Taking the strain out of onchocerciasis?  
A reanalysis of blindness and transmission data does not support the existence of a savanna blinding strain of onchocerciasis in West Africa

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Contents

1. Introduction 4
   1.1 Distribution and disease burden 5
   1.2 *Onchocerca volvulus* strains and *Onchocerca–Simulium* complexes 6
   1.3 Revisiting the forest–savannah strain hypothesis and review aims 11

2. Methods 12
   2.1 Inclusion criteria for studies in West Africa 12
   2.2 Standardization of prevalence by age and sex 13
   2.3 Standardization of incubation period and media for skin snips 14
   2.4 Cytotaxonomic and habitat classifications 15
   2.5 Statistical analyses 16
Revisiting the Strain Hypothesis in Onchocerciasis

3. Results

3.1 All causes of blindness
3.2 Blindness due to onchocerciasis
3.3 Bilateral blindness
3.4 Forest blindness
3.5 Transmission intensity and microfilarial prevalence

4. Discussion

4.1 Transmission intensity and locally-adapted Onchocerca–Simulium populations
4.2 Onchocerca volvulus (and its Wolbachia endosymbiont) genetics
4.3 Age, sex and individual exposure patterns

5. Conclusions and Recommendations

5.1 Implications for onchocerciasis control and elimination programmes
5.2 Future research avenues

Acknowledgements

List of Abbreviations

References

Abstract

Onchocerciasis (also known as ‘river blindness’), is a neglected tropical disease (NTD) caused by the (Simulium-transmitted) filarial nematode Onchocerca volvulus. The occurrence of ‘blinding’ (savannah) and non-blinding (forest) parasite strains and the existence of corresponding, locally-adapted Onchocerca–Simulium complexes were postulated to explain greater blindness prevalence in savannah than in forest foci. As a result, the World Health Organization (WHO) Onchocerciasis Control Programme in West Africa (OCP) focused anti-vectorial and anti-parasitic interventions in savannah endemic areas. In this paper, village-level data on blindness prevalence, microfilarial prevalence, and transmission intensity (measured by the annual transmission potential, the number of infective, L3, larvae per person per year) were extracted from 16 West–Central Africa-based publications, and analysed according to habitat (forest, forest–savannah mosaic, savannah) to test the dichotomous strain hypothesis in relation to blindness. When adjusting for sample size, there were no statistically significant differences in blindness prevalence between the habitats (one-way ANOVA, P = 0.68, mean prevalence for forest = 1.76 ± 0.37 (S.E.); mosaic = 1.49 ± 0.38; savannah = 1.89 ± 0.26). The well-known relationship between blindness prevalence and annual transmission potential for savannah habitats was confirmed and shown to hold for (but not to be statistically different from) forest foci (excluding data from southern Côte d’Ivoire, in which blindness prevalence was significantly lower than in other West African forest communities, but which had been the focus of studies leading to the strain–blindness
hypothesis that was accepted by OCP planners). We conclude that the evidence for a savannah blinding onchocerciasis strain in simple contrast with a non-blinding forest strain is equivocal. A re-appraisal of the strain hypothesis to explain patterns of ocular disease is needed to improve understanding of onchocerciasis epidemiology and disease burden estimates in the light of the WHO 2030 goals for onchocerciasis.

**Keywords:** onchocerciasis; *Onchocerca volvulus*; savannah strain; forest strain; blindness prevalence; microfilarial prevalence; *Simulium damnosum s.l.*; annual transmission potential; West Africa
1. **INTRODUCTION** (14,144 words with abstract, text, refs)

Onchocerciasis, also known as river blindness, is a neglected tropical disease (NTD) caused by the filarial nematode *Onchocerca volvulus* (Leuckart 1893) and is spread from person to person by the bites of infective vector blackflies of the genus *Simulium* Latreille 1802. (The term infective vectors refers to blackflies carrying the parasite stages that are infective to humans, i.e. infective or L3 larvae, which develop within the thoracic muscles of the fly from ingested microfilariae and migrate to the fly’s mouthparts; see Figure 1 for a schematic representation of the parasite’s life cycle.) In West Africa the vectors are members of the *S. damnosum sensu lato* (s.l.) species complex. This complex also has representatives that transmit elsewhere in Africa and in Yemen but in some areas in eastern and central Africa, such as Uganda and Tanzania, the vectors are members of the *S. neavei s.l.* complex. One of the most serious outcomes of onchocerciasis is bilateral blindness, but the infection also causes skin disease (Murdoch et al., 1993), and is associated with excess human mortality (Pion et al., 2002; Little et al., 2004b; Walker et al., 2012) and with epilepsy (Colebunders et al., 2019). Figure 1 also illustrates some of the clinical manifestations of onchocerciasis. Table 1 compares ocular manifestations due to trachoma (caused by the bacterium *Chlamydia trachomatis*) and loiasis (African eye-worm, caused by the filarial nematode *Loa loa*) with those of onchocerciasis. Onchocerciasis ocular and cutaneous morbidity is mainly due to the death of, and subsequent inflammatory responses around, the larval progeny (microfilariae) of the adult worms (reviewed in Bradley et al., 2005), with components from both the filariae and their *Wolbachia pipiensis* endobacteria contributing to the pathogenesis of onchocerciasis (Brattig, 2004). Adult parasites (macrofilariae) reside in *onchocercomata*, i.e. nodules formed by fibrous host tissue around male and female worms, and which can be subcutaneous and palpable, or deep-lying and impalpable (Nnochiri, 1964; Duke, 1993).
1.1 Distribution and disease burden

Onchocerciasis is endemic in 31 sub-Saharan African countries, 11 of which were under the umbrella of the World Health Organization (WHO) Onchocerciasis Control Programme in West Africa (OCP) (World Health Organization, 1995) from 1974 to 2002, and 20 were covered by the WHO African Programme for Onchocerciasis Control (APOC) (Zouré et al., 2014) from 1995 to 2015. In 2005, it was estimated that 37 million people carried *O. volvulus*, with 90 million at risk in Africa, where 99 percent of all cases occur (African Programme for Onchocerciasis Control, 2005; Basáñez et al., 2006). Before the inception of APOC, it was estimated that in the endemic areas under this programme, 404,000 people (0.6%) were blind because of onchocerciasis, and another 889,000 (1.2%) suffered from visual impairment (Coffeng et al., 2013). Modelling of data from the 2013 Global Burden of Disease Study revised estimates of the number of people at risk suggesting that 198 million people live in areas where there is potential for transmission of the parasite (Herrick et al., 2017). This number may further increase as the mapping of low transmission areas is finalized (World Health Organization, 2017). The Global Burden of Disease (GBD) Study 2017 estimated the disability-adjusted life years (DALYs) due to onchocerciasis as 1,340,000 (95% uncertainty interval (UI) = 639,000–2,370,000) in 2017, representing a 7% decrease since 2005, for which year the estimate was 1,442,000 (95%UI = 835,000–2,289,000) (GBD 2017 DALYs and HALE Collaborators, 2018). The most recent estimate of the GBD Study 2019 indicates a further decrease, with 1,230,000 (95%UI = 765,000–1,820,000) DALYs due to onchocerciasis (GBD 2019 Cause and Risk Summaries, 2020). However, the GBD Study estimates only account for years lived with disability (YLD) and do not include years of life lost (YLL) due to premature death caused by infection, blindness or onchocerciasis-
associated epilepsy (Pion et al., 2002; Little et al., 2004b; Walker et al., 2012; Vinkeles Melchers et al., 2018).

