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Indices of onchocerciasis transmission by different members of the *Simulium damnosum* complex conflict with the paradigm of forest and savanna parasite strains

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ABSTRACT

Onchocerciasis in savanna zones is generally more severe than in the forest and pathologies also differ geographically, differences often ascribed to the existence of two or more strains and incompatibilities between vectors and strains. However, flies in the forest transmit more infective larvae than their savanna counterparts, even in sympatry, contradicting expectations based on the forest and savanna strains paradigm. We analysed data on the numbers of *Onchocerca volvulus* larvae of different stages found in 10 different taxonomic categories of the *Simulium damnosum* complex derived from more than 48,800 dissections of flies from Sierra Leone in the west of Africa to Uganda in the east. The samples were collected before widespread ivermectin distribution and thus provide a baseline for evaluating control measures. Savanna species contained fewer larvae per infected or per infective fly than the forest species, even when biting and parous rates were accounted for. The highest transmission indices were found in the forest-dwelling Pra form of *Simulium sanctipauli* (616 L3/1000 parous flies) and the lowest in the savanna-inhabiting species *S. damnosum*/*S. sirbanum* (135) and *S. kilibanum* (65). Frequency distributions of numbers of L1–2 and L3 larvae found in parous *S. damnosum*/*S. sirbanum*, *S. kilibanum*, *S. squamosum*, *S. yahense*, *S. sanctipauli*, *S. leonense* and *S. soubrense* all conformed to the negative binomial distribution, with the mainly savanna-dwelling species (*S. damnosum*/*S. sirbanum*) having less overdispersed distributions than the mainly forest-dwelling species. These infection patterns were maintained even when forest and savanna forms were sympatric and biting the same human population. Furthermore, for the first time, levels of blindness were positively correlated with infection intensities of the forest vector *S. yahense*, consistent with relations previously reported for savanna zones. Another novel result was that conversion rates of L1–2 larvae to L3s were equivalent for both forest and savanna vectors. We suggest that either a multiplicity of factors are contributing to the observed disease patterns or that many parasite strains exist within a continuum.

1. Introduction

Onchocerciasis, caused by the nematode worm *Onchocerca volvulus*, is spread from person to person in much of sub-Saharan Africa by the blackfly *Simulium damnosum* s.l. It has been estimated that in 37 countries worldwide 120,000,000 people are at risk of infection, 99% of whom live in Africa where at least 37,000,000 people are infected (Amazigo et al., 2006; Hotez and Kamath, 2009; WHO, 2009). Symptoms of the disease vary from mild itching caused by sub-dermal microfilariae, serious skin conditions such as leopard skin, sowda and depigmentation, to eye diseases including bilateral blindness. The severe outcome of blindness led to the abandonment of villages, particularly in savanna areas of West Africa, and so extensive areas became unsuitable for agriculture and village life.

The severity of the disease is usually worse in savanna when compared with the pathology of onchocerciasis in the forest, with more microfilariae present in the cornea and anterior chamber of the eye, more anterior uveitis, choroidal retinitis and sclerosing keratitis (Budden, 1963). In a comparison between Sudan-savanna and rain-forest onchocerciasis in Cameroon, Anderson et al. (1974) found more nodules per person and more depigmentation in the forest but less bilateral blindness (2% versus 5.1%). The more severe ocular complications were also reflected in the higher savanna rates for sclerosing keratitis (6.6% in savanna, 1.6% in forest) and iritis. In general, there are more irreversible ocular complications due to

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involvement of the anterior segment of the eye in savanna zones, whereas in the forest anterior lesions are rarer, with the posterior segment more often affected and with some lesions reversible. In the savanna the severity of symptoms such as sclerosing keratitis, advanced iridocyclitis, post-neuritic optic atrophy and choroido-retinitis are related to the intensity of the infection as measured by the Community Microfilarial Load (CMFL) (Remme et al., 1989), but in the forest there is no such clear relationship with CMFL (Dadzie et al., 1990).

The vector *S. damnosum* s.l. is a complex of approximately 60 different sibling species and cytoforms (Adler et al., 2010; Adler and Crosskey, 2011), originally described on the basis of chromosomal variation (Vajime and Dunbar, 1975), which differ in their geographical distribution, ecologies, degree of anthropophily and vectorial efficiency (Adler et al., 2010). The flies' immature stages from egg to pupae are aquatic, with the larvae reliant on fast-flowing stretches of streams and rivers. Emerging female adults seek host blood meals when they may acquire infections by ingesting microfilariae from an infected person. The aquatic stages are targets for vector control achieved by applying larvicides to selected sections of rivers.

Duke and colleagues developed the concept of *Onchocerca–Simulium* complexes involving forest and savanna strains of the parasite (Duke, 1966, 1967, 1970; Duke et al., 1966, 1967; Lewis and Duke, 1966) because (a) patterns of the disease's pathology differed between samples from populations infected in savanna zones of West Africa from those in the forest (see above); (b) the vectors also differed according to bioclimatic zone, and (c) experiments involving taking vectors and patients from one zone to another revealed incompatibilities between vectors and parasites from different zones. Additional support came from experiments involving injecting microfilariae from both forest- and savanna-based patients into rabbits and examining the subsequent pathology (Duke and Anderson, 1972; Duke and Garner, 1973; Garner et al., 1973) and from vector studies showing differences in vectorial efficiencies of forest and savanna flies (Philippon, 1977).

