.C. (2017) *Toxoplasma* growth *in vitro* is dependent on exogenous tyrosine and is independent of AAH2 even in tyrosine-limiting conditions. *Exp. Parasitol.* 176, 52–58
32. Vyas, A. (2013) Parasite-augmented mate choice and reduction in innate fear in rats infected by *Toxoplasma gondii*. *J. Exp. Biol.* 216, 120–126
33. Tong, W.H. *et al.* (2019) Testosterone reduces fear and causes drastic hypomethylation of arginine vasopressin promoter in medial extended amygdala of male mice. *Front. Behav. Neurosci.* 13, 33
34. Flegr, J. (2015) Neurological and neuropsychiatric consequences of chronic *Toxoplasma* infection. *Curr. Clin. Microbiol. Rep.* 2, 163–172
35. Wagner, R.G. and Newton, C.R. (2009) Do helminths cause epilepsy? *Parasite Immunol.* 31, 697–705
36. Kamuyu, G. *et al.* (2014) Exposure to multiple parasites is associated with the prevalence of active convulsive epilepsy in sub-Saharan Africa. *PLoS Negl. Trop. Dis.* 8, e2908
37. Arias, I. *et al.* (2012) Infectious agents associated with schizophrenia: a meta-analysis. *Schizophr. Res.* 136, 128–136
38. Khandaker, G.M. *et al.* (2012) Childhood infection and adult schizophrenia: a meta-analysis of population-based studies. *Schizophr. Res.* 139, 161–168
39. Sasai, M. *et al.* (2018) Host immune responses to *Toxoplasma gondii*. *Int. Immunol.* 30, 113–119
40. Beumer, W. *et al.* (2012) The immune theory of psychiatric diseases: a key role for activated microglia and circulating monocytes. *J. Leukoc. Biol.* 92, 959–975
41. Sullivan, W.J. and Jeffers, V. (2012) Mechanisms of *Toxoplasma gondii* persistence and latency. *FEMS Microbiol. Rev.* 36, 717–733
42. Nawa, H. and Takeji, N. (2006) Recent progress in animal modeling of immune inflammatory processes in schizophrenia: implication of specific cytokines. *Neurosci. Res.* 56, 2–13
43. Tyebji, S. *et al.* (2020) Pathogenic infection in male mice changes sperm small RNA profiles and transgenerationally alters offspring behavior. *Cell Rep.* 31, 107573
44. Smith, G. (2014) Estimating the population attributable fraction for schizophrenia when *Toxoplasma gondii* is assumed absent in human populations. *Prev. Vet. Med.* 117, 425–435
45. McGrath, J. *et al.* (2008) Schizophrenia: a concise overview of incidence, prevalence, and mortality. *Epidemiol. Rev.* 30, 67–76
46. Torrey, E.F. *et al.* (2007) Antibodies to *Toxoplasma gondii* in patients with schizophrenia: a meta-analysis. *Schizophr. Bull.* 33, 729–736
47. Torrey, E.F. *et al.* (2012) *Toxoplasma gondii* and other risk factors for schizophrenia: an update. *Schizophr. Bull.* 38, 642–647
48. Holub, D. *et al.* (2013) Differences in onset of disease and severity of psychopathology between toxoplasmosis-related and toxoplasmosis-unrelated schizophrenia. *Acta Psychiatr. Scand.* 127, 227–238
49. WHO (2018) *Road Traffic Injuries*, World Health Organization
50. WHO (2019) *Latest Suicide Estimates in the World Health Statistics 2019: Monitoring Health for the SDGs*, World Health Organization
51. Torgerson, P. and Mastroiacovo, P. (2013) The global burden of congenital toxoplasmosis: a systematic review. *Bull. World Health Organ.* 91, 501–508
52. Torgerson, P.R. *et al.* (2015) World Health Organization estimates of the global and regional disease burden of 11 foodborne parasitic diseases, 2010: a data synthesis. *PLoS Med.* 12, e1001920
53. Martin-Iguacel, R. *et al.* (2017) Incidence, presentation and outcome of toxoplasmosis in HIV infected in the combination antiretroviral therapy era. *J. Infect.* 75, 263–273
54. McFarland, M.M. *et al.* (2016) Toxoplasmic encephalitis. In *Encephalitis* (1st edn) (Avid Science, ed), pp. 1–51, Avid Science
55. Dunay, I.R. *et al.* (2018) Treatment of toxoplasmosis: historical perspective, animal models, and current clinical practice. *Clin. Microbiol. Rev.* 31, e00057-17