### 1.2 *Onchocerca volvulus* strains and *Onchocerca-Simulium* complexes

In West Africa, blindness due to onchocerciasis has been reported to be more severe in savanna settings than in forest areas; in the former, in particular, it is mostly caused by sclerosing keratitis, but in both habitats blindness is positively correlated with the prevalence and intensity of *O. volvulus* microfilariae in the eye (Budden, 1963; Anderson et al., 1974). In highly endemic villages in Cameroon with equivalent prevalence of nodules located in the head (a predictor of ocular microfilariae and ophthalmological manifestations), the relative risk of having an eye lesion in patients with head nodules compared to those without them was nearly three times as high in the savanna as in the forest (Fuglsang et al., 1976).

These observations, together with vector cross-experimental infection studies reporting that forest blackflies had lower competence for savannah parasites and *vice versa* (Duke, 1966; Duke, 1967; Duke, 1970; Duke et al., 1966; Duke et al., 1967; Lewis and Duke, 1966; Philippon, 1977), led to the notion of parasite strain differences and to the concept of locally adapted *Onchocerca–Simulium* complexes (see Basáñez et al., 2009 for a review). Further evidence in favour of the ‘strain hypothesis’ was provided by injections of *O. volvulus* microfilariae from both savannah- and forest-based patients into eyes of rabbits and subsequent examinations of their effects on ocular pathology (Duke and Anderson, 1972; Duke and Garner, 1973; Garner et al., 1973). In addition, savannah village-level data showed a positive relationship between intensity of transmission, measured by the annual transmission potential (ATP, the number of infective, L3, larvae to which a person living in the village would be exposed during a year) and the prevalence of ocular lesions and blindness in the village (Duke et al., 1975) which, although also present in rainforest
communities (Duke et al., 1972), did not reach the high levels of blindness prevalence reported for savanna settings. A positive linear relationship between intensity of transmission, measured by numbers of L3 infective larvae per 1,000 parous S. yahense flies, and blindness has also been more recently documented with forest data in Liberia (Cheke and Garms, 2013).

Attempts using a variety of techniques have been made to find biomarkers that differ according to O. volvulus strain. Flockhart et al. (1986) compared samples of O. volvulus from forest foci in Côte d’Ivoire and Liberia with samples from savannah foci in Burkina Faso and Sudan using isoenzyme analyses. Of seven enzymes examined, three were invariant and four were polymorphic enzymes, but only three of these showed sufficient variation for inclusion in an analysis of variance of their genotype frequencies, and only one (malate dehydrogenase) showed a substantial percentage difference (28.1%) between the countries. Also, calculations of genetic distances based on allele frequencies revealed that the differences between countries were minimal and it was concluded that “all genetic distances were within the range expected for geographical populations of the same species”. Erttmann et al. (1987) identified a DNA sequence (pFS-1) that hybridised to four samples of forest O. volvulus from Liberia and to a sample from the forest/savannah transition zone in Togo but not to two savannah samples from Mali and a sample from Brazil. Subsequently, Erttmann et al. (1990) reported finding a sequence, termed pSS-1BT which appeared to be specific for savannah O. volvulus. These forest and savannah sequences were from a variable tandemly repeated DNA sequence family of ~150 base pairs (bp) designated O-150. A PCR-based test was developed for strain identification (Meredith et al., 1991), which combined the amplification of the O-150 repeat using species-specific primers and hybridisation with the two strain-specific probes to identify savannah and forest strains. The species-specific probe pOVS134 overlapped the primer sites and the authors redesigned a new species-specific probe, OVS, to identify
Revisiting the Strain Hypothesis in Onchocerciasis

*O. volvulus* species. This test has been widely used (e.g. Toé et al., 1994, 1997; Zimmermann et al., 1992, 1994; Ogunrinade et al., 1999) without confirming unequivocal habitat- or vector-related distinctions. Subsequently, Merriweather and Unnasch (1996) developed another probe (C1 A1-2) for more accurate means of distinguishing *O. volvulus* from the cattle parasite *O. ochengi*. *Onchocerca ochengi*, which is also transmitted by *S. damnosum s.l.*, is the closest relative of *O. volvulus*, having probably switched from cattle to humans less than 5,000 years ago (Morales-Hojas et al., 2006).

Analyses of nuclear DNA, mitochondrial DNA and genes coding for antigenic proteins have suggested that populations of *O. volvulus* can have a mix of habitat-related markers and that they can and do interbreed (Keddie et al., 1999; Choi et al., 2016). This genetic mixing may be readily maintained by the migrations of both vectors (Garms et al., 1979; Cheke and Garms, 1983; Baker et al., 1990) and humans, including traditional transhumance and pastoralism from the Sahel to forests and back (FAO-CIRAD, 2012) and recent economic migration (Zachariah and Condé, 1981), with implications for recrudescence of onchocerciasis (Koala et al., 2017). Nevertheless, recent analyses based on mitochondrial DNA revealed that geographically distinct populations have genetic structure consistent with vector migration patterns (Crawford et al., 2019; Hedtke et al., 2020), knowledge of which could be useful for defining transmission zones (geographical areas where transmission of *O. volvulus* occurs by locally-breeding vectors and which can be regarded as a natural ecological and epidemiological unit for interventions).

Endosymbiont *Wolbachia pipientis* bacteria occur in both the parasite and the vector. Lack of *Wolbachia* seems to be the ancestral condition in *O. volvulus* (Bandi et al., 2001) and, as *Wolbachia* are implicated in the pathogenesis of onchocerciasis (Saint André et al., 2002; Pearlman and Gillette-Ferguson, 2007; Tamarozzi et al., 2011), variation in these
within the parasite (Choi et al., 2016) could account for differences in disease outcomes. Similar variation in Wolbachia within the vectors is yet to be studied in detail but Wolbachia do occur in S. squamosum E (Crainey et al., 2010, 2017) and S. yahense (J.L. Crainey, pers. comm.) and so are also likely to have epidemiological significance. Genetic variation within and between the vectors in West Africa is extensive (Vajime and Dunbar, 1975; Adler et al., 2010) and is well known to have important consequences for the transmission of onchocerciasis (Cheke and Garms, 2013).

Adherence to the forest–savanna strain hypothesis led to the initial vector control operations undertaken by the OCP being restricted to savanna foci (World Health Organization, 1987). After the initiation of the OCP (see O’Hanlon et al., 2016 for a description of the OCP’s initial phases and further extensions), a series of papers compared the village-level prevalence of onchocerciasis-associated ocular lesions as a function of the intensity of skin infection by microfilariae. The latter was measured as the community microfilarial load (CMFL, defined in Remme et al., 1986 as the geometric mean number of microfilariae per skin snip (mf/snip) in those aged ≥20 years) between savanna West African foci (where the parasite was transmitted by S. damnosum sensu stricto (s.str.)/S. sirbanum, Remme et al., 1989) and forest foci with different prevailing vector species (e.g. S. yahense in south-western Côte d’Ivoire, Dadzie et al., 1989; S. sanctipauli in southern Côte d’Ivoire, Dadzie et al., 1990; S. soubrense B (= S. leonense) in southern Sierra Leone, Dadzie et al., 1992). After adjusting for differences in CMFL (typically higher in savanna than in forest communities), these studies concluded that for a given CMFL, the prevalence of ocular lesions and the ‘blinding potential’ of the parasite in forest settings were lower than those in the savannah (with the possible exception of the degraded forest in southern Sierra Leone, where CMFL values were also high). This morbidity paradigm was adopted by Coffeng et al. (2013) to estimate the DALYs due to onchocerciasis in the APOC
area and the health impact of this programme in terms of DALYs averted from its inception in 1995 to its closure in 2015.