The greater severity of the disease and the higher blindness rates in the savanna were reasons why the insecticidal treatments of the WHO Onchocerciasis Control Programme in West Africa (OCP) were initially restricted to the savanna zones (WHO, 1987). Epidemiological data from the OCP also showed that ocular manifestations of the disease were more severe in the savanna than in forest (Dadzie et al., 1989, 1990; Remme et al., 1989; Zimmerman et al., 1992). However, there were also reports of blindness at high levels (e.g. 3.4% in southern Togo) in intermediate forest-savanna mosaic areas (De Sole et al., 1991, 1992; Umeh et al., 1996; Pion et al., 2002), which were influential in decisions to expand the OCP area. Furthermore, the East African forms and that from the Abu Hamed focus in Sudan are not assignable to either typical forest or savanna strains (Fischer et al., 1996; Higazi et al., 2001) and even in West Africa variation in the prevalence of blindness does not accord neatly with the two strains hypothesis (Little et al., 2003). Furthermore there are epidemiological data revealing high blindness rates in forest zones in, for instance, Liberia (up to 8.9%, Frentzel-Beyme, 1973; and 2.4% blind in one or both eyes, Newland et al., 1991) and Cameroon (9 blind eyes found amongst 88 people at Bombe, giving 5.7% assuming a minimum of 5 people involved, Duke et al., 1972) and in deforested areas of Sierra Leone (5.5%, Zimmerman et al., 1992). Thus, there may be a spectrum of different strains as suggested by Fischer and Büttner (2002). They wrote "It appears reasonable to conclude that several different strains of *O. volvulus* occur throughout its large distribution area, but strain differences are not sufficient to explain all the geographic variation of the disease. The human host, biting habits of the vector or environmental factors may also influence the clinical picture of onchocerciasis."

The situation is further complicated by anthropogenic changes such as deforestation that have allowed savanna forms of the fly to penetrate forest areas previously inimical to them in Liberia (Garms et al., 1991), Ghana and Togo (Wilson et al., 2002), Sierra Leone (Baker et al., 1990; Thomson et al., 1996) and Côte d'Ivoire (Adjami et al., 2004). Furthermore, members of the *S. damnosum* complex, but especially the savanna species *S. damnosum* s.str. and *S. sirbanum* undertake long distance movements up to 500 km (Garms et al., 1979; Cheke and Garms, 1983; Baker et al., 1990) and are thus able to bring *O. volvulus* back into areas where it has been thought to be eliminated or under control.

When the OCP began, all interventions relied on vector control but the advent of the microfilaricidal drug ivermectin shifted attention towards the importance of chemotherapy which became an adjunct to, and then a replacement for, vector control. Indeed the successor to OCP, the African Programme for Onchocerciasis Control (APOC) extended the area where onchocerciasis control by chemotherapy was conducted into many forested areas, in recognition of the importance of onchocerciasis even in areas without onchocercal blindness and, finally, APOC is now seeking to eliminate the disease. This ambition is currently controversial in view of increasing evidence of people who do not respond appropriately to the drug, raising fears of incipient resistance (Awadzi et al., 2004a,b; Osei-Atweneboana et al., 2007, 2011; Churcher et al., 2009).

Monitoring the success of ivermectin distribution campaigns has relied on clinical data but it has also been important to check whether any transmission by the vectors is continuing or not in treated areas. In Africa, ivermectin distribution has not led to rapid declines in transmission rates (e.g. see Kutin et al., 2004; Cheke et al., 2006), except in areas that had relatively low disease prevalence at the outset of the chemotherapy (e.g. Diawara et al., 2009). Some of the entomological monitoring that has occurred often relied, regrettably, on PCR-based tests on pooled samples of insects from which detailed transmission indices cannot be calculated.

Any evidence of reductions in transmission can only be confirmed by comparisons with pre-control data. One of the purposes of this paper is to present a review of data on onchocerciasis transmission by different forms of the vector over a wide geographical area in West Africa, and for one focus in East Africa, based on fly samples that we have dissected ourselves, to provide a baseline against which future control activities can be used for comparisons. In addition, during the course of this examination of our data we uncovered previously unrecognised links between transmission indices of the flies and blindness in forest areas. We also re-examine a previous report that both types of vectors ("forest" and "savanna") may be involved in transmitting the disease sympatrically in a manner that we cannot explain in the context of the accepted forest-savanna dichotomy (Garms and Cheke, 1985). Also, our data confirm for some previously unstudied taxa that different vectors have their own typical parasite distribution characteristics which are independent of location and are retained even when a "savanna" fly is sympatric with a "forest" fly. Thus *S. yahense* and members of the *S. sanctipauli* sub-complex tend to have higher mean parasite loads than *S. damnosum*/*S. sirbanum*. The forest forms also have some flies that retain high numbers of parasites, which *S. damnosum*/*S. sirbanum* do not, and the parasite burdens of *S. squamosum* are intermediate between these two groups. The numbers of infective larvae transmitted by *S. yahense* and by most members of the *S. sanctipauli* sub-complex are higher than those transmitted by *S. damnosum*/*S. sirbanum*, yet the latter are responsible for spreading the so-called "blinding" onchocerciasis in savanna zones. This paradox was explained by the two strains hypothesis but it breaks down, as we shall show later, in forest areas where there is much blinding onchocerciasis transmitted by *S. yahense*, for example, and in forest-savanna mosaic zones with high blindness rates where, for instance, the Beffa form of *S. soubrense* retains its high parasite