56. Milne, G. *et al.* (2020) Towards improving interventions against toxoplasmosis by identifying routes of transmission using sporozoite-specific serological tools. *Clin. Infect. Dis.* Published online April 13, 2020. <http://dx.doi.org/10.1093/cid/ciaa428/5819395>
57. Chorlton, S.D. (2017) *Toxoplasma gondii* and schizophrenia: a review of published RCTs. *Parasitol. Res.* 116, 1793–1799
58. Ibrahim, I. *et al.* (2019) Randomized controlled trial of adjunctive Valproate for cognitive remediation in early course schizophrenia. *J. Psychiatr. Res.* 118, 66–72
59. Ingram, W.M. *et al.* (2013) Mice infected with low-virulence strains of *Toxoplasma gondii* lose their innate aversion to cat urine, even after extensive parasite clearance. *PLoS One* 8, e75246
60. Chew, W.K. *et al.* (2012) Significant reduction of brain cysts caused by *Toxoplasma gondii* after treatment with spiramycin coadministered with metronidazole in a mouse model of chronic toxoplasmosis. *Antimicrob. Agents Chemother.* 56, 1762–1768
61. Montazeri, M. *et al.* (2018) Activities of anti-*Toxoplasma* drugs and compounds against tissue cysts in the last three decades (1987 to 2017), a systematic review. *Parasitol. Res.* 117, 3045–3057
62. Loh, F.K. *et al.* (2019) Vaccination challenges and strategies against long-lived *Toxoplasma gondii*. *Vaccine* 37, 3989–4000
63. Xiao, J. and Yolken, R.H. (2015) Strain hypothesis of *Toxoplasma gondii* infection on the outcome of human diseases. *Acta Physiol.* 213, 828–845
64. G. Milne *et al.*, Infectious causation of abnormal host behaviour: *Toxoplasma gondii* and its potential association with Dopey Fox Syndrome. *Front. Psych.*, in press.

65. Kano, S. *et al.* (2020) Host–parasite interaction associated with major mental illness. *Mol. Psychiatry* 25, 194–205
66. Shimokawa, P.T. *et al.* (2016) HLA-DQA1/B1 alleles as putative susceptibility markers in congenital toxoplasmosis. *Virulence* 7, 456–464
67. Xiao, J. *et al.* (2009) Serological pattern consistent with infection with type I *Toxoplasma gondii* in mothers and risk of psychosis among adult offspring. *Microbes Infect.* 11, 1011–1018
68. Zhang, J. and Kai, F.Y. (1998) What's the relative risk?: A method of correcting the odds ratio in cohort studies of common outcomes. *JAMA* 280, 1690–1691
69. Cornfield, J. (1951) A method of estimating comparative rates from clinical data. Applications to cancer of the lung, breast, and cervix. *J. Natl. Cancer Inst.* 11, 1269–1275
70. James, S.L. *et al.* (2018) Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* 392, 1789–1858
71. Wang, X. *et al.* (2014) Meta-analysis of infectious agents and depression. *Sci. Rep.* 4, 4530
72. Chegeni, T.N. *et al.* (2019) Is there any association between *Toxoplasma gondii* infection and depression? A systematic review and meta-analysis. *PLoS One* 14, e0218524
73. Chegeni, T.N. *et al.* (2019) Relationship between toxoplasmosis and obsessive compulsive disorder: A systematic review and meta-analysis. *PLoS Negl. Trop. Dis.* 13, e0007306
74. Gohardehi, S. *et al.* (2018) The potential risk of toxoplasmosis for traffic accidents: A systematic review and meta-analysis. *Exp. Parasitol.* 191, 19–24
75. Chegeni, T.N. *et al.* (2019) Is *Toxoplasma gondii* a potential risk factor for Alzheimer's disease? A systematic review and meta-analysis. *Microb. Pathog.* 137, 103751
76. Palmer, B. (2007) Meta-analysis of three case controlled studies and an ecological study into the link between cryptogenic epilepsy and chronic toxoplasmosis infection. *Seizure* 16, 657–663
77. Ngougou, E.B. *et al.* (2015) Toxoplasmosis and epilepsy – systematic review and meta analysis. *PLoS Negl. Trop. Dis.* 9, e0003525