Whilst the evidence for different parasite strains in African savanna and forest initially relied mostly on epidemiological data, clear differences were also documented in the simuliid vectors. Not only did the *S. damnosum* complex prove to comprise a variety of cytospecies and cytoforms (*Vajime and Dunbar, 1975; Adler et al., 2010*) which had different habitat preferences, but there were also differences in their vectorial abilities and their infection intensities of larval *O. volvulus* stages. *Simulium damnosum s.str. and S. sirbanum*, species typically found in savanna regions, transmit fewer infective larvae per infective fly than do forest-dwelling species such as *S. sanctipauli* (*Cheke and Garms, 2013*). The latter species also have much higher variances of numbers of developing stages of *Onchocerca* larvae per fly, and so the (overdispersed) frequency distributions of numbers of parasites per fly have longer tails. (Overdispersion is defined in comparison to the Poisson distribution, in which the variance is equal to the mean and, therefore, the variance over mean ratio = 1; an overdispersed distribution is characterised by a variance over mean ratio >> 1.) Although these differences may have been ascribed to the parasite strain that each type of vector ingested, more fundamental population biology processes may be at play. For instance, negative density dependence acting on ingested microfilariae has been reported to limit the output of L3 per fly in the savanna species of the *S. damnosum* complex (*Philippon and Bain, 1972; Basáñez et al., 1995*), whereas density independence (leading to a linear relationship between the number of larvae successfully establishing within the vector and microfilarial load) has been described in forest species (*Demanou et al., 2003; Soumbeý-Alley et al., 2004*); reviewed in Basáñez et al. (2009). At a forest–savanna mosaic site in southern Togo, *Garms and Cheke (1985)* noticed that populations of the Beffa form of *S. soubrense*, harboured the “forest” pattern of larval distributions but were occurring
sympatrically with *S. damnosum s.str./S. sirbanum* harbouring “savannah” distributions. Given that the flies were biting the same population of people, it was unlikely that two parasite strains were involved, but rather that there were intrinsic differences between the two vector taxa regarding the specificities of vector–parasite interactions which determine how they respond to ingested microfilariae. Similarly, there have been reports of a lack of consistency with the blinding–non-blinding strain divide with observations of high levels of onchocerciasis-associated blindness in forest–savanna mosaic areas of Nigeria (Umeh et al., 1996) and Cameroon (Pion et al., 2002).

1.3 Revisiting the forest-savannah strain hypothesis and review aims

The simple forest–savanna strain hypothesis was questioned by Cheke and Garms (2013) on the basis of extensive entomological data in relation to blindness prevalence in West Africa. They found that high blindness prevalences in forested parts of Liberia were associated with transmission by forest vectors but that the proportions of first and second stage (L1/L2) *Onchocerca* larvae that succeeded in becoming third stage (L3) infective larvae did not differ between different vectors species (Table 6 of Cheke and Garms, 2013), with the maximum values of such proportions being essentially the same as those of *S. damnosum s.str./S. sirbanum*. This suggests that what differs between vector species is the probability of microfilariae successfully establishing within the fly’s thorax; once established—in a competent vector—the proportion developing does not differ greatly (Basáñez et al., 1995).

Novel research assessing micro-computerised tomography (micro-CT) scans of the peritrophic matrix in both forest and savannah blackflies preserved soon after feeding did not support the hypothesis that forest vectors have thinner peritrophic matrices (Philippon, 1977), which would permit a greater proportion of ingested microfilariae to reach the haemocoele of the flies and ultimately lead to more *Onchocerca* L3 larvae developing than in savannah
vectors (Hall et al., 2020). Cheke and Garms (2013) concluded that a complex of factors may be at play, perhaps including the involvement of Wolbachia endosymbionts in the flies and/or the worms.

In a preliminary study of the data re-analysed here—village-level estimates of blindness prevalence, microfilarial prevalence, and transmission intensity from 16 West–Central Africa studies—Little et al. (2003) concluded that there was no statistically significant difference between the prevalence of blindness in savanna and forest settings. Here we re-examine these epidemiological data and conclude that the evidence for the existence of two distinct savanna and forest *O. volvulus* strains associated, respectively, with more or less severe ocular onchocerciasis, is weak. In particular, we find that prevalence of blindness estimates in the savannah are not significantly different from those in the forest and that in both forest and savannah, blindness prevalence varies with transmission rates. We have largely restricted our analysis to data from West Africa, while including Cameroon from West Central Africa, because the original conception of the strain hypothesis was based on data from this zone.

2. **METHODS**

2.1 **Inclusion criteria for studies in West Africa**

We collated data from studies that were suitable to be included in the analysis on the basis of the following criteria: (a) studies conducted within the geographical area of West Africa but including Cameroon and the island of Bioko, which is in the Gulf of Guinea and is part of Equatorial Guinea; (b) studies conducted before (at baseline) or in the initial phases of the implementation of a control programme, as interventions based on the use of larvicides for vector control (by the OCP) or on mass microfilaricidal treatment (with ivermectin) could
have distorted the results (a maximum of two years from the inception of interventions was allowed for vector control following O’Hanlon et al. (2016); only pre-intervention data at initial endemicity and transmission intensity were used in case of ivermectin distribution); (c) studies including the prevalence of bilateral blindness, the prevalence of skin microfilariae and/or the intensity of transmission (measured by the ATP), and (d) studies including vector identification or conducted in areas where entomological data were also available.

Sixteen publications with data on onchocerciasis from 93 communities in West Africa reporting prevalence of blindness with either associated ATPs or prevalence of microfilaridermia (%mf) were collated (Table 2) and assigned to a habitat (savannah, forest–savannah mosaic or forest). The earliest study was conducted in 1968 and the latest in 1998. Some publications did not include any data on microfilarial density (mf/snip or mf/mg) in the overall population, while others quoted such data or reported CMFLs instead, hence possible effects of microfilarial intensities were not investigated. Only data for which the numbers of patients examined were given in a publication were included. Bilateral blindness was used as the outcome variable. Blindness was defined as either a visual acuity of <3/60 or the inability to count fingers at a distance of one metre (Little et al., 2004a). As the data came from many different studies, it was necessary to standardise them to make the measures comparable, so far as possible.

[Insert Table 2 about here]

### 2.2 Standardization of prevalence by age and sex

The community prevalence of skin microfilariae and the blindness prevalence by both age and sex required standardisation. This is important as both measures are known to increase with cumulative exposure (and therefore age), and to differ significantly between the sexes
Revisiting the Strain Hypothesis in Onchocerciasis

(see Hamley et al., 2019 for relationships between age- and sex-standardised microfilarial prevalence and annual biting rate (ABR) in savannah settings before intervention fitted using the stochastic EPIONCHO-IBM transmission model). Standardising adjusts for different age- or sex-compositions of different villages or study samples. Some of the studies included results that had already been standardised for both age and sex, by either the direct or indirect methods (Kirkwood and Sterne, 2003), while others had either only been standardised for one or the other or neither. In these cases, the data required standardisation using the following process. We first identified a reference population, broken down into age and sex categories; this could be either the census population of the region or country given by the author (indirect standardisation) or it could be estimated by pooling age and sex data from all of the villages in the study (direct standardisation). We then multiplied the prevalence (of microfilaridermia or of blindness) in each (age, sex) category within the study population by the proportion of people in each corresponding category in the standard population. This gave the weighted (mean) prevalence of infection and blindness within each category. Finally, we calculated the age- and sex-standardised prevalence as the sum of these weighted means (Kirkwood and Sterne, 2003).

2.3 Standardization of incubation period and media for skin snips

As the studies analysed covered a span of several decades the methods used varied, particularly in the estimation of microfilarial prevalence. Prevalence of microfilaridermia was estimated by taking small ~1–2 mg skin snips from each individual, with usually at least two snips taken from the same or different regions of the body. Originally this was done using a pin and a razor blade and latterly superseded by a Holth-type corneo-scleral biopsy instrument (Moreau et al., 1978). These skin snip samples were incubated for a period to allow the microfilariae to emerge from the biopsy. It was during this step that the studies
varied most, with the snips incubated from 10 minutes to 24 hours. In several studies the skin snips were also teased to stimulate emergence of microfilariae. All three of these factors needed to be standardised in order to make the samples comparable as the number of positive samples (prevalence) increases with incubation time and varies with incubation medium. Collins et al. (1980) calculated the mean percent of skin snips that became positive after different intervals as a percentage of those that were positive at 24 hours. The percentages according to different intervals (Table 3) were used to adjust prevalence estimates, standardizing to a 24-hour incubation. For those studies in which the samples were teased (and then incubated for 15 minutes) it was assumed, following Demanou et al. (2003), that the number of microfilariae that emerged was approximately half that which would have emerged if incubated for 24 hours without teasing. No adjustments were made for the part of the body that was snipped as these were usually found to be similar (Demanou et al., 2003; but see Bottomley et al., 2016).

[Insert Table 3 about here]

2.4 Cytotaxonomic and habitat classifications

Cytotaxonomic data on the vectors were presented in some of the identified studies. Where such data were not presented, and for samples taken within the area covered by the OCP, the simuliid cytospecies present in the study sites were taken from the maps in Boayke et al. (1998), and the data in Cheke et al. (1987) and Cheke et al. (1992). For other areas not in the OCP such as Liberia, data were taken from Vajime and Dunbar (1975).

The following simuliid species were found in the studies analysed: Simulium damnosum s.str., S. sirbanum, S. squamosum, S. soubrense, S. leonense, Beffa form of S. soubrense, S. sanctipauli, S. yahense, S. mengense.
Decisions on habitat classifications were based on information in the publications, but when there was doubt about how the habitat terms had been used or if no information was provided, the habitat was defined according to the vegetation map of White (1983).

2.5 Statistical analyses

Analysis using a binomial generalised linear model (GLM) (with logit link) showed evidence of severe overdispersion (extra-binomial variation) in the data (residual deviance of 6,820 in 86 degrees of freedom). Correcting this overdispersion by means of a quasibinomial GLM was rejected on the basis of model diagnostic plots, but an analysis of variance (ANOVA) with angular transformation of prevalence (percentage) data had acceptable model diagnostics. The transformation (asin(sqrt(percentage/100))) was very similar to the logit link in the GLM. The effect of the arcsine transformation is similar to the logit, in that it expands the ends of the distribution, but not to the extent that the logit does (Holland, 2017). Charts based on ANOVA models with angular-transformed data show means and standard errors de-transformed from model coefficients, resulting in asymmetrical error bars. In order to take sample sizes into account, the value of the denominator of the authors’ percent blindness calculations was used if reported; otherwise, the total sample size for the whole study was substituted. The analyses thus consist of weighted linear models using angular-transformed percentage data. All analyses were conducted using R (R Core Team, 2016). An explanation of the procedures involved and the associated data file and R-codes are provided in the Supplementary Material available in the online version of this article.
3. RESULTS

3.1 All causes of blindness

Using the raw data, there was no statistically significant difference between prevalence of blindness according to habitat (one-way ANOVA, P = 0.38; Figure 2A). With the raw data weighted by sample size, there was also no statistically significant effect of habitat on the prevalence of blindness (one-way ANOVA, P = 0.68; Figure 2B). Similarly, but with a linear model using angular-transformed percentage data on unweighted data, there was also no significant effect of habitat on the prevalence of blindness (one-way ANOVA, P = 0.53; Figure 2C). This was also the case for a linear model using angular-transformed percentage data weighted by sample size (one-way ANOVA, P = 0.40; Figure 2D).

[Insert Figure 2 about here]

3.2 Blindness due to onchocerciasis

The results above were based on 93 records using data on blindness which included some publications that did not distinguish between blindness due to onchocerciasis and blindness due to other causes. However, amongst the publications, data on 52 communities were specified as being based on onchocerciasis-associated blindness only. When these data were analysed using the weighted linear model method, there was again no evidence of any statistical difference in blindness prevalence according to habitat (one-way ANOVA, P = 0.51; Figure 3).

[Insert Figure 3 about here]
3.3 Bilateral blindness

There are 11 data points for which there was no information to establish whether these data referred to bilateral blindness. These data are from De Sole et al. (1992), who reported data on blindness without specifying if they were referring to bilateral blindness or not. Therefore, firstly the data were analysed assuming that blindness denoted bilateral blindness, but to check the robustness of the results to this assumption, we conducted a further analysis excluding the 11 ambiguous data points (denoted by an asterisk in Table 2). This analysis again indicated no statistically significant difference in blindness prevalence according to habitat (P = 0.17).

3.4 Forest blindness

The forest data are more heterogeneous than the savannah data, as illustrated by Figure 4, in which the forest data are separated according to country. Country was significantly associated with forest blindness prevalence (ANOVA, P < 0.0001). Indeed, the prevalence of blindness in Cameroon is significantly greater than that in Côte d’Ivoire (P = 0.008) and Liberia (Tukey multiple comparisons of means, P = 0.006). This result may reflect differences in the vectors present in each country and/or their transmission abilities in relation to local infection intensities, or other hitherto unidentified factors associated with ocular pathology. Whereas the vectors in the savannah habitats are predominantly *S. damnosum* s.str. and *S. sirbanum*, those for the forest habitats are variable (Table 2). In Cameroon, the forest vectors are *S. squamosum* (forms A, B, C & D; see Mustapha et al., 2004) and *S. mengense*; in Liberia they are *S. soubrense* (sensu Post 1986, formerly *S. sanctipauli* s.str., see Kashan and Garms, 1987) and *S. yahense*; in Côte d’Ivoire they are *S. sanctipauli* and *S. yahense*, with the latter present in a few sites only, and in Bioko the vector was the unique endemic Bioko form of *S. yahense* (Post et al., 2003) before it was eliminated (Traoré et al., 2009).
3.5 Transmission intensity and microfilarial prevalence

In order to examine whether there were any significant effects of transmission intensity rates as measured by ATPs, or prevalence of infection in the community measured by standardised percentages of the population with skin microfilariae (%mf), one-way ANOVA analyses were conducted on the weighted data for all blindness (i.e. not just those specified as due to onchocerciasis only) but excluding data from forest–savanna mosaic settings due to their paucity with associated ATP or %mf data. Given that the blindness prevalence estimates for the forest settings in Côte d’Ivoire were significantly lower than those of other countries’ forest habitats (Figure 4), the forest data were analysed with and without the Côte d’Ivoire data and compared with those for the savannah in Figure 5. The relationship between ATP (annual transmission potential) and angular-transformed percent blindness (blindness prevalence) for all forest data analysed together was not statistically significant (Figure 5A; P = 0.98) but for forest data without those from southern Côte d’Ivoire, the relationship was positive and statistically significant (Figure 5B; P < 0.007); furthermore, its slope was not significantly different from that for the savannah data plotted in Figure 5C (t-test for difference between slopes, P = 0.24), which also have a positive and significant relationship between ATP and blindness prevalence (P = 0.0003).

A similar analysis of the data on microfilarial prevalence and angular-transformed blindness prevalence revealed a statistically significant and positive relationship for the savannah data (Figure 6A; P < 0.0001) but not for the forest data with (Figure 6B; P = 0.49) or without (Figure 6C; P = 0.77) the southern Côte d’Ivoire data.
Revisiting the Strain Hypothesis in Onchocerciasis

[Insert Figure 6 about here]

4. DISCUSSION

4.1 Transmission intensity and locally-adapted *Onchocerca-Simulium* populations

The results, based only on blindness prevalence, do not support the existence of a distinct blinding (savannah) strain of *O. volvulus* in dichotomous contrast with a non-blinding (forest) strain. No statistically significant differences were found between the prevalence of blindness reported from forest, forest–savanna mosaic or savanna habitats. Furthermore, the well-known relationship between transmission rates (ATP, the number of L3/person/year) and blindness prevalence in savanna habitats also holds for forested areas if the data for the forest region of southern Côte d’Ivoire (Dadzie et al., 1990), where blindness prevalence estimates were significantly lower than in other West African countries’ forest foci, are excluded. When the Côte d’Ivoire data are excluded, there is also no difference between the relationships between ATP and blindness prevalence for different habitats (but see Duke et al., 1972 and Duke et al., 1975 for relationships between ATP and prevalence of ocular lesions including blindness in southern Cameroon for forest and savanna, respectively).

The forest zone of southern Côte d’Ivoire was known to have low levels of CMFL, skin lesions and onchocerciasis-associated blindness, with sclerosing keratitis absent. This was attributed to parasite strain differences (Dadzie et al., 1990). However, the main vectors there were unique forms (the Comoé and Sassandra forms) of *S. sanctipauli* (Boakye et al., 1999). Therefore, we suggest that, rather than parasite strain differences accounting for the low blindness levels in the forest sites studied in Côte d’Ivoire, these may be due to the types of vector present, despite the high ATPs recorded in some Côte d’Ivoire forest foci (Table 1).
Perhaps a high ATP, based on dissections of flies, does not always correspond to high transmission to humans. This may be the case if the flies do not feed for long enough to inoculate all of their larvae or if some of the *Onchocerca* larvae found during the studies were not *O. volvulus* but zoophilic species such as *O. ochengi*, which is also transmitted by *S. damnosum s.l.* (Toé et al., 1994).

Toé et al. (1994) reported that ATPs may have been substantially overestimated particularly along the southern border of the OCP (where the forest sites of Côte d’Ivoire were located). Toé et al. (1997) also reported no differences in the numbers of L3 larvae of each ‘strain’ harboured by forest-dwelling blackfly species or in the numbers of forest and savannah larvae found in savannah-dwelling vector species. This supports our conjecture that rather than mere parasite strain differences, there are differences in parasite–host (vector and/or human) interactions and in competence for *O. volvulus* between vector species (which together with vector biting rates and their seasonal fluctuations translate into transmission potentials). Toé et al. (1997) concluded that classical vector–parasite transmission complexes did not play a part in the biology of onchocerciasis transmission in the area of the OCP.

### 4.2 *Onchocerca volvulus* (and its *Wolbachia* endosymbiont) genetics

Claims of molecular differences between forest and savannah *O. volvulus* in support of strain differences (Zimmerman et al., 1992; Zimmerman et al., 1994) were based on a variable tandemly repeated DNA sequence family of ~150 base pairs (bp) designated O-150. The variability of the 150-bp family was used to develop *O. volvulus* strain- and species-specific DNA probes (Meredith et al., 1991).

Subsequent studies, based on other (but still limited) markers such as mitochondrial restriction fragment length polymorphism (RFLP) profiles (Keddie et al., 1999), and nuclear
internal transcribed spacer (ITS) rDNA sequences (Morales-Hojas et al., 2007), indicated a lack of population differentiation between savannah and forest isolates, in conflict with the two-strain hypothesis.

A recent study, aiming to overcome the restrictions imposed by analysis of limited genetic markers, undertook a comprehensive O. volvulus population genomics approach to investigate diversity in the parasite’s nuclear, mitochondrial, and endosymbiotic Wolbachia genomes of 15 isolates from West Africa classified as ‘savannah’ or ‘forest’ according to O-150 (Choi et al., 2016). Six isolates were from savannah sites in eastern Guinea, northwestern Côte d’Ivoire and northern Ghana, and nine from forest locations in Sierra Leone, Liberia, and southern Côte d’Ivoire, Ghana, and Benin. The three genomes indicated a certain degree of population structure, with the nuclear genome analysis providing phylogenetic signals consistent with gene flow and genetic admixture among discrete West African forest and savannah populations (Choi et al., 2016). In particular, there was evidence of unidirectional gene flow from the savannah to the forest. No discernible geographical pattern was detected in the distribution of West African mitochondrial DNA clades, in contrast to the bioclimatic (habitat in our analysis) separation observed in the nuclear genomes. These authors conclude that a simple dichotomy between forest and savannah ‘strains’ is an inadequate description of the parasite variations in West Africa, but remark that the results from the nuclear genome support the notion of geographically- and bioclimatically-structured populations (Choi et al., 2016). In agreement with the mitochondrial results, and in an attempt to evaluate the usefulness of the mitochondrial genome of O. volvulus for delineation of transmission zones, Crawford et al. (2019) reported a lack of (mitochondrial) population structure between forest samples from Côte d’Ivoire and savannah samples from Mali, in contrast to the O-150 population differences between forest and savannah previously reported (Zimmerman et al., 1992).
Higazi et al. (2005), using the same samples as in the Zimmerman et al. (1992) study (from Côte d’Ivoire, Ghana, Guinea, Senegal, Sierra Leone), reported that the median ratio of Wolbachia DNA to O. volvulus nuclear DNA was significantly greater in the ‘severe’ savannah parasite strain than in the ‘mild’ forest parasites despite wide variation among individual parasite samples from each location. More recently, Armoo et al. (2017), analysing samples representing savannah and forest habitats from Côte d’Ivoire, Ghana, Mali and Togo confirmed the observation of substantial variation in Wolbachia genome to O. volvulus nuclear genome copy number ratio within and between the samples analysed, but concluded that this variation was independent of habitat, in agreement with the Wolbachia genome results of Choi et al. (2016). The latter were unable to reveal any identifiable molecular or functional differentiating factor that could be related to pathogenesis; however, further high-resolution polyomics profiling of Wolbachia symbionts, given their central inflammatory role, would be desirable.

4.3 Age, sex and individual exposure patterns

As the results presented here rely solely on blindness prevalence, habitat type, microfilarial prevalence and ATPs, we cannot be sure that there are no other habitat-related features of onchocerciasis that specialists in other disciplines (e.g. ophthalmologists, immunologists, population biologists, social scientists) rather than entomologists could point to. For instance, other factors determining the incidence and prevalence of ocular lesions and ultimately blindness in endemic communities include age- and sex-specific patterns of exposure to vector bites (Filipe et al., 2005) and parasite transmission that may prevail in different foci (Duke and Moore, 1968; Renz et al., 1987a; Bockarie and Davies, 1990) as well as the degree of inter-individual variation in exposure and susceptibility (Hamley et al., 2019). These factors cannot be understood by analysing transmission intensity rates measured by dissection.
of limited fly samples collected during a few weeks when landing on a few individuals (Renz, 1987; Renz and Wenk, 1987; Jacobi et al., 2010). There will be an interplay between processes regulating parasite load in humans, age- and sex-specific exposure patterns, and inter-individual host variation (Hamley et al., 2019; Hamley et al., 2020) that may well depend, in addition to human innate and behavioural or occupational factors, on habitat, vector species and transmission patterns. These may determine patterns of microfilarial prevalence and intensity, and ultimately prevalence of blindness in onchocerciasis-endemic communities along their distributional range.

5. CONCLUSIONS AND RECOMMENDATIONS

Differences in transmission patterns, vector biting habits, host susceptibility, and environmental aspects are among the factors that have been invoked to explain the seemingly higher prevalence of onchocerciasis-associated blindness in the savannah in comparison to that in forest areas of West Africa (Duke, 1981). A principal reason why the OCP decided not to treat vector breeding sites in the forest with larvicidal insecticides at the outset of the vector control programme was because of the assumption that the “blinding strain” was restricted to the savannah (Walsh et al., 1979). The results presented here show that this conclusion is not supported by a re-examination of the epidemiological evidence currently available. It seems possible that the earlier assumptions were biased by the unique situation in southern Côte d’Ivoire, which happened to be the area where much research had been conducted in forest foci during the period leading up to and at the beginning of the OCP (Phillippon, 1977; Quillévéré, 1979; Dadzie et al., 1989; Dadzie et al., 1990).

Intrinsic differences in the pathogenicity of savannah and forest parasites have been proposed to correlate with clinical ocular manifestations which are underpinned by
population genetic structuring into neatly distinct savannah and forest strains (Zimmerman et al., 1992; Zimmerman et al., 1994). However, as we have shown here (and in Cheke and Garms, 2013), a re-appraisal of parasitological, entomological, and ophthalmological data, as well as the results from recent analyses of \textit{O. volvulus} nuclear, mitochondrial and \textit{Wolbachia} genomes (Choi et al., 2016; Armoo et al., 2017; Crawford et al., 2019) suggest a departure from the simple two-strain paradigm.

A re-evaluation of the two-strain hypothesis is advisable if the epidemiology of onchocerciasis is to be fully understood, better modelled (Basáñez et al., 2016), and its disease burden accurately estimated. This burden may have been underestimated as the initial clinico-epidemiological studies on ocular lesions conducted in forest foci of south and south-western Côte d’Ivoire may have not been truly representative of forest ocular onchocerciasis. It also means that the public health impact and cost-effectiveness of reducing blindness due to onchocerciasis by past and ongoing (largely chemotherapeutic) control activities may have been underestimated (Coffeng et al., 2013). To address this is particularly important given the fillip of reaching revised onchocerciasis targets and milestones beyond 2020 (NTD Modelling Consortium Onchocerciasis Group, 2019), and achieving the health-related 2030 United Nations Sustainable Development Goals (World Health Organization, 2020).

5.1 Implications for onchocerciasis control and elimination programmes

The new WHO roadmap for NTDs (2021–2030) has proposed reaching elimination of transmission (EOT) as the target for onchocerciasis. This is in contrast to the goal of elimination as a public health problem (EPHP) proposed for other NTDs such as soil-transmitted helminthiases, schistosomiasis and lymphatic filariasis (World Health Organization, 2020). This means that the interventions that will be necessary to achieve EOT are ‘agnostic’ to the ‘strain’ hypothesis and no longer framed in terms of eliminating
Revisiting the Strain Hypothesis in Onchocerciasis

‘blinding onchocerciasis’. Current intervention tools for achieving EOT largely consist of mass drug administration (MDA) of the endectocide ivermectin, mainly on an annual basis in Africa. Modelling studies have indicated that annual ivermectin MDA will be insufficient for EOT in highly endemic areas (those with high vector biting rates) and that alternative and complementary treatment strategies (ATS) will be essential (Verver et al., 2018; NTD Modelling Consortium Onchocerciasis Group, 2019). Some African countries have adopted biannual ivermectin MDA in some/most of their endemic foci (Katabarwa and Richards, 2014; Katabarwa et al., 2018; Turner et al., 2013), and in 2018, the Food & Drug Administration (FDA) approved the use of moxidectin (another endectocide) for the treatment of human onchocerciasis in patients aged 12 years and older, based on evidence of its superior efficacy to that of ivermectin but comparable safety (see Milton et al., 2020 for a recent moxidectin review). Moxidectin may help to accelerate EOT in hypo- and mesoendemic foci and increase its feasibility in hyperendemic areas (Turner et al., 2015; Milton et al., 2020). However, the microfilaricidal properties of both ivermectin and moxidectin make them unsuitable for MDA delivery (treating of endemic populations without confirming individual infection status) in areas of onchocerciasis–loiasis co-endemicity where the risks of severe adverse events (SAEs, in patients with high L. loa microfilaraemia) outweigh the benefits of onchocerciasis treatment (mainly in areas that are hypoendemic for onchocerciasis) (Cano et al., 2018). In these areas, TaNT protocols, i.e. test-(for loiasis microfilaraemia levels)-and-not-treat (with ivermectin, if such levels exceed a dangerous threshold of >30,000 mf/ml of blood, or >20,000 mf/ml in some studies) have been successfully piloted in Cameroon (Kamgno et al., 2017). Other ATS, such as TTd (test-(for onchocerciasis)-and-treat (with doxycycline)), also piloted in onchocerciasis–loiasis co-endemic areas of Cameroon (Wanji et al., 2019) exploit the fact that (a 4–6 week course) of daily (100–200 mg) doxycycline treatment depletes the Wolbachia endosymbionts of
Onchocerca volvulus (see section 1.2 Onchocerca volvulus strains and Onchocerca–Simulium complexes), exerting a macrofilaricidal and sterilising effect on adult worms (Tamarozzi et al., 2011; Walker et al., 2015), without affecting *L. loa* as the latter does not harbour *Wolbachia* (Taylor et al., 2005; Tamarozzi et al., 2011), circumventing the problem of SAEs, and providing a safe and efficacious curative treatment.

Large-scale and long-term application (weekly for at least 14 years) of aerial larvicidal insecticide treatment of vector breeding sites was effective for vector control in the OCP (Hougard et al., 2001). Although this modality of large-scale vector control is unlikely to be implemented elsewhere, aerial and supplementary ground-based larviciding led to the elimination of the Bioko form of *S. yahense* (Traoré et al., 2009). Therefore, the feasibility, efficacy, cost, environmental considerations (particularly in light of future sustainable management of forest ecosystems and biodiversity), and optimal localization, timing and duration of ground-based focal larviciding (Wanji et al., 2019), or of non-insecticidal community-directed approaches such as ‘slash-and-clear’ (Jacob et al., 2018) need to be further explored and their impact on vector biting rates modelled (Routledge et al., 2018; Smith et al., 2019).

### 5.2 Future research avenues

However agnostic to the onchocerciasis strain hypothesis interventions for control and elimination may need to be, epidemiological models of onchocerciasis transmission should certainly reflect the different features of the vector–parasite interaction observed in savannah and forest habitats, where different members of the *S. damnosum s.l.* complex prevail (Basáñez et al., 2009; Adler et al., 2010; Cheke and Garms, 2013). Given that the operation of density-dependent processes down-regulating parasite establishment in humans and blackfly vectors is a crucial determinant of the resilience of the host–parasite system to
Revisiting the Strain Hypothesis in Onchocerciasis

control interventions (Stolk et al., 2015; Basáñez et al., 2016; Walker et al., 2017; Verver et
al., 2018; Hamley et al., 2019, 2020), it will be important to capture adequately such
processes in savannah and forest environments. Savannah members of *S. damnosum* s.l. (i.e.,
*S. damnosum* s.s./*S. sirbanum*) exhibit limitation of the number of L3 larvae that develop
within the flies as microfilarial load (and hence the number of microfilariae ingested by the
flies) increases (Bain, 1971; Basáñez et al., 1995; Soumbey-Alley et al., 2004), whereas
forest and forest–savannah mosaic members of the complex (e.g. *S. yahense*; *S. squamosum*
B; *S. leonense*) tend to exhibit proportionality (a more linear relationship between
microfilarial counts and the number of larvae that successfully escape the peritrophic matrix
of the flies and/or develop into L3 larvae (Cupp et al., 1986; Demanou et al., 2003; Soumbey-
Alley et al., 2004), being able to harbour higher larval loads per fly (Basáñez et al., 2009;
Adler et al., 2010; Cheke and Garms, 2013). Other differences between the operation of
density-dependent processes in forest and savannah settings have been modelled regarding
parasite establishment and fecundity rates within humans (Duerr et al., 2003, 2004). Yet, the
two mathematical models that are used to inform decision-making in onchocerciasis, namely
EPIONCHO and ONCHOSIM (NTD Modelling Consortium Onchocerciasis Group, 2019)
are only parameterised for savannah settings (Basáñez et al., 2016). If (down-regulating)
density-dependent processes were indeed weaker in forest locales, resilience to interventions
(the ability of the parasite population to bounce back following treatment rounds or to resurge
after stopping MDA) would also be weaker, increasing the feasibility of reaching EOT.

In addition to parasite population regulatory processes, age- and sex-specific exposure
patterns, as well as the degree of heterogeneity in individual exposure may vary between
forest and savannah settings. These factors interact with density dependence to determine the
magnitude of onchocerciasis elimination thresholds (Hamley et al., 2020). Models indicate
that the stronger the degree of heterogeneity in inter-individual exposure, the more difficult
EOT becomes (Winne et al., 2002; Hamley et al., 2019). Interestingly, Budden (1963) suggested that exposure to blackfly bites was much more clustered in the savannah than in the forest (due to differences in transmission seasonality, distribution of breeding sites, and patterns of human settlement and occupation), and that this difference may help to explain epidemiological patterns of microfilarial prevalence, intensity, and ocular manifestations. To illustrate this, Budden (1963) reported than in forest settings of southern Cameroon, skin microfilarial infection was widespread (nearly all villages were affected) and prevalence in adults reached c.100%, yet intensity of infection in the skin and eyes was relatively low. By contrast, in savannah settings, highly exposed individuals would develop high intensities of cutaneous and ocular infection, and resulting ocular lesions. In support of these observations, Gbakima and Barbe (1992) reported that in five hyperendemic villages of Sierra Leone (overall microfilarial prevalence of 62%), the intensity of infection was low and microfilarial invasion of the eye was also low, with corresponding low prevalence of anterior chamber lesions (although that of posterior chamber lesions was relatively high, mainly comprising optic atrophy and choroidoretinitis), with an overall prevalence of blindness of 1.6%.

We, therefore, recommend that transmission models be parameterised and fitted to epidemiological and entomological forest onchocerciasis data (e.g. Duke et al., 1972; Barbiero and Trpis, 1984a, 1984b; Bockarie and Davies, 1990; Bockarie et al., 1990; Fryauff and Trpis, 1987; Trpis, 2006; Wanji et al., 2015) as has been done for savannah data (Hamley et al., 2019), and that differences in age- and sex-specific profiles of exposure and infection across endemic settings be better understood. An approach that may be suitable for estimating exposure to blackfly bites is the development of anti-saliva antibody assays, such as used in sandflies and leishmaniasis (Kostalova et al., 2015; Quinell et al., 2018). Such assays, initially based on titres of antibody responses to crude blackfly salivary antigens, could be used in serological population studies across all age and sex groups and may provide useful
data for estimating age- and sex-dependent exposure as well as heterogeneity in individual-level exposure to blackfly bites. These assays may also be combined with those used for serological monitoring of exposure to *O. volvulus* infection (e.g. Ov16) to provide data that would permit interrogation of modelling assumptions regarding exposure and parasite establishment in forest, forest–savannah mosaic, and savannah settings to better inform onchocerciasis control and elimination efforts across Africa.

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**List of Abbreviations**

**ABR**, annual biting rate; **ANOVA**, Analysis of variance; **APOC**, African Programme for Onchocerciasis Control; **ATS**, Alternative treatment strategies; **asin(sqrt)**, arcsine transformation, also called the arcsine square root transformation, or angular transformation, **ATP**, annual transmission potential; **bp**, base pairs; **CMFL**, community microfilarial load; **CT**, computerised tomography; **DALY**, disability-adjusted life years; **DNA**, deoxyribonucleic acid; **EOT**, elimination of transmission; **EPHP**, elimination as a public health problem; **GBD**, Global Burden of Disease; **GLM**, generalised linear model; **ITS**, internal transcribed spacer; **mf/mg**, microfilariae (of *O. volvulus*) per milligram of skin;
mf/ml, microfilariae (of *Loa loa*) per millilitre of blood; mf/snip, microfilariae (of *O. volvulus*) per skin snip; mg, milligram; L1, sausage-stage larva; L2, pre-infective larva; L3, infective larva; MDA, mass drug administration; NTD, neglected tropical disease; OCP, Onchocerciasis Control Programme in West Africa; rDNA, ribosomal DNA; RFLP, restriction fragment length polymorphism; SAE, severe adverse events (severe drug reactions); S.E., standard error; s.l., *sensu lato*; s.str., *sensu stricto*; TaNT, test (for loiasis microfilaraemia levels) and not treat (with ivermectin); TTd, test (for onchocerciasis) and treat (with doxycycline); UI, uncertainty interval; WHO, World Health Organization; YLD, years lived with disability; YLL, years of life lost; %mf, prevalence of (*O. volvulus*) microfilaridermia.

REFERENCES


Revisiting the Strain Hypothesis in Onchocerciasis


Revisiting the Strain Hypothesis in Onchocerciasis


Revisiting the Strain Hypothesis in Onchocerciasis


Revisiting the Strain Hypothesis in Onchocerciasis


Revisiting the Strain Hypothesis in Onchocerciasis


Revisiting the Strain Hypothesis in Onchocerciasis


**Table 1** Ocular manifestations of trachoma, loiasis and onchocerciasis

<table>
<thead>
<tr>
<th></th>
<th>Trachoma (bacterium: <em>Chlamydia trachomatis</em>)</th>
<th>African eyeworm (filarial nematode: <em>Loa loa</em>)</th>
<th>River blindness (filarial nematode: <em>Onchocerca volvulus</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mode of transmission</strong></td>
<td>Chronic follicular keratoconjunctivitis spread from eye to eye by <em>Musca sorbens</em> flies (mechanical vectors), fingers, fomites, and possibly by coughing and sneezing</td>
<td>Chronic filarial infection transmitted from person to person via the bites of tabanid flies of the genus <em>Chrysops</em> (biological vectors). Main vectors among humans are <em>Ch. silacea</em> and <em>Ch. dimidiata</em></td>
<td>Chronic filarial infection transmitted from person to person via the bites of blackflies of the genus <em>Simulium</em> (biological vectors). Main vectors in Africa are <em>S. damnosum s.l.</em> and <em>S. neavei s.l.</em></td>
</tr>
</tbody>
</table>
| **Disease mechanism and ocular manifestations** | Repeated infections cause chronic inflammation, particularly of the tarsal conjunctiva underlying the upper eyelid producing scarring that leads to eye lashes turning inwards (trichiasis) and rubbing against the cornea, damaging the corneal surface, and leading to corneal opacification and blindness | Passage of the adult worm under the (bulbar or palpebral) conjunctiva of the eye. Migration of the worm under bulbar conjunctiva ranges from 0.5 – >24 hr; accompanied by eye congestion, pruritus and photophobia. Less frequent passage under palpebral conjunctiva is associated with palpebral or periorbital oedema | Disease mechanism and ocular manifestations

*O. volvulus* microfilariae can be found in all eye tissues, from the conjunctiva anteriorly, to the optic nerve posteriorly. Eye disease worsens progressively with parasite exposure and eventually leads to blindness. Ocular lesions range from punctate and sclerosing keratitis (anterior segment) to optic nerve atrophy (posterior segment). Anterior chamber lesions result from repeated inflammatory processes triggered by filarial and *Wolbachia* products released by dying microfilariae. Pathogenesis of retinal lesions may result from autoimmune processes elicited by cross-reactivity between *O. volvulus* antigen Ov39 and human retinal antigen hr44 |
| **Grading and ocular disease progression** | **Simplified WHO Grading System:** Five or more follicles | **Atypical ocular manifestations**

*L. loa* adult worms can be found in anterior (Pan)juveitis, iridocyclitis, vitreous haze, | **Anterior and posterior eye involvement**

MFAC: Microfilariae in anterior chamber | Microfilariae can be seen with a slit lamp in...
<table>
<thead>
<tr>
<th>TF: Trachomatous inflammation follicular</th>
<th>chamber of the eye or extending from anterior to posterior chamber</th>
</tr>
</thead>
<tbody>
<tr>
<td>of &gt;0.5 mm on the upper tarsal conjunctiva</td>
<td>corneal oedema, corneal opacity, macular chorioretinitis, macular degeneration, retinal detachment, unilateral and bilateral blurring/reduction/loss of vision</td>
</tr>
<tr>
<td></td>
<td>the aqueous humour, sticking to the inner surface of the cornea, the endothelium, and in severe cases in the bottom of the anterior chamber, forming a mass, called pseudo-hypopyon. Microfilariae can also penetrate the iris and cause chronic anterior uveitis. This can lead to blindness due to secondary glaucoma or cataract. The pupil shrinks, deforms and sticks to the lens (posterior synechiae)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TI: Trachomatous inflammation intense</th>
<th>Papillary hypertrophy and inflammatory thickening of the upper tarsal conjunctiva obscuring more than half the deep tarsal vessels</th>
</tr>
</thead>
<tbody>
<tr>
<td>PK: Punctate keratitis</td>
<td>Microfilariae can invade the cornea, where as they die, provoke punctuate keratitis, a white (fluffy) inflammatory reaction</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TS: Trachomatous scarring</th>
<th>Presence of scarring in tarsal conjunctiva</th>
</tr>
</thead>
<tbody>
<tr>
<td>SK: Sclerosing keratitis and CO: corneal opacity</td>
<td>It begins at the 3 and 9 o’clock positions of the eye, gradually filling in from the bottom, creating a semilunar</td>
</tr>
</tbody>
</table>
Revisiting the Strain Hypothesis in Onchocerciasis

TT: Trachomatous trichiasis
≥ 1 ingrown eyelash touching the globe; evidence of epilation (eyelash removal)

CO: Corneal opacity
Corneal opacity blurring part of the pupil margin; eventually leading to blindness

Posterior segment
Microfilariae can be seen in the vitreous. The most serious aspects of posterior segment disease are chronic chorioretinitis and optic nerve atrophy. The macula (central vision) is initially spared, but eventually central vision is lost. Optic nerve atrophy leads to blindness


Table 2 Data sources according to country, habitat, vectors, annual transmission potential (ATP), number of people examined, standardised microfilarial prevalence, percent bilateral blindness, causes of blindness and references

<table>
<thead>
<tr>
<th>Country</th>
<th>Habitat</th>
<th>dam</th>
<th>sirh</th>
<th>squa</th>
<th>south</th>
<th>leon</th>
<th>beffa</th>
<th>sanu</th>
<th>yah</th>
<th>max</th>
<th>ATP</th>
<th>No. people exam.</th>
<th>No. +ve for mf</th>
<th>mf prev. (%mf)</th>
<th>Stand. mf prev.</th>
<th>No. people exam. for blindness</th>
<th>No. blind</th>
<th>% blind</th>
<th>Stand. % bilateral blindness</th>
<th>Causes of blindness</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benin</td>
<td>mosaic</td>
<td>1</td>
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**All causes**:

- **Onchocerciasis**
- **All other causes**

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Mas (1995), Cheke et al. (2006), De Sole et al. (1992), Zimmerman et al. (1992), Frentzel-Beyme (1973)
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Frentzel-Beyme (1973)  
Frentzel-Beyme (1973)  
Frentzel-Beyme (1973)  
Frentzel-Beyme (1973)  
Frentzel-Beyme (1973)  
Frentzel-Beyme (1973)  
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Umeh et al. (1996)  
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Zimmerman et al. (1992)  
Zimmerman et al. (1992)  
Zimmerman et al. (1992)  
McMahon et al. (1988)
Revisiting the Strain Hypothesis in Onchocerciasis

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dam = S. damnosum s.str.; sirb = S. sirbanum; squa = S. squamosum; soub = S. soubrense; leon = S. leonense; beffa = Beffa form of S. soubrense; sanc = S. sanctipauli s.l.; yah = S. yahense; men = S. mengense; with 1 = cytoform present; zeroes = cytoform absent; +ve = positive; ATP = annual transmission potential (no. of L3/person/year); exam. = examined; mf = microfilariae; oncho = onchocerciasis; prev. = prevalence (in %); Stand. = standardised; *denotes blindness prevalence which is reported here as ‘bilateral’ blindness’ but for which the authors did not specify if the values refer to bilateral blindness or not (these data were omitted in a subsequent analyses which did not yield different results; see 3.3. Bilateral blindness, in the main text).
Table 3 The percentages of microfilariae (mf) that had emerged after different durations of incubation as a percentage of the total that emerged after 24 hours and the percentages of positive skin snips detected after different durations as a percentage of the total number positive after 24 hours (Collins et al., 1980).

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The data shown correspond to Study I of Collins et al. (1980), in which snips were incubated on microscope slides. After incubation and enumeration of emerged microfilariae, snips were fixed in 10% formalin, serially sectioned at 7µ, stained with haematoxylin and eosin, and the microfilariae remaining in the tissue were counted.
Revisiting the Strain Hypothesis in Onchocerciasis

Figure Legends

Figure 1 (A) Vector (female Simulium damnosum s.l.) and (B) life cycle of Onchocerca volvulus; (C–F) disease manifestations associated with onchocerciasis: (C) head nodule in a child; (D) chronic uveitis with secondary cataract in right eye and sclerosing keratitis in left eye; (E) child leading a blind man; (F) skin depigmentation in the shin (leopard skin). In addition to skin and eye disease, onchocerciasis is also associated with human excess mortality and epilepsy (Little et al., 2004b; Walker et al., 2012; Colebunders et al., 2019). Credits: (A) Rolf Garms; (B) Basáñez et al. (2006) for life cycle and Maria-Gloria Basáñez for microfilariae, L1 larva and L3 larva; (C & F) Simon J. O’Hanlon; (D) Adrian D. Hopkins; (E) Poppy H.L. Lamberton.

Figure 2 Prevalence of blindness (%) according to habitat. (A) raw data with no weights: mean percentage for forest = 2.02 ± 0.36 (S.E.), mosaic = 2.04 ± 0.58, savannah = 2.81 ± 0.47; (B) raw data weighted by sample sizes: mean percentage for forest = 1.76 ± 0.37, mosaic = 1.49 ± 0.38, savannah = 1.89 ± 0.26; (C) angular-transformed data with no weights: mean de-transformed percentage blindness for forest = 1.41 ± 0.30, mosaic = 1.25 ± 0.46, savannah = 1.92 ± 0.47; (D) angular-transformed data, weighted by sample size: mean de-transformed percentage blindness for forest = 1.39 ± 0.32; mosaic = 0.99 ± 0.28; savannah = 1.50 ± 0.24. Error bars are standard errors.

Figure 3 De-transformed weighted mean blindness prevalence for forest, mosaic and savannah habitats based on blindness due to onchocerciasis only. Mean de-transformed blindness prevalence for forest = 0.79 ± 0.36 (S.E.), mosaic = 0.84 ± 0.26, savannah = 1.19 ± 0.23. Error bars are standard errors.

Figure 4 Box-and-whisker plots for the prevalence of blindness in forest habitats only according to country. The horizontal dark black lines within the boxes represent the median blindness prevalence for each country; the lower and upper borders of the box are the lower
(25%) and upper (75%) quartiles of the data; the whiskers represent the minimum and maximum values; marker (open circle in Liberia) represents an outlier.

**Figure 5** Relationships between annual transmission potential (ATP, no. L3/person/year) and blindness prevalence for (A) forest including data from southern Côte d'Ivoire; (B) forest excluding data from southern Côte d'Ivoire; (C) savanna. Dotted lines are 95% confidence intervals. Note logarithmic scale on the x-axis.

**Figure 6.** Relationships between prevalence of microfilariae and prevalence of blindness for (A) forest including data from southern Côte d'Ivoire; (B) forest excluding data from southern Côte d'Ivoire; (C) savanna. Dotted lines are 95% confidence intervals.